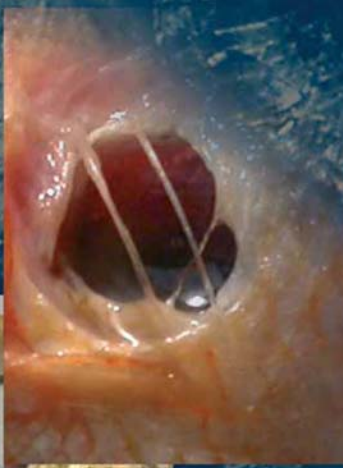
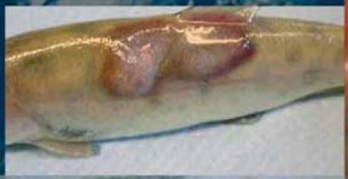
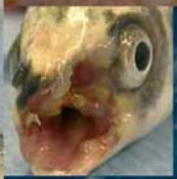
Fourth Edition

BACTERIAL FISH PATHOGENS

Diseases of Farmed and Wild Fish







Brian Austin
Dawn Austin

Springer

PRAXIS

Bacterial Fish Pathogens

Diseases of Farmed and Wild Fish

B. Austin and D. A. Austin

Bacterial Fish Pathogens

Diseases of Farmed and Wild Fish

Fourth Edition

Springer

Published in association with
Praxis Publishing
Chichester, UK

Professor B. Austin
School of Life Sciences
John Muir Building
Heriot-Watt University
Riccarton
Edinburgh
UK

Dr D. A. Austin
Research Associate
Heriot-Watt University
Riccarton
Edinburgh
UK

SPRINGER–PRAXIS BOOKS IN AQUATIC AND MARINE SCIENCES
SUBJECT *ADVISORY EDITOR*: Dr Peter Dobbins Ph.D., CEng., F.I.O.A., Senior Consultant, Marine Devision, SEA, Bristol, UK

ISBN 978-1-4020-6068-7 Springer Dordrecht Berlin Heidelberg New York

Springer is part of Springer-Science + Business Media (springer.com)

A catalogue record of this book is available from the Library of Congress

Printed in Germany

Cover design: Jim Wilkie
Project management: Originator Publishing Services Ltd, Gt Yarmouth, Norfolk, UK

Printed on acid-free paper

Contents

Preface

This fourth edition of *Bacterial Fish Pathogens* is the successor to the original version, first published by Ellis Horwood Limited in 1987, and was planned to fill the need for an up-to-date comprehensive text on the biological aspects of the bacterial taxa which cause disease in fish. The impetus to prepare a fourth edition stemmed initially from discussion with Chinese colleagues when it became apparent that the book was particularly well used and cited (>1,600 citations in China since 1999). Since publishing the third edition, there has been a slowing down in the number of new fish pathogens. However, there has been a steady increase in the number of publications about some aspects of bacterial fish pathogens, including the application of molecular techniques to diagnosis and pathogenicity studies. Consequently, we considered that it is timely to consider the new information in a new edition. The task was made immeasurably easier by the ready availability of electronic journals, which could be accessed from the office. Weeks of waiting for inter-library loans did not feature during the research phase of the project. Our strategy was to include information on new pathogens and new developments on well-established pathogens, such as *Aeromonas salmonicida* and *Vibrio anguillarum*. Because of the deluge of new information, we have needed to be selective, and in particular, we have once again condensed details of the pathology of the diseases, because there are excellent texts already available that cover detailed aspects of the pathological conditions. Nevertheless, this fourth edition will hopefully meet the needs of the readership. As with all the preceding editions, it is emphasised that most of the information still appertains to diseases of farmed, rather than wild, fish.

The scope of the book covers all of the bacterial taxa that have at one time or another been reported as fish pathogens. Of course, it is realised that some taxa are merely secondary invaders of already damaged tissues, whereas others comprise serious, primary pathogens. Shortcomings in the literature or gaps in the overall understanding of the subject have been highlighted.

In preparing the text, we have sought both advice and material from colleagues. We are especially grateful to the following for the supply of photographs:

Dr. J.W. Brunt
Dr. H. Daskalov
Dr. G. Dear
Dr. T. Itano
Dr. V. Jencic
Dr. D.-H. Kim
Dr. A. Newaj-Fyzul
Dr. N. Pieters
Professor M. Sakai
Professor X.-H. Zhang

B. and D. A. Austin
Edinburgh, 2007

To Aurelia Jean

Colour plates

(see colour section between pp. 236 and 237)

4.1 *Aer. salmonicida* subsp. *salmonicida* producing brown, diffusible pigment around the colonies on TSA

6.1 The rainbow trout on the left has bilateral exophthalmia caused by *Ren. salmoninarum*. The second fish is a healthy specimen

6.2 A rainbow trout displaying haemorrhaging in the eye caused by infection with *Lactococcus garvieae*

6.3 A rainbow trout displaying extensive haemorrhaging in the mouth caused by ERM

6.4 A tilapia displaying haemorrhaging around the mouth caused by infection with *Aeromonas* sp.

6.5 Erosion of the mouth of a ghost carp. The aetiological causal agent was *Aer. bestiarum*

6.6 Erosion of the mouth of a carp. The aetiological causal agent was *Aer. bestiarum*

6.7 Erosion and haemorrhaging of the mouth of a ghost carp. The aetiological causal agent was *Aer. bestiarum*

6.8 A tilapia displaying haemorrhaging on the finnage caused by infection with *Aeromonas* sp.

6.9 Extensive erosion of the tail and fins on a rainbow trout. Also, there is some evidence for the presence of gill disease. The aetiological agent was *Aer. hydrophila*

6.10 A saddleback lesion characteristic of columnaris (causal agent = *Fla. columnare*) on a rainbow trout

6.11 A distended abdomen on a rainbow trout with BKD

6.12 Surface haemorrhaging and mouth erosion on a carp which was infected with *Aer. bestiarum*

6.13 Haemorrhagic lesions on the surface of a carp which was infected with *Aer. hydrophila*

6.14 Surface haemorrhaging on a tongue sole (*Cynoglossus semilaevis*) infected with *Edw. tarda*

6.15 Petechial haemorrhages on the surface of an eel with Sekiten-byo

6.16 Surface haemorrhaging on a grayling infected with BKD
6.17 Extensive surface haemorrhaging on a turbot with vibriosis
6.18 Haemorrhaging on the fins and around the opercula of a sea bass. The aetiological agent was *V. anguillarum*
6.19 An ulcer in its early stage of development on a Koi carp. The aetiological agent was atypical *Aer. salmonicida*
6.20 A well-developed ulcer on a Koi carp. The aetiological agent was atypical *Aer. salmonicida*
6.21 An ulcerated goldfish on which the lesion has extended across the body wall, exposing the underlying organs. The aetiological agent was atypical *Aer. salmonicida*
6.22 Carp erythrodermatitis. The aetiological agent is likely to be atypical *Aer. salmonicida*
6.23 An ulcer, caused by *Vibrio* sp., on the surface of olive flounder
6.24 Limited tail erosion and an ulcer on the flank of rainbow trout. The casual agent was considered to be linked to ultramicrobacteria
6.25 An extensive abscess with associated muscle liquefaction in the musculature of rainbow trout. The aetiological agent was *Aer. hydrophila*
6.26 A dissected abscess on a rainbow trout revealing liquefaction of the muscle and haemorrhaging. The aetiological agent was *Aer. hydrophila*
6.27 A furuncle, which is attributable to *Aer. salmonicida* subsp. *salmonicida*, on the surface of a rainbow trout
6.28 A dissected furuncle on a rainbow trout revealing liquefaction of the muscle
6.29 A blood blister on the surface of a rainbow trout with BKD
6.30 Extensive skin erosion around the tail of a rainbow trout. The cause of the condition was not proven
6.31 Mycobacteriosis in yellowtail. Extensive granulomas are present on the liver and kidney
6.32 Nocardiosis in yellowtail. Extensive granulomas are present on the liver and kidney
6.33 Swollen kidneys associated with BKD
6.34 Generalised liquefaction of a rainbow trout associated with infection by *Aeromonas*
6.35 An API-20E strip after inoculation, incubation and the addition of reagents. The organism was a suspected *Aeromonas*
6.36 An API-zym strip after inoculation, incubation and the addition of reagents. The organism is the type strain of *Ren. salmoninarum*
11.1 Red mark disease syndrome (= winter strawberry disease) in rainbow trout. The skin lesions do not usually penetrate to the underlying muscle
11.2 Red mark disease syndrome (= winter strawberry disease) in rainbow trout. With this form of the condition, scales and epidermal cells have been sloughed off
11.3 Red mark disease syndrome (= winter strawberry disease) in rainbow trout. The reddening is often seen in fish of >500 g in weight
11.4 The reddened area associated with red mark disease syndrome (= winter strawberry disease) in >500 g rainbow trout
11.5 The reddened area around the vent associated with red mark disease syndrome (= winter strawberry disease) in >500 g rainbow trout.

Tables

Abbreviations and acronyms

Aer.	*Aeromonas*
AFLP	Amplified Fragment Length Polymorphism
AHL	Acylated Homoserine Lactone
A-layer	The additional surface layer of *Aer. salmonicida*
Arc.	*Arcobacter*
ARISA	Automated Ribosome Intergenic Spacer Analysis
ATCC	American Type Culture Collection, Rockville, Maryland
BHI	Brain Heart Infusion
BHIA	Brain Heart Infusion Agar
BKD	Bacterial Kidney Disease
BLIS	Bacteriocin-Like Substance
BMA	Basal Marine Agar
bp	base pair
Car.	*Carnobacterium*
CBB	Coomassie Brilliant Blue agar
CDC	Centers for Disease Control and Prevention, Atlanta, Georgia
CE	Carp Erythrodermatitis
CFU	Colony-Forming Unit
CgP	Cytidine-phosphate-Guanosine
Chrys.	*Chryseobacterium*
CHSE-214	CHinook Salmon Embryo 214 cell line
Cit.	*Citrobacter*
Cl.	*Clostridium*
CLB	*Cytophaga*-Like Bacteria
CLED	Cystine Lactose Electrolyte-Deficient agar
Cor.	*Corynebacterium*
CpG	Cytidine-phosphate-Guanosine

Cyt.	*Cytophaga*
DNA	DeoxyriboNucleic Acid
ECP	ExtraCellular Product
EDTA	Ethylene Diamine Tetraacetic Acid
Edw.	*Edwardsiella*
ELISA	Enzyme-Linked ImmunoSorbent Assay
En.	*Enterococcus*
Ent.	*Enterobacter*
EPC	Epithelioma Papulosum Cyprini (cell line)
ERM	Enteric RedMouth
Esch.	*Escherichia*
Eu.	*Eubacterium*
FAME	Fatty Acid Methyl Ester
FAT	Fluorescent Antibody Test
FCA	Freund's Complete Adjuvant
FIA	Freund's Incomplete Adjuvant
Fla.	*Flavobacterium*
Fle.	*Flexibacter*
G + C	Guanine plus Cytosine
GCAT	Glycerophospholipid : Cholesterol AcylTransferase
GFP	Green Fluorescent Protein
GMD	Glucose Motility Deeps
H.	*Haemophilus*
Haf.	*Hafnia*
HG	Hybridisation Group
hsp	heat shock protein
i.m.	intramuscular
i.p.	intraperitoneal
iFAT	indirect Fluorescent Antibody Test
IROMP	Iron-Regulated Outer Membrane Protein
ISR	Intergenic Spacer Region
IU	International unit
J.	*Janthinobacterium*
kb	kilobase
kDa	kiloDalton
KDM2	Kidney Disease Medium 2
LAMP	Loop-mediated isothermal AMPlification
LD_{100}	Lethal Dose 100%
LD_{50}	Lethal Dose 50%, i.e. the dose needed to kill 50% of the population
Lis.	*Listeria*
LPS	LipoPolySaccharide
MDa	megaDalton
MHC	Mueller–Hinton agar supplemented with 0.1% (w/v) L-cysteine hydrochloride

MIC	Minimum Inhibitory Concentration
MIS	Microbial Identification System
Mor.	*Moraxella*
mRNA	messenger RNA
MRVP	Methyl Red Voges Proskauer
msa	major soluble antigen (gene)
MSS	Marine Salts Solution
Myc.	*Mycobacterium*
NCBV	Non-Culturable But Viable
NCIMB	National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland
Nec.	*Necromonas*
Noc.	*Nocardia*
ODN	OligoDeoxyNucleotide
OMP	Outer Membrane Protein
ORF	Open Reading Frame
p57	57 kDa protein (of *Ren. salmoninarum*)
Pa.	*Pasteurella*
PAGE	PolyAcrylamide Gel Electrophoresis
PAP	Peroxidase–AntiPeroxidase enzyme immunoassay
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PFU	Plaque Forming Unit
Ph.	*Photobacterium*
PMSF	PhenylMethyl–Sulphonyl Fluoride
Pr.	*Providencia*
Ps.	*Pseudomonas*
QPCR	Quantitative Polymerase Chain Reaction
RAPD	Randomly Amplified Polymorphic DNA
Ren.	*Renibacterium*
RFLP	Restriction Fragment Length Polymorphism
RLO	*Rickettsia*-Like Organisms
ROS	Reactive Oxygen Species
RPS	Relative Percent Survival
rRNA	ribosomal RiboNucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RTFS	Rainbow Trout Fry Syndrome
RTG-2	Rainbow Trout Gonad-2 cell line
Sal.	*Salmonella*
SBL	Striped Bass Larvae
S_D	Dice coefficient
S-layer	Surface layer
SDS	Sodium Dodecyl Sulphate
Ser.	*Serratia*

SKDM	Selective Kidney Disease Medium
SSH	Suppression Subtractive Hybridisation
Sta.	*Staphylococcus*
Str.	*Streptococcus*
TCBS	Thiosulphate Citrate Bile Salts Sucrose Agar
TCID	Tissue Culture Infectivity Dose
TSA	Tryptone Soya Agar
TSB	Tryptone Soya Broth
V.	*Vibrio*
Vag.	*Vagococcus*
VAM	*Vibrio Anguillarum* Medium
vapA	virulence array protein gene A
VHH	*Vibrio harveyi* Haemolysin
VHML	*Vibrio harveyi* Myovirus-Like (bacteriophage)
Y.	*Yersinia*

About the authors

Brian Austin is Dean of the University (Science and Engineering) and Professor of Microbiology in the School of Life Sciences, Heriot-Watt University. From 1975 to 1978 he was Research Associate at the University of Maryland, U.S.A., and from 1978 to 1984 he was Head of Bacteriology at the Fish Diseases Laboratory in Weymouth, U.K. He joined Heriot-Watt University as a Lecturer in Aquatic Microbiology in 1984.

Professor Austin gained a B.Sc. (1972) in Microbiology, a Ph.D. (1975) also in Microbiology, both from the University of Newcastle upon Tyne, and a D.Sc. (1992) from Heriot-Watt University. He was elected F.R.S.A. and Fellow of the American Academy of Microbiology, and is a member of the American Society of Microbiology, Society of Applied Bacteriology, Society of General Microbiology, European Association of Fish Pathologists, and the U.K. Federation of Culture Collections; and has written previous books on bacterial taxonomy, marine microbiology, methods in aquatic bacteriology, methods for the microbiological examination of fish and shellfish, and pathogens in the environment.

Dawn Austin is a Research Associate at Heriot-Watt University, a position she has held since 1986. Prior to this she was Research Assistant at the University of Maryland (1977–1979), Lecturer in Microbiology, University of Surrey (1983–1984), and Research Fellow of the Freshwater Biological Association, The River Laboratory, Dorset (1984–85).

Dr Austin gained a B.S. (1974) from City College, The City University, New York; an M.S. (1979) and a Ph.D. (1982) both from the University of Maryland.

1

Introduction

Representatives of many bacterial taxa have, at one time or another, been associated with fish diseases. However, not all of these bacteria constitute primary pathogens. Many should be categorised as opportunistic pathogens, which colonise and cause disease in already damaged hosts. Here, the initial weakening process may involve pollution or a natural physiological state (e.g. during the reproductive phase) in the life cycle of the fish. There remains doubt about whether some bacteria should be considered as fish pathogens. In such cases, the supportive evidence is weak or non-existent. Possibly, such organisms constitute contaminants or even innocent saprophytes. However, it is readily apparent that there is great confusion about the precise meaning of disease. A definition, from the medical literature, states that:

> "... a disease is the sum of the abnormal phenomena displayed by a group of living organisms in association with a specified common characteristic or set of characteristics by which they differ from the norm of their species in such a way as to place them at a biological disadvantage ..."
>
> (Campbell *et al.*, 1979)

This definition is certainly complex, and the average reader may be excused for being only a little wiser about its actual meaning. Dictionary definitions of disease are more concise, and include "an unhealthy condition" and "infection with a pathogen [= something that causes a disease]". One conclusion is that disease is a complex phenomenon, leading to some form of measurable damage to the host. Yet, it is anticipated that there might be profound differences between scientists about just what constitutes a disease. Fortunately, infection by micro-organisms is one aspect of disease that finds ready acceptance within the general category of disease.

For his detailed treatise on diseases of marine animals, Kinne (1980) considered that disease may be caused by:

- genetic disorders;
- physical injury;
- nutritional imbalance;
- pathogens;
- pollution.

This list of possible causes illustrates the complexity of disease. An initial conclusion is that disease may result from biological (= *biotic*) factors, such as pathogens, and *abiotic* causes, e.g. the emotive issue of pollution. Disease may also be categorised in terms of epizootiology (Kinne, 1980), namely as:

- *Sporadic* diseases, which occur sporadically in comparatively small numbers of a fish population.
- *Epizootics*, which are large-scale outbreaks of communicable disease occurring temporarily in limited geographical areas.
- *Panzootics*, which are large-scale outbreaks of communicable disease occurring over large geographical areas.
- *Enzootics*, which are diseases persisting or re-occurring as low-level outbreaks in certain areas.

The study of fish diseases has concentrated on problems in fish farms (= aquaculture), where outbreaks either begin suddenly, progress rapidly often with high mortalities, and disappear with equal rapidity (= *acute* disease) or develop more slowly with less severity, but persist for greater periods (= *chronic* disease).

This text will deal with the diseases caused by bacteria. However, it is relevant to emphasise that disease is not necessarily caused by single bacterial taxa. Instead, there may well be synergistic interactions between two or more taxa. This possibility is often ignored by scientists. Then, there are the situations in which infectious diseases are suspected but not proven. An example includes red mark syndrome/disease (also known as winter strawberry disease) of rainbow trout in the U.K. where the causal agent is suspected—but not proven—to be bacterial of which *Fla. psychrophilum* or *Aer. hydrophila* are suspected to be the possible aetiological agent.

Disease is usually the outcome of an interaction between the host (= fish), the disease-causing situation (= pathogen) and external stressor(s) (= unsuitable changes in the environment; poor hygiene; stress). Before the occurrence of clinical signs of disease, there may be demonstrable damage to/weakening of the host. Yet all too often, the isolation of bacteria from an obviously diseased fish is taken as evidence of infection. Koch's Postulates may be conveniently forgotten.

So, what are the bacterial fish pathogens? A comprehensive list of all the bacteria, which have been considered to represent fish pathogens, has been included in Table 1.1 (see p. 4). Some genera, e.g. *Vibrio*, include many species that are acknowledged to be pathogens of freshwater and/or marine fish species. Taxa (highlighted by quotation marks), namely "*Catenabacterium*", "*H. piscium*" and "*Myxobacterium*" are of doubtful taxonomic validity. Others, such as *Pr. rettgeri* and *Sta. epidermidis*, are of questionable significance in fish pathology insofar as their recovery from diseased

animals has been sporadic. A heretical view would be that enteric bacteria (e.g., *Providencia*), comprise contaminants from water or from the gastro-intestinal tract of aquatic or terrestrial animals. Many of the bacterial pathogens are members of the normal microflora of water and/or fish. Others have been associated only with clinically diseased or covertly infected (asymptomatic) fish. Examples of these "obligate" pathogens include *Aer. salmonicida* and *Ren. salmoninarum*, the causal agents of furunculosis and bacterial kidney disease (BKD), respectively. In later chapters, it will be questioned whether or not bacteria should be considered as obligate pathogens of fish, at all. It is a personal view that the inability to isolate an organism from the aquatic environment may well reflect inadequate recovery procedures. Could the organism be dormant/damaged/senescent in the aquatic ecosystem; a concept which has been put forward for other water-borne organisms (Stevenson, 1978)?

It is undesirable that any commercially important species should suffer the problems of disease. Unfortunately, the aetiology of bacterial diseases in the wild is often improperly understood. Moreover, it seems that little if anything may be done to aid wild fish stocks, except, perhaps, by controlling pollution of the rivers and seas, assuming that when environmental quality deteriorates this influences disease cycles. In contrast, much effort has been devoted to controlling diseases of farmed fish.

Conclusion

- The list of fish pathogens has extended substantially since 1980. Current interest focuses on the vibrios, CLBs, mycobacteria and streptococci–lactococci.
- A question mark hangs over the significance of some organisms to fish pathology—are they truly pathogens or chance contaminants?
- There has been considerable improvement in the taxonomy of some groups (e.g., vibrios).
- There has been a shift from emphasis on culture-dependent to culture-independent techniques.
- Molecular methods have become commonplace in laboratories involved in the study of fish diseases.

Table 1.1. Bacterial pathogens of freshwater and marine fish

Pathogen	Disease	Host range	Geographical distribution
ANAEROBES			
"*Catenabacterium*" sp.	—	Grey mullet (*Mugil auratus*)	U.S.A.
Clostridiaceae representative			
Clostridium botulinum	Botulism	Redfish (*Sebastes* sp.) Salmonids	Denmark, England, U.S.A.
Eubacteriaceae representative			
Eubacterium tarantellae	Eubacterial meningitis	Striped mullet (*Mugil cephalus*)	U.S.A.
GRAM-POSITIVE BACTERIA— THE "LACTIC ACID" BACTERIA			
Carnobacteriaceae representative			
Carnobacterium piscicola	Lactobacillosis, pseudokidney disease	Salmonids	North America, U.K.
Enterococcaceae representatives			
Enterococcus (Streptococcus) faecalis subsp. *liquefaciens*	—	Rainbow trout (*Oncorhynchus mykiss*), catfish	Italy
Vagococcus salmoninarum	Lactobacillosis, pseudokidney disease, peritonitis, septicaemia	Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout	Australia, France, North America
Lactobacillaceae representative			
Lactobacillus spp.	Lactobacillosis, pseudokidney disease	Salmonids	North America, U.K.
Streptococcaceae representatives			
Lactococcus garvieae (= Enterococcus seriolicida)	Streptococcicosis/ streptococcosis	Many fish species	Australia, Europe, Israel, Japan, Saudi Arabia, Red Sea, South Africa, Taiwan, U.S.A.

Lactococcus piscium	Lactobacillosis, pseudokidney disease	Rainbow trout	North America
Streptococcus dysgalactiae	—	Amberjack (*Seriola dumerili*), yellowtail (*Seriola quinqueradiata*)	Japan
Streptococcus difficilis (= Str. agalactiae)	Meningo-encephalitis	Carp (*Cyprinus carpio*), rainbow trout, silver pomfret (*Pampus argenteus*), tilapia (*Oreochromis* spp.)	Israel, Kuwait, USA
Streptococcus iniae (= Str. shiloi)	Meningo-encephalitis, streptococcicosis/ streptococcosis	Various freshwater and coastal fish species	Australia, Bahrain, Europe, Israel, Japan, Saudi Arabia, South Africa, U.S.A.
Streptococcus milleri	—	Koi carp (*Cyprinus carpio*)	U.K.
Streptococcus parauberis	Streptococcicosis/ streptococcosis	Turbot (*Scophthalmus maximus*)	Spain
AEROBIC GRAM-POSITIVE RODS AND COCCI			
Renibacterium salmoninarum	Bacterial kidney disease (BKD; Dee disease; corynebacterial kidney disease)	Salmonids	Europe, Japan, North and South America
Bacillaceae representatives			
Bacillus spp.	Septicaemia; bacillary necrosis	Various freshwater fish species including catfish (*Pangasius hypophthalmus*)	Nigeria, Vietnam
Bacillus cereus	Branchio-necrosis	Carp (*Cyprinus* sp.), striped bass (*Morone saxatilis*)	U.S.A.
Bacillus mycoides	Ulceration	Channel catfish (*Ictalurus punctatus*)	Poland, U.S.A.
Bacillus subtilis	Branchio-necrosis	Carp	Poland

(*continued*)

Table 1.1 (*cont.*)

Pathogen	Disease	Host range	Geographical distribution
Corynebacteriaceae representatives			
Corynebacterium aquaticum	Exophthalmia	Striped bass	U.S.A.
Coryneform bacteria	"Corynebacteriosis"	Salmonids	England
Micrococcaceae representative			
Micrococcus luteus	Micrococcosis	Rainbow trout	England
Mycobacteriaceae representatives			
Mycobacterium spp. (*Myc. abscessus, Myc. anabanti, Myc. chelonei* subsp. *piscarium, Myc. fortuitum, Myc. gordonae, Myc. marinum, Myc. montefiorense, Myc. neoaurum, "Myc. piscium", "Myc. platypoecilus", Myc. pseudoshottsii, "Myc. ranae", "Myc. salmoniphilum", Myc. shottsii, Myc. scrofulaceum, Myc. simiae, Myc. smegmatis, Myc. ulcerans*	Mycobacteriosis (fish tuberculosis)	Most fish species	Worldwide
Nocardiaceae representatives			
Nocardia spp. (*Noc. asteroides, Noc. salmonicida, Noc. seriolae*)	Nocardiosis	Most fish species	Worldwide
Rhodococcus sp.	Ocular oedema	Chinook salmon (*O. tshawytscha*)	Canada
Rhodococcus erythropolis	?	Atlantic salmon	Norway, Scotland
Planococcaceae representative			
Planococcus sp.	—	Salmonids	England

Staphylococcaceae representatives			
Staphylococcus aureus	Eye disease	Silver carp (*Hypophthalmichthys molitrix*)	India
Staphylococcus epidermidis	—	Gilthead sea bream (*Sparus aurata*), red sea bream (*Chrysophrus major*), yellowtail (*Seriola quinqueradiata*)	Japan, Turkey
Staphylococcus warneri	Ulcerations	Rainbow trout	Spain
GRAM-NEGATIVE BACTERIA			
Aeromonadaceae representatives			
Aeromonas allosaccharophila	—	Elvers	Spain
Aeromonas bestiarum	—		U.S.A.
Aeromonas caviae	Septicaemia	Atlantic salmon (*Salmo salar*)	Turkey
Aeromonas hydrophila (= Aer. liquefaciens, Aer. punctata)	Haemorrhagic septicaemia, motile aeromonas septicaemia, redsore disease, fin rot	Many freshwater fish species	Worldwide
Aeromonas jandaei	—	Eel (*Anguilla* sp.)	Spain
Aeromonas salmonicida (subspecies *achromogenes, masoucida, salmonicida and smithia*) {= *Haemophilus piscium*}	Furunculosis, carp erythrodermatitis, ulcer disease	Salmonids, cyprinids, and marine species (dabs, cod)	Worldwide
Aeromonas sobria	—	Perch (*Perca fluvialitis*), gizzard shad (*Dorosoma cepedianum*)	Switzerland, U.S.A.
Aeromonas veronii biovar sobria	Epizootic ulcerative syndrome	African catfish (*Clarias gariepinus*), rajputi (*Puntius gonionotus*), rui (*Labeo rohita*), catla (*Catla catla*), shole (*Channa striatus*)	Bangladesh

(*continued*)

Table 1.1 (*cont.*)

Pathogen	Disease	Host range	Geographical distribution
Alteromonadaceae representative			
Pseudoalteromonas piscicida	Egg disease	Damselfish	U.S.A.
Shewanella putrefaciens	Septicaemia	Rabbit fish (*Siganus rivulatus*)	Saudi Arabia
Campylobacteriaceae representative			
Arcobacter cryaerophilus	—	Rainbow trout	Turkey
Enterbacteriaceae representatives			
Citrobacter freundii	—	Salmonids, sunfish (*Mola mola*), carp (*Cyprinus carpio*)	Europe, India, U.S.A
Edwardsiella ictaluri	Enteric septicaemia of catfish	Brown bullhead (*Amieurus nebulosus*), channel catfish, freshwater catfish (*Pangasius hypophthalmus*), danio (*Danio devario*), striped catfish (*Pangasius hypophthalmus*)	Indonesia, U.S.A., Vietnam
Edwardsiella tarda (*Paracolobactrum anguillimortiferum, Edw. anguillimortifera*)	Redpest, edwardsiellosis, emphysematous putrefactive disease of catfish	Various freshwater fish species	Japan, Spain, U.S.A.
Escherichia vulneris	Septicaemia	Various freshwater fish species	Turkey
Hafnia alvei	Haemorrhagic septicaemia	Cherry salmon (*O. masou*), rainbow trout	Bulgaria, England, Japan
Klebsiella pneumoniae	Fin and tail disease	Rainbow trout	Scotland
Plesiomonas shigelloides	—	African catfish (*Heterobranchus bidorsalis*), eel, gourami (*Osphyronemus gourami*), rainbow trout, sturgeon (*Acipenser sturio*)	Germany, Portugal, Spain

Pantoea (= Enterobacter) agglomerans	—	Dolphin fish (*Coryphaena hippurus*)	U.S.A.
Providencia (Proteus) rettgeri	—	Silver carp	Israel
Salmonella enterica subsp. *arizonae (= Sal. choleraesuis* subsp. *arizonae = Sal. arizonae)*	Septicaemia	Piarucu (*Arapaima gigas*)	Japan
Serratia liquefaciens	Septicaemia	Arctic charr (*Salvelinus alpinus*), Atlantic salmon, turbot	France, Scotland, U.S.A
Serratia marcescens	—	White perch (*Morone americanus*)	U.S.A.
Serratia plymuthica	—	Rainbow trout	Poland, Scotland, Spain
Yersinia intermedia	—	Atlantic salmon	Australia
Yersinia ruckeri	Enteric redmouth (ERM), salmonid blood spot	Salmonids	Australia, Europe, North and South America
Flavobacteriaceae representatives			
Chryseobacterium balustinum (= Flavobacterium balustinum)	Flavobacteriosis	Marine fish	U.S.A.
Chryseobacterium scophthalmum (= Flavobacterium scophthalmum)	Gill disease, generalised septicaemia	Turbot	Scotland
Flavobacterium branchiophilum	Gill disease	Salmonids	Europe, Korea, Japan, U.S.A.
Flavobacterium columnare (= Flexibacter/Cytophaga columnaris)	Columnaris, saddleback disease	Many freshwater fish species	Worldwide
Flavobacterium hydatis (= Cytophaga aquatilis)	Gill disease	Salmonids	Europe, U.S.A.
Flavobacterium johnsoniae (= Cytophaga johnsonae)	Gill disease, skin disease	Barramundi (*Lates calcarifer*)	Australia, France
	Bacterial gill disease	Salmonids	Europe, U.S.A.

(*continued*)

Table 1.1 (*cont.*)

Pathogen	Disease	Host range	Geographical distribution
Flavobacterium psychrophilum (= *Cytophaga psychrophila*)	Coldwater disease, rainbow trout fry syndrome, necrotic myositis	Salmonids, sea lamprey (*Petromyzon marinus*)	Australia, Europe, Japan, North America
Tenacibaculum maritimum (= *Flexibacter maritimus*)	Bacterial stomatitis, gill disease, black patch necrosis	Many marine fish species	Europe, Japan, North America
Tenacibaculum ovolyticum (= *Flexibacter ovolyticus*)	Larval and egg mortalities	Halibut (*Hippoglossus hippoglossus*)	Norway
"Cytophaga rosea"	Gill disease	Salmonids	Europe, U.S.A.
Sporocytophaga sp.	Saltwater columnaris	Salmonids	Scotland, U.S.A.
Francisellaceae representative			
Francisella sp.	Granulomatous inflammatory disease	Atlantic cod (*Gadus morhua*), hybrid striped bass (*Morone chrysops* × *M. saxatilis*), three-line grunt (*Parapristipoma trilineatum*)	Japan, Norway, USA
Halomonadaceae representative			
Halomonas (= *Deleya*) *cupida*	—	Black sea bream (*Acanthopagrus schlegeli*)	Japan
Moraxellaceae representatives			
Acinetobacter sp.	Acinetobacter disease	Atlantic salmon, channel catfish	Norway, U.S.A.
Moraxella sp.	—	Striped bass	U.S.A.
Moritellaceae representatives			
Moritella marina (= *V. marinus*)	Skin lesions	Atlantic salmon	Iceland
Moritella viscosa	Winter ulcer disease/syndrome	Atlantic salmon	Iceland, Norway, Scotland

Mycoplasmataceae representative			
Mycoplasma mobile	Red disease	Tench (*Tinca tinca*)	U.S.A.
Myxococcaceae representative			
Myxococcus piscicola	Gill disease	Green carp (*Ctenopharyngodon idelluls*)	China
Neisseriaceae representative			
Aquaspirillum sp.	Epizootic ulcerative syndrome	Snakeheads (*Ophicephalus striatus*) and catfish (*Clarias batrachus*)	Thailand
Oxalobacteraceae representative			
Janthinobacterium lividum	Anaemia	Rainbow trout	Scotland
Pasteurellaceae representative			
Pasteurella skyensis	?	Atlantic salmon	Scotland
Photobacteriaceae representatives			
Photobacterium damselae subsp. *damselae (= Photobacterium histaminum)*	Vibriosis	Damsel fish (*Chromis punctipinnis*), redbanded sea bream (*Pagrus auriga*), sharks, turbot, yellowtail	Europe, U.S.A.
Photobacterium damselae subsp. *piscicida (= Pasteurella piscicida)*	Pasteurellosis, pseudotuberculosis	Bluefin tuna (*Thunnus thynnus*), gilt-head sea bream (*Sparus aurata*), sole (*Solea senegalensis*), striped bass (*Morone saxatilis*), white perch (*Roccus americanus*), yellowtail	Europe, Japan, U.S.A.
Piscirickettsiaceae representative			
Piscirickettsia salmonis	Coho salmon syndrome, salmonid rickettsial septicaemia	Salmon, sea bass (*Atractoscion nobilis*)	Canada, Chile, Greece, Norway, Scotland, U.S.A.

(*continued*)

Table 1.1 (*cont.*)

Pathogen	Disease	Host range	Geographical distribution
Pseudomonaceae representatives			
Pseudomonas anguilliseptica	Red spot (Sekiten-byo), winter disease	Rainbow trout, marine fish species, and particularly cod, eels (*Anguilla anguilla*, *A. japonica*), black spot sea bream (*Pagellus bogaraveo*), gilthead sea bream (*Sparus aurata*)	Finland, France, Japan, Portugal, Scotland, Spain
Pseudomonas chlororaphis	—	Amago trout (*Oncorhynchus rhodurus*)	Japan
Pseudomonas fluorescens	Generalised septicaemia	Most fish species	Worldwide
Pseudomonas plecoglossicida	Bacterial haemorrhagic ascites	ayu (*Plecoglossus altivelis*), pejerrey (*Odonthestes bonariensis*)	Japan
Pseudomonas pseudoalcaligenes	Skin ulceration	Rainbow trout	Scotland
Pseudomonas putida	Haemorrhagic ascites, ulceration	ayu, rainbow trout	Japan, Turkey
Vibrionaceae representatives			
Vibrio alginolyticus	Eye disease, septicaemia	Cobria (*Rachycentron canadum*), gilt-head sea bream, grouper (*Epinephelus malabanicus*), sea bream (*Sparus aurata*)	Asia, Europe, Israel
Vibrio anguillarum (= Listonella anguillara)	Vibriosis	Most marine fish species	Worldwide

V. cholerae (non-01)	Septicaemia	Ayu, goldfish (*Carassius aurata*)	Australia, Japan
V. fischeri	—	Gilt-head sea bream, turbot	Spain
V. furnissii	—	Eel	Spain
Vibrio harveyi (= *V. carchariae* and *V. trachuri*)	Eye disease (blindness), necrotising enteritis, vasculitis	Gilt-head sea bream, sea bass, common snook (*Centropomus undecimalis*), horse mackerel (*Trachurus japonicus*), milkfish, red drum (*Sciaenops ocellatus*), sharks (*Carcharhinus plumbeus*, *Negaprion breviorstris*), sole (*Solea senegalensis*), summer flounder (*Paralichthys dentatus*)	Europe (notably Spain), Japan, Taiwan, U.S.A.
V. ichthyoenteri	Intestinal necrosis	Japanese flounder (*Paralichthys olivaceus*), summer flounder	Japan, Korea, USA
V. logei	Skin lesions	Atlantic salmon	Iceland
V. ordalii	Vibriosis	Most marine fish species	Worldwide
V. pelagius	—	Turbot	Spain
V. salmonicida	Coldwater vibriosis, Hitra disease	Atlantic salmon	Canada, Norway, Scotland
V. splendidus	Septicaemia, vibriosis	Corkwing wrasse (*Symphodus melops*), gilt-head sea bream, turbot	Norway, Spain
V. tapetis	Vibriosis	Corkwing wrasse	Norway
V. vulnificus	Septicaemia, ovate pompano (*Trachinotus ovatus*)	Eel	Europe, Japan, P.R.C., U.S.A.
V. wodanis	Winter ulcer disease/syndrome	Atlantic salmon	Iceland, Norway, Scotland

(*continued*)

Table 1.1 (*cont.*)

Pathogen	Disease	Host range	Geographical distribution
Miscellaneous pathogens			
"*Candidatus* Arthromitus"	Summer enteritic syndrome	Rainbow trout	France, Spain
Streptobacillus	—	Atlantic salmon	Ireland
Unidentified	Gill lesions	Rockfish	Japan
Unidentified	*Varracalbmi*	Atlantic salmon	Norway
Unidentified	Ulceration	Rainbow trout	Scotland

Names in quotation marks are not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) or their supplements.

2

Characteristics of the diseases

ANAEROBES

The first report of botulism as a disease of fish was from Denmark, stemming from the work of Huss and Eskilden (1974). These workers showed that botulism was a chronic disease, termed "bankruptcy disease"', of farmed trout, with the causal agent recognised as *Cl. botulinum* type E. Subsequently, the disease was found on one farm of rainbow trout in Great Britain (Cann and Taylor, 1982, 1984) and was similarly identified among farmed coho salmon in the U.S.A. (Eklund *et al.*, 1982). Characteristic disease symptoms seemed to be very vague, but fish have been observed to exhibit sluggish, erratic swimming, appeared to be listless, and may alternately float and sink, before showing temporary rejuvenation. This pattern was repeated until death eventually ensued (Cann and Taylor, 1982).

Eubacteriaceae representative

Eubacterium tarantellae

The term "eubacterial meningitis" was coined for this disease (Winton *et al.*, 1983), which is a neurological condition, in which the infected fish display twirling until death results (Udey *et al.*, 1976). Cells of the organism may be readily observed in sections of brain tissue. There was little, if any, external pathology observed. It is interesting to note from Udey's work that some fish were also infected with other organisms, namely, trematodes (*Bucephalus* sp.) and *Vibrio* spp.; whereas ~20% possessed low numbers of *Myxosoma cephalus* spores in the brain cavity. Therefore, it is relevant to enquire whether this anaerobe represented a primary or secondary pathogen during the outbreak of disease in Biscayne Bay.

GRAM-POSITIVE BACTERIA—THE "LACTIC ACID" BACTERIA

According to Ross and Toth (1974), the abdomens of moribund fish were distended because of the presence of ascitic fluid. However, it was readily admitted that mortalities could not be directly attributed to the lactobacillus "pathogen". The subsequent report of Cone (1982) indicated that the condition was stress-mediated, insofar as it was recognised mostly in post-spawning fish. In these specimens, there was an accumulation of ascitic fluid in the peritoneal cavity, and extensive damage in the liver, kidney and spleen. Fin rot and other external signs of disease were absent. However, petechial haemorrhages in the muscle and hyperaemic air bladder were observed in some fish. The heart and gills appeared normal. According to Hiu *et al.* (1984), the disease occurred in fish >1 year old, which may have undergone stress, namely handling and spawning. Disease symptoms were varied, including septicaemia, the abdomen distended with ascitic fluid, muscle abscesses, blood blisters just beneath the skin and internal haemorrhaging.

Enterococcaceae representatives

Enterococcus (Streptococcus) faecalis subsp. *liquefaciens*

Although a questionmark remains over the accuracy of the identification of this pathogen, its inclusion is justified for the sake of completion. Also, this is the first indication of streptococci as fish pathogens in Croatia. Farmed brown bullhead (*Amiurus nebulosus*) developed deep ulcers predominantly between the dorsal and caudal fins. Internal organs displayed unspecified changes, and fluid was present in the digestive tract. There was haemorrhaging at the anus. Gram-positive cocci were observed in the kidney and liver (Teskeredzic *et al.*, 1993).

Vagococcus salmoninarum

In France, the organism was attributed to significant losses, i.e. up to 50% in a year, in rainbow trout farmed at low water temperature (Michel *et al.*, 1997). Disease signs included listless behaviour, impaired swimming, unilateral exophthalmia, external haemorrhages, petechial haemorrhages on the gills, and enlarged liver and spleen (Michel *et al.*, 1997) and peritonitis (Schmidtke and Carson, 1994).

Streptococcaceae representatives

Lactococcus garvieae

Infection of ayu with this pathogen may be exacerbated by prior infection/infestation with other organisms, such as blood flukes, that may weaken the host (Kumon *et al.*, 2002). Internal signs of disease were absent in golden shiners, although raised lesions were apparent on the body surface (Robinson and Meyer, 1966). Yellowtails were damaged in the liver, kidney, spleen and intestine, and there was a concomitant accumulation of ascitic fluid in the peritoneal cavity (Kusuda *et al.*, 1976a, 1991;

Ugajin, 1981). In rainbow trout, the disease was of sudden onset, and was described as a hyperacute systemic disease (Eldar and Ghittino, 1999). Marine fish showed pronounced enteritis, pale livers and blood in the peritoneal cavity, although the kidneys were apparently unaffected (Plumb *et al.*, 1974).

The pathogen appears to be spreading, and has been detected in wild Red Sea wrasse *Coris aygula* (Colorni *et al.*, 2003).

Lactococcus piscium

The disease condition encompasses lactobacillosis or pseudokidney disease of rainbow trout.

Streptococcus dysgalactiae

This Lancefield group C streptococcus was recovered in Japan from amberjack and yellowtail that had been previously vaccinated with a commercial vaccine against lactococcosis caused by *Lactococcus garvieae* and were displaying necrotic lesions of the caudal peduncle. Some fish revealed splenic hypertrophy (Nomoto *et al.*, 2004).

Streptococcus difficilis (= Str. agalactiae)

Str. difficilis was named as a result of an outbreak of disease in St. Peter's fish (*Tilapia*) and rainbow trout within Israel during 1986. The disease spread rapidly, and caused severe economic losses in the farmed fish (Eldar *et al.*, 1994). Diseased tilapia were lethargic, swam erratically, and showed signs of dorsal rigidity. In rainbow trout, the disease signs were consistent with a septicaemia, with brain damage (Eldar *et al.*, 1994). An organism, identified as *Str. agalactiae*, was recovered from diseased cultured silver pomfret in Kuwait (Duremdez *et al.*, 2004). Here, the disease signs included inappetence, lethargy, swollen abdomen, the stomach and intestine filled with a gelatinous or yellowish fluid, and in some fish slight haemorrhaging in the eye, exophthalmia and corneal opacity. Also, the liver was enlarged, there was evidence of congestion of the kidney and spleen, and fluid occurred in the peritoneal cavity (Duremdez *et al.*, 2004).

Streptococcus iniae (= Str. shiloi)

Str. shiloi was named as a result of an outbreak of disease in St. Peter's fish and rainbow trout within Israel during 1986. The disease spread rapidly, and caused severe economic losses in the farmed fish (Eldar *et al.*, 1994). Diseased tilapia were lethargic, swam erratically and showed signs of dorsal rigidity. In rainbow trout, the disease signs were consistent with a septicaemia, with brain damage including meningitis (Eldar *et al.*, 1994; Eldar and Ghittino, 1999). In separate developments, an organism, subsequently identified as *Str. iniae*, was diagnosed as causing disease in two tanks of hybrid striped bass in a commercial farm, using recirculating freshwater, in the U.S.A. (Stoffregen *et al.*, 1996), in white spotted rabbitfish (*Siganus canaliculatus*) in Bahrain (Yuasa *et al.*, 1999), in barramundi (*Lates calcarifer*) in Australia

(Bromage *et al.*, 1999) and in caged and wild fish from the Red Sea (Colorni *et al.*, 2002).

Str. iniae serotype II infection led to rainbow trout displaying lethargy, discoloration, loss of orientation, bilaterial exophthalmia, corneal opacity and haemorrhaging in the eye, and surface and internal (mostly in the spleen and fat around the intestine) haemorrhaging leading to death (Lahav *et al.*, 2004).

Streptococcus milleri

During 1992, Gram-positive chaining cocci and atypical *Aer. salmonicida* were recovered from newly imported Koi carp, which displayed pronounced surface ulcers of 4–20 mm in diameter on the flank or tail. Internal damage was not recorded (Austin and Robertson, 1993).

Streptococcus parauberis

This form of streptococcosis was originally recognised in farmed turbot (weight: 0.8–2 kg) from five sites in northern Spain during 1993 and 1994 (Doménech *et al.*, 1996). Overall, the farms, which used the same fish food, reported losses of 0.1–5%. Disease signs, which were more severe during summer, included weight loss, haemorrhaging on the anal and pectoral fins, petechial haemorrhages on the abdomen, bilateral exophthalmia, haemorrhaging and pus in the eyes, pale liver, congested kidney and spleen, ascites, and mucohaemorrhagic enteritis (Doménech *et al.*, 1996).

AEROBIC, GRAM-POSITIVE RODS AND COCCI

Renibacterium salmoninarum

In 1935, the disease was reported in the U.S.A., where it occurred in hatchery-reared brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) (Belding and Merrill, 1935). Additional evidence indicated the presence of BKD in chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*) and sockeye salmon (*O. nerka*) (Rucker *et al.*, 1951). Since then, the disease has been reported to occur in 13 species of salmonids in Canada, Chile (especially during transfer of chinook salmon from fresh to seawater; Uribe *et al.*, 1995), England, France, Germany, Iceland, Italy, Spain, the U.S.A. and Yugoslavia (see Fryer and Sanders, 1981; Hoffman *et al.*, 1984; Uribe *et al.*, 1995). During 1997, BKD was demonstrated for the first time in Denmark (Lorenzen *et al.*, 1997). Workers have highlighted the presence of the disease in farmed salmonid stocks but only occasionally has it been found in wild fish populations (Rucker *et al.*, 1951, 1953; Smith, 1964; Pippy, 1969; Evelyn *et al.*, 1973; Wood, 1974; Ellis *et al.*, 1978; Paterson *et al.*, 1979, 1981; Mitchum and Sherman, 1981; Banner *et al.*, 1986). The presence of BKD in Japanese farmed ayu from April to July 2001, possibly reflecting horizontal transmission from masu salmon, has been documented (Nagai and Iida, 2002). BKD may be exacerbated by or exacerbate other conditions: for

example, Weiland *et al.* (1999) documented that Chinook salmon that were traumatised with gas (= gas bubble trauma) died more rapidly than those fish that were only exposed to renibacterium alone. Wire-tagging appears to influence horizontal transmission of the disease insofar as the wounds in the snout caused by tagging can become infected with renibacteria, and lead to the spread to internal organs. Possibly renibacteria are spread with the tagging devices (Elliott and Pascho, 2001). External signs include exophthalmia, lesions in the eyes, swollen abdomen (full of ascitic fluid), blood-filled blisters on the flank and the presence of ulcers/abscesses (Fryer and Sanders, 1981; Hoffman *et al.*, 1984). Internally, lesions may develop in the kidney (this may become swollen), brain (= meningo-encephalitis; Speare, 1997), liver, heart and spleen. The lesions contain a fluidy mass of leucocytes, bacteria and cellular debris (Fryer and Sanders, 1981). A false membrane, covering some internal organs, has been described (Snieszko and Griffin, 1955; Bell, 1961). This membrane, the presence of which may be influenced by water temperature (Smith, 1964), consists of layers of fibroblasts and histiocytes, degenerating leucocytes with macrophages, and fibrin (Wolke, 1975). In Atlantic salmon, petechial haemorrhages have been described on the muscle lining the peritoneum (Smith, 1964). However, *Ren. salmoninarum* may be found in salmonids, e.g. charr and grayling in Alaska, without any evidence of clinical disease (Meyers *et al.*, 1993). Renibacterial cells have been found in intra-abdominal adhesions in Atlantic salmon and coho salmon that may have previously or were considered to have received adjuvant (= adjuvanted vaccine) intraperitoneally (Bruno and Brown, 1999). Evidence suggests an impact of the disease on appetite with infected fish consuming less than their less diseased counterparts (Pirhonen *et al.*, 2000).

Bacillaceae representatives

Bacillus spp.

The initial outbreak of disease led to mortalities of 10–15% of the fish in earthern ponds (Oladosu *et al.*, 1994). Diseased fish were characterised by weakness, lethargy, emaciation and generalised necrotising dermatitis, with death occurring in a few days. Blood-tinged fluid was present in the peritoneal cavity. Petechia and focal necrosis was evident in the liver and kidney. The spleen was enlarged, soft and friable. The myocardium was described as soft and flabby. The stomach was hyperaemic (Oladosu *et al.*, 1994). Gram-positive rods of 1–4 μm in length were observed. It should be emphasised that skin lesions revealed the presence of *Aeromonas* and *Fla. columnare*.

Bacillary necrosis was described in farmed populations of catfish (*Pangasius hypophthalmus*) from the Mekong Delta, Vietnam. Mortalities among fish that did not otherwise respond to treatment were observed, and the disease signs centred on 1–3 mm diameter white necrotic and granulomatous areas in the kidney, liver, spleen and viscera. Apart from the presence of mostly myxosporean parasites, an organism considered as an unspeciated *Bacillus* was recovered (Ferguson *et al.*, 2001).

Bacillus cereus

The organism has been associated with branchionecrosis in common carp (Pychynski *et al.*, 1981) and striped bass (Baya *et al.*, 1992a).

Bacillus mycoides

An epizootic occurred in channel catfish in Alabama during 1992. The fish were darker in colour, inappetent, displayed pale areas or ulcers on the dorsal surface, focal necrosis of the epaxial muscle, and opaque muscle (Goodwin *et al.*, 1994). Histopathological examination revealed the presence of chains of Gram-positive rods.

Bacillus subtilis

The organism has been associated with branchionecrosis in common carp (Pychynski *et al.*, 1981).

Corynebacteriaceae representative

Corynebacterium aquaticum

The organism was associated with 3-year-old striped bass in an experimental aquaculture facility in Maryland during December, 1990. Fish displayed pronounced bilateral exophthalmia, and contained the organism in brain tissue (Baya *et al.*, 1992b). Fish stopped feeding, swam more slowly and died (at this point the eyes were ruptured). Internally, the only disease sign was that the brain was haemorrhagic, and the cranium was full of blood.

Micrococcaceae representative

Micrococcus luteus

Disease signs were consistent with the notion of rainbow trout fry syndrome (RTFS). Thus, moribund fish, in the size range of 0.5–5.0 g, displayed exophthalmia, pale gills, enhanced skin pigmentation and swollen abdomen. Internally, the kidney was swollen, the spleen was pale and elongated, and some ascitic fluid was present in the peritoneal cavity (Austin and Stobie, 1992a).

Mycobacteriaceae representatives

Mycobacterium spp.

Mycobacteriosis (fish tuberculosis) is a chronic progressive disease, with various external signs, including emaciation, inflammation of the skin, exophthalmia, open lesions and ulceration (e.g. Lansdell *et al.*, 1993) and may attack many fish species,

including Atlantic menhaden (Stine *et al.*, 2005), rockfish (Whipps *et al.*, 2003), shortfin molly (Poort *et al.*, 2006), striped bass (Kaattari *et al.*, 2005) and turbot (dos Santos *et al.*, 2002). Internally, greyish white nodules (granulomas) develop on various organs, particularly the liver, kidney, heart and spleen (Dulin, 1979; Van Duijn, 1981). The disease may take several years to progress from the asymptomatic state to clinical illness. Initially, the pigment may fade, and the fish appear sluggish with loss of appetite. If the skin is affected, blood spots develop with the ultimate formation of ulcers. In addition, fin and tail rot and the loss of scales may be seen.

Myc. abscessus was associated with 2–27-month-old Japanese meduka (*Oryzias latipes*), which had been cultured in the U.S.A. for aquatic toxicology testing (Teska *et al.*, 1997), and in milkfish in Taiwan (Chang *et al.*, 2006). During a routine examination, granulomas, notably in the buccal cavity and vent, and a few acid-fast bacteria were noted in <1% of the otherwise healthy fish. On clinically diseased fish, the disease signs would include listlessness, inappetence, swollen abdomen and visible granulomas (Teska *et al.*, 1997). Milkfish displayed epithelial granulomas and red/grey nodules throughout the fish, which experienced 67% mortalities (Chang *et al.*, 2006).

Myc. marinum was first recognised from the liver, spleen and kidney of tropical coral fish kept in the Philadelphia Aquarium (Aronson, 1926). As the name implies, the organism was considered to be only pathogenic to marine fish. But, it is now recognised to infect both marine and freshwater fish and also human beings (Van Duijn, 1981). Currently, *Myc. marinum* is a major constraint on the farming of sea bass in Israel, leading to stunting and therefore loss of market value of the infected fish (Knibb *et al.*, 1993), African catfish in Poland (Antychowicz *et al.*, 2003) and in Chesapeake Bay, Virginia (Gauthier *et al.*, 2004). Here, an epizootic has developed, and fish experimentation has revealed the development of large aggregates of macrophages, which contain phagocytosed bacteria, with *Myc. marinum* contained exclusively within phagosomes. It is relevant to note that the first case of "tuberculosis" reported in wild stocks, was in cod (*Gadus morhua*) landed at Fleetwood (U.K.), although isolation of the pathogen was not achieved (Alexander, 1913).

Myc. montefiorense was recovered sporadically from granulomatous skin lesions in captive moray eels in the U.S.A. (Levi *et al.*, 2003).

Myc. neoaurum has been associated with ocular lesions (oedema, exophthalmia) in Atlantic salmon (Bachman *et al.*, 1990). Nodules may form in the muscle, where they are visible on the outside of the fish. These nodules may burst, releasing bacteria into the aquatic environment. Internally, nodules may develop on the organs, leading to emaciation, or oedema or peritonitis may ensue. Infection may spread to the skeleton, in which case deformities become apparent. Death will ultimately occur (Van Duijn, 1981).

Myc. pseudoshottsii was recovered from an epizootic of mycobacteriosis in striped bass from Chesapeake Bay, Virginia (Rhodes *et al.*, 2005).

Myc. shottsii was described as the cause of an epizootic in striped bass from Chesapeake Bay. Infected fish had granulomatous lesions in the kidney and spleen, and in the skin (Rhodes *et al.*, 2003).

Myc. gordonae was recovered from guppy (*Poecilia reticulata*) in Thailand notably during rainy and/or cold periods. The fish, which experienced substantial mortalities, displayed inappetence, sluggish swimming behaviour, fin erosion, skin ulceration and the presence of systemic granulomas (Sakai *et al.*, 2005).

A range of mycobacteria was recovered from various aquarium fish in Slovenia, and based on molecular methods included *Myc. chelonae*, *Myc. fortuitum*, *Myc. gordonae*, *Myc. marinum*, *Myc. peregrinum* and *Mycobacterium* spp. Of these *Myc. gordonae* and *Myc. peregrinum* are new to fish pathology (Pate *et al.*, 2005). Unfortunately, the authors did not address the pathogenicity of the isolates. Similarly, Rhodes *et al.* (2004) recovered a range of mycobacteria from striped bass in Chesapeake Bay, Virginia, and based on phenotypic traits included *Myc. interjectum*, *Myc. marinum*, *Myc. scrofulaceum*, *Myc. shottsii*, *Myc. szulgai* and *Myc. triplex* (Rhodes *et al.*, 2004b). Again, some of these taxa are new to fish pathology, and deserve further study. The message about the diversity of mycobacteria in Chesapeake Bay fish was reinforced by work with Atlantic menhaden (*Brevoortia tyrannus*), which led to the recovery of *Mycobacterium* spp., from ulcers, and *Myc. fortuitum*, *Myc. gordonae* and *Myc. marinum* from spleen (Stine *et al.*, 2005). In their excellent review, Kaattari *et al.* (2006) discuss all the currently recognised mycobacterial fish pathogens, including the new but not formally named species, *Myc. "chesapeaki"*.

Nocardiaceae representatives

Nocardia spp.

It is appreciated that nocardiosis may be problematical in freshwater (Valdez and Conroy, 1963; Conroy, 1964; Snieszko *et al.*, 1964b; Heuschmann-Brunner, 1965a; Campbell and MacKelvie, 1968; Ghittino and Penna, 1968) and marine fish (Wood and Ordal, 1958), occurring in a range of fish species, including Atlantic salmon (Bransden *et al.*, 2000). Symptoms similar to mycobacteriosis develop in affected fish. All age groups may be infected, with lesions, manifested as small white spots, present in the dermis, muscle, gills and internal organs. *Noc. seriolae* has spread from its initial focal point in Japan, and has, for example, been diagnosed among pond cultured sea bass (*Lateolabrax japonicus*) in Taiwan. Here, the cumulative mortality reached 17.5% within a month, with disease signs including yellow–white nodules of 0.1–0.2 cm in diameter in the gills, heart, kidney, liver and spleen (Chen *et al.*, 2000).

Rhodococcus sp.

In chinook salmon, there was evidence of melanosis and ocular oedema, leading to rupture of the cornea. There was no evidence of involvement of any internal organs (Backman *et al.*, 1990). With Atlantic salmon, the presence of granulomas in the kidney was apparent (Claveau, 1991). Progressive low-level mortalities were recorded.

Rhodococcus erythropolis

Freshwater- and seawater-reared Atlantic salmon, which had been previously intraperitoneally injected with oil-adjuvanted vaccine of more than one manufacturer in Norway (five cases) and Scotland (two cases), experienced losses of 1–35%. The systemic infection centred on the peritoneal cavity, and moribund fish displayed scale loss and occasionally cutaneous haemorrhages in the abdomen and at the base of the fins. In addition, there was some evidence of abdominal distension. In Scotland, bilateral exophthalmia was reported. Internally, there were bloody ascites, splenomegaly, peritonitis, the stomach filled with mucoidal contents, the presence of petechia, and fluid- or pus-filled cavities on the internal organs (Olsen *et al.*, 2006a). Adhesions were present in the peritoneum, consistent with the administration of oil-adjuvanted vaccines (Olsen *et al.*, 2006a).

Planococcaceae representative

Planococcus sp.

The organism has been associated with small off-white (2–4 mm diameter) round recessed spots on the heads of Atlantic salmon (Austin *et al.*, 1988). In large rainbow trout (average weight = 500 g), the only disease sign was the presence of watery kidney and small amounts of ascitic fluid in the peritoneal cavity (B. Austin, unpublished data). In addition, during 1990 the organism was associated with two populations of rainbow trout fry deemed to have RTFS. These animals were anaemic, with pale gills, swollen kidney, pale liver and elongated spleen (Austin and Stobie, 1992a).

Staphylococcaceae representatives

Staphylococcus aureus

Dead animals displayed pronounced eye disease, with the cornea becoming reddish, due to vascularisation, and then opaque. Thereafter, there was degeneration of the eye tissues, leaving a hollow cup. The brain and optic nerves were affected. In addition, diseased fish became lethargic and darker in colour (= melanosis). The internal organs did not appear to be affected (Shah and Tyagi, 1986).

Staphylococcus epidermidis

The initial description of the disease was not exhaustive, but typical signs included exophthalmia, congestion and ulceration on the tail (Kusuda and Sugiyama, 1981). Later, *Sta. epidermidis* was recovered from moribund cultured grass carp in Taiwan (Wang *et al.*, 1996). Here, the fish displayed haemorrhages on the opercula and pelvic fins. Internally, petechial haemorrhages and bloody ascites were observed. Although tapeworms were present in the lumen of the intestine, smears revealed the presence of oval bacteria, which were isolated, and considered to represent *Sta. epidermidis* (Wang *et al.*, 1996). The organism was associated with mortalities of up to 12% in one day coinciding with a sudden increase in water temperature in juvenile gilthead

sea bream in Turkey during 2003. The diseased fish diplayed haemorrhaging on the fins and gills, a slightly distended abdomen with ascites, and anaemic liver (Kubilay and Uloköy, 2004).

Staphylococcus warneri

At a water temperature of 14–16°C, rainbow trout of 50–100 g weight displayed ulcerations on the fins and exophthalmia. The abdomens were distended with ascitic fluid. The kidney was normal, but the liver was discoloured (Gil *et al.*, 2000).

GRAM-NEGATIVE BACTERIA

Aeromonadaceae representatives

The scientific literature abounds with references to aeromonads as fish pathogens. In addition to those discussed below, other species have been mentioned in connection with a role in fish pathology. One example is *Aer. veronii* which has been mentioned in terms of immunisation and an immune response in spotted sand bass (*Paralabrax maculofasciatus*). Infectivity data was presented but whether or not the organism is correctly identified and indeed a fish pathogen must await further study (Merino-Contreras *et al.*, 2001).

Aeromonas allosaccharophila

A description of the exact pathology present in diseased elvers was not provided (Martinez-Murcia *et al.*, 1992).

Aeromonas caviae

In 1991, a septicaemic condition was diagnosed on four Atlantic salmon farms located on the Black Sea in Turkey (Candan *et al.*, 1995). Diseased fish displayed signs of haemorrhagic septicaemia, namely haemorrhages on the body, intestine filled with bloody exudate, enlarged liver and spleen, and liquefying kidney. Subsequently, the organism has been associated with eye disease and haemorrhagic septicaemia in farmed rainbow trout from Kenya (Ogara *et al.*, 1998).

Aeromonas hydrophila

Since its initial recognition as the causal agent of haemorrhagic septicaemia (Sanarelli, 1891; Schäperclaus, 1930; Haley *et al.*, 1967), *Aer. hydrophila* has been recovered as a pathogen from a wide variety of freshwater fish species, including ornamental fish (Hettiarachchi and Cheong, 1994) and occasionally from marine fish, e.g. ulcer disease of cod (Larsen and Jensen, 1977). The aetiological agent has been considered to be the dominant cause of motile aeromonas disease in China (Nielsen *et al.*, 2001), and may well have worldwide distribution. However, some doubt has been expressed over its precise role as a fish pathogen (Heuschmann-Brunner, 1965b; Eurell *et al.*, 1978; Michel, 1981) with some workers contending that it may be merely a secondary invader of already compromised hosts. Conversely, other groups have insisted that *Aer. hydrophila* constitutes a primary pathogen.

Aer. hydrophila has been credited with causing several distinct pathological conditions, including tail/fin rot and haemorrhagic septicaemias (e.g. Hettiarachchi and Cheong, 1994). The organism may be found commonly in association with other pathogens, such as *Aer. salmonicida*, although there is no evidence for the presence of synergistic interactions leading to exacerbated disease conditions. Haemorrhagic septicaemia (also referred to as motile aeromonas septicaemia) is characterised by the presence of small surface lesions (which lead to the sloughing off of scales), local haemorrhages particularly in the gills and vent, ulcers, abscesses, exophthalmia and abdominal distension. Llobrera and Gacutan (1987) described the presence of necrotic ulcers in a variety of fish from the Philippines. Internally, there may be accumulation of ascitic fluid, anaemia and damage to the organs, notably kidney and liver (Huizinga *et al.*, 1979; Miyazaki and Kaige, 1985). Also, redsore disease in bass has been attributed to *Aer. hydrophila* (Hazen *et al.*, 1978). This condition, which may reach epizootic proportions, is characterised by erosion of the scales and pinprick haemorrhages, which may cover up to 75% of the body surface. There is often a high mortality rate. Hettiarachchi and Cheong (1994) described *Aer. hydrophila* as the cause of disease in freshwater ornamental fish in Sri Lanka, with disease signs including the presence of eroded fins, haemorrhages on the skin and at the base of the caudal fin, sloughing scales and haemorrhaging in the intestinal wall.

Aeromonas salmonicida

Historically, *Aer. salmonicida* was thought to have a predilection for salmonids. Over the years, however, the apparent host range of the pathogen has steadily expanded. Thus, infections are known to occur among representatives of several major families of Osteicthys, including Cyprinidae, Serranidae and Anoplopomatidiae, in addition to the Salmonidae (Herman, 1968), and also the Agnatha (Family Petromyzontidae) (Hall, 1963). Non-salmonids which have been documented as suffering from diseases of *Aer. salmonicida* aetiology include minnow and goldfish (Williamson, 1929), carp (Bootsma *et al.* 1977), perch (Bucke, 1979) and bream, roach, dace, chub, tench, pike, bullheads, sculpins and catfish (McCarthy, 1975a), wrasse (Treasurer and Cox, 1991), sea bream (Real *et al.*, 1994) and cultured marbled sole (*Pleuronectes yokohamae*) in Japan [these isolates infected Japanese flounder and spotted halibut (*Verasper variegatus*)] (Kumagai *et al.*, 2006). In some of the fish, particularly the non-salmonids, the disease may manifest itself in a different form to the classical furunculosis, and, the causal agent is often regarded as "atypical".

Traditionally, *Aer. salmonicida* has been known as the causative agent of furunculosis. However, it has become apparent that the pathogen manifests itself with other conditions, notably ulcerative dermatitis (Brocklebank, 1998), and ulcerations, especially in non-salmonids, e.g. in cod (Magnadóttir *et al.*, 2002). Furunculosis, named because of the sub-acute or chronic form of the disease, is recognised by the presence of lesions resembling boils, i.e. furuncles, in the musculature. In fact, the term furunculosis is a misnomer, because the lesions do not resemble those found in the similarly named condition of human beings (McCarthy, 1975a). The name has, however, become established in the fisheries literature, so that

it has been retained for convenience and to avoid the confusion which could result from a new name.

The sub-acute or chronic form of furunculosis, which is more common in older fish, is characterised by lethargy, slight exophthalmia, blood-shot fins, bloody discharge from the nares and vent, and multiple haemorrhages in the muscle and other tissues. Internally, haemorrhaging in the liver, swelling of the spleen and kidney necrosis may occur (Snieszko, 1958a; McCarthy and Roberts, 1980). This form of the disease usually causes low rates of mortality, and fish may survive, although survivors have scar tissue in the vicinity of the furuncles (McCarthy, 1975a). Oddly enough, the chronic form of the disease is not the most frequently occurring, nor is the presence of furuncles the most typical symptom of the disease (Snieszko, 1958a).

The acute form of furunculosis, which is most common particularly in growing fish and adults, is manifested by a general septicaemia accompanied by melanosis, inappetence, lethargy and small haemorrhages at the base of the fins. This form of the disease is of short duration, insofar as the fish usually die in 2–3 days, and causes high mortalities. The bacteria occur in the blood, disseminated throughout the tissues, and in the lesions. Internally, haemorrhaging occurs over the abdominal walls, viscera and heart. The spleen may appear enlarged. The acute disease is of sudden onset, with few, if any, external signs (McCarthy, 1975a).

McCarthy and Roberts (1980) discussed a third clinical form of furunculosis, termed peracute furunculosis, which is confined to fingerling fish. The infected animals darken in colour, and may quickly die with only slight external symptoms, such as mild exophthalmia. Haemorrhages may occur at the base of the pectoral fin, if the fish manage to survive for long-enough periods. Losses in farmed stock may be extremely high (Davis, 1946).

Yet another form of furunculosis was discussed by Amlacher (1961), i.e. intestinal furunculosis. The symptoms were described as inflammation of the intestine, and anal inversion. This description is similar to a report by Herman (1968) of chronic furunculosis, i.e. low, relatively constant rate of mortality with intestinal inflammation and variable haemorrhages.

In addition to furunculosis, *Aer. salmonicida* has been implicated in several conditions, especially of non-salmonids, e.g. wrasse (Laidler *et al.*, 1999), Arctic charr (*Salvelinus alpinus*) and grayling (*Thymallus thymallus*) (Pylkkö *et al.*, 2005). The best known of these conditions is undoubtedly carp erythrodermatitis (CE). Fijan (1972), who is credited with the name of the disease, demonstrated that CE was caused by a transmissible, antibiotic-sensitive organism, which manifested itself as predominantly a skin infection. Bootsma *et al.* (1977) isolated a small, Gram-negative, rod-shaped organism from skin lesions in mirror carp in Yugoslavia. This organism was subsequently identified as an "atypical" strain of *Aer. salmonicida*. CE was described as a sub-acute to chronic contagious skin disease, which varied in its morbidity and mortality (Bootsma *et al.*, 1977). It appears that the infection often starts at the site of injury to the epidermis. A haemorrhagic inflammatory process then develops between the epidermis and dermis. This red inflammatory zone gradually extends as the infection spreads. The breakdown of tissue leads to the formation of a central ulcer, which may occur in any location on the body surface, although it is most

frequently located on the flanks. Infected fish exhibit inappetence, and appear darker in colour. Secondary invasion of the ulcer by fungi or other bacteria is common. If the fish recovers, the healed ulcer is recognisable as a grey–black scar. Frequently, contraction of the collagen of the scar tissue can result in serious deformity, which reduces the commercial value of the fish (Fijan, 1972). In some instances, CE may also result in generalised septicaemia and death. Unlike furunculosis, which usually occurs only when water temperatures exceed 16°C, CE may occur at all water temperatures.

More recently, *Aer. salmonicida* has been reported to cause a cutaneous ulcerative disease in ornamental fish, especially goldfish (*Carassius auratus*), where the condition is referred as goldfish ulcer disease, which is a stress-mediated condition associated with the atypical form of the pathogen (Dror *et al.*, 2006). However, the disease has been known for a long time previously. Ulcer disease of cyprinids, in general, has occurred in widely separated geographical locations, including the U.S.A., Japan and England (Shotts *et al.*, 1980). Mawdesley-Thomas (1969) studied, in detail, an outbreak of an ulcerative disease of goldfish, and recovered *Aer. salmonicida*. Symptoms included lethargy, loss of orientation, and abnormal swimming behaviour. The ulcers were of various sizes and depths, and some fish died shortly after infection. Secondary invasion of the ulcers by *Saprolegnia* was observed. More recently, we have recovered an extremely fastidious form of the pathogen from ulcerated carp, goldfish and roach in England. Here, there was evidence of secondary invasion of the ulcers by *Aer. hydrophila*. According to McCarthy and Roberts (1980), ulcer disease differed from CE in the following ways:

(a) the ulcers were deeper and more extensive;
(b) renal and splenic changes, such as those found in sub-acute furunculosis of salmonids, were present at an earlier stage in the course of the disease than in CE (where internal lesions were restricted to later stages in cases where septicaemia developed).

Another variation of *Aer. salmonicida* infection, termed "head ulcer disease", has been described in Japanese eels (Hikada *et al.*, 1983; Ohtsuka *et al.*, 1984; Kitao *et al.*, 1984). An atypical strain of *Aer. salmonicida* was implicated as the aetiological agent. The progression of this disease is worthy of note because the pathogen is apparently capable of causing mortalities solely as a result of localised proliferation, with no evidence for the development of a generalised septicaemia. Results of natural and laboratory-based infections of eels with head ulcer disease revealed that *Aer. salmonicida* was not recovered in significant numbers from internal organs, i.e. brain, kidney or spleen or, indeed, blood (Ohtsuka *et al.*, 1984; Nakai *et al.*, 1989a). However, the pathogen proliferated substantially in the muscle of eels (Nakai *et al.*, 1989a). Similar, localised ulcerative infections caused by atypical *Aer. salmonicida* have been recognised in goldfish (Elliott and Shotts, 1980) and carp (Csaba *et al.*, 1981a).

Aeromonas sobria

Organisms, identified as *Aer. sobria*, have been isolated from wild-spawning gizzard shad (*Dorosoma cepedianum*) in Maryland during 1987 (Toranzo *et al.*, 1989) and from farmed perch (*Perca fluviatilis*) in Switzerland (Wahli *et al.*, 2005). Moribund gizzard shad did not display any external or internal signs of disease. The perch displayed skin lesions and fin rot.

Aeromonas veronii biovar sobria

The organism was recovered from epizootic ulcerative syndrome, which is characterised by the presence of large ulcers all over the fish leading to death often within a week (Rahman *et al.*, 2002a).

Alteromonadaceae representatives

Pseudoalteromonas piscicida

The organism was associated with whitening of the egg cases, followed by mortalities within 24 h among eggs of damsel fish, *Amphiprion clarkii* and *Amblyglyphidodon curacao* (Nelson and Ghiorse, 1999).

Shewanella putrefaciens

During spring of 1985, a disease occurred which resulted in high mortalities in rabbitfish, *Siganus rivulatus*, farmed in sea cages in the Red Sea. From diseased animals, a Gram-negative bacterium was recovered, which was capable of re-infecting healthy fish (Saeed *et al.*, 1987). To date, the disease has not been described in any other fish species, or, for that matter, elsewhere.

Disease signs included lethargy, discoloration, exophthalmia, haemorrhaging and necroses on the body and mouth, and fin damage. Internal damage was not reported (Saeed *et al.*, 1987).

Campylobacteriaceae representative

Arcobacter cryaerophilus

Diseased rainbow trout were recovered from three fish farms in Turkey during 1997 and 1998. The reported disease signs included deformation of the upper jaw, darkened or alternatively pale pigment, fin rot, pale gills, haemorrhaging in the musculature, haemorrhaging and bloody fluid in the intestine, skin ulcerations, damaged spleens and swollen kidney (Aydin *et al.*, 2000, 2002).

Enterobacteriaceae representatives

Citrobacter freundii

Diseased sunfish displayed erratic swimming, inappetence, eroded skin, surface haemorrhages, enteritis, deep red spleen, pale liver and tumorous masses (granulomas) on the kidney (Sato *et al.*, 1982). Similar signs have been observed in salmonids within the U.K. (B. Austin, unpublished data). In the initial disease outbreak, 25/29 animals (= 86% of the total) died (Sato *et al.*, 1982).

Edwardsiella ictaluri

Although *Edw. ictaluri* is primarily a disease of catfish, with time other fish groups have been reported to become infected: for example, brown bullhead (*Amieurus nebulosus*) in the U.S.A. (Iwanowicz *et al.*, 2006). The disease has spread from its original focal point in the U.S.A. to include cultured striped catfish (*Pangasius hypophthalmus*) in Sumatra, Indonesia (Yuasa *et al.*, 2003) and catfish in Vietnam (Crumlish *et al.*, 2002). It is apparent that there is a great variability in the clinical signs associated with the disease. Just prior to death, the fish may hang listlessly in an almost vertical position at the water surface, spin rapidly in circles, or exhibit spiral swimming. External signs, often absent in fish over 15 cm in length, include the presence of petechial (pin-prick) haemorrhages on the skin in the vicinity of the throat and mouth, pale gills, exophthalmia and open lesions on the head, particularly on the frontal bone of the skull between the eyes and on the lateral body surface. Internally, there may be swelling of the kidney and spleen, haemorrhaging and necrotic areas in the liver, blood-filled ascitic fluid in the peritoneum, and petechial haemorrhages throughout the internal muscle walls (Hawke, 1979). In Vietnam, the disease is referred to as "bacillary necrosis of *Pangasius*" with which there are irregular white lesions on the kidney, liver and spleen, and the involvement of parasites and bacteria including *Bacillus* and *Edw. ictaluri* (Crumlish *et al.*, 2002).

Edwardsiella tarda

Excellent desriptions of the clinical manifestation of *Edw. tarda* infections in channel catfish were published by Meyer and Bullock (1973). These authors reported that with mild infections the only external signs of disease are the presence of small cutaneous lesions of approximately 3–5 mm in diameter, which are located in the postero-lateral region of the body. With progression of the disease, abscesses develop in the muscle of the body and tail. These abscesses may enlarge, and develop into gas-filled hollow areas. From the surface, these are seen as poorly pigmented, convex swollen areas which, if punctured, emit a foul odour. This condition has given rise to the name emphysematous putrefactive disease of catfish. Although catastrophic losses of catfish have not been recorded, the disease has a severe economic effect on infected sites. When infected fish enter processing plants, the noxious odours effectively stop production by necessitating disinfection and deodorisation. Thus, heavy financial losses to the processors may result from the presence of only a small number of infected animals (Meyer and Bullock, 1973).

Examination of naturally diseased tilapia has indicated a range of symptoms including loss of pigmentation, the presence of a swollen abdomen filled with ascitic fluid, protruded haemorrhaged anus and opaqueness to the eyes. Internally, small white nodules may be observed in the gills, kidney, liver and spleen, and occasionally the intestine. These nodules are packed with bacteria (Kubota *et al.*, 1981).

Diseased turbot in Spain revealed the presence of eye tumefaction, haemorrhaging, inflammation (kidney, liver and spleen) and ascites (Padrós *et al.*, 2006).

Escherichia vulneris

Clinical signs included haemorrhagic lesions on the skin, pale gills, digestive tract full of bloody exudate, haemorrhaging in the gonads, and yellow liver with hyperaemic areas (Aydin *et al.*, 1997).

Hafnia alvei

The disease was described as a haemorrhagic septicaemia. Fish became darker, moved slowly, developed exophthalmia, haemorrhaging in the eye and petechial haemorrhages on the body surface. Internally, petechial haemorrhages were apparent on the spleen (enlarged) and kidney (hyperaemic). The disease appeared in fish after transportation or during cultivation in inappropriate conditions (Gelev and Gelev, 1988).

A second outbreak occurred in cherry salmon (*O. masou*) on Japanese farms. Here, the disease was characterised by melanosis, swollen abdomen and slow swimming. There were grey/white furuncles on the kidney (Teshima *et al.*, 1992).

Klebsiella pneumoniae

The organism was recovered from the diseased tails and fins of 12 rainbow trout in Scotland. No other disease signs were noted (Daskalov *et al.*, 1998).

Pantoea agglomerans

Pronounced haemorrhaging was noted in the eyes of dead and moribund animals. Haemorrhages were also recorded in the musculature. Otherwise, there was an absence of disease signs in the internal organs (Hansen *et al.*, 1990).

Plesiomonas shigelloides

Symptoms included emaciation, reddening of the anus with yellow exudation, petechial haemorrhages in the internal muscle wall, and sometimes the accumulation of ascitic fluid in the peritoneal cavity (Cruz *et al.*, 1986). Inappetence was noted by Klein *et al.* (1993).

Providencia rettgeri

The fish displayed large red ulcers on the abdomen, base of the pectoral fin and around the head. At 20–23°C, mortalities occurred in 3 days, whereas at 12–20°C, the deaths ensued in 8 days (Bejerano *et al.*, 1979).

Salmonella enterica subsp. *arizonae*

A pirarucu was deemed to have died of a septicaemic condition. Externally, there was minimal evidence of disease, with the eyes (corneas) displaying opacity. A bloody exudate was found in the body cavity. Lesions and congestions were recorded in the mucus membranes of the stomach and intestine (Kodama *et al.*, 1987).

Serratia liquefaciens

Moribund fish did not display any external signs of disease. Yet, internally the kidney was swollen, and nodules were present on both the kidney and spleen; the liver appeared speckled, and some ascitic fluid was present in the peritoneal cavity. Initially, it was considered likely that the animals were infected with *Ren. salmoninarum*, the causal agent of BKD, but the presence of Gram-negative bacteria and complete absence of any Gram-positive micro-organisms precluded this possibility (McIntosh and Austin, 1990b). In a separate development during 1990, the organism was attributed to low-level mortalities in turbot, farmed in floating cages in France. With this outbreak, the disease signs included swelling and liquefaction of the kidney and spleen, which were also characterised by the presence of yellowish nodules (Vigneulle and Baudin-Laurencin, 1995). In 1999, Arctic charr in the U.S.A. were also found to be infected with the pathogen (Starliper, 2001). The external signs centred on a slight redness and swelling around the anus, but internally there was evidence of severe hemorrhaging with bloody ascites.

Serratia marcescens

It should be emphasised that the organisms were recovered only from apparently healthy fish, which were devoid of overt signs of disease (Baya *et al.*, 1992c).

Serratia plymuthica

Nieto *et al.* (1990) noted that diseased fish did not display any external or internal clinical signs. However, in Scotland the diseased fish possessed extensive skin lesions over the entire flank, from the operculum to the tail (Austin and Stobie, 1992b). In Poland, the organism has been recovered from 42 Atlantic salmon, rainbow trout and sea trout farms since 1996 (Grawinski and Antychowitz, 2001).

Yersinia intermedia

Affected Atlantic salmon were of 40–50 g in weight, and were held at a water temperature of 5°C. Disease signs included lazy movement with the fish congregating

at the surface of the water, darkening of the body pigment, tail erosion, haemorrhaging on the flank and abdominal inflammation (Carson and Schmidtke, 1993).

Yersinia ruckeri

The disease is mostly restricted to salmonids (Bullock and Snieszko, 1975) within the geographical locations of North and South America (Ross *et al.*, 1966; Stevenson and Daly, 1982; Bravo and Kojagura, 2004), Denmark (Dalsgaard *et al.*, 1984), Great Britain (Austin, 1982; Roberts, 1983), France (Lesel *et al.*, 1983), Germany (Fuhrmann *et al.*, 1983), Italy (Busch, 1978), Ireland (McCormick and McLoughlin, 1993), Norway (Richards and Roberts, 1978) and Australia (Bullock *et al.*, 1978a; Llewellyn, 1980). With the recent upsurge in the number of cases of the disease, particularly in Europe, it would appear that the disease is still spreading. So far, it has been a severe problem mostly in rainbow trout, although outbreaks of disease have been reported among populations of brown trout, brook trout, chinook salmon and coho salmon (Dulin *et al.*, 1976) and Atlantic salmon and Pacific salmon (Bullock *et al.*, 1978a). A few non-salmonid fish species have also been reported to harbour the pathogen, and these include channel catfish (Danley *et al.*, 1999), emerald dace (*Notropis atherinoides*; Mitchum, 1981), goldfish (McArdle and Dooley-Martin, 1985), carp (B. Austin, unpublished observation), lake herring or cisco (*Coregonus artedii*; Bullock and Anderson, 1984) and minnows (*Pimephales promelas*) (Michel *et al.*, 1986a). Also, sea bass and turbot appear to be susceptible (Vigneulle, 1990). In the case of rainbow trout, there is experimental evidence that significantly lower mortalities occur with increases of salinity. Thus, mortalities were reduced from 96.5% in freshwater to 75% in 9‰ salinity (Altinok and Grizzle, 2001a). Much excellent work has certainly been accomplished, but there are many basic facets of the biology of the pathogen which remain unknown or unclear.

The name of the disease, i.e. enteric redmouth (ERM), is fairly descriptive insofar as one of the most common symptoms is reddening of the mouth and throat, which is caused by subcutaneous haemorrhaging (Busch, 1973). Other external signs include inflammation and erosion of the jaws and palate, melanosis, haemorrhaging around the base of the fins, bilateral exophthalmia and a tendency for sluggishness (Fuhrmann *et al.*, 1983; Bullock and Anderson, 1984; Danley *et al.*, 1999). In channel catfish, the disease was characterised by swollen haemorrhaged rings around the eyes and raised haemorrhaged areas overlying the frontal foramens (Danley *et al.*, 1999). Internally, there may be haemorrhaging in the muscle, body fat and in the intestine, which may also contain a yellow fluid. A generalised bacteraemia occurs in the principal organs, with slight enlargement of the kidney and spleen. The disease has been held responsible for greater financial loss of the trout-farming industry in western areas of the U.S.A. than any other disease (Hester, 1973). Onset of an epizootic is often gradual with resulting heavy losses (Newman and Majnarich, 1982). It is relevant to note that a second disease, known as salmonid blood spot, in Australia (Llewellyn, 1980) is attributable to the same aetiological agent (Green and Austin, 1982).

Flavobacteriaceae representatives

Many of these organisms cause gill disease, which may be characterised histologically as hyperplasia (swelling) of the gill epithelia. Frequently, the condition in juvenile fish involves fusion at the distal tips of adjoining gill lamellae. The involvement of hyperplasia-inducing agents has also been implicated in some cases by *Flavobacterium* spp. (Kudo and Kimura, 1983a, b).

Chryseobacterium scophthalmum

This organism caused lethargy, gill hyperplasia, haemorrhaging in the gills, distended abdomen, surface haemorrhaging and extensive internal haemorrhaging (Mudarris and Austin, 1989, 1992).

Flavobacterium columnare

Columnaris has been recognised to have worldwide distribution in a wide range of freshwater fish, including Arctic charr, bass, black bullheads, carp, channel catfish, chub, eel, goldfish, killifish, loach, perch, rainbow trout, roach, Atlantic salmon, chinook salmon, sheatfish, squawfish, tilapia, white crappie, whitefish and white-suckers (Nigrelli, 1943; Nigrelli and Hutner, 1945; Wakabayashi and Egusa, 1966; Ajmal and Hobbs, 1967; Fijan, 1969; Bowser, 1973; Wobeser and Atton, 1973; Chun, 1975; Bootsma and Clerx, 1976; Ferguson, 1977; Farkas and Olàh, 1980; Kuo *et al.*, 1981; Morrison *et al.*, 1981; Chen *et al.*, 1982; Koski *et al.*, 1993; Welker *et al.*, 2005). The disease was considered to be sufficiently serious to warrant inclusion in the list of notifiable fish diseases, as defined by the (British) Diseases of Fish Act, 1937. However, columnaris was omitted from the 1983 Act.

Infection with *Fla. columnare* may result in several discrete disease conditions. In young fish, there is often negligible pathology before death ensues. The gill is usually the major site of damage. Typically, congestion (blockage) of the blood vessels supplying the gills occurs, with dissociation of the surface epithelium of the lamellae from the capillary bed. There may be scattered areas of haemorrhaging (Pacha and Ordal, 1967). In adult fish, the lesions may occur on the gills, skin and/or in the musculature. Systemic infections may develop (Wolke, 1975). Skin discoloration/fading and muscle lesions have been documented on neon tetra (*Paracheirodon innesi*) (Michel *et al.*, 2002). Gill lesions consist normally of yellow–orange areas of necrosis. These start usually at the periphery of the gill, and extend towards the base of the gill arch. Eventually, extensive erosion may completely destroy the gill filament (Pacha and Ordal, 1970). On the body, small lesions start as areas of pale discoloration at the base of the dorsal fin or occasionally at the base of the pelvic fin, and lead to deterioration of the fins. These areas increase in size and may become as large as 3–4 cm in diameter, covering as much as 20–25% of the total surface area of the fish. This may have the characteristic appearance of a saddle, and hence the descriptive term, "saddleback". Frequently, the skin becomes completely eroded away, exposing the underlying muscle. Large numbers of bacteria are present at the advancing edge of the lesion. It is not uncommon for the fish to die within

48 h of the appearance of the skin discoloration (Pacha and Ordal, 1970; Becker and Fujihara, 1978; Morrison *et al.*, 1981).

Flavobacterium johnsoniae

In the initial outbreak in Queensland, Australia, 2–5% mortalities occurred in farmed barramundi, *Lates calcarifer*, over a 2-week period when the water temperature was 27–28°C. The fish were listless, anorexic, and displayed elevated scales and superficial erosion of the skin (particularly on the posterior flank). Some fish presented eroded pectoral fins and lower jaws (Carson *et al.*, 1993).

Flavobacterium psychrophilum

Although this organism is mostly associated with infections in salmonids, other fish species have been infected, for example sea lamprey (Elsayed *et al.*, 2006), and ayu has been challenged successfully by immersion (Miwa and Nakayasu, 2005). Mostly, this organism causes skin lesions, which are often described as saddle-like lesions, containing myriads of organisms, near the dorsal fin. The fish may darken and, in advanced cases, develop bacteraemia with the pathogen ramifying throughout the animal (Wood and Yasutake, 1956; Winton *et al.*, 1983; Lehmann *et al.*, 1991).

Tenacibaculum maritimum

Principal signs of disease caused by *T. maritimum* include mouth erosions, gill erosion and tail rot (Handlinger *et al.*, 1997), especially in juvenile fish. In older animals, lesions develop initially as grey–white cutaneous areas on the fins, head and trunk. These lesions degenerate into ulcers (Hikida *et al.*, 1979; Wakabayashi *et al.*, 1984). Black patch necrosis of Dover sole in Scotland and bacterial stomatitis (= mouth rot) of Atlantic salmon in Canada has been attributed to this pathogen (Bernardet *et al.*, 1990; Ostland *et al.*, 1999).

Tenacibaculum ovolyticum

T. ovolyticum led to mortalities among halibut eggs and larvae. The chorion became dissolved, and the underlying zona radiata was damaged by exotoxins resulting in puncturing of the egg, leakage of cell constituents and larval death (Hansen *et al.*, 1992).

Francisellaceae representative

Francisella sp.

Mature farmed cod, with an average weight of 3 kg, and which were contained in sea cages in Norway, developed mortalities in July 2005 when the water temperature was 14.5°C. Mortalities peaked in August, and over the 5-month period to November ~40% of the stock had died. Initially, clinical signs of disease were not noted, but later the fish were observed to be swimming sluggishly, were generally inappetent, and

became emaciated. Some fish displayed dermal haemorrhagic nodules, and corneal opacity and perforation. There were white nodules in the heart, kidney, liver (swollen) and spleen (swollen). The intestinal mucosa was thickened. Some fish had bloody ascites (Olsen *et al.*, 2006).

In Japan, the marine fish species, three-line grunt (*Parapristipoma trilineatum*) were observed with white granulomas. Intracellular bacteria were found in the kidney and spleen from which DNA was extracted and small subunit rRNA amplified by PCR, and sequenced. The outcome was 97.3–98.5% homology to *Francisella*, with *Francisella philomiragia* as the closest neighbour (Kamaishi *et al.*, 2005).

In a third study, *Francisella*-like bacteria were associated with mortalities in freshwater-reared (water temperature = 20–29°C) hybrid striped bass. Here, the fish became lethargic, darker pigmented and demonstrated skin haemorrhages over the abdomen, mild to moderate bilateral exophthalmia, swollen kidney and spleen, and interstitial granulomas (Ostland *et al.*, 2006).

Halomonadaceae representative

Halomonas (= Deleya) cupida

During April to June 1984, heavy mortalities occurred in black sea bream, *Acanthopagrus schlegeli*, fry (up to 14 days after hatching) in hatcheries in western Japan. Generally, the fish were too small to discern disease signs (Kusuda *et al.*, 1986).

Moritellaceae representatives

Moritella marina

The organism was associated with shallow skin lesions of Atlantic salmon farmed in Iceland at low temperatures, i.e. ~10°C (Benediktsdóttir *et al.*, 1998).

Moritella viscosa

Ulcers, of indeterminate cause, have been appearing on the flanks of Atlantic salmon in seawater during winter (= winter ulcer disease), principally in Iceland and Norway (Salte *et al.*, 1994; Lunder *et al.*, 1995; Benediktsdóttir *et al.*, 1998), and more recently in Scotland. Since its first recognition, a view has emerged that two new vibrios, *V. wodanis* and *Moritella viscosa*, are responsible (Benediktsdóttir *et al.*, 2000). *Moritella viscosa* was subsequently recovered from two diseased (with skin lesions) farmed Atlantic cod in Norway (Colquhoun *et al.*, 2004).

Moraxellaceae representatives

Acinetobacter sp.

During Autumn 1978 when the water temperature was between 8 and 11°C, an outbreak of disease occurred in a group of 60 sexually mature Atlantic salmon. The fish, each of 5–12 kg in weight, were wild stock from the River Surma, Norway,

and were held in brackish water during the occurrence of disease. During the 5-week period of disease, the cumulative mortalities amounted to 92% of the population. However, only about 40% of the animals displayed clinical signs of disease, namely hyperaemia of the dermal blood vessels, and haemorrhaging in the scale packets, with severe oedema extending into the lower epidermis in the vicinity of the base of the fins. Ulceration developed. Lesions appeared in the kidney, liver and spleen, and small haemorrhages occurred in the air bladder and on the visceral peritoneal surfaces (Roald and Hastein, 1980).

Moraxella sp.

During winter 1987, mortalities were recorded among juvenile striped bass, *Morone saxatilis*, in the Potomac River, Maryland. Gills of diseased fish were affected with the parasites *Trichodina* and *Ergasilus*. In addition, a reo-like virus and a bacterium were recovered from some individuals. Large haemorrhagic lesions and missing scales occurred on the dorsal surface of the 11 affected fish. Haemorrhages were apparent in the swim bladder. The liver was enlarged, pale and mottled in appearance. Membranous material appeared to connect the liver with the body wall (Baya *et al.*, 1990b).

Mycoplasmataceae representative

Mycoplasma mobile

The mycoplasma was associated with "red disease", a condition in the gills of tench (*Tinca tinca*) (Kirchhoff *et al.*, 1987).

Neisseriaceae representative

Aquaspirillum sp.

There has been a report of putative *Aquaspirilllum* sp., along with *Aer. hydrophila*, *Pseudomonas* sp. and *Streptococcus* sp., being associated with a disease, termed epizootic ulcerative syndrome, in snakeheads and catfish obtained from two fish farms in Thailand (Lio-Po *et al.*, 1998). However, the evidence for the involvement of *Aquaspirillum* is not convincing.

Oxalobacteraceae representative

Janthinobacterium lividum

During 1991, purple-pigmented, Gram-negative rod-shaped bacteria were associated with mortalities at two fish farms. At one site in Scotland, moribund rainbow trout (size range = 0.5–1.0 g) were diagnosed with RTFS. The second site in Northern Ireland also experienced high mortalities (~35% of the stock) in rainbow trout fry of 0.2–0.5 g in size, two to three weeks after the introduction of feeding. At this site, the rise in mortalities coincided with a change from the use of spring to river water

(Austin *et al.*, 1992b). In addition, during January 1992 we found similar purple-pigmented bacteria to be associated with skin lesions on larger rainbow trout (100–200 g in weight), which were otherwise debilitated with ERM. It is relevant to note that this fish population had received prolonged and varied chemotherapy.

Small fish, considered to be displaying RTFS, became lethargic, displayed exophthalmia, pale gills, enhanced skin pigmentation, swollen abdomen and (sometimes) skin lesions. Internally, the kidney was swollen, the spleen was pale and elongated, and some ascitic fluid was present in the peritoneal cavity (Austin *et al.*, 1992b). On the larger rainbow trout, the organism was associated with surface lesions. In particular, the skin was sloughed off along the entire flank of the animals, from operculum to tail, exposing the underlying (necrotic) muscle.

Pasteurellaceae representative

Pasteurella skyensis

The organism was recovered from four separate incidences of disease among farmed Atlantic salmon in Scotland during summer over a 4-year period from 1995–1998. The fish displayed inappetence (Jones and Cox, 1999).

Photobacteriaceae representatives

Photobacterium damselae subsp. *damselae*

The organism was associated initially with ulcerative lesions along the flank of blacksmith (*Chromis punctipinnis*), one of the damsel fish. These ulcers were noted in Summer and Autumn among fish populations in the coastal waters of southern California. Surveys of wild fish populations led to a conclusion that the ulcers were restricted to species of damsel fish. Additional information pointed to a role in human pathogenicity, insofar as the organism has been isolated from human wounds (Love *et al.*, 1981). Subsequent work demonstrated this organism in sharks (Grimes *et al.*, 1984a; Fujioka *et al.*, 1988), turbot (Fouz *et al.*, 1991, 1992), yellowtail (Sakata *et al.*, 1989) and red-banded sea bream (*Pagrus auriga*; Labella *et al.*, 2006).

Characteristic skin lesions, i.e. ulcers, are formed, particularly in the region of the pectoral fin and caudal peduncle. These ulcers may reach a size of 5–20 mm in diameter. Typically, muscle lysis occurs. The results of histopathological examination suggests the presence of granulomatous ulcerative dermatitis.

Photobacterium damselae subsp. *piscicida*

This disease has been responsible for heavy losses among menhaden and striped mullet in Galveston Bay, Texas (Lewis *et al.*, 1970). However, it is in Japan that the disease has become of considerable economic importance, causing significant losses in farmed yellowtail (Egusa, 1983). Since its initial recognition in yellowtails, pasteurellosis appears to have spread to other fish species, including gilthead sea bream (Balebona *et al.*, 1998), red sea bream (Yasunaga *et al.*, 1983), black sea bream

(Muroga *et al.*, 1977a; Ohnishi *et al.*, 1982), Atlantic bluefin tuna (Mladineo *et al.*, 2006) and sole (*Solea senegalensis*) (Zorrilla *et al.*, 1999). More recently, the disease seems to have spread to farmed and wild fish stocks in the Mediterranean area, notably Croatia, France, Italy and Spain (Magariños *et al.*, 1992; Mladineo *et al.*, 2006).

Essentially, pasteurellosis is a septicaemia in which acute cases exhibit only a few pathological signs. Internally, granulomatous-like deposits, which have led to the coining of the descriptive name of pseudotuberculosis, may develop on the kidney and spleen. These deposits comprise many greyish-white bacterial colonies of 0.5–1.0 mm^2 in size (Kusuda and Yamaoka, 1972). Purulent material may accumulate in the abdominal cavity (Lewis *et al.*, 1970).

Piscirickettsiaceae representative

Piscirickettsia salmonis

Although the pathogen was initially associated with salmon, there has been a spread to other groups, including sea bass (McCarthy *et al.*, 2005; Arkush *et al.*, 2005). Also, the geographical range has spread from Chile to North America and Europe, including Greece (McCarthy *et al.*, 2005) and Scotland (Birkbeck *et al.*, 2004). Infected fish gathered at the surface of cages, became sluggish and were inappetent. External signs included melanosis, epidermal indurations, and paleness of the gills, which was indicative of anaemia. The haemacrits fell to $\leq$27%. Internally, haemorrhages were evident on the abdominal walls, visceral fat and on the air bladder. A mottled liver and swollen spleen was apparent in heavily infected animals. The kidney was inflamed and swollen. The intestine was full of yellowish mucoid material (Schäfer *et al.*, 1990; Branson and Diaz-Munoz, 1991). Initially, the disease was not considered to occur during the freshwater stage of fish culture, Instead, mortalities have been noted to begin 6–12 weeks after transfer of fish from fresh to seawater (Fryer *et al.*, 1992). However, in a later development the pathogen was recovered from the freshwater stage of coho salmon and rainbow trout (Bravo, 1994; Gaggero *et al.*, 1995).

Rickettsia-like organism (RLO)

Chern and Chao (1994) reported that the RLO caused mass mortalities in tilapia, with disease signs including the presence of white nodules and microscopic granulomas on all the organs, and an enlarged spleen. Subsequently, Comps *et al.* (1996) described a small coccoid organism from the brain of juvenile sea bass obtained from the South of France. These were derived from a population which suffered 20% mortalities. Also, an RLO has been identified among grouper in Taiwan (Chen *et al.*, 2000a), tilapia (Mauel *et al.*, 2003) and white sea bass in the U.S.A. (Chen *et al.*, 2000) and farmed Atlantic salmon in Tasmania and Canada (Cusack *et al.*, 2002). Moribund grouper displayed dark lesions, splenomegaly (with white nodules), necrosis in the liver, kidney and spleen, and the blood was thin (Chen *et al.*, 2000a). However, it is difficult from the literature to determine the significance of the Tasmanian RLO to fish (Corbeil *et al.*, 2005).

Pseudomonadaceae representatives

Pseudomonas anguilliseptica

Originally observed in eels, when the disease was referred to as Sekiton byo (= red spot), the pathogen has been identified in a wide range of other species, including Baltic herring (*Clupea harengus membras*) (Lönnström *et al.*, 1994), gilthead sea bream (Doménech *et al.*, 1999), black-spot sea bream (*Pagellus bogaraveo*) (López-Romalde *et al.*, 2003), orange-spotted grouper (*Epinephelus coioides*) (Al-Marzouk, 1999) and cod (Ferguson *et al.*, 2004). Typically, the disease manifests itself by the presence of petechial (pin-prick) haemorrhages in the skin of the mouth region, opercula and ventral side of the body. Haemorrhaging in the eye has been seen in infected Baltic herring (Lönnström *et al.*, 1994). Reddening of the fins (as with vibriosis or *Aer. hydrophila* infections) does not usually occur. Small petechial haemorrhages may develop in the peritoneum, and the liver may be pale and haemorrhaged. The kidney may be soft and liquefying. Alternatively, in some cases of disease, there may be a dearth of internal signs of distress (Wakabayashi and Egusa, 1972). Winter disease of gilthead sea bream, in which affected fish displayed slow erratic swimming before sinking to the bottom of the water and dying, has been linked to this pathogen (Doménech *et al.*, 1999). Other disease signs included abdominal distensions on some animals, haemorrhaged kidney, pale liver, and the intestine full of yellowish exudate. Low-level mortalities, albeit in the absence of external signs of disease, were reported in black-spot sea bream in Spain (López-Romalde *et al.*, 2003).

Pseudomonas chlororaphis

In moribund fry, it was observed that symptoms included the presence of distended abdomen with ascitic fluid, and haemorrhages on the body surface (Hatai *et al.*, 1975).

Pseudomonas fluorescens

It has been reported to cause disease in a wide range of fish species, including silver carp (*Hypophthalmichthys molitrix*) and bighead (*Aristichthys nobilis*) (Csaba *et al.*, 1981b; Markovic *et al.*, 1996), goldfish (*Carassius auratus*) (Bullock, 1965), tench (*Tinca tinca*) (Ahne *et al.*, 1982), grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) (Bauer *et al.*, 1973), unnamed species of carp (Schäperclaus, 1959; Schäperclaus and Brauer, 1964; Heuschmann-Brunner, 1978) and rainbow trout (Li and Flemming, 1967; Li and Traxler, 1971, Sakai *et al.*, 1989a). Generally, *Ps. fluorescens* is associated with fin or tail rot in which the infected area is eroded away (Schäperclaus, 1979). In tench fry, high mortalities (up to 90% of the population) have been reported, in which visual signs of disease included haemorrhagic lesions on the skin and at the base of the fins. Ascitic fluid accumulated in the peritoneal cavity, and petechial haemorrhages were evident in the gills, kidney, liver and in the lumen and submucosa of the gut, i.e. a typical generalised bacterial septicaemia (Ahne *et al.*, 1982). Similar symptoms were apparent in silver carp

and bighead (Csaba *et al.*, 1981). Stress, including a lowered water temperature, may trigger outbreaks of disease (Markovic *et al.*, 1996). With rainbow trout the presence of ulcers at haemorrhages on the gills and fins were reported (Sakai *et al.*, 1989a).

Pseudomonas plecoglossicida

A new bacterial disease emerged during the 1990s, and caused mass mortalities in pond-cultured ayu in Japan (Wakabayashi *et al.*, 1996). The term bacterial haemorrhagic ascites is descriptive, with affected ayu displaying lesions in the gills, heart, intestine, kidney, liver and spleen. In particular, lesions in the spleen and haematopoietic tissues were profound. Those in the kidney, liver and spleen were necrotic. Abscesses were apparent in the liver. In contrast, the brain did not reveal the presence of any lesions (Kobayashi *et al.*, 2004).

Pseudomonas pseudoalcaligenes

The fish displayed extensive skin lesions, which extended over the entire flank from the operculum to the tail. The skin and underlying muscle to a depth of approximately 1 mm were totally eroded (Austin and Stobie, 1992b).

Pseudomonas putida

Altinok *et al.* (2006) described exophthalmia, melanosis and ulcers on the dorsal surface of rainbow trout in growout ponds in Turkey. The internal organs appeared normal, but the intestine was full of yellowish liquid.

Vibrionaceae representatives

Apart from the taxa discussed below, a study of the type strains of newly described and some older known vibrios and their ECPs revealed that *V. brasiliensis* ($LD_{50} = \sim 2 \times 10^4$), *V. corallilyticus* ($LD_{50} = 7.5 \times 10^1 - 2.5 \times 10^3$), *V. ezurae* ($LD_{50} = 7.3 \times 10^3$), *V. fortis* ($LD_{50} = \sim 10^2$), *V. kanaloaei* ($LD_{50} = < 2 \times 10^2$), *V. neptunius* ($LD_{50} = 10^2$), *V. rotiferianus* ($LD_{50} = < 10^2 - 5.0 \times 10^5$) and *V. tubiashi* ($LD_{50} = 2.5 \times 10^2$) were pathogenic in laboratory-based experiments with rainbow trout with mortalities of up to 100% (Austin *et al.*, 2005). It will be interesting to see if these organisms become recognised as pathogens in aquaculture.

Vibrio alginolyticus

From the description of Colorni *et al.* (1981) and Austin *et al.* (1993), the disease may be classified as a typical bacterial septicaemia. Infected fish were observed to become sluggish, the skin darkened, scales loosened and sloughed off, and ulcers developed. The liver, capillaries in the intestinal wall, air bladder and peritoneum became congested. Simultaneously, the intestine and gall bladder became distended with clear fluid and bile, respectively. Anaemia and gill rot were also reported. Austin *et al.* (1993) attributed the organism with gill disease leading to progressive low-level mortalities in turbot, which were maintained at supra-optimal temperatures in a

recirculating aquarium. The later study of Lee (1995) attributed the organism with causing exophthalmia and corneal opaqueness in grouper; signs that are reminiscent of the pathology caused by *V. harveyi*. Woo *et al.* (1995) and Ye *et al.* (1997) considered that *V. alginolyticus* was responsible for heavy mortalities in silver sea bream (*Sparus sarba*) in Hong Kong. The organism has also been associated with disease in gilthead sea bream in Spain (Balebona *et al.*, 1998).

Vibrio anguillarum

"Red-pest" (referred to historically as *pestis rubra anguillarum* and *erysipelosis anguillarum*) caused catastrophic losses among eels held in seawater sites within Italy during the 18th and 19th centuries. The excellent description of an outbreak of "red-pest" in eels during 1718 is undoubtedly the first reference to a bacterial fish disease in the European literature (Bonaveri, 1761). For a detailed account of the early narratives, reference is made to the splendid review of Drouin de Bouville (1907). However, confusion may result from the multiplicity of names used to describe the disease. Thus, references may be found to "salt-water furunculosis" (Rucker, 1963), "boil-disease" (Kubota and Takakuwa, 1963) and "ulcer-disease" (Bagge and Bagge, 1956), as well as to the universally accepted name of "vibriosis".

Apt but gory descriptions have been made about the nature of vibriosis in fish. To microbiologists, the disease may be regarded as yet another haemorrhagic septicaemia. Typically, infected fish show skin discoloration, the presence of red necrotic lesions in the abdominal muscle, and erythema (bloody blotches) at the base of the fins, around the vent and within the mouth (in this respect there is a resemblance to ERM, caused by *Yersinia ruckeri*). The gut and rectum may be distended, and filled with clear viscous fluid. Exophthalmia may be evident (Anderson and Conroy, 1970).

In Pacific salmon fingerlings, a bacteraemia occurs in the initial stages of disease. From histological examination, it may be concluded that there are pathological changes in the blood, connective tissue, gills, kidney, liver (an anaemia) and posterior gastro-intestinal tract, and swelling in the spleen. The bacterial cells appear to be uniformly distributed throughout the affected tissues, although the greatest concentration is in the blood (Tajima *et al.*, 1981; Ransom *et al.*, 1984). Usually, infected fish become inactive, cease feeding (this may cause problems for chemotherapy) and suffer heavy mortalities.

Fin rot is another condition attributed to *V. anguillarum*. For example, the pathogen has been blamed for causing fin rot in juvenile turbot principally in northern China (Lei *et al.*, 2006). Here, the infection led to mortalities of 90–100% (Lei *et al.*, 2006).

V. cholerae (non-O1)

Petechial haemorrhages developed on the body surface. Internally, there was congestion of the organs (Muroga *et al.*, 1979; Kiiyukia *et al.*, 1992). Reddacliff *et al.* (1993) reported that septicaemia developed in infected goldfish.

V. fischeri

Most diseased fish possessed whitish nodules on the skin (dorsal surface), haemorrhagic ulceration, and tumours involving the pancreas and bile duct. Within a year, 39% losses occurred in the fish population (Lamas *et al.*, 1990).

V. furnissii

There has been an indication that *V. furnissii* may be associated with eel disease in Spain (Esteve, 1995). However, isolates were recovered from water rather than diseased eels. Therefore, the association with fish pathology is dubious.

Vibrio harveyi (including *V. carchariae* and *V. trachuri*)

V. carchariae was originally isolated from a dead sandbar shark (*Carcharhinus plumbeus*) which died at the National Aquarium in Baltimore, Maryland, in 1982 (Grimes *et al.*, 1984a). Subsequently, a similar organism was recovered from lemon sharks (*Negraprion brevirostris*) (Colwell and Grimes, 1984). Grimes *et al.* (1984b) and Colwell and Grimes (1984) described the disease as a "vasculitis". Infected animals became lethargic, stopped feeding, appeared disorientated and developed necrotic subdermal cysts. On postmortem examination, encephalitis, meningitis, kidney necrosis, vasculitis, and unspecified liver and spleen damage were noted. Evidence has been forthcoming that the pathogen is more serious in compromised than healthy hosts (Grimes *et al.*, 1985). A similar organism has been isolated from a chronic skin ulcer on a shark (Bertone *et al.*, 1996). *V. carchariae* was associated with gastro-enteritis leading to heavy mortalities among cultured groupers (*Epinephelus coioides*) during 1993 in Taiwan (Yii *et al.*, 1997).

A parallel development involved gamefish, namely common snook (*Centropomus undecimalis*). These were found to suffer with opaque white corneas within 24 h of capture in Florida (Kraxberger-Beatty *et al.*, 1990). From such damaged specimens, *V. harveyi* was recovered. Common snoop developed opaque white corneas within 24 h of capture. In the absence of treatment, blindness resulted. With a second species, jack crevalle (*Caranx hippos*), deep dermal lesions were noted in wild specimens, which were also captured in Florida. Internal abnormalities were not recorded (Kraxberger-Beatty *et al.*, 1990). In another example from an aquarium in Barcelona, eye lesions in the short sunfish (*Mola mola*)—due to biting by other fish—were colonised by *V. harveyi* (Hispano *et al.*, 1997). Other diseases include:

- flounder infectious necrotising enteritis, which is characterised by distended abdomens filled with opaque fluid, enteritis, necrosis of the posterior intestine (in extreme cases, this was detached from the anus and exiting via the vent), reddening in the vicinity of the anus, lethargy and inappetence, has been documented in farmed summer flounder in Rhode Island (Soffientino *et al.*, 1999; Gauger *et al.*, 2006).
- Skin ulcers and haemorrhaging in the vicinity of the mouth and fins has been reported in sole (*Solea senegalensis*) in Spain (Zorrilla *et al.*, 2003).

- Infectious gastro-enteritis, which has been reported in cultured red drum (*Sciaenops ocelatus*) from Taiwan (Liu *et al.*, 2003). Disease signs included swollen intestine containing yellow fluid.

A disease, resembling vibriosis and equated to a new species *V. trachurii*, has been long associated with Japanese horse mackerel (*Trachurus japonicus*) especially during summer when the seawater temperature exceeds 25°C (Iwamoto *et al.*, 1995). Infected fish displayed erratic swimming, darkened in colour, developed pronounced bilateral exophthalmia and developed haemorrhages on the internal organs. However, the organism is now recognised as a synonym of *V. harveyi* (Thompson *et al.*, 2002).

V. ichthyoenteri

Since 1971 opaque intestines and intestinal necrosis accompanied by high mortalities have been reported in Japanese and Korean hatcheries rearing Japanese flounder (Ishimaru *et al.*, 1996; Kim *et al.*, 2004).

V. logei

An organism, with similarities to *V. logei*, was associated with shallow skin lesions of Atlantic salmon farmed in Iceland at low temperatures, i.e. ~10°C (Benediktsdóttir *et al.*, 1998).

V. ordalii

Essentially, the disease may be categorised as a haemorrhagic septicaemia. However, there are subtle differences in the pathologies of the diseases caused by *V. anguillarum* and *V. ordalii*. In the case of *V. ordalii* in Pacific salmon, there is a tendency for the formation of micro-colonies in the skeletal and heart muscle, gill tissue, and in both the anterior and posterior regions of the gastro-intestinal tract (Ransom, 1978; Ransom *et al.*, 1984). Moreover, bacteraemia developed much later in the disease cycle than with *V. anguillarum*. Perhaps, this accounted for the lower numbers of bacterial cells in the blood. A further difference concerned the marked decrease in the numbers of leucocytes in the blood, i.e. leucopenia (Ransom, 1978; Harbell *et al.*, 1979; Ransom *et al.*, 1984).

V. pelagius

An epizootic of juvenile farmed turbot in northwest Spain occurred during January and February 1991 when the water temperature was 12–15°C, with fish displaying eroded dorsal fins and tail, haemorrhages at the base of the fins, haemorrhages on the internal organs and intestines full of mucus liquid (Angulo *et al.*, 1992). The total losses amounted to 3% of the turbot population. Subsequently, larval turbot were described with swollen and necrotic secondary gill lamellae, sloughing off of the

intestinal mucosa and necrosis of the haematopoietic tissues of the kidney (Villamil *et al.*, 2003).

V. salmonicida

With the tremendous increases in production of Atlantic salmon in Norway, it was perhaps inevitable that at some time a new or emerging disease would cause havoc to the industry. Thus, in 1979 such a "new" disease appeared in salmon farms located around the island of Hitra, south of Trondheim in Norway. In 1983, the disease appeared in Stavanger and, in particular, the large number of fish farms in the Bergen region. The disease, coined coldwater vibriosis or Hitra disease (Egidius *et al.*, 1981), occurs mainly during the period of late Autumn to early Spring. The disease is now widespread throughout Norway, and there are some reports of outbreaks in Scotland, Shetland (Bruno *et al.*, 1985) and Canada. The disease resembles a generalised haemorrhagic septicaemia. Externally, haemorrhaging may be evident around the abdomen (Holm *et al.*, 1985). Internally, there is often evidence of anaemia, haemorrhaging on the organs, swim bladder, abdominal wall and posterior gastrointestinal tract (Poppe *et al.*, 1985; Holm *et al.*, 1985; Egidius *et al.*, 1986).

V. splendidus

During 1987, a disease occurred in cultured turbot in northwest Spain. During the outbreak, there was a continuous low-level mortality amounting to 4% of the total stock. Infected fish contained a virus, deemed to be a reovirus, and a bacterium, which was considered to resemble *V. splendidus* (Lupiani *et al.*, 1989). Interestingly, a similar organism has been recovered from diseased Atlantic salmon in Scotland (B. Austin, unpublished data), turbot (including larval turbot in Spain; Thomson *et al.*, 2005), sea bass in Norway (Myhr *et al.*, 1991) and gilthead sea bream in Spain (Balebona *et al.*, 1998). Diseased turbot displayed swollen abdomen and haemorrhaging in the mouth, at the anus and base of the fins. The swimming behaviour was not unusual. Internally, the stomach and intestine were swollen, and filled with a mucoid liquid. Haemorrhaging was apparent on the walls of the peritoneal cavity, which also contained a reddish liquid. The liver was pale (Lupiani *et al.*, 1989; Angulo *et al.*, 1994).

V. splendidus 1 and *V. campbellii*-like organisms have been implicated with acute mortalities in turbot (*Colistium nudipinnis*) and brill (*C. guntheri*) in New Zealand (Diggles *et al.*, 2000). Here, the disease signs included inappetence, erratic swimming, distended abdomen, distended stomach and intestine, which contained clear fluid, haemorrhaging around and at the base of the fins, necrosis and sloughing off of the mucosa from the stomach and intestine, haemorrhaging and necrosis in the kidney and liver, and some vacuolation in the brain and spinal cord. A suggested link was made to adverse water quality and inadequate diet (Diggles *et al.*, 2000).

V. splendidus was recovered from the kidney of dead and moribund corkwing wrasse in Norway. The disease signs centred on inappetence, reduced swimming activity, and in some cases surface ulceration (Jensen *et al.*, 2003).

V. tapetis

V. tapetis was recovered from the kidney of dead and moribund corkwing wrasse in Norway. The disease signs centred on inappetence, reduced swimming activity, and in some cases surface ulceration (Jensen *et al.*, 2003).

V. vulnificus

Between 1975 and 1977, in Japan there were serious outbreaks of disease among cultured eels from six separate localities (Muroga *et al.*, 1976a, b; Nishibuchi and Muroga, 1977, 1980; Nishibuchi *et al.*, 1979, 1980). The disease has certainly spread to Europe, with incidences in Spain (Biosca *et al.*, 1991; Amaro *et al.*, 1992), The Netherlands and England (B. Austin, unpublished data). This is a haemorrhagic condition characterised by redness on the body, notably flank and/or tail. In advanced cases, pathological changes may be observed in the gastro-intestinal tract, gills, heart, liver and spleen (Miyazaki *et al.*, 1977). Superficially, the disease resembles classical vibriosis. During 2005, the pathogen was recognized as the cause of high levels of mortality in farmed ovate pompano (*Trachinotus ovatus*) in the P.R.C., with disease signs including external haemorrhaging and ulcers, and haemorrhaging gills, intestine and liver (Li *et al.*, 2006). A new serogroup, termed *V. vulnificus* biotype 2 serovar A, was recognised in Spain during 2000 and in Denmark by 2004, and affected eels of 5–10 g in weight producing severe disease signs including extensive haemorrhaging and necrosis (Fouz *et al.*, 2006).

V. wodanis

Ulcers, of indeterminate cause, have been appearing on the flanks of Atlantic salmon in seawater during winter (= winter ulcer disease), principally in Iceland and Norway (Salte *et al.*, 1994; Lunder *et al.*, 1995; Benediktsdóttir *et al.*, 1998), and more recently in Scotland. Since its first recognition, a view has emerged that two new vibrios, *V. wodanis* and *Moritella viscosa*, may be responsible (Benediktsdóttir *et al.*, 2000).

MISCELLANEOUS PATHOGENS

"*Candidatus* Arthromitus"

The condition, which affects rainbow trout during summer (water temperature $\geq 15^{\circ}C$), has been recognised in France and Spain, and may result from climatic and stress conditions. It is characterised by the huge populations of segmented, filamentous bacteria in the digestive tract, and daily losses of 0.5–1% (Michel *et al.*, 2002a). Affected fish are lethargic and inappetent, and yellow, mucoid faeces may extend from the vent. The digestive tract is haemorrhagic and oedematous, and filled with mucoid material (Michel *et al.*, 2002a).

UNIDENTIFIED GRAM-NEGATIVE RODS

The human and animal pathology literature abounds with references to hard-to-identify or unidentified pathogens. An example from fish pathology concerns a hitherto unknown intracellular bacterial pathogen of farmed Atlantic salmon in Ireland (Palmer *et al.*, 1994). During 1992–1993 when the water temperature was 8–9 and 15–16°C, fish became lethargic, swam close to the water surface and displayed loss of balance. Apart from an infestation of salmon lice, the fish revealed the presence of petechia or haemorrhagic areas on the abdominal walls, petechia on the pyloric caeca and swim bladder, congestion of the kidney and spleen, splenomegaly, and kidney swelling. The fore and hind guts contained white mucus. Some fish had pale friable livers, pale spleens, visceral adhesions, and false membranes in the peritoneum (Palmer *et al.*, 1994).

Another unidentified Gram-negative organism was linked to a previously undescribed condition, coined *Varracalbmi* (= bloody eye), in Norwegian farmed Atlantic salmon. The disease, which occurred in northern Norway during 1989–1992, was described as a haemorrhagic, necrotising, pyogranulomatous inflammation of the eye, being termed panophthalmitis. Lethargy, deep ulcers, necrosis, and haemorrhagic, granulomatous, pyogenic visceral organs occurred (Valheim *et al.*, 2000). Mortalities of only 2.5% were reported (Valheim *et al.*, 2000).

Gram-negative bacteria were associated with a mass mortality of cultured rockfish (*Sebastes schlegeli*) in Japan during the spring of 2001, with the disease signs reflecting aneurysms, hyperplasia, lamellar fusion, haemorrhaging and necrosis of the gills. Some fish revealed bacterial invasion of the kidney, myocardium and spleen. Yet, cultures were not recovered for further work (Kobayashi *et al.*, 2005).

An unusual Gram-negative bacterial culture was linked to ulceration (singular circular ulcers of 10–15 mm in diameter on the flank) in Scottish farmed rainbow trout (Austin *et al.*, 2003).

3

Characteristics of the pathogens: Gram-positive bacteria

Taxonomists rarely consider the ecological relevance of their findings. Similarly, ecologists ignore taxonomy, which is perceived to be old-fashioned and/or boring.

Within the realms of fish diseases, it is apparent that the names of bacterial species are often used with little supporting evidence to justify the use of the names. Also, many studies are based on the examination of single isolates the relevance of which to fish pathology or science in general is doubtful. With the advent of molecular-based methods, the description of new taxa is often based on minimal phenotypic data, which poses problems for determining reliable diagnostic traits.

ANAEROBES

Although only two species of anaerobic bacteria, namely *Clostridium botulinum* and *Eubacterium tarantellae*, have been implicated as fish pathogens, it is considered likely that detailed biological investigations may reveal that anaerobes cause more widespread problems than has been hitherto realised. In the first place, diagnostic laboratories do not normally use anaerobic methods. Therefore, it is unlikely that isolation of an offending anaerobic pathogen would ever be achieved. Consequently, the cause of disease may not be recognised, or maybe attributed to an aerobic secondary invader. Although there is no evidence that anaerobic pathogens have been missed, there are puzzling cases of mortalities among fish populations for which the aetiological agent has never been isolated. Of course, this could reflect the use of inappropriate methods. It is recognised, however, that anaerobes occur in aquatic sediments (Davies, 1969; Rouhbakhsh-Khaleghdoust, 1975) and in the gastro-intestinal tract of fish (Sakata *et al.*, 1978, 1980; Trust *et al.*, 1979; Austin, 2006), where they would be readily available for initiation of a disease cycle. Trust and colleagues

recovered *Actinomyces*, *Bacteroides*, *Fusobacterium* and *Peptostreptococcus* from grass carp, gold fish and rainbow trout. Sakata *et al.* (1980) described two groups of anaerobes from the intestines of freshwater fish, i.e. ayu, goldfish and tilapia, in Japan. These Gram-negative, non-motile, asporogenous rods were considered to be representatives of the family Bacteroidaceae. It is questionable whether or not these bacteria will be recognised as fish pathogens in the future.

Clostridiaceae representative

Clostridium botulinum

Descriptions of fish-pathogenic clostridia have tended towards extreme brevity. However, it is clear that the outbreaks of botulism in fish have been caused by predominantly *Cl. botulinum* type E.

Clostridium botulinum

Cultures comprise anaerobic, chemo-organotrophic, Gram-positive, non-acid-fast rods, of 3.4–7.5 × 0.3–0.7 mm in size, which are motile by means of peritrichous flagella. It is important to note that care should be taken in interpreting Gram-stained smears, because cells may appear Gram-negative with age. Oval, sub-terminally positioned endospores are formed, which have characteristic appendages and exosporia. The cell wall contains diaminopimelic acid. Surface colonies are 1–3 mm in diameter, slightly irregular with lobate margins and raised centres, and translucent to semi-opaque with a matt appearance. Poor to moderate growth occurs in cooked meat broth, but abundant growth occurs in broth containing fermentable carbohydrates at the optimum temperature of 25–30°C. Lecithinase, lipase, neurotoxins and haemolysins, but not catalase, caseinase, H_2S, indole or urease, are produced. Gelatinase is not usually produced by *Cl. botulinum* type E. Nitrates are not reduced, nor is the Voges Proskauer reaction positive. Some carbohydrates, such as fructose and glucose, but not aesculin, cellobiose, dulcitol, glycogen, inulin, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbose, starch, sucrose and xylose, are fermented to acetic and butyric acids. The G+C ratio of the DNA is in the range of 26 to 28 mol % (Cato *et al.*, 1986).

Eubacteriaceae representative

Eubacterium tarantellae

It was reported initially by Udey *et al.* (1976) that a novel anaerobic organism was capable of producing a neurological disease in striped mullet (*Mugil cephalus*). This conclusion resulted from an investigation into major fish mortalities in Biscayne Bay, Florida. The anaerobe was subsequently elevated to species status, as *Eubacterium tarantellus* (Udey *et al.*, 1977), and then corrected to *Eu. tarantellae* (Trüper and

de'Clari, 1997). It seems likely that *Catenabacterium*, previously described as an anaerobic pathogen of fish (Henley and Lewis, 1976), should probably be equated with *Eu. tarantellae* (Udey *et al.*, 1977).

Eubacterium tarantellae

On BHIA, the organism produces flat, translucent colonies, approximately 2–5 mm in diameter, which are colourless, rhizoidal and slightly mucoid. These contain long, unbranched, filamentous, Gram-positive, asporogenous rods, which fragment into smaller bacilli of 1.3–1.6 × 1.0–17.0 μm. Good growth occurs at 25 to 37°C. All isolates degrade blood (β-haemolysis) and lecithin, but not aesculin, gelatin or starch. Catalase, H_2S and indole are not produced; nitrates are not reduced; and carbohydrates are generally not fermented. However, there is some evidence for the production of acid from fructose, glucose and lactose, but not from aesculin, amygdalin, arabinose, cellobiose, maltose, mannitol, mannose, melezitose, raffinose, rhamnose, salicin, starch, sucrose, trehalose or xylose.

Unfortunately, the G + C ratio of the DNA was not determined. From these characteristics, Udey *et al.* (1977) proposed that the organisms should be classified in a new species, as *Eubacterium tarantellus*. Clearly, the fish isolates possess the general characteristics of *Eubacterium*, i.e. Gram-positive, anaerobic, asporogenous, chemoorganotrophic, non-motile, catalase-negative rods, which grow well at 37°C (Moore and Holdeman-Moore, 1986). A comparison between the descriptions of *Eu. limosum* and *Eu. tarantellae* reveals that, among comparative tests, there are similarities (Table 3.1). In fact, the only major differences concern hydrolysis of aesculin and acid production from lactose and mannitol.

GRAM-POSITIVE BACTERIA—THE "LACTIC ACID" BACTERIA

Carnobacteriaceae representative

Carnobacterium piscicola (and the lactobacilli)

There have been only a few reports citing *Lactobacillus* spp. as fish pathogens. The initial work was by Ross and Toth (1974), who described mortalities among 3-year-old rainbow trout in a hatchery in California. The term pseudokidney disease was coined by these workers to distinguish the condition from BKD. However, it is not clear what pathological significance, if any, could be attributed to the lactobacilli, because pure cultures were incapable of reproducing infection. Nevertheless, lactobacilli were again implicated in an infection of female 2–3-year-old rainbow trout from a hatchery in Newfoundland, Canada (Cone, 1982). However, this was a mixed bacterial infection involving primarily lactobacilli but also *Aer. hydrophila*, *Ps. fluorescens* and Enterobacteriaceae representatives. There is no report about any pathogenicity experiments using the Canadian *Lactobacillus* strain; therefore, its precise

Table 3.1. Comparison of *Eubacterium limosum* with *Eu. tarantellae*

Character	*Eu. limosum*[a]	*Eu. tarantellae*[b]
Catalase production	–	–
Growth at 37°C	+	+
Degradation of:		
Aesculin	+	–
Blood	NS	+
DNA	NS	+
Gelatin	V	–
Lecithin	NS	+
Starch	–	–
Production of acid from:		
Adonitol	+	NS
Aesculin	–	–
Amygdalin	–	–
Arabinose	–	–
Cellobiose	–	–
Dulcitol	–	NS
Fructose	+	+
Glucose	+	+
Lactose	–	+
Maltose	–	–
Mannitol	+	–
Mannose	–	–
Melezitose	–	–
Raffinose	–	–
Rhamnose	–	–
Salicin	–	–
Starch	–	–
Sucrose	–	–
Trehalose	–	–
Xylose	–	–

[a] From Moore and Holdeman-Moore (1986)
[b] From Udey *et al.* (1977)
NS = Not stated
V = Variable result

role as a fish pathogen is open to question. From these two initial reports, it is doubtful whether *Lactobacillus* should be regarded as a true fish pathogen. However, additional lactic-acid producing organisms have been isolated from the kidneys of moribund rainbow trout (B. Austin, unpublished data). Moreover, these isolates produced clinical disease upon intraperitoneal injection into salmonids. Similar isolates have been studied by Professor Stemke (pers. commun.; Table 3.2). Hiu *et al.* (1984) proposed a new species, namely *Lactobacillus piscicola*, to accommodate a

Table 3.2. Characteristics of fish-pathogenic lactobacilli

Character	Isolates of Starliper *et al.* (1992)	Isolates of Austin and G. Stemke[a]	*Car. piscicola*
Production of:			
Arginine dihydrolase	−	+	+
Catalase	+/−	−	−
β-galactosidase	−	−	−
H_2S	−	−	−
Indole	−	−	ND
Lysine decarboxylase	−	−	ND
Ornithine decarboxylase	−	−	ND
Oxidase	−	−	−
Phenylalanine deaminase	−	−	ND
Phosphatase	−	+	ND
Gluconate oxidation	−	−	−
Methyl red test	+	+	ND
Nitrate reduction	−	−	−
Degradation of:			
Aesculin	+	+	+
Blood	+ (α)	−	ND
Casein	ND	+	ND
Cellulose, chitin, DNA	ND	−	ND
Elastin, gelatin	ND	−	ND
Hypoxanthine, lecithin	ND	−	ND
RNA, sodium hippurate	ND	−	ND
Starch	−	−	ND
Tween 20	ND	−	ND
Tween 40	ND	+	ND
Tween 60, Tween 80	ND	−	ND
Tyrosine, xanthine	ND	−	ND
Growth at 37°C	−	−	+
Production of acid from:			
Arabinose	+	−	−
Fructose, glucose	+	+	+
Galactose	−	−	+
Glycerol	−	+	+
Lactose, xylose	−	−	−
Maltose, mannitol	+	+	+
Sucrose	+	+	+
G + C ratio of the DNA	−	35.2–36.5 mol %	34–36 mol %

[a] Unpublished data
ND = Not determined

group of 17 isolates recovered from diseased chinook salmon, cut-throat trout and rainbow trout. Similar isolates were recovered from juvenile salmonids and carp in Belgium and France (Michel *et al.*, 1986b) and from striped bass and catfish in the U.S.A. (Baya *et al.*, 1991). *Lactobacillus piscicola* became subsequently re-classified as *Carnobacterium piscicola* (Collins *et al.*, 1987). In addition, fish-pathogenic lactobacilli, with similarities to *Lactobacillus alimentarius* and *Lactobacillus homohiochi*, have been recovered from post-spawning rainbow trout in the U.S.A. (Starliper *et al.*, 1992; Table 3.2).

Carnobacterium piscicola

On TSA, *Car. piscicola* produces small, round, entire, shiny, opaque colonies that develop within 48 h. These contain non-motile, non-acid-fast, Gram-positive, fermentative cocco-bacilli or rods of approximately 1.1–1.4 × 0.5–0.6 μm in size. Other phenotypic traits of the lactobacilli include the inability to produce catalase (catalase-positive isolates were described by Starliper *et al.*, 1992), H_2S, indole, gelatinase, urease, the Voges Proskauer reaction, arginine hydrolysis, or lysine or ornithine decarboxylases or reduce nitrates. However, the isolates produce acid from fructose, galactose, glucose, glycerol, inulin, lactose, maltose, mannitol, melibiose, salicin, starch, sucrose and trehalose, but not raffinose, sorbitol or xylose (Schmidtke and Carson, 1994). There are a few differences between the Californian and Canadian isolates, namely hydrolysis of aesculin and acid production from lactose. Growth occurs at 10°C but not 40°C, in 6.5% (w/v) sodium chloride, and at pH 9.6.

However, in most respects all the isolates were markedly similar. By a comparison of these phenotypic traits with conventional identification schemes, it is apparent that an identification of *Lactobacillus* or *Streptococcus* could result. Furthermore, the photomicrographs of cells, published by Ross and Toth (1974), could be interpreted as chains of cocci rather than short rods, which would be more in keeping with an identification of *Streptococcus*. The isolates recovered from England and Canada, by Austin and Stemke, respectively, possessed more of the characteristics attributable to *Lactobacillus* or the related genera. Cultures comprised Gram-positive, non-motile, fermentative rods of approximately 3.0 × 1.0 μm in size, forming round, raised, entire white colonies on tryptone soya agar after incubation for 48 h at 20°C. Growth occurred at 4–26°C but not 37°C, and in 2.5% but not 7.5% (w/v) sodium chloride. A resemblance to *Car. piscicola* is readily apparent. Indeed, the only differences reflect growth at 37°C and acid production from lactose. Both the Canadian isolates of Stemke and the U.S. isolates of Hiu *et al.* (1984) and Starliper *et al.* (1992) produced lactic acid from the fermentation reactions. Clearly, it seems that all these lactobacilli may belong in the same taxon, i.e. *Car. piscicola*. However, it will be necessary for further study to determine the true relationship of the English and Canadian isolates to those of Hiu *et al.* (1984).

The cell wall peptidoglycan of *Car. piscicola* was found to comprise diaminopimelic acid, alanine and glutamic acid, but no lysine. DNA:DNA hybridisation revealed negligible, i.e. 10%, homology with the reference cultures of *Lactobacillus acidophilus*, *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus salivarius* and *Lactobacillus yamanashiensis*, compared with 70% re-association between isolates of *Car. piscicola* (Hiu *et al.*, 1984). Thus, in their publication it was concluded that the fish pathogens were most closely related to *Lactobacillus yamanashiensis* subsp. *yamanashiensis* in terms of G+C ratio of the DNA and fermentation profile. Subsequently, Collins *et al.* (1987) demonstrated closer relationships with other carnobacteria.

GRAM-POSITIVE COCCI IN CHAINS

General comments

Since the first publication in 1958, there has been considerable confusion about the number of and the nature of the bacterial species involved in streptococcicosis/streptococcosis. Thus, at various times the fish-pathogenic streptococci have been linked with *Str. agalactiae*, *Str. dysgalactiae*, *Str. equi*, *Str. equisimilis*, *Str. (=En.) faecium*, *Str. pyogenes* and *Str. zooepidemicus*. In addition, we have found that *Enterococcus faecalis* NCTC 775^{T}, *En. faecium* NCTC 7171^{T}, *Lactococcus lactis* NCFB 604, *Str. mutans* NCFB 2062 will cause similar diseases in Atlantic salmon and rainbow trout. Certain traits of the causal agent(s) have been repeatedly emphasised as having supposedly taxonomic significance. In particular, the ability to attack blood has been highlighted. Thus, fish-pathogenic strains have been described, at one time or another, as either α- (Kusuda *et al.*, 1976a; Al-Harbi, 1994) or β-haemolytic (Robinson and Meyer, 1966; Minami *et al.*, 1979; Kitao *et al.*, 1981; Ugajin, 1981; Iida *et al.*, 1986) or as non-haemolytic (Plumb *et al.*, 1974; Cook and Lofton, 1975; Iida *et al.*, 1986). Superficially, this information could infer heterogeneity among the pathogens, although some well-established taxa, e.g. *Str. agalactiae*, contain both α- and β-haemolytic strains; Table 3.3). Nevertheless, many characteristics are shared by the majority of the fish pathogens. Yet, there is also some variance in the overall descriptions reported by different groups of workers (Table 3.3). For example, Boomker *et al.* (1979) reported that isolates, recovered from the Transvaal in South Africa, grew on MacConkey agar and at 45°C, hydrolysed sodium hippurate, and produced acid from a range of carbohydrates, including galactose, glucose, lactose, maltose, salicin, starch and trehalose, but not from arabinose, glycerol, inulin, mannitol, raffinose, sorbitol, sucrose or xylose. In contrast, Japanese isolates did not grow at 45°C or hydrolyse sodium hippurate (Minami *et al.*, 1979; Kitao *et al.*, 1981; Ugajin, 1981). Of course, such differences may reflect the lack of standardisation in the testing regimes or, indeed, point to heterogeneity in the species composition of the organisms.

A comparison of the characteristics of fish-pathogenic streptococci and lactobacilli with the results of the comprehensive taxonomy study by Bridge and Sneath

Table 3.3. Characteristics of fish-pathogenic lactobacilli and streptococci

Character	1	2	3	4	5	6	7	8	9	10	11	12	13
Production of arginine dihydrolase	−	NS	NS	NS	+	NS	NS	+	−	+	+	NS	+
Methyl red test	+	NS	NS	NS	NS	NS	NS	NS	NS	+	+	NS	+
Voges Proskauer reaction	−	−	NS	NS	+	NS	NS	NS	NS	−	−	+	+
Degradation of:													
Arginine	NS	−	NS	NS	−	−	NS	+	+	+	+	+	NS
Blood (haemolysis)	α	α/−	−	−	α	β	β	α	β	β	β	NS	−
Sodium hippurate	NS	NS	−	+	−	−	+	−	−	−	−	−	NS
Starch	−	NS	−	−	−	+	NS	−	+	+	+	NS	−
Growth at/in:													
10°C	NS	−	−	−	+	−	−	+	−	−	−	+	−
45°C	−	−	+	−	+	−	+	+/−	−	−	−	+	−
pH 9.6	NS	NS	+	NS	+	−	NS	+	−	−	−	+	+
6.5% (w/v) sodium chloride	+	−	NS	−	+	−	NS	+	−	−	−	+	−
10% bile salts	NS	NS	NS	NS	NS	NS	NS	NS	+	+	NS	+	NS
40% bile salts	NS	−	NS	−	+	−	NS	+	−	−	NS	+	NS
0.1% methylene blue	NS	NS	−	−	+	−	NS	+	−	+	−	NS	NS

Acid production from:													
Arabinose	+	+	+	−	−	−	−	−	−	−	−	−	−
Galactose	−	NS	NS	NS	NS	NS	+	NS	NS	NS	+	NS	−
Glycerol	−	NS	+	−	−	−	−	−	+	−	−	−	NS
Lactose	−	+/−	+	−	−	−	+	+	+	−	−	−	NS
Mannitol	+	+	NS	NS	+	NS	−	+	+	+	−	+	−
Salicin	+	+	+	−	+	+	+	+	+	+	+	+	−
Sorbitol	−	+	+	−	+	−	−	−	−	−	−	−	−
Starch	NS	NS	NS	NS	NS	NS	+	NS	NS	NS	+	NS	NS
Sucrose	+	+	+	+	−	+	−	−	+	+	+	−	+
Trehalose	+	NS		−+	+	+	+	+	+	+	+	+	−

1 = *Lactobacillus* (Starliper *et al.*, 1992), 2 = *Lactobacillus* (Cone, 1982), *3* = *Streptococcus* (Hoshina *et al.*, 1958), 4 = *Streptococcus* (Robinson and Meyer, 1966), 5 = *Streptococcus* (Kusuda *et al.*, 1976a), 6 = *Streptococcus* (Onishi and Shiro, 1978), *7* = *Streptococcus* (Boomker *et al.*, 1979), *8* = *Streptococcus* (Minami, 1979), 9 = *Streptococcus* (Minami *et al.*, 1979), 10 = *Streptococcus* (Kitao *et al.*, 1981), 11 = *Streptococcus* (Ugajin, 1981), 12 = *Streptococcus* (Kitao, 1982a), 13 = *Streptococcus* (Baya *et al.*, 1990c).

(1983) revealed that the isolates, described by Hoshina *et al.* (1958), Robinson and Meyer (1966), Boomker *et al.* (1979), Minami *et al.* (1979), Cone (1982) and Kitao (1982a), approximated to *En. faecalis*, *Str. equinus*, *Str. lactis*, *Str. casseliflavus*, pediococci and the "aerococcal" group, respectively. Subsequently, some of these taxa have been re-classified in the genus *Enterococcus*. However, the organisms recovered by Kusuda *et al.* (1976a), Onishi and Shiro (1978), Minami (1979), Kitao *et al.* (1981) and Ugajin (1981) did not match the descriptions of any of the 28 phena defined by Bridge and Sneath (1983).

With such information, it could readily be assumed from the early literature that streptococcicosis is a syndrome caused by more than one species. To some extent, geographical differences have been implied. For example, the South African isolates of Boomker *et al.* (1979) have been described as comprising unidentified Lancefield group D *Streptococcus*; Japanese isolates linked with, but were not identical to, *En. faecalis* and *En. faecium* [note: the type strain of *En. faecium* has been determined to be pathogenic to salmonids in laboratory-based infectivity experiments; B. Austin, unpublished data]; whereas American strains approximate to the description of *Str. agalactiae* (Kusuda and Komatsu, 1978). It is interesting that isolates from cases of streptococcicosis in rainbow trout farmed in Italy were originally linked with *En. faecalis* and *En. faecium* (Ghittino and Prearo, 1992, 1993) before taxononic re-appraisal, as indicated below.

Serology, although indicating a multiplicity of serotypes, has confirmed that the fish pathogens are indeed *bona fide* representatives of *Streptococcus/Enterococcus*. Thereafter, serological techniques have not improved the understanding of the precise taxonomic status of the strains. The organisms described by Cook and Lofton (1975) and considered as identical to those of Plumb *et al.* (1974) were identified as group B type 1_b *Streptococcus* by the Centers for Disease Control and Prevention, Atlanta, Georgia. Also, Baya *et al.* (1991) identified their isolates as group B. However, Boomker *et al.* (1979) regarded the isolates as group D. To further complicate the issue, Kitao *et al.* (1981) reported a new serotype in Japan, which did not react with specific antisera to Lancefield groups A, B, C, D, E, F, G, H, K, L, N, O and MG; this conclusion was also reached by Kitao (1982a) and Kusuda *et al.* (1982).

Enterococcaceae representatives

Enterococcus (Streptococcus) faecalis subsp. *liquefaciens*

We are not satisfied with the identification of these isolates. However, in the absence of an alternative, details are included here (Teskeredzic *et al.*, 1993).

Enterococcus faecalis subsp. *liquefaciens*

Yellow colonies of 1–2 mm in diameter were obtained from kidney and liver on TSA. Colonies comprised Gram-positive cocci which did not produce catalase, H_2S or indole and were negative for the methyl red test and Voges Proskauer

reaction. Nitrates were reduced. DNA and gelatin were attacked. Citrate was utilised.

Although there are insufficient data for a meaningful comparison with other taxa, it is interesting to note that streptococci are normally associated with the production of white colonies.

Vagococcus salmoninarum

In 1968, a so-called lactobacillus was recovered from diseased adult rainbow trout in Oregon and later subjected to detailed taxonomic examination. This isolate, designated OS1-68T, has become the type strain of *Vagococcus salmoninarum* (Wallbanks *et al.*, 1990). Further isolates have been studied by Schmidtke and Carson (1994).

Vagococcus salmoninarum

Cultures comprise short or oval non-motile, facultatively anaerobic, Gram-positive rods, which produce H_2S but not arginine dihydrolase or catalase. Aesculin and blood (α-haemolysis), but not sodium hippurate or urea, are degraded. Nitrates are not reduced. The Voges Proskauer reaction is negative. Acid is produced from amygdalin, arbutin, N-acetylglucosamine, cellobiose, fructose, β-gentiobiose, glucose (gas is not produced), maltose, mannose, α-methyl-D-glucoside, ribose, salicin, starch, sucrose, D-tagatose and trehalose, but not from D- or L-arabinose, D- or L-arabitol, adonitol, dulcitol, erythritol, D- or L-fucose, galactose, gluconate, glycogen, glycerol, 2 or 5-keto-gluconate, inulin, inositol, lactose, D-lyxose, melibiose, melezitose, methyl-xyloside, methyl-D-mannoside, mannitol, rhamnose, raffinose, sorbose, sorbitol, D-turanose, D- or L-xylose or xylitol. Growth occurs at 5 to 30°C but not 40°C, and at pH 9.6. The major cellular fatty acids are of the straight-chain saturated and mono-unsaturated types. The G+C ratio of the DNA has been calculated as 36.0–36.5 mol %.

Based on an examination of only one culture of *Vag. salmoninarum*, it was established that 96.3% homology with *Vag. fluvialis* was recorded in the 1,340-nucleotide region of the 16S rRNA. Slightly lower homology values of 94.5%, 94.1%, 94.0%, 93.8% and 93.7% were obtained with *En. durans*, *Car. divergens*, *En. avium*, *Car. piscicola* and *Car. mobile*, respectively. Despite the very high similarity to *Vag. fluvialis*, strain 051-68T was described in a new species, as *Vagococcus salmoninarum*. It remains for the examination of further isolates to determine the level of genetic variability within the taxon.

Streptococcaceae representatives

Lactococcus garvieae (= Enterococcus seriolicida)

The first attempt at clarifying the taxonomic status of the causal agents of streptococcicosis/streptococcosis was the landmark publication of Kusuda *et al.* (1991), who described a new species, i.e. *Enterococcus seriolicida*, to accommodate 12 isolates recovered from eels and yellowtail in Japan.

Lactococcus garvieae

Cultures were described as comprising non-motile, facultatively anaerobic, Gram-positive cocci in short chains, which do not produce catalase, H_2S, indole or oxidase. Blood is degraded (α-haemolysis). Aesculin and arginine are hydrolysed, but not so casein, gelatin or sodium hippurate. Acid is produced from a wide range of carbohydrates, namely aesculin, cellobiose, D-fructose, galactose, D-glucose, maltose, mannitol, D-mannose, salicin, sorbitol and trehalose, but not from adonitol, D-arabinose, glycerol, glycogen, inositol, lactose, melezitose, melibiose, raffinose, L-rhamnose, starch, sucrose or D-xylose. The methyl red and tetrazolium reduction tests and the Voges Proskauer reaction are positive, but not nitrate reduction. Growth occurs at 10–45°C but not 50°C, in 0–6.5% (w/v) sodium chloride, and at pH 4.5–9.6. The G + C ratio of the DNA is 44 mol %. The organisms do not belong to Lancefield groups A, B, C, D, E, F, G, H, K, L, M, N or O.

Interestingly, in the original publication describing *En. seriolicida*, only low DNA homology values were obtained with reference species of *Enterococcus*. Indeed, the greatest DNA homology, i.e. 24%, was with *En. hirae* (Kusuda *et al.*, 1991). Perhaps, it was inevitable that the association of these fish pathogens with *Enterococcus* would be challenged. Thus, it was determined that *En. seriolicida* was really identical (77% DNA : DNA homology) with a previously described lactococcus, namely *Lactococcus garvieae* (Teixeira *et al.*, 1996). This view has been reinforced by others. For example, Pot *et al.* (1996) detailed research by SDS-PAGE of whole-cell proteins, concluding that *En. seriolicida* was closely related to *Lactococcus garvieae*. Also, Eldar *et al.* (1996) reached the same conclusion after studying the type strains phenotypically and by DNA : DNA hybridisation. The taxon is certainly homogeneous (Kawanishi *et al.*, 2006), as verified by RFLP (Eyngor *et al.*, 2004), although three groupings along geographical lines were recognised by RAPD, which should have value for epizootiology (Ravelo *et al.*, 2003). However, *Lactococcus garvieae* appears to be similar, in terms of phenetic data, to *Lactococcus lactis* (Zlotkin *et al.*, 1998). This similarity could result in mis-identification of fresh isolates.

Lactococcus piscium

The taxonomy of the group N streptococci has undergone extensive revision. On the basis of 23S rRNA–DNA hybridisation and superoxide dismutase studies, the genus

Lactococcus was defined to accommodate these organisms. A group of fish-pathogenic lactococci/group N streptococci have been studied, and named as a new species, i.e. *Lactococcus piscium* (Williams *et al.*, 1990).

Lactococcus piscium

From the available information, it is apparent that cultures comprise Gram-positive, non-motile, facultatively anaerobic short (ovoid) rods, which are catalase-negative, grow at 5 to 30°C, produce acid from amygdalin, L-arabinose, arbutin, N-acetylglucosamine, cellobiose, D-fructose, galactose, β-gentiobiose, gluconate, glucose, lactose, maltose, D-mannose, mannitol, melibiose, melezitose, D-raffinose, ribose, salicin, sucrose, trehalose, D-turanose and D-xylose, but not adonitol, D-arabinose, D or L-arabitol, dulcitol, erythritol, D and L-fucose, glycogen, glycerol, inositol, inulin, 2 and 5-ketogluconate, D-lyxose, α-methyl-D-glucoside, α-methyl-D-mannoside, β-methyl-xyloside, rhamnose, L-sorbose, sorbitol, D-tagatose, xylitol and L-xylose. Aesculin and starch (slow, weak reaction) but not arginine are degraded. H_2S is not produced. The long-chain cellular fatty acids are considered to be of the straight-chain saturated, mono-unsaturated and cyclopropane-ring types. The major acids correspond to hexadecanoic acid, Δ_{11}-octadecanoic acid and Δ_{11}-methylenoctadecanoic acid. The G + C ratio of the DNA is calculated as 38.5 mol %.

Streptococcus dysgalactiae

This organism was recovered in Japan from fish that had been previously vaccinated with a commercial lactococcosis product. In addition, the organism has been associated with severe necrosis of the caudal peduncle and mortalities of amberjack and yellowtail in Japan (Nomoto *et al.*, 2006).

Streptococcus dysgalactiae

White colonies develop in 48–72 h at 25°C. Cultures comprise Lancefield serological group C, catalase-negative, α-haemolytic on sheep blood (β-haemolytic after prolonged incubation), Gram-positive cocci which form long chains. Electron microscopy reveals fimbriae-like structures surrounding the cell wall. Growth does not occur at 10 or 45°C, at pH 9.6 or in 6.5% (w/v) sodium chloride. Resistance is not recorded to 40% bile salts. The Voges Proskauer test is negative. Esculin and sodium hippurate are not hydrolysed. β-glucuronidase, α-glucosidase, acid and alkaline phosphatase, and leucine arylamidase are produced, but not pyrolidonylamidase, α- or β-galactosidase. Acid is produced at 37°C from trehalose and amygdalin, but not from arabinose, inulin, lactose, mannitol, raffinose or sorbitol (Nomoto *et al.* 2004, 2006).

Identification was confirmed by the results of 16S rDNA sequencing (Nomoto *et al.* 2004, 2006).

Streptococcus difficilis (= Str. agalactiae)

Str. difficilis was described to accommodate what was perceived to be a new species of fish pathogen causing meningo-encephalitis in cultured fish, which was first recognised in Israel during 1984 (Eldar *et al.*, 1994, 1995b). The initial work with diseased fish resulted in the recognition of two groups of streptococci; the separation being achieved by use of API 50 CH and API 20 STREP, and by growth and haemolysis characteristics (Eldar *et al.*, 1994). A fairly unreactive non-haemolytic mannitol-negative group was labelled as *Str. difficile* (Eldar *et al.*, 1994), and the specific epithet corrected to *difficilis*, i.e. *Str. difficilis* (Euzéby, 1998), whereas a second more reactive α-haemolytic, mannitol-positive group became known as *Str. shiloi.*

Streptococcus difficilis

Colonies on BHIA are 1 mm in diameter, and non-pigmented after incubation aerobically for 24 h at 30°C. Cultures comprise fermentative, catalase-negative, Gram-positive cocci of varying diameters in small chains, which do not grow at 10, 37 or 45°C, or in 40% bile or 6.5% (w/v) sodium chloride, but do grow at pH 9.6. The isolates attack (produce acid from) N-acetyl-glucosamine, D-fructose, D-glucose, maltose, D-mannose, ribose and saccharose, but not adonitol, aesculin, amygdalin, L- or D-arabinose, L- or D-arabitol, arbutin, cellobiose, dulcitol, erythritol, L- or D-fucose, galactose, gentiobiose, M-D-glucoside, glycerol, glycogen, inositol, inulin, lactose, melibiose, D-raffinose, rhamnose, salicin, sorbitol, L-sorbose, starch, turanose, xylitol, L- or D-xylose or M-xyloside. Alkaline phosphatase, arginine dihydrolase and leucine arylamidase are produced, but not α- nor β-galactosidase, pyrrolidonylarylamidase nor β-glucuronidase. The Voges Proskauer reaction is positive (Eldar *et al.*, 1994). Bovine blood is not attacked.

These isolates were considered to belong to a separate and distinct DNA homology group, with DNA relatedness between members of 89–100% (Eldar *et al.*, 1994). The level of DNA relatedness with *Str. shiloi* (= *Str. iniae*) was 17% (Eldar *et al.*, 1994). Then, whole-cell protein electrophoresis revealed that the type strain of *Str. difficilis* was indistinguishable from *Str. agalactiae* (Vandamme *et al.*, 1997), which has also been named as a fish pathogen (e.g. Evans *et al.*, 2002). Moreover, it was determined that *Str. difficilis*, which was originally regarded as serologically untypeable (Eldar *et al.*, 1994), cross reacted with group B *Streptococcus*, namely capsular polysaccharide antigen type Ib (Vandamme *et al.*, 1997). Certainly, there were biochemical differences between *Str. difficilis* and *Str. agalactiae*. Yet, in terms of biochemistry, *Str. difficilis* was similar to other group B, type Ib streptococci.

Streptococcus iniae

Streptococcus iniae was initially recovered from an Amazon freshwater dolphin, *Inia geoffrensis* (Pier and Madin, 1976). The association with fish diseases came when it was described as a cause of mortality in tilapia hybrids (*Tilapia nilotica* × *T. aurea*) (Perera *et al.*, 1994) and later in dusky spinefoot (*Siganus fuscenscens*) (Sugita, 1996) and hybrid striped bass (Stoffregen *et al.*, 1996). Then, it was realised that on the basis of DNA:DNA hybridisation, i.e. 77–100% DNA homology, *Str. iniae* was synonymous with *Str. shiloi*, which had been previously named as the causal agent of a septicaemic condition in cultured fish, which occurred in Israel in 1984 (Eldar *et al.*, 1994, 1995a, b). This change in the taxonomy was confirmed by others (e.g. Teixeira *et al.*, 1996). The organism was recovered from rainbow trout, which had been previously vaccinated with a streptococcus vaccine, with the conclusion that a new serotype had emerged (Bachrach *et al.*, 2001). Indeed, a study of 26 Israeli and 9 other isolates using phenotypic, RAPD, and AFLP and 16S rDNA sequencing revealed a new variant among the Israeli cultures (Kvitt and Colorni, 2004). *Str. iniae* serotype II, which differed in arginine hydrolase activity, was described as the cause of disease in rainbow trout initially within Israel and then the U.S.A. (Bachrach *et al.*, 2001; Barnes *et al.*, 2003; Lahav *et al.*, 2004). Two phenotypes were recognised serologically among cultures from Japan (mostly from flounder), with differences reflecting the presence or absence of polysaccharide capsule (Kanai *et al.*, 2006).

Streptococcus iniae (= Str. shiloi)

Colonies on BHIA are 1 mm in diameter, and non-pigmented after incubation aerobically for 24 h at 30°C. Cultures comprise fermentative, catalase-negative [virulent cultures are encapsulated; Barnes *et al.*, 2003], Gram-positive cocci in pairs and chains (some degree of pleomorphism has been observed), which grow at 37°C but not at 10 or 45°C, or in 40% bile or 6.5% (w/v) sodium chloride, but do grow at pH 9.6. Isolates attack (produce acid from) N-acetyl-glucosamine, aesculin, arbutin, cellobiose, D-fructose, gentiobiose, D-glucose, glycogen, maltose, mannitol, D-mannose, melezitose, ribose, salicin, starch and trehalose, but not adonitol, amygdalin, L- or D-arabinose, L- or D-arabitol, dulcitol, erythritol, L- or D-fucose, galactose, gluconate, glycerol, inositol, inulin, lactose, melibiose, D-raffinose, rhamnose, sorbitol, L-sorbose, tagatose, turanose, xylitol, or L- or D-xylose. Alkaline phosphatase, arginine dihydrolase, β-glucuronidase, leucine arylamidase and pyrrolidonylarylamidase are produced, but not α- or β-galactosidase. The Voges Proskauer reaction is negative. α-haemolysis is recorded for bovine blood (Eldar *et al.*, 1994).

By serology using streptoccocal-specific antisera, the original isolates equated with *Str. shiloi* were untypeable. Moreover, these isolates were considered to belong to a separate and distinct DNA homology group, with DNA relatedness between members of 89–100% (Eldar *et al.*, 1994). Some phenotypic differences were noted

between fish and human isolates; however, molecular techniques did not discriminate the two sets of cultures (Dodson *et al.*, 1999).

Streptococcus milleri

Two cultures were obtained from kidney samples in ulcerated Koi carp (Austin and Robertson, 1993). The following characteristics were displayed:

Streptococcus milleri

Cultures contain catalase- and oxidase-negative fermentative cocci in chains, that produce acid and alkaline phosphatase, arginine dihydrolase, chemotrypsin, esterase (caprylate and lipase), β-galactosidase, leucine and valine arylamidase and pyrrolidonylarylamidase but not cystine arylamidase, α-fucosidase, α-galactosidase, α- and β-glucosidase, β-glucuronidase, H_2S, indole, α-mannosidase, nitrate reductase, trypsin or tryptophan deaminase. The methyl red and bile aesculin tests are negative. Aesculin, arginine, casein and horse blood (weakly β-haemolytic) are degraded, but not DNA, gelatin, sodium hippurate, starch or urea. Growth occurs in 0–1.5% but not 8% (w/v) sodium chloride and on MacConkey agar. Citrate is utilised. Acid is produced from N-acetyl-glucosamine, amygdalin, D-fructose, D-glucose, inositol, maltose, D-mannose, α-methyl-D-glucoside, ribose, saccharose, sucrose, D-trehalose, xylitol and xylose, but not from L-arabinose, glycogen, inulin, lactose, D-mannitol, D-melibiose, raffinose, rhamnose or sorbitol.

An identification to *Str. milleri* (probability of a correct identification = 98%) resulted from use of the Bacterial Identifier Program (Bryant and Smith, 1991). By means of the API 20 STREP system, an identification of *Lactococcus lactis* resulted (probability of a correct identification = 96.7%). Yet, from the published description there was a closer fit with *Str. milleri*. The only discrepancies concerned utilisation of citrate, and acid production from inositol, xylitol and xylose. The isolates from Koi carp were positive for these tests.

Streptococcus parauberis

During 1993 and 1994, low-level (0.1–5%) mortalities occurred in farmed turbot of 0.8 to 2 kg in size in northern Spain (Doménech *et al.*, 1996). Isolates were obtained and identified by phenotypic (Rapid ID32 and API 50CH systems) and genotypic data (16S rRNA sequencing) as *Str. parauberis*; an organism known previously as *Str. uberis* genotype II. Eighteen isolates recovered from diseased turbot in northwest Spain (Galicia) were subjected to ribotyping and RAPD analyses with the data demonstrating marked homogeneity among the cultures (Romalde *et al.*, 1999).

Streptococcus parauberis

After overnight incubation, pure cultures produce whitish slightly α-haemolytic colonies of 1.5 to 2 mm in diameter. These contain non-motile, Gram-positive short rods/cocco-bacilli in pairs or short chains, which produce alkaline phosphatase, α-galactosidase, β-glucuronidase and pyrrolidonyl arylamidase, but not catalase, indole or catalase, grow at 10 to 37°C but not at 4 or 45°C, in 4.5 but not 6.5% (w/v) sodium chloride or at pH 9.6 or on MacConkey agar, and degrade arginine and hippurate (some strains).

There was a 100% sequence homology between the fish isolates and *Str. parauberis*. The only reliable difference between the turbot isolates and the type strain concerned the action on D-raffinose which was negative for the latter (Doménech *et al.*, 1996).

AEROBIC, GRAM-POSITIVE RODS AND COCCI

Aerobic, heterotrophic, Gram-positive rods and cocci have received great attention from fisheries microbiologists, largely because of the severity of the diseases caused by pathogenic representatives. There are nine genus groupings which will be considered in this chapter, namely *Bacillus*, *Corynebacterium*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Planococcus*, *Rhodococcus*, *Renibacterium* and *Staphylococcus*.

Renibacterium salmoninarum

Bacterial kidney disease (BKD, Dee disease, corynebacterial kidney disease, salmonid kidney disease) was described initially in 1930 for a condition in Atlantic salmon (*Salmo salar*) found in the Rivers Dee and Spey in Scotland (Mackie *et al.*, 1933; Smith, 1964). Histological examination of fixed material revealed the presence of large numbers of Gram-positive rods in lesions. At various times, the causal agent of BKD has been linked with *Corynebacterium* (Ordal and Earp, 1956; Smith, 1964, Sanders and Fryer, 1978; Austin and Rodgers, 1980), *Brevibacterium* (Smith, 1964), *Listeria* (Bullock *et al.*, 1975), *Lactobacillus* (Vladik *et al.*, 1974) and *Rickettsia* (Snieszko and Griffin, 1955). Subsequently, it was appreciated that the organisms were sufficiently unique to warrant separate species status, so *Corynebacterium salmoninus* was described (Sanders and Fryer, 1978). With further information, these authors realised that the pathogen belonged in a new, as yet undescribed genus and, therefore, proposed *Renibacterium*. Thus, the causal agent of BKD became classified as *Renibacterium salmoninarum* (Sanders and Fryer, 1980).

The initial difficulties experienced in culturing the pathogen contributed significantly to the uncertainty over its precise taxonomic status. Early work emphasised a few morphological features, namely the presence of small ($0.3–1.5 \times 0.1–1.0$ μm), Gram-positive, asporogenous, non-motile, non-acid-fast rods, which frequently occurred in pairs. Evidence of pleomorphism, metachromatic granules and a "coryneform" appearance (Ordal and Earp, 1956; Smith, 1964) led to the initial,

tenuous association with the coryneform group of bacteria, namely *Corynebacterium*. It is interesting to note that the later investigation of Young and Chapman (1978) did not substantiate the "coryneform" morphology. However, transmission electron microscopy of negatively stained cells, obtained from 28-day-old cultures on growth medium, i.e. KDM2 (see Chapter 5), revealed the presence of pleomorphism and intracellular vacuoles/granules (B. Austin, unpublished data). By using FAT on kidney smears from coho salmon, Cvitanich (2004) observed small short rods, termed bar forms because of their staining reaction in FAT, which could not be cultured and were not virulent.

Earp (1950) and Ordal and Earp (1956) demonstrated catalase and proteolytic activity, and realised that there was a growth requirement for cysteine. Additional attributes of the organism were slowly realised; in particular, Smith (1964) indicated the temperature range of growth, i.e. most rapid at 15°C, slow at 5 and 22°C, and not at all at 37°C, and determined an inability to degrade gelatin. During the period of the late 1970s to early 1980s, a wealth of knowledge was accumulated on *Renibacterium*. A low genetic diversity among North American isolates has been indicated from multilocus enzyme electrophoresis using 44 enzymes (Starliper, 1996). Thus, from 40 isolates, 21 electrophoretic types were recognised. Grayson *et al.* (1999) highlighted the inability of conventional systems to differentiate among *Renibacterium* isolates, and investigated molecular methods that might be useful to identify intraspecific variation. The outcome was the differentiation of isolates by RAPD according to host and geographical location.

Renibacterium salmoninarum

Characteristically, *Ren. salmoninarum* produces cream (non-pigmented), shiny, smooth, round, raised, entire, 2-mm diameter colonies on KDM2 after incubation at 15°C for 20 days. Subclinical infections may lead to two colony types, the smooth colonies described above and a thin film of growth, the latter of which does not develop on SKDM (Hirvelä-Koski *et al.*, 2006). Old cultures, i.e. 12 weeks, may become extremely granular or crystalline in appearance. Indeed, a transverse section through such colonies will reveal the presence of a few Gram-positive rods embedded in a crystalline matrix. Subculturing at this stage often leads to the development of more crystalline "colonies". It is thought that the material is principally cystine, which has been precipitated from the medium. For some strains, a uniformly turbid growth occurs in broth, but for others, a sediment may develop. The cell wall peptidoglycan of renibacteria contains D-alanine, D-glutamic acid, glycine and lysine as the diamino acids (Fiedler and Draxl, 1986). The principal cell wall sugar is glucose, but arabinose, mannose and rhamnose are also present (Sanders and Fryer, 1980). Here, there is a discrepancy with the more recent work of Kusser and Fiedler (1983). These authors reported that the principal cell wall sugar is galactose, with lesser amounts of N-acetyl-glucosamine, rhamnose and N-acetyl-fucosamine. This is a curious anomaly insofar as the same strain, i.e. the type strain (ATCC 33209), is common to both studies. Mycolic acids are absent. Methyl-branched fatty acids form over 92% of the total fatty acid

component of the cells, with 12-methyltetradecanoic (anteiso-C_{15}), 13-methyldecanoic (iso-C_{15}) and 14-methylhexadecanoic (anteiso-C_{17}) as the major components. Straight-chain fatty acids generally account for 1% of the total fatty acids, and unsaturated fatty acids are not detected at all. Over 81% of the total fatty acids are composed of the lower melting point anteiso acids, which may contribute to membrane fluidity at low temperatures. Unsaturated menaquinones with nine isoprene units are present. All strains contain diphosphatydylglycerol, two major and six or seven minor glycolipids and two unidentified minor phospholipids (Embley *et al.*, 1983). Although renibacteria were considered to be serologically homogeneous (Bullock *et al.*, 1974; Getchell *et al.*, 1985), two antigenic groups have been described (Bandín *et al.*, 1992). These groups have been defined after analyses of membrane proteins, which determined the presence of 57 kDa and 30 kDa molecules in the respective groups. The G + C ratio of the DNA has been calculated as 53.0 ± 0.46 mol % by Sanders and Fryer (1980) and as 55.5 mol % by Banner *et al.* (1991). Additional characteristics of *Ren. salmoninarum* have been included in Table 3.4.

The exact taxonomic position of *Ren. salmoninarum* is uncertain. However, the numerical phenetic study of Goodfellow *et al.* (1985) confirmed the homogeneity of the taxon, and demonstrated its dissimilarity to *Lactobacillus* and *Listeria* (*Lis. denitrificans*). The results of the chemotaxonomy study discussed above also indicated the unique position of renibacteria. On the basis of the fatty acid data, *Renibacterium* is distinguishable from *Corynebacterium sensu stricto* and other representatives of mycolic-acid-containing taxa, which have predominantly straight-chain and monounsaturated fatty acids. In short, the data indicate that *Renibacterium* is distinct from other Gram-positive organisms (Embley, 1983; Embley *et al.*, 1983; Goodfellow *et al.*, 1985), although its relationship to *Cor. (Actinomyces) pyogenes* needs clarification. On the basis of 16S rRNA cataloguing, *Ren. salmoninarum* was considered to comprise a member of the actinomycete subdivision, being related to *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Jonesia*, *Micrococcus*, *Promicromonospora*, *Stomatococcus* and *Terrabacter* (Stackebrandt *et al.*, 1988; Gutenberger *et al.*, 1991).

Bacillaceae representatives

Bacillus spp.

During 1989 to 1991, low-level mortalities (10–15%) were noted in farmed populations of *Clarias carpis*, *Clarias gariepinus*, *Clarias nigrodigitatus*, *"Heteroclarias"* and *Heterobanchus bidorsalis* in Nigeria (Oladosu *et al.*, 1994).

Bacillus sp.

Using nutrient agar plates with an incubation temperature of 37°C, cream, rough opaque colonies may be obtained. These colonies comprise non-motile, fermenta-

Table 3.4. Characteristics of *Renibacterium salmoninarum*[a]

Character	Response
Production of:	
Acid and alkaline phosphatase	+
Butyrate esterase	−
Caprylate esterase	+
Catalase	+
Chymotrypsin	−
Cystine arylamidase	−
α-fucosidase	−
α- and β-galactosidase	−
β-glucosaminidase	−
α-glucosidase	+
β-glucosidase	−
β-glucuronidase	−
Leucine arylamidase	+
α-mannosidase	+
Myristate esterase	−
Oxidase	−
Trypsin	+
Valine arylamidase	−
Nitrate reduction	−
Degradation of:	
Adenine, aesculin, arbutin, chitin, chondroitin, DNA	−
Casein, Tributyrin, Tween 40 and 60	+
Elastin, gelatin, guanine, hyaluronic acid, hypoxanthine	−
Lecithin, RNA, starch, testosterone Tween 80, tyrosine	−
Xanthine	−
Acid production from sugars	−
Growth on/at:	
pH 7.8	+
0.025% (w/v) bile salts, 0.001% (w/v) methylene blue	−
0.0001% (w/v) crystal violet, 0.00001% (w/v) nile blue	+
0.005% (w/v) phenol, 1% (w/v) potassium thiocyanate	−
1% (w/v) sodium chloride	+ (poor)
0.01% (w/v) sodium selenite, 0.001% (w/v) thallous acetate	−
Utilisation of:	
4-umbelliferyl-acetate, 4-umbelliferyl-butyrate	+
4-umbelliferyl-βD-cellobiopyranoside monohydrate	−
4-umbelliferyl-elaidate, 4-umbelliferyl-α-L-arabinopyranoside	−
4-umbelliferyl-2-acetamido-2-deoxy-β-D-galactopyranoside	−
4-umbelliferyl-β-L-fucopyranoside	−
4-umbelliferyl-heptanoate, 4-umbelliferyl-laurate	+
4-umbelliferyl-nonanoate, 4-umbelliferyl-oleate	+
4-umbelliferyl-palmitate	−
4-umbelliferyl-propionate	+

[a] From Embley (1983) and Goodfellow *et al.* (1985)

tive, Gram-positive rods of 1–4 μm in length, which contain central and oval endospores. The cells grow at 45°C but not 50°C, and are not haemolytic.

From these data, a link with *Bacillus* was made. However, there is insufficient information to achieve a proper identification.

A second report appertained to bacillary necrosis among catfish in Vietnam. The bacteria were described as comprising 1 mm diameter, cream-coloured colonies on TSA after 24 h incubation at 28°C. The cells were Gram-variable, long thin motile, oxidase-positive rods, that were unreactive towards sugars, and grew at 15–37°C. H_2S was produced, and gelatin was attacked. Curiously, there was not any mention about the presence of endospores. However, by 16S rRNA sequencing, the nearest match at 95% homology was *Bacillus fumarioli* (Ferguson *et al.*, 2001).

Bacillus cereus

There has been ocasional mention of *B. cereus* as a fish pathogen (Pychynski *et al.*, 1981; Baya *et al.*, 1992a). However, the supporting evidence is weak.

Bacillus mycoides

Cultures were considered to possess the key characteristics of *B. mycoides*, as follows:

Bacillus mycoides

Cultures are rhizoidal and contain non-motile, Gram-positive rods with oval endospores. Parasporal crystals are not observed. Indole is not produced. The Voges Proskauer reaction is positive. Blood (haemolysis), casein, gelatin, lecithin and tyrosine are degraded. Acid is produced from D-glucose. Resistance is recorded to penicillin. Growth does not occur at 45°C (Goodwin *et al.*, 1994).

Bacillus subtilis

There has been one reference to *B. subtilis* as a fish pathogen (Pychynski *et al.*, 1981). However, the supporting evidence is weak.

Corynebacteriaceae representatives

Corynebacterium aquaticum

So far, *Corynebacterium aquaticum* has been associated with fish diseases on only one occasion, namely bilateral exophthalmia and cerebral haemorrhaging in striped bass (Baya *et al.*, 1992b). Characteristics of the organism were, as follows:

Corynebacterium aquaticum

Colonies are 1–3 mm in diameter and exhibit a yellow non-diffusible pigment after incubation at 25°C for 48 h. Cultures comprise motile, non-spore-forming, non-acid-fast, slightly pleomorphic (club shapes and angular arrangements), Gram-positive rods, which are neither fermentative nor oxidative, and produced alkaline phosphatase, catalase, β-galactosidase, α-glucosidase, pyrazinamidase, pyrrolidonyl arylamidase, but not N-acetyl-β-glucosaminidase, arginine dihydrolase, β-glucuronidase, H_2S, indole, lysine or ornithine decarboxylase, oxidase or phospholipase. Aesculin, blood (β-haemolysis; only at 37°C), casein and gelatin are degraded, but not urea. Nitrates are not reduced. Citrate is not utilised, nor is acid produced from any of the carbohydrates examined. The Voges Proskauer reaction is positive. Growth occurs at 4–42°C and in 0–5% but not 8% (w/v) sodium chloride.

Identification was achieved using the API-Coryne system, and comparison with the type culture of *Cor. aquaticum* ATCC 14665. The fish isolate and reference culture agglutinated with antisera prepared against both strains. Discrepancies with the named reference culture included growth at 4°C and in 5% (w/v) sodium chloride, degradation of casein and gelatin, nitrate reduction, pyrrolidonyl arylamidase, and N-acetyl-β-glucosaminidase (Baya *et al.*, 1992b). Also, the fish isolate differed from the reference culture in the precise composition of the membrane proteins, as determined by western blotting. However, both cultures shared a 68 kDa major antigenic protein (Baya *et al.*, 1992b).

Coryneform bacteria

Occasional mention has been made of the role of coryneforms as fish pathogens. Ajmal and Hobbs (1967) referred to *Corynebacterium* infections in rudd, salmon and trout. However, there may have been confusion with BKD, the aetiological agent of which used to be regarded as *Corynebacterium* but is now classified as *Ren. salmoninarum*. Nevertheless, during a routine examination of apparently healthy rainbow trout, Austin *et al.* (1985) recovered an organism with some of the salient features of *Ren. salmoninarum*. Cultures were subsequently assigned to the coryneform group of bacteria.

Coryneforms

Growth occurs on BHIA and plate count agar, weakly on CLED, but not on MacConkey agar or TCBS. Characteristically, isolates are non-motile, non-acid-fast rods of 0.75 × 1.5–3.0 μm in size, which contain darkly stained intracellular granules. Growth occurs at 15 and 30°C but not at 4 or 37°C, and in 0% and weakly in 2% (w/v) sodium chloride but not at all in 4% (w/v) sodium chloride. Catalase is produced, but not arginine dihydrolase, β-galactosidase, H_2S, indole,

lysine or ornithine decarboxylase, oxidase, phenylalanine deaminase or phosphatase. The methyl red test and Voges Proskauer reaction are negative. Nitrates are reduced to nitrites, weakly. Aesculin is degraded, but not blood, DNA, gelatin, lecithin or urea. Sodium citrate is utilised slowly. Acid is not produced from glucose.

However, it must be emphasised that the status of this organism to fish pathology is uncertain.

Micrococcaceae representative

Micrococcus luteus

Conroy (1966) described a single outbreak of disease, termed micrococcosis, in farmed rainbow trout from Argentina. However, the identification of that aetiological agent is uncertain. Nevertheless, during 1990, a "micrococcus" was associated with diseased rainbow trout fry in the U.K. (Austin and Stobie, 1992a). On a rainbow trout farm, deemed to harbour RTFS, large (~2 μm diameter), Gram-positive cocci, displaying a characteristic tetrad arrangement, were recovered from moribund fish.

Micrococcus luteus

The cultures (eight in total were examined) comprise yellow-pigmented non-motile, oxidative, Gram-positive cocci, which display a characteristic tetrad arrangement. Acid and alkaline phosphatase, catalase, esterase, leucine arylamidase, lipase, oxidase and phosphoamidase are produced, but not so α- or β-galactosidase, H_2S, indole, lysine or ornithine decarboxylase or tryptophane deaminase. Nitrates are not reduced, nor is the Voges-Proskauer reaction positive. Casein and gelatin are degraded.

From these characteristics, it was apparent that the organisms matched the description of *Micrococcus luteus* (Kocur, 1986).

Mycobacteriaceae representatives

Mycobacterium spp.

The first report of acid-fast bacteria in freshwater fish (carp) was published by Bataillon *et al.* (1897). This was followed over a decade later by an observation in marine fish (von Betegh, 1910). Interest in infections caused by acid-fast bacteria continued with the isolation of *Myc. fortuitum* from diseased neon fish (*Paracheirodon innesi*) in 1953, although its identification was not reported until six years later (Ross and Brancato, 1959). To date, mycobacteriosis (a term suggested by Parisot and Wood, 1960) has been observed in >150 species of marine and freshwater fish (Nigrelli and Vogel, 1963). The aetiological agents have been classified, at various

times, into a wide assortment of species including *Myc. anabanti*, *Myc. chelonei*, *Myc. chelonei* subsp. *piscarium*, *Myc. fortuitum*, *Myc. marinum*, *Myc. piscium*, *Myc. platypoecilus*, *Myc. ranae*, *Myc. salmoniphilum*, *Myc. scrofulaceum* and *Myc. simiae*. In addition, *Myc. neoaurum* has been recovered as mixed culture growth from Atlantic salmon with ocular lesions (Backman *et al.*, 1990). Unspeciated *Mycobacterium* have also been reported by Kusuda *et al.* (1987). Also, possible new species have been found (e.g. Heckert *et al.*, 2001). Descriptions of fish-pathogenic mycobacteria are generally poor (e.g. Gomez *et al.*, 1993; Hatai *et al.*, 1993). Many publications have been based on purely morphological descriptions, which have resulted from the examination of histological sections. It has been established that the pathogens are Gram-positive, acid-fast, non-motile, pleomorphic rods of approximately 1.5–2.0 × 0.25–0.35 μm in size (Dulin, 1979). They produce pale-cream to yellow/orange colonies on solid media. The optimum temperature for growth is 25°C, although some isolates grow well at 37°C. From these descriptions and a lack of molecular genetic data, it is difficult to determine whether the isolates belong in *Mycobacterium* or *Nocardia*. Conceivably the problem arises from the inherent difficulty in isolating the organisms, and a lack of interest among workers. Of the nomenspecies listed above, it is relevant to note that the taxonomic validity of *Myc. piscium* is in doubt (Van Duijn, 1981), *Myc. anabanti* and *Myc. platypoecilus* are regarded as synonyms of *Myc. marinum* (Van Duijn, 1981), and the slow-growing *Myc. salmoniphilum* is synonymous with *Myc. fortuitum* (Gordon and Mihm, 1959). Therefore, from the early literature, it would appear that *Myc. fortuitum* and *Myc. marinum* were the only *bona fide* species of fish-pathogenic mycobacteria, which could be differentiated, as follows:

	Myc. fortuitum	*Myc. marinum*[a]
Nitrate reduction	+	−
Production of nicotinamidase	−	+
Production of pyrazinamidase	−	+

[a] Data from Runyon *et al.* (1974)

A limited range of phenotypic tests were used to study the pathogen equated with *Myc. abscessus* (Teska *et al.*, 1997).

Mycobacterium abscessus

Described as homogeneous, the pathogen produces arylsulphatase, catalase and pyrazinamidase, degrades Tween 80 and urea, grows in 7 days and in 6.5% (w/v) sodium chloride, 2% (w/v) thiophenecarboxylic acid and on MacConkey agar (without crystal violet), but does not reduce nitrate, accumulates niacin and is negative for iron uptake (Teska *et al.*, 1997).

Six isolates, recovered between 1964 and 1982, were recognised as a new subspecies, i.e. *Myc. chelonei* subsp. *piscarium*, by Arakawa and Fryer (1984). Essentially, the testing regime was quite extensive. Colonies on Ogawa medium were off-white with a smooth texture.

Mycobacterium chelonei subsp. *piscarium*

These comprise pleomorphic, acid-fast, weakly Gram-positive rods 1–4 × 0.3–0.6 μm in size. Neither branching nor aerial hyphae have been observed. Growth occurs at 10 and 30°C, but not all at 37°C, weakly in 3% (w/v) sodium chloride but not at all in 5% (w/v) sodium chloride, and on MacConkey agar and potassium tellurite agar, and in 250 μg/ml of azoguanine, 5 μg/ml of ethambutol, 250 μg/ml of hydroxylamine, 0.1% (w/v) malachite green, 0.01% (w/v) methyl violet, 0.2% (w/v) picric acid, 0.01% (w/v) pyronin B, 1% (w/v) sodium deoxycholate and 0.1% (w/v) sodium nitrate, and variably in 0.01% (w/v) chlorophenol red and 20 μg/ml of sodium azide. Acid phosphatase, aryl sulphatase and catalase are produced, but not acetamidase, benzamidase, isonicotinamidase, nicotinamidase, pyrazinamidase or succinimidase. *p*-aminobenzoate, *p*-aminosalicylate, sodium salicylate and urea are attacked, but not allantoin, sodium hippurate or Tweens. Acid is produced from glucose and mannose, but not arabinose, dulcitol, fructose, galactose, inositol, mannitol, rhamnose, sorbitol, sucrose, trehalose or xylose. Neither sodium benzoate, sodium malonate nor sodium oxalate are utilised as the sole source of carbon, but variable reponses may be recorded with sodium citrate, sodium fumarate and sodium succinate. L-serine and sodium L-glutamate but not acetamide, benzamide, glucosamine hydrochloride or nicotinamide are utilised as the sole source of carbon and nitrogen. Mycolic acids are present. The G + C ratio of the DNA is in the range of 61–65 mol %; with an average value of 63 ± 1.7 mol % (Arakawa and Fryer, 1984).

The fish isolates were considered to be related to *Myc. chelonei*, although somewhat distinct from the current subspecies, i.e. *chelonei* and *abscessus*. For example, the fish isolates were unable to grow at 37°C, produce nicotinamidase or pyrazinamidase and degrade sodium hippurate or produce acid from trehalose, in contrast to the two validly published subspecies. However, there was an overall phenotypic similarity in excess of 85%. Thus, from this similarity together with the results of mycolic acid determination (the fish isolates were identical with *Myc. chelonei* subsp. *chelonei* and *Myc. chelonei* subsp. *abscessus* when examined by two-dimensional thin-layer chromatography of acid methanolysates), it was proposed to establish a new subspecies, i.e. *Myc. chelonei* subsp. *piscarium*. Nevertheless, to date this subspecies has not been formally proposed in the refereed scientific literature.

Myc. chelonae is slowly increasing in significance in farmed fish. For example, *Myc. chelonae* isolates, not allocated to subspecies, were recovered from diseased Atlantic salmon in two farms in Shetland (Bruno *et al.*, 1998).

Myc. gordonae was described as comprising acid-fast rod-shaped cells, which was identified presumptively by 16S rRNA sequencing (Sakai *et al.*, 2005).

Myc. montefiorense was regarded as a cause of granulomatous skin lesions in moray eels (Levi *et al.*, 2003).

Mycobacterium montefiorense

Cultures comprise slow-growing (20 weeks at 25°C), non-chromogenic acid-fast coccobacilli (on blood agar) or rods (on Middelbrook agar), which do not produce catalase or arylsulphatase, do not reduce nitrate, degrade Tween 80 or urea, or grow at >30°C (growth occurred at 25°C) or in 5% (w/v) NaCl (Levi *et al.*, 2003).

By examination of the *hsp65* gene (97.4% similarity to *Myc. triplex*), small-subunit rRNA genes, rRNA spacer regions and phenotypic traits, the organism was linked to *Myc. triplex*, but was regarded as sufficiently distinct to justify description as a new species, i.e. *Myc. montefiorense* (Levi *et al.*, 2003).

Myc. neoaurum was described by Backman *et al.*, 1990):

Mycobacterium neoaurum

Contains yellow-pigmented acid-fast rods, which virtually fail to be stained by the Gram's method. Growth occurs on blood agar at room temperature (but not at 37°C) in 5–7 days, but not in 5% (w/v) sodium chloride. Aryl sulphatase is produced. Resistance is recorded to penicillin. The cell wall chemotype is IVA. Glycolated muramic acids, mycolic acids and MK-9, as the predominant isoprenoid quinone, are present (Backman *et al.*, 1990).

Myc. pseudoshottsii (Rhodes *et al.*, 2005).

Mycobacterium pseudoshottsii

Cultures develop rough colonies of 1–3 mm in diameter on Middlebrook 7H10 agar after incubation for 2 months at 23°C, which slowly become pale yellow to golden in the light, and comprise (clumping) acid-fast cocco-bacilli, which produce niacin and urease but not arylsulphatase, β-galactosidase or pyrazinamidase, but do not reduce nitrates or attack Tween 80, and grow slightly at 30°C, not at all at 37°C. Resistance is recorded to 1 mg/ml of isoniazid. Cultures do not grow on Löwenstein–Jensen medium or MacConkey agar (Rhodes *et al.*, 2005).

Myc. shottsii was named after the examination of 21 isolates recovered from mycobacteriosis among striped bass in Chesapeake Bay, Virginia (Rhodes *et al.*, 2003).

Mycobacterium shottsii

Comprises slow-growing, non-pigmented, acid-fast aggregating cocco-bacilli that grow at 23, less at 30 and none at all at 37°C to produce small (0.5–1.0 mm), rough flat colonies becoming umbonate upon aging with slightly irregular margins after 4–6 weeks on Middlebrook 7H10 agar, that produce niacin and urease but not arylsulphatase, β-galactosidase or pyrazinamidase, do not reduce nitrate or attack Tween 80, and are resistant to isoniazid (1 μg/ml) and *p*-aminosalicylic acid. Susceptibility is recorded to ethambutol, ethionamide, kanamycin, rifampicin and streptomycin. Growth does not occur on Löwenstein–Jensen medium or on MacConkey agar with 5% (w/v) NaCl. Mycolic acids are present, and comprise eight peaks with similarities to the *Myc. tuberculosis* complex (Rhodes *et al.*, 2003).

The results of 16S rRNA sequencing linked the organisms to *Mycobacterium*, and bore some affinity to *Myc. marinum* and *Myc. ulcerans* (similarity = 99.2%).

Nocardiaceae representatives

Nocardia spp.

There has been some confusion between distinguishing infections caused by *Mycobacterium* and *Nocardia*, the latter leading to nocardiosis. Thus, it is often difficult to determine from largely histological reports the genus to which an acid-fast pathogen belongs. Conroy and Valdez (1962) isolated tubercle bacilli from neon fish (*Paracheirodon innesi*), which were also pathogenic to paradise fish (*Macropodus opercularis*) and three-spot gouramis (*Trichogaster trichopterus*), but not to goldfish. These organisms were subsequently identified by Dr. R.E. Gordon as *Nocardia asteroides*. A second species, *Noc. kampachi*, was described as a causal agent of nocardiosis, in yellowtail farmed in Japan, by Kariya *et al.* (1968) and Kubota *et al.* (1968). However, the name was not validated. Instead, *Noc. seriolae* was formally proposed, as a causal agent of fish nocardiosis (Kudo *et al.*, 1988).

Nocardia asteroides

There is limited information available about the characteristics of fish-pathogenic strains of *Noc. asteroides*.

Nocardia asteroides

Most of the description appertains to morphological characters. For example, it has been observed that cultures undergo a complete life cycle, including

germination from resting microcysts, simple and complex fission, and branching. Thus, nocardias may appear in coccal to oval forms, and as long, slender, multiseptate rods. All these stages have been seen in infected fish (Van Duijn, 1981). From Valdez and Conroy (1963), it would appear that the organisms reduce nitrates and degrade starch but not gelatin or urea. Neither H_2S nor indole is produced. The methyl red test and Voges Proskauer reaction are negative. Acid is produced from glucose, but not from any other carbohydrate tested.

Nocardia seriolae

A better description exists for *Noc. kampachi* (Kariya *et al.*, 1968; Kubota *et al.*, 1968; Kusuda *et al.*, 1974). However, in retrospect it would appear that Kariya and coworkers exercised considerable taxonomic intuition in elevating the isolates into a new species, i.e. *Noc. kampachi*. Unfortunately, the results of G + C determinations were not reported. Moreover, it is perhaps surprising that the original authors did not provide detailed reasons explaining why *Noc. kampachi* should be regarded as distinct from other species of *Nocardia*. This is especially relevant as there is some resemblance between the description of *Noc. kampachi* and *Noc. caviae* (Table 3.5). Nevertheless, a more recent publication has formally proposed another nomenspecies, i.e. *Noc. seriolae*, which effectively replaces *Noc. kampachi* (Kudo *et al.*, 1988).

Nocardia seriolae

The isolates of *Noc. seriolae* contain meso-diaminopimelic acid, arabinose and galactose, suggesting chemotype IVA. The major components of the cellular fatty acids are *n*-$C_{16:0}$, *n*-$C_{16:1}$ and *n*-$C_{18:1}$; 10-methyl-$C_{19:0}$ is also present as a major component in four of the five isolates examined. Iso- and anteiso-branched acids have not been detected. The total number of carbon atoms in the mycolic acids is from 44 to 58. The predominant isoprenoid quinone is tetrahydrogenated menaquinone with eight isoprene units. The G + C ratio of the DNA is 66.8–67.4 mol %.

Nocardia salmonicida (= Streptomyces salmonis = Streptoverticillium salmonis)

Streptomycoccis in fish was described initially by Rucker (1949), who classified the aetiological agent in *Streptomyces*, as *Streptomyces salmonicida*. This was emended initially to *Verticillomyces salmonicida* (Shinobu, 1965), then to *Streptoverticillium salmonicida* (Baldacci *et al.*, 1966), *Streptoverticillium salmonis* (Locci *et al.*, 1969) *Streptomyces salmonis* (Witt and Stackebrandt, 1990) and finally to *Noc. salmonicida* (Isik *et al.*, 1999). In general, a dearth of information exists about this fish pathogen. Essentially, since the work of Rucker (1949), the disease has received limited attention. Therefore, it is difficult to decide whether or not streptomycoccis represents a genuine problem.

Table 3.5. Characteristics of nocardias

Character	*Nocardia asteroides*[a]	*Noc. caviae*[a]	*Noc. kampachi*[b]	*Noc. salmonicida*[c]	*Noc. seriolae*[d]
Gram-positive rods and cocci	+	+	+	+	+
(Weakly) acid-fast staining	+	+	+	+	ND
Aerial hyphae	+	+	+	+	+
Motility	−	−	−	−	−
Growth at 10°C	V	V	−	−	−
Production of:					
Catalase	V	+	+	+	ND
H_2S	ND	ND	+	ND	ND
Indole	ND	ND	−	ND	ND
Oxidase	−	−	−	ND	ND
Nitrate reduction	+	+	+	+	ND
Degradation of:					
Casein	−	−	−	−	−
Gelatin	−	−	−	ND	ND
Hypoxanthine	−	+ (weak)	+ (weak)	−	−
Starch	ND	ND	+	−	ND
Tyrosine	−	−	+ (weak)	+	−
Urea	+ (weak)	+ (weak)	−	+	−
Xanthine	−	+	−	−	−
Acid production from:					
Fructose	+	+	+	−	ND
Glucose, glycerol	+	+	+	+	+
Utilisation of:					
Adonitol, arabinose	−	−	−	ND/−	ND
Cellobiose	−	V	−	−	ND
Dextrin, maltose	V	V	−	ND	ND
Dulcitol, glycogen	−	−	−	−	ND
Fructose, glucose	+	+	+	+	ND
Glycerol	+	−	V	ND	ND
Inositol	−	+	−	−	ND
Inulin	−	−	V	−	ND
Lactose, salicin	−	−	−	ND	ND
Mannitol	V	+	−	+	ND
Mannose	+	V	V	ND	ND
Rhamnose	V	−	−	−	ND
Sodium acetate	+	+	+	−	ND
Sodium benzoate	−	−	−	−	−
Sodium citrate	−	V	+	+	+

(*continued*)

Table 3.5 (*cont.*)

Character	*Nocardia asteroides*[a]	*Noc. caviae*[a]	*Noc. kampachi*[b]	*Noc. salmonicida*[c]	*Noc. seriolae*[d]
Utilisation of:					
Sodium lactate	+	+	+	–	ND
Sodium malate	+	+	+	+	ND
Sodium malonate	–	–	v	–	ND
Sodium propionate	+	+	+	+	ND
Sodium pyruvate	+	+	+	ND	ND
Sodium tartrate	–	–	V	–	–
Sorbitol, xylose	–	–	–	+/ND	ND
Starch, trehalose	V	V	–	ND	ND

[a] from Goodfellow (1971)
[b] from Kusuda *et al.* (1974)
[c] from Isik *et al.* (1999)
[d] from Kudo *et al.* (1988)
ND = Not determined
V = Variable response

Nocardia salmonicida

The organism produces brick-red to orange-pigmented substrate mycelia with white (with pink and yellow shades) aerial mycelia. On primary isolation, the colonies are small and, initially, smooth, but later they develop aerial mycelia that appear velvety. Characteristically, the aerial mycelia produce whorls (verticils) at frequent intervals, giving an appearance of barbed wire. The Gram-positive, catalase-positive non-motile mycelia contain L-diaminopimelic acid (DAP) and glycine but not meso-DAP, arabinose or galactose in the cell wall (i.e. Type I). Melanin, but generally not H_2S, is produced. Aesculin, DNA, gelatin, starch, testosterone, Tween 20 (some isolates), Tween 20, tyrosine and urea are degraded, but not adenine, casein, cellulose, chitin, elastin, guanine, hypoxanthine, starch, uric acid, xanthine or xanthan. Nitrates are reduced. Growth occurs at 12° and 30°C but not at 35°C, in 53% (w/v) sodium chloride, 0.0001% (w/v) bismuth citrate, 0.00001% (w/v) crystal violet, 0.01% (w/v) phenol and 0.01% (w/v) potassium tellurite, but not in or 0.01% (w/v) malachite green or 0.01% (w/v) sodium azide. Acid is produced from glucose, glycerol, inositol, ribose and trehalose, and slightly from sucrose, but not from arabinose, cellulose, erythritol, fructose, galactose, maltose, mannose, mannitol, raffinose, rhamnose, trehalose or xylose. Butyrate, citrate, fumarate, D-fructose, D(+)-glucose, malate, D(+)-mannitol, L-proline, propionate, D(+)-sorbitol and succinate are utilised, but not amygdalin, D- and L-arabinose, arbutin, D(+)-cellobiose, dulcitol, D(+)-galactose, glycogen, *m*-inositol, inulin, D(+)-melezitose, D(+)-raffinose, L-rhamnose, acetamide, acetate, anthranilic acid, benzoate, 1,4-butanediol, 2,3-butanediol, hippurate, 4-hydroxybenzoate, lactate, malonate, 2-octanol, pimelic acid or tatrate (Williams *et al.*, 1985; Isik *et al.*, 1999). The major cellular fatty acids are

hexdecanoic, octadecanoic, octadeanoic and 10-methyloctadecanoic acid. The G + C ratio of the DNA is 67 mol % (Isik *et al.*, 1999).

Rhodococcus sp.

In Canada, farmed Chinook salmon, *Oncorhynchus tshawytscha*, have been found with exophthalmia, from which Gram-positive bacteria were recovered. These organisms have been cultured, and identified as *Rhodococcus* (Backman *et al.*, 1990). Subsequent examination of similar isolates from Atlantic salmon has also suggested an affinity with *Rhodococcus* (Claveau, 1991).

One colony type was equated with *Mycobacterium* whereas the second was regarded as *Rhodococcus*.

Rhodococcus sp.

Isolates comprise non-acid-fast, facultatively anaerobic, Gram-positive rods (2–3 × 0.6 μm in size) [slightly club-shaped], which grow aerobically at room temperature (but not at 37°C) on blood agar in 3–4 days. The cell wall components include meso-diaminopimelic acid, arabinose and galactose, which equates with chemotype IVA, N-glycolated muramic acid, mycolic acids, and MK-8 as the predominant isoprenoid menaquinone. Neither catalase nor oxidase is produced. Urea is degraded. Xylose is fermented, but not so glucose, lactose, maltose or sucrose. Acid is produced from *meso*-inositol but not from dulcitol, mannitol or sorbitol. Growth occurs in 5% (w/v) sodium chloride.

Rhodococcus erythropolis

Rhodococcus erythropolis

Cultures comprise round, shiny off-white colonies that contain strictly aerobic, non-haemolytic, Gram-positive rods that produce catalase but not oxidase, grow well at 30 rather than 15°C and not at all at 4 or 37°C, and grow less well in the presence of only 1.5% (w/v) NaCl. N-acetyl-glucosamine, adipate, D-arabitol, gluconate, glucose, glycerol, inositol, malate, mannitol, phenylacetate, sorbitol, sucrose and trehalose are utilised, but not aesculin, amygdalin, D- and L-arabinose, caprate, cellobiose, dulcitol, D- and L-fucose, galactose, β-gentibiose, glycogen, inulin, 2-keto-gluconate, lactose, D-lyxose, maltose, D-mannose, melezitose, melibiose, α-methyl-D-glucoside, β-methyl xyloside, α-methyl-D-mannoside, D-raffinose, rhamnose, salicin, L-sorbose, starch, D-tagatose, D-turanose, D- and L-xylose (Olsen *et al.*, 2006a)

16S rDNA sequencing revealed 99.9% and 100% homology between Scottish and Norwegian isolates and *Rhodococcus erythropolis*, respectively (Olsen *et al.*, 2006a).

Planococcaceae representative

Planococcus sp.

Since 1988, there has been a steady increase in the incidence of motile, Gram-positive cocci, tentative *Planococcus*, associated with diseases of Atlantic salmon and rainbow trout in the U.K. In some cases, it appeared that the Gram-positive cocci were inhabiting fish that had previously received extensive chemotherapy to control diseases caused by Gram-negative bacteria.

> *Planococcus* sp.
>
> Cultures comprise motile (single polar flagellum), often paired Gram-positive cocci of 1–2 μm in diameter, which possess a strictly aerobic metabolism for glucose, and produce catalase, β-galactosidase and oxidase, but not gelatinase, H_2S, indole or lysine decarboxylase. Growth occurs at 37°C and in 0–15% (w/v) sodium chloride.

From these traits and despite a freshwater rather than a marine origin, it was considered that the organisms belonged in the genus *Planococcus*, possibly related to *P. citreus* (Hao and Komagata, 1985).

Staphylococcaceae representatives

Staphylococcus aureus

During 1982 and 1983, mortalities occurred among silver carp, *Hypophthalmichthys molitrix*, at a farm in India. These mortalities were associated with eye disease, from which Gram-positive cocci were recovered, and identified as *Staphylococcus aureus* (Shah and Tyagi, 1986).

> *Staphylococcus aureus*
>
> The cultures were described as comprising Gram-positive cocci, which produce coagulase and phosphatase, degrade blood (β-haemolysis) and DNA, and ferment glucose and mannitol. Zones of opalescence develop around (black) colonies on Baird-Parker's agar.

From this description, an identification of *Staphylococcus aureus* was achieved (Shah and Tyagi, 1986). However, it is apparent that there are insufficient data to differentiate between *Sta. aureus* and *Sta. intermedius* (Kloos and Schleifer, 1986).

Staphylococcus epidermidis

The only reports of fish-pathogenic strains of *Staphylococcus epidermidis* have emanated from Japan where, from July 1976 to September 1977, severe epizootics occurred

in farmed yellowtail (*Seriola quinqueradiata*) and red sea bream (*Chrysophrus major*) (Kusuda and Sugiyama, 1981; Sugiyama and Kusuda, 1981a, b). From these outbreaks, six isolates, identified as *Sta. epidermidis*, were recovered.

Staphylococcus epidermidis

All cultures comprise non-motile, Gram-positive, fermentative spherical cells of approximately 0.6–1.8 μm in diameter, which form white to white/yellow colonies on BHIA. The cells occur singly, in pairs, and in irregular clusters. Catalase, β-galactosidase and phosphatase are produced, but not arginine dihydrolase, coagulase, H_2S, indole, lysine or ornithine decarboxylase or oxidase. Nitrates are reduced. The methyl red test and Voges Proskauer reaction are positive. Blood (β-haemolysis), gelatin, sodium hippurate, tributyrin and urea are degraded, but not starch, Tween 80, tyrosine or xanthine. Growth occurs at 45°C and in 0–15% (w/v) sodium chloride. Neither citrate, mucic acid nor D-tartrate is utilised (Sugiyama and Kusuda, 1981a).

These isolates matched closely the species description of *Sta. epidermidis* (Kloos and Schleifer, 1986), and approximated to biotypes II, V and VI of Baird-Parker (1963, 1965). It is curious that a diverse range of serotypes, i.e. five serotypes, were recognised (Sugiyama and Kusuda, 1981a). The G+C content of the DNA was not assessed.

Staphylococcus warneri

Staphylococcus warneri

Yellow colonies develop on Sal Mannitol medium. Cells comprise catalase-positive, oxidase-negative, facultatively anaerobic, Gram-positive cocci, which produce arginine dihydrolase, β-glucosidase and urease, but not alkaline phosphatase, and does not reduce nitrates. Acid is produced from sucrose and trehalose, but not from L-arabinose, lactose, mannitol, mannose, raffinose, ribose or xylose (Gil *et al.*, 2000).

The characteristics of the organism were considered by Gil *et al.* (2000) to match the description of *Sta. warneri*.

MISCELLANEOUS GRAM-POSITIVE BACTERIAL PATHOGEN

"*Candidatus* Arthromitus"

The digestive tract of fish was observed to be full of filamentous, Gram-positive/variable organisms with metachromatic granules and endospores (Michel *et al.*, 2002a).

4

Characteristics of the pathogens: Gram-negative bacteria

Some Gram-negative bacteria have been described as fish pathogens with little if any supportive evidence. Consequently, a question mark hangs over whether or not such organisms should be regarded as *bona fide* pathogens. In the following text, it will become apparent that varying amounts of information are available for the Gram-negative bacterial fish pathogens. For some, e.g. *Aer. salmonicida*, a wealth of information is available. Unfortunately, for others, such as *Aer. bestiarum*, barely enough information is available to enable scientists to recognise fresh isolates.

Aeromonadaceae representatives

Aeromonas is classified in the family Aeromonadaceae (Colwell *et al.*, 1986).

Aeromonas allosaccharophila

During an examination of 16S rRNA sequences of motile aeromonads, two isolates, which were originally recovered from diseased elvers in Spain during 1988, were considered as sufficiently distinct from existing species to warrant description as a new species, *Aer. allosaccharophila* (Martinez-Murcia *et al.*, 1992). However, it is recognised that isolates are phenetically heterogeneous (Huys *et al.*, 2001).

Aeromonas allosaccharophila

Cultures comprise Gram-negative, motile, fermentative rods, which produce catalase, β-galactosidase, indole, lysine decarboxylase and oxidase but not H_2S, reduce nitrates, degrade casein, DNA, egg yolk, gelatin, starch and Tween 80 but not elastin, sodium dodecyl sulphate or urea, and grow in 0–3% (w/v) sodium

chloride, at 4–42°C and at pH 9.0. The Voges Proskauer reaction is negative. Acid is produced from D-cellobiose, D-galactose, glucose (plus gas), glycerol, maltose, D-mannitol, D-mannose and D-trehalose, but not from adonitol, arbutin, dulcitol, *m*-erythritol, *m*-inositol, lactose, salicin, D-sorbitol or D-xylose. A wide range of compounds are utilized as sole sources of carbon for energy and growth, including L-arabinose, L-arginine, D-cellobiose, fumarate, D-galactose, D-gluconate, L-glutamate, glycerol, L-histidine, maltose, D-mannitol, D-mannose, L-proline, succinate, sucrose and D-trehalose, but not L-alanine, γ-aminobutyrate, L-citrulline, dulcitol, *m*-erythritol, ethanol, D-glucuronate, L-glutamine, glycine, DL-3-hydroxybutyrate, *m*-inositol, α-ketoglutarate, lactose, L-leucine, propionate, putrescine, salicin or L-serine. Susceptibility has been recorded to chloramphenicol, erythromycin, fosfomicin, gentamicin, kanamycin, nalidixic acid, nitrofurantoin, oxolinic acid, polymyxin B and rifampicin, but not to ampicillin, streptomycin, sulphadimethoxine or trimethoprim. The G + C content of the DNA is 59.6 mol % (Martinez-Murcia *et al.*, 1992).

The basis of allocating the isolates to a new species stemmed from the examination of 16S rRNA sequences, where homology values of >97.7% were exhibited to other validly described *Aeromonas* species (Martinez-Murcia *et al.*, 1992). Three isolates were found to be highly related, i.e. 70–100%, by DNA:DNA hybridisation (Esteve *et al.*, 1995a). On the basis of AFLP fingerprinting, *Aer. allosaccharophila* has been determined to be genetically related to *Aeromonas* HG 8/10 (Huys *et al.*, 1996).

Aeromonas bestiarum

Aer. bestiarum appears to be an example of a taxon, which emerged from the taxonomic chaos surrounding the understanding of *Aer. hydrophila* (Ali *et al.*, 1996). Originally, classified in DNA HG 2 (*Aer. hydrophila*), isolates have apparently been recovered from diseased fish (Huys *et al.*, 1996). However, negligible information is available about the characteristics of the supposed fish-pathogenic cultures.

Aeromonas caviae

Cultures were identified by recourse to the scheme in Popoff (1984).

Aeromonas caviae

Cultures produce arginine dihydrolase, β-galactosidase and indole, but not H_2S, lysine or ornithine decarboxylase or tryptophan deaminase, degrade aesculin, blood and gelatin, but not urea, ferment amygdalin, arabinose, glucose, mannitol, sorbitol and sucrose, but not inositol, melibiose or rhamnose, utilise potassium cyanide, but not citrate, grow in 0% (w/v) sodium chloride, and do not reduce nitrates (Candan *et al.*, 1995).

Aeromonas hydrophila

The significance of *Aer. hydrophila* and its synonyms *Aer. formicans* and *Aer. liquefaciens* has been overshadowed by *Aer. salmonicida*. Nevertheless, since its initial recognition as the causal agent of haemorrhagic septicaemia (Sanarelli, 1891; Schäperclaus, 1930; Haley *et al.*, 1967), *Aer. hydrophila* has been recovered as a pathogen from a wide variety of freshwater fish species (e.g. Pathiratne *et al.*, 1994) and occasionally from marine fish, e.g. ulcer disease of cod (Larsen and Jensen, 1977). The aetiological agent may be considered to have worldwide distribution. However, some doubt has been expressed over its precise role as a fish pathogen (Heuschmann-Brunner, 1965b; Eurell *et al.*, 1978; Michel, 1981) with some workers contending that it may be merely a secondary invader of already compromised hosts. Conversely, other groups have insisted that *Aer. hydrophila* constitutes a primary pathogen. Yet, with improvements in the taxonomy of the "motile" aeromonads (see Carnahan and Altwegg, 1996), it is speculative about whether or not the fish isolates belong as *Aer. hydrophila* or in any of the other *Aeromonas* Hybridisation Groups. To some extent, the improvements in aeromonad taxonomy may reflect the sudden emergence of other taxa as fish pathogens. Certainly, there is marked phenotypic, serological and genotypic heterogeneity within the descriptions of fish-pathogenic *Aer. hydrophila* (MacInnes *et al.*, 1979; Leblanc *et al.*, 1981; Allen *et al.*, 1983a).

Aeromonas hydrophila

The fish pathogens comprise Gram-negative, straight, chemo-organotrophic (fermentative) rods of approximately 0.8–1.0 × 1.0–3.5 μm in size, which are motile by single polar flagella. Arginine dihydrolase, catalase, β-galactosidase, indole, lysine decarboxylase (a variable response may occur), cytochrome-oxidase and phosphatase are produced, but not H_2S, ornithine decarboxylase, phenylalanine or tryptophan deaminase. Nitrates are reduced to nitrates without the production of gas. The Voges Proskauer reaction is positive, but not so the methyl red test. Growth occurs in 0–4% (w/v) but not 5% (w/v) sodium chloride, at 5–37°C and in potassium cyanide. Aesculin, blood (β-haemolysis; by some isolates), casein, DNA, gelatin, lecithin, RNA, starch and Tween 80 are degraded, but not pectin or urea. There is resistance to the vibriostatic agent O/129. *N*-acetyl-β-D-galactosamine, L-alanine, L-arabinose, *p*-arbutin, DL-lactate, D-mannitol, putrescine, D-serine, salicin and D-sucrose are utilised as the source carbon course for energy and growth, but not D-cellobiose, DL-isocitrate, β-alanine, 4-aminobutyrate or urocanic acid. Sodium citrate is utilised by some isolates. Acid is produced from cellobiose (a variable response), fructose, galactose, glucose (acid and gas), glycerol (a variable response), lactose (a variable response), maltose, mannitol, salicin, sucrose and trehalose, but not from adonitol, dulcitol, erythritol, inositol, raffinose, rhamnose, sorbitol or xylose (Paterson, 1974; Larsen and Jensen, 1977; Allen *et al.*, 1983a; Popoff, 1984). The G + C ratio of the DNA falls in the range of 58–61.6 mol % (Larsen and Jensen, 1977; MacInnes *et al.*, 1979; Huys *et al.*, 2002).

In contrast to the usual characteristics of *Aer. salmonicida*, the majority of isolates of *Aer. hydrophila* are capable of growth at 37°C and are, indeed, motile. Some isolates have also been determined to produce diffusible brown pigments, as does *Aer. salmonicida*, which could superficially confuse diagnosticians (Ross, 1962; Paterson, 1974, Allen *et al.*, 1983a, b; Austin *et al.*, 1989). Santos *et al.* (1991) serotyped 62 motile *Aeromonas* spp. from rainbow trout. Of these, 55 isolates (89% of the total) were distributed between 17 serogroups, of which O3, O6, O11 and O19 were dominant. Moreover, 40 (63% of the total) of these isolates were pathogenic to fish. Nevertheless, antigenic cross-reactivity with *Aer. salmonicida* and *Aer. sobria* has been noted (Leblanc *et al.*, 1981). However, Shaw and Hodder (1978) reported that the core region of the LPS of *Aer. hydrophila* was distinct from that of *Aer. caviae* and *Aer. sobria*.

Aeromonas jandaei

Aer. jandaei has been reported as pathogenic to eel in Spain (Esteve *et al.*, 1993, 1994; Esteve, 1995). Initially, eight isolates were recovered using unstated procedures during 1987 and 1988. Whereas initially the method of identification was not stated (Esteve *et al.*, 1994), a subsequent numerical taxonomy study equated isolates with *Aer. jandaei* (Esteve, 1995).

Aer. jandaei

Cultures are motile Gram-negative rods, that produce arginine dihydrolase, indole and lysine decarboxylase, but not ornithine decarboxylase, degrade casein, chitin, DNA, gelatin, starch and Tween 80, grow in 0%, but not 6% (w/v) sodium chloride and at 4–42°C, and produce acid from galactose, glycogen, mannose, sucrose and trehalose, but not arabinose, lactose, melibiose, raffinose, rhamnose or salicin.

Aeromonas salmonicida

Aer. salmonicida, which comprises the so-called non-motile taxon, is one of the most important fish pathogens because of its widespread distribution, diverse host range and economically devastating impact on cultivated fish, particularly the valuable salmonids. More has been written about *Aer. salmonicida* than any other bacterial fish pathogen. Comprehensive reviews, i.e. McCraw (1952), Herman (1968), McCarthy and Roberts (1980) and an excellent textbook (Bernoth *et al.*, 1997) have adequately summarised the available knowledge on the pathogen in its context as a fish pathogen. The following narrative will emphasise taxonomic aspects which have not been considered adequately by others.

Aer. salmonicida is one of the oldest described fish pathogens. It is generally accepted that the first authentic report of the organism was by Emmerich and Weibel (1894), who isolated the pathogen from diseased brown trout, obtained from a hatchery in Germany, and named it "Bacillus der Forellenseuche" or bacillus of

trout contagious disease. Because of the importance of the organism, some authors have examined the early literature seeking evidence for the occurrence of furunculosis prior to 1894 (Williamson, 1928; McCarthy and Roberts, 1980). However, although several reports exist which suggest that furunculosis occurred earlier, it has proved impossible because of poor descriptions to be certain that the bacterial isolates in question were *Aer. salmonicida* (Forel, 1868; Fabre-Domerque, 1890; Fischel and Enoch, 1892). In North America, the first report of furunculosis was made by Marsh (1902) who described an organism, which was named as *Bacillus truttae*. This caused an epizootic in hatchery fish in Michigan.

The placement of *Aer. salmonicida* in the bacterial taxonomic hierarchy should be put into context. Like so many of the bacterial taxa described in the 19th century, the organism has undergone a series of changes in its classification. Shortly after Emmerich and Weibel (1894) reported it, the pathogen was placed in the genus *Bacterium*, as *Bacterium salmonicida*, by Lehmann and Neumann (1896). This was probably its best known epithet before its eventual transfer to the genus *Aeromonas*. To confuse the issue, however, *Bacillus devorans* (Zimmermann, 1890), *Bacterium salmonica* (Chester, 1897), *Bacterium truttae* (Marsh, 1902) and *Bacillus salmonicida* were also names assigned to the pathogen in the past. With the publication of the seventh edition of *Bergey's Manual of Determinative Bacteriology* in 1957, the pathogen was transferred to the genus *Aeromonas*, then placed in the family Pseudomonadaceae. Later, the genus *Aeromonas* was moved again, to the family Vibrionaceae and more recently to its own family, i.e. the Aeromonadaceae (Colwell *et al.*, 1986). The initial re-classification was based primarily on the work of Griffin *et al.* (1953a). This team undertook the first detailed characterisation of the organism, providing the information necessary to formulate the description, which resulted in its re-classification. However, the division of *Aer. salmonicida* strains into subspecies remains an ongoing bone of taxonomic contention. The description of *Aer. salmonicida* subsp. *pectinolytica* opens a new chapter in the understanding of the organism insofar as this is the first subspecies which is not associated directly with fish diseases (Pavan *et al.*, 2000). Instead, the isolates were recovered from a polluted river in Argentina (Pavan *et al.*, 2000). To date, there is not any evidence to link this subspecies with fish pathogenicity.

Two aspects concerned with the status of *Aer. salmonicida* in the bacterial taxonomic hierarchy require discussion. One centres on the intraspecific relationships of *Aer. salmonicida* strains; the other is involved with questions that have been raised regarding the retention of the species in the genus *Aeromonas*.

Aer. salmonicida has been known by its present name since the 1950s when, on the basis of work by Griffin *et al.* (1953a), Snieszko (1957) in his contribution on the genus *Aeromonas* in the seventh edition of *Bergey's Manual of Determinative Bacteriology* assigned the pathogen to this genus, where it has remained. It is curious, in view of its seriousness as a pathogen, that it was over 50 years from the initial discovery of the organism to the characterisation and description of *Aer. salmonicida*. Griffin *et al.* (1953a) provided the first detailed description of the organism, and were of the opinion that attempts to recognise and identify isolates were being hampered by a lack of a complete description. This resulted in confusion due to disagreement

concerning the physiological and biochemical characterisation of the organism. >From the results of a study of ten isolates, it was concluded that *Bacterium salmonicida* was extremely consistent in its general cultural and biochemical traits, and that problems in the past had arisen primarily from the use of media which varied in composition among laboratories. Although Griffin *et al.* (1953a) ended their report by recommending the re-classification of *Bacterium salmonicida* to the newly created genus *Aeromonas*, as *Aer. salmonicida*, no definite reasons were given for this move. However, with time, additional data have accumulated, and the homogeneity and authenticity of the taxon has generally been supported.

Subsequent investigators have re-examined the homogeneity of the taxon, using conventional and numerical phenetic methods (Eddy, 1960; Ewing *et al.*, 1961; Schubert, 1961; Smith, 1963; Eddy and Carpenter, 1964; Popoff, 1969). Thus, the traditional description of *Aer. salmonicida* is of non-motile, fermentative, Gram-negative rods, which produce a brown, water-soluble pigment on tryptone-containing agar, which do not grow at 37°C, and which produce catalase and oxidase (Table 4.1). The circular chromosome is 4,658 ± 30 kb (Umelo and Trust, 1998). Cells are found in, and are pathogenic to, salmonids and increasingly other fish species. Traditionally, the lack of motility has been accepted as one of the reliable diagnostic traits used for the division of the aeromonads. However, this criterion has been challenged by the report of McIntosh and Austin (1991a) of motility (by polar flagella) in a strain of *Aer. salmonicida* subsp. *salmonicida* grown at elevated temperatures, i.e. 30–37°C. The appearance of motility was also accompanied by variation in sugar fermentation patterns, the loss of ability to degrade complex molecules and an increase in antibiotic resistance. Further evidence for a motile mode of existence of *Aer. salmonicida* was provided by the recovery of eight atypical isolates from ulcers (but not from kidney tissue) on goldfish, carp and roach. The ulcerated fish were obtained from aquaria, garden ponds and rivers in England (Austin, 1993). Interestingly, these isolates did not dissociate into different colony types, but grew at 37°C. Of course, there is always the concern that motile contaminants may have been present in cultures which were predominantly *Aer. salmonicida*. However, the isolation of flagella genes, *flaA* and *flaB*, which coded for unsheathed polar flagella at low frequency, has clinched the argument that *Aer. salmonicida* can be motile under certain circumstances (Umelo and Trust, 1997).

Certain traits, such as pigment production, captured the attention of fisheries scientists particularly because they were readily observable. In an examination of pigment production, Griffin *et al.* (1953b) showed that its development was dependent upon medium composition, insofar as tyrosine or phenylalanine was deemed to be essential. This was confirmed by O'Leary *et al.* (1956). However, it was initially assumed that this pigment was related to melanin, although subsequent investigation has refuted this possibility. Thus, Donlon *et al.* (1983) discovered that biosynthesis of the pigment from tyrosine differed substantially from melanogenesis and not 3,4-dihydroxyphenylalanine as would have been expected of melanin synthesis. Although production of the brown, water-soluble pigment constitutes a major diagnostic feature of *Aer. salmonicida* (Fig. 4.1—see colour section; Martin-Carnahan and Joseph, 2005), caution is advised against relying too heavily on the presence of pigment,

Table 4.1. Characteristics of *Aeromonas salmonicida*[a]

	Aer. salmonicida subsp.				
Character	*achromogenes*	*masoucida*	*pectinolytica*	*salmonicida*	*smithia*
Production of:					
Brown, diffusible pigment	−	−	+	+	+
Arginine dihydrolase	+	+	v	v	−
Catalase	+	+	+	+	+
β-galactosidase	+	+	+	+	+
H_2S	−	−	+	V	+
Indole	−	−	+	−	−
Lysine decarboxylase	−	+	−	V	−
Ornithine decarboxylase	−	−	−	−	−
Oxidase	+	+	+	+	+
Phenylalanine deaminase	ND	ND	ND	−	−
Phosphatase	ND	ND	ND	−	+
Fermentative metabolism	+	+	+	+	+
Gluconate oxidation	−	+	−	−	−
Methyl red test	−	+	ND	V	−
Motility	−	−	−	−	−
Nitrate reduction	+	+	+	+	ND
Voges Proskauer reaction	−	+	V	−	−
Degradation of:					
Aesculin	−	+	−	V	−
Blood (β-haemolysis)	−	+	V	+	−
Casein	+	−	ND	+	+
Chitin	+	−	ND	−	−
DNA	+	+	+	+	+
Elastin	ND	ND	−	+	−
Gelatin	−	+	+	+	+
Lecithin	ND	ND	ND	+	−
Polypectate	ND	ND	+	ND	ND
RNA	+	+	ND	+	+
Starch	ND	ND	ND	+	+
Tweens	+	+	ND	+	−
Tyrosine	ND	ND	ND	+	−
Urea	−	−	−	−	ND
Xanthine	−	−	ND	−	ND
Growth at/on:					
4–5°C	V	V	ND	V	+
30°C	+	+	+	+	−
37°C	−	−	+	−	−
Cystine lactose electrolyte-deficient agar	−	−	ND	−	ND

(*continued*)

Table 4.1 (*cont.*)

Character	*Aer. salmonicida* subsp.				
	achromogenes	*masoucida*	*pectinolytica*	*salmonicida*	*smithia*
Growth at/on (*cont.*):					
MacConkey agar	+	+	ND	+	−
Potassium cyanide	−	−	V	−	ND
TCBS agar	−	−	ND	−	ND
0–2% (w/v) NaCl	+	+	ND	+	+
3% (w/v) NaCl	V	V	ND	V	−
4% (w/v) NaCl	−	−	ND	−	−
Utilisation of sodium citrate	−	−	+	−	−
Production of acid from:					
Adonitol	−	−	−	−	ND
Amygdalin	−	−	−	−	ND
Arabinose	−	+	+	+ (slow)	ND
Cellobiose	−	−	+	−	−
Dulcitol	−	−	−	−	ND
Erythritol	ND	ND	−	−	ND
Fructose	ND	ND	ND	+	ND
Galactose	+	+	ND	+	−
Glucose	+	+	+	+	V
Glycerol	ND	ND	+	−	−
Glycogen	ND	ND	ND	+	ND
Inulin	ND	ND	ND	−	ND
Lactose	−	−	+	−	−
Maltose	+	+	ND	+	−
Mannitol	−	+	ND	+	−
Mannose	ND	+	ND	+	ND
Melezitose	ND	ND	ND	−	ND
Melibiose	ND	ND	−	−	ND
Raffinose	−	−	−	−	−
Rhamnose	−	−	−	−	−
Salicin	V	V	−	+ (slow)	ND
Sorbitol	−	−	+	−	−
Sucrose	+	+	+	−	V
Trehalose	+	+	ND	+	−
Xylose	−	−	−	−	ND
G + C ratio of the DNA (mol %)	ND	ND	ND	57–59	56

[a] Based on Griffin *et al.* (1953a), Schubert (1967a, b, 1974), McCarthy (1977a, 1980), Austin *et al.* (1989) and Pavan *et al.* (2000).
V = Variable result
ND = Not done.

insofar as there are variations among pigmented strains in the quantity of compound produced and of the time needed for its appearance (Horne, 1928; Mackie and Menzies, 1938). In addition, non-pigmented variants may arise (Wiklund *et al.*, 1993), particularly upon subculture (Duff and Stewart, 1933; Evelyn, 1971a). It has also been observed that other *Aeromonas* species, namely *Aer. hydrophila* and *Aer. media*, may produce such pigments when grown on media containing tryptone (see Paterson, 1974; Allen *et al.*, 1983a). Obviously, this questions the reliability of using pigment production as a differential characteristic. To further complicate the issue, the existence of achromogenic or slowly pigmenting strains of *Aer. salmonicida* have been described.

Another intriguing trait of *Aer. salmonicida* is the ability to dissociate into different colony types, i.e. rough, smooth and G-phase (intermediate) colonies. This phenomenon was extensively studied by Duff (1937), and will be discussed further in connection with its relevance to pathogenicity (see Chapter 9). Electron microscopy demonstrated that "rough" and "smooth" forms were attributed to the presence or absence of an extracellular layer (= the A-layer), respectively.

The notion of homogeneity could be dispelled by the results of PFGE of 44 isolates of *Aer. salmonicida* subsp. *salmonicida*, which generated 30 different profiles and 40 distinct types (Chomarat *et al.*, 1998). However, numerical analysis using the SD coefficient revealed that all the isolates were genomically related (Chomarat *et al.*, 1998). Yet, using 17 typical, 39 atypical and three type strains, RAPD and PFGE analyses suggested heterogeneity across all the strains—notably atypical isolates—but confirmed that typical *Aer. salmonicida* (including the type strain of *Aer. salmonicida* subsp. *salmonicida*) was homogeneous (O'Hici *et al.*, 2000).

Atypical isolates of Aeromonas salmonicida

When compared with the so-called motile aeromonads, the description of *Aer. salmonicida* suggests a very homogeneous group of organisms. Alas, as so often happens in biology, a multitude of exceptions have disturbed the apparent idyllic situation. "Atypical" strains deviate from the classical description of the taxon over a number of biochemical, physiological and genetic properties, e.g. AFLP fingerprints, making typing difficult (Hirvelä-Koski *et al.*, 1994; Austin *et al.*, 1998; Wiklund and Dalsgaard, 1998; Dalsgaard *et al.*, 1998; Høi *et al.*, 1999; Lund *et al.*, 2002). For example, Japanese isolates of so-called atypical *Aer. salmonicida* were recovered in four groups, which were defined after 16S rDNA sequencing. There was not any host specificity with these groups (Yamada *et al.*, 2000). The most common reasons for describing isolates as "atypical" are:

- lack of weak or slow pigment production (Nakatsugawa, 1994; Koppang *et al.*, 2000);
- catalase negativity (Kaku *et al.*, 1999);
- oxidase negativity (Wiklund and Bylund, 1993; Wiklund *et al.*, 1994; Wiklund and Dalsgaard, 1995; Pedersen *et al.*, 1994, 1996a; Kaku *et al.*, 1999);
- nutritional fastidiousness, i.e. for blood or blood products (Austin, 1993);

- slow growth, i.e. $\geq$5 days to obtain visible colonies (Austin, 1993; Kaku *et al.*, 1999);
- different hosts from salmonids, i.e. cyprinids (e.g. Austin, 1993; Kaku *et al.*, 1999) and marine fish, including shotted halibut (*Eopsetta grigorjewi*; Nakatsugawa, 1994), dab, plaice and flounder (*Platichthys flesus*) (Wiklund *et al.*, 1994; Wiklund and Dalsgaard, 1995), common wolf fish (*Anarhichas lupus*) (Hellberg *et al.*, 1996), turbot (*Scophthalmus maximus*) (Pedersen *et al.*, 1994), greenling (*Hexagrammos otakii*), Japanese flounder (*Paralichthys olivaceus*) and Schlegel's black rockfish (*Sebastes schlegeli*) (Iida *et al.*, 1997), where the disease is often ulceration.

With the last mentioned example, the justification for describing the isolates as atypical was based on the host rather than the characteristics of the cultures.

One of the earliest indications that aberrant strains occurred was provided by Smith (1963), who examined six isolates of non-pigmented cultures, which were clustered as Group I from a numerical taxonomy study. These organisms were related at the 75.6% similarity level to typical pigment-producing isolates. Smith (1963) proposed a separate new species for Group I, i.e. with the specific epithet *achromogenes*, although the recommendation was not adopted. A second, non-pigmented group was recognised by Kimura (1969a) as *Aer. salmonicida* subsp. *masoucida*. This subspecies differed from typical strains on account of indole production, Voges Proskauer reaction, H_2S and lysine decarboxylase production, and fermentation of sucrose (Table 7.1). In the eighth edition of *Bergey's Manual of Determinative Bacteriology*, Schubert (1974) regarded these non-pigmented isolates as *Aer. salmonicida* subsp. *achromogenes* and *Aer. salmonicida* subsp. *masoucida*, respectively. Typical strains were classified by him as *Aer. salmonicida* subsp. *salmonicida*. This classification into subspecies has been retained in the recent literature (Martin-Carnahan and Joseph, 2005). This is interesting, because in an earlier publication (Popoff, 1970), it was contended that subspecies *achromogenes* and *masoucida* were more closely related to *Aer. hydrophila* than to *Aer. salmonicida*. In fact, Paterson *et al.* (1980) suggested that "*masoucida*" bridged the gap between typical *Aer. salmonicida* cultures and *Aer. hydrophila*, insofar as the subspecies possessed similar physiological and growth characteristics to the latter. However, *Aer. salmonicida* subsp. *masoucida* is non-motile, sensitive to *Aer. salmonicida* bacteriophages, possesses an antigenic component specific to *Aer. salmonicida*, and has a DNA homology of 103% with *Aer. salmonicida* (MacInnes *et al.*, 1979). The relationship between the subspecies is certainly not sacrosanct, insofar as they could be combined or kept separate according to which methods happen to be in vogue (Austin *et al.*, 1998). For example, by PCR there would be good reason to consider combining subspecies *achromogenes* and *masoucida*, a view which is not substantiated by ribotyping and RAPD analyses (Austin *et al.*, 1998). Phenetic data suggested that there would be a case for combining subspecies *masoucida* with *salmonicida*, and subspecies *achromogenes* with *Haemophilus piscium*. Indeed, examination of the small-subunit rRNA gene sequences revealed a profound (99.9%) homology of an authentic strain of *Haemophilus piscium* with *Aer. salmonicida* subsp. *salmonicida* (Thornton *et al.*,

1999). Yet, methods point to the comparative uniqueness of subspecies *smithia* (Austin *et al.*, 1998).

Certainly, a species subdivided into four subspecies should not be considered as unworkable; however, the classification is complicated by other factors. Thus, the existence of aberrant strains from a wide range of fish hosts and geographical locations is well established, and new reports are continually being made. According to McCarthy (1980), the existence of such strains is no doubt more common than even their documentation in the published literature suggests. As Mawdesley-Thomas (1969) pointed out, there is no sound reason why a pathogen which affects one family of freshwater fishes should not infect others. He contended that emphasis had been placed on food and game fish, and that only the absence of detailed investigations of fish diseases generally had given the false impression that each fish has its own specific set of diseases. McCarthy (1980) and McCarthy and Roberts (1980) made the valid point that the original description of atypical strains, as reported by Schubert (1974), was based upon data for only a few isolates. These authors submitted that revision is now both possible and necessary. According to a comparative phenetic and genotypic analysis of 29 atypical isolates, in addition to 144 other *Aeromonas* spp., McCarthy (1977a) delineated four phenetic groups. Of these, one cluster comprised typical isolates of *Aer. salmonicida*, a second group was composed of atypical isolates of *Aer. salmonicida* derived from salmonids including representatives of subspecies *achromogenes* and *masoucida*, a third group contained atypical isolates (*Aer. salmonicida*) from non-salmonids, and the fourth group was equated with *Aer. hydrophila*. Not surprisingly, the typical isolates of *Aer. salmonicida* formed an extremely compact group, which could not be readily differentiated from the atypical strains. However, the results of numerical phenetic analyses were not unequivocally confirmed by guanine plus cytosine (G + C) ratio determinations or DNA : DNA homology studies. McCarthy and Roberts (1980) proposed that, from the results of their studies, there should be three subspecies of *Aer. salmonicida*, as follows:

Group 1 *Aer. salmonicida* subsp. *salmonicida*.
Group 2 *Aer. salmonicida* subsp. *achromogenes* (incorporating subsp. *masoucida*).
Group 3 *Aer. salmonicida* subsp. *nova*.

However, the "Approved Lists of Bacterial Names" (Skerman *et al.*, 1980) retained the classification of Schubert (1974), namely of subspecies *achromogenes*, *masoucida* and *salmonicida*. Thus, a growing consensus of opinion suggested that it was timely to elevate certain of the better characterised atypical isolates to subspecies status; the problem concerns the number and composition of such groups (Austin *et al.*, 1998). On the basis of DNA : DNA reassociation studies, Belland and Trust (1988) also supported the rationale of McCarthy and Roberts (1980) to create a new subspecies, i.e. *Aer. salmonicida* subsp. *nova*, to accommodate atypical isolates from non-salmonids. Yet, the subspecies was not formally proposed. Moreover, they also agreed with the suggestion of McCarthy and Roberts (1980) to combine the subspecies *achromogenes* and *masoucida*. In another (numerical) taxonomy and DNA : DNA hybridisation study, Austin *et al.* (1989) elevated a group of 18 non- or

slowly pigmenting "atypical" isolates into a new subspecies, as *Aer. salmonicida* subsp. *smithia*. In addition to the phenotypic studies, there is an increasing trend to employ molecular genetic techniques to elucidate inter- and intraspecific relationships within the genus. Thus, DNA:DNA and RNA:DNA hybridisation, 16S RNA cataloguing, and 5S and 16S rRNA sequencing techniques have been used. Work on DNA homologies by MacInnes *et al.* (1979) revealed that all isolates of *Aer. salmonicida* (including *Aer. salmonicida* subsp. *masoucida*) possessed very high homologies, i.e. 96–106%, when hybridised against a representative strain of *Aer. salmonicida* subsp. *salmonicida*. Indeed, these authors concluded that the non-motile aeromonads comprise a genetically homogeneous taxon. In this study, the culture of *Aer. salmonicida* subsp. *masoucida* hybridised at 103% with *Aer. salmonicida* subsp. *salmonicida*. MacInnes and co-workers determined that this homology level was also achieved with a strain of *Aer. salmonicida* subsp. *salmonicida*, which was an ultraviolet induced mutant of NCIMB (= National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland) 74. An interpretation was reached, therefore, that *Aer. salmonicida* subsp. *masoucida* and, perhaps, some of the biochemically atypical isolates do not warrant separate subspecies status, insofar as they may be merely mutants of other well-recognised groups. However, we emphasise that dramatic conclusions should not be made from an examination of only 11 isolates. It is interesting to note, however, that McCarthy (1980) also reported, as a result of the genotypic part of his analyses, that typical and atypical isolates were very closely related, with minimal divergence. It is a pity that the exact homology values were not presented.

In a study of 26 typical and atypical isolates by DNA:DNA re-association methods, Belland and Trust (1988) found that typical isolates were recovered in a homogeneous group, whereas the atypical representatives were more diverse. Of these, one biotype consisted of isolates obtained from goldfish (obtained from a wide geographical range), whereas the second group accommodated isolates derived from carp in Europe. From the results of a numerical taxonomic and DNA:DNA hybridisation study, Austin *et al.* (1989) made similar conclusions regarding the homogeneity of typical isolates of *Aer. salmonicida*. However, using 16S rRNA sequencing techniques, Martínez-Murcia *et al.* (1992) reported that subspecies *achromogenes* and *masoucida* were indistinguishable, and only differed from subspecies *salmonicida* by two bases.

There is overwhelming evidence that the so-called "atypical" isolates are distinct from typical *Aer. salmonicida* (e.g. Hänninen and Hirvelä-Koski, 1997; Austin *et al.*, 1998; Wiklund and Dalsgaard, 1998; Umelo and Trust, 1998). Yet, it has so far proved to be impossible to include the atypical isolates into a meaningful classification. Moreover, there has been incongruence reported between the results of molecular (PCR, RAPD and ribotyping and phenotypic methods, in terms of group membership (Austin *et al.*, 1998). Høi *et al.* (1999) recognised 4 PCR groups among 205 atypical isolates. The problems of inter-laboratory differences and lack of standardisation in test methods has been highlighted by Dalsgaard *et al.* (1998). Some studies have indicated homogeneity among atypical isolates; a sentiment which is not endorsed by others. For example, Kwon *et al.* 1997) carried out a RAPD analysis of 29 atypical isolates from 8 species of fish in Japan, and concluded that the

profiling was identical, thereby indicating genetic homogeneity. However, heterogeneity was apparent between these atypical isolates and reference cultures from the validly described subspecies (Kwon *et al.*, 1997); a notion which has been confirmed by others (e.g. Austin *et al.*, 1998). After studying 51 isolates from Finland by ribotyping, plasmid profiling and phenotyping, it was concluded that pigment-producing strains could be separated from achromogenic cultures. Also, oxidase-negative isolates were distinct from oxidase-positive atypical isolates in terms of ribotypes and phenotypes (Hänninen and Hirvelä-Koski, 1997).

There have been several reports of acid production in sucrose fermentation tests among typical isolates (see Fryer *et al.*, 1988; Wiklund *et al.*, 1992). This is relevant because hitherto this was one of the tests used to differentiate typical from atypical isolates of the pathogen (Martin-Carnahan and Joseph, 2005).

Plasmid profiles of Aeromonas salmonicida

Plasmids carried by typical (14 strains) and atypical (11 strains) forms of *Aer. salmonicida* have also provided additional genetic evidence for the classification of typical and atypical isolates into separate taxa (Belland and Trust, 1989). These workers found that typical isolates possessed a very homologous plasmid content comprised of a single large (70–145 kb) plasmid and three low molecular weight plasmids. Livesley *et al.*, (1997) reported that 5 plasmids were most common among the 18 isolates examined; Giles *et al.* (1995) found 4 or 6 plasmids with 4 smaller plasmids of 4.3–8.1 kb being often observed in isolates from the Atlantic coast of Canada, but 6 plasmids of 4.2–8.9 kb among cultures from the Pacific coast of Canada. A total of 23 plasmids and 40 different plasmid profiles were recognised among 124 isolates from Denmark, Norway, Scotland and North America (Nielsen *et al.*, 1993). An earlier theme was repeated insofar as all isolates had one large plasmid of 60–150 kb, and two low molecular weight plasmids of 5.2 and 5.4 kb. In addition, two plasmids of 5.6 and 6.4 kb were frequently present (Nielsen *et al.*, 1993). A larger investigation of 383 isolates over a 6-year period concluded that 1–4 plasmids of 52–105 mDa were inevitably present, casting doubt on the relevance of plasmid typing for epizootiology (Sørum *et al.*, 1993b). Again, oxytetracycline- and streptomycin-resistant isolates from the Atlantic and Pacific coasts contained 4 or 6 plasmids, with 4 smaller plasmids of 4.3 to 8.1 kb being often observed. Some slight variation in plasmid content was noted between sources of the isolates (Giles *et al.*, 1995). Atypical isolates possessed two to four different plasmid types (Belland and Trust, 1989). Moreover, there was a correlation between plasmid composition and source of the atypical isolates. This observation may prove useful in epizootiological studies, where plasmid content of atypical isolates could serve as useful markers (Belland and Trust, 1989). In a subsequent investigation of 113 cultures of atypical isolates from a wide range of geographical locations, 7 groupings were defined; 18 cultures did not have any common plasmid profile. Of interest, the two type strains NCIMB 1110 and ATCC 27013 were recovered in different groups. For some groups, i.e. I, III, V and VII (these isolates were catalase-negative), there was an association with the origin of the cultures, i.e. the location of the farm. Also, some differences in

phenotype were apparent between members of some groups. Again, the value for epizootiological investigations was stressed (Sørum *et al.*, 2000).

The taxonomic dilemma

Using molecular techniques, a consistent view about the genetic relatedness of the long-established subspecies of *Aer. salmonicida* emerges. The outstanding dilemma concerns the poor correlation between phenetic and genotypic data (Austin *et al.*, 1989). This problem needs to be addressed before the definitive classification of *Aeromonas* results. Nevertheless, it may be concluded that, unlike the atypical isolates, *Aer. salmonicida* subsp. *salmonicida* is extremely homogeneous; a conclusion which is supported by phenotypic and molecular data (Austin *et al.*, 1989; Dalsgaard *et al.*, 1994; Hänninen *et al.*, 1995; Miyata *et al.*, 1995; Umelo and Trust, 1998).

Despite the existence of typical and atypical representatives of *Aer. salmonicida*, the current theme in operation for speciation has proved tenable. Nevertheless, new "atypical" isolates, which do not fit into existing classifications of *Aer. salmonicida*, are regularly reported. Debate has centred on the relationship of *Aer. salmonicida* within the genus *Aeromonas*. In the study by Eddy (1960), attention was focused on the inability of *Aer. salmonicida* to produce 2,3-butanediol from glucose, and the absence of motility, both characters of which contravened the genus description of Kluyver and van Niel (1936). However, Eddy did not dispute the retention of *Aer. salmonicida* within the genus. Instead, this placement was challenged by Smith (1963), who expressed doubt as to whether *Aer. salmonicida* belonged in the genus *Aeromonas*. Her recommendation was the establishment of a new genus, i.e. *Necromonas*, with two species, namely *Nec. salmonicida* for the typical isolates and *Nec. achromogenes* for the non-pigmented strains. The evidence appertained to the morphological, biochemical and metabolic traits of 42 isolates of "*Bacterium salmonicida*", 6 non-pigmented pathogens and 42 other bacterial cultures. Thus, there were pronounced differences between *Bacterium salmonicida* and other *Aeromonas* cultures. It emphasised, for instance, that the production of gas from glucose was an important genus characteristic. Although many previous reports had stated that the pathogen produced gas from glucose (Griffin *et al.*, 1953a; Eddy, 1960, 1962; Ewing *et al.*, 1961; Schubert, 1961), the hundreds of isolates examined at the Marine Laboratory, Aberdeen, between 1953 and 1962 produced either very little or no gas from glucose. Instead, they produced gas from mannitol (Smith, 1963). As for the production of 2,3-butanediol from glucose, Smith (1963) contended that previously this test required a tedious procedure, and consequently was often not applied to presumptive aeromonads. However, in her laboratory, *Bacterium salmonicida* isolates did not so produce the compound. Due to such discrepancies with the genus description, it was proposed that the species should be removed from the genus *Aeromonas* and placed in a new genus, i.e. *Necromonas*. Although Smith's proposal was not formally adopted, it should be mentioned that Cowan (1974) followed her suggested classification, by including *Nec. salmonicida* in the diagnostic tables. However, it is our opinion that the deviations of *Aer. salmonicida* from the initial genus description of *Aeromonas* should, for several reasons, be viewed less stringently than may be to the approval of

some taxonomic purists. For example, it is often a difficult decision in bacterial systematics as to how much variation to allow within the definition of a species or a genus, before the line is drawn and relationships, or lack of, declared. If examples are taken from the characteristics of motile aeromonads, it is certainly the case that discrepancies occur for some members of these species as regards agreement with the genus description (Holder-Franklin *et al.*, 1981; Allen *et al.*, 1983b). Schubert (1974) included the production of 2,3-butanediol (a generic trait) as occurring in *some* species. Non-motility is also taken into account, and carbohydrates are cited as being broken down to acid or to acid and gas. These modifications to the genus description thus eliminate the major objections of Smith (1963) regarding the retention of *Aer. salmonicida* within the genus *Aeromonas*. In addition, subsequent serological and bacteriophage sensitivity data have provided strong evidence for a relationship between *Aer. salmonicida* and the motile aeromonads. The existence of a common antigen between *Aer. hydrophila* and *Aer. salmonicida* subsp. *masoucida* and some other strains of *Aer. salmonicida* was demonstrated by Kimura (1969b) and Paterson *et al.* (1980). In an examination of the specificities of aeromonad extracellular antigens, Liu (1961) observed that cross-reactions occurred between *Aer. salmonicida* and the motile aeromonads. Liu suggested that the absence of serological cross-reactions with other Gram-negative bacteria indicated that organisms belonging to the genus *Aeromonas* comprise a distinct group. Studies, using bacteriophages, demonstrated the sensitivity of some *Aer. hydrophila* cultures to *Aer. salmonicida* bacteriophages, whereas organisms not belonging to *Aeromonas* showed complete resistance to the virus (Popoff, 1971a, b). In particular, studies employing molecular genetic techniques support the retention of *Aer. salmonicida* in the genus *Aeromonas*. MacInnes *et al.* (1979) determined that percentage DNA homologies of motile aeromonads with *Aer. salmonicida* subsp. *salmonicida* ranged between 31 and 80%, whereas those hybridised against *Aer. hydrophila* were between 31 and 100%. The *Aer. salmonicida* strains exhibited a relatively high degree of homology when hybridised against *Aer. hydrophila*. Thus, the values for 10 out of 11 isolates were in the range of 51 to 69%. From these results, MacInnes *et al.* (1979) concluded that non-motile aeromonads demonstrated a legitimate genetic relationship to the motile species of *Aeromonas*. In fact, some motile strains shared a higher level of sequence homology with *Aer. salmonicida* than with the reference motile aeromonads which according to MacInnes could be attributed to the smaller genome size of *Aer. salmonicida*. In another investigation, 56–65% binding between *Aer. salmonicida* and *Aer. hydrophila* DNA strands was recorded (McCarthy, 1978). These homology values indicate strong genetic relationships between the principal *Aeromonas* species (Paterson *et al.*, 1980; Belland and Trust, 1988).

It is well established that *Aer. salmonicida*, as a species, is phenotypically distinct from its motile counterparts. The increasing evidence from genetic and other molecular biology studies pertaining to intrageneric relationships between *Aer. salmonicida* and other aeromonad species, however, appear to support the retention of the pathogen within the genus. Certainly, the situation is not aided by ongoing manoeuvres in the classification of the motile aeromonads. Therefore, since the arrangement of the genus is in transition, and yet to be definitively

resolved, we believe that nothing would be gained by the re-classification of *Aer. salmonicida*.

To reiterate, it appears to be the consensus of opinion that the area in need of further work is the intraspecific relationships between typical and atypical isolates. It is anticipated that such work would improve the classification of the genus *Aeromonas*.

Serology

Additional approaches to ascertaining the intra- and interspecific relationships of *Aer. salmonicida* have been adopted. These include serological techniques and bacteriophage typing. Certainly, the antigenicity of *Aer. salmonicida* has been the focus of much attention, primarily with a view to its significance in vaccine development. Unfortunately, the early investigations of Williamson (1929) were halted by the persistent agglutination of the strains in saline. This problem was circumvented by Blake and Anderson (1930), who employed complement fixation for the examination of 82 isolates of *Aer. salmonicida*. All of these isolates gave a positive response. Ewing *et al.* (1961) examined agglutinin absorption with reference to "O" and "H" antigens. They concluded that the 21 strains examined were related to each other, but also to a strain of *Aer. hydrophila* (O-antigen suspensions prepared using the 21 cultures reacted to ca. 25% of the titre of the O-antiserum prepared with *Aer. hydrophila*). Other researchers have also found serological homogeneity among strains of *Aer. salmonicida*, but some degree of cross-reactivity with *Aer. hydrophila*. For example, common antigens among two *Aer. salmonicida* strains, as determined by gel-diffusion, and cross-reactions between *Aer. salmonicida* antiserum and three out of four isolates of *Aer. hydrophila*, but not of *Aer. salmonicida* strains and *Aer. hydrophila* antiserum, was reported by Liu (1961). Karlsson (1962), using the antigenic properties of a haemolysin from *Aer. salmonicida*, found no serological differences among the six strains tested. Although these six strains did not cross-react with other aeromonads recovered from humans, it was later established that there were indeed common thermolabile antigens between *Aer. salmonicida* and other *Aeromonas* species as assessed using precipitin, agglutination and double-diffusion precipitin tests. Bullock (1966) also found evidence of cross-reactions between soluble antigens of *Aer. salmonicida* and *Aer. hydrophila*. Serological cross-reactions between casein-precipitating enzymes of these two *Aeromonas* species were reported by Sandvick and Hagan (1968). Within the *Aer. salmonicida* group, Popoff (1969) found no serological differences among large numbers of typical pigment-producing isolates. Indeed, Popoff (1984), citing the work of Karlsson (1964), Spence *et al.* (1965) and his own previous studies, concluded that *Aer. salmonicida* is a serologically homogeneous species. Several workers have, in contrast, reported antigenic differences within the species (Duff, 1939; Liu, 1961; Klontz and Anderson, 1968; Kimura, 1969b). Paterson *et al.* (1980) considered that these conflicting findings reflected the choice of cultures, because comparable methodology had been used throughout. This group also reported results similar to those of Kimura (1969a, b), suggesting that *Aer. salmonicida* may be separated serologically into two groups based upon antigenicity of a

given strain. In their studies, *Aer. salmonicida* NCIMB 1110 and 1102 and *Aer. salmonicida* subsp. *masoucida* contained an extra antigenic component, termed the "c" component. Kimura (1969b) demonstrated the heat sensitivity of an additional antigenic component (shared with *Aer. hydrophila*) in the subspecies *masoucida*. Klontz and Anderson (1968) observed smears prepared from 24 cultures of *Aer. salmonicida* with three antisera, by means of an iFAT. They postulated the existence of at least seven different serotypes, based upon non-reactivity of certain strains with one or more of the antisera. However, McCarthy and Roberts (1980) questioned the suitability of this technique for serological analysis of laboratory cultures, due to the potential for technical difficulties.

McCarthy and Rawle (1975) carried out an extensive serological study of both thermolabile and thermostable somatic antigens of *Aer. salmonicida* and their relationship to other bacteria. They employed whole-cell agglutination and double cross-absorption of smooth strains, and passive haemagglutination and double cross-absorption of rough colony types. It was determined that cross-reaction titres for both antigen types were, in general, high, and cross-reactions between the *Aer. hydrophila* isolate and three out of six thermostable *Aer. salmonicida* antisera were very weak. In contrast, a strain of *V. anguillarum* and *Ps. fluorescens* gave no reaction with the *Aer. salmonicida* antisera. The passive haemagglutination method was more sensitive than whole-cell agglutination, as titres obtained for positive reactions were 10-fold higher in the former. When a double-diffusion method was employed to study cell-free extracts, prepared from the bacteria used for the somatic antigen study, strong cross-reactions among *Aer. salmonicida*, *Aer. hydrophila* and *V. anguillarum*, and, to a lesser extent, with *Ps. fluorescens* occurred. McCarthy and Rawle (1975) concluded that no qualitative differences in serological composition among *Aer. salmonicida* strains had been demonstrated, but noted that laboratory maintenance of some *Aer. salmonicida* cultures resulted in progressive loss of serological reactivity, giving negative responses with their antisera. Therefore, they suggested that, when embarking on serological (or vaccination) studies, it is important to include only fresh isolates. Hahnel *et al.* (1983) used micro-agglutination and double-diffusion precipitin tests to study serological relatedness among virulent and avirulent forms of eight isolates of *Aer. salmonicida* subsp. *salmonicida*. No serological differences were detected in the virulent isolates, but antigenic differences were observed between the virulent and avirulent form of each culture. Thus, in double-diffusion precipitin tests, the antigens of virulent sonicated cells formed an additional precipitin line when compared with the homogeneous avirulent form.

Bacteriophage typing

Bacteriophages have been used to study taxonomic relatedness between strains. The first isolation of bacteriophage specific for *Aer. salmonicida* was made by Todd (1933), although the usefulness for typing purposes was not demonstrated until the work of Popoff (1971a, b). It is considered that phage typing has value in epizootiological studies (Popoff, 1984; Bast *et al.*, 1988; Belland and Trust, 1989). Essentially, the bacteriophages may be divided into three morphological groups and

ten serological types (Popoff, 1984). Thus, using a set of eight phages, Popoff (1971b) recognised 14 phage types. Also, Paterson *et al.* (1980) studied phage sensitivity as a means of determining relationships between typical and atypical cultures. Pigmented and achromogenic as well as aggregating and non-aggregating strains showed a high sensitivity to two out of the three bacteriophages. In a further investigation by Rodgers *et al.* (1981), 27 groups of *Aer. salmonicida* were defined on the basis of sensitivity patterns to 18 bacteriophage isolates. Significantly, the morphological characteristics of the host bacterium, i.e. whether a rough, smooth or G-phase form, influenced attachment of the bacteriophage. This was apparently attributed to the varying quantities of LPS in the cell wall of the different morphological types.

Haemophilus piscium

What about *Haemophilus piscium*, the causal agent of ulcer disease? The name was coined by Snieszko *et al.* (1950). However, the detailed taxonomic study of Kilian (1976) showed that the organism did not belong in the genus *Haemophilus*. In particular, the strains did not exhibit requirements for haemin or NAD, which contrasted with the genus description. *H. piscium* differed from the type species, *H. influenzae*, in the inability to reduce nitrate or alkaline phosphatase and to grow at 37°C, together with a relatively high G + C ratio of the DNA. Unfortunately, Kilian did not establish the most appropriate taxonomic position of the pathogen. The validity of the taxonomic position was similarly questioned by Broom and Sneath (1981), as a result of a detailed numerical taxonomic study. The low similarity of *H. piscium* to other *Haemophilus* spp., i.e. only 65%, suggested that the organism should be excluded from the genus. From examination of DNA, biochemical, serological and bacteriophage sensitivity data, it is apparent that *H. piscium* represents an atypical, achromogenic variant of *Aer. salmonicida* (Paterson *et al.*, 1980). Evidence for this conclusion consists of the G + C ratio of the DNA (55.1 mol %), which is well within the range reported for *Aer. salmonicida* by McCarthy (1978). Moreover, Paterson *et al.* (1980) regarded *H. piscium* to be serologically indistinguishable from *Aer. salmonicida*. Also, the pathogen was sensitive to several *Aer. salmonicida* bacteriophages, and exhibited biochemical reactions similar to those expected for some achromogenic variants of *Aer. salmonicida*. Trust *et al.* (1980a) also concluded, on the basis of bacteriophage sensitivity, that *H. piscium* is, in fact, atypical *Aer. salmonicida*. Thus, a virus that produced lysogeny in *Aer. salmonicida*, but displayed no such activity in *Aer. hydrophila*, caused plaque formation in several isolates of *H. piscium*. On the basis of one strain, Austin *et al.* (1998) concurred with the view that *H. piscium* should probably be classified with *Aer. salmonicida*. However, its precise relationship to the four subspecies reflected the nature of the phenotypic and molecular methods used.

Aeromonas sobria

Evidence has been presented for *Aeromonas sobria* as a fish pathogen. Yet, fish-pathogenic isolates have often been mis-identified, whereas *bona fide Aer. sobria* has not been harmful to salmonids (Austin *et al.*, 1989). However, organisms,

considered as *Aer. sobria*, have been isolated from wild-spawning gizzard shad (*Dorosoma cepedianum*) in Maryland during 1987 (Toranzo *et al.*, 1989).

Aer. sobria

Cultures comprise motile (single polar flagellum) fermentative Gram-negative rods, which produce arginine dihydrolase, catalase, β-galactosidase, indole, lysine decarboxylase and oxidase, but not H_2S or ornithine decarboxylase. Blood, casein, gelatin, starch and Tween 80 are degraded, but not so aesculin, elastin or urea. Acid is produced from glucose (with gas), mannitol and sucrose, but not from arabinose, inositol, rhamnose, salicin or sorbitol. Nitrates are reduced, and the Voges Proskauer reaction is positive. Growth occurs in 0–5%, but not 10% (w/v) sodium chloride and on TCBS. Resistance has been recorded to ampicillin, novobiocin and the vibriostatic agent, O/129. The G + C ratio of the DNA is 60.4 mol % (Toranzo *et al.*, 1989).

Overall, there was good agreement with the description of *Aer. sobria* (Martin-Carnahan and Joseph, 2005). In particular, the important differentiating traits included production of acid from glucose (with gas), mannitol and sucrose, but not salicin, and the inability to degrade aesculin (Martin-Carnahan and Joseph, 2005). The only difference to the species description centred on the production of H_2S.

Aeromonas veronii biovar sobria

Aer. veronii has been implicated as a potential fish pathogen but only in laboratory-based experiments, where intramuscular injection of 10^7 cells/ml resulted in muscle necrosis in Atlantic salmon (McIntosh and Austin, 1990b). In a subsequent study, identification was achieved after examination of 14 isolates by FAME and AFLP fingerprinting, and biochemical profiling using the API 20E, API 20NE and Phene-Plate system (Rahman *et al.*, 2002a).

Alteromonadaceae representative

Pseudoalteromonas piscicida

An isolate, coined Cura-d, from *Amblyglyphidodon curacao* eggs was identified by 16S rDNA sequencing as *Pseudoalteromonas piscicida*.

Pseudoalteromonas piscicida

Colonies on marine 2216E agar were 3–6 mm in diameter and orange to dark orange in colour (the centres were often white) with the pigment diffusing into the agar. Cells comprised oxidative, Gram-negative, polarly flagellated rods, which utilised fructose, maltose, mannose and sucrose, but not L-threonine,

and contained intracellular granules, but not poly-β-hydroxybutyrate. Growth occurs at 40°C (Nelson and Ghiorse, 1999).

Shewanella putrefaciens

During Spring of 1985, a disease occurred which resulted in high mortalities in rabbitfish, *Siganus rivulatus*, farmed in sea cages in the Red Sea. From diseased animals, a Gram-negative bacterium was recovered, which was capable of re-infecting healthy fish (Saeed *et al.*, 1987). To date, the disease has not been described in any other fish species, or, for that matter, elsewhere.

Shewanella putrefaciens

Cultures comprise motile Gram-negative rods, which are neither fermentative nor oxidative for glucose, but which grow at 15–42°C, in 0.85–9.0% (w/v) sodium chloride, at pH 6.2–9.6, and on MacConkey agar. Catalase, H_2S, ornithine decarboxylase and oxidase are produced, but not arginine dihydrolase, β-galactosidase, indole, lysine decarboxylase, or phenylalanine or tryptophan deaminase. Gelatin but not urea is attacked. Nitrates are reduced, and citrate is utilised. The Voges Proskauer reaction is negative. Acid is not produced from amygdalin, arabinose, glucose, inositol, mannitol, melibiose, rhamnose, sorbitol or sucrose. Growth occurs at 37°C, and in 7% but not 0% (w/v) sodium chloride (Saeed *et al.*, 1987).

From the results of the API 20E rapid identification system, it was considered that the pathogen was *Ps. (= Alteromonas) putrefaciens*, a taxon which has been subsequently re-classified as *Shewanella putrefaciens* (MacDonell and Colwell, 1985).

Campylobacteriaceae representative

Arcobacter cryaerophilus

According to Aydin *et al.* (2002), the cultures were identified according to the results in *Bergey's Manual of Systematic Bacteriology*, and there does appear to be a close agreement in the characteristics.

Arcobacter cryaerophilus

The small, white colonies comprise aerobic, Gram-negative, motile rods that produce catalase and oxidase, but not alkaline phosphatase, arginine dihydrolase, H_2S, indole or urease, reduce nitrates to nitrites, do not attack aesculin, gelatin, starch or Tween 20 or 80, grow at 14–42°C and in potassium cyanide, but not in 3% (w/v) sodium chloride, do not produce acid from adonitol, arabinose, dulcitol, erythritol, fructose, galactose, glucose, glycogen, inositol, inulin, lactose, maltose,

mannitol, mannose, melibiose, raffinose, rhamnose, salicin, sorbitol, sorbose, sucrose, trehalose or xylose, or utilise citrate (Aydin *et al.*, 2002).

Enterobacteriaceae representatives

In addition to the organisms detailed below, there has been fleeting mention of a fish-pathogenic role for *Erwinia* sp. (Starr and Chatterjee, 1972) and *Salmonella typhimurium* (Morse *et al.*, 1978).

Citrobacter freundii

Historically, there have been brief references to *Cit. freundii* as a fish pathogen (see Conroy, 1986). Yet, definitive evidence was not forthcoming until a report of the organism as a pathogen of sunfish, *Mola mola* in a Japanese aquarium (Sato *et al.*, 1982). Subsequently, *Cit. freundii* has been implicated with disease in Atlantic salmon and rainbow trout in Spain and the U.S.A. (Baya *et al.*, 1990a; Sanz, 1991) and with carp in India (Karunasagar *et al.*, 1992). In addition, the organism has been recovered in mixed culture from diseased salmonids in the U.K. (B. Austin, unpublished data). Consequently, it appears that *Cit. freundii* is an emerging fish pathogen.

Citrobacter freundii

Cultures contain Gram-negative, motile, fermentative rods, which produce catalase, β-galactosidase and H_2S, but not arginine dihydrolase, indole, lysine or ornithine decarboxylase, oxidase or tryptophan deaminase. Neither gelatin nor urea is attacked, nor is citrate utilised. The Voges Proskauer reaction is negative. Acid is produced from arabinose, glucose, mannitol, melibiose, rhamnose, sorbitol and sucrose, but not from amygdalin or inositol.

From these traits, mostly obtained with the API 20E rapid identification system, an identification as *Cit. freundii* was obtained (Sato *et al.*, 1982; Baya *et al.*, 1990a; Sanz, 1991). Certainly, these characteristics match the description of *Cit. freundii*, except in the inability to utilise citrate (Frederiksen, 2005).

Edwardsiella ictaluri

Enteric septicaemia of catfish was initially recognised in 1976 among populations of pond-reared fingerlings and yearling fish in Alabama and Georgia (Hawke, 1979). Later, reports described its presence in Mississippi, Arkansas, Idaho, Colorado, Indiana and Maryland. All the published reports point to the presence of a very homogeneous group of bacteria phenotypically, including use of isozyme analysis (Starliper *et al.*, 1988; Bader *et al.*, 1998), although subgroups may be recognised by molecular methods. Thus, Bader *et al.* (1998) recognised 4 subgroupings among 20 by use of arbitrary primed PCR.

Edwardsiella ictaluri

Cultures comprised Gram-negative, rod-shaped, fermentative organisms (strains with a limited tolerance for oxygen have been recovered from channel catfish in the U.S.A.; Mitchell and Goodwin, 2000), which are motile by peritrichous flagella. Catalase, β-galactosidase (a variable response has been recorded between various laboratories) and lysine and ornithine decarboxylase are produced, but not H_2S, indole, oxidase or phenylalanine deaminase. The methyl red test is positive, but not so the Voges Proskauer reaction. Nitrates are reduced. Growth occurs in 1.5%, but not 2% (w/v) sodium chloride. The optimum growth temperature is between 20 and 30°C, which coincides with the water temperature during severe outbreaks of the disease. Blood is degraded, but not casein, DNA, elastin, gelatin, Tween 20, 40, 60 and 80 or urea. Acid is produced from fructose, galactose, glycerol, maltose, mannose and ribose, but not from adonitol, aesculin, amygdalin, arabinose, arbutin, cellobiose, dulcitol, erythritol, inositol, inulin, lactose, melezitose, raffinose, rhamnose, salicin, sodium malonate, sorbitol, sorbose, starch or sucrose (Waltman *et al.*, 1986). The G+C ratio of the DNA is 53 mol % (Hawke *et al.*, 1981).

Cryptic plasmids, i.e. pCL1 and pCL2 of 5.7 kb and 4.9 kb respectively, have been found in isolates of *Edw. ictaluri* (Lobb *et al.*, 1993). However, an isolate from green knife fish differed insofar as four plasmids, of 3.1, 4.1, 5.7 and 6.0 kb, were present. Of these, the 4.1 and 5.7 kb plasmids hybridised strongly (Lobb *et al.*, 1993).

Similarities have been observed with *Edw. tarda,* except that isolates of *Edw. ictaluri* did not produce H_2S or indole, or ferment glucose with the production of gas at 37°C. Moreover, the isolates did not agglutinate with antiserum to *Edw. tarda* (Hawke, 1979). However, in terms of DNA relatedness, the causal agent of enteric septicaemia of catfish was determined to be most similar to *Edw. tarda*, i.e. 56–62% DNA homology, but sufficiently distinct to warrant separate species recognition (Hawke *et al.*, 1981). It remains for further work to elucidate the relationship, if any, with *Edwardsiella* phenon 12 of Johnson *et al.* (1975).

Edwardsiella tarda (= Paracolobactrum anguillimortiferum, Edw. anguillimortifera)

Whereas *Edw. ictaluri* has remained confined within the broadly defined geographical limits of the U.S.A., *Edw. tarda* has emerged with some frequency in the Far East, particularly Japan and Taiwan (Wakabayashi and Egusa, 1973; Miyazaki and Egusa, 1976; Kou, 1981). The history of edwardsiellosis, as the disease is called, may be traced to two separate parallel developments, i.e. those of Wakabayashi and Egusa (1973) in Japan, and Meyer and Bullock (1973) in the U.S.A. The situation is further complicated by an earlier report of Hoshina (1962), who described *Paracolobactrum anguillimortiferum* as a pathogen of pond-cultured eels. This organism is probably synonymous with *Edw. tarda.*

The organisms from fish match closely the description of *Edw. tarda* (Ewing *et al.*, 1965; Cowan, 1974; Farmer and McWhorter, 1984), insofar as they are fairly

reactive (Table 4.2), Gram-negative rods, motile by means of peritrichous flagella. The original isolates of Meyer and Bullock (1973) were identified definitively as *Edw. tarda* by the CDC. Thereafter, each successive report emphasised the similarity to the species description of Ewing *et al.* (1965), highlighting the homogeneity of the fish isolates. In fact, it is astonishing how little variation has been recorded in the phenotypic characters, despite recovery from as far afield as Japan (Wakabayashi and Egusa, 1973; Nakatsugawa, 1983) and the U.S.A. (Amandi *et al.*, 1982). The only difference reflects serology. Thus, four serotypes (A, B, C and D) have been recognised from O-agglutination analyses of 270/445 isolates recovered from eel ponds in Japan during 1980 and 1981 (Park *et al.*, 1983). Among the cultures, serotype B predominated (22–35% of the total), followed by serotype A (13–17% of the isolates), serotype C (4–13% of the isolates) and serotype D (2–4% of the total). Most of the isolates in serotype A were derived from kidney samples. Moreover, this group was by far the most virulent as assessed from experimental infections of eels, loach and tilapia (Park *et al.*, 1983) and natural outbreaks in Japanese flounder (Rashid *et al.*, 1994a,b). It has been argued that isolates from turbot in Spain constitute a separate serological group (Castro *et al.*, 2006).

The G + C ratio of the DNA was calculated as 59 mol % (Amandi *et al.*, 1982).

Confusion has enveloped the taxonomic status of this pathogen, insofar as the "Approved Lists of Bacterial Names" (Skerman *et al.*, 1980) include two specific epithets for what is apparently the same organism, *Edw. tarda* and *Edw. anguillimortifera*. *Edw. tarda* was proposed initially by Ewing *et al.* (1965), and it would appear that there was a lack of familiarity with the earlier work of Hoshina (1962), who described *Paracolobactrum anguillimortiferum*. The characteristics of these two nomenspecies are identical (Table 4.2). Recognising the efforts of Hoshina (1962), Sakazaki and Tamura (1975) proposed that *Paracolobactrum anguillimortiferum* should be reclassified in the genus *Edwardsiella*, as *Edw. anguillimortifera*. Furthermore, Sakazaki and Tamura (1975) emphasised that priority should be attached to the specific epithet of *anguillimortiferum* rather than *tarda*. Thus, two names have resulted for what is surely the same organism. Clearly, this is an unacceptable situation and, despite the pioneering work of Hoshina (1962), it is our contention that, to avoid further confusion in medical and veterinary microbiology, the name of *tarda* should be retained.

Escherichia vulneris

An organism, subsequently identified as *Esch. vulneris*, was first isolated in 1994 from naturally infected balloon moly (*Poecilia* sp.), silver moly (*Poecilia* sp.) and Caucasian carp (*Carassius* sp.) from Turkey (Aydin *et al.*, 1997).

Escherichia vulneris

Cultures comprise Gram-negative, motile, fermentative rods, which produce arginine dihydrolase, catalase and lysine decarboxylase, but not H_2S, indole, oxidase or ornithine decarboxylase, degrade aesculin and blood, but not casein,

Table 4.2. Characteristics of *Edwardsiella tarda*[a] and *Paracolobactrum anguillimortiferum*[b]

Character	*Edw. tarda*	*Paracolobactrum anguillimortiferum*
Fermentative metabolism	+	+
Production of:		
Arginine dihydrolase	−	NS
Catalase	+	+
β-galactosidase	−	NS
H_2S	+	+
Indole	+	+
Lysine decarboxylase	+	NS
Ornithine decarboxylase	+	NS
Oxidase	−	NS
Phenylalanine deaminase	−	NS
Methyl red test	+	NS
Nitrate reduction	+	+
Voges Proskauer reaction	−	−
Degradation of:		
Aesculin, casein, gelatin	−	−
Tributyrin, urea	−	NS
DNA, elastin, lecithin	−	−
Tween 20, 40, 60 and 80	−	−
Chitin	−	−
Blood	+	+
Utilisation of:		
Sodium citrate, sodium malonate	−	NS
Growth at:		
42°C	+	+
3% (w/v) sodium chloride	+	+
8% (w/v) sodium chloride	−	−
Acid production from:		
Adonitol, aesculin, erythritol	−	NS
Arabinose, cellobiose, dulcitol	−	−
Fructose, galactose, glycerol	+	NS
Glucose, maltose	+	+
Inositol	−	NS
Lactose, mannitol, raffinose	−	−
Mannose	+	NS
Rhamnose, salicin, sorbitol	−	−
Sucrose, trehalose, xylose	−	−

[a] From Sakazaki and Tamura (1975), Wakabayashi and Egusa (1973), Amandi *et al.* (1982) and Nakatsugawa (1983).
[b] From Hoshina (1962)
NS = Not stated

DNA, gelatin, starch or urea, reduce nitrates, utilise malonate, and produce acid from arabinose, glucose, lactose, maltose, mannitol, melibiose, rhamnose, salicin, trehalose and xylose, but not adonitol, cellobiose, dulcitol, glycerol, inositol, sorbitol or sucrose. The methyl red test is positive, but the Voges Proskauer reaction is negative. Growth occurs at 37°C, but not at 5 or 42°C.

The organisms coincided with the description of *Esch. vulneris* (Brenner *et al.*, 1982).

Hafnia alvei

In 1988, an apparently new *Brucella*-like bacterium was described as a pathogen of rainbow trout in Bulgaria (Gelev and Gelev, 1988). Although antigenic relationships to *Brucella abortus* were reported, the organism displayed marked similarities to the Enterobacteriaceae. Subsequent investigation led to a realisation that the organism was, in fact, *Hafnia alvei* (Gelev *et al.*, 1990). Subsequently, *Haf. alvei* was recognised as the cause of mortalities among cherry salmon (*O. masou*) in Japanese farms (Teshima *et al.*, 1992). Isolates matched the species description, with the exception of lack of motility and utilisation of D-tartrate.

Hafnia alvei

The isolate comprises small coccoid or slightly elongated, motile, Gram-negative rods which produce catalase, β-galactosidase and lysine and ornithine decarboxylase, but not arginine dihydrolase, H_2S, indole or oxidase. The methyl red, nitrate reduction and citrate utilisation tests and Voges Proskauer reaction (weakly positive) are positive, but not malonate utilisation or phenylalanine deaminase. Neither aesculin, DNA, gelatin, lipids nor urea is degraded. Acid is produced from arabinose, cellobiose, glucose (but not gas), glycerol, maltose, mannose, mannitol, rhamnose, trehalose and xylose, but not from adonitol, dulcitol, erythritol, lactose, melibiose, raffinose, salicin, sorbitol or sucrose. Growth occurs at 41°C.

It was deemed that there was a common antigenic determinant in the LPS with *Brucella abortus* and *Y. ruckeri* (Gelev and Gelev, 1988). However, the presence of motility and the negative reaction in the oxidase test precludes a relationship with *bona fide Brucella* taxa. There was 82–100% re-association between the fish isolates and *Haf. alvei* (Gelev *et al.*, 1990).

Klebsiella pneumoniae

Twelve pure cultures were obtained. The characteristics were as follows:

Klebsiella pneumoniae

Cultures comprise fermentative, Gram-negative, encapsulated rods that produce catalase but not arginine dihydrolase, H_2S, indole, lysine or ornithine

decarboxylase, oxidase nor phenylalanine deaminase, produce acid from arabinose, cellobiose, glucose, maltose and raffinose, reduce nitrates to nitrite, and do not degrade gelatin (Daskalov *et al.*, 1998).

Pantoea agglomerans

There has been some debate over the precise taxonomic relationship of the organism, which has been classified as *Enterobacter agglomerans*, *Erwinia herbicola* and now as *Pantoea agglomerans* (see Grimont and Grimont, 2005). Nevertheless, an organism corresponding to the description of *Pantoea agglomerans* was recovered from dead mahi-mahi (100 g) (dolphin fish; *Coryphaena hippurus*). Juvenile mahi-mahi (average weight = 1 g) were transported from Florida to Bermuda for ongrowing in sea cages. During January 1986, mortalities were noted with dead specimens displaying marked haemorrhaging in the eyes. From these animals, *Pantoea agglomerans* was recovered (Hansen *et al.*, 1990).

There was excellent agreement between the characteristics of the fish isolate and the description of *Pantoea agglomerans* (Grimont and Grimont, 2005).

Pantoea agglomerans

Cultures comprise pale yellow pigmented, motile, fermentative, Gram-negative rods, which produce catalase and β-galactosidase, but not arginine dihydrolase, H_2S, indole, lysine or ornithine decarboxylase, or oxidase. The methyl red test, Voges Proskauer reaction and nitrate reduction test are positive. Gelatin, but not blood, chitin or starch, is degraded. Acid is produced from glucose, D-mannitol and sucrose. Citrate is utilised. Growth occurs at 4–37°C, but not at 40°C, and in 0–6%, but not 8% (w/v), NaCl (Hansen *et al.*, 1990).

Plesiomonas shigelloides

Although there have been unauthenticated verbal reports that *Plesiomonas shigelloides* may be pathogenic to fish, it was not until the summer of 1984 that the organism was definitely recovered from diseased rainbow trout in northern Portugal (Cruz *et al.*, 1986). Subsequently, the organism was recovered in Germany from catfish, which had originated in Nigeria, sturgeon fingerlings, which had been sent from Russia, gourami imported from Thailand and eels (Klein *et al.*, 1993). Many of the characteristics of the pathogen were derived from use of the API 20E rapid identification system (Cruz *et al.*, 1986) and API 20NE system (Klein *et al.*, 1993).

Plesiomonas shigelloides

Colonies contain motile, fermentative, Gram-negative rods, which produce arginine dihydrolase, catalase, β-galactosidase, indole, lysine and ornithine decarboxylase and oxidase, but not H_2S, phenylalanine deaminase, tryptophan deaminase or urease. Nitrates are reduced to nitrite, but negative reactions are

recorded for gelatin liquefaction, the methyl red test, utilisation of citrate or malonate, and the Voges Proskauer reaction. Acid is produced from glucose, inositol and trehalose, but not from adonitol, aesculin, amygdalin, L-arabinose, dulcitol, lactose, mannitol, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose or xylose. Growth occurs at 22 and 37°C, but not at 4°C, and in 0% (w/v) sodium chloride (Cruz *et al.*, 1986; Klein *et al.*, 1993).

It should be emphasised that phylogenetic studies have indicated that the taxon should really belong in the genus *Proteus* as *Proteus shigelloides* (MacDonell and Colwell, 1985).

Providencia rettgeri

During 1976, there was a mass mortality among farmed silver carp (*Hypophthalmichthys molitrix*) in Israel. From moribund animals, *Pr. rettgeri* was isolated. To date, this has been the only report implicating this organism as a fish pathogen (Bejerano *et al.*, 1979).

Providencia rettgeri

Cultures comprise fermentative, Gram-negative rods, which are motile by means of peritrichous flagella. Catalase, indole, phenylalanine deaminase and tryptophan deaminase are produced, but not β-galactosidase, H_2S or oxidase. DNA and urea are degraded, but not casein, gelatin, starch or the Tweens. The methyl red test is positive. Nitrates are reduced. Acid is produced from adonitol, aesculin, erythritol, galactose, inositol, mannitol, mannose, melezitose, rhamnose and salicin, but not from amygdalin, arabinose, cellobiose, dulcitol, glycerol, glycogen, inulin, lactose, maltose, melibiose, raffinose, ribose, sorbitol, sucrose, trehalose or xylose. The G + C ratio of the DNA is 39.2 mol % (Bejerano *et al.*, 1979).

Generally, the isolates matched the description of *Pr. rettgeri* (Cowan, 1974; Johnson *et al.*, 1975; McKell and Jones, 1976; Penner, 2005)

Salmonella enterica subsp. *arizonae* (*Salmonella choleraesuis* subsp. *arizonae* = *Salmonella arizonae*)

In a previous study by Austin *et al.* (1982), a possible taxonomic relationship was discussed between *Y. ruckeri* and *Sal. choleraesuis* subsp. *arizonae*. Therefore, it is ironic that the latter organism has now been involved in fish pathogenicity. Thus, *Sal. enterica* subsp. *arizonae* was recovered during 1986 from a single dead pirarucu, *Arapaima gigas*, which had been exhibited in an aquarium at Sapporo, Japan. To date, this is the only report implicating *Sal. enterica* subsp. *arizonae* as a fish pathogen (Kodama *et al.*, 1987).

The culture was identified biochemically as *Sal. enterica* subsp. *arizonae*. Yet, agglutination was not recorded with commercial *Salmonella* antisera, although not specifically a product to *Sal. enterica* subsp. *arizonae* (Kodama *et al.*, 1987).

Salmonella enterica subsp. *arizonae*

Cultures contain Gram-negative, fermentative, motile rods, which produce catalase, β-galactosidase, H_2S and lysine decarboxylase, but not indole or oxidase. Neither aesculin, blood, casein, gelatin, starch nor urea is degraded. Citrate is utilised, nitrate is reduced and the methyl red test is positive. The Voges Proskauer reaction is negative. Acid is produced from glucose (plus gas), lactose, maltose, mannitol, raffinose, sorbitol, sucrose, trehalose and xylose, but not arabinose or salicin. Growth occurs at 15–41°C in 0–6% (w/v) NaCl and on MacConkey agar (Kodama *et al.*, 1987).

With the exception of acid production from arabinose, raffinose and sucrose, there is good agreement with the description of *Sal. enterica* subsp. *arizonae* (Popoff and Le Minor, 2005).

Serratia liquefaciens

During Spring and early Summer of 1988, heavy mortalities, i.e. up to 30% of the stock, were noted among three separate populations of Atlantic salmon (*Salmo salar* L.), two of which (average weight = 450 g) were in Scottish marine cage sites, and the third population (average weight = 25 g) was in freshwater. Microscopic examination of formalin-fixed kidney sections indicated the presence of Gram-negative bacteria which exhibited bipolar staining. These organisms were recovered as dense, pure culture growth from diseased tissues, and were examined bacteriologically (McIntosh and Austin, 1990b). Since the initial occurrence, the disease has been recognised on another two marine sites in Scotland during 1990. Also in 1990, the organism was recognised as the cause of low, continuous mortalities among farmed turbot in France (Vigneulle and Baudin-Laurençin, 1995).

Serratia liquefaciens

Cultures comprise Gram-negative, fermentative, catalase-positive rods of 2–3 μm in length which, in contrast to the normal characteristics of the Enterobacteriaceae (Grimont and Grimont, 2005a), are motile by single polar flagella. Intracellular, bipolar staining properties are exhibited. Catalase, β-galactosidase and lysine and ornithine decarboxylase are produced, but not H_2S, indole or tryptophan deaminase. The results of the oxidase test are variable, i.e. from negative to weakly positive, depending to some extent on the age of the culture. The methyl red test and Voges Proskauer reaction is negative. Nitrates are not reduced. Blood (β-haemolysis), casein, DNA, lecithin (weak), tributyrin (slow), Tween 20, 40, 60 and 80, and tyrosine are degraded, but not chitin, elastin, starch or urea. Growth

occurs at 4–37°C, in 0–5% (w/v), but not 7% (w/v) sodium chloride, and on deoxycholate citrate agar, eosin methylene blue agar and MacConkey agar, but not in 40% (v/v) bile salts. Sodium citrate, but not sodium malonate, is utilised. Acid is produced from amygdalin, arabinose, inositol, mannose, melibiose and saccharose, but not rhamnose. Acid and gas are produced from fructose, galactose, glucose, maltose, mannitol, melibiose, raffinose, sorbitol, sucrose, trehalose and xylose. The G + C ratio of the DNA is 55 mol % (McIntosh and Austin, 1990b).

Initially, it was considered that the organisms bore similarities to *Aer. veronii* (Hickman-Brenner *et al.*, 1987) and *Ser. liquefaciens* (Grimont and Grimont, 2005a). Similarities with the former included the presence of Gram-negative, fermentative, catalase- (and oxidase-) positive rods, which were motile by single polar flagella, and with a G + C ratio of the DNA of 55 mol %. Indeed, the only discrepancies with the definition of *Aer. veronii* concerned some sugar fermentation reactions. Yet, similarities were also apparent with *Ser. liquefaciens*. However, the use of the API 20E rapid identification system (profile = 5305363 or 5305367) and whole-cell agglutination, using a polyclonal antiserum raised against the type strain, confirmed an identification as *Ser. liquefaciens*.

Serratia marcescens

The initial association of *Ser. marcescens* with fish diseases stemmed from a publication by Clausen and Duran-Reynals (1937). More detailed work waited for over half a century when in July 1990 during a survey of white perch (*Morone americanicus*) from the Black River, a tributary of Chesapeake Bay (Virginia), a red-pigmented organism, i.e. *Ser. marcescens*, was recovered, and, on subsequent examination, deemed to be potentially pathogenic for fish. It was considered likely that the presence of the organisms reflected the polluted nature of the river (Baya *et al.*, 1992c).

Serratia marcescens

Cultures comprise red- (prodigiosin-) pigmented, fermentative, motile, Gram-negative rods, which produce catalase, β-galactosidase and lysine and ornithine decarboxylase, but not arginine dihydrolase, H_2S, indole, oxidase or tryptophan deaminase. Nitrate is reduced; but the methyl red test and the Voges Proskauer reaction are negative. Growth occurs at 4–45°C, and in 0–8% (w/v) sodium chloride. Blood (sheep), casein, gelatin, starch and Tween 80 are degraded, but not so urea. A wide range of carbohydrates are attacked, including amygdalin, fructose, galactose, glycerol, inositol, maltose, mannitol, mannose, salicin, sorbitol, sucrose and trehalose, but not so arabinose, cellobiose, dulcitol, lactose, melibiose, raffinose, rhamnose or xylose (Baya *et al.*, 1992c).

From these characteristics, it is apparent that there is reasonable agreement with the description of *Ser. marcescens* (Grimont and Grimont, 2005a).

Serratia plymuthica

During September to December 1987, a new pathogen was associated with diseased rainbow trout fingerlings (average weight = 7 g) in a hatchery in northwestern Spain. Progressive, low-level mortalities (cumulative total = 35% of the stock) were reported, with which there appeared to be a correlation with rainfall (Nieto *et al.*, 1990). Then, in 1992 a similar organism was associated with skin lesions in farmed rainbow trout in Scotland (Austin and Stobie, 1992b). In this disease outbreak, there was an association with pollution by domestic sewage, i.e. leakage from a septic tank.

Serratia plymuthica

Nieto *et al.* (1990) reported that cultures comprise red- (prodigiosin-) pigmented, fermentative, non-motile, Gram-negative rods, producing catalase and β-galactosidase, but not arginine dihydrolase, H_2S, indole, lysine or ornithine decarboxylase, or oxidase. The nitrate reduction, citrate utilisation and Voges Proskauer reaction tests are positive. A negative response is recorded for the methyl red test. Gelatin is degraded, but not so blood, casein or urea. Acid is produced from L-arabinose, D-fructose, D-galactose, D-glucose (but not gas), inositol, D-maltose, D-mannitol, D-mannose, melibiose and sucrose, but not from D-adonitol, lactose, L-rhamnose or D-sorbitol.

From these results, it was considered that the cultures resembled *Ser. plymuthica* and *Ser. rubidaea* (Grimont and Grimont, 2005a). Yet, as a result of acid production from inositol, but not adonitol, it was considered that the pathogen should be assigned to *Ser. plymuthica*. Similar traits were reported by Austin and Stobie (1992b).

Yersinia intermedia

Characteristics matched the species description (Farmer and Kelly, 1991) at 36°C, except that the fish isolate utilised sodium citrate and was weakly positive in the Voges Proskauer reaction (Carson and Schmidtke, 1993).

Yersinia intermedia

The culture comprises fermentative, motile (at 25°C but not 36°C) cells that produced β-galactosidase and indole, but not arginine dihydrolase, H_2S, lysine or ornithine decarboxylase or oxidase. Aesculin and urea are degraded. The methyl red test and Voges Proskauer reaction (at 36°C but not 25°C) are positive. Nitrates are reduced. Acid was produced from glycerol, inositol, mannitol, melibiose (at 36°C but not 25°C), rhamnose, sorbitol, sucrose, trehalose and

xylose, but not adonitol or lactose. Sodium citrate is utilised at 36°C, but not 25°C (Carson and Schmidtke, 1993).

Yersinia ruckeri

Enteric redmouth (ERM, Hagerman redmouth disease, redmouth, salmonid blood spot) was initially diagnosed as a systemic infection among farmed rainbow trout in the Hagerman Valley of Idaho during the early 1950s, and subsequently described in detail by Ross *et al.* (1966).

Yersinia ruckeri

The organisms comprise a homogeneous group of fermentative, Gram-negative, slightly curved rods of 1.0 × 2.0–3.0 μm in size, which are motile usually by means of 7 or 8 peritrichously arranged flagella. Catalase, β-galactosidase, and lysine and ornithine decarboxylase are produced, but not H_2S, indole, oxidase, phenylalanine deaminase or phosphatase. The methyl red test is positive, but not the Voges Proskauer reaction. Nitrates are reduced. Gelatin and Tween 20, 40 and 60 are degraded, but not aesculin, chitin, DNA, elastin, pectin, tributyrin, Tween 80 or urea. Growth occurs in 0–3% (w/v) sodium chloride. Sodium citrate is utilised. Acid is produced from fructose, glucose, maltose, mannitol and trehalose, but not from inositol, lactose, raffinose, salicin, sorbitol or sucrose. The G + C content of the DNA is 47.5–48.5 mol % (Ewing *et al.*, 1978).

The precise taxonomic position of the causal agent of ERM has intrigued bacteriologists since the initial isolation of the organism. Ross *et al.* (1966) realised that heated O-antigens prepared from 14 cultures of the ERM organism agglutinated strongly (titre = 1:320 or 1:640) with the corresponding antigens of *Sal. enterica* subsp. *arizonae* O group 26, and weakly (titre = 1:20) with O group 29. Conversely, there was no reaction with O-antigens prepared from *Salmonella*, *Ent. liquefaciens*, *Citrobacter* or *Serratia*. In addition, this team pointed to the biochemical similarities with *Ent. liquefaciens*, *Ser. marcescens* subsp. *kiliensis* and *Sal. enterica* subsp. *arizonae*. Furthermore, Stevenson and Daly (1982) indicated serological cross-reactions with *Haf. alvei*. However, after an examination of the phenotypic and molecular traits of 33 isolates, Ewing *et al.* (1978) elevated the pathogen to species status, as *Y. ruckeri*. It is interesting to note that the pathogen was included in the genus *Yersinia* because of only a 30–31% DNA homology to *Y. enterocolitica* and *Y. pseudotuberculosis*. This compares with DNA homologies of 24–28% and 31% with *Ser. marcescens* and *Ser. liquefaciens*, respectively (Steigerwalt *et al.*, 1976). Therefore, from the DNA hybridisation experiments, it is difficult to determine the reasons for including the ERM organism with *Yersinia* rather than *Serratia*. However, it would be relevant to extend the existing data bases by comparing the homology with *Sal. enterica* subsp. *arizonae*. This is especially relevant because Green and Austin (1982) have shown a greater phenotypic relationship of *Y. ruckeri* with

Sal. enterica subsp. *arizonae* than with *Y. enterocolitica* or *Y. pseudotuberculosis*. In fact, the causal agent of ERM may belong in a new genus of the Enterobacteriaceae, an idea which has been mooted by Bercovier and Mollaret (1984). The distinctiveness was confirmed by an examination of phylogeny based on 16S rRNA sequences, when *Y. ruckeri* formed a distinct node, albeit associated with other yersinias (Ibrahim *et al.*, 1993).

Whereas the taxon is phenotypically homogeneous, it is serologically diverse insofar as five serotypes have been recognised (O'Leary, 1977; Bullock *et al.*, 1978a; Stevenson and Airdrie, 1984a). Three serotypes have been referred to colloquially as the "Hagerman strain" (the most common and the most virulent), the "Big Creek strain" (relatively avirulent) and the "Australian strain" (appears to be avirulent) (Busch, 1981). These serotypes have been designated as Type 1 (Hagerman), Type 2 (O'Leary) and Type 3 (Australian) (Bullock and Anderson, 1984) and the newly described Serovars IV and V (Stevenson and Airdrie, 1984a). Serotype 2 may be distinguished from Serotype 1 by its ability to ferment sorbitol (O'Leary, 1977). However, caution needs to be advocated since this may well be a plasmid-mediated trait. Nevertheless, serotypes/serovars 2, 3, 4 and 5 appear to be highly related in terms of DNA homology (De Grandis *et al.*, 1988). Also, two LPS profiles have been recognised among 23 Portuguese isolates, corresponding to serotypes O1 and O3. Greater heterogeneity was recorded for OMP, with 7 profiles recognised, and there were 10 ribotypes, 6 of which accommodated serotype O1 isolates (Sousa *et al.*, 2001).

During the early 1990s, in England a new form of the pathogen was recovered among rainbow trout that had been previously vaccinated by immersion with commercial enteric redmouth vaccines. This form, which was initially considered to share some of the characteristics of *Haf. alvei*, was confirmed as *Y. ruckeri* by 16S rRNA sequencing (homology = 100%), and was regarded as a new biogroup. In contrast to the species description, cultures often appeared to be non-motile, and were positive for the Voges Proskauer reaction. There was only weak agglutination to sera prepared against serovar I and II (Austin *et al.*, 2005a). Similar non-motile variants were also recovered from previously vaccinated rainbow trout in Spain (Fouz *et al.*, 2006).

Flavobacteriaceae representatives

Numerous reports have centred around the role of Gram-negative chromogens as agents of fish disease. Genera, which have been mentioned frequently, include *Cytophaga*, *Flavobacterium*, *Flexibacter*, *Myxobacterium*, *Myxococcus* and *Sporocytophaga*. The common factor is that these genera comprise difficult-to-identify species, a taxonomic re-evaluation of which has at long last occurred. Moreover, from the early literature it is often uncertain into which of these genera unknown isolates should have been placed. In particular, the distinction between *Cytophaga* and *Flexibacter* was confusing (Christensen, 1977; Allen *et al.*, 1983b). The authenticity of *Flavobacterium* has been questioned repeatedly, insofar as it became a recipient of problematical pigmented bacteria. Fortunately, more recent work has improved the taxonomy of *Flavobacterium* (Holmes *et al.*, 1984; Bernardet *et al.*,

1996). *Myxobacterium*, considered to be a causal agent of gill disease (Ghittino, 1967; Wood, 1968; Bullock and McLaughlin, 1970; Ashburner, 1978), is not included in the "Approved Lists of Bacterial Names" (Skerman *et al.*, 1980) or their supplements. Therefore, this genus lacks taxonomic meaning. It is possible that the organisms identified as *Myxobacterium* (and referred to as myxobacters) belong in either *Cytophaga*, *Flavobacterium* or *Flexibacter*.

Historically, interest in chromogenic, Gram-negative bacteria started with a publication by Davis (1922), who reported serious mortalities (columnaris) among warm-water fish, namely small mouth bass and common perch, from the Mississippi River. These fish were held at the U.S. Biological Station at Fairport, Iowa, when the disease occurred. Davis recognised two important features of the disease, namely that occurrence was primarily in injured (damaged/stressed) fish, and that the water temperature was high, i.e. >21.1°C. Unfortunately, Davis did not succeed in isolating the pathogen. In fact, this was not achieved for two decades, until Ordal and Rucker (1944) succeeded in 1943 during an outbreak in hatchery-reared sockeye salmon.

Fish-pathogenic flexibacters have been recognised (see Masumura and Wakabayashi, 1977; Hikida *et al.*, 1979; Pyle and Shotts, 1980, 1981; Wakabayashi *et al.*, 1984). For example, during 1976 and 1977, a bacterial disease developed in juvenile (usually ≤60 mm in length) red sea bream and black sea bream maintained at marine sites in Hiroshima Prefecture, Japan. The outbreak occurred 1–2 weeks after the fish were transported from the hatchery to sea cages. An organism was isolated by Masumura and Wakabayashi (1977), and later considered to be a new species of *Flexibacter*, for which the name of *Fle. marinus* was coined (Hikida *et al.*, 1979). This organism was subsequently re-named as *Fle. maritimus* (Wakabayashi *et al.*, 1986) and thence as *Tenacibaculum maritimum* (Suzuki *et al.*, 2001). However, it has been suggested that this taxon is synonymous with *Cyt. marina* (Holmes, 1992). *Fle. ovolyticus* has been named as a new pathogen of halibut eggs and larvae (Hansen *et al.*, 1992), and then reclassified as *Tenacibaculum ovolyticum* (Suzuki *et al.*, 2001). Additional reports of fish-pathogenic *Flexibacter* spp. have been noted. In particular, the genus has been found in cases of fin rot (Bullock and Snieszko, 1970; Schneider and Nicholson, 1980) and general "myxobacterial" diseases (e.g. Pyle and Shotts, 1980, 1981).

Flavobacterium became the second genus of yellow-pigmented, Gram-negative, fish pathogens to be recognised, with the description of *Fla. balustinum* by Harrison and Sadler (1929). This taxon was re-classified into a newly established genus, as *Chryseobacterium balustinum* (Vandamme *et al.*, 1994). However, we believe that this organism was really a fish spoilage agent rather than a pathogen, insofar as the only work describing "infectivity" referred to dead not living fish. Historically, the organism was first described as being a problem on freshly landed halibut, on which it produced a yellowish slime (Harrison and Sadler, 1929). It would seem likely that the first *bona fide* report of fish-pathogenic flavobacteria was by Bein (1954), who described *Fla. piscicida* as the causal agent of mass mortalities (referred to as "red tide") in marine fish from Florida. Incidence of the disease appeared to be associated with the proliferation of phytoplankton and may, consequently, be considered as

influenced by water quality. The name of *Fla. piscicida* does not appear in the "Approved Lists of Bacterial Names" (Skerman *et al.*, 1980) or their supplements, and is, therefore, of dubious taxonomic standing.

A filamentous, Gram-negative organism, isolated from gill lesions in rainbow trout and Yamame salmon, formed the basis of an article by Kimura *et al.* (1978a). These isolates from Japan, together with similar strains recovered from Oregon, were included in a detailed investigation by Wakabayashi *et al.* (1980). The outcome was recognition of a novel group of *Flavobacterium*, i.e. *Fla. branchiophila* (Wakabayashi *et al.*, 1989). This name has now been corrected to *Fla. branchiophilum* (von Graevenitz, 1990), and its authenticity verified (Bernardet *et al.*, 1996). *Fla. branchiophilum* appears to be spreading as a fish pathogen in the Far East, e.g. in Korea (Ko and Heo, 1997).

There have been other reports of "flavobacteriosis" caused by unknown species of *Flavobacterium* (e.g. Brisou *et al.*, 1964; Roberts, 1978; Acuigrup, 1980a; Farkas, 1985). Whereas there has been reticence to equate these organisms with existing nomenspecies (or to name new species), the descriptions of the aetiological agents have been quite reasonable. For example, Acuigrup (1980a) discussed flavobacteriosis among coho salmon held in Spanish seawater sites. This disease, which resembled a generalised septicaemia, caused 20–25% mortality in the fish population during the Summer of 1978. The subsequent description of the organism was superior to the initial work with *Fla. balustinum* or *Fla. piscicida.*

Cytophagas became implicated as fish pathogens with the work on coldwater (low-temperature) disease. The causal agent was initially isolated and described by Borg (1948), and subsequently named as *Cyt. psychrophila* (Borg, 1960), and then to *Fla. psychrophilum* (Bernardet *et al.*, 1996). Coldwater disease affects predominantly juvenile salmonid fish, notably coho salmon in the northwest U.S.A., and is most prevalent in winter and spring when the water temperature is $<10^{\circ}C$. Cranial and vertebral lesions may occur (Kent *et al.*, 1989). More recently, the organism has been associated with systemic disease in eels and cyprinids in Europe (Lehmann *et al.*, 1991) and with an anaemic condition of juvenile rainbow trout in Chile (Bustos *et al.*, 1995) and Europe (e.g. Lorenzen *et al.*, 1991), referred to as rainbow trout fry syndrome (RTFS) (Baudin-Laurençin *et al.*, 1989; Lorenzen *et al.*, 1991).

A causal mention was given initially to the role of *Cyt. johnsonae* and *Cyt. rosea* as fish pathogens (Christensen, 1977). *Cyt. johnsonae* has emerged as a problem in Australia. However, only scant information is available about *Cyt. rosea*; therefore, this organism will not be considered further.

An organism was recovered initially from the gills of diseased, hatchery-reared salmon, trout and suckers in Michigan (Strohl and Tait, 1978). Thirteen isolates were recovered, and although similarities were noted to organisms previously described by Borg (1960), Pacha and Porter (1968) and Anderson and Conroy (1969), it was decided to elevate them into a new species, as *Cyt. aquatilis* (Strohl and Tait, 1978), and thence to *Fla. hydatis* (Bernardet *et al.*, 1996). It must be emphasised that Strohl and Tait (1978) did not prove that the organisms were capable of causing disease. Nevertheless, we have recovered similar organisms from outbreaks of gill disease in farmed rainbow trout from England.

Cytophaga sp. has been associated with skin and muscle lesions on Atlantic salmon in the U.S.A. (Kent *et al.*, 1988), and a previously undescribed *Cytophaga*-like bacterium (CLB) has been associated with a gill and systemic disease in turbot (Mudarris and Austin, 1989). This organism was described as a new species, as *Fla. scophthalmum* (Mudarris *et al.*, 1994), which was re-classified to *Chrys. scophthalmum* (Vandamme *et al.*, 1994).

Little is known about the role of *Sporocytophaga* as a fish pathogen. Mixed infections attributed to *Sporocytophaga* and *V. anguillarum* occurred as surface lesions, termed saltwater columnaris, on salmon and trout held in marine conditions (Wood, 1968). However, apart from discussing the presence of microcysts which began to form at 2–7 days, there is little information about this suspected pathogen (Pacha and Ordal, 1970).

Chryseobacterium balustinum (= Flavobacterium balustinum)

The original description was extremely brief, referring to the growth of colonies at very low temperatures, i.e. 1–3°C, rod-shaped micromorphology, motility and the ability to degrade gelatin (Harrison and Sadler, 1929). The revised description of *Flavobacterium* excludes motile organisms (Holmes *et al.*, 1984); therefore, the validity of *Fla. balustinum* was doubtful. The organisms were re-classified to *Chryseobacterium*, as *Chrys. balustinum* on the basis of rRNA clustering (Vandamme *et al.*, 1994). Interestingly, the extensive list of characters in *Bergey's Manual of Systematic Bacteriology* (Holmes *et al.*, 1984) precluded reference to motility or, for that matter, gliding movement among representative strains of any species. Other characteristics of the taxon included:

Chryseobacterium balustinum

The presence of yellow-pigmented, mucoid colonies comprising non-motile, oxidative rods of 1.0–1.8 × 0.5 μm in size. Catalase, indole, oxidase and phosphatase, but not β-galactosidase, arginine dihydrolase, H_2S or phenylalanine deaminase, are produced. Nitrates are reduced. Aesculin, casein, DNA, gelatin, tributyrin, Tween 20 and 80, but not starch, tyrosine or urea, are degraded. Growth occurs at 37°C, but not at 5 or 42°C. This is in contrast to the original work of Harrison and Sadler (1929), who reported growth at almost the freezing point of water. Acid is produced from ethanol and glucose, but not from arabinose, cellobiose, lactose, mannitol, raffinose, salicin, sucrose or xylose. The G + C ratio of the DNA is 33.1 mol %.

Chryseobacterium scophthalmum (= Flavobacterium scophthalmum)

Chryseobacterium scophthalmum

Colonies (2–3 mm in diameter after 48 h at 25°C) contain orange-pigmented (flexirubin pigment), uniformly shaped, short, fermentative, Gram-negative rods of

approximately 2.0 × 0.8 μm in size. Gliding movement is exhibited. Catalase and oxidase are produced, but not H_2S, indole, or lysine or ornithine decarboxylase. Nitrates are not reduced. The Voges Proskauer reaction is negative. Aesculin, casein, DNA, gelatin, tributyrin and tyrosine are degraded, but not chitin or starch. Acid is produced from cellobiose and glucose, but not arabinose, mannitol, raffinose, sucrose or xylose. Thin sections reveal the presence of a thick (43.5 nm) cell envelop, which could be mistaken for an extracellular layer. The G + C ratio of the DNA is 31.7–34.2 mol % (Mudarris and Austin, 1989; Mudarris *et al.*, 1994).

From these characteristics, the 50 isolates from turbot were equated with a new species of *Flavobacterium*, as *Fla. scophthalmum* (Mudarris *et al.*, 1994). With subsequent improvements in taxonomy, the species was transferred to the newly established genus *Chryseobacterium*, as *Chrys. scophthalmum* (Vandamme *et al.*, 1994).

Flavobacterium sp.

The organisms, discussed by Acuigrup (1980a), were characterised as follows:

Flavobacterium sp.

The isolates produce yellow–orange colonies after 24 h (the temperature of incubation was not stated). Cultures comprise motile, pleomorphic, Gram-negative rods, which produce arginine dihydrolase and oxidase, but not β-galactosidase, H_2S, indole or lysine or ornithine decarboxylase, and are neither oxidative nor fermentative. The Voges Proskauer reaction is positive. Gelatin is degraded. Acid is produced from amygdalin, arabinose, inositol, mannitol, melibiose and rhamnose, but not from glucose or lactose. Sodium citrate is not utilised (Acuigrup, 1980a).

Most of the data originate from use of an unstated API product, possibly the API 20E rapid identification system. The presence of motility contrasts with the revised definition of *Flavobacterium* (Holmes *et al.*, 1984). Therefore, it appears that the organisms are not *bona fide Flavobacterium*, but probably represent a closely allied taxon.

Flavobacterium branchiophilum

Flavobacterium branchiophilum

This pathogen forms yellow, translucent, smooth colonies of 0.5–1.0 mm in diameter after incubation at 18°C for 5 days. Isolates (10 isolates from Oregon, 5 from Japan and 1 from Hungary; Farkas, 1985) comprise slender, strictly aerobic, non-motile, Gram-negative rods of 5–8 × 0.5 μm in size, which are surrounded by pili (Heo *et al.*, 1990). Usually, they occur as short chains, each comprising 2 to 3 cells. Catalase and oxidase are produced, but not H_2S or indole. Nitrates are not

reduced. Casein, gelatin, lecithin, starch, tributyrin, Tweens and tyrosine are degraded, but not cellulose or chitin (Bernardet *et al.*, 1996). Growth occurs at 5 and 30°C, but not at 37°C, and in 0 and 0.1% (w/v) sodium chloride. It is noteworthy that Japanese isolates grow at 30°C, whereas American strains do not. Acid is produced from a wide range of compounds, including cellobiose, fructose, glucose, inulin, maltose, melibiose, raffinose, sucrose and trehalose, but not from adonitol, arabinose, dulcitol, galactose, inositol, lactose, mannitol, salicin, sorbitol or xylose (Ko and Heo, 1997). The G+C ratio of the DNA is 29–34 mol %. Another geographical difference concerns serology, insofar as the Japanese isolates are antigenically distinct from the American and Hungarian cultures (Huh and Wakabayashi, 1989).

Flavobacterium columnare (= Flexibacter/Cytophaga columnaris)

Flavobacterium columnare

Colonies appear to be flat, spreading, yellowish-green (flexirubin-type pigment), with rhizoidal edges, and contain slender, strictly aerobic, Gram-negative rods of 4–8 × 0.5–0.7 μm in size, which move by gliding. Catalase, H_2S and oxidase are produced, but not indole, or lysine or ornithine decarboxylase. Nitrates are reduced. The methyl red test and Voges Proskauer reaction are negative. Casein, gelatin and tributyrin are degraded, but not aesculin, agar, cellulose, chitin, starch and tyrosine. Growth occurs at 4–30°C (some isolates grow also at 37°C), and in 0–0.5% (w/v), but not 1% (w/v), sodium chloride (Bernardet, 1989; Bernardet and Grimont, 1989a; Bernardet *et al.*, 1996). Dead bacterial cells, notably *Escherichia coli*, are lysed. Carbohydrates are generally not utilised. Moreover, acid is not produced from arabinose, cellobiose, glucose, lactose, mannitol, raffinose, salicin, sucrose or xylose. The G + C ratio of the DNA is 32.9–35.9 mol % (Wakabayashi and Egusa, 1966; Pacha and Porter, 1968; Pacha and Ordal, 1970; Chun, 1975; Bootsma and Clerx, 1976; Morrison *et al.*, 1981). Four major serological groups and one miscellaneous group have been recognised among the 325 strains examined by Anacker and Ordal (1959). Moreover, separate and distinct DNA homology groups have been described (Song *et al.*, 1988).

The taxonomic status of the pathogen has undergone radical change since the pioneering work of Davis (1922). Davis coined the name *Bacillus columnaris* because, in wet preparations of infected material, the bacteria congregated in column-like masses. According to Ordal and Rucker (1944), pure cultures of the organism exhibited gliding, and therefore should be associated with the myxobacteria. The taxonomy became further complicated by the observation in cultures of oval and spherical structures, which were thought to be microcysts (this notion was subsequently rejected). This led to a re-classification, as *Chondrococcus columnaris*. Shortly afterwards, Garnjobst (1945) suggested that the pathogen was actually a *Cytophaga*, and thus the transition was made to *Cytophaga columnaris*. With further deliberation,

the organism was transferred to *Flexibacter*, as *Flexibacter columnaris* (Leadbetter, 1974), and from phylogenetic data as *Fla. columnare* (Bernardet *et al.*, 1996).

The intraspecific diversity of 10 isolates was examined by means of 16S rRNA gene sequencing and RFLP, and three genomic groups recognised of which most cultures were recovered in Group 1. Interestingly, the 3 isolates in Group 3 contained a ~370 bp fragment that was absent from the other cultures (Schneck and Caslake, 2006). Arias *et al.* (2004) evaluated 30 fish isolates and reference cultures by 16S rDNA sequencing, AFLP and intergenic spacer region sequencing, and defined two predominating genomovars (I and II) although the intergenic spacer region (ISR) sequencing revealed a higher diversity among genomovar I representatives, and by AFLP 22 profiles were recognised. Of interest, 4 isolates from tilapia in Brazil were recovered in a separate group, albeit related to genomovar I and II. Using a similar approach and 30 Finnish isolates plus the type strain, Suomalainen *et al.* (2006a) reported the outcome was that the isolates were recovered in a genomovar although 8 ARISA profiles were defined of which three were similar. Overall, these genetic approaches should have value in epizootiological studies.

Flavobacterium hydatis (= Cytophaga aquatilis)

Flavobacterium hydatis (= Cytophaga aquatilis)

Following incubation at 20°C for 14 days, yellow–orange colonies develop, which contain Gram-negative, facultatively anaerobic rods of 8.0×0.5 µm in size. Copious quantities of extracellular slime are produced. Cells demonstrate gliding movement. Microcysts and fruiting bodies are absent. Catalase is produced, but not H_2S, indole, lysine or ornithine decarboxylase, oxidase or phenylalanine deaminase, and the methyl red test and Voges Proskauer reaction are negative. A wide range of complex molecules are degraded, including aesculin, blood, casein, DNA, gelatin, pectin, starch, tributyrin, Tween 40, 60 and 80, and tyrosine (slowly), but not cellulose or urea. Some strains attack chitin. Growth occurs at 5 to 35°C, but not 42°C, in 0–2% (w/v) sodium chloride, which is an indication that the organism is unlikely to be present in full-strength seawater. Growth occurs also at pH 5.5–11.0. Nitrates are reduced to ammonia. Acid is produced from arabinose, cellobiose, glucose, lactose, mannitol, raffinose, sucrose and xylose. The G + C content of the DNA is 33.7 mol %. Chemical analyses have established that the major pigments are similar to the "flexirubin" of *Fle. elegans* (Strohl and Tait, 1978).

On the basis of the micromorphology, gliding movement, G + C ratio and the ability to degrade complex molecules, this group was considered to represent a previously undescribed species of *Cytophaga*, for which the name of *Cyt. aquatilis* was proposed (Strohl and Tait, 1978). However, on the basis of phylogenetics, the species was transferred to *Flavobacterium*, as *Fla. hydatis* (Bernardet *et al.*, 1996).

Flavobacterium johnsoniae (= Cytophaga johnsonae)

Fla. johnsoniae was identified by an examination of phenotypic tests, and associated with mortalities in salmonids (Rintamäki-Kinnunen *et al.*, 1997). The characteristics for the fish isolates were as follows:

Flavobacterium johnsoniae

Colonies are yellow (flexirubin pigment) and contain Gram-negative, gliding filaments. Catalase, β-galactosidase and oxidase are produced, but not H_2S or indole. Nitrates are reduced. Acid is produced from glucose. Growth occurs at 10–30°C, but not at 37°C, and in 0.5–1.0% (w/v) sodium chloride. Casein, chitin, DNA, gelatin, starch, tributyrin, Tween 20, Tween 40 and tyrosine (with pigment production) are degraded, but not Tween 80. Ammonium, asparagine, glutamate, potassium nitrate and urea are utilised as sole sources of nitrogen (Carson *et al.*, 1993).

Flavobacterium psychrophilum (= Cytophaga psychrophila = Flexibacter psychrophilum)

The best of the descriptions of this organism stems from the work of Pacha (1968), Bernardet and Kerouault (1989), Bernardet and Grimont (1989) and Schmidtke and Carson (1995). Opinion is that this pathogen comprises a phenotypically homogeneous group (Madsen and Dalsgaard, 2000; Madetoja *et al.*, 2001). Plasmid profiling was carried out to evaluate genetic variability among 104 isolates, most of which were recovered from Japan. Most (72/104 = 69%) possessed plasmids of 2.8, 3.4, 4.1 and 5.6 kb. The 3.4 kb plasmid was mostly recovered from cultures obtained from rainbow trout; the 4.1 and 5.6 kb plasmids were from Japanese isolates (Izumi and Aranashi, 2004).

Flavobacterium psychrophilum

Cultures produce non-diffusible, yellow-pigmented (flexirubin pigment) colonies with thin, spreading margins. Cells are strictly aerobic, Gram-negative, slender, flexible rods of 1.5–7.5 × 0.75 μm in size. With increasing age, the cells appear to be shorter. As with *Fla. hydatis*, gliding movement is exhibited, and fruiting bodies and microcysts are absent. Catalase is produced, oxidase may appear to be positive, but H_2S, indole, and lysine and ornithine decarboxylase are not produced. Nitrates are not reduced, and the Voges Proskauer reaction is negative. Casein, gelatin and tributyrin are degraded; tyrosine is attacked by some isolates, and aesculin, chitin, starch and xanthine not at all. Generally, growth occurs at 4–23°C, but not at 30°C, and in 0.8%, but not 2% (w/v), sodium chloride. This demonstrates clearly that the organisms are suited to low-temperature, freshwater environments. The organisms are capable of degrading autoclaved cells of *Esch. coli* (Pacha, 1968). The G + C ratio of the DNA is 32.5–34 mol %. The original 10

isolates of Pacha (1968) were deemed to be serologically homogeneous. Yet, later work identified two major serogroups, with O1 accommodating isolates from Japan and the U.S.A. and O2 comprising only Japanese cultures (Wakabayashi *et al.*, 1994). This number increased to three, with European isolates included in one major serogroup (Lorenzen and Olesen, 1997). Seven antigenic types have been recognised among Finnish isolates, with results suggesting a new serogroup (Madetoja *et al.*, 2001). Furthermore, a novel serotype has been described for cultures recovered from amago in Japan (Izumi *et al.*, 2003). Several ribotypes have been recognised among 85 isolates, with most harbouring one or more plasmids (11 plasmid profiles defined) (Chakroun *et al.*, 1998). In comparison, 13 ribotypes were recognised among Finnish cultures (Madetoja *et al.*, 2001). PCR and RFLP led to the recognition of genetic heterogeneity among 242 isolates (Izumi *et al.*, 2003a).

On the basis of the phenotypic characteristics, the conclusion was reached that the causal agent of coldwater disease could be equated with the genus *Cytophaga*, as defined by Stanier (1942), but was sufficiently distinct from existing species to warrant description as a new species. Hence, *Cyt. psychrophila* was named (Borg, 1960). However, Lewin and Lounsberry (cited in Leadbetter, 1974) disagreed with this notion, and proposed that the organisms would be better classified in the genus *Flexibacter*, as *Fle. aurantiacus*. Bernardet and Grimont (1989) agreed with the opinion that the organisms belong in the genus *Flexibacter*, but proposed *Fle. psychrophilum*. Then, with general improvements in the understanding of the yellow-pigmented bacteria resulting from phylogenetic data, the taxon was transferred to *Flavobacterium*, as *Fla. psychrophilum* (Bernardet *et al.*, 1996). It is agreed that the taxon comprises a homogeneous group of bacteria (Lorenzen *et al.*, 1997).

Flavobacterium piscicida

Only a poor description of *Fla. piscicida* exists in the fisheries literature. Therefore, further discussion will not be attempted.

Flexibacter spp.

The fish pathology literature abounds with references to unspeciated *Flexibacter*. Pyle and Shotts (1980, 1981) studied 17 strains for phenotypic traits, as determined by use of the API 20E rapid identification system, and by DNA homology. The conclusion reached was that the isolates from warm-water fish were distinct to those recovered from cold-water fish. At least three separate groups were recognised. However, whether or not these organisms belong in existing species or represent new taxa must await further study.

Tenacibaculum maritimum (= Flexibacter maritimus = Cytophaga marina)

Tenacibaculum maritimum

A very homogeneous group (Bernardet *et al.*, 1994), with cultures forming pale yellow, flat, thin colonies, containing strictly aerobic, Gram-negative rods of 2–30 × 0.5 μm in size, which occasionally form filaments of up to 100 μm in length. In older cultures, cells become spherical (~0.5 μm in diameter). Microcysts do not occur. Gliding movement is a characteristic feature of all isolates. Catalase and oxidase are produced, but not H_2S or indole. Nitrates are not reduced. The methyl red test is negative. Casein, gelatin, tributyrin and tyrosine are degraded, but not aesculin, agar, cellulose, chitin, starch or urea. Growth occurs from 14.6–34.3°C, and at pH 6–9. There are requirements for potassium chloride and sodium chloride. Acid is not produced from arabinose, cellobiose, glucose, lactose, mannitol, raffinose, salicin, sucrose or xylose. Isolates are capable of lysing dead cells of *Aer. hydrophila*, *Edw. tarda*, *Esch. coli* and *V. anguillarum*, but not *Bacillus subtilis*. The G + C ratio of the DNA is 31.3–32.5 mol % (Hikida *et al.*, 1979; Wakabayashi *et al.*, 1986). All isolates share a common antigen (Wakabayashi *et al.*, 1984).

From these traits, Hikida *et al.* (1979) proposed that the organism belonged in an as yet undescribed species of *Flexibacter*, for which the name of *Fle. marinus* was mooted. Subsequently, Wakabayashi *et al.* (1986) formally proposed the name of *Fle. maritimus* to accommodate the pathogen. Independently, Reichenbach (1989) proposed the name of *Cyt. marina* for the same organism. However, the organism was subsequently reclassified in a newly described genus *Tenacibaculum*, as *T. maritimum* (Suzuki *et al.*, 2001). The intraspecific diversity of 29 fresh isolates and 3 reference cultures has been addressed using RAPDs with the outcome that two principle (and distinct serological) groups were recognised, of which one contained all the cultures from sole and gilthead sea bream, and the second group comprised isolates from Atlantic salmon, turbot and yellowtail. Interestingly, the reference strains were not recovered with the fresh isolates, and may reflect the problem of using archived cultures which have been long removed from their natural habitat (Avendaño-Herrera *et al.*, 2004b). A further publication by this group (Avendaño-Herrera *et al.*, 2004c, 2005a) defined three serotypes, i.e. O1, O2 and O3, which were regarded as host-specific, with serotype O1, O2 and O3 comprising isolates from sole and gilthead sea bream, turbot and sole, respectively.

Tenacibaculum ovolyticum (= Flexibacter ovolyticus)

The 35 isolates were described as comprising:

Tenacibaculum ovolyticum

Pale yellow-pigmented, long, slender (0.4 × 2–20 μm), Gram-negative rods, which demonstrate gliding movement. Catalase and oxidase are produced, but not so

arginine dihydrolase, β-galactosidase, H_2S, indole or lysine or ornithine decarboxylase. Nitrates are reduced. DNA, gelatin, Tween 80 and tyrosine, but not agar, cellulose, chitin, starch or urea, are degraded. Acid is not produced aerobically from arabinose, cellobiose, glucose, lactose, mannitol, raffinose, salicin, sucrose or xylose. Growth occurs at 4–30°C, but not 35°C, and in >3% (w/v) sodium chloride. The G+C ratio of the DNA was reported as 31.0 mol % (Hansen *et al.*, 1992).

On the basis of DNA:DNA hybridisation, there is 26–42% homology with *T. maritimum*. Certainly, the strains are distinct from *T. maritimum*, but it would be relevant to enquire about possible relationships with other cytophagas–flexibacteria.

Sporocytophaga spp.

Negligible information is available about these Gram-negative, microcyst-forming organisms (see Pacha and Ordal, 1970).

Francisellaceae representative

Francisella sp.

Francisella sp.

Cultures initially developed small, opaque colonies on cysteine heart agar at 22°C, but not at 37°C. These colonies comprised non-motile, small (variable sizes), Gram-negative cysteine requiring strictly aerobic, catalase-negative (or weakly positive), oxidase-negative, non-haemolytic, facultatively intracellular coccobacilli that were examined by 16S rRNA sequencing for which the nearest match was *Francisella philomiragia* (99% identity) and several apparent *Francisella* from tilapia in Taiwan and three-lined grunt in Japan (Olsen *et al.*, 2006). It was considered that the isolate from Atlantic cod was more fastidious that *Francisella philomiragia* (Olsen *et al.*, 2006).

Separately, intracellular bacteria were found in the kidney and spleen of three-line grunt from which DNA was extracted and small-subunit rRNA amplified by PCR, and sequenced. The outcome was 97.3–98.5% homology to *Francisella*, with *Francisella philomiragia* as the closest neighbour (Kamaishi *et al.*, 2005). Originally, *Francisella philomiragia* was classified in *Yersinia* as *Y. philomiragia*, which comprised bacteria first recovered from dying muskrat in Utah (Jensen *et al.*, 1969). The link to *Yersinia* resulted from the micro-morphology of the cells and supposed DNA relatedness to *Y. pestis* (Ritter and Gerloff, 1966). However, subsequent evaluation led to a transfer to *Francisella* (Hollis *et al.*, 1989).

Halomonadaceae representative

Halomonas (= Deleya) cupida

Homogenates of black sea bream revealed the presence of *Halomonas (= Deleya) cupida (= Alcaligenes cupidus)*, *V. alginolyticus* and *V. nereis* (Kusuda *et al.*, 1986).

H. cupida

Cultures comprise motile, Gram-negative, salt-requiring rods, which grow at 10–25°C, but not at 4°C and 30°C, and are unreactive in the oxidative–fermentative test. Arginine dihydrolase, catalase, and lysine and ornithine decarboxylase are produced, but not H_2S, indole or oxidase. Nitrates are reduced, but the methyl red test and Voges Proskauer reaction are negative. Haemolysis is recorded to eel erythrocytes, but not those of sheep or yellowtail. Sensitivity is recorded to the vibriostatic agent, O/129. Acid is produced from adonitol, galactose, lactose, maltose, mannitol, L-rhamnose, D-sorbitol, salicin, sucrose (weak) and trehalose, but not from fructose or inulin.

From these characteristics, a close relationship to *Alcaligenes cupidus* was noted (see Kersters and De Ley, 1984). The only discrepancy concerned acid production from fructose. However, this taxon was re-classified as *Deleya cupida* (Baumann *et al.*, 1983), and by inference to *Halomonas cupida* on the basis of 16S rRNA sequencing (Dobson and Franzmann, 1996).

Moraxellaceae representatives

Acinetobacter sp.

A bacterium, resembling *Acinetobacter*, was recovered from sexually mature Atlantic salmon, comprising wild stock from the River Surma, Norway (Roald and Hastein, 1980).

Acinetobacter sp.

Cultures comprise round, raised, translucent, mucoid colonies of 1.5 mm in diameter within 48 h incubation at 22°C. Colonies contain fairly unreactive, short, plump, facultatively anaerobic, non-motile, Gram-negative rods of 1.6–1.8 × 0.8–1.2 μm in size. Catalase and oxidase are produced, but not arginine dihydrolase, β-galactosidase, H_2S, indole, lysine or ornithine decarboxylase or tryptophan deaminase. Blood (haemolysis) is degraded, but not gelatin or urea. Nitrates are not reduced. The methyl red test and Voges Proskauer reaction are negative. Sodium citrate is not utilised. Acid is produced from galactose, maltose and mannose, but not from adonitol, amygdalin, arabinose, cellobiose, glucose, inositol, lactose, mannitol, melibiose, raffinose, rhamnose, saccharose, salicin, sorbitol or xylose. Unfortunately, the G + C ratio of the DNA has not been determined.

From the available information, Roald and Hastein (1980) considered that the pathogen corresponded to an as yet unnamed species of *Acinetobacter*. Although this appears to be a sound decision, *bona fide Acinetobacter* spp. should not produce oxidase (Juni, 2005). In fact, this ability belongs to the morphologically similar *Moraxella* (Juni and Bøvre, 2005) and *Neisseria* (Vedros, 1984). There is some degree of resemblance between the fish pathogen and *Mor. atlantae* and *Mor. osloensis*, although one of the general traits of moraxellae is an inability to produce acid from carbohydrates. Thus, as a compromise solution it would appear that the fish pathogen should be classified in the area loosely bounded by *Acinetobacter*, *Moraxella* and *Neisseria*.

Moraxella sp.

A bacterium was recovered as pure culture growth from internal organs of juvenile striped bass, and equated with *Moraxella* (Baya *et al.*, 1990b).

Moraxella sp.

Cultures comprise non-motile, short (0.8–1.0 × 1.3 μm in size), non-fermentative, paired rods with pronounced bipolar staining. Catalase and oxidase are produced, but not so arginine dihydrolase, β-galactosidase, H_2S, indole or lysine or ornithine decarboxylase. Sheep's blood is degraded, but not gelatin or urea. Citrate utilisation, nitrate reduction and the Voges Proskauer reaction are negative. Acid is produced weakly from galactose and mannose, but not from amygdalin, arabinose, glucose, inositol, lactose, maltose, mannitol, rhamnose, salicin, sorbitol or sucrose.

Whereas similarities to *Moraxella* were noted (Juni and Bøvre, 2005), the organism strongly resembled the *Acinetobacter* described by Roald and Hastein (1980). Indeed, the major differences concerned acid production from maltose. Clearly, the precise taxonomic position of both organisms must await further study.

Moritellaceae representatives

Moritella marina (= V. marinus)

Nineteen Icelandic and one Norwegian isolate from shallow skin lesions on Atlantic salmon, and the type strain of *V. marinus* NCIMB 1144 were identified as *V. marinus* after an examination of phenotypic data and analyses by numerical taxonomy (Benediktsdóttir *et al.*, 1998). On the basis of 16S rRNA sequencing the taxon was transferred to a newly established genus, as *Moritella marina* (Urakawa *et al.*, 1998). However, apart from emphasising a relationship to *Shewanella*, the authors did not make any comment about family membership.

Moritella marina

Cultures produce lysine decarboxylase and oxidase, but not arginine dihydrolase or ornithine decarboxylase, reduce nitrates, are positive for the methyl red test but not the Voges Proskauer test, degrade blood (β-haemolysis), chitin, DNA, gelatin, lipids and starch, do not produce acid from carbohydrates except *N*-acetyl glucosamine, maltose, mannitol, mannose and ribose, is resistant to the vibriostatic agent, O/129, and grow at 4–20°C, but not at 25°C.

Moritella viscosa (= V. viscosus)

Two groups of psychrotrophic bacteria were recovered from Atlantic salmon with so-called winter ulcer disease/syndrome (Lunder *et al.*, 2000). One homogeneous group was determined to be closest to *Moritella marina* (43% re-association by DNA : DNA hybridisation), and hence was named as *V. viscosus*. However, by 16S rDNA sequencing, the closest match was *Moritella* HAR 08 and HAR 013 (Lunder *et al.*, 2000) and *Moritella marina* (99.1% sequence homology). Hence, the organism was re-classified to *Moritella*, as *Moritella viscosa* (Benediktsdóttir *et al.*, 2000).

Moritella viscosa

Colonies on bovine blood agar containing 2% (w/v) NaCl of up to 1 mm in diameter after 24 h at 15°C or 22°C are described as viscous (and adhere to the medium), translucent and grey in colour that contain motile, fermentative, Gram-negative rods that produce alkaline phosphatase, caprylate esterase, catalase, lysine decarboxylase and oxidase, but not arginine dihydrolase, chemotrypsinase, α-fucosidase, indole, α- or β-galactosidase, α-mannosidase or ornithine decarboxylase, degrade bovine blood (β-haemolysis), casein, DNA, gelatin, lecithin, starch, Tween 80 and urea, but not aesculin, alginate, produce acid from galactose and glucose, but not L-arabinose, cellobiose, glycerol, inositol, lactose, mannitol, mannose, melibiose, raffinose, L-rhamnose or xylose, grow at 15°C (and also survive freezing) in 1–4% (w/v) NaCl, are sensitive to the vibriostatic agent, O/129, and are negative for the methyl red test and Voges Proskauer reaction. All isolates harbour one or more plasmids. The G + C ratio of the DNA is 42.5 mol % (Lunder *et al.*, 2000).

Mycoplasmataceae representative

Mycoplasma mobile

Cell wall defective/deficient bacteria, i.e. L-forms and mycoplasmas, have been associated with fish diseases. Since the initial recovery of a motile mycoplasma from fish (Kirchhoff and Rosengarten, 1984), a new species, i.e. *Mycoplasma mobile*, has been described (Kirchhoff *et al.*, 1987). In addition, there is an increasing awareness

of L-forms in fish diseases. To date, L-forms have been described for *Aer. salmonicida* and *Y. ruckeri* (McIntosh and Austin, 1990a).

Mycoplasma mobile

Cultures produce "fried-egg" colonies of 10–500 μm diameter on Hayflick medium, which contains horse or bovine serum, after incubation at an unstated temperature for 2–6 days. Colonies contain filterable (through the pores of 0.45 μm pore size filters), Gram-negative, conical or flask-shaped, wall-less cells with distinctive terminal structures. The cells demonstrate marked ability to adhere to and glide on glass, plastic, erythrocytes and tissue culture cells. Growth occurs at 4–30°C, but not at 37°C. Catalase (weak), oxidase (weak) and phosphatase (weak) are produced. Blood is degraded, but not arginine, casein, gelatin or urea. Acid is produced fermentatively from arabinose, fructose, galactose, glucose, lactose, maltose and mannose. 2,3,5-Triphenyltetrazolium chloride and potassium tellurite (weakly) are reduced, but not methylene blue. Gluconate is not oxidised, nor is phenylalanine deaminated. The G + C ratio of the DNA is 22.4–24.6 mol % (Kirchhoff *et al.*, 1987).

Serologically, the tench isolate was distinct from all other validly described species of *Acholeplasma* and *Mycoplasma*. From the traits listed above it was deemed that the organism formed a new species, for which the name of *Mycoplasma mobile* was coined (Kirchhoff *et al.*, 1987).

Myxococcaceae representative

Myxococcus piscicola

There has been one report of gill disease (in 1972) caused by a supposed new species of the fruiting organism *Myxococcus*, for which the name of *Myxococcus piscicola* was suggested (Xu, 1975). This organism was associated with an epizootic in grass carp (*Ctenopharyngodon idelluls*) fingerlings held in ponds in Wuhan, China. Conical fruiting bodies were produced on agar. These fruiting bodies were surrounded by a thin membrane, without peduncle or branches (Xu, 1975).

Oxalobacteraceae representative

Janthinobacterium lividum

Pure cultures have been recovered and characterised:

Janthinobacterium lividum

Cultures comprise purple-pigmented, motile, Gram-negative, strictly aerobic rods, which produce arginine dihydrolase, catalase and oxidase, but not β-galactosidase,

indole or tryptophan deaminase. Nitrates are reduced, and the Voges Proskauer reaction is positive. Gelatin, but not urea, is degraded. Growth occurs at 4–30°C but not 37°C, and in 0–2%, but not 3% (w/v), sodium chloride. Caprate, citrate, malate, maltose, mannitol, mannose and phenylacetate are utilised, but not *N*-acetyl-glucosamine or adipate.

From these traits, the organisms were identified as typical (Table 4.3; from Northern Ireland) and atypical (Table 4.3; from Scotland) *Janthinobacterium lividum* (Sneath, 1984; Logan, 1989).

Pasteurellaceae representative

Pasteurella skyensis

Four isolates were obtained from diseased Atlantic salmon (Birkbeck *et al.*, 2002).

Pasteurella skyensis

Cultures on blood-containing medium comprise non-motile, facultatively anaerobic, pleomorphic, Gram-negative, catalase-negative rods that produce esterase (lipase), indole, leucine arylamidase, lysine and ornithine decarboxylase, oxidase (weakly), acid and alkaline phosphatase and naphthol-ASBI-phosphohydrolase, but do not produce arginine dihydrolase, β-galactosidase, urease, the Voges Proskauer reaction or reduce nitrate. Acid is produced from glucose, lactose, maltose, mannitol, mannose and trehalose, but not from adonitol, arbutin, dulcitol, galactose, inositol, inulin, melibiose, raffinose, rhamnose, salicin, sucrose, sorbitol or xylose. Haemolytic activity is at best weak. There is a requirement for blood and 1.5% (w/v) for growth. Growth occurs at 14–32°C, but not at 37°C. The G + C ratio of the DNA is 39–41 mol % (Birkbeck *et al.*, 2002).

The phenotypic tests were used to link the pathogen to the Pasteurellaceae, and the results of 16S rRNA sequencing confirmed the association to the family with *Pa. phocoenarum* (97.1% homology) being regarded as the closest neighbour phylogenetically.

Photobacteriaceae representatives

Photobacterium damselae subsp. *damselae*

The validity and distinctiveness of *Ph. damselae* has been confirmed, with isolates homogeneous by BIOLOG-GN fingerprints, API 20E profiles and LPS profiles, but heterogeneous by ribotyping and serology (four serogroups were defined) (Austin *et al.*, 1997).

Table 4.3. Differential characteristics of *J. lividum* recovered from moribund and dead rainbow trout fry. A comparison has been made with taxa which accommodate purple-pigmented bacteria[a]

Character	*Chromobacterium violaceum*[b]	*Iodobacter fluviatile*[b]	*J. lividum*[b]	Atypical *J. lividum*[b]	Isolates from Ireland	Isolates from Scotland
Gelatinous colonies	−	−	V	+	+	+
Oxidative (O)–fermentative (F) metabolism of glucose	F	F	O	O	O	O
Growth at:						
4°C	−	+	+	+	+	+
37°C	+	−	−	−	−	−
Degradation of:						
Aesculin	−	−	+	−	+	−
Arginine	+	−	−	−	−	−
Production of acid from:						
L-arabinose	−	−	+	+	+	+
Gluconate	+	+	−	−	+	+
Glycerol	+	−	+	−	+	−

V = Variable result

[a] From Austin *et al.* (1992b)

[b] From Logan (1989)

Photobacterium damselae subsp. *damselae*

Cultures comprise facultatively anaerobic, Gram-negative, weakly motile (by one or more unsheathed polar flagella) rods. Arginine dihydrolase, catalase and oxidase are produced, but not β-galactosidase, H_2S, indole, lysine or ornithine decarboxylase or phenylalanine deaminase. Chitin, DNA, starch and urea, but not corn oil (lipids) or gelatin, are degraded. The methyl red test and Voges Proskauer reaction are positive. Nitrates are reduced. Growth occurs in 1–6% (w/v), but not 0% or 7% (w/v), sodium chloride. Acid is produced from D-glucose, maltose and mannose, but not D-adonitol, arabinose, cellobiose, dulcitol, erythritol, inositol, lactose, mannitol, melibiose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylose. Acetate, citrate and malonate are not utilised. Sensitivity is recorded to the vibriostatic agent, O/129. The G + C ratio (for one strain) is 43 mol %.

Although Love *et al.* (1981) did not publish detailed reasons for the dissimilarity of "*V. damsela*" to other species of *Vibrio*, they did mention that DNA:DNA hybridisation studies had been completed. Unfortunately, the results were not published. This situation was corrected by Grimes *et al.* (1984b), who demonstrated low DNA homology values with other vibrios. Therefore, it is not surprising that the pathogen was re-classified out from *Vibrio*, initially to *Listonella* (MacDonell and Colwell, 1986), then to *Photobacterium*, as *Photobacterium damsela* (Smith *et al.*, 1991) and finally corrected to *Ph. damselae* (Trüper and De'Clari, 1997).

Photobacterium damselae subsp. *piscicida* (= *Pasteurella piscicida*)

During the summer of 1963, an epizootic was reported in white perch and striped bass in the upper region of the Chesapeake Bay, Virginia. From the diseased fish, 30 cultures of an organism were recovered which possessed some of the salient features of *Pasteurella* (Snieszko *et al.*, 1964a). Hence, the condition was termed "pasteurellosis". However, the literature became confusing, insofar as the disease is also referred to in Japan as "pseudotuberculosis" because of the distinctive pathology. There are also some reports indicating the presence of fish-pathogenic *Pasteurella* in Great Britain (Ajmal and Hobbs, 1967) and Norway (Håstein and Bullock, 1976). However, it is possible that these organisms should have been identified as atypical *Aer. salmonicida* (see Paterson *et al.*, 1980). The results of many investigations led to the conclusion that the pathogen consists of a phenotypically and serologically homogeneous taxon (e.g. Magariños *et al.*, 1992), but genetically heterogeneous, as determined by results of subtractive hybridisation (Juíz-Río *et al.*, 2005). By ribotyping of 29 isolates, 2 major ribotypes were recognised which effectively separated European and Japanese isolates. A third ribotype accommodated an unique strain (Magariños *et al.*, 1997b).

Photobacterium damselae subsp. *piscicida*

Cultures comprise fairly unreactive, Gram-negative, non-motile, fermentative rods of 0.5 × 1.5 μm in size, with pronounced bipolar staining. Pleomorphism may be evident, especially in older cultures. Catalase and oxidase are produced, but not alanine deaminase, β-galactosidase, H_2S, indole, lysine or ornithine decarboxylase or phenylalanine deaminase. Nitrates are not reduced. The methyl red test is strongly positive, whereas the Voges Proskauer reaction is weakly positive. Arginine and Tween 80 are degraded, but not blood, casein, chitin, gelatin, starch or urea. Growth occurs at 25–30°C, but not 10 or 37°C, in 0.5–3.0% (w/v) sodium chloride and at pH 5.5–8.0, but not on MacConkey agar or in potassium cyanide broth. Uniform turbidity is recorded in broth cultures. Sodium citrate is not utilised. Acid is produced from fructose, galactose, glucose (weak) and mannose, but not amygdalin, arabinose, dulcitol, inositol, lactose, maltose, mannitol, melibiose, rhamnose, salicin, sorbitol, sucrose or trehalose. Unfortunately, the G + C ratio of the DNA has not been determined for any *bona fide* strains. So far, only one serotype has been recognised. In general, the organism possesses one heat-stable and four heat-labile somatic antigens, and three heat-labile extracellular antigens (presumably enzymes) (Kusuda *et al.*, 1978a). The LPS has been found to comprise <1% protein, 18–24% sugar and 34–36% fatty acids. The sugar component includes hexose, heptose, pentose, 6-deoxyhexose, 2-keto-3-deoxyoctonate and hexosamine. The fatty acids include lauric acid, 3-hydroxy lauric acid, myristic acid and palmitic acid (Salati *et al.*, 1989a, b; Hawke *et al.*, 2003).

The morphology and physiology of this pathogen led Snieszko *et al.* (1964a) to suspect a similarity to the genus *Pasteurella*. This view was reinforced by cross-precipitin reactions with *Pasteurella (Yersinia) pestis*. From this deduction, Janssen and Surgalla (1968) realised that, from an examination of 27 isolates, the organism was very homogeneous and different from existing species of *Pasteurella*. Therefore, the name of *Pa. piscicida* was coined. Independently, Kusuda gave it an alternative name, i.e. *Pa. seriola*, but quickly realised its synonymy with *Pa. piscicida*, which was accorded preference. However, *Pa. piscicida* was not included in the "Approved Lists of Bacterial Names" (Skerman *et al.*, 1980) or their supplements. Consequently, the name of *Pa. piscicida* lacked taxonomic validity.

A detailed taxonomic evaluation based on small-subunit rRNA sequencing and DNA:DNA hybridisation revealed that the organism was highly related to *Ph. damsela* (there was >80% relatedness of the DNA), and it was proposed that the organism be accommodated in a new subspecies, as *Ph. damsela* subsp. *piscicida* (Gauthier *et al.*, 1995), the epithet of which was corrected to *damselae* (Trüper and De'Clari, 1997), as *Ph. damselae* subsp. *piscicida*. AFLP analysis revealed that the two subspecies are indeed distinct and separate entities (Thyssen *et al.*, 2000).

To complicate matters, there is controversy over interpretation of the Gram-staining reaction. The majority opinion is that the organism is Gram-negative. However, Simidu and Egusa (1972) considered that cells displayed Gram-variability

when young, i.e. in 12–18 h cultures incubated at 20–25°C. In addition, they presented photographic evidence which showed that cells shortened with age. In fact, the suggestion was made that the pathogen is related to *Arthrobacter*. It is ironic that a similar phenomenon, concerning the interpretation of Gram-stained smears, was reported by Kilian (1976) and Broom and Sneath (1981) for *Haemophilus piscium*, the causal agent of ulcer disease.

Piscirickettsiaceae representative

Piscirickettsia salmonis

Degenerate or obligately parasitic bacteria, i.e. chlamydias and rickettsias, have been long established as pathogens of invertebrates, and sporadically mentioned in connection with fish diseases (Wolf, 1981). Yet, firm evidence of their role in fish pathology has not been forthcoming until an upsurge of interest in Chile. Thus, since 1989 a disease coined "coho salmon syndrome", Huito disease (Schäfer *et al.*, 1990) or salmonid rickettsial septicaemia (Cvitanich *et al.*, 1991) has been observed in coho salmon, chinook salmon, Atlantic salmon and rainbow trout, with a spread to Atlantic salmon in Norway (Olsen *et al.*, 1997) and white sea bass in California (Arkush *et al.*, 2005). Losses fluctuated between 3–7% of stock per week, the cumulative mortalities reaching 90%. The organism was formally recognised as a new taxon, for which the name of *Piscirickettsia salmonis* was proposed (Fryer *et al.*, 1992). The problem of purifying the bacteria from tissue culture cells was addressed by use of 30% percoll in which bacteriophage-like particles were observed by TEM (Yuksel *et al.*, 2001) and resolved by use of iodixanol (= Optiprep) as substrate for differential centrifugation gradients which together with DNase1 digestion led to sufficiently pure, i.e. 99%, bacteria for DNA work (Henríquez *et al.*, 2003). Analysis of 16S rRNA revealed that Irish isolates formed two groupings whereas Canadian, Norwegian and Scottish cultures clustered together (Reid *et al.*, 2004). The possibility of genetic differences between isolates was examined with a view to explaining reasons for differences in virulence and mortality rates. By electrophoretic analysis of the internal transcribed spacer region of 11 Chilean isolates, two groupings were recognised (Casanova *et al.*, 2003).

Piscirickettsia salmonis

The pathogen is a pleomorphic, non-motile, Gram-negative, predominantly coccoid (and ring forms) organism of variable size (0.5×1.5–$2.0\ \mu m$), occurring intracellularly as individuals, pairs or groups. Electron microscopy reveals that each organism is bound by two membrane layers; a characteristic trait of the Rickettsiales, and possibly the tribe Erlichiae.

A single isolate, designated LF-89, was studied in detail by Fryer *et al.* (1992). The 16S rRNA conformed to the gamma subdivision of the Proteobacteria. Moreover, LF-89 did not show any specific relationship to any of 450 bacterial 16S rRNA

sequences held on file. Nevertheless, similarities were apparent with *Wolbachia persica* (similarity = 86.3%) and *Coxiella burnetii* (similarity = 87.5%) than to representatives of *Ehrlichia*, *Rickettsia* or *Rochalimaea*. In short, it was deemed that the salmonid pathogen was sufficiently novel to warrant description in a new genus of the family Rickettsiaceae. The organism recovered from white sea bass was reported to have a 96.3–98.7% 16S rDNA homology with *Pis. salmonis* (Arkush *et al.*, 2005), which is low for a confirmed identity.

Rickettsia-like organisms

An increasing number of publications have described rickettsia-like organisms (RLO) as causal agents of disease (e.g. Rodger and Drinan, 1993; Chen *et al.*, 1994; Khoo *et al.*, 1995; Palmer *et al.*, 1997; Jones *et al.*, 1998; Corbeil *et al.*, 2005). Whether or not these organisms correspond with *Piscirickettsia salmonis* has not been always established. For example, an RLO was reported as causing disease in tilapia from Taiwan during October 1992 to February 1993 (Chern and Chao, 1994). The pathogen was described as a Gram-negative rod of $0.86 \pm 0.32 \times 0.63 \pm 0.24$ μm in size, and thought likely to be a representative of the Rickettsiaceae (Chern and Chao, 1994).

A Tasmanian isolate from Atlantic salmon was distinct from *Piscirickettsia* in terms of sequence alignment of the 16S rRNA, and for the present is regarded as an RLO (Corbeil *et al.*, 2005).

Pseudomonadaceae representatives

Pseudomonas anguilliseptica

Evidence suggests that isolates are homogenous (López-Romalde *et al.*, 2003).

Pseudomonas anguilliseptica

A homogeneous group of Gram-negative, asporogenous rods, which are motile by means of single polar flagella. Electron microscopy of 18-hour-old cultures on TSA reveal the presence of long, slightly curved rods with rounded ends. The size of these cells has been estimated as $5–10 \times 0.8$ mm. In addition, many bizarre forms have been observed. Fluorescent pigment is not produced. There is no reaction in the oxidative–fermentative test. Catalase and oxidase are produced, but not arginine dihydrolase, β-galactosidase, H_2S or indole. Nitrates are not reduced. Gelatin, Tween 20 (variable result) and Tween 80 are degraded, but not blood, DNA, starch (variable result) or urea. Acid is not produced from arabinose, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sucrose or xylose. Citrate is utilised by some isolates. Growth occurs at 5–30°C but not 37°C, in 0–4% (w/v) sodium chloride, and at pH 5.3–9.7. The G + C ratio of the DNA is 56.5–57.4 mol % (Wakabayashi and Egusa, 1972; Muroga *et al.*, 1977b; Nakai and Muroga, 1982; Stewart *et al.*, 1983; López-Romalde *et al.*, 2003).

On the basis of phenotypic traits, evidence suggests that isolates are homogenous (López-Romalde *et al.*, 2003). However, other approaches have detected some variation. Thus, a comprehensive examination of 96 isolates indicated the presence of two antigenic groups. Type I was not agglutinated in unheated antisera (this was prepared against heat-killed cells), although clumping (agglutination) of the cells subsequently occurred after the antiserum was heated to 100°C for 2 h (or 121°C for 30 min). Type II lacked this inhibition. It was speculated that this thermolabile agglutination-inhibiting antigen corresponds to the so-called K-antigens of coliforms (Nakai *et al.*, 1981, 1982a, b). Molecular traits based on PFGE have revealed four types among 54 isolates from sea bream in Portugal and Spain (Blanco *et al.*, 2002). Results with RAPD revealed two groups related to the host of origin of the cultures, with most of the isolates from eels in one cluster and the second grouping comprising isolates from other fish species (López-Romalde *et al.*, 2003).

From the phenotypic traits, Wakabayashi and Egusa (1972) concluded that the causal agent of Sekiten-byo corresponded to a new centre of variation within "Group III" or "Group IV" of the genus *Pseudomonas*. This opinion was reached because the pathogen was Gram-negative, rod-shaped, motile by polarly located flagella, insensitive to the vibriostatic agent (O/129), and produced catalase and oxidase, but not acid from glucose or, for that matter, diffusible (fluorescent) pigment. Because the strains were dissimilar to other fish-pathogenic pseudomonads, namely *Ps. fluorescens*, a new taxon was proposed, i.e. *Ps. anguilliseptica*. We are sceptical about the validity of this proposal because the description could equally fit *Alcaligenes* or *Deleya* as well as *Pseudomonas* (see Cowan, 1974; Kersters and De Ley, 1984; Palleroni, 2005). In some respects, the G + C ratio and the inability to produce acid in peptone water sugars is more conducive to the concept of *Alcaligenes* or *Deleya*, although the pathogen is clearly distinct from existing nomenspecies (Kersters and De Ley, 1984). Moreover, it may not be ruled out that the causal agent of Sekiten-byo should be classified in a newly described genus. Certainly, the distinctive micromorphology adds weight to this supposition. Maybe this explains the pronounced dissimilarity of *Ps. anguilliseptica* to other species of *Pseudomonas* as revealed by analyses of fatty acids and outer-membrane proteins (Nakajima *et al.*, 1983).

Pseudomonas chlororaphis

To date, there has been only one report of *Pseudomonas chlororaphis* as a fish pathogen. This involved a heavy mortality among farmed Amago trout (*Oncorhynchus rhodurus*) in Japan (Hatai *et al.*, 1975). For the present, it is uncertain whether *Ps. chlororaphis* represents an emerging problem, or a secondary (opportunistic) invader of already diseased hosts.

The isolates matched the description of *Ps. chlororaphis*, insofar as cultures comprised Gram-negative motile rods, which produced distinctive colonies. These produced green pigment, which crystallised as needles in the colonies (Stanier *et al.*, 1966; Palleroni, 1984). Other phenotypic traits were not reported, although the authors inferred that further tests had been carried out, and that these agreed with the definition of *Ps. chlororaphis*.

Pseudomonas fluorescens

Ps. fluorescens is a dominant component of the freshwater ecosystem (Allen *et al.*, 1983b). At various times, *Ps. fluorescens* has been considered as a fish spoilage organism (Shewan *et al.*, 1960), a contaminant or secondary invader of damaged fish tissues (Otte, 1963), as well as a primary, but poor pathogen (Roberts and Horne, 1978). All the published descriptions of the organism (e.g. Bullock, 1965; Csaba *et al.*, 1981b; Ahne *et al.*, 1982) agree closely with the definition of *Ps. fluorescens* (Stanier *et al.*, 1966; Palleroni, 2005).

Pseudomonas fluorescens

Cultures comprise Gram-negative, oxidative, arginine dihydrolase-, catalase- and oxidase-producing rods, which are motile by polar flagella. Growth occurs at 4°C, but not at 42°C. Fluorescent pigment (fluorescein) and gelatinase, but not β-galactosidase, H_2S, indole, amylase or urease, are produced. The Voges Proskauer reaction is negative. Citrate is utilised, and acid is produced from arabinose, inositol, maltose, mannitol, sorbitol, sucrose, trehalose and xylose, but not from adonitol or salicin.

It seems likely that other fish-pathogenic pseudomonads, as discussed by Li and Flemming (1967) and Li and Traxler (1971), correspond to *Ps. fluorescens*.

Pseudomonas plecoglossicida

The pathogen was regarded as having phenetic similarities with *Ps. putida* biovar A, but on the basis of 16S rRNA sequencing was regarded as distinct, and elevated into a new species, as *Ps. plecoglossicida* (Nishimori *et al.*, 2000).

Pseudomonas plecoglossicida

The 6 cultures (a brown-pigmented culture has been subsequently recovered; Park, 2000a) examined comprise a homogeneous group of strictly aerobic, Gram-negative, motile (several polar flagella) rods that produce catalase and oxidase, and reduce nitrate to nitrite, and grow at 10–30°C, but not at 4 or 41°C, in 0–5% (w/v) NaCl. Arginine dihydrolase is produced, but not lysine or ornithine decarboxylase. Blood is degraded, but not gelatin, lecithin, starch or Tween 80. Caprate, citrate, D-fructose, 2-ketogluconate, L-alanine, glucose, D-malate, propylene glycol, L-lysine, succinate and L-citrulline are utilised, but not L-arabinose, *m*-inositol, mannitol, D-mannose, sucrose, D-tartrate, testosterone, trehalose, L-tryptophan or D-xylose. A weak fluorescent pigment is produced on King medium B. The G + C ratio of the DNA is 62.8 mol % (Nishimori *et al.*, 2000).

DNA:DNA hybridisation levels were <50% with reference to *Pseudomonas* spp. (Nishimori *et al.*, 2000).

Pseudomonas pseudoalcaligenes

During 1992, at a site in the U.K. rainbow trout (average weight = 100 g) were observed with extensive skin lesions and signs of enteric redmouth (from which *Yersinia ruckeri* was recovered). From these animals, an organism with the key characteristics of *Ps. pseudoalcaligenes* was recovered (Austin and Stobie, 1992b).

Pseudomonas pseudoalcaligenes

The cream-coloured colonies (with a "gummy" consistency) comprise motile, oxidative (alkali is produced), short, Gram-negative rods, which produce arginine dihydrolase, catalase, ornithine decarboxylase and oxidase, but not β-galactosidase, H_2S, indole, lysine decarboxylase or tryptophan deaminase, degrade gelatin, tyrosine (with the production of melanin) and Tween 80, but not DNA, starch or urea, and grow at 15 and 25°C, but not at 4 or 40°C. Acid is produced from arabinose and glucose, but not from amygdalin, inositol, mannose, melibiose, rhamnose or sorbitol. Citrate is utilised. The Voges Proskauer reaction is negative.

The organism matched the description of *Pseudomonas* (Palleroni, 2005), and approximated *Ps. pseudoalcaligenes* as determined from the probability matrix of Holmes *et al.*, (1986). The only discrepancies concerned the degradation of gelatin and starch, and the production of ornithine decarboxylase.

Pseudomonas putida

There has been a casual mention of *Ps. putida* as a fish pathogen in Japan (Muroga, 1990). The organism was recovered from diseased (the disease was described as bacterial haemorrhagic ascites) ayu in Japan, and equated with *Pseudomonas* (Wakabayashi *et al.*, 1996). Similarities were noted to *Ps. putida*, but there was an absence of fluorescent pigment and a lack of agglutination with antiserum prepared to the type strain (of *Ps. putida*). The profile, obtained with the API 20NE rapid identification system, was "1140452" (Wakabayashi *et al.*, 1996). In a subsequent study, Altinok *et al.* (2006) identified a pathogen as *Ps. putida* on the basis of 16S rRNA sequencing (homology = 99.8%) and phenotypic characteristics.

Pseudomonas putida

Cultures are fluorescent, motile, Gram-negative rods, which produce arginine dihydrolase and oxidase, but not β-galactosidase, H_2S or indole, grow at 4, but not 41°C, do not attack aesculin, gelatin or urea, and produce acid from arabinose, capric acid, glucose, malate, potassium gluconate and trisodium citrate, but not adipic acid, maltose, mannitol, D-mannose, rhamnose, sorbitol or sucrose (Altinok *et al.*, 2006).

Vibrionaceae representatives

Vibrios have emerged as the scourge of marine fish and shellfish. Renewed interest has resulted in the description of new species and a better understanding of the biology of long-recognised taxa. To date, many species have been described as fish pathogens. In addition, hard-to-speciate *Vibrio* have been regularly recovered (e.g. Yasunobu *et al.*, 1988; Masumura *et al.*, 1989; Muroga *et al.*, 1990). There is controversy over the role of *V. parahaemolyticus* as a fish pathogen, and we are not entirely satisfied with the evidence; therefore, for the purpose of this chapter it has been concluded that the organism does not constitute a *bona fide* fish pathogen. One article has mentioned challenging tilapia with *V. parahaemolyticus*, but there was insufficient information about the authenticity of the isolates (Balfry *et al.*, 1997). However, organisms with intermediate characteristics between *V. alginolyticus* and *V. parahaemolyticus* have been recovered from diseased milkfish in the Philippines (Muroga *et al.*, 1984a). A recent publication has suggested that *V. campbellii*, *V. nereis* and *V. tubiashii* may be associated with disease in gilthead sea bream in Spain (Balebona *et al.*, 1998). However, confirmation is desirable before these taxa become recognised as *bona fide* fish pathogens.

In the taxonomic study of Austin *et al.* (1997), a single isolate from Atlantic salmon in Tasmania was equated with *V. aestuarianus* using the diagnostic scheme of Alsina and Blanch (1994a, b). By phenotypic means, more isolates were associated with disease in gilthead sea bream in Spain (Balebona *et al.*, 1998).

Vibrio alginolyticus

The most extensive study of the role of *V. alginolyticus* as a fish pathogen concerned the observations of mortalities in farmed sea bream (*Sparus aurata*) in Israel. Mortalities were recorded after extensive handling of the fish (Colorni *et al.*, 1981). However, these workers were unable to reproduce the infection under laboratory conditions, which casts some doubt on the importance of this organism in fish pathology. Nevertheless, there is additional evidence linking this organism with a pathogenic mode. Lee (1995) recovered one isolate, which was identified phenotypically, from diseased grouper (*Epinephelus malabaricus*). Also, occasional isolations have been made from ulcers (Akazawa, 1968). Isolates have been recovered from diseased gilthead sea bream in Spain (Balebona *et al.*, 1998). Additionally, *V. alginolyticus* has been reported as a secondary invader of sea mullet suffering with "red spot" (Burke and Rodgers, 1981). *V. alginolyticus* has also been implicated with mortalities in cultured black sea bream fry (Kusuda *et al.*, 1986). Therefore, the implication is that *V. alginolyticus* constitutes an opportunistic invader of already damaged tissues, or a weak pathogen of stressed fish.

Vibrio alginolyticus

Typically, swarming growth develops on the surface of solid media. Cultures comprise motile, fermentative, Gram-negative rods that produce catalase, H_2S, indole, lysine and ornithine decarboxylase, and oxidase, but not arginine

dihydrolase or β-galactosidase. Blood (haemolysis), chitin, gelatin, lipids, starch and urea are degraded, but not aesculin. Nitrates are reduced. The methyl red test and Voges Proskauer reaction are positive. Acid is produced from glycerol, maltose, mannitol, mannose, salicin and sucrose, but not arabinose, inositol or lactose. Growth occurs at 37°C, and in 7%, but not 0% or 10% (w/v), sodium chloride. Sensitivity is recorded to the vibriostatic agent, O/129. The G + C ratio of the DNA is 45–47 mol %.

Generally, fresh isolates matched the species description of *V. alginolyticus* (Farmer III *et al.*, 2005).

Vibrio anguillarum (= Listonella anguillara/anguillarum)

The causal agent of "red-pest" in eels was first isolated by Canestrini (1893), who designated the organism as *Bacterium anguillarum*. A further case among eels in Sweden during 1907 was investigated by Bergman (1909), and it was directly attributable to this scientist that the name of *V. anguillarum* was coined.

The taxonomy of the pathogen has had a chequered history, which culminated in the description of a second species, i.e. *V. ordalii*. This accommodated strains previously regarded as biotype II of *V. anguillarum* (Schiewe, 1981). However, there is cross-reactivity with the LPS of *V. anguillarum* serogroup O2 and *V. ordalii* (Muthiara *et al.*, 1993). The complexity in the taxonomic understanding of the pathogen began with the recognition by Nybelin (1935) of two biotypes. These were differentiated on the basis of a few biochemical reactions. A further group, i.e. biotype C, was recognised by Smith (1961). These biotypes were distinguished as follows:

Type A Known as *V. anguillarum forma typica*, produced indole and acid from mannitol and saccharose.

Type B Referred to as *V. anguillarum forma anguillicida*, did not produce indole or acid from mannitol or saccharose.

Type C Coined as *V. anguillarum forma ophthalmica*, produced acid from mannitol and saccharose, but did not produce indole.

Two further biotypes, i.e. D and E, were later described, both of which produced indole, but not acid from mannitol. Biotype D, but not E, produced acid from saccharose. In view of modern thought and approaches on bacterial taxonomy, these descriptions are inadequate. Nevertheless, they heralded an appreciation of heterogeneity within the species. Type C deserves special mention because this was proposed for Japanese strains derived from rainbow trout, and labelled as *V. piscium* var. *japonicus* (David, 1927; Hoshina, 1956). Interestingly, they were originally recognised as dissimilar to *V. anguillarum*. Alternatively, it must be conceded that the separate name may have reflected ignorance of the existence of *V. anguillarum* as described by Bergman (1909). The relationship of these so-called biotypes with *V. ichthyodermis*, as described by Wells and ZoBell (1934), and the organism tentatively assigned as

V. anguillicida (Nishibuchi and Muroga, 1977), needs clarification. Indeed, the latter was considered to resemble both *V. anguillarum* and *V. fischeri*.

The multiplicity of studies of Harrell *et al.* (1976), Ohnishi and Muroga (1976), Håstein and Smith (1977), Schiewe *et al.* (1977), Baumann *et al.* (1978), Kusuda *et al.* (1979), Ezura *et al.* (1980), Lee *et al.* (1981), Kaper *et al.* (1983) and West *et al.* (1983) demonstrated very clearly the heterogeneity within *V. anguillarum*. Håstein and Smith (1977) distinguished two subgroups after a principal components analysis on data collected for 163 isolates and 28 tests. A similar conclusion, i.e. two subgroupings, was voiced by Schiewe *et al.* (1977), Baumann *et al.* (1978), Ezura *et al.* (1980) and Lee *et al.* (1981). Of the 50 isolates studied by numerical taxonomy, Kusuda *et al.* (1979) defined three groups. These were equated with *V. anguillarum* (divided into three subgroups), a group closely related to *V. parahaemolyticus*, and a cluster considered to have affinity with *V. ichthyodermis*. Kaper *et al.* (1983) recognised four homogeneous phena among isolates received as *V. anguillarum*. This view was reinforced in a later study by West *et al.* (1983). Pazos *et al.* (1993) studied 46 isolates of *V. anguillarum*-like organisms from diseased fish and shellfish and the environment by numerical phenetic methods, and recognised 4 phena. The apparent heterogeneity was reinforced by the results of ribotyping, with 44 ribotypes recognised among isolates of *V. anguillarum* (Olesen and Larsen, 1993). Yet, Austin *et al.* (1995a, 1997) recognised a single taxon, homogeneous by ribotyping—isolates were recovered in a single ribotype—and OMP patterns, but heterogeneous in terms of LPS profiles, plasmid composition, serogrouping, and BIOLOG-GN fingerprints and API 20E profiles.

The taxon was re-classified to *Listonella*, as *Listonella anguillara* (MacDonell and Colwell, 1985). However, the name change was not widely accepted. Consequently, the organism is still regarded as *V. anguillarum*.

Vibrio anguillarum

Cultures comprise cream-coloured (a water-soluble, brown-pigmented culture has been reported in Japanese flounder; Sakai *et al.*, 2006a), round, raised, entire, shiny colonies (dissociation into two or three colony types may occur; Austin *et al.*, 1996) comprising short (0.5×1.5 μm), fermentative, Gram-negative rods, which are motile by single polar flagella. Arginine dihydrolase, catalase, β-galactosidase, indole and oxidase, but not H_2S, lysine or ornithine decarboxylase, phenylalanine deaminase or urease, are produced. Along with most other vibrios, sensitivity is displayed to the vibriostatic agent, O/129. A positive result is usually recorded for the Voges Proskauer reaction, but not for the methyl red test. Chitin, gelatin, DNA, lipids and starch, but not aesculin, are degraded. Nitrates are reduced. Growth occurs at 15–37°C, and in 0.3–3.0% (w/v), but not 0% and 7% (w/v), sodium chloride. Citrate, malonate and tartrate are utilised. The organisms produce acid from amygdalin, arabinose, cellobiose, galactose, glycerol, maltose, mannitol, sorbitol, sucrose and trehalose, but not from adonitol, dulcitol, erythritol, inositol, lactose, melibiose, raffinose, rhamnose, salicin or xylose. Cultures comprise a single, dominant ribotype. The G + C ratio of the DNA is 45.6–46.3

mol % (Smith, 1961; Kiehn and Pacha, 1969; Evelyn, 1971b; Muroga *et al.*, 1976a, b; Schiewe *et al.*, 1981; Austin *et al.*, 1995a; Farmer III *et al.*, 2005).

It has been observed that some so-called *bona fide* isolates of *V. anguillarum* possess fascinating micromorphologies, insofar as broth cultures appear to contain two types of cells. Apart from the typical, short, motile rods, we have observed very small, highly motile cells, some of which are capable of passing through 0.22 μm porosity filters. From transmission electron microscopy, we believe that these cells comprise extremely small, spherical bodies, each attached to a single polar flagellum. The significance of these small cells is unclear, but they may represent a stage in a life cycle, a laboratory artefact, or some form of survival mechanism.

The results of serology further complicated the understanding of *V. anguillarum* (Bolinches *et al.*, 1990). The establishment of serotypes has traversed species (or phenetic) boundaries. Initially, three serotypes were recognised for isolates from northwestern (U.S.A.), Europe, and the Pacific Northwest (U.S.A.) (Pacha and Kiehn, 1969). This was supported by the work of Japanese scientists (Aoki *et al.*, 1981; Muroga *et al.*, 1984b). With further study, the number of serotypes increased to six (Kitao *et al.*, 1983). Thus, in a mammoth study of 267 isolates from ayu, eel and rainbow trout, Kitao and co-workers defined serotypes A, B, C, D, E, and F as a result of cross-agglutination and cross-absorption tests with thermo-stable "O" (somatic) antigens. The majority (243) of these Japanese isolates were recovered in serotype A. It is noteworthy, however, that avirulent isolates were not recovered in any of these serotypes (Muroga *et al.*, 1984b). This reflects the nature of the LPS in the cell wall, which accounts for both the nature of the serotype (Johnson, 1977; Aoki *et al.*, 1981) and the immunogenicity. Later, Sørensen and Larsen (1986) reported the presence of 10 O-antigen serotypes, based upon examination of 495 isolates, representatives of which shared a common 40 kDa protein (Simón *et al.*, 1996). A common 47 kb plasmid was reported by Giles *et al.* (1995). Serovars (= serotypes/serogroups) O1 and O2 contained plasmids, but there was no apparent correlation between the presence of such extrachromosomal DNA and biochemical properties (Larsen and Olsen, 1991), although serogroup O1 was regarded as biochemically homogeneous (Pedersen and Larsen, 1995). The same 10 serogroups together with 6 non-typeable groups were described by Olsen and Larsen (1991). The number of serogroups was now increased to 16, i.e. O1 to O16 (Grisez and Ollevier, 1995).

A consensus view would be that serogroup O1 has dominated both in the number of isolates available for study and the relative importance to fish pathology (Austin *et al.*, 1995a; Pedersen *et al.*, 1996a, c). The homogeneity of serogroup O1 has been established (Austin *et al.*, 1995a), yet data have pointed to variability among isolates. For example, after studying 75 isolates of serogroup O1, 8 plasmid profiles—with one predominating—and 6 ribotypes were recognised (Skov *et al.*, 1995). An even larger examination of 103 isolates of serogroup O1 recognised 15 plasmid profiles (Pedersen and Larsen, 1995). PFGE had high discriminatory power, recognising 35 profiles.

It is a personal view that isolates of serogroup O2 have seemed to be more aggressive than serogroup O1. Serogroup O2, which has been subdivided into

serogroup O2a and O2b, has revealed heterogeneity in LPS profiles—6 different profiles have been recognised among 129 isolates (Tiainen *et al.*, 1997). By western blotting and slide agglutination, four different patterns have emerged. By comparing LPS profiling, western blotting and slide agglutination, 9 different groupings were formed (Tiainen *et al.*, 1997). A view was expressed that additional subgroups within serogroup O2 remain to be described (Tiainen *et al.*, 1997).

V. cholerae (non-O1)

During the summer of 1977, an epizootic occurred in a wild population of ayu in the River Amano, Japan. From diseased animals, an organism conforming to the description of *V. cholerae* was isolated (Muroga *et al.*, 1979; Kiiyukia *et al.*, 1992). Subsequently, *V. cholerae* was associated with a disease of goldfish in Australia (Reddacliff *et al.*, 1993).

Vibrio cholerae

Cultures comprise small (1.5–3.0 × 0.7–1.0 μm in size), Gram-negative, fermentative rods, which are motile by single polar flagella. Growth occurs in 0–6% (w/v) sodium chloride, at 10–42°C, and at pH 7–10. Catalase, β-galactosidase, indole, lysine decarboxylase and oxidase are produced, but not arginine dihydrolase, H_2S, ornithine decarboxylase or phenylalanine deaminase. Aesculin, blood (haemolysis), chitin, gelatin, lipids and starch, but not urea, are degraded. The methyl red test and Voges Proskauer reaction are positive. Nitrates are reduced. Citrate, fructose, galactose, glucose, maltose, sucrose and tartrate are utilised, but not adonitol, arabinose, cellobiose, dulcitol, inositol, inulin, malonate, mannose, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol or xylose. Acid is produced from sucrose, but not lactose. Growth occurs at 37°C and in 0%, but not 7% (w/v), sodium chloride. Sensitivity is recorded to the vibriostatic agent, O/129. The G + C ratio of the DNA is 47–49 mol % (Muroga *et al.*, 1979; Yamanoi *et al.*, 1980; Kiiyukia *et al.*, 1992).

The phenotypic traits published by Muroga *et al.* (1979) and Kiiyukia *et al.* (1992) were in close agreement with the description of *V. cholerae* (Farmer III *et al.*, 2005). Indeed, there were only two discrepancies with the characteristics of the "El Tor" biotype, i.e. production of ornithine decarboxylase and utilisation of mannose (these were reported as negative for the fish-pathogenic isolates). However, the fish isolates did not react by slide agglutination with antisera to *V. cholerae*. Therefore, the conclusion was that the fish-pathogenic isolates comprised *V. cholerae* non-O1. It should be emphasised, however, that the fish isolates demonstrated 86% DNA : DNA homology with a reference strain of *V. cholerae*. This coincided well with the intraspecific homology values of *V. cholerae* (Citarella and Colwell, 1970). Again, there was reasonably close agreement between *V. cholerae* and the isolates studied by Reddacliff *et al.* (1993). Yet, a detailed taxonomic study by Austin *et al.* (1997) reported heterogeneity among 6 isolates received as *V. cholerae*, insofar as they were

recovered as single-member ribotype clusters, and displayed diverse BIOLOG-GN fingerprints and API 20E profiles. However, 5 cultures corresponded with *V. cholerae* serogroups, namely O8, O9, O23, O32 and O63.

V. fischeri

During Autumn 1988, visceral tumours (neoplasia) and skin papillomas were observed in juvenile turbot, farmed in northwest Spain. Although viral involvement was suspected, bacteria were evident in the majority of affected fish. The bacteria approximated the description of *V. fischeri*, albeit with similarities to *V. harveyi* (Lamas *et al.*, 1990). However, detailed characteristics of the cultures were not presented. Interestingly, other isolates have been recovered from gilthead sea bream, also in Spain (Balebona *et al.*, 1998).

Vibrio harveyi (= V. carchariae = V. trachuri)

A comparative newcomer to the growing list of vibrio fish pathogens, *V. carchariae* was originally isolated from a dead sandbar shark (*Carcharhinus plumbeus*) which died at the National Aquarium in Baltimore, Maryland, in 1982 (Grimes *et al.*, 1984a). Subsequently, a similar organism was recovered from lemon sharks (*Negraprion brevirostris*) (Colwell and Grimes, 1984). Yii *et al.* (1997) reported *V. carchariae* as the cause of gastro-enteritis and mortalities in grouper. One isolate, EmI82KL, was noted to be motile, did not auto-agglutinate, but was haemolytic to grouper, rabbit, sheep and tilapia blood. In a separate development, gamefish, namely common snook (*Centropomus undecimalis*), were found to suffer with opaque, white corneas within 24 h of capture in Florida (Kraxberger-Beatty *et al.*, 1990). From such damaged specimens, *V. harveyi* was recovered. Twelve cultures were recovered from corneas, and were identified as *V. harveyi* as a result of biochemical tests and DNA:DNA hybridisation (90–94% DNA homology with the type strain of *V. harveyi*). Certainly, the characteristics of the isolates were in good accord with the species description of *V. harveyi* (Farmer III *et al.*, 2005). Subsequently, there has been a gradual awareness of the increasing significance of *V. harveyi* as a killer of marine fish and penaeids. Saeed (1995) blamed *V. harveyi* with causing mortalities in cultured brown spotted grouper (*Epinephelus tauvina*) and silvery black porgy (*Acanthopagrus cuvieri*) in Kuwait. Then, as a result of DNA:DNA hybridisation, Ishimaru and Muroga (1997) determined that pathogenic vibrios recovered from milkfish in Japan were indeed *V. harveyi*. The organism has also been recovered from ocular lesions in the short sunfish (*Mola mola*) in Spain (Hispano *et al.*, 1997).

The synonymy of *V. harveyi* and *V. carchariae* was realised by Pedersen *et al.* (1998) as a result of phenotypic and genotypic studies, and by Gauger and Gómez-Chairri (2002) from 16D rDNA sequencing. In terms of taxonomic standing, *harveyi* has precedence, and therefore this name will be used in preference to *carchariae*.

Vibrio harveyi

V. harveyi shows similarities to *V. alginolyticus*, principally because of the presence of swarming on agar medium. Essentially, cultures comprise pleomorphic, fermentative, Gram-negative rods (1.0–1.6 × 0.5-0.7 μm in size), which are motile by polar and/or lateral flagella. Catalase, indole, lysine and ornithine decarboxylase and oxidase are produced, but not arginine dihydrolase. The Voges Proskauer reaction is negative. Nitrates are reduced. Alginate, blood, DNA, gelatin and lecithin are degraded, but not aesculin, casein, cellulose, pectin or starch. Many compounds are utilised, including arabinose, aminobutyrate, cellobiose, ethanol, glucose, glycine, α-ketoglutarate, propanol, sucrose and trehalose, but not inositol or lactose. Growth occurs in 3–8%, but not 0% or 10% (w/v), sodium chloride, and at 11–40°C. Sensitivity is demonstrated to 150 μg, but not to 10 μg, of the vibriostatic agent, O/129. The G + C ratio of the DNA is 45–47 mol %. The only differences with the description of *V. carchariae* centre on the degradation of starch (*V. carchariae* = +), and growth in 7% (w/v) sodium chloride (*V. carchariae* = −). The G + C ratio of the DNA of *V. carchariae* is 47 mol %.

Results of DNA:DNA homology experiments showed a high degree of homology with *Ph. damselae*, at 88% re-association. Eight RAPD and 13 ribotypes were recognised by Pujalte *et al.* (2003), Phenotypic and genotypic traits point to relatedness between *V. harveyi* and *V. campbellii* (Gomez-Gil *et al.*, 2004). Moreover, these authors considered that some cultures of *V. harveyi* should more correctly have been identified as *V. campbellii*. This raises the question about the role of *V. campbellii* in fish pathology.

V. ichthyoenteri

V. ichthyoenteri was described as a result of an examination of seven isolates from flounder larvae (Ishimaru *et al.*, 1996).

V. ichthyoenteri

Cultures are non-pigmented on marine 2216E agar, but produce yellow colonies on TCBS, which contain Gram-negative, fermentative rods, which are motile by single polar flagella. Catalase and oxidase are produced, but not arginine dihydrolase, β-galactosidase, H_2S, indole or ornithine decarboxylase. Nitrates are reduced. Neither agar, chitin, gelatin, lipids nor starch are degraded. Polyhydroxybutyrate is not accumulated intracellularly. Growth occurs at 15–30°C, but not at 4 or 35°C, and in 1–6%, but not 0 or 8% (w/v), sodium chloride. Acid is produced from fructose, D-glucose, maltose, D-mannose, sucrose and trehalose, but not from adonitol, L-arabinose, D-cellobiose, dulcitol, erythritol, D-galactose, glycerol, inulin, inositol, lactose, D-mannitol, melibiose, raffinose, L-rhamnose, salicin or D-sucrose. Neither D-cellobiose, citrate, D-gluconate, L-leucine nor D-xylose

are utilised. Sensitivity is recorded to the vibriostatic agent, O/129. The G+C ratio of the DNA is 43–45 mol %.

V. logei

Fifteen Icelandic and one Norwegian isolates from shallow skin lesions in Atlantic salmon were considered to be similar to *V. logei* (Benediktsdóttir *et al.*, 1998).

Vibrio logei

Cultures do not produce arginine dihydrolase, indole or lysine or ornithine decarboxylase. Blood (haemolysis) and chitin are degraded, but not starch. Acid is produced from *N*-acetyl glucosamine, glycerol, maltose, mannose, ribose, sucrose and trehalose.

V. ordalii

The establishment of a new species to accommodate strains previously classified as *V. anguillarum* biotype II, i.e. *V. ordalii* (Schiewe, 1981; Schiewe *et al.*, 1981), generated an awareness that vibriosis could be caused by more than one bacterial taxon. Disease caused by *V. ordalii* has been documented in Japan (e.g. Muroga *et al.*, 1986) and the Pacific Northwest, U.S.A. *V. ordalii* was homogeneous by plasmid profiling, ribotyping and serogrouping, accommodated two LPS groups, and was heterogeneous by BIOLOG-GN fingerprints and API 20E profiles (Austin *et al.*, 1997).

Vibrio ordalii

Cultures comprise fermentative, Gram-negative, curved rods of 2.5–3.0 × 1.0 μm in size, motile by means of single polar flagella. Growth occurs quite slowly, insofar as 4–6 days incubation at 22°C are required for the production of off-white, circular, convex colonies of 1–2 mm in diameter on seawater agar. *V. ordalii* is, however, not especially active. Catalase and oxidase are produced, but not arginine dihydrolase, β-galactosidase, H_2S, indole, lysine or ornithine decarboxylase, or phenylalanine deaminase. DNA, chitin (by some isolates) and gelatin are degraded, but not aesculin, lipids, pectate, starch or urea. Nitrates are reduced by some isolates. The methyl red test and Voges Proskauer reaction are negative. Tartrate is utilised. Only a few carbohydrates, e.g. galactose (variable result), maltose, mannitol and sucrose are attacked with the production of acid. Negative responses are recorded for adonitol, arabinose, cellobiose, dulcitol, erythritol, glycerol, inositol, lactose, melibiose, raffinose, rhamnose, salicin, sorbitol, trehalose and xylose. Growth occurs at 15–22°C, but not at 37°C, and in 0.5–3.0%, but not 0% or 7% (w/v), sodium chloride. Sensitivity is recorded to the vibriostatic agent, O/129. The G+C ratio of the DNA is 43–44 mol % (Schiewe, 1981; Schiewe *et al.*, 1981; Austin *et al.*, 1997).

Results of DNA:DNA hybridisation studies have confirmed the homogeneity and validity of *V. ordalii*, with intraspecific homologies of approximately 80%. There is only a 58–59% association with *V. anguillarum* (Schiewe *et al.*, 1981). *V. ordalii* contains plasmids (Tiainen *et al.*, 1995), but the profile is inevitably different from *V. anguillarum*. In one study, Schiewe and Crosa (1981) determined that 11 isolates of *V. ordalii* contained a common plasmid type (pMJ101) with a molecular weight of 20 mDa. Indeed, this plasmid (= ~32 kb) is common to all *V. ordalii* isolates (Pedersen *et al.*, 1996b).

There is serological (antigenic) cross-reactivity between *V. ordalii* and *V. anguillarum* serogroup O2 (Chart and Trust, 1984).

V. pelagius

Four isolates were obtained in pure culture, and described as (Angulo *et al.*, 1992):

Vibrio pelagius

The cultures comprise Gram-negative, motile, fermentative rods that produce catalase, β-galactosidase (variable), indole (variable) and oxidase, but not arginine dihydrolase, H_2S, or lysine or ornithine decarboxylase, reduce nitrates, degrade alginate (variable), gelatin, starch, Tween 80 and urea (variable), produce acid from mannitol, mannose, trehalose and sucrose (variable), but not L-arabinose, arbutin, inositol, salicin or sorbitol, and are sensitive to the vibriostatic agent O/129, ampicillin and novobiocin. The methyl red test is generally positive. The Voges Proskauer reaction gives a variable response. Growth occurs in 6% (w/v), but not 0% or 10% (w/v), sodium chloride, at 35°C, but not 4 or 42°C. However, the isolates do not agglutinate with O-antigens of *V. pelagius* ATCC 25916.

It is noteworthy that the fish isolates differed from the reference culture of *V. pelagius* ATCC 25916 in indole production, urea degradation and whole-cell agglutination, which must cast some doubt on the validity of the original identification.

V. salmonicida

An organism, named *V. salmonicida*, has been recovered from diseased salmon (Egidius *et al.*, 1986), and the validity and distinctiveness confirmed (Austin *et al.*, 1997).

Vibrio salmonicida

Cultures contain motile (~9 polar flagella), fermentative, Gram-negative, curved, pleomorphic rods of 2–3 × 0.5 μm in size. Catalase and oxidase are produced, but not arginine dihydrolase, β-galactosidase, H_2S or indole. Nitrates are not reduced, nor is the Voges Proskauer reaction positive. Citrate is not utilised. Neither blood, chitin, gelatin, lipids nor urea are degraded. *N*-acetylglucosamine, glucose, glyc-

erol (slowly), maltose, ribose, sodium gluconate and trehalose are utilised, but not adonitol, amygdalin, D- or L-arabinose, D- or L-arabitol, arbutin, D-cellobiose, dulcitol, erythritol, D- or L-fucose, inositol, β-gentobiose, lactose, D-lycose, D-mannose, melezitose, melibiose, D-raffinose, rhamnose, salicin, sorbitol, sucrose, L-sorbose, D-tagatose, D-turanose or D- or L-xylose. Growth occurs at 1–22°C, optimally at 15°C, but not at 37°C, and in 0–4%, but not 7% (w/v), sodium chloride. Sensitivity is displayed to the vibriostatic agent, O/129, but not to novobiocin. The G + C ratio of the DNA is 44 mol % (Holm *et al.*, 1985; Egidius *et al.*, 1986).

Strains have been divided into four different categories on the basis of plasmid profiles (Wiik *et al.*, 1989). The plasmids were 2.6, 3.4 and 24 mDa in size, with the largest plasmid being common to all four groups. There was no apparent difference in biochemical traits among these four plasmid groups. In a separate study, Sørum *et al.* (1990) described plasmids of 2.8, 3.4, 21 and 61 mDa from isolates recovered from Atlantic salmon and cod. These authors reported 11 plasmid profiles for *V. salmonicida*. A similar plasmid composition has been indicated for isolates from the Faroe Islands (Nielsen and Dalsgaard, 1991). Comparing isolates from Canada, the Faroe Islands, Norway and Shetland, Sørum *et al.* (1993a) noted a similarity in plasmid profile, with three plasmids of 2.8, 3.4 and 21 mDa revealed. Furthermore, a conclusion has been reached that all strains carry plasmids (Valla *et al.*, 1992). Differences have also been implied by serological studies, which have indicated the presence of two serotypes.

DNA hybridisation of four cultures confirmed homogeneity (DNA homology = 82–100%), but low relatedness to *V. anguillarum* (30%), *V. ordalii* (34%) or *V. parahaemolyticus* (40%) (Wiik and Egidius, 1986). Although these data were used to justify the uniqueness of *V. salmonicida*, the relationship to other representatives of the Vibrionaceae was not considered. The validity of the species is not, however, questioned, and in the detailed study of Austin *et al.* (1997) *V. salmonicida* formed a discrete taxon among the other fish-pathogenic vibrios.

V. splendidus

A detailed taxonomic study by Austin *et al.* (1997) of 22 isolates revealed that fish-pathogenic isolates (these were non-pathogenic in laboratory-based infectivity experiments with Atlantic salmon and rainbow trout) were markedly heterogeneous, being recovered in 7 ribotype clusters, many API 20E profiles and 6 BIOLOG-GN groups (the type strain was recovered as a single-member cluster). However, only two serogroups (there were some cross-reactions with *V. pelagius* antiserum) and one LPS profile was recognised. With such heterogeneity, it is difficult to decide whether or not any fish isolates actually constitute *bona fide V. splendidus*. Notwithstanding, an attempt will be made to present a consensus view of the phenotypic traits associated with so-called *V. splendidus*:

Vibrio splendidus

Cultures contain motile, fermentative, Gram-negative rods, which produce arginine dihydrolase (some isolates), catalase, β-galactosidase, lysine decarboxylase and oxidase, but not H_2S, indole, ornithine decarboxylase or phenylalanine deaminase, degrade blood (β-haemolysis), chitin, gelatin, lipids and starch, but not aesculin or urea, reduce nitrates, demonstrate positivity for the methyl red test, but not the Voges Proskauer reaction, are sensitive to the vibriostatic agent, O/129, grow at 37°C and in 3% (w/v), but not 0 or 7% (w/v), sodium chloride, utilise citrate, β-gentiobiose, glucose, ribose and sorbitol, but not malonate or sucrose, and produce acid from glucose (no gas), maltose and mannose, but not from arabinose, inositol, mannitol, sorbitol or sucrose. The G + C ratio of the DNA is 45.7–47.8 mol % (Jensen *et al.*, 2003).

Agglutination was recorded with antisera prepared against whole cells of *V. splendidus* and strongly to O-antigen of *V. splendidus* and *V. tubiashii*. Weak agglutination was recorded against O-antigen of *V. anguillarum* and *V. ordalii* (Lupiani *et al.*, 1989). It is considered that the pathogen showed similarities to both *V. anguillarum* (including *V. ordalii*) and *V. splendidus*. Differences from *V. splendidus* (biotype II) included arginine dihydrolase, β-galactosidase and indole production, the Voges Proskauer reaction, degradation of gelatin and urea, and acid production from arabinose, mannitol and sorbitol (Baumann *et al.*, 1984). Consequently, an identification as *V. splendidus* can only be regarded as tentative, pending further investigation. However, the isolate recovered from corkwing wrasse by Jensen *et al.* (2003) was equated with *V. splendidus* on the basis of phylogenetic and DNA : DNA hybridisation (78.4% with the type strain) relationships.

V. tapetis

A culture was obtained from diseased corkwing wrasse in Norway. The characteristics were as follows:

Vibrio tapetis

The culture contained motile, Gram-negative, fermentative rods that produced oxidase, but not arginine dihydrolase, and required NaCl. The Voges Proskauer reaction was negative. Growth did not occur at 37°C. Blood was not degraded. Glucose, ribose and sucrose were utilised, but not mannitol or sorbitol. The G + C ratio of the DNA is 43.8 mol % (Jensen *et al.*, 2003).

There was 78.4% DNA : DNA re-association with a named reference culture of *V. tapetis* (Jensen *et al.*, 2003).

V. vulnificus

A group of bacteria was recovered, which appeared to belong to the same taxon. The closest neighbour was thought to be *V. anguillarum* type B (= *V. anguillarum forma anguillicida*), according to the description of Nybelin (1935). However, this group lacks taxonomic meaning, and consequently it was agreed to resurrect the name of *V. anguillicida* (after Bruun and Heiberg, 1935) to accommodate the organisms (Muroga *et al.*, 1976 a, b; Nishibuchi *et al.*, 1979). Some excellent detective work by Tison *et al.* (1982) led to the realisation that the organisms, in fact, approximated *V. vulnificus* (= lactose-fermenting vibrios). A new biotype, i.e. biogroup 2, was duly established. However, the biotype concept was considered no longer appropriate (Arias *et al.*, 1997a, b), and it was subsequently deemed preferable to refer to the organisms as a serovar rather than a biotype (Biosca *et al.*, 1997a). Nevertheless, *V. vulnificus* biotype 2 serovar A was subsequently described (Fouz *et al.*, 2006).

From an examination of 80 cultures, eel isolates were separated from others by ribotyping (Arias *et al.*, 1997a, b). Moreover, AFLP was very discriminatory (Arias *et al.*, 1997a, b).

Vibrio vulnificus

Isolates comprise short, Gram-negative, fermentative rods, motile by means of single polar flagella. Arginine dihydrolase, catalase and oxidase are produced, but not β-galactosidase, indole (indole-producing cultures have been recovered from eels in Denmark; Dalsgaard *et al.*, 1999), or lysine or ornithine decarboxylase. Casein, blood, lecithin and Tween 80 are degraded, but not gelatin or urea. Nitrates are reduced. The Voges Proskauer reaction is positive. Acid is produced from a wide range of compounds including D-amygdalin, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, glycogen, maltose, mannose, melibiose, starch and trehalose, but not from adonitol, arabinose, dulcitol, inositol, inulin, mannitol, raffinose, L-rhamnose, D-sorbitol, sucrose (cultures from the P.R.C. were positive for sucrose) or D-xylose. Tartrate is utilised. Growth occurs at 20 and 37°C, but not at 5 or 42°C, and in 0.5–5.0%, but not 0% (w/v), sodium chloride. Sensitivity is recorded to the vibriostatic agent, O/129. The G + C ratio of the DNA is 45.7–47.8 mol % (Tison *et al.*, 1982; Amaro *et al.*, 1992).

There are some differences between the species description of *V. vulnificus* and the fish isolates. For example, the latter do not produce indole, ornithine decarboxylase, acid from mannitol or sorbitol, or grow at 42°C. These differences add weight to assigning the fish pathogens to a new biogroup. However, the results of DNA:DNA hybridisation, which show 90% homology between *V. vulnificus* and the group of fish pathogens (Tison *et al.*, 1982), confirm taxonomic (species) relatedness.

Results of serology have clearly demonstrated that isolates of *V. vulnificus* contain heterologous surface antigens. It must be emphasised, however, that the fish isolates are distinctly homogeneous (Nishibuchi and Muroga, 1980). There are common soluble intracellular antigens, which are homologous in all isolates. In

particular, there is a *V. vulnificus*-specific antigen, which may have value in the development of rapid diagnostic tests.

V. wodanis

Two groups of bacteria were recovered from Atlantic salmon with so-called winter ulcer disease/syndrome (Lunder *et al.*, 2000), of which one comprised a comparatively heterogeneous assemblage of cultures, i.e. *V. wodanis*.

V. wodanis

The taxon is regarded as not especially homogeneous, phenotypically. Nevertheless, the yellow, opaque colonies contain Gram-negative, motile, fermentative rods that produce alkaline phosphatase, caprylate esterase, catalase, indole and oxidase, but not arginine dihydrolase, chemotrypsinase, α-fucosidase, α- or β-glucosidase, lysine decarboxylase, α-mannosidase, ornithine decarboxylase, degraded bovine blood (β-haemolysis only in the presence of 2% w/v NaCl), DNA, starch and Tween 80, but not aesculin, casein or lecithin, grow at 4–25°C, but not 30°C, in 1–4% (w/v) NaCl, produce acid from galactose, glycerol, glucose and mannose, but not L-arabinose, cellobiose, inositol, lactose, melibiose, raffinose, L-rhamnose or xylose, and are susceptible to the vibriostatic agent, O/129. The methyl red test is positive, but not the Voges Proskauer reaction. The G + C ratio of the DNA is 40.0 mol % (Lunder *et al.*, 2000).

By DNA : DNA hybridisation and 16S 3RNA sequencing, the closest neighbour was *V. logei* with 57% re-association (Lunder *et al.*, 2000) and 98.8% sequence homology (Benediktsdóttir *et al.*, 2000), respectively.

MISCELLANEOUS PATHOGENS

Streptobacillus

A possibly unique organism has been recovered from seawater-farmed Atlantic salmon in Ireland. The organism, which occurred intracellularly in tissues, was considered to be related to *Streptobacillus moniliformis* and fusobacteria on the basis of 16S rRNA analyses (Maher *et al.* (1995).

UNNAMED BACTERIA

There is an increasing awareness of diseases caused by apparently unique bacteria. For example, Sorimachi *et al.* (1993) and Iida and Sorimachi (1994) described jaundice in yellowtail, attributed to an unknown filamentous bacterium of 4–6 μm

in length, which grew only in L15 medium and Eagles MEM medium each supplemented with 10% (v/v) foetal calf serum at 23–26°C.

The unknown Gram-negative cocco-bacilli of Palmer *et al.* (1994) were characterised as follows:

Unknown Gram-negative organism of Palmer et al. (1994)

Cultures produce small colonies of 0.3 mm in diameter after incubation aerobically for 10–14 days. Anaerobic incubation results in larger colonies of 0.6 mm in diameter. Improved growth results by the addition of 0.5 mg of L-cysteine hydrochloride/l. Colonies are off-white, convex and granular, and contain non-motile, Gram-negative, β-haemolytic cocco-bacilli. Alkaline phosphatase (weakly positive/variable), arginine dihydrolase, H_2S (weakly positive/variable), indole (weakly positive/variable) and lipases are produced, but not α-glucosidase, *N*-acetyl glucosamine, catalase, β-galactosidase, β-glucuronidase, lysine decarboxylase or oxidase. Nitrates are not reduced. The Voges Proskauer reaction is negative. Neither aesculin, gelatin, Tween 80 nor urea is attacked. Citrate is not utilised. Fructose, glucose and maltose are fermented, but not glycogen, lactose, mannitol, mannose, ribose, sucrose or xylose. Growth occurs at 10°C, but not at 37°C, and in 1–4%, but not 5% (w/v), sodium chloride.

The organism was linked tenuously to the Neisseriaceae and Pasteurellaceae.

The unnamed organism associated with *Varracalbmi* was described as follows:

Causal agent of Varracalbmi

The small, i.e. 1 mm in diameter, colonies, which grow anaerobically into the agar medium, comprise Gram-negative non-motile slender fermentative rods sometimes arranged as short chains (when grown in broth), which produce arginine dihydrolase, oxidase but not catalase, indole or lysine or ornithine decarboxylase, attack starch (weakly) and lecithin (weakly) but not aesculin, casein, chitin, gelatin, Tween 20 or 80 or urea, and are non-haemolytic (α-haemolysis was recorded after a week), require NaCl, and grow at 4–22°C, do not reduce nitrates, but are sensitive to the vibriostatic agent, O/129. Growth occurs with galactose, glucose, glycerol, lactose, maltose, mannitol, mannose and sorbitol, but not arabinose, cellobiose, erythritol, melibiose, raffinose or rhamnose (Valheim *et al.*, 2000).

The authors considered that the organism is linked to the Pasteurellaceae or Vibrionaceae (Valheim *et al.*, 2000), and this seems appropriate.

The unnamed Gram-negative organism from ulcerated rainbow trout was linked to *Ultramicrobacterium* by 16S rRNA sequencing (homology = 95%) (Austin *et al.*, 2003).

There has been a trend away from the conventional phenotypic approach of characterising fish pathogens to molecular methods. Whereas the use of new technologies is to be encouraged, an ongoing dilemma remains concerning the authenticity of isolates. Also, too many conclusions seem to result from the examination of too few isolates.

5

Isolation/Detection

There is no single technique suitable for the recovery of all known bacterial fish pathogens. Scientists need to use a combination of methods and incubation conditions to achieve pure cultures.

To an extent, the range of media to be used is governed by personal choice and experience (Table 5.1). The formula of commonly used media is included in an appendix (Appendix 5.1) at the end of the chapter.

- For marine fish, it is advisable to include media prepared with seawater, e.g. seawater/marine 2216E agar (for example, as supplied by Difco).
- For marine fish with damaged gills, low nutrient agar, such as cytophaga agar (see Appendix 5.1) prepared in seawater, should be used.
- For freshwater fish, the routine use of TSA and BHIA (as supplied by Difco or Oxoid) is recommended.

Although most of the pathogens are aerobic, it is worthwhile remembering that *Cl. botulinum* and *Eu. tarantellae* are anaerobic. For the former, Robertson's meat broth (Appendix 5.1) should be used for isolation, whereas with the latter, BHIA is satisfactory. For some aerobic organisms, i.e. *Acinetobacter*, atypical *Aer. salmonicida* and *Ph. damselae* subsp. *piscicida*, the use of media supplemented with blood aids isolation.

Table 5.1. Methods of isolation for bacterial fish pathogens

Medium[a]	Temperature of incubation (°C)	Pathogen
Aeromonas selective medium	?	*Aer. veronii* biovar sobria
BHIA	20–37°C	*Cit. freundii*, *Cor. aquaticum*, *Edw. ictaluri*, *Edw. tarda*, *Haf. alvei*, *Halomonas cupida*, *Lactococcus garvieae*, *Lactococcus piscium*, *Planococcus* sp., *Sal. enterica* subsp. *arizonae*, *Ser. liquefaciens*, *Ser. marcescens*, *Sta. aureus*, *Sta. epidermidis*, *Streptococcus* spp., *Y. ruckeri*
BHIA	22–24°C (anerobically)	*Eu. tarantellae*
BHIA supplemented with 5–10% (v/v) blood	20–25°C	*Cit. freundii*, *Haf. alvei*, *Ph. damselae*, *Salmonella enterica* subsp. *arizonae*
BHIA supplemented with 3% (w/v) NaCl	15–25°C	*Shewanella putrefaciens*
BHIA supplemented with 10% (v/v) foetal calf serum and 1% (w/v) NaCl	22°C	*Streptobacillus*
Blood agar	15–37°C	Streptococci, *Vag. salmoninarum*, *Y. intermedia*
Blood agar	15 or 22°C	*Rhodococcus erythropolis*
Blood agar supplemented with 1.5 % (w/v) NaCl	15°C	*V. splendidus*, *V. tapetis*
Blood agar supplemented with 0.5–1.5 % (w/v) NaCl	22–25°C	*Acinetobacter* sp., *Ph. damselae* subsp. *piscicida*, *V. logei*, *Moritella marina*, *Pasteurella skyensis*
Campylobacter-selective agar	25°C	*Campylobacter cryaerophilus*
CBB	15–25°C	*Aer. salmonicida*
Cystine heart agar supplemented with 1% (w/v) haemoglobin	?	*Francisella* sp.

Cytophaga agar	18–20°C	*Fla. branchiophilum*, *Fla columnare*, *Fla. hydatis*, *Fla. johnsoniae*, *T. ovolyticum*, *Fla. psychrophilum*
Cytophaga agar prepared in sea water	20°C	*Chrys. scophthalmum*, *T.. maritimum*, *Myxococcus piscicola*, *Sporocytophaga* sp. (possibly)
Glucose asparagine agar	25–37°C	*Noc. salmonicida*
Hayflick medium	Room temperature	*Mycoplasma mobile*
KDM2/SKDM	15°C	*Ren. salmoninarum*
Löwenstein–Jensen medium/Dorset egg medium	15–22°C	*Mycobacterium* spp., *Nocardia* spp.
Marine agar	28°C	*Pseudoalteromonas piscicida*
Middlebrook 7H10 agar	30°C	*Myc. pseudoshottsii*, *Myc. shottsii*
Middlebrook medium	25°C	*Myc. montefiorense*
Nutrient agar	20–25°C	*Haf. alvei*, *Ps. anguilliseptica*, *Ps. chlororaphis*, *V. cholerae*
Nutrient agar	37°C	*Bacillus* spp.
1% Ogawa-egg medium	30°C	*Myc. gordonae*
0.1% (w/v) peptone and agar prepared in sea water	20–25°C	*Pseudoalteromonas piscicida*
Robertson's meat broth	30°C (anaerobically)	*Cl. botulinum*
Seawater agar (marine 2216E agar)	15–25°C	*Ph. damselae* subsp. *piscicida*, *V. alginolyticus*, *V. anguillarum*, *V. cholerae*, *V. fischeri*, *V. harveyi*, *V. ordalii*, *V. vulnificus*
Skimmed milk agar	15–25°C	*Janthinobacterium* sp., *Micrococcus luteus*, *Planococcus* sp.
TCBS	15–25°C	*Ph. damselae*, *V. alginolyticus*, *V. anguillarum*, *V. fischeri*, *V. harveyi*, *V. ordalii*, *V. pelagius* and *V. splendidus*

(*continued*)

Table 5.1 (*cont.*)

Medium[a]	Temperature of incubation (°C)	Pathogen
Tissue culture (salmonid cell line)	12–21°C	*Francisella* sp., *Piscirickettsia salmonis*
TSA possibly supplemented with 1–2% (w/v) NaCl	15–25°C	*Aer. allosaccharophila*, *Aer. caviae*, *Aer. hydrophila*, *Aer. salmonicida*, *Aer. sobria*, *Bacillus* sp., *Car. piscicola*, *Cit. freundii*, *En. faecalis* subsp. *liquefaciens*, *Pantoea agglomerans*, *Esch. vulneris*, *Flavobacterium* sp., *J. lividum*, *Klebsiella pneumoniae*, *Lactobacillus* spp., *Micrococcus luteus*, *Moraxella* sp., *Planococcus* sp., *Plesiomonas shigelloides*, *Pr. rettgeri*, *Ps. fluorescens*, *Ps. pseudoalcaligenes*, *Rhodococcus* sp., *Ser. marcescens* *Ser. plymuthica*, *Sta. warneri*, *V. harveyi*, *V. pelagius*, *V. salmonicida*, *V. splendidus*, *V. vulnificus*, *Y. ruckeri*
TSA supplemented with 10% (v/v) horse serum	Unspecified	*Aquaspirillum*
Yeast extract glucose agar	25°C	*Vag. salmoninarum*

[a] Most of these media may be obtained from Difco and/or Oxoid

ANAEROBES

Clostridiaceae representative

Clostridium botulinum

Isolation may be achieved by use of straightforward anaerobic techniques. Samples of intestinal contents, which probably support a resident anaerobic microflora, and internal organs should be homogenised in 1% (w/v) peptone phosphate buffer at pH 7.0, and diluted five-fold. These diluted samples should be inoculated into 100 ml or 200 ml aliquots of Robertson's meat broth (see Appendix 5.1), with subsequent anaerobic incubation at 30°C for up to 6 days. Thereupon, the presence of *Cl. botulinum* and its toxicity may be assessed (Cann *et al.*, 1965a, b; Cann and Taylor, 1982). In addition, sterile culture filtrates may be injected into mice and/or fish to assess the presence of toxic factors (Cann and Taylor, 1982). However, the recovery of *Cl. botulinum* from intestinal samples should not be used as the sole reason for diagnosing botulism, in view of its possible widespread presence in this habitat (see Trust *et al.*, 1979).

Eubacteriaceae representative

Eubacterium tarantellae

Samples of tissues should be plated on BHIA, whereupon bacterial cultures will develop after 7 days anaerobic incubation at room temperature. Additionally, tissues may be inoculated into Brewer's thioglycollate medium (Appendix 5.1; Udey *et al.*, 1977).

GRAM-POSITIVE BACTERIA—THE "LACTIC ACID" BACTERIA

Carnobacteriaceae representatives

Carnobacterium piscicola (and the lactobacilli)

The use of TSA or BHIA with incubation at 22–24°C for 48 h has been advocated (Ross and Toth, 1974; Cone, 1982; Hiu *et al.*, 1984).

Enterococcaceae representative

Vagococcus salmoninarum

Schmidtke and Carson (1994) used Oxoid blood agar base supplemented with 7% (v/v) defibrinated sheep blood with incubation at 25°C for 48 h to recover cultures from brain, kidney, peritoneum, spleen and testes.

Streptococcaceae representatives

Recovery is straightforward, involving use of bovine blood tryptose agar (Naudé, 1975; Roode, 1977; Boomker *et al.*, 1979), Columbia agar (Appendix 5.1), 5% (v/v) defibrinated sheep blood agar (Doménech *et al.*, 1996), Todd–Hewitt broth (Appendix 5.1), Todd–Hewitt agar (Nomoto *et al.* 2004), nutrient agar supplemented with rabbit blood (Kitao *et al.*, 1981), TSA (Teskeredzic *et al.*, 1993; Michel *et al.*, 1997), 10% (v/v) horse blood in Columbia agar (Oxoid), 10% (v/v) horse serum in Columbia agar (Austin and Robertson, 1993), yeast extract glucose agar (Appendix 5.1; Michel *et al.*, 1997) or BHIA (Minami *et al.*, 1979; Ugajin, 1981; Kusuda *et al.*, 1991; Eldar *et al.*, 1994). Media may be supplemented with 1% (w/v) sodium chloride (Austin and Robertson, 1993). Inoculated media should be inoculated with diseased tissue, notably from the kidney, and incubated at 15–37°C for up to 7 days (usually 48 or 72 h) when "dull grey" colonies approximately 1–2 mm in diameter develop. These colonies contain cocci in chains. It must be emphasised that an incubation temperature of 37°C is in excess of the normal growth temperature of many fish species, notably salmonids. This indicates that the organisms may well have been derived from warm-blooded animals, and may, therefore, constitute a public health risk.

Heart infusion agar supplemented with thallium acetate and oxolinic acid or with colistin sulphate and oxolinic acid was evaluated for the selective recovery of *Str. iniae* from Japanese flounder and the fish farm environment. Defibrinated horse blood was also added to determine haemolysin pattern. The result was recovery of *Str. iniae* from brain, intestine and kidney of diseased fish (Nguyen and Kanai, 1999).

AEROBIC GRAM-POSITIVE RODS AND COCCI

Renibacterium salmoninarum

Cultivation of the causal agent of BKD *in vitro* from chinook salmon was not achieved until Earp (1950) used a nutrient-rich medium, containing fish extract, glucose, yeast extract and bovine serum/meat infusion, with incubation at 15 or 20°C. An improvement resulted from use of minced chick embryos in 1% (w/v) agar or on Dorset egg medium (Earp *et al.*, 1953). Nevertheless, growth was generally poor, even after prolonged incubation periods of $\geq$14 days. To prove that the growth was of the pathogen, Earp successfully inoculated the bacterial culture into healthy chinook salmon, and eventually recovered it again from the resultant kidney lesion. Continuing this pioneering work, Ordal and Earp (1956) supplemented Dorset egg medium with 0.05–1.0% (w/v) L-cysteine, tryptone and yeast extract, and succeeded in isolating the pathogen in 3–4 weeks following incubation at 17°C. The outcome of this work was the formulation of cysteine blood agar with which Koch's postulates were fulfilled (Appendix 5.1; Ordal and Earp, 1956). Foetal calf serum was substituted for human blood in a modification proposed by Evelyn *et al.* (1973). This was developed further by removing sodium chloride and substituting peptone for tryptone and beef extract (KDM2; Appendix 5.1; Evelyn, 1977); this medium is now used commonly for growth of the BKD organism. In a parallel development, Wolf and

Dunbar (1959) used Mueller–Hinton agar supplemented with 0.1% (w/v) L-cysteine hydrochloride (MHC) to culture the pathogen, although success with this medium did not occur with Smith (1964). However, Bullock *et al.* (1974) confirmed the value of MHC, although this has been subsequently contended by Evelyn (1977). Serum-rich KDM2 was considered to be superior to serum-deficient MHC, indicating the benefit of serum for the cultivation of the BKD organism. This was further supported by Paterson *et al.* (1979), who supplemented MHC with 10% (v/v) foetal calf serum, and successfully used the medium for isolating the pathogen from Atlantic salmon. Daly and Stevenson (1985a) proposed replacing serum with charcoal, which serves as a detoxicant (Appendix 5.1—charcoal agar). However, these media, being extremely rich in composition, are generally suitable for the growth of many aerobic, heterotrophic bacteria. Moreover, fast-growing organisms may rapidly outcompete and overgrow the slower-growing BKD organism. A solution was proposed by Evelyn (1977), who advocated the use of a drop-plating technique (essentially, this is analogous to dilution plates, which dilute out potential interference by fast-growing heterotrophs). In a later report, Evelyn (1978) recommended the use of peptone (0.1% w/v)–saline (0.85% w/v) as a diluent to remove any inhibiting factors against the pathogen, which may be present in kidney tissue (Evelyn *et al.*, 1981; Austin, 1986). Some inconsistencies in the performance of KDM2 were attributed to variation in the composition of the commercial peptone (Evelyn and Prosperi-Porta, 1989). To overcome this inconsistency when single lots of peptone were not available, two possible modifications were suggested. First, a "nurse" culture technique was reported. This technique accelerated the growth of the BKD organism, and increased the sensitivity at which the pathogen could be detected. The technique, which was based on satellitism or cross-feeding, involved inoculating the nutritionally fastidious pathogen next to a non-fastidious feeder—the nurse organism. Evelyn *et al.* (1989) placed drops of a dense suspension of a stock culture of the BKD organism (= the nurse organism) onto the centre of KDM2 plates. Samples, suspected of containing the pathogen, were placed as 25 µl drops around the periphery of the nurse culture. With incubation, the nurse culture grew rapidly, presumably modified the conditions in the KDM2, and thus enhanced the growth of the pathogen in the periphery. For example, colonies of the BKD organism were observable after incubation for 19 days, compared with 25 days for the conventional approach. The second modification involved supplementing KDM2 agar with a small amount of spent (KDM2) broth that was previously used for growing the pathogen. In both cases, it seems that an unknown metabolite serves as a growth stimulant (Evelyn *et al.*, 1989, 1990).

Until the advent of selective isolation techniques, initial isolation of the pathogen from fish tissues was an uncertain affair, prone to contamination by fast-growing aerobic heterotrophs. With this in mind, a selective isolation medium, SKDM (Appendix 5.1; Austin *et al.*, 1983a) was devised, which proved to be effective for isolation of the pathogen from dilute samples. SKDM permitted the recovery of the pathogen from seeded river water, and from the kidney and faeces of experimentally infected fish (Embley, 1983; Austin and Rayment, 1985). In contrast, the pathogen was not recovered on corresponding KDM2 plates, which were completely overgrown by other bacteria. Clearly, selective media, such as SKDM, should prove

useful in further ecological studies on the causal agent of BKD. In a comparison of KDM2, SKDM and the charcoal-containing derivative, it was determined that the selective medium (SKDM) was most effective for the primary isolation of the pathogen from Atlantic salmon (Gudmundsdóttir *et al.*, 1991). In this comparison of positive samples, 91%, 60% and 35% were positive on SKDM, the charcoal containing derivative and KDM2, respectively. Clearly, the selective medium enhanced significantly the ability to recover the pathogen. Moreover, serum was more advantageous than charcoal as a medium supplement. However, long incubation periods of 12–19 weeks were necessary to recover colonies on the media from dilute samples (Benediktsdóttir *et al.*, 1991). In a subsequent comparison of media for the recovery of *Ren. salmoninarum* from head kidney of rainbow trout, the best recovery was on KDM2 supplemented with 10% (v/v) spent medium (used previously for the growth of the pathogen) followed by SKDM, and then KDM2 with charcoal (Chambers and Barker, 2006).

The question concerning the necessary growth requirements for the pathogen was addressed in a detailed study by Embley *et al.* (1982). These workers formulated a rich, semi-defined medium devoid of serum (Appendix 5.1—semi-defined medium), which was suitable for the cultivation of cells, but not for the initial isolation of cells from infected fish tissues. The semi-defined medium was used, however, to obtain biomass destined for lipid analyses (Embley *et al.*, 1983), and inocula for nutritional and physiological studies. Subsequently, Shieh (pers. commun.) described a complex blood-free medium, which permits the growth of the pathogen.

With the increasing use and sophistication of molecular techniques, their use for culture-independent detection systems are becoming increasingly used in human and veterinary medicine. A nested RT-PCR has shown promise for the detection of mRNA from viable cells of *Ren. salmoninarum* from fish tissues (kidney and ovarian fluid) with detection limits stated to be 1–10 bacterial cells (Cook and Lynch, 1999).

Bacillaceae representatives

Bacillus spp.

Oladosu *et al.* (1994) relied on nutrient agar and incubation at the comparatively high temperature of 37°C for an unspecified period to isolate *Bacillus* spp.

Bacillus mycoides

Material from ulcers, brain, kidney, liver and necrotic muscle were inoculated onto a range of media, including 5% (v/v) sheep blood in blood agar base, Mueller–Hinton agar (for example, as supplied by Difco or Oxoid) and BHIA with incubation at an unspecified temperature for an unstated duration (Goodwin *et al.*, 1994). Raised, rhizoidal colonies with filamentous, swirling patterns developed.

Corynebacteriaceae representative

Corynebacterium aquaticum

Brain tissue samples were plated onto BHIA and TSA with incubation at 25°C for 48–72 h (Baya *et al.*, 1992b).

Micrococcaceae representative

Micrococcus luteus

Cultures were recovered following incubation of swabbed material (kidney, spleen and ascitic fluid) on skimmed milk agar with incubation at 25°C for 48–72 h (Appendix 5.1; Austin and Stobie, 1992a).

Mycobacteriaceae representatives

Mycobacterium spp.

With many cases of mycobacteriosis, there is no attempt made at isolation of the pathogen. Yet, great, scientific conclusions seem to result from the examination of only histological material. Nevertheless, attempts at isolating the aetiological agent often fail, indicating a fastidiousness on the part of the pathogen. Some success occurs by inoculating pieces of infected tissue (especially kidney, liver or spleen) on standard mycobacterial media, including Petragnani, Löwenstein–Jensen, Middlebrook 7H10 and Dorset egg media (see Appendix 5.1), or even blood agar, BHIA or TSA, whereupon growth may occur in 2–28 days at incubation temperatures of 15–30°C in aerobic or microaerophilic, i.e. 3–5% carbon dioxide, conditions (Dulin, 1979; Lansdell *et al.*, 1993). Most difficulty surrounds the recovery of mycobacteria from marine fish species. Clearly, more effort is required to understand the precise nutritional requirements of these organisms. *Myc. abscessus* was not isolated on Löwenstein–Jensen medium. Instead, Middlebrook 7H10 medium was modified by the addition of 10 μg/ml of amphotericin B, 500 μg/ml of chloramphenicol, 5 μg/ml of gentamicin or 30 μg/ml of cephalothin—either singly or in combination. Diseased fish were immersed in the modified Middlebrook 7H10 broth for 1 h at room temperature, before homogenisation, and inoculation of modified Middlebrook 7H10 agar with incubation at 25°C for 14–28 days (Teska *et al.*, 1997). Middlebrook 7H10 agar with incubation at 23°C for 4–6 weeks, but not Löwenstein–Jensen medium, permitted the recovery of *Myc. shottsii* (Rhodes *et al.*, 2003). In comparison, *Myc. montefiorense* was isolated on blood agar and Middlebrook medium after incubation at 25°C for up to 20 weeks (Levi *et al.*, 2003). To recover *Myc. gordonae*, Sakai *et al.* (2005) dipped the spleen and liver of infected guppies into 2% (w/v) sodium hydroxide for 20 min, and then inoculated 1% Ogawa egg medium with incubation at 30°C for one month.

Nocardiaceae representatives

Nocardia spp.

Essentially, the same isolation methods as for mycobacteria, e.g. use of Löwenstein–Jensen medium, have been employed with *Nocardia*. The initial development of colonies usually occurs within 21 days at 18–37°C (Valdez and Conroy, 1963; Conroy, 1964; Snieszko *et al.*, 1964b; Heuschmann-Brunner, 1965a; Campbell and MacKelvie, 1968; Ghittino and Penna, 1968).

A loop-mediated isothermal amplification (LAMP) technique, which is a modern molecular approach for rapidly amplifying DNA with a high degree of specificity, has been proposed for the rapid and sensitive detection of *Noc. seriolae* amplifying up to 10^3 CFU/ml (this was 10-fold more sensitive than PCR) (Itanno *et al.*, 2006).

Nocardia salmonicida

The organism may be isolated on glucose asparagine agar, glycerol asparagine agar and yeast extract malt extract agar, Emerson agar, Bennett agar or nutrient agar, following incubation aerobically at 25–37°C for an undisclosed period (Appendix 5.1; Rucker, 1949).

Rhodococcus sp.

Dense growth of two colony types was obtained from diseased tissue following inoculation of blood agar, MacConkey agar (Appendix 5.1) and TSA with incubation at room temperature (19°C) for up to 14 days. Both colony types were also recovered from the kidney and spleen of chinook salmon with ocular lesions (Backman *et al.*, 1990; Claveau, 1991).

Rhodococcus erythropolis

Kidney and sometimes ascitic fluid was inoculated onto 4% (v/v) bovine or horse blood agar with incubation at 15 and 22°C for 7 days (Olsen *et al.*, 2006a).

Planococcaceae representative

Planococcus sp.

The pathogen was recovered from kidney swabs following incubation on BHIA, skimmed milk agar or TSA at 25°C for up to 7 days. Cultures comprised off-white to yellow, raised, shiny colonies, which were 1–2 mm in diameter after 48 h (Austin *et al.*, 1988).

Staphylococcaceae representatives

Staphylococcus aureus

Eye and brain tissue revealed the presence of bacteria. However, the precise isolation procedures were not stated (Shah and Tyagi, 1986).

Staphylococcus epidermidis

The pathogen was successfully cultured on BHIA following incubation at 37°C for 24 h (Kusuda and Sugiyama, 1981). However, the use of such a high temperature is puzzling, and it is conceivable that mesophiles, preferring lower growth temperatures, may have been overlooked.

Staphylococcus warneri

Growth was achieved from the kidney and liver of diseased fish on TSA with incubation at 22–25°C for 48–72 h (Gil *et al.*, 2000).

GRAM-NEGATIVE BACTERIA

Aeromonadaceae representatives

Aeromonas allosaccharophila

The precise isolation method was not described. However, it was mentioned that growth occurred after an unstated period on TSA at 4–42°C (Martinez-Murcia *et al.*, 1992).

Aeromonas caviae

Diseased tissues were homogenised in 0.1% (w/v) peptone, before inoculation of TSA and blood agar with incubation at 22°C for 48 h (Candan *et al.*, 1995).

Aeromonas hydrophila

This is quite straightforward, involving use of kidney swabs with non-selective media, such as nutrient agar or TSA, or selective media, namely Rimler–Shotts medium (Appendix 5.1; Shotts and Rimler, 1973) or peptone beef extract glycogen agar (Appendix 5.1; McCoy and Pilcher, 1974) with incubation at 20–25°C for 24–48 h. Typically, on non-selective media, cream, round, raised, entire colonies of 2–3 mm diameter develop within 48 h at 25°C.

Aeromonas salmonicida

Under ordinary circumstances, i.e. in cases of classical furunculosis caused by "typical" strains of *Aer. salmonicida*, the pathogen may be readily recovered from diseased fish, especially from surface lesions and the kidney, by use of standard, non-selective, bacteriological agar media. TSA has been commonly used for this purpose. On TSA, the occurrence of colonies surrounded by a dark-brown water-soluble pigment after incubation at 20–25°C for 3–4 days, is considered as indicative of the presence of *Aer. salmonicida*. However, it must be remembered that non-pigmented or slowly pigmented strains of the pathogen occur, and also that some other bacteria produce diffusible brown pigments, e.g. *Aer. hydrophila* and *Aer. media*. In addition, if the fish have succumbed to secondary infection with other micro-organisms, isolation of *Aer. salmonicida* becomes much more difficult because of overgrowth by other bacteria. Thus, growth of *Aer. salmonicida* may be suppressed or pigment production inhibited. For these reasons, McCarthy and Roberts (1980) recommended that a minimum of six fish should be sampled from any disease outbreak where the presence of *Aer. salmonicida* is suspected. Also, they suggested that samples destined for bacteriological examination should be taken from skin lesions, in all stages of development. In a similar vein, Daly and Stevenson (1985b) concluded that it is advisable to sample other organs, i.e. heart, liver and spleen, in addition to the kidney in order to increase the chances of detecting *Aer. salmonicida*. Indeed, these workers found that 45% (14/31) of successful isolations of the pathogen from brown trout, *Salmo trutta*, were from organs other than kidney. Likewise, in a study to detect carrier rates of the pathogen, Rose *et al.* (1989) concluded that sampling of just the kidney might result in an underestimate of the numbers of fish harbouring the pathogen. Thus, they recommended examining the intestine as well as the kidney for the presence of *Aer. salmonicida*. The problem is with the asymptomatic carriers from which recovery of the pathogen is notoriously difficult without stressing the fish (Cipriano *et al.*, 1997).

In an attempt to improve the chances of recovering *Aer. salmonicida* from lake trout in a hatchery experiencing an outbreak of furunculosis, an enrichment procedure using TSB was evaluated by Daly and Stevenson (1985b). This entailed placing swabbed material, derived from the organs of diseased animals, into TSB with incubation at 26°C for 48 h. Then, the resulting broth cultures were streaked for single-colony isolation onto plates of TSA. The results indicated that the recovery of *Aer. salmonicida* was twice that of using the conventional direct plating of swabbed material onto TSA.

The use of BHIA for isolation and maintenance of *Aer. salmonicida* has been recommended by some groups. In fact, we have observed that a greater proportion of rough-type colonies (this trait is associated with virulence) of *Aer. salmonicida* were recovered on BHIA than TSA.

In several instances, media supported with blood have been employed for the isolation of the pathogen, especially atypical isolates from cyprinids. McCarthy (1977a), however, stated that unsupplemented media, e.g. TSA, should by used in preference to blood-containing media, but no explanation was given, other than that the observations had resulted from extensive personal experiences.

A more recent addition to the media employed for the isolation of *Aer. salmonicida* is CBB (Appendix 5.1), as developed originally by Udey (1982). CBB was evaluated as a differential and presumptive medium for use in the identification of *Aer. salmonicida* in clinical specimens (Markwardt *et al.*, 1989). The results showed that CBB was effective in differentiating *Aer. salmonicida* among mixed bacterial populations obtained from asymptomatic fish. In laboratory-based experiments, CBB was also successful in differentiating *Aer. salmonicida* colonies from mixed cultures containing *Aer. hydrophila* or *Y. ruckeri*. Here, *Aer. salmonicida* colonies were dark blue. An interesting development concerned the ability to detect *Aer. salmonicida* within 72 h by filtering 100 ml amounts of water through 0.45 μm pore size Millipore cellulose acetate and nitrate filters, and incubating the filters on CBB (Ford, 1994). However, it is apparent that the *Aer. salmonicida* cells must possess the A-layer for the differentiating capacity of CBB to be effective. Also, other aquatic organisms, such as the purple-pigmented *Chromobacterium* and *Janthinobacterium*, may produce dark-blue colonies on CBB. Nevertheless, CBB is a promising addition to the narrow range of media which may be used for the primary isolation of *Aer. salmonicida*.

The stress-induced furunculosis test to detect covertly infected fish has been very successful, and involves intramuscular injection with corticosteroid, namely 20 mg prednisolone acetate/kg of fish followed by increasing the water temperature typically from 12 to 18–20°C. Cultures of *Aer. salmonicida* may be then recovered on TSA or CBB within 3–6 days (Bullock and Stuckey, 1975b; McCarthy, 1977b; Smith, 1991; Bullock *et al.*, 1997). Using such stressed rainbow trout, culturing was the most sensitive method for detecting *Aer. salmonicida* (detected 40 positives out of 80 followed by a direct FAT (detected 6 positive) and then a commercial ELISA system (detected 6 positives)) (Bullock *et al.*, 1997). Overall, the culture of gill and mucus was more sensitive (39 positives out of 80 fish examined) than kidney and spleen (18 positives) (Bullock *et al.*, 1997).

In the case of CE and goldfish ulcer disease, the pathogen appears to be located more or less exclusively in the skin lesions. Thus, Bootsma *et al.* (1977) used an inoculating wire, which was plunged below the transparent epidermis at the edge of the ulcer, into the haemorrhagic zone. A loopful of the resulting material was streaked onto agar media. According to Bootsma, satisfactory growth occurred on tryptone-containing media supplemented with serum. Although the enrichment of a culture medium by the addition of serum has been deduced as necessary for the initial recovery of some fastidious strains, notably non-pigmented cultures, Bootsma *et al.* (1977) determined that the fastidiousness of the isolates decreased during maintenance *in vitro*. In our experience, strains associated with non-salmonid fish are extremely difficult to isolate. We have greatest success with blood agar (blood agar base [Oxoid] supplemented with 10% v/v horse, sheep or bovine blood), which is inoculated and incubated at 15–18°C for up to 7 days. Even with this method, *Aer. salmonicida* is recovered from only a small proportion of the clinically diseased fish. This begs the question about the reasons for culturability, when the pathogen is recovered from only a proportion of obviously infected animals. Microscopy will often reveal a greater number of bacterial cells than might be deduced from the results

of plating experiments. Perhaps, as has been argued with L-forms, a threshold number of bacterial cells need to be present to enable some to be capable of producing growth in broth or on solid medium. Also, the definition of growth needs to be carefully considered, insofar as the basic criterion reflects observations with the naked eye, i.e. turbidity in broth or clearly visible colonies. The limited growth of micro-colonies may well be missed by classical bacteriological methods.

Aeromonas sobria

Pure culture growth was obtained from kidney, liver and spleen of moribund animals following inoculation of TSA with incubation at 22°C for possibly one or two days (Toranzo *et al.*, 1989).

Aeromonas veronii biovar sobria

Scrapings from the ulcer were inoculated on to *Aeromonas*-selective medium containing 5 μg/ml of ampicillin with unspecified incubation conditions (Rahman *et al.*, 2002a).

Alteromonadaceae representatives

Pseudoalteromonas piscicida

Individual diseased eggs were placed on marine agar with incubation at 28°C for 2 days (Nelson and Ghiorse, 1999).

Shewanella putrefaciens

Bacteria were isolated from the kidney, liver and spleen following inoculation onto BHIA supplemented with 3% (w/v) sodium chloride, with incubation at an unspecified temperature (presumed to be ≥37°C) for an undetermined period (Saeed *et al.*, 1987).

Campylobacteriaceae representative

Arcobacter cryaerophilus

Growth occurred on *Campylobacter*-selective agar and enriched TSA with incubation at 25°C for 1–7 days (Aydin *et al.*, 2002).

Enterobacteriaceae representatives

Citrobacter freundii

Pure culture growth was recovered from kidney on rabbit blood agar (Sato *et al.*, 1982). Subsequently, similar organisms have been obtained from kidney homogen-

ates spread over the surface of BHIA and TSA with incubation at 25 or 37°C for 24–48 h.

Edwardsiella ictaluri

Isolation has been readily achieved from kidney, liver, spleen, intestine, brain and skin or muscle lesions by inoculation of material into BHIA or blood agar. Following incubation at 26°C for 48 h, smooth circular (2 mm diameter), slightly convex, entire, non-pigmented colonies develop (Hawke, 1979). A selective medium has been described by Shotts and Waltman (1990) [Appendix 5.1].

Edwardsiella tarda

Isolation of *Edw. tarda* from diseased fish is a straightforward procedure involving the use of commonly available media, such as TSA (Meyer and Bullock, 1973; Alcaide *et al.*, 2006) or BHIA (Amandi *et al.*, 1982). On such media, small, round (0.5 mm in diameter), raised, transparent colonies develop in 48 h at 24–26°C (Meyer and Bullock, 1973). The use of thioglycollate broth followed by subculturing on BHIA has also been used successfully (Appendix 5.1; Amandi *et al.*, 1982). Indeed, this two-step enrichment procedure has proved to be more sensitive than BHIA used alone. In one experiment, this two-step procedure enabled the recovery of *Edw. tarda* from 19% of a group of chinook salmon, compared with only 2% recovery on BHIA used alone (Amandi *et al.*, 1982).

LAMP has been proposed for the rapid and sensitive detection of *Edw. tarda* (Savan *et al.*, 2004). Developed to detect the pathogen in Japanese flounder kidney and spleen (and seawater), results were achieved in 45 min. An indication of sensitivity is that the LAMP technique was positive for seawater containing 3.2×10^2 CFU of *Edw. tarda*.

Escherichia vulneris

Isolates were recovered on TSA with incubation at 25°C for 48 h (Aydin *et al.*, 1997).

Hafnia alvei

Cultures were obtained from the internal organs and subcutaneous tissues at the base of the pelvic fins on BHIA and nutrient agar supplemented with 10% (v/v) sheep blood following incubation at an unspecified temperature for 48 h, whereupon small, round, smooth colonies developed. Teshima *et al.* (1992) isolated the organism on heart infusion agar following incubation at 30°C for 2 days.

Klebsiella pneumoniae

Pure cultures were obtained after shaking tails and pectoral fins in 0.9% (w/v) saline for 5 min, and thereafter spreading 0.1 ml volumes on TSA with incubation at 15°C for 7 days (Daskalov *et al.*, 1998).

Plesiomonas shigelloides

This was accomplished by inoculating samples of kidney and liver onto plates of TSA with incubation at 22–37°C for an unspecified period whereupon round, raised, off-white, circular colonies developed (Cruz *et al.*, 1986).

Pantoea agglomerans

Pale-yellow colonies were recovered from kidney and blood on TSA supplemented with 2% (w/v) NaCl following incubation at 16°C for 7 days. Subculturing was possible on marine 2216E agar (Hansen *et al.*, 1990).

Providencia rettgeri

Pure culture growth was recovered from the heart, kidney and base of the lesions following inoculation of nutrient agar, blood agar and TSA with incubation at 15–35°C for an unspecified period (Bejerano *et al.*, 1979).

Salmonella enterica subsp. *arizonae*

Samples of internal organs were inoculated onto plates of blood agar, BHIA and MacConkey agar with incubation at 25°C for 48 h. Thereupon, an organism was recovered as pure culture growth from heart, kidney, liver and spleen (Kodama *et al.*, 1987).

Serratia liquefaciens

Isolation was readily achieved from kidney by inoculation of swabbed material onto BHIA and TSA with incubation at 25°C for 24–48 h (McIntosh and Austin, 1990b).

Serratia marcescens

Pure cultures resulted from inoculation of kidney swabs onto plates of BHIA and TSA with incubation at 22°C for 48–72 h (Baya *et al.*, 1992c).

Serratia plymuthica

Pure culture growth was recovered from the kidney and liver following inoculation of TSA with incubation at 22°C for 7 days (Austin and Stobie, 1992b).

Yersinia intermedia

Blood samples were cultured on Oxoid blood agar base supplemented with 7% (v/v) defibrinated sheep's blood at 18°C for 7 days (Carson and Schmidtke, 1993). After 48 h, the kidneys of two fish revealed the presence of dense growth, equated with *Yersinia intermedia*. In addition, other bacteria were recovered including CLB and *Ps. fluorescens*.

Yersinia ruckeri

The pathogen may be readily recovered from kidney on routine bacteriological media, e.g. BHIA or TSA, following incubation at 20–25°C for 48 h whereupon round, raised, entire, shiny, off-white colonies of 2–3 mm diameter develop (e.g. Ross *et al.*, 1966). Three selective media have been devised (Appendix 5.1; Waltman and Shotts, 1984; Rodgers, 1992; Furones *et al.*, 1993). Tween 80 hydrolysis, which occurred commonly among isolates from the U.S.A., resulted in the precipitation of insoluble calcium salts around colonies on Waltman–Shotts medium. However, according to Hastings and Bruno (1985), there were some limitations insofar as there is some growth variation among *bona fide* strains of *Y. ruckeri*. Moreover, non-motile isolates which did not degrade Tween 80 have been recovered in Europe, e.g. Germany (Klein *et al.*, 1994). Ribose ornithine deoxycholate medium of Rodgers (1992) appears to have overcome the problems of the earlier formulation, and laboratory use points to its value for the recovery of *Y. ruckeri*. Furones *et al.* (1993) supplemented TSA with 1% (w/v) SDS, 100 μg/ml of coomassie brilliant blue and 100 μg/ml of Congo red. On this medium, *Y. ruckeri* produced a creamy deposit around the colonies.

Flavobacteriaceae representatives

Generally, the aetiological agents may be readily recovered from diseased tissues on low-nutrient media, with incubation at 10–25°C for 4–14 days. From "halibut jelly", which was used to culture *Chrys. balustinum* as yellow–green ("fluorescent") pigmented colonies (Harrison and Sadler, 1929), scientists have devised many suitable media, of which cytophaga agar (Appendix 5.1; after Anacker and Ordal, 1959) has received greatest use. This is suitable for the isolation of *Fla. hydatis*, *Fla. johnsoniae* (specifically with incubation at 27°C for 7 days; Carson *et al.*, 1993), *Fla. psychrophilum*, *Fla. branchiophilum* and *Fla. columnare*. An improved growth medium for *Fla. psychrophilum* has been published with the outcome that more rapid and luxuriant growth occurred; the recipe was based on cytophaga agar/broth supplemented with galactose, glucose, rhamnose and skimmed milk (Appendix 5.1; Daskalov *et al.*, 1999). Then, Cepeda *et al.* (2004) reported that a glucose, salt, tryptone and yeast extract based medium, coined FLP, was superior for speed and abundance of growth of the pathogen (Appendix 5.1). An alternative approach was to add 5 μg/l of the aminoglycoside antibiotic tobramycin, which was stated to improve recovery of *Fla. psychrophilum* especially from the external surfaces of carrier ayu (Kumagai *et al.*, 2004). Caution has been suggested over the source of ingredients used to make some media. For example, the importance of the brand of beef extract (the Difco product was reported as superior to Gibco or Oxoid) for the growth of *Fla. psychrophilum* on cytophaga agar was highlighted by Lorenzen (1993). Cytophaga agar, prepared in 70% seawater, is ideal for recovery of marine organisms, such as *T. maritimum* (Appendix 5.1; Hikida *et al.*, 1979) and *T. ovolyticum* (Hansen *et al.*, 1992). Other isolation media have been described, including skimmed milk agar, CP medium (Carlson and Pacha, 1968) and Pacha and Ordal's medium (Pacha and Ordal,

1967) for *Fla. hydatis* (Strohl and Tait, 1978), TSA supplemented with 0.5–3.0% (w/v) sodium chloride for *Flavobacterium* spp. (Acuigrup, 1980a), Bootsma and Clerx's medium for *Fla. columnare* (Appendix 5.1; Bootsma and Clerx, 1976), TCY medium for *T. maritimum* (Appendix 5.1; Hikida *et al.*, 1979) and medium K for *Chrys. scophthalmum* (Appendix 5.1; Mudarris and Austin, 1989). Anderson and Conroy (1969) described a seawater-based medium for *Sporocytophaga*-like organisms (Appendix 5.1).

Two selective media containing antibiotics have been devised (Appendix 5.1). These were formulated for the selective recovery of *Fla. columnare* (Fijan, 1969) and myxobacteria (Hsu *et al.*, 1983), respectively. On these media, the pathogens produce characteristically yellow–orange-pigmented colonies.

The problem of overgrowth/outcompetition of the pathogen by saprophytes has been raised in connection with the recovery of the causal agent of flavobacteriosis with the advice that serial dilutions of the (external) samples are needed before plating techniques are used in order to maximise the possibility of recovering the actual disease-causing agent (Tiirola *et al.*, 2002).

A PCR based on the 16S-23S rDNA intergenic spacer region has demonstrated promise for the detection of *Fla. columnare* from catfish in terms of specificity and sensitivity (capable of detecting 7 CFU). The technique was regarded as more sensitive than culturing (Welker *et al.*, 2005).

Francisellaceae representative

Francisella spp.

Initially, the organism was grown in cell cultures at 20°C. Subculturing onto cysteine heart agar at 22°C resulted in dense growth of small, opaque colonies, which increased to 2–3 mm in diameter (Olsen *et al.*, 2006). However, Kamaishi *et al.* (2005) managed to recover an organism from the spleen of an infected three-line grunt using cysteine heart agar supplemented with 1% (w/v) haemoglobin.

Halomonadaceae representative

Halomonas (= Deleya) cupida

Although the wisdom of using homogenates of whole fish for bacteriological examination may be debated, it is probably an acceptable compromise with fry. Nevertheless, care needs to be taken when interpreting results insofar as bacteria may be derived from the normal surface/intestinal microflora as well as from diseased tissues. In this investigation, cultures were obtained from homogenates following inoculation of BHIA with incubation at 25°C for 24 h (Kusuda *et al.*, 1986).

Moraxellaceae representatives

Acinetobacter sp.

Pure cultures were recovered on 5% (w/v) blood agar supplemented with 0.5% (w/v) sodium chloride, following incubation at 22°C for 48 h. Thus, blood, kidney, liver, spleen and ulcers in the muscle appeared to contain dense bacterial populations. Pure cultures were obtained, which were capable of reproducing the infection during laboratory-based studies (Roald and Hastein, 1980).

Moraxella sp.

Pure culture growth of round, raised, translucent, mucoid colonies developed in 48 h of incubation at 22°C of kidney, liver and pancreas tissue on TSA (Baya *et al.*, 1990b).

Moritellaceae representatives

Moritella marina

Benediktsdóttir *et al.* (1998) used 5% (v/v) horse blood agar supplemented with 1.5% (w/v) sodium chloride with incubation at 15°C for 7 days.

Neisseriaceae representative

Aquaspirillum sp.

Lio-Po *et al.* (1998) used TSA supplemented with 10% (v/v) horse serum and cytophaga agar at an unspecified temperature and duration to recover *Aquaspirillum*, *Aer. hydrophila* and *Streptococcus* from diseased animals.

Oxalobacteraceae representative

Janthinobacterium lividum

Homogenates of whole fish (prepared in quarter-strength Ringer's buffer; Oxoid) and, where possible, loopfuls of kidney, liver, spleen, ascitic fluid and material from surface lesions were spread over the surface of a variety of media, including blood agar (5% v/v bovine blood in Gibco blood agar base), cytophaga agar, KDM2, L-F medium (Appendix 5.1) and TSA, with incubation aerobically at 22°C for up to 14 days. Purple-pigmented colonies were apparent after 3 days.

Pasteurellaceae representative

Pasteurella skyensis

Isolation from kidney was on TSA supplemented with 1.5% (w/v) sea salts and 5% (v/v) defibrinated horse blood aerobically at 20°C for 48 h when small, grey colonies

resulted. After initial isolation, culturing was possible on TSA (or Columbia agar) supplemented with 1.5% (w/v) sodium chloride and 10% (v/v) citrated sheep or horse blood (Birkbeck *et al.*, 2002).

Photobacteriaceae representatives

Photobacterium damselae subsp. *damselae*

Isolation may be readily achieved by swabbing ulcerative material onto the surface of BHIA supplemented with 5% (v/v) sheep blood, or TCBS (Appendix 5.1) with incubation at 25°C for an unspecified period (probably 2–5 days) (Fujioka *et al.*, 1988; Sakata *et al.*, 1989; Fouz *et al.*, 1991).

Photobacterium damselae subsp. *piscicida*

The organism may be isolated by inoculating swabs of kidney and/or spleen material onto marine 2216E agar (Difco), nutrient agar or blood agar, with incubation at 25°C for 48–72 h. An improved liquid medium has been described, which may be solidified by the addition of 1% (w/v) agar (Appendix 5.1; Hashimoto *et al.*, 1989). On conventional media, shiny, grey–yellow, entire, convex colonies develop, which are approximately 1–2 mm in diameter after 72 h (Kusuda and Yamaoka, 1972). Another approach, which has met with success, has enabled the recovery of *Ph. damselae* subsp. *piscicida* from water in the vicinity of fish. The method involved filtering 250 ml volumes of water through 0.45 μm cellulose nitrate filters, before transfer (of the filters) to 2216E agar supplemented with 1% (w/v) mannitol and 0.5% (w/v) phenol red. The non-fermenting *Ph. damselae* subsp. *piscicida* produced red colonies (Reali *et al.*, 1997). With this method, the pathogen was detected on sea bass 8 days before the outbreak of disease.

Piscirickettsiaceae representative

Piscirickettsia salmonis

Isolation of the pathogen from the kidney of infected fish was possible in the cytoplasm of salmon cell lines (including CHSE-214) with incubation at 12–21°C (optimally at 15–18°C), whereupon a cytopathic effect was demonstrated in 5–6 days (Fryer *et al.*, 1990). The cell sheet was completely lysed in 14 days. Growth did not occur on bacteriological media, including BHIA, blood agar, mycoplasma medium, charcoal yeast extract agar, Loeffler medium (Appendix 5.1) or Mueller–Hinton agar.

Pseudomonadaceae representatives

Pseudomonas anguilliseptica

Isolation of *Ps. anguilliseptica* is readily achieved from blood, kidney, liver and spleen samples by use of nutrient agar supplemented with 10% (v/v) horse blood or nutrient agar containing 0.5% (w/v) sodium chloride, and adjusted to a pH of 7.4. Incubation

should be at 20–25°C for at least 7 days, when small (≤1mm in diameter), round, raised, entire, shiny, pale-grey colonies develop (Wakabayashi and Egusa, 1972).

Pseudomonas chlororaphis

This may be accomplished by inoculating homogenates, prepared from the entire fish, onto the surface of nutrient agar plates, with incubation at 25°C for 5 days. It is surprising that this simple method enabled the recovery of pure cultures, because contaminants may be expected from the intestine and body surface (Hatai *et al.*, 1975).

Pseudomonas fluorescens

Ps. fluorescens was recovered from most organs as pure culture growth on standard bacteriological media, such as Pseudomonas F agar (Appendix 5.1), blood agar, TSA and nutrient agar, following incubation at 22–28°C for 24–28 h (Csaba *et al.*, 1981b; Ahne *et al.*, 1982).

Pseudomonas pseudoalcaligenes

Ps. pseudoalcaligenes was recovered as mixed cultures with *Serratia plymuthica* from the ascitic fluid and surface lesions following incubation on TSA at 22°C for 3–4 days (Austin and Stobie, 1992b).

Vibrionaceae representatives

Generally, vibrios may be isolated on marine 2216E agar (supplied by Difco) with incubation at 25°C for 2–7 days (e.g. Ishimaru *et al.*, 1996).

Vibrio alginolyticus

This may be readily achieved from blood by inoculation onto TSA prepared with seawater, TCBS or seawater agar with incubation at 15–25°C for 2–7 days. The precise conditions employed by Colorni *et al.* (1981) were not stated. However, this group succeeded in isolating *V. alginolyticus*, *V. anguillarum* and *V. parahaemolyticus* from blood, and long, thin, flexible rods from cases of gill rot. In addition, we have isolated pure culture growth of *V. alginolyticus* from moribund eels.

Vibrio anguillarum

The pathogen may be readily recovered from infected tissue by use of TSA (Traxler and Li, 1972), nutrient agar (Muroga *et al.*, 1976a, b) and BHIA (Tajima *et al.*, 1981) supplemented with sodium chloride at 0.5–3.5% (w/v), seawater agar and TCBS (Bolinches *et al.*, 1988), with incubation at 15–25°C for periods of up to 7 days.

The presumptive identification of *V. anguillarum* has been achieved using a specially designed medium, designated VAM, which combined bile salts with a high sodium chloride concentration, ampicillin, sorbitol and a high pH (Appendix 5.1;

Alsina *et al.*, 1994). On VAM, *V. anguillarum* produced bright-yellow colonies with yellow haloes. Its usefulness was attested by its ability to recognise the majority, i.e. 197/227 = 87%, of *V. anguillarum* isolates. However, some erroneous results occurred insofar as VAM recognised 3/66 = 4% of other vibrios as *V. anguillarum* (Alsina *et al.*, 1994).

Vibrio cholerae (non-O1)

This may be achieved by inoculating kidney material (from swabs) onto the surface of nutrient agar plates, with incubation at 25°C for an undisclosed period (presumably 2–5 days) (Muroga *et al.*, 1979; Kiiyukia *et al.*, 1992).

Vibrio fischeri

By use of TSA supplemented with 2% (w/v) sodium chloride, marine 2216E agar (Difco) and TCBS (Oxoid), virtual pure culture growth of bacteria was recovered from the kidney and liver, following incubation at an unstated temperature for an unspecified duration (Lamas *et al.*, 1990).

Vibrio harveyi

Samples of kidney and liver were inoculated into thioglycollate broth (Appendix 5.1), followed by single-colony isolation on plates of TSA supplemented with 1% (w/v) sodium chloride (Grimes *et al.*, 1984a). Yii *et al.* (1997) used TSA supplemented with 2% (w/v) sodium chloride and TCBS with intestinal fluid from diseased grouper. Generally, the use of TSA, MacConkey agar, TCBS and cytophaga agar were advocated, with incubation at 25–35°C for 24–48 h. Thus according to Kraxberger-Beatty *et al.* (1990), the eyes were removed and homogenised in sterile saline, loopfuls of the material being streaked onto the agar-containing media. It should be emphasised that attempts were not made to surface-sterilise the eyes. Therefore, the resulting homogenate would have also contained aquatic (saprophytic) bacteria, as well as any potential pathogen.

It is unclear from the article describing *V. trachuri* how the organisms were isolated from fish (Iwamoto *et al.*, 1995).

Vibrio logei

Benediktsdóttir *et al.* (1998) used 5% (v/v) horse blood agar supplemented with 1.5% (w/v) sodium chloride with incubation at 15°C for 7 days.

Vibrio ordalii

As with *V. anguillarum*, isolation involves use of seawater agar and TCBS with incubation at 15–25°C for up to 7 days (Ransom, 1978; Ransom *et al.*, 1984).

Vibrio pelagius

Samples from diseased tissues were inoculated onto TSA supplemented with 2% (w/v) sodium chloride, marine 2216E agar and TCBS with incubation at 25°C for 48 h (Angulo *et al.*, 1992).

Vibrio salmonicida

Microscopy suggests that bacteria are rampant throughout infected fish, and especially in the blood and kidney of moribund and freshly dead specimens. Pure cultures may be readily recovered from blood and kidney samples on TSA supplemented with 1.5% (w/v) sodium chloride following incubation at 15°C for up to 5 days (Holm *et al.*, 1985; Egidius *et al.*, 1986). Colonies are small, i.e. $\leq$1 mm in diameter, round, raised, entire and translucent. Inexperienced personnel could easily miss the colonies upon cursory glances at inoculated plates. To some extent, the organism is fragile, and will quickly die at supra-optimum temperatures or by failure to carry out regular subculturing.

Vibrio splendidus

Kidney (anterior), liver, spleen and fluid from within the peritoneal cavity contained bacterial populations, which grew on TSA supplemented with 2% (w/v) sodium chloride and on TCBS with incubation at 22°C for 48 h (Lupiani *et al.*, 1989). Jensen *et al.* (2003) used nutrient agar supplemented with 5% (v/v) sheep blood and 1.5% (w/v) NaCl, which was inoculated with kidney tissue and incubated aerobically at 15°C for up to 7 days.

Vibrio tapetis

Jensen *et al.* (2003) used nutrient agar supplemented with 5% (v/v) sheep blood and 1.5% (w/v) NaCl, which was inoculated with kidney tissue and incubated aerobically at 15°C for up to 7 days.

Vibrio vulnificus

Use of standard bacteriological media, such as seawater agar and TSA supplemented with sodium chloride, and incubation for up to 7 days at 20–25°C is sufficient to obtain cultures of the pathogen (Muroga *et al.*, 1976a, b; Nishibuchi and Muroga, 1977, 1980; Nishibuchi *et al.*, 1979, 1980).

MISCELLANEOUS PATHOGENS

"Candidatus Arthromitus"

Isolation of bacteria with the morphological characteristics of "*Candidatus* Arthromitus" was not achieved (Michel *et al.*, 2002a).

Streptobacillus

Brain heart infusion supplemented with 10% (v/v) foetal calf serum and 1% (w/v) sodium chloride with incubation for 10 days at 22°C was used to recover the novel *Streptobacillus*-like organism (Maher *et al.*, 1995).

UNIDENTIFIED GRAM-NEGATIVE ROD

The intracellular, Gram-negative cocco-bacilli required serum or blood for growth, which was accomplished on 7% (v/v) horse blood agar and 10% (v/v) foetal calf serum medium after 4–14 days incubation at 15 or 22°C (Palmer *et al.*, 1994).

The unnamed organism associated with *Varracalbmi* grew at 22°C on 5% (v/v) citrated bovine blood agar supplemented with 2% (w/v) NaCl from inocula obtained from dermal lesions, kidney and liver (Valheim *et al.*, 2000).

APPENDIX 5.1 MEDIA USED FOR THE ISOLATION AND GROWTH OF BACTERIAL FISH PATHOGENS

Anderson and Conroy's medium for *Sporocytophaga*-like organisms

5.0% (w/v)	enzymic digest of fish muscle
0.1% (w/v)	peptone
0.1% (w/v)	yeast extract
0.9% (w/v)	agar
pH	7.0

This medium is prepared in seawater.

Bootsma and Clerx's medium for *Flavobacterium columnare*

0.05% (w/v)	casitone
0.05% (w/v)	yeast extract
1.0% (w/v)	agar
pH	8.0

Brewer's thioglycollate medium

0.1% (w/v)	Lab-lemco
0.2% (w/v)	yeast extract
0.5% (w/v)	peptone
0.5% (w/v)	dextrose
0.5% (w/v)	sodium chloride
0.11% (w/v)	sodium thioglycollate
0.0002% (w/v)	methylene blue
0.1% (w/v)	agar No. 1
pH	7.2

Sterilise at 121°C/15 min.

Charcoal agar; for the growth of *Renibacterium salmoninarum*

1.0% (w/v)	peptone
0.05% (w/v)	yeast extract
0.1% (w/v)	L-cysteine hydrochloride
0.1% (w/v)	activated charcoal
1.5% (w/v)	agar
pH	6.8

Sterilise at 121°C for 15 min (the charcoal may be placed in dialysis tubing prior to sterilisation in order to obtain a clear broth medium).

Columbia agar

2.3% (w/v)	special peptone
0.1% (w/v)	starch
0.5% (w/v)	sodium chloride
1% (w/v)	agar No. 1
pH	7.3

Sterilise at 121°C/15 min.

Coomassie brilliant blue agar (CBB)

0.01% (w/v)	coomassie brilliant blue dye [C.I. 42655]

TSA

Sterilise at 121°C/15 min.

Cysteine blood agar

0.3% (w/v)	beef extract
0.1% (w/v)	L-cysteine hydrochloride
20% (v/v)	human blood
0.5% (w/v)	sodium chloride
0.05% (w/v)	yeast extract
1.5% (w/v)	agar

Cytophaga agar (Anacker and Ordal, 1959)

0.05% (w/v)	tryptone
0.05% (w/v)	yeast extract
0.02% (w/v)	sodium acetate
0.02% (w/v)	beef extract
0.9% or 1.0% (w/v)	agar
pH	7.2–7.4

Sterilise at 121°C for 15 min.

Improved growth medium for *Fla. psychrophilum* (Daskalov *et al.*, 1999)

Cytophaga agar/broth supplemented with:

0.05% (w/v)	D(+) galactose
0.05% (w/v)	D(+) glucose
0.05% (w/v)	skimmed milk powder

The supplements were prepared as 10% (w/v) solutions, and filtered separately through 0.22 μm porosity filters, and added to molten, cooled medium.

Dorset egg medium

75% (v/v)	fresh egg mixture (whites and yolks)
0.25% (w/v)	Lab-lemco powder
0.25% (w/v)	peptone
0.125% (w/v)	sodium chloride

Crystal violet may also be incorporated to suppress contaminants
Inspissate (~80°C for 1 hour).

Emerson agar

0.4% (w/v)	beef extract
0.1% (w/v)	yeast extract
0.4% (w/v)	peptone
1% (w/v)	dextrose
0.25% (w/v)	sodium chloride
2% (w/v)	agar
pH	7.0

Sterilise at 121°C/15 min.

***Flavobacterium columnare* selective medium** (Fijan, 1969)

0.02% (w/v)	beef extract
0.05% (w/v)	tryptone
0.05% (w/v)	yeast extract
0.02% (w/v)	sodium acetate
0.9% (w/v)	agar
pH	7.2–7.4

Sterilise at 121°C/15 min, allow to cool, and add filter-sterilised antibiotic solutions: 5 μg/ml of neomycin sulphate and 10 IU/ml of polymyxin B.

FLP medium (for *Fla. psychrophilum*; Cepeda *et al.*, 2004**)**

0.05% (w/v)	glucose
0.02% (w/v)	$CaCl_2 . 2H_2O$
0.05% (w/v)	$MgSO_4 . 7H_2O$
0.4% (w/v)	tryptone
0.04% (w/v)	yeast extract
1% (w/v)	agar
pH	7.2–7.4

Sterilise at 121°C/15 min.

Kidney disease medium 2 (KDM2)

1.0% (w/v)	peptone
0.05% (w/v)	yeast extract
0.1% (w/v)	L-cysteine hydrochloride
1.5% (w/v)	agar
pH	6.5

Sterilise at 121°C for 15 min, cool to 45°C, and add 20% (v/v) sterile foetal calf serum.

L-F medium

1.0% (w/v)	brain heart infusion
10.0% (w/v)	sucrose
0.5% (w/v)	yeast extract
1.0% (w/v)	agar No. 3

Sterilise at 115°C/20 min
10% (v/v) horse serum, inactivated by heating at 60°C/30 min
Cool the medium and warm the horse serum to ~50°C, mix and pour as plates.

Loeffler (serum) medium

75% (v/v)	horse serum
0.25% (w/v)	Lab-lemco powder
0.25% (w/v)	peptone
0.25% (w/v)	dextrose
0.125% (w/v)	sodium chloride distilled water to 1 l.

Löwenstein–Jensen medium

0.15% (w/v)	potassium hydrogen phosphate
o.15% (w/v)	magnesium sulphate hydrated
0.037% (w/v)	magnesium citrate
0.22% (w/v)	asparagine
0.73% (v/v)	glycerol
1.83% (w/v)	potato starch
60.97% (v/v)	fresh egg mixture (whites and yolks)
0.024% (w/v)	malachite green

Inspissate (~80°C for 1 hour)

MacConkey agar

2% (w/v)	peptone
1% (w/v)	lactose
0.5% (w/v)	bile salts
0.5% (w/v)	sodium chloride
0.0075% (w/v)	neutral red
1.3% (w/v)	agar
pH	7.4

Sterilise at 121°C/15 min.

Medium K (Mudarris and Austin, 1989)

0.5% (w/v)	beef extract
0.6% (w/v)	casein
0.2% (w/v)	tryptone
0.1% (w/v)	yeast extract
0.1% (w/v)	calcium chloride
1.5% (w/v)	agar
pH	7.0

Sterilise at 121°C/15 min.

Middlebrook 7H10 agar

0.05% (w/v)	ammonium sulphate
0.15% (w/v)	potassium phosphate
0.15% (w/v)	sodium phosphate
0.04% (w/v)	sodium citrate
0.0025% (w/v)	magnesium sulphate
0.00005% (w/v)	calcium chloride hydrated
0.0001% (w/v)	zinc sulphate
0.0001% (w/v)	cupric sulphate
0.05% (w/v)	L-glutamic acid
0.5% (v/v)	glycerol
0.04% (w/v)	ferric ammonium citrate
0.0001% (w/v)	pyridozine
0.00005% (w/v)	biotin
0.000025% (w/v)	malachite green
1.5% (w/v)	agar
pH	6.6

Sterilise at 121°C for 15 min, cool to 50–55°C and add supplements of either bovine albumin—fraction V, glucose and beef catalase (to 0.5% w/v, 0.2% w/v and 0.0003% w/v, respectively); oleic acid, bovine albumin—fraction V, glucose , beef catalase and sodium chloride (to 0.005% v/v, 0.05% w/v, 0.2% w/v, 0.0004% w/v and 0.085% w/v, respectively); or oleic acid, bovine albumin—fraction V, glucose, beef catalase, sodium chloride and Triton (to 0.005% v/v, 0.05% w/v, 0.02% w/v, 0.0004% w/v, 0.085% w/v and 0.025% v/v, respectively).

Myxobacterium selective medium (Hsu *et al.*, 1983)

0.3% (w/v)	casein
0.2% (w/v)	tryptone
0.05% (w/v)	yeast extract
0.03% (w/v)	calcium chloride
1.0% (w/v)	agar
pH	7.0

Sterilise at 121°C/15 min, allow to cool to ~50°C, and add filter-sterilised antibiotic solution: 10 µg/ml of erythromycin, 10 µg/ml of neomycin sulphate or 256 IU/ml of polymyxin sulphate (this may be replaced by colistin sulphate).

Peptone beef extract glycogen agar (PBG)

1% (w/v)	beef extract
0.5% (w/v)	glucose
1% (w/v)	peptone
0.5% (w/v)	sodium chloride
0.004% (w/v)	bromothymol blue
1.5% (w/v)	agar
2% (w/v)	agar (for overlay)

Sterilise separately at 121°C/15 min. The basal medium is used for pour plates, after which the water agar is used as an overlay.

Petragnani medium

900 ml	whole milk
36 g	potato flour
500 g	potato
1,200 ml	whole egg (whites and yolks)
70 ml	glycerol
1.2 g	malachite green
pH	7.2

***Photobacterium damselae* subsp. *piscicida* medium** (Hashimoto *et al.*, 1989)

1% (w/v)	casamino acids/polypeptone
0.5% (w/v)	yeast extract
0.2% (w/v)	galactose
1% (w/v)	sodium glutamate
0.5% (w/v)	magnesium acetate

Agar may be added to solidify the medium.

Pseudomonas F agar

1% (w/v)	tryptone
1% (w/v)	proteose peptone
0.15% (w/v)	dipotassium phosphate
0.15% (w/v)	magnesium sulphate
1.% (w/v)	agar
1% (v/v)	glycerol
pH	7.0

Sterilise at 121°C/15 min.

Ribose ornithine deoxycholate medium for the isolation of *Yersinia ruckeri* (Rodgers, 1992)

0.3% (w/v)	yeast extract
0.1% (w/v)	sodium deoxycholate
0.5% (w/v)	sodium chloride
0.68% (w/v)	sodium thiosulphate
0.08% (w/v)	ferric ammonium citrate
0.75% (w/v)	maltose
0.375% (w/v)	ribose
0.375% (w/v)	ornithine hydrochloride
0.1% (w/v)	sodium dodecyl sulphate
0.008% (w/v)	phenol red
1.25% (w/v)	agar
pH	7.4

10 ml of a filtered (0.22 μm) solution containing 0.5 g sucrose/ml should be added after the basal medium has been autoclaved (121°C/15 min) and cooled to 50°C.

Rimler–Shotts medium (Shotts and Rimler, 1973)

0.05% (w/v)	L-lysine hydrochloride
0.65% (w/v)	L-ornithine hydrochloride
0.35% (w/v)	maltose
0.68% (w/v)	sodium thiosulphate
0.03% (w/v)	L-cysteine hydrochloride
0.003% (w/v)	bromothymol blue
0.08% (w/v)	ferric ammonium citrate
0.1% (w/v)	sodium deoxycholate
0.0005% (w/v)	novobiocin
0.3% (w/v)	yeast extract
0.5% (w/v)	sodium chloride
1.35% (w/v)	agar
pH	7.0

After boiling to dissolve the ingredients, the medium is not sterilised further.

Robertsons meat broth (= cooked meat medium)

45.4% (w/v)	heart muscle
1% (w/v)	peptone
1% (w/v)	Lab-lemco powder
0.2% 9w/v)	dextrose
0.5% (w/v)	sodium chloride
pH	7.2

Sterilise at 121°C/15 min.

Selective flexibacter medium (Bullock *et al.*, 1986)

0.2% (w/v)	tryptone
0.05% (w/v)	yeast extract
0.3% (w/v)	gelatin
1.5% (w/v)	agar

Sterilise at 121°C for 15 min, cool to 45°C and add filter-sterilised neomycin sulphate (0.0004% w/v).

Selective kidney disease medium (SKDM)

1.0% (w/v)	tryptone
0.05% (w/v)	yeast extract
0.005% (w/v)	cycloheximide
1.0% (w/v)	agar
pH	6.8

Sterilise at 121°C for 15 min, cool to 50°C, add sterile foetal calf serum to 10% (v/v), and filter-sterilised solutions of L-cysteine hydrochloride (0.1% w/v), D-cycloserine (0.00125% w/v), polymyxin B sulphate (0.0025% w/v) and oxolinic acid (0.00025% w/v).

Semi-defined medium for *Renibacterium salmoninarum*

Amount/1 l	*Ingredient*	*Preparation*
10 g	Tryptone	
10 ml	Mineral salts	Contains per 200 ml: EDTA dihydrate (disodium salt), 100 mg;
	Solution	$MgCl_2$, 4 g; $CaCl_2.2H_2O$, 1.4 g; $FeCl_2.6H_2O$, 100 mg; $ZnSO_4.7H_2O$, 100 mg; $MnSO_4.4H_2O$, 100 mg; $CuSO_4.5H_2O$, 50 mg; $CoCl_2.6H_2O$, 50 mg; $Na_2MoO_4.2H_2O$, 50 mg
10 ml	Nitrogen compounds	Contains per 100 ml: uracil, 50 mg; guanine, 50 mg; adenine, 50 mg; xanthine, 50 mg
1 mg each	Vitamins	Nicotinic acid, riboflavin, thiamine, calcium pantothenate
2 mg each	Vitamins	Pyridoxal HCl, pyridoxine HCl (prepared after Rogosa *et al.*, 1961)
20 ml	Buffer (pH 6.8)	Contains per 100 ml: K_2HPO_4, 15 g; KH_2PO_4, 15 g; sterilised at 121°C/15 min
8 ml	Cysteine HCl	Prepared immediately prior to addition as a solution (12.5% [w/v]) in N NaOH to give a pH of 6.8; filter-sterilised
2 ml	Glucose	Prepared as a solution (50% [w/v]) in distilled water; sterilised at 115°C/20 min

Note: tryptone, mineral salts solution and nitrogen compounds are added to distilled water, the pH adjusted to 6.8 with N NaOH, and the medium dispensed in 190 ml amounts in 800 ml capacity Erlenmeyer flasks. Sterilisation is at 121°C/15 min. After cooling to ~50°C, the pre-sterilised buffer, glucose, cysteine HCl and vitamin solutions are added to a final volume of 200 ml. Agar to 1% (w/v) may be incorporated, as necessary.

Shotts and Waltman medium for the isolation of *Edwardsiella ictaluri* (Shotts and Waltman, 1990)

1% (w/v)	tryptone
1% (w/v)	yeast extract
0.125% (w/v)	phenylalanine
0.12% (w/v)	ferric ammonium citrate
0.0003% (w/v)	bromothymol blue
0.1% (w/v)	bile salts
1.5% (w/v)	agar
980 ml	distilled water

Dissolve by boiling, cool to 50°C and adjust pH to 7.0, sterilise at 121°C for 15 min, cool to 50°C and add mannitol (filter-sterilised) to 0.35% (v/v) and colistin sulphate to 10 μg/ml.

Skimmed milk agar

0.05% (w/v)	yeast extract
1.0% (w/v)	skimmed milk powder
1.0% (w/v)	agar No. 3
pH	7.2

Sterilise at 115°C/15 min.

TCY medium (Hikida *et al.*, 1979)

0.1% (w/v)	casamino acids
0.1% (w/v)	tryptone
0.02% (w/v)	yeast extract
0.1% (w/v)	calcium chloride
1.08% (w/v)	magnesium chloride
0.07% (w/v)	potassium chloride
3.13% (w/v)	sodium chloride
pH	7.0–7.2

Thioglycollate broth

0.05% (w/v)	L-cystine
0.25% (w/v)	sodium chloride
0.5% (w/v)	dextrose
0.5% (w/v)	yeast extract
1.5% (w/v)	pancreatic digest of casein
0.05% (w/v)	sodium thioglycollate
pH	7.1

Sterilise at 121°C/15 min

Thiosulphate citrate bile salt sucrose agar (TCBS)

0.5% (w/v)	yeast extract
1.0% (w/v)	peptone
1.0% (w/v)	sodium thiosulphate
1.0% (w/v)	sodium citrate
0.8% (w/v)	ox bile
2.0% (w/v)	sucrose
1.0% (w/v)	sodium chloride
0.1% (w/v)	ferric citrate
0.004% (w/v)	bromothymol blue
0.004% (w/v)	thymol blue
1.4% (w/v)	agar
pH	8.6

After boiling to dissolve the ingredients the medium will not require further sterilisation.

Todd–Hewitt broth

1% (w/v)	infusion from 450 g of fat-free minced beef
2% (w/v)	tryptone
0.2% (w/v)	dextrose
0.2% (w/v)	sodium bicarbonate
0.2% (w/v)	sodium chloride
0.04% (w/v)	disodium phosphate, anhydrous
pH	7.8

Sterilise at 115°C/10 min.

***Vibrio anguillarum* medium (VAM)**

1.5 (w/v)	sorbitol
0.4% (w/v)	yeast extract
0.5% (w/v)	bile salts
3.5% (w/v)	sodium chloride
0.001% (w/v)	ampicillin
0.004% (w/v)	cresol red
0.004% (w/v)	bromothymol blue
1.5% (w/v)	agar-distilled water to 1 l
pH	8.6

Heat to dissolve, do not autoclave.

Waltman–Shotts medium for the isolation of *Yersinia ruckeri* (Waltman and Shotts, 1984)

0.2% (w/v)	tryptone
0.2% (w/v)	yeast extract
1.0% (v/v)	Tween 80
0.5% (w/v)	sodium chloride
0.01% (w/v)	calcium chloride hydrated
0.0003% (w/v)	bromothymol blue
1.5% (w/v)	agar
pH	7.4

Sterilise at 121°C for 15 min, cool to 50°C and add filter-sterilised sucrose to 0.5% (w/v).

Yeast extract glucose agar (Michel *et al.*, 1997)

2.5% (w/v)	nutrient broth No. 2 [Oxoid]
0.3% (w/v)	yeast extract
0.5% (w/v)	glucose
1.5% (w/v)	agar
pH	6.8

***Yersinia ruckeri*-selective medium** (Furones *et al.*, 1993)

TSA

1% (w/v)	SDS
100 µg/ml	coomassie brilliant blue
100 µg/ml	Congo red

6

Diagnosis

Historically, scientists have seemed loath to make rapid diagnoses, preferring to adopt laborious testing regimes. Why do scientists bother to identify the precise cause of a disease, when the information is often not useful for control purposes? Yet, there have been dramatic improvements in diagnostics, encompassing recent developments in molecular biology.

Diagnosis may often appear to be as much art as science, with a multitude of preferred methods adorning the notebooks of most diagnosticians. Diagnosis is sometimes achieved on purely histological material, with little effort made to isolate the pathogens. This is especially true of some of the acid-fast bacterial fish pathogens. When isolation of the pathogen is attempted, it is a common fault that diagnosis proceeds with emphasis on old-fashioned biochemical tests designed originally for bacteria important to human medicine. Consequently, a superficial glance at many laboratories would suggest that diagnostic techniques for fish pathogens need to be brought up to date. The astute diagnostician synthesises all available information before reaching a judgement. Useful information includes:

- Gross clinical signs of disease on individual fish.
- Internal abnormalities apparent during post-mortem examination.
- Histopathological examination of diseased tissues.
- Bacteriological examination of tissues (this requires special dexterity to avoid contamination by the normal bacterial microflora present on the surface and in the intestinal tract of fish, and in water; special contamination problems may be encountered with the examination of small fish, such as fry).

GROSS CLINICAL SIGNS OF DISEASE

The appearance of every sick fish tells a story, which fits into the proverbial jigsaw pattern of disease diagnosis. Good observation uncovers many useful clues. To an extent, the external appearance of the animal may be ignored by the eager diagnostician in the rush to attack the specimen with scalpel and swabs. Formalin and bacteriological media may be the order of the day. Of course, the same is not true elsewhere in veterinary and human medicine where diagnosis is often achieved by apparently cursory glances at the wretched individual.

Fish may display many behavioural and physical changes, some of which give valuable clues as to the nature of the disease (Table 6.1). It should be emphasised, however, that many symptoms are common to a multitude of bacterial diseases. Consequently, in the following discussion symptoms have been categorised in clear groups rather than splitting them into a plethora of esoteric detail. Thus, many external signs of disease are recognised which are pertinent to the bacterial fish pathogens (Table 6.1).

Sluggish behaviour

With some infections, notably those caused by *Bacillus* sp., *Cl. botulinum*, *Cor. aquaticum*, *Fla. johnsoniae*, *Haf. alvei*, *J. lividum*, *Mycobacterium* spp., *Myc. abscessus*, *Nocardia* spp., *Piscirickettsia salmonis*, *Shewanella putrefaciens*, *Str. difficilis*, *Str. iniae*, *V. anguillarum*. *V. ordalii*, *V. splendidus*, *V. tapetis* and *Y. intermedia*, the fish may become very inactive, and often cease feeding. Flatfish—for example, turbot—lie listlessly on the bottom of the tank, whereas salmonids float just below the surface of the water. In this state, death may quickly follow.

Twirling, spiral or erratic movement

This is often indicative of neurological damage. The offending pathogens include *Cit. freundii*, *Cl. botulinum*, *Edw. ictaluri*, *Eu. tarantellae*, *Lactococcus garvieae*, *Str. difficilis*, *Str. iniae* and *V. harveyi*.

Faded pigment

With this condition, attributed to a reduction in melanin content, the fish become very pale. This may occur with infections caused by *Arc. cryaerophilus*, *Edw. tarda*, *Mycobacterium* spp. and *Nocardia* spp.

Darkened pigment/melanosis

An enhancement of pigment results from infection with *Bac. mycoides*, *Arc. cryaerophilus*, *Fla. psychrophilum*, *Haf. alvei*, *J. lividum*, *Lactococcus garvieae*, *Lactococcus piscium*, *Micrococcus luteus*, *Piscirickettsia salmonis*, *Rhodococcus* sp., *Streptococcus* spp., *V. alginolyticus*, *V. harveyi*, *Y. intermedia* and *Y. ruckeri*.

Table 6.1. External signs of disease associated with the bacterial fish pathogens

Pathogen	Disease sign																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Acinetobacter spp.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Aer. caviae	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Aer. hydrophila	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	+	+	−	−	+	−	−	−	−	−	+	−	−	−	−
Aer. salmonicida	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−	−	−	+	−	−	−	−
Aer. veronii subsp. *sobria*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Arc. cryaerophilus	−	−	+	+	−	−	−	−	−	+	−	−	+	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−
Bacillus spp.	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−
Bac. cereus	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bac. mycoides	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−
Car. piscicola	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	−	+	−	−	−	−	−	−	−	−
Chrys. scophthalmum	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Cit. freundii	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	+	−	−	−	−
Cl. botulinum	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Cor. aquaticum	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Edw. ictaluri	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Edw. tarda	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−
En. faecalis subsp. *liquefaciens*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−
Esch. vulneris	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Eu. tarantellae	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fla. branchiophilum	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fla. columnare	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Fla. hydatis	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fla. johnsoniae	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−
Fla. psychrophilum	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Haf. alvei	+	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
J. lividum	+	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−

(*continued*)

Table 6.1 (*cont.*)

Pathogen	Disease sign																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Klebsiella pneumoniae	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lactococcus garvieae	−	+	−	+	+	+	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lactococcus piscium	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	−	+	−	−	−	−	−	−	−	−
Micrococcus luteus	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−		−	−	−	−	−	−	−	−	−
Moraxella spp.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Moritella marina	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Moritella viscosa	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Mycobacterium spp.	+	−	+	−	+	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−
Myc. abscessus	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Myc. marinum	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Myc. neoaurum	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mycoplasma mobile	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Myxococcus piscicola	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nocardia spp.	+	−	+	−	+	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−
Pantoea agglomerans	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Ph. damselae	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−		−	−	−	−	−	−	−	−	−
Piscirickettsia salmonis	+	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Planococcus sp.	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−
Plesiomonas shigelloides	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	−	−	−
Pr. rettgeri	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−		−	−	−	−	−	−	−	−	−
Ps. anguilliseptica	−	−	−	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−		−	−	−	−	−	−	−	−	−
Ps. chlororaphis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Ps. fluorescens	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Ps. pseudoalcaligenes	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Ren. salmoninarum	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−
Rhodococcus sp.	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sal. enterica subsp. *arizonae*	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Ser. plymuthica	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Shewanella putrefaciens	+	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Sporocytophaga sp.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sta. aureus	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sta. epidermidis	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Sta. warneri	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Str. difficilis	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Str. dysgalactiae	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Str. iniae	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Str. milleri	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Str. parauberis	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−
T. maritimum	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Vag. salmoninarum	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	+	−	+	−	−	−	−	−	−	−	−
V. alginolyticus	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−		−	−	−	−	−	−	−	−	−
V. anguillarum	+	−	−	−	−	−	+	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
V. cholerae	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
V. fischeri	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
V. harveyi	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
V. logei	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
V. ordalii	+	−	−	−	−	−	+	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
V. pelagius	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
V. salmonicida	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
V. splendidus	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−	+	−	+	−	−	−	−	−
V. tapetis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−
V. vulnificus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Y. intermedia	+	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Y. ruckeri	−	−	−	+	+	+	+	+	−	−	−	−	−	+	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−

1 = sluggish behaviour; 2 = twirling, spiral or erratic movement; 3 = faded pigment; 4 = darkened pigment; 5 = eye damage/exophthalmia; 6 = haemorrhaging in the eye; 7 = haemorrhaging in the mouth; 8 = erosion of the jaws/mouth; 9 = haemorrhaging in the opercular area; 10 = gill damage; 11 = white nodules on the gills/skin; 12 = white spots on the head; 13 = fin rot; 14 = haemorrhaging at base of fins; 15 = haemorrhaging on the fins; 16 = tail rot; 17 = saddle-like lesions on dorsal surface; 18 = distended abdomen; 19 = haemorrhaging on the surface/muscle; 20 = necrotising dermatitis; 21 = ulcers; 22 = external abscesses; 23 = furuncles; 24 = blood-filled blisters on flank; 25 = protruded anus; 26 = haemorrhaging around the vent; 27 = emaciation; 28 = inappetence; 29 = stunted growth; 30 = sloughing of skin; 31 = dorsal rigidity; 32 = necrotic lesions on caudal peduncle.

Eye damage—exophthalmia ("pop-eye")/corneal opacity/rupture

The presence of eye damage, i.e. bulging eyes (= exophthalmia; Figure 6.1 [see colour section]) and corneal opacity possibly leading to corneal rupture, occurs with infections by *Aer. caviae*, *Aer. hydrophila*, *Cor. aquaticum*, *Edw. ictaluri*, *Haf. alvei*, *J. lividum*, *Lactococcus garvieae*, *Lactococcus piscium*, *Micrococcus luteus*, *Mycobacterium* spp., *Myc. neoaurum*, *Nocardia* spp., *Ren. salmoninarum*, *Rhodococcus* sp., *Sal. enterica* subsp. *arizonae*, *Shewanella putrefaciens*, *Sta. aureus*, *Sta. epidermidis*, *Sta. warneri*, *Streptococcus* spp., *Str. parauberis*, *Vag. salmoninarum*, *V. alginolyticus*, *V. harveyi* and *Y. ruckeri*.

Haemorrhaging in the eye

This is characterised by the presence of generalised haemorrhaging (Figure 6.2 [see colour section]) or blood spots in the eye. The causal agents include *Pantoea agglomerans*, *Haf. alvei*, *Lactococcus garvieae*, *Ps. anguilliseptica*, *Streptococcus* spp., *Str. parauberis* (with pus in the eye) and *Y. ruckeri* ("salmonid blood spot").

Haemorrhaging in the mouth

Essentially, generalised haemorrhaging (Figures 6.3, 6.4 [see colour section]) or discrete blood spots become apparent on or around the mouth; a phenomenon attributed to infection by *Aeromonas* sp., *Ps. anguilliseptica*, *Shewanella putrefaciens*, *V. anguillarum*, *V. ordalii* and *Y. ruckeri*.

Erosion of the jaws/mouth

This condition occurs principally with infections by *Aer. bestiarum/hydrophila* (Figures 6.5–6.7 [see colour section]), *Fla. johnsoniae*, *T. maritimum* and *Y. ruckeri*.

Haemorrhaging in the opercula region/gills

This occurs with infections by *Chrys. scophthalmum*, *Lactococcus garvieae*, *Ps. anguilliseptica*, *Sta. epidermidis*, *Streptococcus* spp. and *Vag. salmoninarum*.

Gill damage

This includes the presence of swollen gill lamellae, anaemia/pale gills, clubbing and gill rot, which are symptoms of gill disease, caused by *Bac. cereus*, *Bac. mycoides*, *Arc. cryaerophilus*, *Chrys. scophthalmum*, *Esch. vulneris*, *Fla. branchiophilum*, *Fla. columnare*, *Fla. hydatis*, *J. lividum*, *Micrococcus luteus*, *Mycoplasma mobile*, *Myxococcus piscicola*, *Piscirickettsia salmonis*, *Planococcus* sp., *V. alginolyticus*, *V. anguillarum* and *V. ordalii*.

White nodules on the gills/skin

This is one of the characteristics of infections by *Edw. tarda* and *V. fischeri.*

White spots on the head

This occurs with infection by *Planococcus* sp.

Fin rot/damage

The presence of badly damaged (rotted) fins may be a sign of infection by *Aer. hydrophila*, *Arc. cryaerophilus*, *Klebsiella pneumoniae*, *Mycobacterium* spp., *Nocardia* spp., *Ps. fluorescens*, *Shewanella putrefaciens* and *V. pelagius.*

Haemorrhaging at the base of fins

The presence of clusters of tiny haemorrhagic lesions at the base of the fins is associated with disease caused by *Aer. hydrophila*, *Lactococcus garvieae*, *Ps. fluorescens*, *Streptococcus* spp., *V. anguillarum*, *V. ordalii*, *V. pelagius*, *V. splendidus* and *Y. ruckeri.*

Haemorrhaging on the fins

This occurs with infections caused by *Aeromonas* sp. (Figure 6.8 [see colour section]), *Sta. epidermidis* and *Str. parauberis.*

Tail rot/erosion

This occurs with infection by *Aeromonas* sp. (Figure 6.9 [see colour section]), *T. maritimum*, *Klebsiella pneumoniae*, *Mycobacterium* spp., *Nocardia* spp., *Ps. fluorescens* and *Y. intermedia.*

Saddle-like lesions on the dorsal surface (columnaris, saddleback disease)

This condition develops with infections by *Fla. columnare* (Figure 6.10 [see colour section]), *Fla. psychrophilum* and *Sporocytophaga* sp.

Distended abdomen

This condition, where the abdomen is often filled with ascitic fluid, should not be confused with overfeeding. The presence of a distended abdomen is a sign of many diseases, including those caused by *Aer. hydrophila*, *Aer. salmonicida*, *Car. piscicola*, *Chrys. scophthalmum*, *Edw. ictaluri*, *Edw. tarda*, *J. lividum*, *Lactobacillus* spp., *Lactococcus garvieae*, *Lactococcus piscium*, *Micrococcus luteus*, *Myc. abscessus*, *Ps.*

chlororaphis, *Ps. fluorescens*, *Ren. salmoninarum* (Figure 6.11 [see colour section]), *Sta. warneri*, *Streptococcus* spp., *Vag. salmoninarum* and *V. splendidus*.

Haemorrhaging on the surface and in the muscle

This is a common condition with infections by *Aer. caviae*, *Aer. bestiarum* (Figure 6.12 [see colour section]), *Aer. hydrophila* (Figure 6.13 [see colour section]), *Aer. salmonicida*, *Arc. cryaerophilus*, *Car. piscicola*, *Chrys. scophthalmum*, *Cit. freundii*, *Esch. vulneris*, *Edw. ictaluri*, *Edw. tarda* (Figure 6.14 [see colour section]), *Pantoea agglomerans*, *Fla. columnare*, *Haf. alvei*, *Lactobacillus* spp., *Lactococcus piscium*, *Moraxella* sp., *Mycobacterium* spp., *Nocardia* spp., *Ph. damselae* subsp. *piscicida*, *Ps. anguilliseptica* (Figure 6.15 [see colour section]), *Ps. chlororaphis*, *Ps. fluorescens*, *Ren. salmoninarum* (Figure 6.16 [see colour section]), *Shewanella putrefaciens*, *Str. parauberis*, *Vag. salmoninarum*, *Vibrio* sp. (Figure 6.17 [see colour section]), *V. anguillarum* (Figure 6.18 [see colour section]), *V. cholerae*, *V. salmonicida*, *V. vulnificus* and *Y. intermedia*.

Necrotising dermatitis

This condition has been associated with infection by *Bacillus* spp. and unknown causes.

Ulcers

The presence of ulcers (or surface erosion) on fish occurs with infections caused by *Acinetobacter* sp., *Aer. salmonicida* (Figures 6.19–6.22 [see colour section]), *Aer. veronii* biovar sobria, *Bac. mycoides*, *Cit. freundii*, *En. faecalis* subsp. *liquefaciens*, *Moritella marina*, *Moritella viscosa*, *Mycobacterium* spp., *Nocardia* spp., *Ph. damselae*, *Pr. rettgeri*, *Ren. salmoninarum*, *Sta. epidermidis*, *Sta. warneri*, *Str. milleri*, *Vibrio* sp. (Figure 6.23 [see colour section]), *V. alginolyticus*, *V. fischeri*, *V. harveyi*, *V. logei*, *V. splendidus*, *V. tapetis* and unidentified bacteria loosely associated with ultramicrobacteria (Figure 6.24 [see colour section]).

External abscesses

These may be present with infections by *Aer. hydrophila* (Figures 6.25, 6.26 [see colour section]), *Car. piscicola*, *Edw. tarda*, *Lactobacillus* spp., *Lactococcus piscium*, *Ren. salmoninarum* and *Vag. salmoninarum*.

Furuncles (or boils)

These are characteristic of infections by *Aer. salmonicida* (Figures 6.27, 6.28 [see colour section]).

Blood-filled blisters on the flank

These have been described in infections by *Car. piscicola*, *Lactobacillus* spp., *Lactococcus piscium*, *Ren. salmoninarum* (Figure 6.29 [see colour section]) and *Vag. salmoninarum*.

Protruded anus/vent

This is symptomatic of infection by *Edw. tarda* and *Plesiomonas shigelloides*.

Haemorrhaging around the vent

This occurs in infections caused by *En. faecalis* subsp. *liquefaciens*, *Plesiomonas shigelloides*, *V. anguillarum*, *V. ordalii* and *V. splendidus*.

Necrotic lesions on the caudal peduncle

This occurs with *Str. dysgalactiae*

Emaciation (this should not be confused with starvation)

This has been described for mycobacteriosis and nocardiosis, and infections by *Bacillus* spp., *Fla. johnsoniae* and *Str. parauberis*.

Inappetence

This is a common sign of many infections caused by bacterial fish pathogens. Nevertheless, loss of appetite is particularly associated with diseases caused by *Aer. hydrophila*, *Aer. salmonicida*, *Bac. mycoides*, *Cit. freundii*, *Cor. aquaticum*, *Myc. abscessus*, *Pasteurella skyensis*, *Piscirickettsia salmonis*, *Plesiomonas shigelloides*, *V. splendidus* and *V. tapetis*.

Stunted growth

This condition has been reported with *Myc. marinum*.

Sloughing off of skin/external surface lesions

This condition has occasionally been reported in infections attributed to *Fla. johnsoniae*, *J. lividum*, *Ps. pseudoalcaligenes*, *Ser. plymuthica* and unknown causes (Figure 6.30 [see colour section]).

Dorsal rigidity

This condition has been reported with infections attributed to *Str. difficilis* and *Str. iniae*.

INTERNAL ABNORMALITIES APPARENT DURING POST-MORTEM EXAMINATION

Careful internal observation of diseased fish may reveal the presence of clearly discernible abnormalities (Table 6.2).

Skeletal deformities

These have been noted with infection by *Myc. neoaurum*.

Gas-filled hollows in the muscle

These are generally evil-smelling, and characteristic of *Edw. tarda* infections.

Opaqueness in the muscle

This condition has been described with infections by *Bac. mycoides*.

Ascitic fluid in the abdominal cavity

This may be responsible for abdominal swelling, a trait common of many bacterial diseases including those caused by *Aer. hydrophila*, *Aer. salmonicida*, *Bacillus* spp., *Car. piscicola*, *Edw. ictaluri*, *Edw. tarda*, *Flavobacterium* sp., *J. lividum*, *Lactobacillus* spp., *Lactococcus garvieae*, *Lactococcus piscium*, *Micrococcus luteus*, *Planococcus* sp., *Ps. chlororaphis*, *Ps. fluorescens*, *Ps. pseudoalcaligenes*, *Ren. salmoninarum*, *Ser. liquefaciens*, *Ser. plymuthica*, *Sta. epidermidis*, *Sta. warneri*, *Streptococcus* spp., *Str. parauberis*, *Vag. salmoninarum* and *V. alginolyticus*.

Peritonitis

Peritonitis is a feature of infections by *Myc. neoaurum* and *Vag. salmoninarum*.

Petechial (pin-prick) haemorrhages on the muscle wall

These are common to many diseases, including those caused by *Acinetobacter* sp., *Aer. hydrophila*, *Aer. salmonicida*, *Edw. ictaluri*, *Lactobacillus* spp., *Lactococcus piscium*, *Piscirickettsia salmonis*, *Ps. fluorescens*, *Ren. salmoninarum*, *Vag. salmoninarum*, *V. anguillarum*, *V. ordalii* and *Y. ruckeri*.

Haemorrhaging in the air bladder

This has been described for *Acinetobacter*, *Moraxella* and *Piscirickettsia salmonis* infections.

Liquid in the air bladder

This has been associated with "vibriosis", caused by *V. alginolyticus*.

White nodules (granulomas) on/in the internal organs

These have been reported in some diseases, notably those caused by *Cit. freundii*, *Edw. tarda*, *Fla. piscicida*, *Mycobacterium* spp. (Figure 6.31 [see colour section]), *Myc. neoaurum*, *Nocardia* spp. (Figure 6.32 [see colour section]), *Ph. damselae* subsp. *piscicida*, *Ser. liquefaciens* and the PLO.

Yellowish nodules on the internal organs

These have been noted with infections by *Ser. liquefaciens*.

Nodules in the muscle

The presence of nodules has been reported with infections caused by *Myc. neoaurum*.

Swollen and/or watery kidney

This occurs principally in infections caused by *Aer. caviae*, *Arc. cryaerophilus*, *Car. piscicola*, *Edw. ictaluri*, *J. lividum*, *Lactococcus piscium*, *Micrococcus luteus*, *Piscirickettsia salmonis*, *Planococcus* sp., *Ren. salmoninarum* (Figure 6.33 [see colour section]), *Ser. liquefaciens* and *Str. parauberis*.

False membrane over the heart and/or kidney

This has been attributed only to bacterial kidney disease, which is caused by *Ren. salmoninarum*.

Haemorrhaging/bloody exudate in the peritoneum

This may occur with *Fla. piscicida*, *Lactococcus garvieae*, *Ps. anguilliseptica*, *Ps. fluorescens*, *Sal. enterica* subsp. *arizonae*, *V. alginolyticus*, *V. anguillarum*, *V. ordalii* and *V. splendidus* infections.

Table 6.2. Internal signs of disease

Taxon	Disease sign																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Acinetobacter sp.	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Aer. caviae	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	+	−	+	−	−
Aer. hydrophila	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Aer. salmonicida	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Arc. cryaerophilus	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	−	+	−
Bacillus spp.	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−
Car. piscicola	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
Chrys. scophthalmum	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Cit. freundii	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−
Cor. aquaticum	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−
Edw. ictaluri	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
Edw. tarda	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Esch. vulneris	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	+	−
Fla. columnare	−	−	−	−	(+)	−	−	−	−	−	−	−	(+)	−	−	−	−	−	−	−	(+)	−	(+)	−	−
Fla. piscicida	−	−	−	−	(+)	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Fla. psychrophilum	−	−	−	−	(+)	−	−	−	−	−	−	−	(+)	−	−	−	−	−	−	−	(+)	−	(+)	−	−
Haf. alvei	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
J. lividum	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−
Lactococcus garvieae	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	+	−	−
Lactococcus piscium	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
Micrococcus luteus	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−
Moraxella sp.	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Mycobacterium spp.	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Myc. neoaurum	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Nocardia spp.	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Ph. damselae subsp. *piscicida*	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Piscirickettsia salmonis	−	−	−	−	−	−	+	+	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−

Planococcus sp.	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	+	−	−
PLO	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Ps. anguilliseptica	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−
Ps. chlororaphis	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ps. fluorescens	−	−	−	−	+	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−
Ps. pseudoalcaligenes	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Ren. salmoninarum	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−	+	−	+	+	−	−	−	−	−	−
Sal. enterica subsp. *arizonae*	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Ser. liquefaciens	−	−	−	−	+	−	−	−	−	+	+	−	+	−	−	−	−	−	−	−	+	−	−	−	−
Ser. plymuthica	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Sta. epidermidis	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−
Sta. warneri	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Str. difficilis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Str. iniae	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Str. parauberis	−	−	−	−	+	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	+	−	+	−	−
Vag. salmoninarum	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−
V. alginolyticus	−	−	−	−	+	−	−	−	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−
V. anguillarum	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−
V. cholerae	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
V. harveyi	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
V. ichthyoenteri	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
V. ordalii	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−
V. pelagius	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−
V. salmonicida	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
V. splendidus	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	+	−	+
Y. ruckeri	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−

1 = skeletal deformity; 2 = intestinal necrosis; 3 = hyperaemic stomach; 4 = gas-filled hollows in the muscle; 5 = ascitic fluid; 6 = peritonitis; 7 = petechial haemorrhages on muscle wall; 8 = haemorrhaging in the air bladder; 9 = liquid in the air bladder; 10 = white nodules (granulomas) in/on internal organs; 11 = yellowish nodules on the internal organs; 12 = nodules in the muscle; 13 = swollen and/or watery kidney; 14 = haemorrhaging and/or bloody exudate in the peritoneum; 15 = swollen intestine; 16 = false membrane over heart and/or kidney; 17 = blood in the cranium; 18 = haemorrhaging in/on the internal organs; 19 = brain damage; 20 = emaciation; 21 = pale elongated/swollen spleen; 22 = swollen liver; 23 = pale mottled/discoloured liver; 24 = yellowish liver, 25 = presence of tumours.
(+) = weakly positive.

Swollen intestine, possibly containing yellow or bloody fluid/gastro-enteritis

This develops sometimes in infections attributed to *Aer. caviae*, *Arc. cryaerophilus*, *Cit. freundii*, *Esch. vulneris*, *Lactococcus garvieae*, *Piscirickettsia salmonis*, *Str. parauberis*, *V. alginolyticus*, *V. harveyi*, *V. pelagius*, *V. splendidus* and *Y. ruckeri*.

Intestinal necrosis and opaqueness

This condition has been documented with *V. ichthyoenteri*.

Hyperaemic stomach

This develops in infections by *Bacillus* spp.

Haemorrhaging in/on the internal organs

This is common to most bacterial fish diseases, including those caused by *Acinetobacter* sp., *Aer. hydrophila*, *Aer. salmonicida*, *Bacillus* spp., *Car. piscicola*, *Chrys. scophthalmum*, *Cor. aquaticum*, *Edw. ictaluri*, *Edw. tarda*, *Esch. vulneris*, *Flavobacterium* sp., *Haf. alvei*, *Lactobacillus* spp., *Lactococcus garvieae*, *Lactococcus piscium*, *Ps. anguilliseptica*, *Ps. fluorescens*, *Ps. pseudoalcaligenes*, *Ren. salmoninarum*, *Ser. plymuthica*, *Streptococcus* spp., *V. alginolyticus*, *V. anguillarum*, *V. cholerae*, *V. harveyi*, *V. ordalii*, *V. pelagius*, *V. salmonicida* and *Y. ruckeri*.

Brain damage

In particular, brain damage has been associated with diseases caused by *Cor. aquaticum*, *Ren. salmoninarum*, *Str. difficilis* and *Str. iniae*.

Blood in the cranium

This has been reported with infection by *Cor. aquaticum*.

Emaciation

This is characteristic of mycobacteriosis and nocardiosis, and with infections by *Bacillus* spp.

Pale, elongated/swollen spleen

This occurs with infections by *Aer. caviae*, *Bacillus* spp., *Arc. cryaerophilus*, *J. lividum*, *Micrococcus luteus*, *Planococcus* sp., *Piscirickettsia salmonis*, the PLO, *Ser. liquefaciens*, *Str. parauberis* and *Vag. salmoninarum*.

Pale (possibly mottled/discoloured) liver

This condition occurs with many bacterial fish pathogens, including *Aer. caviae*, *Cit. freundii*, *Lactococcus garvieae*, *Moraxella* sp., *Planococcus* sp., *Sta. warneri*, *Str. parauberis* and *V. splendidus*.

Yellowish liver (with hyperaemic areas)

This occurs with infection by *Arc. cryaerophilus* and *Esch. vulneris*.

Swollen liver

This has been noted in infection by *Vag. salmoninarum*.

Generalised liquefaction

This can occur as a result of infection with many bacterial pathogens, including *Aeromonas* spp. (Figure 6.34 [see colour section]) and *Vibrio* spp.

The presence of tumours

Although it is unlikely that bacteria are directly involved with tumorous conditions, there is some indication that *V. fischeri* is implicated as a possible secondary invader to viruses.

HISTOPATHOLOGICAL EXAMINATION OF DISEASED TISSUES

Although many histological procedures may be routinely used, it is important for the bacteriologist that Gram-stained sections should be prepared. Thus, the presence of any offending bacterial pathogen will quickly be recognised. Microscopic examination of Gram-stained material will enable the determination of the basic staining reaction and micromorphology of the pathogen. Possibilities include the presence of Gram-positive or Gram-negative rods (spore-bearing or asporogenous), cocci and mycelium. For Gram-positive organisms, the acid-fast stain will help in the recognition of *Mycobacterium*, *Nocardia* and possibly *Rhodococcus*.

A simplistic approach for the diagnosis of columnaris has involved the observation of wet preparations, prepared from lesion material, which were supposed to demonstrate the presence of "column"-like masses of bacteria (Snieszko, 1958b). Undoubtedly, use of this technique led to mis-diagnosis with other myxobacteria. However, a recent development, which is meeting with some success, is the microscopic examination of spleen squashes from fish considered to be suffering with RTFS (Sarti *et al.*, 1992). Here, bacteria can be readily seen in the infected animals.

BACTERIOLOGICAL EXAMINATION OF TISSUES

Although detailed in Chapter 5, it is worth re-iterating that by use of a comparatively small range of media—a couple of incubators adjusted to 15°C and 20–25°C, an anaerobic jar, and standard aseptic techniques—the majority of the bacterial fish pathogens may be readily recovered as pure culture growth. It should be emphasised, however, that in some asymptomatic "carrier" states, e.g. BKD, only a scant number of colonies may develop. Nevertheless, in the majority of clinical cases of disease, it should be possible to obtain dense growth.

Tissues to be sampled

Generally, examination should be made of any damaged tissues. Experience has taught us that it is always advisable to include a kidney sample, which often permits the most satisfactory isolation of the pathogen. Quite simply it is adequate to sample the material by means of swabbing. The swabs should then quickly be used to inoculate the bacteriological media.

Culturing *Aeromonas salmonicida*

Diagnosis of *Aer. salmonicida* is readily achieved by culturing techniques, usually on TSA or BHIA (the preferred medium in the view of many scientists) in which case "typical" isolates produce a characteristic brown, diffusible pigment. Also, CBB may be employed as a differential medium (Markwardt *et al.*, 1989). However, as a primary isolation medium, CBB appears to be less sensitive and gives a poor recovery of the pathogen compared with BHIA (B. Austin, unpublished data).

A special case for diagnosis—BKD

Confusion surrounds which of the available methods is most suitable for detecting and thus diagnosing the presence of BKD. Essentially, the opposing opinions include those who favour serology and those who recommend cultivation. Gene probes are also entering the armoury of the diagnostician (Etchegaray *et al.*, 1991). It is difficult for most microbiologists to determine which of the alternative approaches is correct. Historically, diagnosis of BKD was achieved histologically, by the presence of Gram-positive cocco-bacilli in kidney tissue. However, the reliability was impaired by the presence of melanin granules and other morphologically similar bacteria (Chen *et al.*, 1974). A subsequent derivative has been a histochemical technique using Lillie's allochrome to stain glycogen in the bacteria (Bruno and Munro, 1982). Again, interference could result from the presence of other morphologically similar glycogen-containing bacteria.

The early spate of interest in culturing techniques improved diagnosis, but the effectiveness was marred by the apparently slow growth of the organisms, i.e. up to 6 weeks at 15°C. In fact, recent evidence suggests that 19 weeks may be necessary for the initial incubation period. Therefore, there was widespread attention focused on

serological procedures as being the saviour of diagnosticians. This view has been reinforced by the current emphasis on western blots (e.g. Lovely *et al.*, 1994). But, which is more efficient—culturing or serology—at detecting renibacterium? Comparing kidney and ovarian fluid from broodstock Atlantic salmon, the selective medium, SKDM (see Austin *et al.*, 1983a) detected a higher number of positive BKD samples than iFAT, which in turn was more sensitive than ELISA or western blots (Griffiths *et al.*, 1996). Interestingly, renibacteria were found in either kidney or ovarian fluid, but not both. However, the use of culturing in SKDM broth followed by western blotting increased the sensitivity beyond the maximum level recorded by SKDM alone (Griffiths *et al.*, 1996). This is interesting because the benefit of this broth stage in increasing the detection rate for renibacterium paralleled an observation with peptone water during the 1970s. Here, we found that pre-incubation of kidney, spleen and more importantly heart tissue in peptone water enhanced the level of resulting colonies, and therefore positivity, on solid medium. Another study concluded that ELISA, using a polyclonal rather than a monoclonal [this was less sensitive] antiserum, was more sensitive than SKDM. Here, SKDM detected *Ren. salmoninarum* in 45% of kidney samples, compared with ELISA, which found that 50% of the kidneys were positive (Jansson *et al.*, 1996).

A special case—*Piscirickettsia salmonis*

Isolation in cell culture or detection in acridine orange stained smears or by iFAT has been advocated (Lannan and Fryer, 1991; Lannan *et al.*, 1991).

IDENTIFICATION OF BACTERIAL ISOLATES

The most common shortcomings in diagnosis of fish diseases concern the identification of bacterial isolates. There are two schools of thought, namely those that rely on serology *and* those relying on phenotypic tests.

SEROLOGY

We will preface further discussion by wholeheartedly endorsing a view that reliable diagnoses occur only with monospecific antisera to assure the homologous reaction between antigen and antibody. The development of monoclonal antibodies has improved diagnoses by standardising serological tests, i.e. by means of defined reagents (Goerlich *et al.*. 1984), and enhanced the reliability of ELISA, iFAT and immunohistology for the detection of pathogens, such as *Mycobacterium* spp. and *Ren. salmoninarum* (Adams *et al.*, 1995). In contrast, it may be argued that the more conventional polyclonal antibodies have generated contradictory results. Also, the extent of any cross-reactions with polyclonal antibodies has not been adequately determined. Goerlich *et al.* (1984) noted that a monoclonal antibody raised against a typical virulent isolate of *Aer. salmonicida* (i.e. the strain possessed an A-layer)

reacted only with virulent cultures, but not with avirulent cells (i.e. those lacking an A-layer).

Nevertheless, tentative diagnoses, especially of asymptomatic infections (Busch and Lingg, 1975; Hansen and Lingg, 1976; Johnson *et al.*, 1974) may result from use of polyclonal antibodies in any of a multiplicity of serological procedures, including FAT, whole-cell (slide) agglutination, precipitin reactions, complement fixation, immunodiffusion, antibody-coated latex particles (this is akin to human pregnancy testing), co-agglutination using antibody-coated staphylococcal cells, passive haemagglutination, immuno-India ink technique (Geck) or ELISA (e.g. Saeed and Plumb, 1987). The serological techniques are described below.

Fluorescent antibody technique (FAT)

There are two variations to this test, namely the direct and indirect methods. FAT has found use for the diagnosis of many fish diseases, especially in laboratories (Eurell *et al.*, 1978), for which it is regarded as a highly effective procedure (Kawahara and Kusuda, 1987). For example, α- and β-haemolytic streptococcal isolates may be readily differentiated by FAT (Kawahara and Kusuda, 1987). iFAT has a proven track record with the diagnosis of ERM (Johnson *et al.*, 1974). FAT appears to be useful for detecting *T. maritimum* in fish tissues (Baxa *et al.*, 1988a). Moreover, Lorenzen and Karas (1992) detailed an immunofluorescence technique for detecting *Fla. psychrophilum* in the spleen of rainbow trout suffering with RTFS. In addition, iFAT with monoclonal antibodies [and an enzyme immunoassay—also useful for *Edw. tarda* (Rogers, 1981)] have shown promise for the recognition of *Edw. ictaluri* (Rogers, 1981; Ainsworth *et al.*, 1986).

For the direct method (see Bullock *et al.*, 1980 and Smibert and Krieg, 1981 for further details), fluorescein isothiocyanate is conjugated with whole or with the IgG fraction of the antiserum. Two-fold dilutions (1:5 and 1:8) are prepared in PBS (0.1236% [w/v]) Na_2HPO_4, anhydrous; 0.018% (w/v) $NaH_2PO_4.H_2O$; 0.85% (w/v) NaCl; pH 7.6) and used to standardise the "conjugate". Thus, a bacterial suspension (containing ca. 10^8 cells/ml in PBS) is pipetted onto grease-free microscope slides, air-dried and fixed at 60°C for 2 min (or fixed in 95% ethanol for 1 min., and then air-dried). The "conjugate" is pipetted onto the slide and left in a moist chamber for 5 min. to react (room temperature, i.e. 15–20°C, is adequate). Subsequently, the excess antiserum is removed by draining, before the slide is thoroughly rinsed in PBS (for *ca.* 10 min). The slide is air-dried, and the smear covered with a drop of buffered glycerol (Difco; at pH 9.0) before overlayering with a coverslip. This should be quickly examined with a fluorescence microscope. The optimum dilution of conjugate is determined, from the doubling dilutions, by rating the degree of fluorescence from 0 to 4+ (after Jones *et al.* 1978). The "use" dilution of the conjugated antiserum is 50% of the highest dilution, which gives maximum fluorescence. With this information, the diagnosis may proceed for unknown cultures. However, it is always important to include positive and negative controls.

For iFAT (see Laidler, 1980), bacterial smears are prepared and fixed (as above). Doubling dilutions of rabbit antiserum are prepared, and 20 µl aliquots added to the

bacterial smears. These are placed in a moist chamber, left for 30 min to react, and washed for 30 min in two changes of PBS. After air-drying, the smears are covered with a suitable dilution of fluorescein-labelled sheep anti-rabbit globulin (Wellcome), incubated for 30 min in the moist chamber, rinsed thoroughly in PBS, air-dried and mounted in buffered glycerol. Examination with a fluorescence microscope should proceed as quickly as possible.

iFAT (Bullock and Stuckey, 1975b; Mitchum *et al.*, 1979; Paterson *et al.*, 1979; Laidler, 1980) and FAT (Bullock *et al.*, 1980) have been developed for the diagnosis of BKD. Improvements in the FAT included a 60 min staining time with the fluorescent antibody (Cvitanich, 1994). A further refinement involved concentrating samples—in this case coelomic fluid from spawning chinook salmon—on membrane filters, which were used with FAT (Elliott and McKubben, 1997). This modification was regarded as more sensitive than FAT on smears (Elliott and McKubben, 1997). The iFAT has found use for detecting asymptomatic or overt cases of BKD (Bullock and Stuckey, 1975b; Lee and Gordon, 1987), although the technique is not always as sensitive and reliable as culturing (Armstrong *et al.*, 1989). Indeed, Paterson *et al.* (1979) pointed to the enzootic nature of BKD in one Canadian river. These workers reported asymptomatic infections in 33.4% of 456 Atlantic salmon parr and 35.1% of 37 adult salmon in the Margaree River. Seemingly, iFAT was more sensitive than the examination of Gram-stained kidney tissue or cultivation on Mueller–Hinton agar supplemented with 10% foetal calf serum and 0.1% L-cysteine hydrochloride (Paterson *et al.*, 1979). iFAT incorporating monoclonal antibodies has successfully recognised *Str. iniae* in tissues (Klesius *et al.*, 2006). Kawahara and Kusuda (1987) reported that FAT was superior to culturing for the diagnosis of *Aer. hydrophila* (and atypical *Aer. salmonicida*) infections in eels. Moreover, FAT was more successful than culturing for detecting *Aer. hydrophila* in mixed infections. FAT has been found useful for diagnosing *Edw. tarda* and determining the presence of the pathogen in infected fish tissues (Amandi *et al.*, 1982). In contrast to other views, Horiuchi *et al.* (1980) considered FAT as being extremely valuable for field diagnoses, albeit of *Edw. tarda*. A relevant development has been the use of a rapid iFAT to simultaneously detect two pathogens, i.e. *Edw. ictaluri* and *Fla. columnare*, using fluorochromes with two different spectra properties, Alexa Fluor 488 and 594 emitting green and red fluorescence, respectively (Panangala *et al.*, 2006).

Whole-cell agglutination

This is a quick and easy technique, which provides much useful data. Whole-cell agglutination is used widely as a diagnostic tool, for example with the fish-pathogenic streptococci (Kitao *et al.*, 1979; Kitao, 1982a) and *Edw. tarda* (Amandi *et al.*, 1982). Pacha (1968) implied that whole-cell agglutination reactions were effective for differentiating *Fla. psychrophilum* from other (unnamed) myxobacteria. Also, together with ELISA, the approach allowed a useable typing scheme to be devised (Mata *et al.*, 2002). Whole-cell agglutination was also effective for the detection of *Fla. columnare* (Morrison *et al.*, 1981), and possibly for *T. maritimum* (Wakabayashi *et al.*, 1984). Eurell *et al.* (1978) considered the effectiveness of slide agglutination

especially for field use in the recognition of *Aer. hydrophila* infections, whereas tube- and macro-agglutination were useful in laboratories. The technique is effective with *Aer. salmonicida*, but only for smooth (non-auto-agglutinating) colonies (Rabb *et al.*, 1964). This is a pity since the majority of isolates recovered from clinical cases of disease are, in fact, rough and auto-agglutinating (McCarthy, 1976). Also, *Ps. anguilliseptica* may be rapidly diagnosed by slide agglutination. However, slight cross-reactions may also occur with *Ps. putida* and *V. anguillarum*. These interfering cross-agglutinations may be effectively eliminated by using diluted antiserum (Nakai *et al.*, 1981). An interesting development concerns the detection of a thermolabile *V. anguillarum* O-antigen, termed the k-1 antigen by slide agglutination (Tajima *et al.*, 1987a). So far, the data indicate that this antigen is specific to *V. anguillarum*.

Essentially, a drop of bacterial suspension (*ca.* 10^8 cells/ml, prepared in 0.85% [w/v] saline) is added to a microscope slide. This is followed by adding a drop of antiserum (use a range of dilutions), with gentle mixing for 2 min. A positive response is indicated by clumping of the cells. The reaction may also be carried out in microtitre wells, using serial dilutions of antisera (Toranzo *et al.*, 1987). Of course, it must be emphasised that the reliability reflects the specificity of the antiserum.

Precipitin reactions and immunodiffusion

The value of these tests for diagnosis has been overshadowed by other techniques, such as FAT and whole-cell agglutination. For detailed discussion, the reader should consult Kimura *et al.* (1978b) and Smibert and Krieg (1981). Nevertheless, an immunodiffusion test for BKD, based on the detection of soluble antigens in infected tissues, was developed by Chen *et al.* (1974) and discussed further by Kimura *et al.* (1978b). Use of this method together with more classical agglutination reactions on 10 isolates led to the conclusion by Bullock *et al.* (1974) that the causal agent of BKD was antigenically homogeneous. Following examination of over 50 isolates, we concur with this conclusion. Immunodiffusion was, of course, much quicker than cultivation, with diagnosis, achieved on the basis of specific precipitin lines, taking no more than 24 h.

Complement fixation

This technique probably has greater value for fish virology than for fish bacteriology; it has been described by Ahne (1981).

Antibody-coated latex particles

The so-called latex agglutination test has found widespread use for diagnosis of ERM (e.g. Hansen and Lingg, 1976), furunculosis and vibriosis (including Hitra disease). Commercial kits have found success, despite some cross-reactions (Romalde *et al.*, 1995). However, the technique may be readily adapted for most bacterial fish pathogens. As originally described for *Aer. salmonicida* by McCarthy (1975a, b), the test

involves the use of globulins from hyper-immune serum (titre $\geq$1:5,000) and sensitised latex. The globulins are precipitated by the addition of saturated ammonium sulphate to the antiserum, and the precipitated proteins are sedimented by centrifugation. They are subsequently re-dissolved in 0.9% (w/v) saline, dialysed overnight at 4°C against three changes of saline, and, after centrifugation, the supernatant, which contains the globulins, is stored at −20°C until required. The latex particles (0.81 mm diameter; Difco) are sensitised in globulin solution at 37°C for 2 h. For the test, 200 µl of the antigen (bacterial suspension in glycine-buffered saline, i.e. 0.73% [w/v] glycine and 1% [w/v] NaCl; pH 8.2; supplemented with 1% [w/v] Tween 80) is mixed for 2 min with an equal volume of sensitised latex on a clean glass plate. A positive result is indicated by clumping of the latex. The technique may be used for pure or mixed cultures and tissue. Thus, positive diagnoses may ensue from tissues unsuitable for culturing, e.g. fish stored at −20°C for 14 days, 5°C for 7 days or from formalin-fixed material (McCarthy, 1975a, b). As before, positive and negative controls are necessary.

Co-agglutination with antibody-sensitised staphylococci

Reported for *Aer. salmonicida* and *Ren. salmoninarum*, this technique is similar to the latex test (Kimura and Yoshimizu, 1981, 1983, 1984). Essentially, *Sta. aureus* (ATCC 12598) is suspended in 0.5% formalin–PBS for 3 h at 25°C to inactivate the cells, and washed three times in fresh PBS. The cells are mixed with antiserum in the ratio of 10:1 and incubated at 25°C for 3 h. An equal volume of a boiled bacterial suspension and the sensitised staphylococci are mixed on a glass slide. Following incubation in a moist chamber at room temperature for up to 2 h, a positive response is indicated by clumping of the cells. The advantages of this technique concern its simplicity and reliability. Moreover, it was considered suitable for deployment in field conditions.

The co-agglutination test of Kimura and Yoshimizu (1981) showed considerable promise for rapid detection of BKD, i.e. within 2 hours. The anti-*Renibacterium* antibody coated staphylococcal cells are reacted with the supernatant from heated (i.e. 100°C for 30 min) kidney tissues. Unlike iFAT/FAT, it does not require an expensive fluorescence microscope, and would, therefore, be more suited to field conditions.

Passive agglutination

For rough colonies of *Aer. salmonicida*, which were unsuitable for use with whole-cell agglutination (because of auto-agglutination), McCarthy and Rawle (1975) recommended the mini-passive agglutination test. This technique involves the use of sheep erythrocytes sensitised with *Aer. salmonicida* O-antigen (extracted with hot physiological saline). This reacts with dilute anti-*Aer. salmonicida* immune serum, assuming that the antigen is present. The obvious advantages of this method concern its application to the detection of both rough and smooth colonies. However, McCarthy and Rawle (1975) cautioned that false negative results may sometimes be obtained with cultures that have been maintained in laboratory conditions for prolonged

periods. Hence, old cultures may not be suitable for use in serological studies (or, for that matter, vaccine production!).

Immuno-India ink technique (Geck)

Another rapid technique, which allows diagnosis within 15 min, is the India ink immunostaining reaction as developed initially by Geck (1971). This is a microscopic technique, in which the precise mode of action is unknown, although Geck suggested that it could be regarded as an immuno-adsorption method. The technique has been described only for use with *Aer. salmonicida* (McCarthy and Whitehead, 1977). A drop of bacterial suspension is smeared onto a clean (de-fatted) microscope slide, air-dried and heat-fixed. The smear is covered with a 1:1 mixture of India ink and antiserum, before incubation in a moist chamber for 10 min at room temperature. Subsequently, the mixture is removed by washing with ferric chloride (0.00001% w/v), and the slide air-dried prior to microscopic examination. A positive result is indicated by the presence of cells, clearly outlined with India ink.

Enzyme-linked immunosorbent assay (ELISA)

This is a technique which is becoming widely adopted for the detection and diagnosis of bacterial fish pathogens, some commercial kits having been developed. This is a useful technique, which has already gained widespread use in human and veterinary medicine. Essentially, there is a requirement for a specific antiserum, an enzyme, e.g. alkaline phosphatase or horseradish peroxidase, and a substrate, e.g. *o*-phenylene-diamine (for use with alkaline phosphatase) (see Austin *et al.*, 1986). A positive result is indicated by a colour change, which may be recorded quantitatively with a specially designed reader. Variations of the technique have been published, and include indirect ELISA, indirect blocking ELISA and competitive ELISA (e.g. Swain and Nayak, 2003).

Hsu *et al.* (1991) described a monoclonal antibody based ELISA which appears to be effective for the diagnosis of BKD. This system detected 0.05–0.1 μg of antigen/ml within a few hours. An ELISA was developed, which successfully identified *Ph. damselae* subsp. *piscicida*, albeit in artificially infected fish tissue within 4 h. By visually recording the ELISA the threshold for positivity was 10^5 cells/ml. However, use of a reader cut this level to only 10^3 cells/ml (Bakopoulos *et al.*, 1997a). A sensitivity limit of 10^4–10^5 cells/well was detailed for the *Aer. hydrophila* system devised by Sendra *et al.* (1997). Rapid identification of *Fla. psychrophilum* and *Fla. branchiophilum* was achieved by ELISA, which detected $\geq 1 \times 10^4$ cells/ml from infected spleen (Rangdale and Way, 1995) and 1×10^3 cells/ml from gills (= the threshold value) (MacPhee *et al.*, 1995), respectively. By use of ELISA, a useful typing scheme was devised for *Fla. psychrophilum*, recognising seven serogroups with host specificity (Mata *et al.*, 2002). Similarly, an ELISA was developed for *V. vulnificus* and field-tested, the results of which indicated a sensitivity of 10^4–10^5 cells/well, and an ability to detect non-culturable cells (Biosca *et al.*, 1997b). We have successfully married monoclonal antibodies to *Aer. salmonicida* with ELISA for a

test, which has proven suitable for use on fish farms. Indeed, experiments demonstrated that reliable diagnoses were achieved within 30 min (Austin *et al.*, 1986). It is noteworthy that ELISA systems appear to be more sensitive than culturing for the detection of *Aer. salmonicida* (Hiney *et al.*, 1994).

An indirect ELISA has been effective at detecting *Y. ruckeri* (Cossarini-Dunier, 1985) and for determining the presence of antibodies to *Edw. ictaluri* in fish serum (Waterstrat *et al.*, 1989; Swain and Nayak, 2003). A development of this approach involved the use of tissue homogenisation (using 0.5% v/v Triton X-100 in 0.05 M PBS [pH 7.2]), filtration and then the ELISA (Earlix *et al.*, 1996). This approach was used successfully to detect asymptomatic carriers, and permitted live bacteria to be filtered from 1 g quantities of tissue slurries, with a sensitivity of <10 CFU/g of tissue. The filter–ELISA system detected *Edw. ictaluri* in 80% of 98 channel catfish compared with a detection of 24% by culturing (Earlix *et al.*, 1996).

A monoclonal-based ELISA was successful and specific in detecting *Piscirickettsia* in infected fish tissues (Aguayo *et al.*, 2002).

Immunohistochemistry

Immunohistochemistry, based on avidin–biotin complexes, has identified *V. salmonicida* in fixed tissues (Evensen *et al.*, 1991). Also, a peroxidase–antiperoxidase immunohistochemical technique appears to be useful for differentiating *Y. ruckeri* (Jansson *et al.*, 1991). Complications with the interpretation of slides by interference with melanin and/or melanomacrophages resulted in the adoption of melanin-bleaching with 3.0 g/l potassium permanganate for 20 min followed by 1% (w/v) oxalic acid for 1.5 min before immunostaining, which removed some of the problems of interpreting the presence/absence of *Ren. salmoninarum* (Morris *et al.*, 2002).

Immunomagnetic separation of antigens

A comparatively novel approach concerns the recovery of *Aer. salmonicida* cells with immunomagnetic beads coated with monoclonal antibodies to LPS coupled with culturing (Nese and Enger, 1993). Thus, the cells are recovered by serological procedures for culturing on routine bacteriological media. Magnetic beads incorporating polyclonal antibodies with an enzyme immunoassay have found success for the rapid diagnosis of pasteurellosis, in which the commercial kit had a detection limit of 10^4 cells albeit with a problem of specificity, i.e. other photobacteria—namely *Ph. damselae* subsp. *damselae* and *Ph. histaminum*—were also detected (Romalde *et al.*, 1999a).

WHICH METHOD IS BEST?—THE SAGA OF BKD

Pascho *et al.* (1987) compared five techniques for the detection of *Ren. salmoninarum* in coho salmon. The conclusion was that the ELISA was most sensitive,

followed by FAT, filtration–FAT, culturing, counterimmuno-electrophoresis and immunodiffusion. This view was echoed by results from Meyers (1993), who regarded ELISA as more sensitive than FAT. Specifically, FAT did not detect *Ren. salmoninarum* in 80% of the samples positive by ELISA. However, a complication was that in the same study FAT detected *Ren. salmoninarum* in 28% of the samples negative by ELISA (Meyers *et al.*, 1993). The comparative benefit of a membrane–filtration FAT over ELISA was illustrated when the former detected *Ren. salmoninarum* in 66/103 (=64%) ovarian samples compared with 40/103 (=39%) of positives with the latter (Pascho *et al.*, 1998). The membrane–filtration FAT was capable of detecting $\geq$25 renibacterial cells/ml of ovarian fluid, whereas the ELISA was not consistent in detection at levels of $\leq 1.3 \times 10^4$ cells/ml (Pascho *et al.*, 1998). Yet, both methods were inferior to a nested PCR amplifying a 320 bp (base pair) fragment of the p57 antigen. This PCR detected *Ren. salmoninarum* in all of the ovarian samples (Pascho *et al.*, 1998).

Following an examination of 1,239 kidney samples, Gudmundsdóttir *et al.* (1993) considered that a double-sandwich ELISA was more sensitive than culturing on SKDM. Yet, Bandín *et al.* (1996) reported the comparatively high cut-off for ELISA of $\sim 10^6$ bacteria/g of tissue. An amount of 0.3 μg of antigen/ml was noted by Olea *et al.* (1993). Sakai *et al.* (1989b, c) favoured the indirect dot blot assay (Sakai *et al.*, 1989b, c; Sakai and Kobayashi, 1992) (involving peroxidase and diaminobenzidine tetrahydrochloride as enzyme and substrate, respectively), which detected 10^2 cells/g of kidney tissue, over iFAT, co-agglutination, microscopy by Gram-stain, immunodiffusion and latex agglutination. Confirmatory diagnoses were made using dot blot and western blot assays (Sakai *et al.*, 1990; see also Olivier *et al.*, 1992). Certainly, Griffiths *et al.* (1991) highlighted the value of western blots over FAT and culturing for the detection of renibacteria. Immunohistochemistry is another approach which is gaining popularity for the efficient detection of BKD. In particular, the indirect peroxidase technique, as applied to tissue sections, was deemed to be more sensitive than iFAT or Gram-staining (Hoffman *et al.* 1989). Further work has also highlighted the value of the peroxidase–antiperoxidase immunohistochemical technique for the detection of *Renibacterium* (Jansson *et al.*, 1991).

The developments of serological methods for the detection and/or diagnosis of BKD must be examined sceptically because the reports preceded detailed taxonomic study of the organisms. It is unclear how workers knew that the aetiological agent possessed a unique antigenic profile, distinguishing it from other Gram-positive organisms. Unfortunately, the reliability of serological methods may now be questioned insofar as cross-reactions with apparently unrelated organisms have been recognised. Bullock *et al.* (1980) observed large bacteria, in faecal samples of brook trout, which fluoresced with antiserum to *Renibacterium*. After studying authentic representatives of 44 Gram-positive bacterial taxa and 101 cultures from fish and water, Austin and Rayment (1985) reported false positive reactions with coryneform bacteria obtained from fish, a fish-pathogenic *Mycobacterium* spp., and *Rothia dentocariosa*. Yoshimizu *et al.* (1987) noted a cross-reaction between *Pseudomonas* and *Ren. salmoninarum* in iFAT. A 60 kDa heat shock protein (hsp60) of *Chlamydia*

psittaci migrated with the 57 kDa protein of *Ren. salmoninarum*, and may explain the cross-reactivity of polyclonal renibacterium antiserum (Wood *et al.*, 1995). Of course, antisera can be made more specific by cross-absorbing with these organisms. Reinforcing a separate article (Toranzo *et al.*, 1993) by using western blots, Bandín *et al.* (1993) reported a common antigen, i.e. the 57 kDa protein, between *Cor. aquaticum*, *Car. piscicola* and *Ren. salmoninarum*. Also, it was noted that some isolates of *Ren. salmoninarum* did not produce the 57 kDa protein (Bandín *et al.*, 1993). This is interesting because, using the same strains, McIntosh *et al.* (1996) could not find the 57 kDa protein in *Cor. aquaticum* or *Car. piscicola*. Also in contrast to Bandín and coworkers, *Ren. salmoninarum* strain K57 was found to produce the 57 kDa protein. Brown *et al.* (1995) produced evidence that bacteria other than renibacterium could cross-react with antiserum to *Ren. salmoninarum*. Moreover, these workers used PCR and confirmed the conclusion of McIntosh *et al.* (1996) that *Cor. aquaticum* and *Car. piscicola* lacked the p57 antigen.

Investment may be placed in the development of monoclonal antibodies, which should be totally specific for *Renibacterium* (Arakawa *et al.*, 1987; Wiens and Kaattari, 1991). Evelyn (1978) contradicted the Utopian opinion of serology, by reporting that culturing was more sensitive than fluorescent antibody techniques for the detection of renibacteria in kidney tissue by a factor of 10:1. This theme was continued in a later study (Evelyn *et al.*, 1981) when experiments were undertaken to determine whether or not there was correlation between culturing and fluorescent antibody based diagnoses of the BKD carrier state. Again, culturing was reported as more sensitive than fluorescent antibody methods (Evelyn *et al.*, 1981). Nevertheless, from the work of Paterson and colleagues, it could not be explained what was present in the fish which gave a positive fluorescence test but which could not be cultured. Explanations include the presence of dead cells which retain the ability to fluoresce, anaerobes which would require specialized isolation procedures, fastidious aerobes, damaged, dormant or inhibited cells of renibacteria, or even inanimate particles which microscopically could be mistaken for bacteria. Obviously, caution is needed in interpreting serological diagnoses. Whenever possible, culturing should be used for confirmation. Certainly, the selective medium (SKDM; Austin *et al.*, 1983a) has proved useful in isolating *Renibacterium* from mixed cultures. In addition, during a comparative exercise with KDM2, SKDM consistently enabled a greater recovery of cells from infected fish. In some cases, scant growth of only one or two colonies were recovered from kidney tissue on SKDM, although the parallel KDM2 plates were devoid of any growth (Austin *et al.*, 1983a). Suspect cultures of renibacteria were subsequently confirmed by the characteristic profile on API-zym and other phenotypic traits, namely catalase production and inability to produce oxidase.

Whereas debate has centred over the most effective means of detecting BKD, it may be concluded that effective diagnosis should encompass a multiplicity of methods. These include isolation and characterisation, and serology on infected tissue. It is proposed that clinical cases of disease should be examined by FAT and culturing methods. Asymptomatic cases should be the subject of full bacteriological examination.

WHICH METHOD IS BEST?—FURUNCULOSIS

In a comparative study of serodiagnostic techniques, Sakai *et al.* (1986) reported that iFAT and the peroxidase–antiperoxidase enzyme immunoassay (PAP) were more sensitive (capable of detecting 10^3 CFU/ml) than the latex agglutination and co-agglutination techniques. These required 10^7 CFU/ml for positive results to be recorded. Nevertheless, with latex agglutination and co-agglutination techniques more (15/15 = 100%) positive samples were detected than by iFAT (10/15 = 67%) or PAP (1/15 = 73%).

MOLECULAR TECHNIQUES

There is evidence that molecular techniques have been used with increasing regularity for bacterial pathogens. A timely overview of PCR with emphasis on validation of the techniques and problems relating to diagnosis has been published (Hiney and Smith, 1998). A noteworthy advance resulted from the use of PCR technology to identify *Mycobacterium* spp. in sea bass (Knibb *et al.*, 1993) and *Myc. chelonei* in a cichlid oscar (*Astronotus ocellatus*) (McCormick *et al.*, 1995). A PCR followed by reverse cross blot hybridisation identified *Mycobacterium* sensitively (to 100 fg of DNA, which equated to 20 mycobacterial cells) to the species level (Puttinaowarat *et al.*, 2002).

A PCR using a 1100 bp fragment has distinguished *Lactococcus garvieae* from *Lactococcus lactis* (Zlotkin *et al.*, 1998). In terms of sensitivity, the PCR detected *Lactococcus garvieae* in 1 µl of fish plasma. Another publication reported a PCR based on a 709 bp fragment that was specific for *Lactococcus garvieae*, enabling a positive result to be obtained in 4 h (Aoki *et al.*, 2000). A multiplex PCR has reportedly been developed, and successfully recognised—from culture and fish tissues—the fish-pathogenic lactococci–streptococci, i.e. *Lactococus garvieae*, *Str. difficilis*, *Str. iniae* and *Str. parauberis* with a sensitivity for the purified DNA of 30 pg, 12.5 pg, 25 pg and 50 pg, respectively (Mata *et al.*, 2004).

León *et al.* (1994a, b) published details of a PCR assay using a 149 bp DNA sequence, which was sensitive enough to detect 22 renibacterial cells even in tissue, and of sufficient specificity to recognise *Ren. salmoninarum*, but not *Aer. hydrophila*, *Aer. salmonicida*, *Car. piscicola*, *Fla. columnare*, *V. anguillarum*, *V. ordalii* or *Y. ruckeri*. Then, this group detailed a 2,282 bp DNA fragment that appeared to be responsible for internalisation of renibacteria, at least into CHSE tissue culture cells (Maulén *et al.*, 1996). A nested reverse transcription PCR of 16S rRNA sequences successfully detected 1–10 renibacterial cells in ovarian fluid, but was unreliable with kidney (Magnússon *et al.*, 1994). Although sensitive, this system took 1–2 days to carry out. In a further development, McIntosh *et al.* (1996) devised a simplified PCR invoking the 376 bp region of the gene encoding the 57 kDa surface antigen. This system had a minimum detection limit of 5×10^3 renibacterial cells/ml in rainbow trout lymphocytes. Two 24-base oligonucleotide primers used to amplify a 501 bp region of the gene encoding the 57 kDa soluble protein (p57) formed the basis

of a PCR which was capable of detecting two renibacterial cells within individual salmonid eggs (Brown *et al.*, 1994). This PCR was considered to have value for the screening of broodstock for the presence of BKD (Brown *et al.*, 1994). A nested PCR amplifying a 320 bp fragment of the p57 antigen was suitable for detecting *Ren. salmoninarum* in ovarian samples (Pascho *et al.*, 1998). Terminal RFLP permitted the detection of ~30 CFU/mg of artificially inoculated kidney tissue (Nilsson and Strom, 2002). A QPCR was developed to detect the pathogen in chinook salmon, and correlated well with ELISA at high levels of infection (Powell *et al.*, 2005).

A clone, pRS47, of 5.1 kb (kilobase), was used to develop a specific DNA probe (Hariharan *et al.*, 1995). In a dot blot assay, the biotinylated pRS47/*Bam*HI insert probe hybridised with only DNA from three strains of *Ren. salmoninarum*, but not with *Arthrobacter protophormiae*, *Aer. salmonicida*, *Cor. aquaticum*, *Car. piscicola*, *Micrococcus luteus*, *Ps. fluorescens*, *V. anguillarum*, *V. ordalii* or *Y. ruckeri*. With kidney tissue from fish challenged with *Ren. salmoninarum*, the dot blot assay was regarded as sensitive as culture and FAT. With the latter technique, samples negative by dot blot assay and culturing revealed the presence of ≤ 1 fluorescing object, presumed to be a bacterium)/50 microscope fields. Consequently, this probe was regarded as having potential for the diagnosis of BKD (Hariharan *et al.*, 1995).

A PCR detected 10^2 CFUs of *Noc. seriolae* in yellowtail (Miyoshi and Suzuki, 2003).

The technology has been extended to the aeromonads including *Aer. hydrophila* (Chu and Lu, 2005) and *Aer. salmonicida* (e.g. Mooney *et al.*, 1995; Miyata *et al.*, 1996; Oakey *et al.*, 1998). Barry *et al.* (1990) suggested that such probes have the potential to detect the pathogen in environmental and clinical samples. These workers found that specific probes for micro-organisms could be developed, even if only 2 bp differences existed in the target sequence. Hiney *et al.* (1992) continued with developmental work leading to the isolation of a DNA fragment specific to *Aer. salmonicida*, which when incorporated into a polymerase chain reaction technique enabled a sensitivity of detection of approximately two cells of *Aer. salmonicida*. Mooney *et al.* (1995) examined the blood from 61 wild Atlantic salmon from 3 rivers in Ireland, and recorded 87% positives, with 100 genome equivalents/fish, using a specific DNA probe for *Aer. salmonicida*. Høie *et al.* (1997) designed primers and probes from 16S rRNA and plasmid DNA; the former of which amplified *Aer. hydrophila*, *Aer. salmonicida* subsp. *achromogenes*, *Aer. salmonicida* subsp. *masoucida* and atypical isolates, whereas the latter detected only *Aer. salmonicida* subsp. *achromogenes* and *Aer. salmonicida* subsp. *salmonicida*. Based on an examination of 100 ml volumes of kidney suspension and gill swabs, the PCR detected 20 and 200 CFU in 10 µl of PCR template by 16S rRNA and plasmid primers, respectively. The numbers corresponded to 10^3 and 10^4 CFU in 100 ml of kidney suspension, respectively (Høie *et al.*, 1997). A conclusion was reached that the PCR detected *Aer. salmonicida* more often than culturing. Terminal RFLP permitted the detection of ~30 CFU/mg of artificially inoculated kidney tissue (Nilsson and Strom, 2002). A possible drawback for use of DNA probes, however, has been proposed by Hennigan *et al.* (1989). Using four DNA probes in combination with seven restriction enzymes and seven strains of *Aer. salmonicida*, their data suggested that the DNA sequences of the species are very

strongly conserved. They emphasised that the use of DNA probe technology to identify different strains of *Aer. salmonicida* may be limited.

In a comparison of the sensitivity of culturing with DNA probes, the former detected *Aer. salmonicida* from the kidney of only dead or moribund farmed Atlantic salmon smolts in Ireland whereas probe technology allied to a PCR assay was capable of recognising the pathogen in water, faeces and effluent (O'Brien *et al.*, 1994). The benefit of species-specific primers and a nested PCR was demonstrated over universal eubacterial primers when the detection limit improved from 1.4×10^4 CFU/reaction to <14 CFU/sample (Taylor and Winton, 2002). The importance of the primer set was further highlighted in a comparative study by Byers *et al.* (2002a, b).

Specific DNA hybridisation probes for *Ph. damselae* subsp. *piscicida* offer promise for the future. Already, initial studies with a ^{32}P-labelled DNA probe indicated a minimum detection limit of 3.9 ng of DNA or 10^5 bacterial cells (Zhao and Aoki, 1989). Aoki *et al.* (1997) emphasised the value of a 629 bp DNA fragment from the subspecies-specific plasmid pZP1 for PCR. Kvitt *et al.* (2002) reported a detection limit of 0.35 pg, which equated to ~40 bacterial cells. A nested PCR detected 10 fg to 1 pg of DNA, which was considered to be equivalent to 20 to 200 cells, being sensitive enough to detect the pathogen in asymptomatic fish (Osorio *et al.*, 1999). In comparison, the PCR–RFLP approach detected <180 fg of purified DNA, and was useful for use with sea bream and sea bass (Zappulli *et al.*, 2005). In one case, it was necessary to discriminate subspecies *piscicida* from *damselae*, which was achieved using TCBS on which the former did not grow (Rajan *et al.*, 2003). However, this had been previously resolved by Osorio *et al.* (2000), who used multiplex–PCR to detect and differentiate subsp. *damselae* and *piscicida*. The former produced two amplification products, i.e. of 267 (fragment of 16S rRNA) and 448 bp (fragment of *ureC* gene) whereas the latter revealed only the 267 bp product. This suggests—and was confirmed by dot blot hybridisation—that subsp. *piscicida* lacks the *ureC* gene.

A PCR has been developed for the detection of *Ps. anguilliseptica*, with a detection limit of 170–200 cells/PCR tube within 8 h (Blanco *et al.*, 2002).

Molecular methods have been invoked to improve the identification of *V. anguillarum*. Using partial 16S rRNA sequences, a specific 16S rRNA oligonucleotide probe detected a minimum of 5×10^3 cells/ml in culture or tissue extracts (Rehnstam *et al.*, 1989). Detection of 1–10 bacterial cells in culture or 10–100 cells (equivalent to 2×10^3 to 2×10^4 cells/g of tissue) in turbot tissue per PCR reaction was detailed by Powell and Loutit (1994a) who used a 310 bp DNA fragment as a probe for *V. anguillarum*. This system detected 100 (but not 10) ng of purified genomic DNA of most serogroups (but not serogroup O7) of *V. anguillarum*, but not did react with other vibrios. Using the species-specific Gonzalez *et al.* (2003). probe in combination with membrane filtration, *V. anguillarum* could be detected in water (Powell and Loutit, 1994b). In a parallel development, an oligonucleotide (VaV3) detected 150 ng by DNA:DNA slot blot hybridisation. The system did not cross-react with other species, and was capable of detecting 8 out of 10 serogroups of *V. anguillarum* (Martínez-Picardo *et al.*, 1994). A high level of specificity and sensitivity (detection limit $= 4.0 \times 10^3$ cells/ml) involved the use of the *toxR* gene for the

detection of *V. harveyi*; the PCR of which took <5 h to enact and selectively recognised 20 authentic representatives of the taxon (including cells in diseased fish tissues), but not representatives of other vibrios (Pang *et al.*, 2006).

PCR technology offers promise for the detection of *Piscirickettsia salmonis* (e.g. Heath *et al.*, 2000; Mauel and Fryer, 2001; Venegas *et al.*, 2004), and a system has already been designed which is capable of detecting 1 tissue culture infectious dose (Mauel *et al.*, 1996) and 1–10 genome equivalents (competitive PCR; Heath *et al.*, 2000). Subsequently, a PCR was described that was effective with a few millilitres of serum (Marshall *et al.*, 1998). The benefit of this system is that the test could be carried out on live fish, such as valuable broodstock. A *Taq*Man PCR was specific and sensitive (0.5 $TCID_{50}$/ml) (Corbeil *et al.*, 2003).

PCR-targeted 16S rRNA has been successful with *Fla. branchiophilum*, *Fla. columnare* and *T. maritimum* (Toyama *et al.*, 1994, 1996; Avendaño-Herrera *et al.*, 2004d; Yeh *et al.*, 2006) although freezing–thawing destroyed the cells leading to the DNA being undetectable by PCR (Suomalainen *et al.*, 2006). With *T. maritimum*, a detection limit of 10^2–10^4 cells/reaction tube was reported (Avendaño-Herrera *et al.*, 2004). A nested PCR gave even greater sensitivity, detecting 1–250 cells/PCR reaction, particularly in the skin and mucus (Avendaño-Herrera *et al.*, 2004). This is compatible with the sensitivity of 75 CFU/g reported for the nested PCR by Cepeda *et al.* (2003). Also, LAMP was used successfully to diagnose *Fla. columnare* (Yeh *et al.*, 2006). In addition, a PCR was sensitive enough to detect 1.5 CFU/PCR reaction tube of *Fla. psychrophilum* from apparently healthy coho salmon eggs and juvenile ayu (Izumi and Wakabayashi, 1997). *In situ* hybridization using digoxigenin-labelled 16S rDNA probes led to the detection of *Fla. psychrophilum* in experimentally (after immersion or subcutaneous injection—in gills, heart, kidney, muscle and spleen, but not in the brain, intestine, liver, pyloric caeca or stomach) and naturally (intestine, liver, pancreas, pyloric caeca and stomach) infected ayu (Liu *et al.*, 2001). A PCR targeting 16S rRNA has shown promise for the specific (negative for other flavobacteria) and sensitive (~100 cells) detection of *Fla. columnare* from culture and fish tissues within 5–8 h. A nested PCR increased sensitivity to 10 bacterial cells in asymptomatic fish (Bader *et al.*, 2003). Similarly, PCR technology has proven to be successful for the detection of *Fla. psychrophilum* and *T. maritimum*, and was considered to be more sensitive than culturing (Wiklund *et al.*, 2000; Avendaño-Herrera *et al.*, 2004). In experiments using brain tissue seeded with the pathogen, the detection limit was 0.4 CFU/PCR tube, which corresponded to 17 CFU/g of brain tissue. Using normal freshwater, i.e. containing a mixed microbial population, seeded with *Fla. psychrophilum*, the detection limit was 1.7 CFU/PCR tube, which corresponded to 110 CFU/ml of water. The PCR detected the pathogen in water from a fish farm, whereas culturing did not reveal the presence of the organism (Wiklund *et al.*, 2000). A nested PCR enabled a detection limit of 1 cell/PCR tube, which was equivalent to 10 cells/g of spleen and 5 cells/g of ovarian fluid (Baliarda *et al.*, 2002). Ovarian fluid eggs and gill washings and benthic diatoms were used with a nested PCR by Taylor (2004) and Izumi *et al.* (2005), respectively, with again commendable results. A *Taq*Man-based PCR used primers amplifying a 971 bp fragment of the 16S rRNA, and detected 1.1 pg of *Fla. psychrophilum* DNA, which equated with

4.7 CFU/PCR reaction (del Cerro *et al.*, 2002). Terminal RFLP permitted the detection of ~30 CFU of *Fla. psychrophilum*/mg of artificially inoculated kidney tissue (Nilsson and Strom, 2002). The benefit of species-specific primers and a nested PCR was demonstrated over universal eubacterial primers when the detection limit improved from 1.4×10^5 CFU/reaction to <14 CFU/sample (Taylor and Winton, 2002).

There is evidence that molecular techniques are finding use for the identification of infections caused by *Y. ruckeri* (e.g. Argenton *et al.*, 1996; Taylor and Winton, 2002; Sakai *et al.*, 2006). A PCR was successful in detecting *Y. ruckeri* in artificially and naturally diseased trout tissues, with a sensitivity of 60–65 cells/PCR tube (Gibello *et al.*, 1999). The value of PCR was echoed by Altinok *et al.* (2001), who detected the pathogen in the blood of rainbow trout within 1 h of immersion in a suspension containing 4.5×10^6 CFU of *Y. ruckeri*/l. Indeed, the approach was more reliable than culturing at detecting the organism (Altinok *et al.*, 2001). Detection of the *yruR*/*yruI* genes involved with quorum-sensing has been regarded as sensitive (i.e. 1 pg; 12 CFU) specific for the 6 isolates of *Y. ruckeri* tested, but not to representatives of 5 other *Yersinia* species (Temprano *et al.*, 2001). Others have proposed a PCR and RFLP targeting the *aroA* gene (Yugueros *et al.*, 2001). A nested PCR had a detection limit of 1.4×10^5 CFU/reaction. However, use of species-specific primers improved detection to <14 CFU/sample (Taylor and Winton, 2002).

Real time PCR, which produces a result in 4–5 h, has been developed for *Edw. ictaluri*, and detected the equivalent of 2.5 cells using DNA samples from cultures and fish blood (Bilodeau *et al.*, 2003).

Sequencing of the 16S rDNA is becoming an accepted procedure for the identification of bacteria, including fish pathogens. The technique has been instrumental in the recognition of new pathogens, including *Str. dysgalactiae* (Nomoto *et al.*, 2004), and confirmed the presence of *Lactococcus garvieae* in Taiwan (Chen *et al.*, 2002).

An ideal situation would involve techniques that could recognise and differentiate between multiple diseases, and this has been achieved with multiplex PCR. Del Cerro *et al.* (2002) detected simultaneously *Aer. salmonicida*, *Fla. psychrophilum* and *Y. ruckeri* in fish tissues, recognising the equivalent of 6, 0.6 and 27 CFU, respectively. Similarly, González *et al.* (2004) used a multiplex PCR and DNA microarray, and achieved the simultaneous and differential diagnosis of *Aer. salmonicida*, *Ph. damselae* subsp. *damselae*, *V. anguillarum*, *V. parahaemolyticus* and *V. vulnificus*, with a minimum detection limit of <20 fg per reaction, which equates to 4–5 bacterial cells. Matsuyama *et al.* (2006) developed a low-density oligonucleotide DNA array for the detection and discrimination of multiple *Photobacterium* and *Vibrio* spp. within a day, albeit with some cross-hybridisation reported. These workers designed a low-density oligonucleotide DNA array between the 16S and 23S ribosomal DNA leading to the development of three oligonucleotide probes, which were immobilized on nylon membranes. The low-density oligonucleotide DNA arrays were amplified by PCR, hybridised and the specific signals produced with alkaline phosphatase-conjugated anti-digoxigenin-labelled PCR products (Matsuyama *et al.*, 2006).

PHENOTYPIC TESTS

For many pathogens emphasis has been placed on conventional phenotypic tests for diagnosis. For example, Boulanger *et al.* (1977) highlighted the value of confirming the Gram-staining reaction, fermentative metabolism of glucose, and production of arginine dihydrolase, catalase and oxidase, but not of lysine or ornithine decarboxylase. Caution is advocated should consideration be given to using commercially available diagnostic kits. The API 20E (Figure 6.35 [see colour section]) and API-zym (Figure 6.36 [see colour section]) systems, and more recently the API 20NE API 50CH, API 50L, Biolog-GN, Enterotubes and RapidID 32 systems (Meyer and Bullock, 1973; Amandi *et al.*, 1982) have made an inroad into routine diagnostic laboratories. Whereas some systems, e.g. Biolog-GN, are clearly adaptable to and useful for environmental isolates, others have been developed specifically for a given use, usually medically important bacteria. The consequence is that the supporting identification schemes may be inappropriate. Many workers have used the API 20E system for the identification of fish-pathogenic bacteria, and the technique has been considered useful be some (e.g. Santos *et al.*, 1993). For example, by inference the API 20E rapid identification system may be useful for diagnosing *Flavobacterium* sp. (Acuigrup, 1980a). Kent (1982) reported that *V. ordalii* produces a characteristic profile in API 20E, i.e.

− − − − − − − − − − + + + − − − + − − − +

Kent (1982) reported the API 20E profile for *V. anguillarum* as:

+ v − − + − − − v + + + + − + − + − + v +

whereas Maugeri *et al.* (1983) published a slightly different pattern, i.e.

v + − − v − − − v v v + + − − − v − + v +

where "v" indicated a variable result.

Kent (1982) reported that *Ph. damselae* subsp. *piscicida* gave positive responses in the API 20E rapid identification system for arginine dihydrolase and weak acid production from glucose, all other tests being negative. Of course, it is necessary to modify the protocol for use with marine bacteria. Thus, it was essential to suspend cultures in 2–3% (w/v) saline rather than distilled water, and the inoculated test strips were incubated at 25°C (not 37°C) for up to 48 h. Maugeri *et al.* (1983) considered that it was essential to carry out some additional tests with putative *V. anguillarum*, namely motility and sensitivity to O/129, in order to confirm that the isolates were indeed motile and were inhibited by the vibriostatic agent. However, some cultures may be resistant to the action of O/129 (Muroga *et al.*, 1979) and appear to be non-motile. The latter phenomenon may result from exposure to the partial inhibitory activity of some antimicrobial compounds.

The API 20E system weighs heavily on the use of sugar fermentation reactions, which may be influenced by the presence of plasmids. The profile(s) for *Aer. hydrophila* are similar to those of *Aer. allosaccharophila* and *Aer. sobria*, whereas *T. maritimum* and *Ps. anguilliseptica* may be indistinguishable by the API 20E rapid

identification system. *Y. ruckeri* may be confused with *Haf. alvei*. Toranzo *et al.* (1986) compared the API 20E rapid identification system for *Aer. hydrophila* with Kaper's medium (Kaper *et al.*, 1979) and conventional biochemical tests. Kaper and co-workers formulated a single-tube medium, which was suitable for determining motility, inositol and mannitol fermentation, ornithine decarboxylase and deamination, and the production of H_2S and indole. Thus, *bona fide* isolates of *Aer. hydrophila* gave an alkaline reaction on the top of the medium, acid production in the butt, motility, and indole, but not H_2S production (H_2S production may occur on the top). Toranzo and colleagues pointed to shortcomings of the API 20E system, insofar as many environmental isolates were mis-identified or not listed by the published profile index. In contrast, Kaper's medium was effective for fast, presumptive identification. Problems were encountered with the reliability of some conventional biochemical tests, notably the Voges Proskauer reaction, fermentation and gas production from arabinose, gelatinase production, and the lysine decarboxylase test. Ironically, these tests have also been considered to be correlated with virulence in motile aeromonads. Nevertheless, it is accepted that the API 20E and API 20NE systems have a role in the diagnosis of bacterial fish pathogens. Consequently, diagnostic schemes based on these systems are included in Tables 6.3 and 6.4. In addition, API-zym is very useful for diagnosing *Ren. salmoninarum* (See Table 6.5), which gives a characteristic profile:

$$- + - + - + - - + - + + - - - + - - +/- - -$$

A diagnostic scheme for use with Biolog-GN has been included as Table 6.6.

Diagnoses may also be achieved by means of diagnostic schemes based on reactions in conventional phenotypic tests. The procedure may be automated, as with the Abbott Quantum II system (Teska *et al.*, 1989), and involves spectrophotometric readings at 492.6 nm, with a sample cartridge containing 20 inoculated biochemical chambers.. Alternatively, specially constructed diagnostic tables may be used, such as presented in Tables 6.7 and 6.8.

Diagnosis of botulism has been accomplished by isolation of *Cl. botulinum* from diseased tissues and, more importantly, by demonstrating the presence of circulating toxin (particularly to *Cl. botulinum* type E) in the blood of moribund fish (Cann and Taylor, 1982).

Presumptive identification of *Lactococcus garvieae*-like organisms has been made following growth on bile (40%)–aesculin agar (see Facklam and Moody, 1970), with hydrolysis of aesculin and by the characteristic growth on eosin–methylene blue agar (lactose is not fermented).

Bacillus mycoides could be distinguished from other bacilli, as follows (Goodwin *et al.*, (1994):

	Presence of parasporal crystals	Rhizoidal growth	Motility	Growth at 45°C
Bacillus mycoides	−	+	−	−
Bacillus anthracis	−	−	−	+
Bacillus cereus	−	−	+	+
Bacillus thuringiensis	+	−	+	+

Table 6.3. Profiles of fish pathogens obtained with the API 20E rapid identification system. Results were recorded after incubation at 15–37°C for 24–48 h

Taxon:	API 20E test no.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Acinetobacter–Moraxella spp.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Aer. allosaccharophila	+	v	+	v	v	−	−	−	+	−	+	+	+	−	−	v	+	v	−	v	+
Aer. hydrophila	+	+	v	−	v	−	−	−	+	v	+	+	+	−	−	−	+	−	v	v	+
Aer. salmonicida	−	+	v	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	+
Aer. sobria	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	+	−	−	−	+
Cit. freundii	+	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	+	+	−	+	−
Edw. ictaluri	+	−	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Edw. tarda	−	−	+	+	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−
Fla. branchiophilum	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	+	+	−	−	−
Fla. columnare	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Fla. hydatis	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	+	−
Fla. psychrophilum	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	(+)
T. maritimum	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Haf. alvei	−	−	+	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−
Halomonas cupidus	−	+	+	+	−	−	−	−	−	−	−	−	+	−	+	+	(+)	−	−	−	−
J. lividum	−	+	−	−	+	−	−	−	−	+	+	(+)	+	−	−	−	−	−	−	−	+
Klebsiella pneumoniae	+	−	v	−	+	−	v	−	−	+	−	+	+	+	+	+	+	+	+	+	−
Pantoea agglomerans	+	−	−	−	+	−	−	−	−	+	+	+	+	−	−	−	+	−	−	−	−
Ph. damselae subsp. *damselae*	−	+	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−	+
Ph. damselae subsp. *piscicida*	−	−	−	−	−	−	−	−	−	(+)	−	(+)	−	−	−	−	−	−	−	−	+
Plesiomonas shigelloides	+	+	+	+	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	+
Pr. rettgeri	−	−	−	−	−	−	+	+	+	−	−	+	+	+	−	+	−	−	−	−	−
Ps. anguilliseptica	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Ps. fluorescens	−	+	−	−	+	−	−	−	−	−	+	(+)	+	+	+	−	+	−	−	+	+
Sal. enterica subsp. *arizonae*	+	−	+	−	+	+	−	−	−	−	+	+	−	+	−	+	−	−	−	−	−

(*continued*)

Table 6.3 (*cont.*)

Taxon:	API 20E test no.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Ser. liquefaciens	+	−	+	+	+	−	−	−	−	+	−	+	+	+	−	−	+	+	+	+	−
Ser. marcescens	+	−	+	+	+	−	v	−	−	v	+	+	+	v	+	−	+	v	+	−	−
Ser. plymuthica	+	−	−	−	+	−	−	−	−	+	+	+	+	+	−	−	+	−	−	+	−
Shewanella putrefaciens	−	−	−	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
V. alginolyticus	−	−	+	−	v	−	−	−	+	−	v	+	+	−	−	−	+	−	−	−	+
V. anguillarum	+	+	−	−	+	−	−	−	+	+	+	+	+	−	+	−	+	−	+	+	+
V. cholerae	+	−	+	+	v	−	−	−	+	v	+	+	+	−	−	−	+	−	−	−	+
V. harveyi	−	−	+	+	−	−	−	−	+	−	+	+	−	−	−	−	+	−	−	−	+
V. ordalii	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	+
V. salmonicida	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+
V. splendidus	−	+	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−	+
V. vulnificus	+	−	v	−	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	−	+
Y. intermedia	+	−	−	+	−	−	+	−	+	−	−	+	+	v	+	+	+	+	+	v	−
Y. ruckeri	+	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−

1 = β-galactosidase; 2 = arginine dihydrolase; 3 = lysine decarboxylase; 4 = ornithine decarboxylase; 5 = citrate utilisation; 6 = H_2S production; 7 = urease production; 8 = tryptophan deaminase; 9 = indole production; 10 = Voges Proskauer reaction; 11 = gelatin hydrolysis; 12 = acid from glucose; 13 = acid from mannitol; 14 = acid from inositol; 15 = acid from sorbitol; 16 = acid from rhamnose; 17 = acid from sucrose; 18 = acid from melibiose; 19 = acid from arabinose; 20 = oxidase production.

+, − and v correspond to >80, >20 and 21–79% of positive results, respectively.

( ) indicates weakly positive results.

Table 6.4. Differential characteristics of some fish pathogens obtained with the API 20NE rapid identification system. Results were recorded after incubation at 30°C for 24–48 h

Taxon[a]	API 20NE test No.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Acinetobacter calcoaceticus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	−
Aer. hydrophila	+	v	+	+	−	+	+	+	+	v	+	+	+	+	+	+	−	+	v	−	+
Aer. salmonicida subsp. *achromogenes/masoucida*	+	v	−	−	−	v	v	−	v	−	v	v	−	−	−	−	−	−	−	−	+
Aer. salmonicida subsp. *salmonicida*	+	v	v	v	−	+	+	−	+	−	v	+	+	+	+	−	−	+	−	−	+
Aer. sobria	+	v	+	+	−	−	+	+	+	−	+	+	+	+	+	+	−	+	+	−	+
Ph. damselae	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	+
Plesiomonas shigelloides	+	+	+	+	−	−	−	+	v	−	−	−	v	v	v	v	−	v	−	−	+
Ps. chlororaphis	−	−	−	−	−	v	−	−	+	+	+	+	−	−	+	v	−	+	+	−	v
Ps. fluorescens	v	−	−	v	−	−	v	−	+	v	+	+	v	−	+	+	−	+	+	−	+
Ps. plecoglossicida	+	−	−	+	−	−	+	−	+	−	−	−	+	−	+	+	−	+	+	+	+
Shewanella putrefaciens	+	−	−	−	−	v	+	−	−	−	−	−	+	−	−	v	−	+	−	−	+
V. alginolyticus	+	+	+	−	−	v	+	−	v	−	v	v	v	v	v	−	−	+	−	−	+
V. cholerae	+	+	+	−	−	−	+	+	+	−	v	v	v	+	+	−	−	+	+	−	+

[a] +, − and v correspond to ≥80, ≥20 and 21–79% of positive results, respectively.

Table 6.5. Distinguishing profiles of Gram-positive bacteria as obtained with API zym[a]

Taxon (and source of strains)	API zym test no.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Actinomyces viscosus (ATCC 15987)	−	−	−	+	−	+	+	−	−	−	+	+	+	+	−	−	+	−	−	+
Aerococcus viridans subsp. *homari* (NCIMB 1119)[b]	−	+	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Arthrobacter aurescens (NCIMB 8912)	−	−	−	+	−	+	−	+	+	−	+	+	−	+	+	+	+	−	+	−
Arthrobacter crystallopoietes (ATCC 15481)	−	−	−	+	−	+	+	−	+	−	−	+	−	−	−	−	−	−	−	−
Arthrobacter globiformis (NCIMB 8907)	−	−	−	+	−	+	+	+	+	−	+	+	−	+	−	+	+	−	+	−
Arthrobacter nicotianae (NCIMB 9458)	−	−	−	+	−	+	+	−	+	−	−	+	−	−	−	+	−	+	+	−
Bacillus cereus (CCM 2010)	−	+	+	+	−	+	+	−	+	+	+	+	−	−	−	+	−	−	−	−
Bacillus licheniformis (ATCC 9945)	−	+	+	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−
Bacillus megaterium (CCM 2007)	−	+	+	+	−	+	−	−	−	+	+	+	+	+	−	−	−	−	−	−
Bacillus polymyxa (ATCC 12321)	−	+	+	+	−	+	−	−	−	+	−	+	+	+	−	−	−	−	−	−
Bacillus sphaericus (ATCC 10208)	−	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
Brevibacterium flavum (ATCC 13826)	−	+	−	+	−	+	+	−	+	−	+	+	−	−	+	−	+	−	−	−
Cor. acnes (NCTC 737)	−	−	−	+	−	−	−	−	(+)	−	−	+	−	−	−	−	−	−	−	−
Cor. pyogenes (NCTC 5224)	−	+	−	+	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−
Cor. xerosis (NCTC 7929)[b]	−	+	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
En. faecalis (CCM 1875)	−	−	−	+	−	+	−	−	−	+	+	+	−	+	−	+	−	−	−	−
En. faecium (CCM 2801)[b]	−	+	+	+	−	+	−	−	(+)	−	−	+	−	−	−	−	−	−	−	−
Kurthia zopfii (CCM 3478)	−	−	−	(+)	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
Lactobacillus casei (CCM 1753)	−	−	+	+	−	+	+	−	−	−	+	+	−	+	−	−	+	−	−	−
Lactobacillus curvatus (NCIMB 9710)	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−
Lactobacillus brevis (NCDO 1749)	−	−	(+)	+	−	+	+	−	−	−	+	+	+	+	−	+	+	−	−	−
Lactobacillus sp. (pseudokidney disease, 3 isolates)	−	−	(+)	+	−	+	(+)	−	−	(+)	+	−	(+)	−	−	+	+	−	−	−
Listeria denitrificans (ATCC 14870)	−	−	−	+	−	+	−	−	−	−	−	+	+	+	+	+	+	−	−	−
Listeria grayi (CCM) 5887)	−	−	(+)	+	−	(+)	(+)	−	−	−	−	+	−	−	−	−	−	−	−	−
Listeria murrayi (CCM 5990)	−	−	+	+	−	+	−	−	−	(+)	−	+	−	−	−	−	−	−	−	−
Microbacterium lacticum (NCIMB 8450)	−	−	−	+	−	+	+	−	−	−	+	+	−	+	−	−	−	−	−	−
Micrococcus luteus (NCIMB 9278)	−	+	−	+	−	+	−	−	−	+	+	+	−	−	−	+	−	−	−	−

Myc. aquae (Körmendy)	−	+	+	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−
Myc. fortuitum (Körmendy)	−	+	+	+	−	+	+	−	+	−	+	+	−	−	−	−	−	−	−	−
Myc. marinum (Körmendy)	−	+	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−
Myc. smegmatis (Körmendy)	−	+	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−
Mycobacterium sp. (Ashburner, SC 744)	−	+	+	+	−	+	−	−	−	−	+	+	−	−	−	+	−	−	−	−
Noc. asteroides (ATCC 14759)	−	+	+	+	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−
Noc. corallina (ATCC 4273)	−	+	+	+	−	+	+	+	−	−	−	+	−	−	−	−	−	−	−	−
Planococcus citreus (NCIMB 1493)	−	−	−	+	−	+	+	+	(+)	(+)	−	+	−	+	−	+	−	−	−	−
Propionibacterium acnes (CCM 3343)	−	−	−	(+)	−	+	−	−	−	−	−	+	−	+	−	−	−	−	+	−
Ren. salmoninarum (48 isolates)	−	+	−	+	−	+	−	−	+	−	+	+	−	−	−	+	−	−	(+)	−
Rothia dentocariosa (ATCC 17931)	−	−	−	+	−	+	+	−	+	−	−	+	−	−	−	+	−	−	−	−
Sta. epidermidis (NCIMB 2699)	−	+	(+)	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−
Str. agalactiae (CCM 6187)	−	+	−	+	−	−	−	−	−	−	+	+	−	−	−	(+)	−	−	−	−
Str. dysgalactiae	−	+	−	−	−	+	(−)	−	−	−	+	(+)	−	−	+	+	−	−	−	−
Streptomyces griseus (ATCC 23345)	−	+	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−
Presumptive coryneform (laboratory isolate 198)	−	−	+	+	−	+	−	−	−	−	+	+	−	−	−	+	−	−	−	−

1 = control, 2 = alkaline phosphatase, 3 = esterase (butyrate), 4 = esterase (caprylate), 5 = lipase (myristate), 6 = leucine arylamidase, 7 = valine arylamidase, 8 = cystine arylamidase, 9 = trypsin, 10 = chemotrypsin, 11 = acid phosphatase, 12 = phosphoamidase, 13 = α-galactosidase, 14 = β-galactosidase, 15 = β-glucuronidase, 16 = α-glucosidase, 17 = β-glucosidase, 18 = *N*-acetyl-β-glucosaminidase, 19 = α-mannosidase, 20 = α-fucosidase.

[a] 15°C/18 h.

[b] Distinguish by results of the Gram-staining reaction.

Sources: ATCC, American Type Culture Collection; CCM, Czechoslovak Collection of Micro-organisms; NCDO, National Collection of Dairy Organisms; NCIMB, National Collection of Industrial and Marine Bacteria; NCTC, National Collection of Type Cultures; Dr. L.D. Ashburner, Freshwater Fisheries Research Station, Victoria, Australia; Dr. B. Körmendy, Central Veterinary Institute, Hungary.

Table 6.6. Characteristics of selected taxa by Biolog-GN

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Aer. caviae	−	v	+	+	+	v	−	+	−	+	v	+	−	+	−	+	+	+	−	v	v	+	+	+	−	+	+	−	−	+	+	+	+	−	+	+	+	−	−
Aer. hydrophila	−	v	+	+	+	+	+	+	−	v	−	−	−	+	−	+	v	+	−	v	v	+	+	+	−	+	+	−	−	+	+	+	+	−	+	+	v	v	−
Aer. salmonicida	−	−	+	+	+	+	−	+	−	−	−	−	−	+	−	+	−	+	−	−	−	+	v	+	−	−	+	−	−	v	−	−	−	−	+	v	−	−	−
Aer. sobria	−	−	+	+	+	+	−	+	−	−	−	−	−	+	−	+	v	+	−	v	v	+	+	+	−	+	+	−	−	+	+	+	−	−	+	v	v	v	v
Cit. freundii	−	−	+	+	+	+	+	+	−	+	v	v	−	+	+	+	v	+	v	+	v	+	+	+	v	+	+	v	+	+	v	+	−	−	+	+	+	v	+
Edw. tarda	−	−	+	+	−	−	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	+	−	+	−	v	+	−	−	−	−	−	−	−	+	+	+	v	v
Esch. vulneris	−	−	+	+	v	v	−	+	−	+	v	+	−	+	−	+	+	+	v	+	v	+	+	+	+	+	+	+	+	+	v	+	v	−	+	+	+	−	−
Haf. alvei	−	v	+	+	+	+	+	+	v	+	v	v	−	+	+	+	+	+	v	v	v	+	+	+	−	v	+	v	v	+	v	+	v	v	+	+	+	−	−
Klebsiella pneumoniae	−	−	+	+	v	v	−	+	+	v	+	v	−	+	+	+	+	+	+	v		v	+	+	v	+	+	+	+	+	+	+	+	−	−	v	+	+	v	+
Pantoea agglomerans	−	−	+	+	v	v	v	+	v	+	+	v	−	+	v	+	+	+	+	v	v	+	+	+	v	+	+	v	+	+	+	+	v	v	+	+	v	+	v	
Ph. damselae	−	−	+	+	−	−	v	+	−	−	−	v	−	+	−	+	v	+	−	−	−	+	−	+	−	−	+	−	−	−	−	v	−	−	+	+	v	−	−
Plesiomonas shigelloides	−	−	−	v	−	−	v	+	−	−	−	−	−	−	−	v	−	+	+	−	−	+	−	v	v	+	−	−	−	−	−	+	−	−	+	+	v	−	−
Pr. rettgeri	−	−	v	+	v	v	v	+	v	−	v	−	v	+	−	+	v	+	+	−	−	+	v	+	−	v	v	−	−	v	v	v	−	v	+	+	+	+	+
Ps. fluorescens	−	−	v	v	v	v	−	v	v	v	v	−	−	−	+	−	v	v	+	+	−	−	v	+	+	−	−	v	−	−	v	v	v	−	v	+	v	v	+	+
Ps. pseudoalcaligenes	−	−	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	v	v
Ser. liquefaciens	−	−	+	+	v	v	+	+	v	+	v	v	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	+	+	+	v	+	+	+	+	+
Ser. marcescens	−	v	+	+	+	+	+	+	+	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	v	+	+	v	v	+	+	+	+	+	+	+	+	+	+
Ser. plymuthica	−	−	+	+	+	+	−	+	−	+	v	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	+	+	+	−	+	+	+	+	+
V. alginolyticus	−	+	+	+	+	v	−	+	−	−	v	−	−	+	−	−	+	+	−	−	−	+	+	+	−	v	+	−	−	+	+	+	v	−	+	+	+	−	−
V. harveyi	−	+	v	+	+	−	+	+	−	−	−	+	−	+	−	+	−	+	−	−	−	+	+	+	−	−	+	−	−	v	+	+	−	−	+	−	−	−	−
V. vulnificus	−	−	v	+	−	v	v	+	−	−	−	+	−	+	−	v	+	+	−	−	−	+	v	+	−	v	+	−	−	v	v	+	−	−	+	v	v	−	−
Y. intermedia	−	−	+	+	v	v	+	+	−	+	+	+	−	+	+	+	+	+	+	−	−	+	+	+	−	+	+	−	−	+	+	+	−	−	+	v	+	−	v
Y. ruckeri	−	−	+	+	−	−	v	+	−	−	v	−	−	+	−	+	v	+	−	−	−	+	+	+	−	v	+	−	−	+	−	+	−	−	+	v	+	−	−

Taxon	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
Aer. caviae	+	−	v	+	−	−	+	v	−	−	−	−	+	−	+	−	v	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	v	+	−	+	−
Aer. hydrophila	v	−	−	+	−	−	v	−	−	−	−	−	v	−	v	−	−	−	−	−	+	+	+	−	v	+	+	+	+	+	+	+	+	v	−	−	v	−	v	−
Aer. salmonicida	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	v	−	v	v	+	+	+	v	+	v	+	−	−	v	−	−	−	−
Aer. sobria	−	−	−	+	−	−	−	−	−	−	−	−	v	−	v	−	−	−	−	−	+	+	+	−	v	v	+	+	+	+	+	+	+	v	−	v	−	−	v	−
Cit. freundii	+	−	+	+	v	+	+	+	−	−	−	+	v	−	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	v	−	+	−
Edw. tarda	+	−	−	+	−	+	+	−	−	−	−	v	+	−	+	−	−	−	−	−	+	+	+	+	−	−	−	v	+	+	+	+	+	−	−	−	−	−	+	−
Esch. vulneris	+	+	+	+	v	+	v	−	−	−	−	−	−	−	+	v	−	v	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	v	v
Haf. alvei	+	+	+	+	+	+	+	−	−	v	−	v	v	−	+	v	−	−	−	−	+	+	+	+	v	v	+	+	+	+	+	+	+	+	−	v	+	+	+	−
Klebsiella pneumoniae	+	v	+	+	−	+	v	−	−	v	−	−	v	−	+	v	−	v	+	−	+	+	+	+	v	+	+	+	+	+	+	+	+	+	−	−	v	v	+	−
Pantoea agglomerans	v	v	+	+	v	+	v	v	−	−	−	−	v	−	+	v	v	v	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	v	−	+	−
Ph. damselae	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	v	−	−	−	−	−	+	+	+	+	v	−	−	−	−	−	−	−
Plesiomonas shigelloides	−	−	−	+	−	−	−	−	−	−	−	−	v	−	−	−	−	−	−	−	+	+	v	−	−	−	−	−	+	+	+	v	v	−	−	−	−	−	v	−
Pr. rettgeri	+	v	−	+	v	−	v	−	−	+	−	v	+	−	v	−	v	−	−	−	+	+	+	−	v	v	v	v	+	+	+	+	+	+	+	+	v	+	+	+
Ps. fluorescens	+	v	v	+	v	v	v	+	v	v	+	v	+	v	+	+	+	+	v	−	+	v	+	v	v	+	+	+	+	+	+	−	v	+	+	v	+	−	+	+
Ps. pseudoalcaligenes	+	−	−	−	−	−	+	+	+	−	+	+	+	v	+	−	+	−	−	v	+	+	+	−	+	+	+	v	+	v	+	−	−	v	v	v	v	v	+	+
Ser. liquefaciens	+	+	+	+	+	+	v	v	−	v	−	−	+	−	+	v	−	v	−	−	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	−	+	v	+	v
Ser. marcescens	+	v	+	+	+	+	+	+	v	+	−	+	+	−	+	v	+	v	v	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Ser. plymuthica	+	*v*	+	+	+	+	−	−	−	+	−	−	+	−	+	−	−	+	−	−	+	+	−	*v*	−	+	+	+	+	+	+	+	+	+	*v*	−	−	−	+	−
V. alginolyticus	v	−	−	+	−	−	v	−	−	−	−	v	v	−	+	−	+	−	−	−	+	v	+	−	v	+	+	+	+	+	+	+	+	+	v	v	v	−	+	−
V. harveyi	−	−	−	+	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	v	−	−	+	+	−	+	+	+	v	−	−	−	−	−	−	−
V. vulnificus	−	−	−	v	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	+	−	v	−	−	−	v	v	v	v	+	v	v	−	−	−	−	−	−	−
Y. intermedia	v	+	+	+	−	+	−	−	+	−	−	−	v	−	v	−	−	−	−	−	+	v	v	+	+	v	+	+	+	v	+	+	+	v	−	−	−	−	v	−
Y. ruckeri	−	−	+	+	−	v	v	−	−	−	−	−	−	−	+	−	−	−	−	−	+	v	v	v	−	−	+	+	+	v	+	v	+	−	−	−	−	−	+	−

(*continued*)

Table 6.6 (*cont.*)

Taxon	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
Aer. caviae	+	+	+	−	v	+	+	+	+	−	−	−	−	+	+	−	−
Aer. hydrophila	+	+	v	−	−	+	+	+	+	−	−	−	−	+	+	+	+
Aer. salmonicida	v	+	−	−	−	+	+	+	+	−	−	−	−	+	−	−	−
Aer. sobria	+	+	v	−	−	+	+	+	+	−	−	−	−	+	+	+	+
Cit. freundii	+	+	v	−	−	+	+	+	+	−	v	+	−	+	+	+	+
Edw. tarda	+	+	+	−	−	−	+	+	+	−	−	−	−	+	+	+	+
Esch. vulneris	v	+	+	−	−	v	+	+	+	−	−	−	−	+	+	+	+
Haf. alvei	+	+	+	−	v	v	+	+	+	v	+	−	−	+	+	+	+
Klebsiella pneumoniae	+	+	+	−	v	v	+	+	+	−	v	−	−	+	+	+	+
Pantoea agglomerans	v	+	v	−	v	+	+	+	+	−	−	−	−	+	+	+	+
Ph. damselae	−	v	−	−	−	−	+	+	+	v	−	−	−	+	+	+	+
Plesiomonas shigelloides	−	v	−	−	−	−	+	+	+	v	−	−	−	+	+	−	−
Pr. rettgeri	+	+	v	v	−	+	+	+	+	−	−	−	−	+	+	+	+
Ps. fluorescens	−	+	+	−	v	v	v	−	−	−	+	+	−	v	−	−	−
Ps. pseudoalcaligenes	−	+	+	−	v	v	v	−	−	−	+	+	−	v	−	−	−
Ser. liquefaciens	+	+	+	−	v	+	+	+	+	−	+	−	−	+	+	+	+
Ser. marcescens	+	+	+	−	+	+	+	+	+	−	+	−	−	+	+	+	+
Ser. plymuthica	+	+	v	−	−	+	+	+	+	−	v	−	−	+	+	+	+
V. alginolyticus	v	+	+	−	−	+	+	+	+	v	−	v	−	+	v	+	+
V. harveyi	+	+	+	+	+	−	+	+	+	−	−	−	−	+	+	+	+
V. vulnificus	−	v	−	−	−	v	+	+	v	−	−	−	−	v	−	v	v
Y. intermedia	+	+	v	−	−	v	+	+	+	v	−	−	−	+	+	+	+
Y. ruckeri	−	+	+	−	−	−	+	+	+	−	−	−	−	+	*v*	−	−

+, − and v correspond to ≥80%, ≥20% and 21–79% of positive responses, respectively.
1 = water; 2 = α-cyclodextrin; 3 = dextrin; 4 = glycogen; 5 = Tween 40; 6 = Tween 80; 7 = *N*-acetyl-D-galactosamine; 8 = *N*-acetyl-D-glucosamine; 9 = adonitol; 10 = L-arabinose; 11 = D-arabitol; 12 = cellobiose; 13 = *i*-erythritol; 14 = D-fructose; 15 = L-fucose; 16 = D-galactose; 17 = gentiobiose; 18 = α-D-glucose; 19 = *m*-inositol; 20 = α-lactose; 21 = lactulose; 22 = maltose; 23 = D-mannitol; 24 = D-mannose; 25 = D-melibiose; 26 = β-methyl glucoside; 27 = psicose; 28 = D-raffinose; 29 = L-rhamnose; 30 = D-sorbitol; 31 = sucrose; 32 = D-trehalose; 33 = turanose; 34 = xylitol; 35 = methyl pyruvate; 36 = mono-methyl-succinate; 37 = acetic acid; 38 = *cis*-aconitic acid; 39 = citric acid; 40 = formic acid; 41 = D-galactonic acid lactone; 42 = D-galacturonic acid; 43 = D-gluconic acid; 44 = D-glucosaminic acid; 45 = D-glucuronic acid; 46 = α-hydroxy butyric acid; 47 = β-hydroxy butyric acid; 48 = β-hydroxy butyric acid; 49 = *p*-hydroxy phenylacetic acid; 50 = itaconic acid; 51 = α-keto-butyric acid; 52 = α-keto-glutaric acid; 53 = α-keto-valeric acid; D,L-lactic acid; 55 = malonic acid; 56 = propionic acid; 57 = quinic acid; 58 = D-saccharic acid; 59 = sebacic acid; 60 = succinic acid; 61 = bromosuccinic acid; 62 = succinamic acid; 63 = glucuronamide; 64 = alaninamide; 65 = D-alanine; 66 = L-alanine; 67 = L-alanyl-glycine; 68 = L-asparagine; 69 = L-aspartic acid; 70 = L-glutamic acid; 71 = glycyl-L-aspartic acid; 72 = glycyl-L-glutamic acid; 73 = L-histidine; 74 = hydroxy-L-proline; 75 = L-leucine; 76 = L-ornithine; 77 = L-phenyl-alanine; 78 = L-proline; 79 = L-pyroglutamic acid; 80 = D-serine; 81 = L-serine; 82 = L-threonine; 83 = D,L-carnitine; 84 = γ-aminobutyric acid; 85 = urocanic acid; 86 = inosine; 87 = uridine; 88 = thymidine; 89 = phenylethylamine; 90 = putrescine; 91 = 2-aminoethanol; 92 = 2,3-butanediol; 93 = glycerol; 94 = D,L-α-glycerol phosphate; 95 = glucose-1-phosphate; 96 = glucose-6-phosphate.

Table 6.7. Diagnostic traits of the Gram-positive bacterial fish pathogens

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Bacillus spp.	c	r	−	+	−	−	−	+	F	?	?	?	?	−	?	−	?	?	?	+	?	?	?	?
Bacillus mycoides	w	r	−	+	−	−	−	+	?	?	?	?	?	−	?	+	?	?	?	+	?	?	?	?
Car. piscicola	w	r	−	−	−	−	−	+	F	−	?	?	−	−	−	−	?	−	+	+	+	+	?	−
Cl. botulinum[b]	w[a]	r	−	+	−	+	−	−	−	−	?	?	−	−	−	+	?	−	?	+	?	?	?	?
Cor. aquaticum	y	r	−	−	−	−	−	+	−	+	?	+	−	−	−	+(β)	?	−	+	+	+	?	?	−
Eu. tarantellae	w	r	−	−	−	−	−	−	−	−	?	?	?	−	−	+	?	?	?	+	?	?	?	?
Lactococcus garvieae	w	c	−	−	−	−	−	+	F	−	?	?	−	−	−	+	?	?	+	+	+	+	?	+
Lactococcus piscium	w	c	−	−	−	−	−	+	F	−	?	?	?	−	−	?	+	?	+	−	?	?	?	−
Micrococcus luteus	y	c*	−	−	−	−	−	+	O	+	−	−	+	−	−	?	?	?	?	?	+	?	?	?
Myc. abscessus	−	r	−	−	+	−	−	+	O	+	?	?	?	−	?	?	?	+	?	?	?	+	?	?
Myc. chelonei subsp. *piscarium*	w	r	−	−	+	−	−	+	O	+	?	?	−	−	?	?	?	+	+	−	?	−	+	−
Myc. fortuitum	w	r	−	−	+	−	−	+	O	+	?	?	−	−	?	?	?	+	?	+	?	?	+	?
Myc. marinum	w	r	−	−	+	−	−	+	O	−	?	?	−	−	?	?	?	+	?	v	−	−	−	?
Myc. montefiorense	w	c/r	−	−	+	−	−	+	?	−	?	?	?	−	−	?	?	−	?	−	?	−	?	?
Myc. neoaurum	y	r	−	−	+	−	−	+	O	?	?	?	?	−	?	?	?	?	?	−	+	−	?	?
Myc. pseudoshottsii	y	c/r	−	−	+	−	−	+	?	(+)	?	+	?	−	?	?	?	+	?	−	?	?	?	?
Myc. shottsii	w	c/r	−	−	+	−	−	+	?	(−)	−	−	?	−	?	?	?	+	?	−	?	?	?	?
Noc. asteroides	w	c/r	(+)	−	+	−	−	+	O	+	?	?	−	−	−	?	+	−	?	+	?	?	−	−
Noc. seriolae	w	c/r	−	−	+	−	−	+	O	+	?	?	−	−	+	?	−	−	?	?	?	?	?	?
Planococcus sp.	y	c	−	−	−	+	−	+	O	+	?	+	+	−	−	?	?	?	?	+	+	+	?	?
Ren. salmoninarum[c]	w	r	−	−	−	−	+	+	−	+	−	−	−	−	−	?	−	?	+	−	+	−	?	−
Rhodococcus sp.	re	r	−	−	+	−	−	+	F	−	?	?	−	−	?	?	?	+	?	−	+	?	?	+
Rhodococcus erythropolis	c	r	−	−	?	−	−	+	O	+	?	?	−	−	−	−	?	?	?	−	+	−	?	?
Sta. aureus	y	c	−	−	−	−	−	+	F	+	?	?	?	+	?	+(β)	?	?	?	+	−	?	?	?
Sta. epidermidis	w	c	−	−	−	−	−	+	F	+	?	+	−	−	−	+(β)	−	+	?	+	+	+	?	?
Sta. warneri	(y)	c	−	−	−	−	−	+	F	+	?	?	−	−	?	?	?	+	?	?	?	?	?	?
Str. difficilis	w	c	−	−	−	−	−	+	F	−	−	−	?	−	?	−	−	?	−	−	?	−	?	−
Str. dysgalactiae[d]	w	c	−	−	−	−	−	+	F	−	−	−	?	−	?	+(β)	?	?	−	+	?	−	?	−

(*continued*)

Table 6.7. (*cont.*)

Str. iniae[d]	w	c	−	−	−	−	−	+	F	−	−	−	?	−	?	+(α)	+	?	−	+	?	−	?	−
Str. milleri	w	c	−	−	−	−	−	+	F	−	−	+	−	−	−	+(β)	−	−	?	?	+	?	?	−
Str. parauberis	w	c	−	−	−	−	−	+	?	−	+	?	?	−	?	+(α)	?	?	+	+	?	−	?	?
Noc. salmonicida	o/r	m	+	−	−	+	−	+	O	+	?	?	?	−	−	?	−	+	?	−	−	−	−	−
Vag. salmoninarum	w	c/r	−	−	−	−	−	+	F	−	?	?	?	−	+	+(α)	?	−	+	−	?	?	?	−

1 = colony pigmentation; 2 = rods-cocci; 3 = presence of aerial hyphae; 4 = presence of endospores; 5 = acid-fast staining reaction; 6 = motility; 7 = requirement for L-cysteine hydrochloride; 8 = growth in air; 9 = oxidative–fermentative metabolism of glucose; 10 = catalase production; 11 = α-galactosidase production; 12 = β-galactosidase production; 13 = oxidase production; 14 = coagulase production; 15 = H_2S production; 16 = blood degradation (haemolytic activity); 17 = starch degradation; 18 = urea degradation; 19 = growth at 10°C; 20 = growth at 37°C; 21 = growth in 0% (w/v) sodium chloride; 22 = growth in 6.5% (w/v) sodium chloride; 23 = growth in 0.001% (w/v) crystal violet; 24 = acid production from sorbitol.
(+) = weakly positive result; v = variable results; ? = not stated; c = cocci; r = rods; m = mycelium; O = oxidative metabolism; F = fermentative metabolism

[a] colony pigmentation: w, y, re and o correspond to off-white/white, yellow/golden, red and orange, respectively.

[b] Confirm by presence of toxin.

[c] Confirmatory profile with API-zym.

[d] Differentiate serological or by sequencing of the 16SrDNA gene.

Sta. epidermidis and *Sta. warneri* may be distinguished by the lack of the former to produce acid from lactose and mannose.

Table 6.8. Diagnostic traits of the Gram-negative bacterial fish pathogens

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Acinetobacter sp.	w	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	?	−	?	?	?
Aer. allosaccharophila	w	−	−	−	−	+	F	?	+	+	+	+	?	−	?	+	+	+	+	+	+
Aer. caviae	w	−	−	−	−	+	F	+	+	+	+	−	+	−	+	+	?	+	+	+	+
Aer. hydrophila	w	−	−	−	−	+	F	v	+	+	+	−	−	v	+	+	+	+	+	+	+
Aer. jandaei	w	−	−	−	−	+	F	+	?	+	+	+	?	+	?	+	+	?	+	+	+
Aer. salmonicida subsp. *achromogenes*	w	−	−	−	−	−	F	+	+	+	+	−	−	−	−	+	+	+	−	−	+
Aer. salmonicida subsp. *masoucida*	w	−	−	−	−	−	F	+	+	+	+	+	−	+	+	+	+	+	+	−	+
Aer. salmonicida subsp. *salmonicida*	b	−	−	−	−	−	F	+	+	−	+	−	−	−	+	+	+	+	+	−	+
Aer. salmonicida subsp. *smithia*	b	−	−	−	−	−	F	−	+	−	+	−	−	−	−	+	+	−	−	−	+
Aer. sobria	w	−	−	−	−	+	F	+	+	+	+	+	?	+	+	+	+	?	+	+	+
Arc. cryaerophilus	w	−	−	−	−	+	−	−	?	−	+	?	−	−	?	−	−	−	+	+	/
Chrys. balustinum	y	−	−	−	−	−	O	−	−	+	+	+	?	?	?	+	−	?	+	+	?
Chrys. scophthalmum	o	−	−	−	+	−	F	?	?	−	+	−	?	−	?	+	−	?	?	?	?
Cit. freundii	w	−	−	−	−	+	F	−	+	−	−	−	?	−	?	−	?	?	+	+	+
Edw. ictaluri	w	−	−	−	−	+	F	−	−	−	−	+	+	−	+	−	?	+	+	+	+
Edw. tarda	w	−	−	−	−	+	F	−	−	+	−	+	+	−	(+)	−	?	+	+	+	+
Esch. vulneris	w	−	−	−	−	+	F	+	?	−	−	+	+	−	+	−	−	+	+	+	?
Fla. branchiophilum	y	−	−	−	−	−	O	?	?	−	+	?	?	?	?	+	+	?	+	−	+
Fla. columnare	y	−	−	−	+	−	O	?	?	−	+	−	−	−	?	+	−	?	+	(+)	+
Fla. hydatis	y	−	−	−	+	−	O	?	?	−	−	+	−	−	+	+	+	?	+	+	+
Fla. johnsoniae	y	−	−	−	+	−	?	?	+	−	+	?	?	?	?	+	+	?	+	−	−
Fla. psychrophilum	y	−	−	−	+	−	−	?	?	−	−	−	?	−	?	+	−	?	−	−	+
T. maritimum	y	−	−	−	+	−	O	?	?	−	+	?	?	−	?	+	−	?	+	−	−
T. ovolyticum	y	−	−	−	+	−	O	−	−	−	+	−	?	?	?	+	−	−	−	−	
Haf. alvei	w	−	−	−	−	+	F	−	+	−	−	+	+	+	?	−	?	?	+	+	+
Halomonas cupida	w	−	−	−	−	+	−	+	?	−	−	+	−	−	+	?	?	−	−	−	
J. lividum	p	−	−	−	−	+	O	+	−	−	+	?	?	+	?	+	?	+	+	−	+
Klebsiella pneumoniae	w	−	−	−	−	+	F	−	?	−	−	−	?	?	?	−	?	+	+	+	+

(*continued*)

Table 6.8. (*cont.*)

Moraxella sp.	w	−	−	−	−	−	O		−	−	−	+	−	?	−	+	−	?	+	?	?	?
Moritella marina	w	−	−	−	−	+	F		−	?	?	+	?	+	−	+	+	+		−	−	−
Moritella viscosa	−	−	−	−	−	+	F		−	?	−	+	+	−	−	+	+	+	?	−	−	−
Mycoplasma mobile	?	−	+	−	+	−	?		?	?	?	+	?	?	?	+	−	?	+	+	−	?
Myxococcus piscicola	y	+	−	−	+	−	?		?	?	?	?	?	?	?	?	?	?	?	?	?	+
Pantoea agglomerans	y	−	−	−	−	+	F		−	+	−	−	−	+	+	−	+	−	?	+	+	?
Ph. damselae	w	−	−	−	−	+	F		+	−	+	+	−	+	+	?	−	+	+	+	+	−
Ph. damselae subsp. *piscicida*	w	−	−	−	−	−	F		−	−	−	+	+	+	+	−	−	−		+	−	−
Piscirickettsia salmonis	?	−	−	+	−	−	−		?	?	?	?	?	?	?	?	?	?	?	?	?	?
Plesiomonas shigelloides	w	−	−	−	−	+	F		+	+	+	+	+	?	−	?	−	?	?	+	+	+
Pr. rettgeri	w	−	−	−	−	+	F		−	−	+	−	−	+	−	?	−	−	−	+	+	+
Pasteurella skyensis	?	−	−	−	−	−	F		−	−	+	(+)	+	?	−	(+)	?	?	+	+	−	−
Pseudoalteromonas piscicida	o	−	−	−	−	+	O		?	?	?	?	?	?	?	?	?	?	?	+	+	−
Ps. anguilliseptica	w	−	−	−	−	+	O		−	−	−	−	−	?	?	−	+	−		+	−	+
Ps. chlororaphis	g/fl	−	−	−	−	+	O		+	?	?	+	?	?	?	?	+	?	?	+	?	+
Ps. fluorescens	w/fl	−	−	−	−	+	O		v	−	−	+	−	?	−	?	+	−		+	+	+
Ps. plecoglossicida	w/fl	−	−	−	−	+	O		+	+	?	+	−	?	?	+	−	−	?	+	?	+
Ps. pseudoalcaligenes	w	−	−	−	−	+	O	(alk)	+	−	−	+	−	?	−	?	+	−	−	+	−	+
Sal. enterica subsp. *arizonae*	w	−	−	−	−	+	F		?	+	−	−	+	+	−	−	−	−	+	+	+	+
Ser. liquefaciens	w	−	−	−	−	+	F		−	+	−	(−)	+	−	−	+	+	−	+	+	+	+
Ser. marcescens	r	−	−	−	−	+	F		−	+	−	−	+	−	+	+	+	+	+	+	+	+
Ser. plymuthica	r	−	−	−	−	+	F		−	+	−	−	−	−	+	−	+	?	+	+	+	+
Shewanella putrefaciens	w	−	−	−	−	+	−		−	−	−	+	−	?	−	?	−	?	−	+	+	−
Sporocytophaga sp.	y	+	−	−	+	−	O		?	?	?	?	?	?	?	?	?	?		?	?	−
V. alginolyticus	w	−	−	−	−	+	F		−	+	+	+	+	+	+	+	+	+	+	+	+	−
V. anguillarum	w	−	−	−	−	+	F		−	+	v+	−	−	+	?	+	+	+	+	+	−	
V. cholerae	w	−	−	−	−	+	F		−	+	+	+	+	+	+	+	+	+	?	+	+	+
V. harveyi	w	−	−	−	−	+	F		−	−	+	+	+	?	−	+	+	−	−	+	+	−
V. ichthyoenteri	w	−	−	−	−	+	F		−	−	−	+	?	?	?	?	−	−	+	+	−	+
V. logei	w	−	−	−	−	+	F		−	?	−	+	−	?	−	+	−	−	+	−	−	−

V. ordalii	w	−	−	−	−	+	F	−	−	−	+	−	−	−	?	+	−	+	(+)	−	−
V. pelagius	w	−	−	−	−	+	F	−	v	v	+	−	+	v	?	+	+	?	+	+	?
V. salmonicida	w	−	−	−	−	+	F	−	−	−	+	?	?	−	−	−	?		−	−	+
V. splendidus	w	−	−	−	−	+	F	+	−	−	+	−	+	+	+	−	+	+	+	+	−
V. tapetis	w	−	−	−	−	+	F	−	?	?	+	?	?	−	−	−	?	?	?	−	−
V. vulnificus	w	−	−	−	−	+	F	−	−	−	+	+	+	−	+	+	+	+	+	+	−
V. wodanis	y	−	−	−	−	+	F	−	?	+	+	−	+	−	(+)	+	+	?	−	−	−
Y. intermedia	w	−	−	−	−	+	F	−	+	+	−	−	+	+	?	?	?	?	+	+	?
Y. ruckeri	w	−	−	−	−	+	F	−	+	−	−	+	+	−	?	+	?	+	+	+	+

1 = colony pigmentation; 2 = presence of microcysts; 3 = filterability through 0.45 μm pore filters; 4 = growth only occurs in tissue culture; 5 = gliding; 6 = motility by flagella; 7 = oxidative–fermentative metabolism of glucose; 8 = arginine dihydrolase production; 9 = β-galactosidase production; 10 = indole production; 11 = oxidase production; 12 = lysine decarboxylase production; 13 = methyl red test; 14 = Voges Proskauer reaction; 15 = degradation of blood; 16 = gelatin degradation; 17 = starch degradation; 18 = acid production from maltose; 19 = growth at 30°C; 20 = growth at 37°C; 21 = growth in 0% (w/v) NaCl.

b, fl, g, p, r, w and y correspond to brown, fluorescent, green, purple, red, cream/white and yellow pigment, respectively.

+, −, v, ?, (+) and (−) correspond to positive (≥80% of positive responses), negative (≤20% of positive responses), variable (21–79% of positive responses), unstated, weakly positive and weakly negative result, respectively.

O, F, alk = oxidative or fermentative metabolism, or production of alkali, respectively.

A simple chemical technique has been described which may readily delineate *Nocardia* from *Mycobacterium* (Kanetsuna and Bartoni, 1972). Assuming that pure cultures are available, the bacteria are saponified in 2.5% (w/v) potassium hydroxide in a 1:1 (v/v) mixture of methanol and benzene at 37°C for 24 h. Crude mycolic acids from *bona fide* mycobacteria may be subsequently precipitated by addition of an equal volume of ethanol to an ethereal solution of the extracted lipids. Mycobacteria give rise to copious quantities of white precipitate of melting point between 45 and 70°C, whereas nocardias produce negligible amounts, which do not melt below 150°C (Kanetsuna and Bartoli, 1972).

Aer. allosaccharophila isolates may be identified by the examination of key phenotypic characters. In particular, the utilisation of L-arabinose and L-histidine as sole carbon sources, acid production from D-mannitol, D-melibiose, D-raffinose, L-rhamnose, salicin and sucrose, and the Voges Proskauer reaction were considered differential (Martinez-Murcia *et al.*, 1992). However, a word of caution is necessary, insofar as the organisms which clearly demonstrated genetic homogeneity were markedly heterogeneous phenotypically. This would complicate diagnoses.

Aer. salmonicida may be distinguished from other fish pathogens on the basis of a small number of phenotypic tests, notably the Gram-staining reaction (small Gram-negative rods), motility (usually appears to be non-motile), growth at 37°C (usually a negative response), fermentative metabolism, catalase and oxidase production (both positive) and acid production from sucrose and xylose (both negative; recently, acid production from sucrose has been attributed to some isolates [Wiklund *et al.*, 1992]). These tests will result in a provisional identification of *Aer. salmonicida* (McCarthy, 1976). In addition, it is recommended that pathogenic isolates should be examined for degradation of gelatin (positive), starch (positive) and urea (negative), arginine dihydrolase (positive), gluconate oxidation (negative) and ornithine decarboxylase production (negative). Unfortunately, this apparently simple state of affairs may be complicated by the increasing presence of "atypical" isolates, particularly in non-salmonid fish. In particular, these may be non- or slow-pigmenting.

Diagnosis of *Y. ruckeri* may be achieved by isolation of the pathogen, such as on the selective media of Waltman and Shotts (1984) or Rodgers (1992), and thence identification. According to Waltman and Shotts (1984), 53/60 isolates hydrolysed Tween 80, but none fermented sucrose. Therefore, typically on the selective medium, *Y. ruckeri* colonies were green with a zone of hydrolysis (indicated by the presence of insoluble calcium salts) around them. Unfortunately, in our experience with this medium U.K. isolates rarely hydrolysed Tween 80. Therefore, interpretations should be made carefully.

Wakabayashi and Egusa (1972) proposed an identification scheme for *Ps. anguilliseptica* based on a small number of phenotypic traits, principally motility, growth at 37°C, presence of soluble pigment, production of H_2S, indole and oxidase, nitrate reduction, gelatin degradation, susceptibility to the vibriostatic agent (O/129), and the ability to attack glucose. According to these workers, the tests were sufficient to differentiate *Ps. anguilliseptica* from *Ps. fluorescens*, *Ps. alcaligenes*, *V. anguillarum*, *Aer. liquefaciens (= Aer. hydrophila)*, *Ph. damselae* subsp. *piscicida* and *H. piscium*.

A simplified diagnostic test for *V. anguillarum*, involving "glucose motility deeps" (GMD) has been reported (Walters and Plumb, 1978). Essentially, GMD is a much modified version of the oxidation–fermentation test medium, comprising:

Phenol red broth base (Difco)	1.6% (w/v)
Glucose	1.0% (w/v)
Yeast extract	0.3% (w/v)
Agar	0.3% (w/v)

Stab-inoculated media are incubated at 25°C for 24–48 h, when acid production and motility (indicated as a carrot-like diffuse growth around the stab mark) are recorded. It remains for further work to confirm the specificity of the reaction for *V. anguillarum*.

Colony morphology and pigmentation

This should be recorded from "young" colonies, i.e. shortly after growth is initially detected. The presence of aerial hyphae may be assessed with a stereo-microscope. The presence of pigment should be assessed from basal medium supplemented with 5–10% (w/v) skimmed milked powder (Oxoid).

The Gram-staining reaction

With smears from young cultures, this reaction serves also to determine the presence of rods, cocci, mycelia, microcysts and endospores. For convenience, we recommend the use of commercially available staining and decolorising solutions, such as those marketed by Difco. Heat-fixed smears should be stained for 1 min with crystal violet, washed in tap water, covered with Gram's iodine for 1 min, re-washed, decolorised by a few seconds in acetone–alcohol, and counterstained for 30 sec in safranin. The smears are washed thoroughly, and gently blotted dry, prior to microscopic examination preferably at a magnification of ×1,000.

The acid-fast staining reaction

This reaction highlights the presence of *Mycobacterium*, *Nocardia* and possibly *Rhodococcus*. Heat-fixed smears may be flooded with carbol fuchsin, and heated until the steam rises by means of wafting a source of heat (from a Bunsen burner or cotton wool plug soaked with alcohol) underneath the slide. After 5 min, the stain is washed away with tap water, and the smear decolorised with acid–alcohol until only a faint pinkish tint remains. The slide is re-washed, before applying a methylene blue counterstain for 30 sec. Following re-washing with tap water, the slide is gently blotted dry and examined by oil immersion (Doetsch, 1981).

Motility

In our experience, wet preparations prepared from barely turbid suspensions are most satisfactory when viewed by phase contrast microscopy at ×400 magnification.

Gliding motility

This may be assessed from the development of spreading growth on low-nutrient (cytophaga) agar. It should be differentiated from locomotion by means of flagella.

Filterability through the pores of 0.45 μm pore size porosity filters

The ability of cells to pass through the pores of 0.45 μm pore size porosity filters is indicative of the presence of L-forms and mycoplasmas. Thus, the bacterial suspension is filtered, and the filtrate applied to a suitable growth medium. Growth within 7 days is indicative of filterability.

The ability to grow only in fish cell cultures

Viruses and rickettsias are only capable of growth in suitable cell cultures.

Aerobic or anaerobic requirements for growth

These are apparent after incubating inoculated media aerobically and anaerobically.

Catalase production

This is recorded by effervescence within 1 min from 3% (v/v) hydrogen peroxide following application of a bacterial colony. Quite simply, the "young" colony may be scraped with a thin glass rod and transferred to a drop of hydrogen peroxide on a glass slide.

Fluorescent (fluorescein) pigment production

This is assessed by the presence of a fluorescent, green pigment seen under ultraviolet light after 7 days incubation on the medium of King *et al.* (1954).

Growth at 10, 30 and 37°C

Growth at 30 and 37°C should be recorded within 72 h incubation on basal medium. At 10°C, the media should be retained for up to 14 days.

Growth on 0% and 6.5% (w/v) sodium chloride and on 0.001% (w/v) crystal violet

This is reported after 7 and 14 days incubation on suitably modified basal medium.

Requirement for 0.1% (w/v) L-cysteine hydrochloride

This is essentially a requirement for the growth of *Ren. salmoninarum*. Inoculated media should be incubated at 15°C, and examined at weekly intervals for up to 16 weeks.

Oxidation–fermentation test

This involves the measurement of acid production from glucose metabolism under aerobic and/or anaerobic conditions in the basal medium of Hugh and Leifson (1953). The production of an alkaline reaction is indicated by a deep blue colour which develops, usually in the open tube. For marine organisms, it is necessary to use the modified medium of Leifson (1963). The presence of acid, indicated by a colour change to yellow, should be recorded after incubation for 1, 2 and 7 days.

Indole production

This is recorded after 7 days incubation in 1% (w/v) peptone water. For marine organisms, this should be prepared MSS (2.4% [w/v] NaCl; 0.7% [w/v] $MgSO_4.7H_2O$; 0.075% [w/v] KCl; after Austin *et al.*, 1979). A positive response is indicated by a red coloration following the addition of a few drops of Kovacs reagent.

α-Galactosidase production

One of the most reproducible methods is to record α-galactosidase production from the API-zym system after incubation for 48 h at 15 or 25°C.

β-Galactosidase production

This involves use of the medium of Lowe (1962). Inoculated medium is incubated for 7 days, whereupon a positive response is indicated by a yellow coloration. For marine organisms, the medium should be prepared in MSS.

Production of arginine dihydrolase and lysine decarboxylase

We recommend use of the medium described by Møller (1955). Essentially, inoculated medium is incubated for 7 days, when a positive reaction is indicated by a purple coloration. With marine organisms, the medium should be prepared in MSS.

Urease production

Using the medium of Stuart *et al.* (1945), a positive response develops as a reddish coloration within 28 days. For marine organisms, it is suggested that the medium is supplemented with 2.4% (w/v) sodium chloride.

Methyl red test and Voges Proskauer reaction

These may be recorded after 7 days incubation in MRVP broth (Difco). Following the addition of a few drops of methyl red, a bright red coloration indicates a positive methyl red test. The Voges Proskauer reaction is recorded after use of commercially available reagents. A positive reaction is indicated by a red coloration which develops within 18 h (usually within 1 h) after the addition of the reagents. As before, with marine organisms the medium may be prepared in MSS.

Degradation of blood

This should be recorded within 7 days as zones of clearing around colonies on basal medium supplemented with 5% (v/v) defibrinated sheep's blood.

Degradation of gelatin

This is detected after 7 days incubation by the addition of saturated ammonium sulphate solution to the medium of Smith and Goodner (1958). A positive result is indicated by zones of clearing around the bacterial growth. For marine organisms, the medium should be supplemented with MSS.

Degradation of starch

Basal medium supplemented with 1% (w/v) soluble starch is streaked, and incubated at 15–25°C. After 7 days, the starch plates are flooded with an iodine solution (e.g. Difco Gram's iodine). The degradation of starch is indicated by a clear area surrounded by a blue/black background.

Acid production from maltose and sorbitol

The use of Andrade or phenyl red–peptone water supplemented with maltose or sorbitol is advocated (see Cowan, 1974). This medium contains 1% (w/v) bacteriological peptone 0.5% (w/v) sodium chloride (for marine organisms this amount should be increased to 2%), 1% (w/v) maltose and Andrade or phenyl red indicator. The filter-sterilised (0.22 μm pore size porosity filter) maltose solution should be added to the basal medium after autoclaving, and the completed medium dispensed into test tubes. The production of acid is indicated by the development of a pink colour within 48 h at 25–37°C.

Production of hydrogen sulphide

Many methods have been developed to detect the production of hydrogen sulphide. We have found success with triple sugar iron agar (Oxoid), which should be prepared as slopes in test tubes. Following incubation of the inoculated media at 15–25°C for

up to 7 days, the production of hydrogen sulphide is indicated by blackening of the agar.

Coagulase test

We recommend a simple test using citrated plasma (of rabbit, sheep, donkey or ox). The bacterial culture should be emulsified (to form a dense suspension of $\sim 5 \times 10^8$ cells/ml) in a drop of 0.9% (w/v) saline on a clean grease-free microscope slide. This suspension is then carefully mixed with one drop of citrated plasma. A positive result, which is indicated by clumping of the bacterial cells, is apparent within 2–3 min.

Most of the above-mentioned phenotypic tests have been derived from medical microbiology. Nevertheless, careful attention to detail will generate useful data about bacterial fish pathogens. Undoubtedly, more modern methods will eventually enter the realms of fish microbiology. These methods may include the development of highly reliable rapid techniques, such as offered by high-pressure liquid chromatography and mass-spectrometry. Moreover, lipid analyses could be adapted further for fisheries work. Serological techniques, such as those involving ELISA and monoclonal antibodies, are steadily entering the domain of the fish disease diagnosticians. In addition, molecular genetic techniques, notably gene probe technology, are under evaluation in several laboratories.

OTHER TECHNIQUES

A novel diagnostic approach concerns determination of plasmid profiles for *Edw. ictaluri* (Lobb and Rhodes, 1987; Speyerer and Boyle, 1987).

Chemotaxonomic characters, namely whole-cell fatty acid profiles and a commercial system, i.e. MIS–Microbial ID, have been used with *Fla. columnare* (Shoemaker *et al.*, 2005). The dominant fatty acids included 11-methyl-dodecanoic acid, 13-methyl-tetradecanoic acid, pentadecanoic acid, 14-methyl-pentadecanoic acid, 3-hydroxy-13-methyl tetradecanoic acid, 15-methyl-*cis*-9-hexadecanoic acid, 3-hydroxy-14-methyl pentadecanoic acid, 15-methyl-*cis*-9-hexadecanoic acid, 3-hydroxy-14-methyl pentadecanoic acid and 3-hydroxy-15-methyl hexadecanoic acid (Shoemaker *et al.*, 2005).

Development of a bacteriophage-typing scheme may be of considerable value for diagnosis in the future. Reference is made here to a collection of tailed icosahedral bacteriophages which are specific to *Y. ruckeri* (Stevenson and Airdrie, 1984b).

The use of microwave radiation (700 W energy from a domestic microwave) has been suggested for *Pis. salmonis* (Larenas *et al.*, 1996).

Colour section

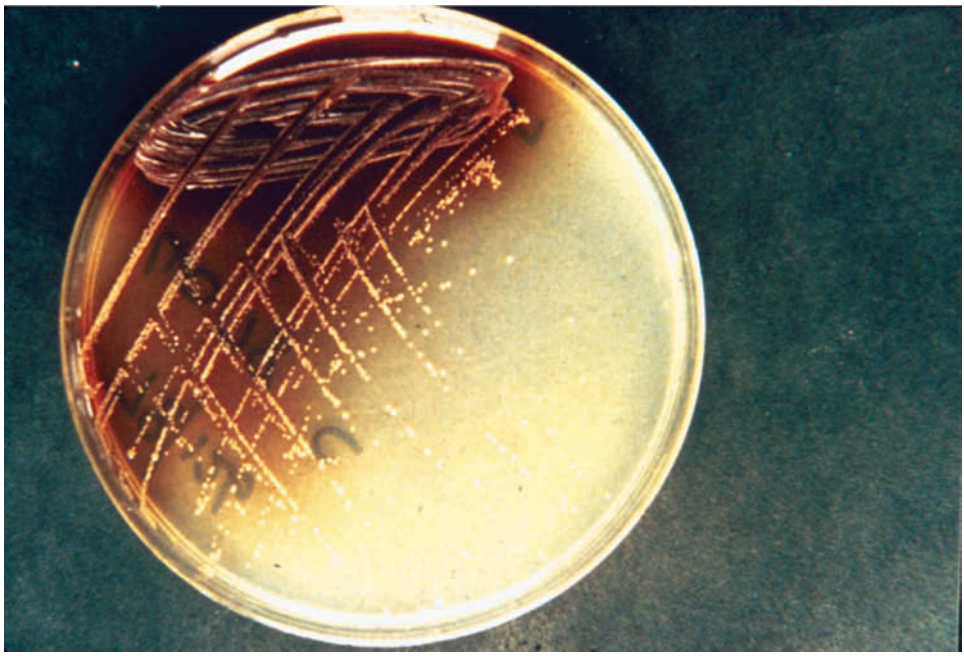


Figure 4.1. *Aer. salmonicida* subsp. *salmonicida* producing brown diffusible pigment around the colonies on TSA.

Figure 6.1. The rainbow trout on the left has bilateral exophthalmia caused by *Ren. salmoninarum*. The second fish is a healthy specimen.

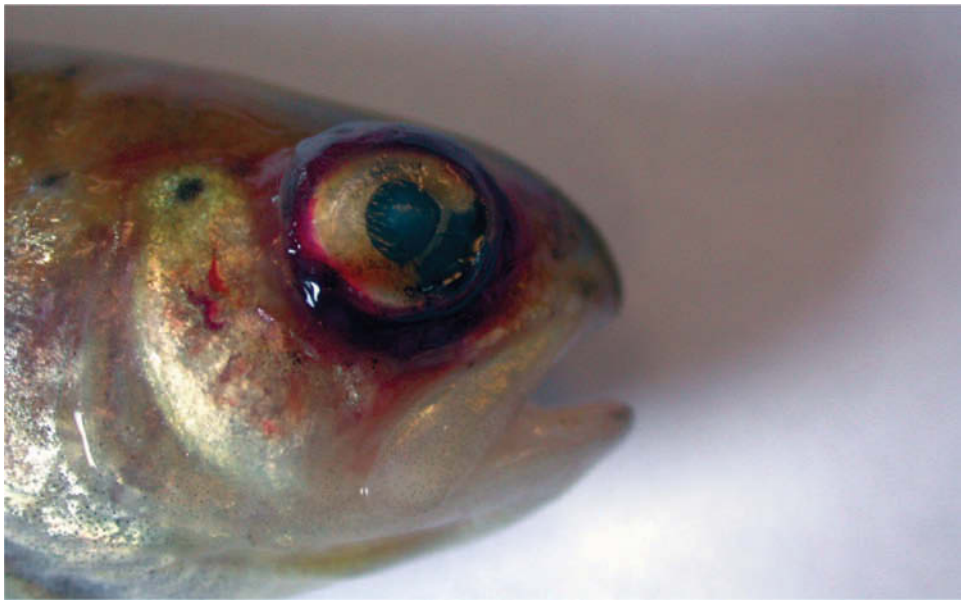


Figure 6.2. A rainbow trout displaying haemorrhaging in the eye caused by infection with *Lactococcus garvieae*. Photograph courtesy of Dr. J.W. Brunt.

Figure 6.3. A rainbow trout displaying extensive haemorrhaging in the mouth caused by ERM. Photograph courtesy of Dr. V. Jencic.

Figure 6.4. A tilapia displaying haemorrhaging around the mouth caused by infection with *Aeromonas* sp. Photograph courtesy of Dr. A. Newaj-Fyzul.

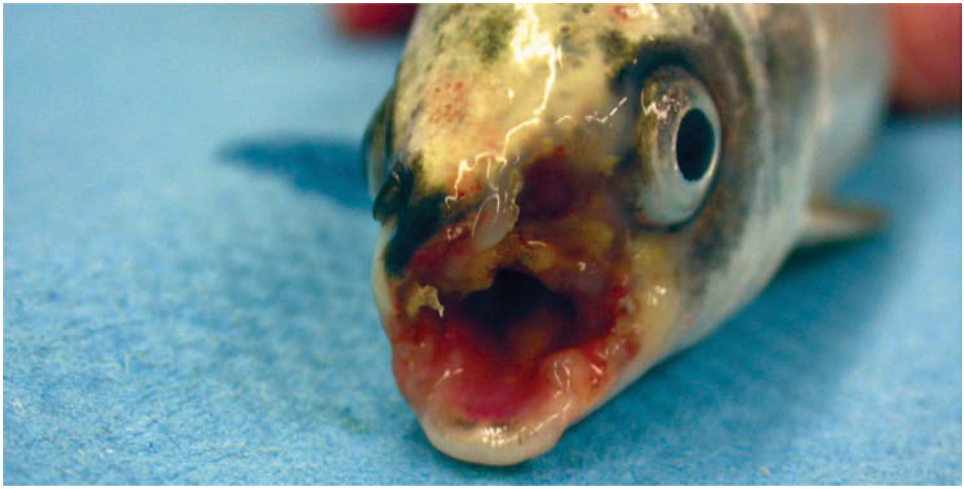


Figure 6.5. Erosion of the mouth of a ghost carp. The aetiological causal agent was *Aer. bestiarum.*

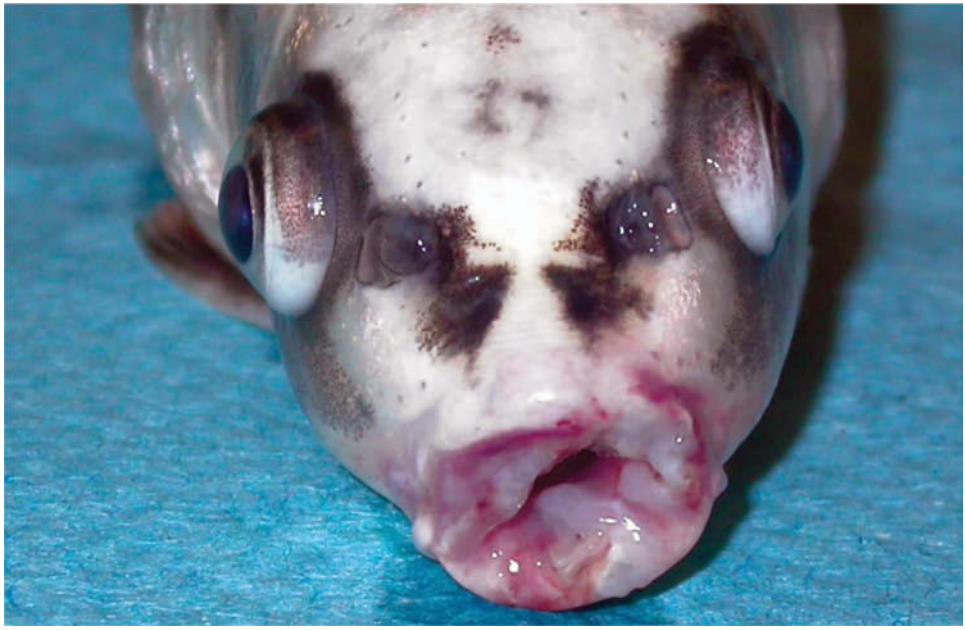


Figure 6.6. Erosion of the mouth of a carp. The aetiological causal agent was *Aer. bestiarum*.

Figure 6.7. Erosion and haemorrhaging of the mouth of a ghost carp. The aetiological causal agent was *Aer. bestiarum*.

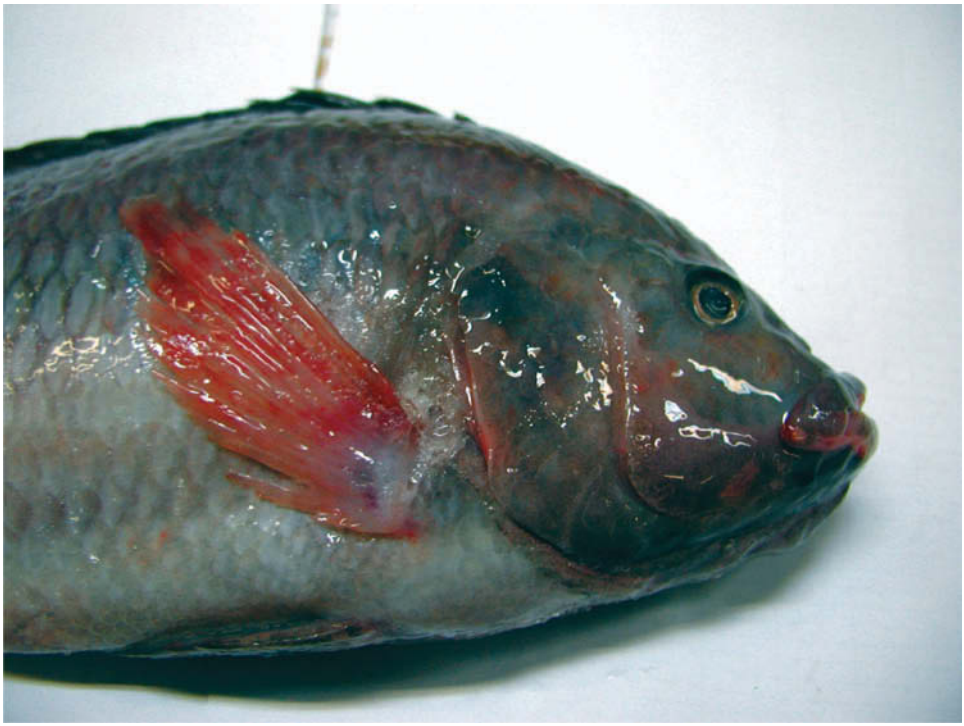


Figure 6.8. A tilapia displaying haemorrhaging on the finnage caused by infection with *Aeromonas* sp. Photograph courtesy of Dr. A. Newaj-Fyzul.

Figure 6.9. Extensive erosion of the tail and fins on a rainbow trout. Also, there is some evidence for the presence of gill disease. The aetiological agent was *Aer. hydrophila*. Photograph courtesy of Dr. N. Pieters.

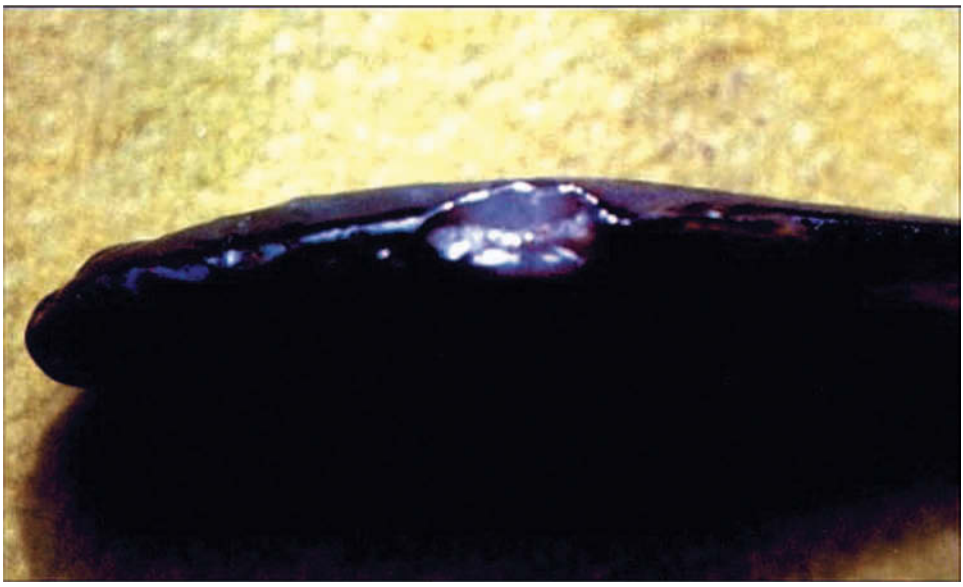


Figure 6.10. A saddleback lesion characteristic of columnaris (causal agent = *Fla. columnare*) on a rainbow trout. Photograph courtesy of Dr. V. Jencic.

Figure 6.11. A distended abdomen on a rainbow trout with BKD.

Figure 6.12. Surface haemorrhaging and mouth erosion on a carp which was infected with *Aer. bestiarum*.

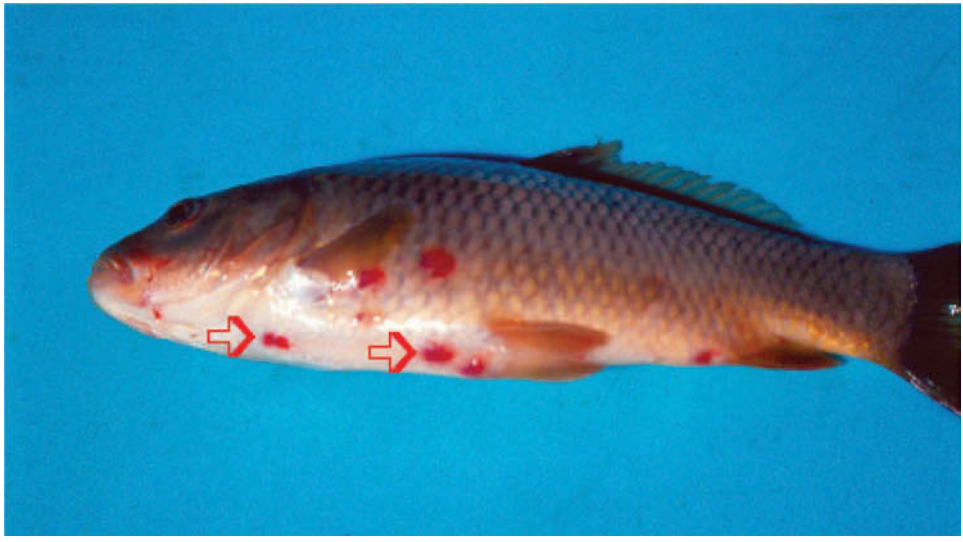


Figure 6.13. Haemorrhagic lesions on the surface of a carp which was infected with *Aer. hydrophila*. Photograph courtesy of Dr. H. Daskalov.

Figure 6.14. Surface haemorrhaging on a tongue sole (*Cynoglossus semilaevis*) infected with *Edw. tarda*. Photograph courtesy of Professor X.-H. Zhang.

Figure 6.15. Petechial haemorrhages on the surface of an eel with Sekiten-byo. Photograph courtesy of Dr. G. Dear.

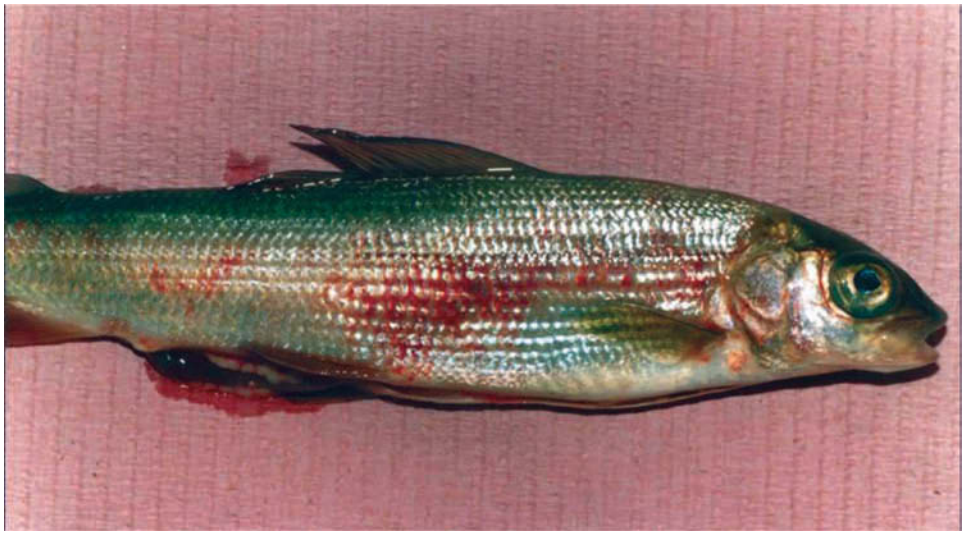


Figure 6.16. Surface haemorrhaging on a grayling infected with BKD. Photograph courtesy of Dr. V. Jencic.

Figure 6.17. Extensive surface haemorrhaging on a turbot with vibriosis. Photograph courtesy of Professor X.-H. Zhang.

Figure 6.18. Haemorrhaging on the fins and around the opercula of a sea bass. The aetiological agent was *V. anguillarum*. Photograph courtesy of Dr. V. Jencic.

Figure 6.19. An ulcer in its early stage of development on a Koi carp. The aetiological agent was atypical *Aer. salmonicida*.

Figure 6.20. A well-developed ulcer on a Koi carp. The aetiological agent was atypical *Aer. salmonicida*.

Figure 6.21. An ulcerated goldfish on which the lesion has extended across the body wall, exposing the underlying organs. The aetiological agent was atypical *Aer. salmonicida*.

Figure 6.22. Carp erythrodermatitis. The aetiological agent is likely to be atypical *Aer. salmonicida*. Photograph courtesy of Dr. H. Daskalov.

Figure 6.23. An ulcer, caused by *Vibrio* sp., on the surface of olive flounder. Photograph courtesy of Dr. D.-H. Kim.

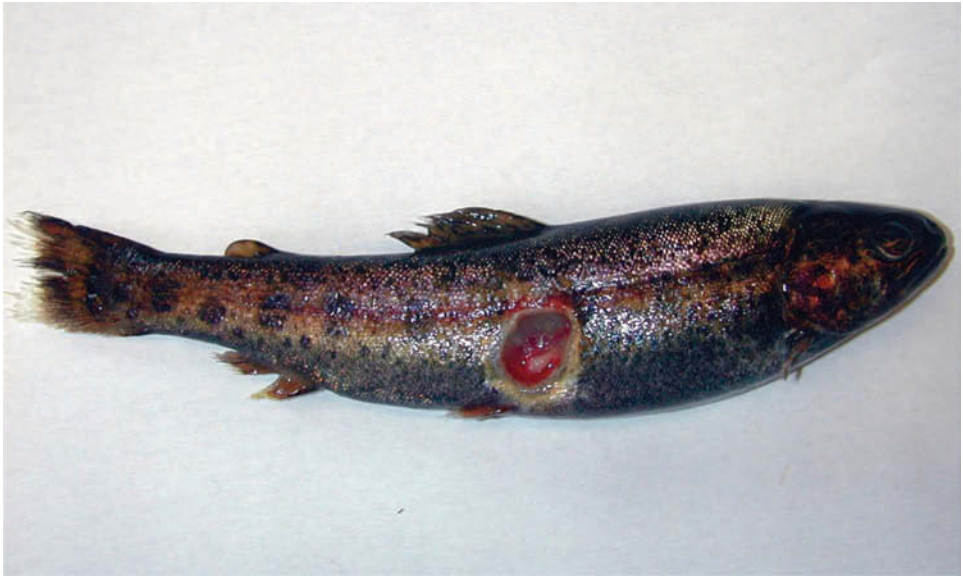


Figure 6.24. Limited tail erosion and an ulcer on the flank of rainbow trout. The casual agent was considered to be linked to ultramicrobacteria.

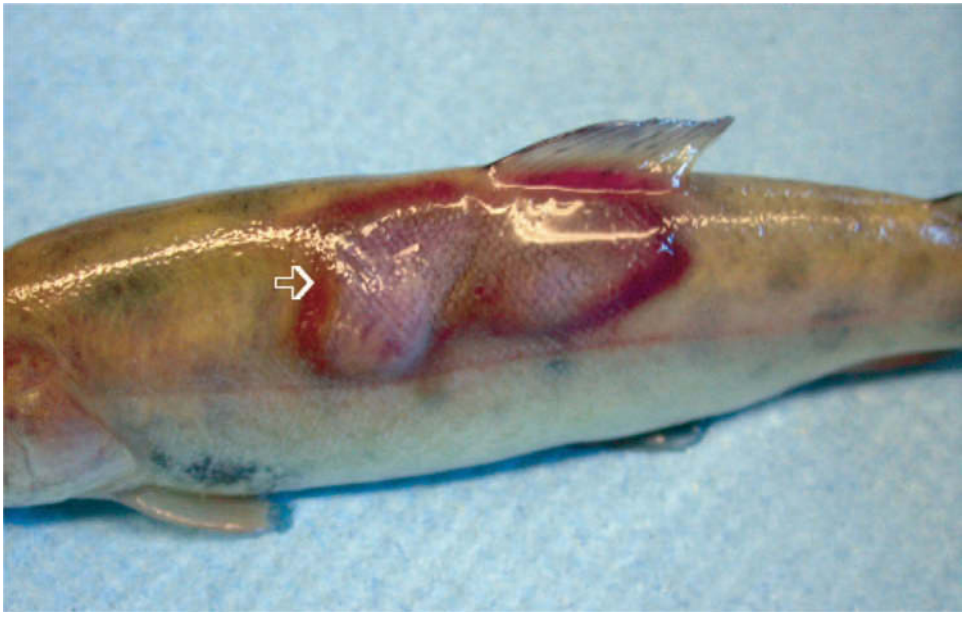


Figure 6.25. An extensive abscess with associated muscle liquefaction in the musculature of rainbow trout. The aetiological agent was *Aer. hydrophila*. Photograph courtesy of Dr. A. Newaj-Fyzul.

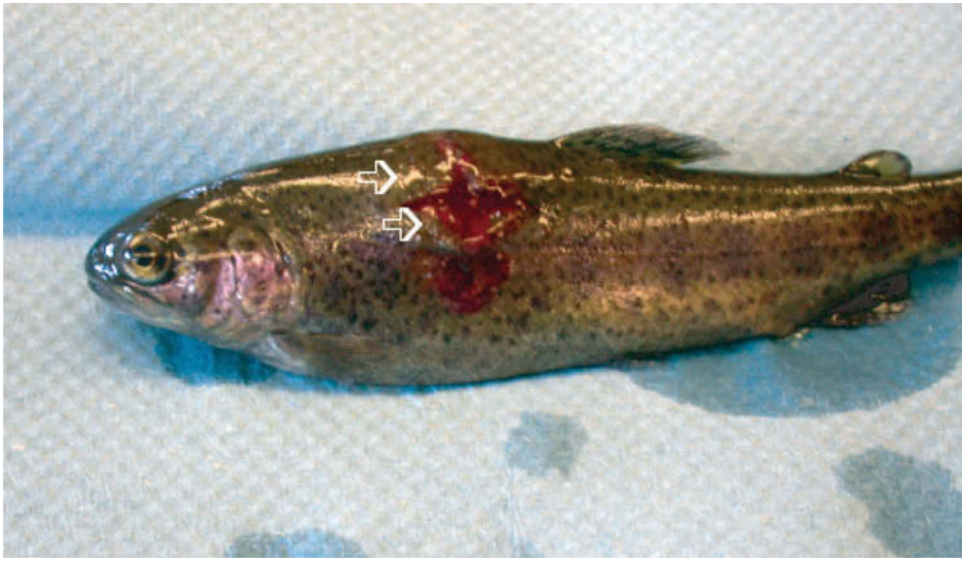


Figure 6.26. A dissected abscess on a rainbow trout revealing liquefaction of the muscle and haemorrhaging. The aetiological agent was *Aer. hydrophila*.

Figure 6.27. A furuncle, which is attributable to *Aer. salmonicida* subsp. *salmonicida*, on the surface of a rainbow trout.

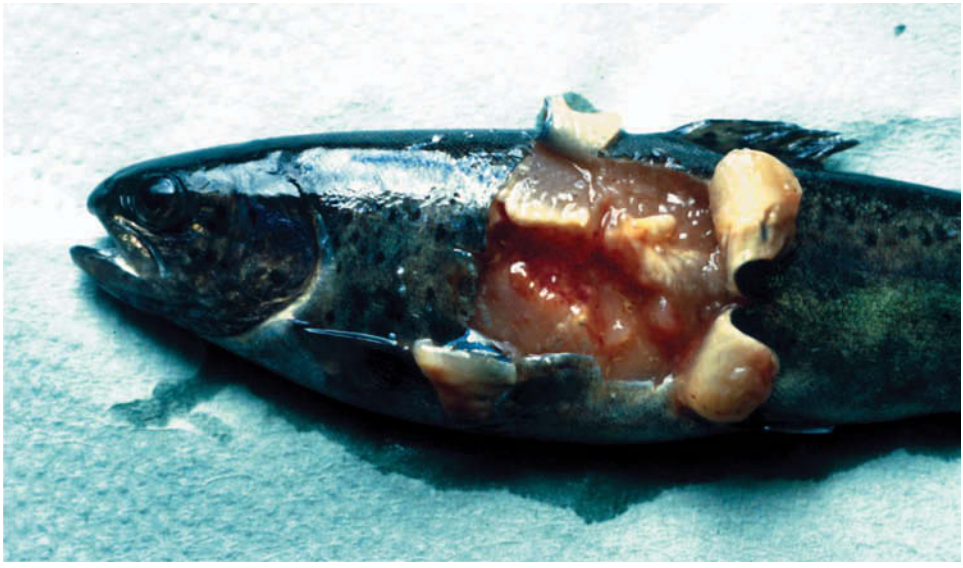


Figure 6.28. A dissected furuncle on a rainbow trout revealing liquefaction of the muscle.

Figure 6.29. A blood blister on the surface of a rainbow trout with BKD.

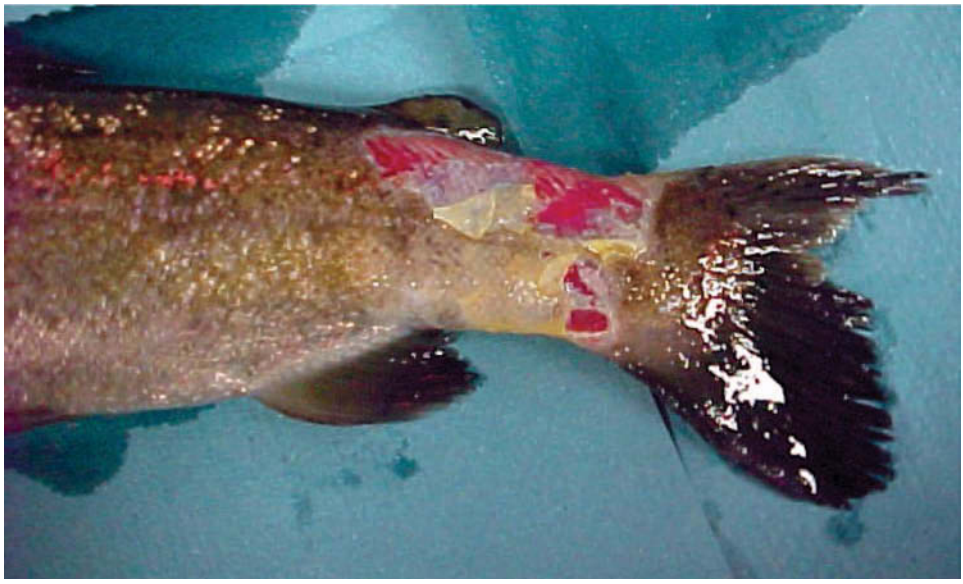


Figure 6.30. Extensive skin erosion around the tail of a rainbow trout. The cause of the condition was not proven.

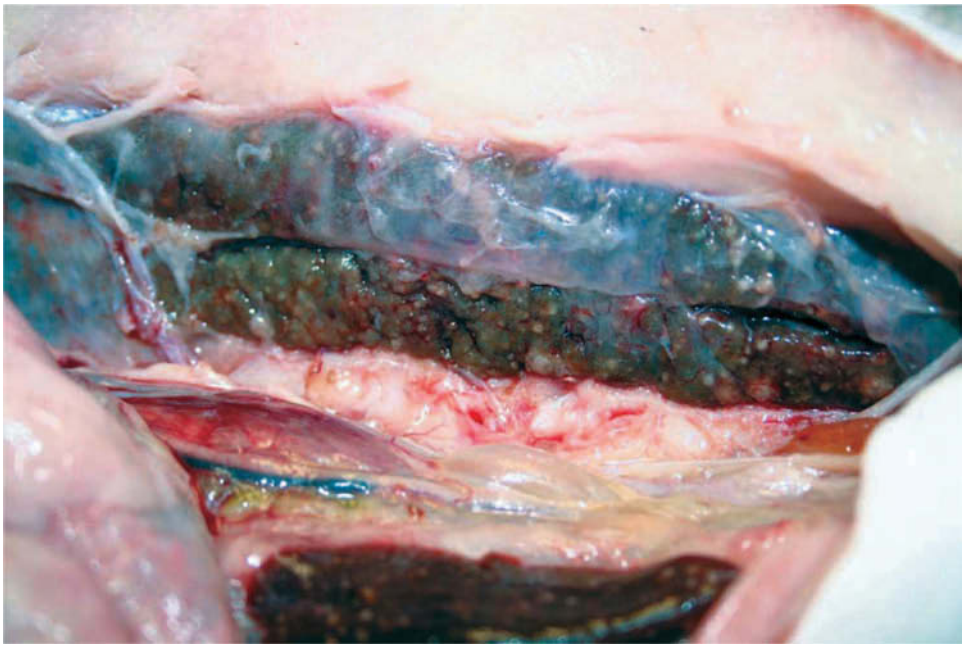


Figure 6.31. Mycobacteriosis in yellowtail. Extensive granulomas are present on the liver and kidney. Photograph courtesy of Dr. T. Itano.

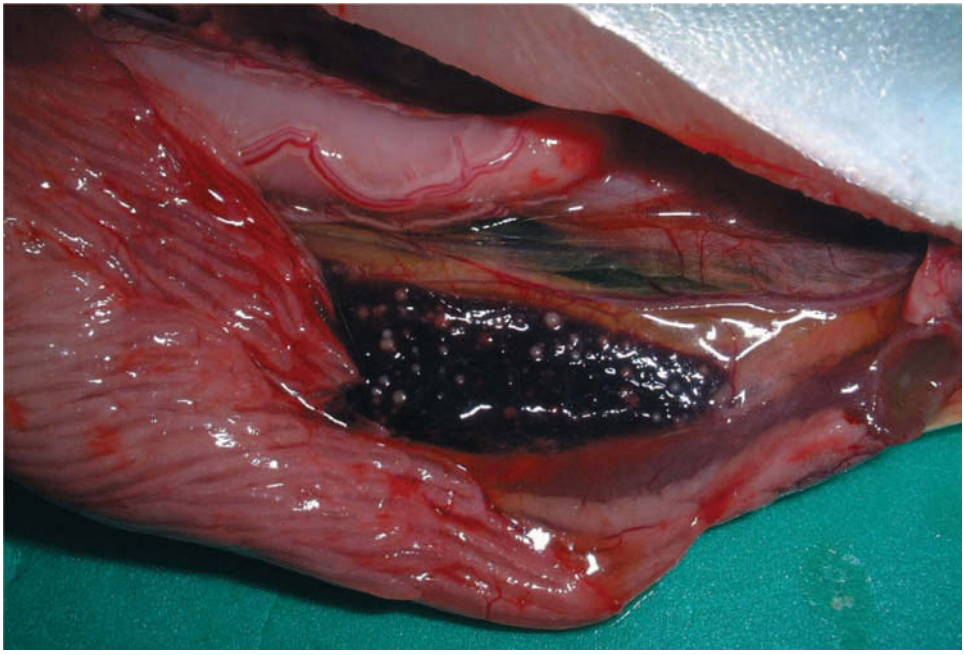


Figure 6.32. Nocardiosis in yellowtail. Extensive granulomas are present on the liver and kidney. Photograph courtesy of Dr. T. Itano.

Figure 6.33. Swollen kidneys associated with BKD.

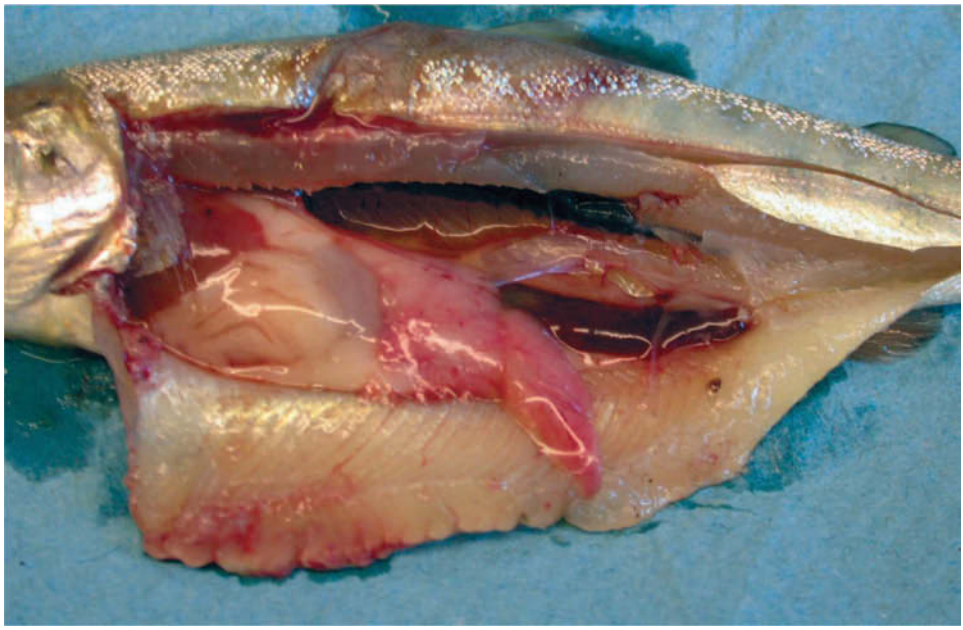


Figure 6.34. Generalised liquefaction of a rainbow trout associated with infection by *Aeromonas*.

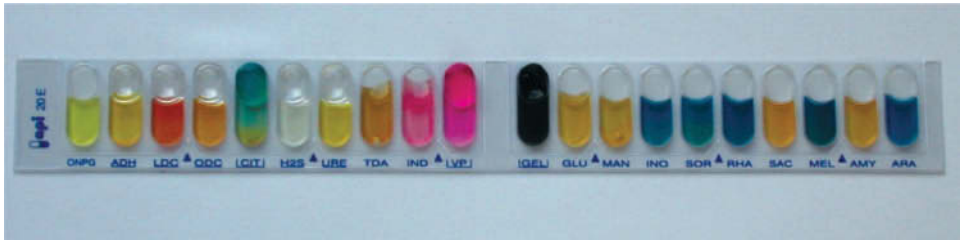


Figure 6.35. An API 20E strip after inoculation, incubation and the addition of reagents. The organism was a suspected *Aeromonas*.

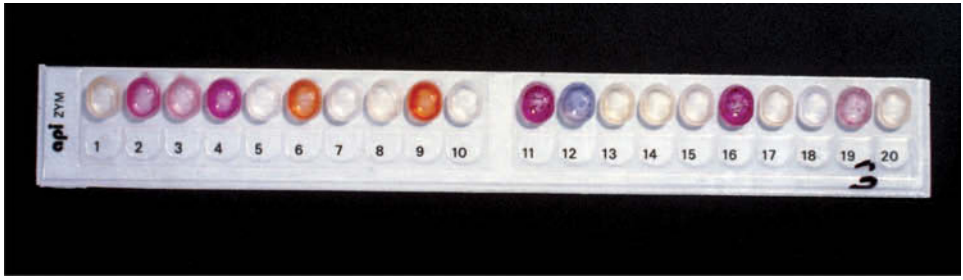


Figure 6.36. An API zym strip after inoculation, incubation and the addition of reagents. The organism is the type strain of *Ren. salmoninarum*.

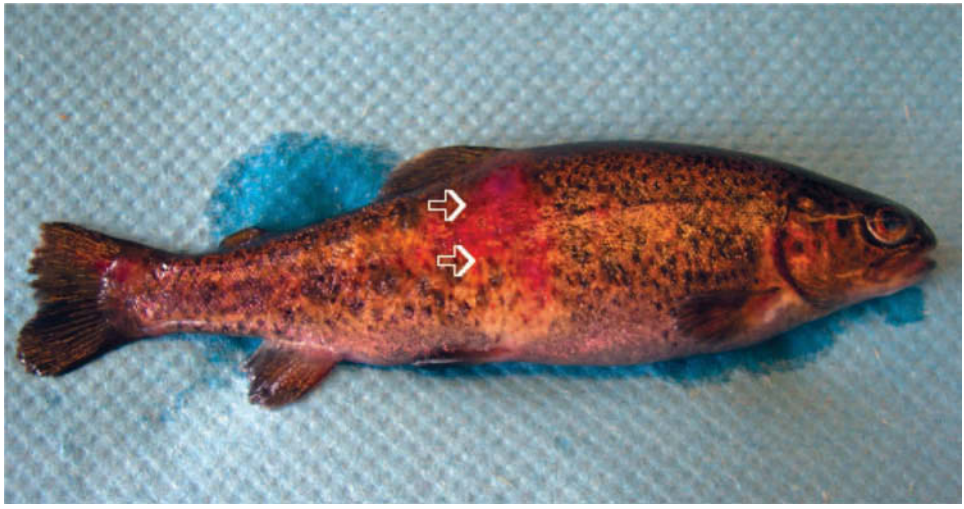


Figure 11.1. Red mark disease syndrome (= winter strawberry disease) in rainbow trout. The skin lesions do not usually penetrate to the underlying muscle.

Figure 11.2. Red mark disease syndrome (= winter strawberry disease) in rainbow trout. With this form of the condition, scales and epidermal cells have been sloughed off.

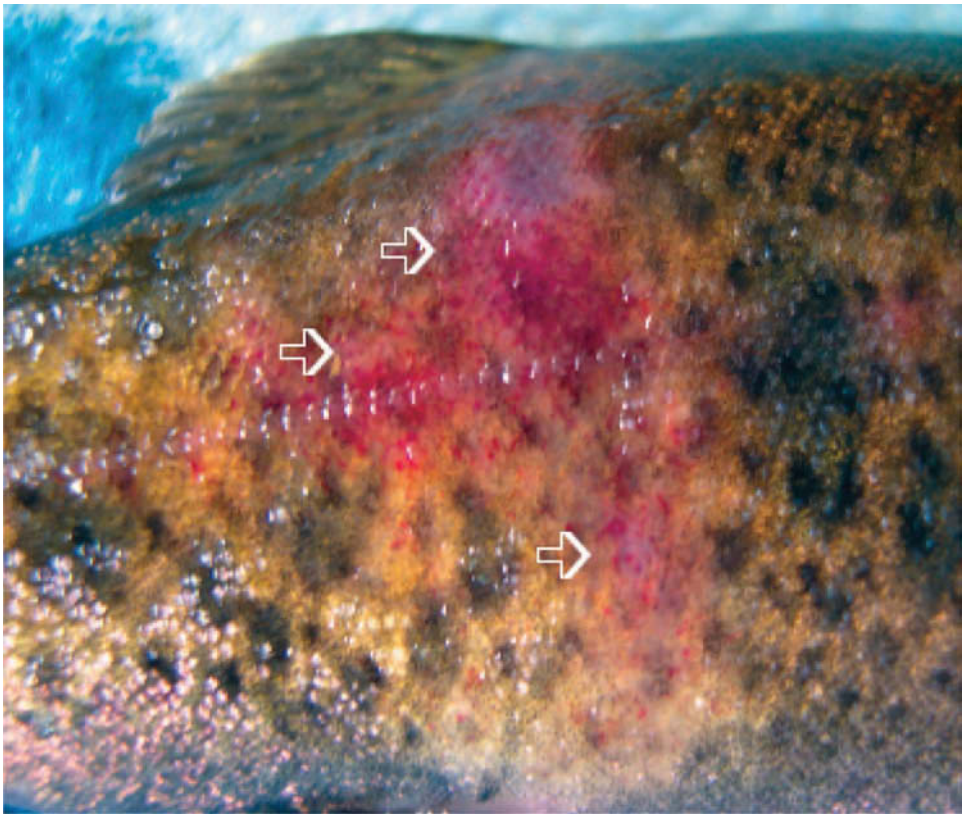


Figure 11.3. Red mark disease syndrome (= winter strawberry disease) in rainbow trout. The reddening is often seen in fish of >500 g in weight.

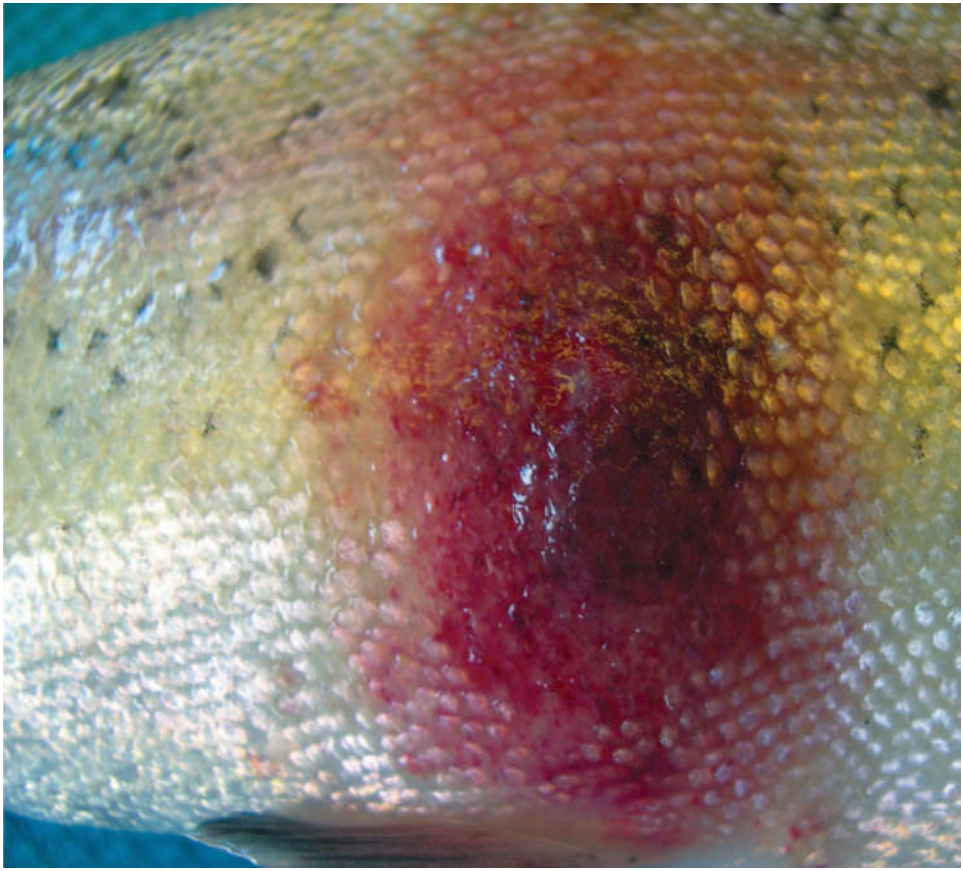


Figure 11.4. The reddened area associated with red mark disease syndrome (= winter strawberry disease) in >500 g rainbow trout.

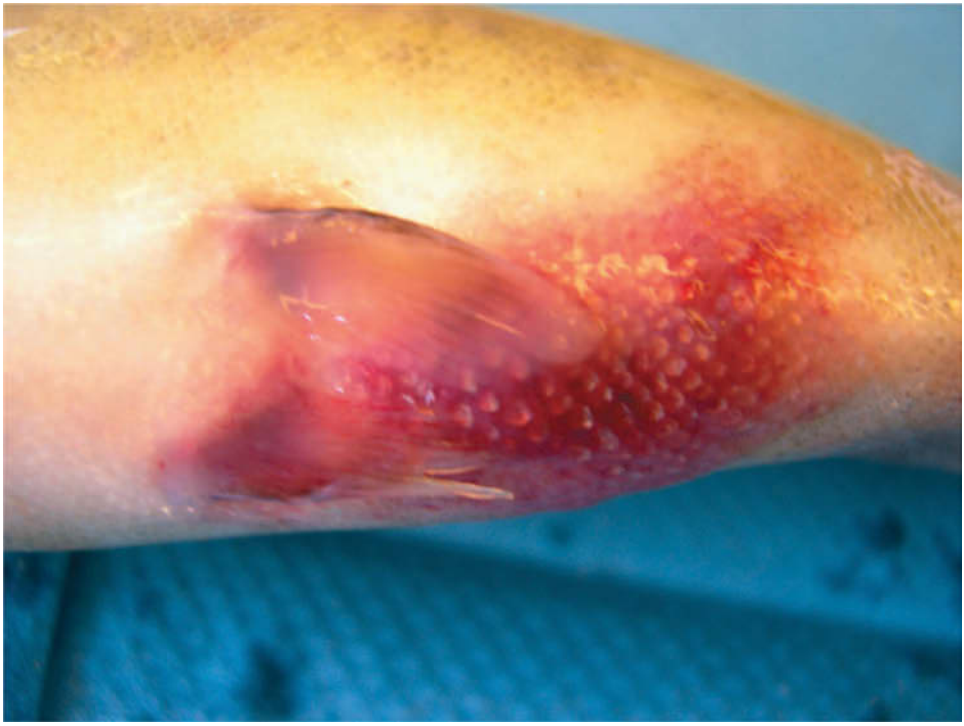


Figure 11.5. The reddened area around the vent associated with red mark disease syndrome (= winter strawberry disease) in >500 g rainbow trout.

7

Epizootiology: Gram-positive bacteria

The reservoir of many Gram-positive bacterial fish pathogens is unknown. Whereas some groups, e.g. streptococci, occur in polluted waters, other organisms, e.g. *Ren. salmoninarum*, seem to be restricted to fish. How do such organisms spread between separate fish populations?

ANAEROBES

Clostridiaceae representative

Clostridium botulinum

Cl. botulinum is widespread in soil, marine and freshwater sediments and in the gastro-intestinal tract of man and other animals, including fish (Bott *et al.*, 1968; Cato *et al.*, 1986). In one study of 530 trout in Danish earth ponds, *Cl. botulinum* type E was discovered to occur in 5–100% of the fish in winter, and in 85–100% of the population in late summer (Huss *et al.*, 1974a). It was supposed that the principal source of contamination with this organism was from minced trash fish used as feed, although soil and water could also be involved (Huss *et al.*, 1974a). Moreover, it was considered likely that clostridia become established in the mud and bottom-living invertebrates in trout ponds (Huss *et al.*, 1974b). In Britain, it has been determined from an examination of 1,400 trout collected from 17 fish farms that the incidence of *Cl. botulinum* in whole fish and viscera was 9.4% and 11.0%, respectively. Nevertheless, *Cl. botulinum* lingers in the fish farm environment for considerable periods following outbreaks of disease. Thus, at the English trout farm which experienced botulism, the organism (possibly as endospores) was recovered for a year after the outbreak of disease. The numbers ranged from 1 to 800 organisms/g of sediment, compared with <1/g at an unaffected control site (Cann and Taylor, 1982). There are no data available to assess the level of contamination in wild fish stocks (Cann *et al.*,

1975). Similarly, there is no evidence to suggest that trout contaminated with *Cl. botulinum* could comprise a human health hazard (Bach *et al.*, 1971).

Eubacteriaceae representative

Eubacterium tarantellae

So far, the organism has only been recovered from the brain of mullet and 10 other unnamed species of estuarine fish caught in Biscayne and Florida Bay. It has not been found outside this area. Moreover, the inability to grow in 2% (w/v) sodium chloride implies that the organism is likely to be restricted to estuarine environments (Udey *et al.*, 1977). These authors consider that isolates recovered from moribund fish, caught off the Texas coast and tentatively identified as *Catenabacterium* (Henley and Lewis, 1976), also belong in *Eubacterium*, as *Eu. tarantellae*. Therefore, the range would appear to be restricted to the warmer waters of the southern part of the U.S.A. It is uncertain whether or not the organism occurs in water, or indeed as part of the resident microflora of fish, although Trust *et al.* (1979) isolated eubacteria from the intestinal tract of three fish species. Therefore, it is conceivable that *Eu. tarantellae* could comprise part of the anaerobic microflora of the digestive tract, although it will be necessary for further study to clarify this point.

GRAM-POSITIVE BACTERIA—THE "LACTIC ACID" BACTERIA

Carnobacteriaceae representative

Carnobacterium piscicola (and the lactobacilli)

So far, it would appear that the disease is confined to Europe and North America. However, it is unclear whether fish are the natural hosts for *Car. piscicola* and other lactobacilli, or if they comprise part of the natural aquatic microflora.

Streptococcaceae representatives

Streptococcicosis (= streptococcosis) was initially described among populations of rainbow trout farmed in Japan (Hoshina *et al.*, 1958). Since then, the disease has increased in importance, with outbreaks occurring in yellowtails (Kusuda *et al.*, 1976a; Kitao *et al.*, 1979), coho salmon (Atsuta *et al.*, 1990), Jacopever (*Sebastes schlegeli*) (Sakai *et al.*, 1986), Japanese eels (Kusuda *et al.*, 1978b), ayu and tilapia (Kitao *et al.*, 1981). The disease, also known as "pop-eye", has assumed importance in rainbow trout farms in Australia, Israel, Italy and South Africa (Barham *et al.*, 1979; Boomker *et al.*, 1979; Carson and Munday, 1990; Ceschia *et al.*, 1992; B. Austin, unpublished data) and in Atlantic croaker (*Micropogon undulatus*), blue fish (*Pomatomus saltatrix*), channel catfish, golden shiner (*Notemigonous chrysoleuca*), hardhead (sea) catfish (*Arius felis*), menhaden (*Brevoortia patronus*), pinfish (*Lagodon rhomboides*), sea trout (*Cynoscion regalis*), silver trout (*Cynoscion nothus*), spot (*Leiostomus xanthurus*), stingray (*Dasyatis* sp.), striped bass (*Morone saxatilis*) and

striped mullet (*Mugil cephalus*) in the U.S.A. (Robinson and Meyer, 1966; Plumb *et al.*, 1974; Cook and Lofton, 1975; Baya *et al.*, 1990c). The disease may also have occurred sporadically in Great Britain and Norway. There is good evidence that streptococcicosis is problematical in both farmed and wild fish stocks.

There is evidence that the pathogens abound throughout the year in the aquatic environment, occurring in water, mud and in the vicinity of fish pens (Kitao *et al.*, 1979). Some seasonality has been recorded, with higher numbers present in seawater during the summer. In contrast, greatest numbers were isolated in mud during autumn and winter (Kitao *et al.*, 1979). This is interesting information, but unfortunately the authors do not comment further about the reasons for the presence of streptococci in the aquatic environment. Conceivably, the organisms may have been released from infected fish and were being merely retained in the water and underlying sediment. Alternatively, with the inconclusive taxonomic status of the fish-pathogenic streptococci, it is difficult to conclude that any environmental isolates correspond precisely to the description of the pathogens. Therefore, any environmental isolates could be merely indicators of an unsanitary condition and not necessarily imply the presence of fish-pathogenic strains. However, this evades the question about the precise source of infection. Minami (1979) determined that streptococci, with similarities to the fish pathogens, were present in fresh and frozen fish used for yellowtail diets. This worker reported that the isolates were pathogenic, and could survive for over 6 months in the frozen state. The suggestion was made, therefore, that the contaminated diets served as an important source of infection. The importance of food-borne infection was further highlighted by Taniguchi (1982a, b, 1983).

It is recognised that streptococcicosis may be transmitted by contact with infected fish. In this context, Robinson and Meyer (1966) transmitted the disease by co-habiting an infected golden shiner with healthy specimens of the same species. The healthy fish succumbed to streptococcicosis, and died within 5 days.

Bacteriophages of *Lactococcus garvieae* have been found in seawater and sediment, but after defining 14 phage types (among 111 isolates), it was concluded that there was not any correlation between phage type and geographical source of the isolates (Park *et al.*, 1998).

Work has demonstrated that *Str. parauberis* has the potential to survive in the marine environment in dormant, i.e. non-cultured form, after an initial culturable phase that lasted for approximately 1 and 6 months in water and sediment, respectively (Currás *et al.*, 2002). The addition of nutrients to the experimental microcosms led to a return to a culturable state (Currás *et al.*, 2002). Also, *Str. parauberis* has been associated with raw milk and bovine mastitis (Doménech *et al.*, 1996).

AEROBIC GRAM-POSITIVE RODS AND COCCI

Renibacterium salmoninarum

To date, there has been no evidence to suggest that *Renibacterium* is a component of the normal aquatic microflora. Indeed, in one study, water and sediment from 56 fish

farms were examined for the presence of renibacteria, but to no avail (Austin and Rayment, 1985). Twelve days after experimentally infecting Chinook salmon with a high-challenge dose, which led to infections with high numbers of renibacterial cells as determined by ELISA and FAT, the pathogen could be detected in the water (McKibben and Pascho, 1999). Survival experiments confirmed that *Renibacterium* could survive in fish tank sediment/faecal material for up to 21 days in the absence of any fish. However, the organism was not at any time recovered from the overlying water, suggesting that renibacteria have an affinity with organic matter. Longer survival times of 13 weeks in river, but not ground, water were reported by Hirvelä-Koski (2004). The question regarding survival of the pathogen in water was the topic of detailed experimentation. This confirmed earlier work that laboratory-grown cultures were short-lived in river water. In the absence of indigenous water-borne organisms, i.e. using filter-sterilised river water, renibacterial cells survived for 28 days, after which there was a rapid decline in numbers. Essentially, these data show that renibacteria have the potential to survive outside of fish for limited periods, although in water it is probably unable to compete with members of the normal aquatic microflora (Austin and Rayment, 1985). In one study, the pathogen was found only in association with asymptomatic and clinically diseased fish (Austin and Rayment, 1985). In another investigation, it was determined that the blue mussel (*Mytilus edulis*) cleared and killed most *Ren. salmoninarum* cells from seawater (Paclibare *et al.*, 1994). However, some renibacterial cells could be found in mussel faeces during settling. Yet, it was conceded that mussels were unlikely to pose a realistic threat to fish farms regarding the survival and spread of renibacterium. But, what if infected mussels are transferred to clean seawater? The answer according to Paclibare *et al.* (1994) was that the mussel cleared *Ren. salmoninarum* from within them upon transfer to clean sites. Clearly, early studies may have been hampered by lack of a suitable selective medium. Nevertheless, the use of SKDM has not, as yet, produced any definite evidence to suggest a non-fish reservoir for the organism (Austin *et al.*, 1983a; Embley, 1983; Austin and Rayment, 1985). The precise source of infection is unclear, but may include clinically or asymptomatically diseased fish (Wood and Wallis, 1955; Wolf, 1966; Bucke, 1978; Mitchum *et al.*, 1979; Paterson *et al.*, 1979; Fryer and Sanders, 1981). The organism has been recovered from faeces of both cultured and wild salmonid stocks. According to Balfry *et al.* (1996), *Renibacterium* is shed from faeces, and may survive in seawater for a week. Attention has also been focused on the role of eggs in the transmission of BKD ("vertical" transmission) (Allison, 1958; Wolf, 1966; MacLean and Yoder, 1970; Mitchum *et al.*, 1979; Lee and Evelyn, 1989). Allison (1958) indicated the involvement of eggs when BKD occurred following transfer of ova from an infected site. Similarly, Bullock *et al.* (1978b) implicated disinfected eggs of chinook salmon in the spread of the disease. Moreover, the preliminary data of Paterson *et al.* (1981) pointed to the presence of *Renibacterium* within fertilised eggs. Evelyn *et al.* (1984) demonstrated the presence of renibacteria in 11.6–15.1% of eggs from a coho salmon which was infected with BKD, such that the coelomic fluid was cloudy because of high numbers of the organism. These authors suggested that *Ren. salmoninarum* was present in the yolk of the eggs, even after treatment with erythromycin (Evelyn *et al.*, 1986a). Of greater significance was

the finding that iodophors were ineffective at preventing intra-ovum infections. Clearly, this has profound implications for control of the disease.

The manifestation of the disease is complicated by certain environmental factors, including water hardness (Warren, 1963), temperature, salinity and diet. Belding and Merrill (1935) were the first workers to describe the seasonal nature of BKD, with a correlation between water temperature and level of mortality. Earp *et al.* (1953) found that BKD occurred over a wide range of water temperatures from 8 to 18°C. Most epizootics occurred in the autumn and winter, i.e. during periods of declining water temperatures. However, most mortalities occurred at higher temperatures, a conclusion which has been echoed by Austin (1985), although the reverse has also been reported (Sanders *et al.*, 1978). At low temperatures, the effect was the continual loss of small numbers of fish (Snieszko and Griffin, 1955). This is quite a feat for a supposedly unreactive organism!

BKD has been diagnosed in fish following movement from fresh to seawater (Earp *et al.*, 1953; Bell, 1961). Indeed, the disease may be of paramount importance in the ability to acclimatize to seawater (Frantsi *et al.*, 1975) and the survival of salmonids in the oceanic environment (Fryer and Sanders, 1981).

Data obtained with ELISA have shown that *Ren. salmoninarum* occurs commonly, in the absence of pathological signs of BKD, in wild fish, i.e. Artic charr and brown trout in Iceland (Jónsdóttir *et al.*, 1998). Thus, the route of transmission to aquaculture may be from wild fish.

The effect of nutritional (dietary) status on the development of BKD is only partially understood. Some diets, notably those containing corn gluten (Wedemeyer and Ross, 1973) or lipid (Austin, 1985), enhanced the disease. Nutritional studies with Atlantic salmon have shown that levels of vitamin A, iron and zinc are lowered in BKD-infected fish (Paterson *et al.*, 1981). Subsequent experiments in which fish were administered diets rich in trace elements resulted in reduced incidences of BKD. This theme should be exploited further for control purposes.

In fish culture, *Ren. salmoninarum* appears to be a most unaggressive organism, generally devoid of much production of exoenzymes (exotoxins). Yet, it causes such a severe problem in salmonids. With lack of evidence to the contrary, it is our hypothesis that the organism is a normal resident of some fish, in (or on) which it exists, probably in fairly low numbers. Conceivably, it may be a normal resident of kidney tissue, forming a synergistic or controlled parasitic relationship with the host, possibly in the macrophages. Alternatively, it may be a normal resident of the digestive tract (Austin, 1986). To continue the scenario, we postulate that at times of stress to the host, such as sub-clinical infections, damage to the digestive tract, starvation, kidney damage or temperature shock, the organism is able to migrate to the kidney (if not already there) and multiply. This would lead ultimately to the condition known as BKD. The problems with recovery of the organism, particularly from asymptomatic fish, may be explained within the realms of this concept. Evelyn and co-workers (Evelyn, 1978; Evelyn *et al.*, 1981) have considered the presence of inhibitors in the kidney that suppress the development of *Renibacterium* on solid medium. Could these unnamed compounds control the growth and development of the organism in healthy fish? This remains a possibility. However, there are other equally plausible

explanations, namely dormancy, damage, or the presence of altered—osmotically fragile—cells. The renibacteria may normally be in a dormant or altered phase within the fish and, thus, would require to be triggered back into activity in order to produce colonies. Alternatively, renibacteria may be in some way damaged in the fish, and require repair before being able to produce colonies. This parallels the problem of damaged coliform bacteria in the aquatic environment (Olson, 1978). Of course, it is also possible that the media are deficient in certain essential nutrients, necessary for the replication of *Renibacterium*. A long lag phase, which has been suggested by Embley (1983), would be necessary for the organism to adjust to the new environment of the laboratory medium, prior to replication. Any or all these possibilities could apply to *Renibacterium*. Careful thought is necessary to unravel many of the mysteries still surrounding the biology of this pathogen.

Corynebacteriaceae representative

Corynebacterium aquaticum

Apart from fish, the organism was recovered from water and the "scum" forming at the air–water interface on the tank walls (Baya *et al.*, 1992b).

Mycobacteriaceae representatives

Mycobacterium spp.

Very little is known about the epizootiology of fish-pathogenic mycobacteria. Undoubtedly, the reservoir for the organism is the aquatic environment (Beran *et al.*, 2006), although the factors that lead to the development and spread of the disease condition are unknown. To illustrate the potential spread of the disease, in Oregon as many as 26% of hatchery fish may be infected (Arakawa and Fryer, 1984). Possibly, transmission may be by ingesting contaminated food or debris (Dulin, 1979). Infection, via the intra-ovarian route, has been demonstrated for the Mexican platyfish (*Xiphophorus maculatus*) (Conroy, 1966). However, other investigators have ruled out vertical (i.e. egg) transmission as a means of spreading the disease (Ross and Johnson, 1962; Wood, 1974).

Nocardiaceae representatives

Nocardia spp.

Nocardia spp. (including *Noc. asteroides*) occur in freshwater and soil. With the observation that *Noc. kampachi* grows in up to 4.5% (w/v) sodium chloride, it was suggested that the normal habitat is terrestrial or limnetic. Moreover, in survival experiments, the organism remained viable in clean seawater for only a few days, although in polluted conditions viability was considerably extended (Kariya *et al.*, 1968). The inference, therefore, is that fish become infected from the natural environment. Presumably these diseased animals serve as a reservoir for further infection.

Rhodococcus sp.

It was considered that feeding with crude fish offal may have been the cause of infection (Claveau, 1991).

Rhodococcus erythropolis

There was a link between the administration of oil-adjuvanted vaccines intraperitoneally. Olsen *et al.* (2006a) speculated that the oil may be a source of nutrition and may give protection to the rhodococci. However, the precise source/origin of the organism was not determined.

Staphylococcaceae representatives

Staphylococcus epidermidis

Sugiyama and Kusuda considered that the bacteria originated from water (or fish) rather than from human beings, because of the pronounced antigenic differences to human—Tachikawa—strains (Sugiyama and Kusuda, 1981b). This seems to be a likely possibility in view of other ecological studies which have clearly demonstrated the presence of *Sta. epidermidis* in the aquatic environment (e.g. Gunn *et al.*, 1982).

"*Candidatus* Arthromitus"

Possibly, the organism comprises part of the normal microflora of the digestive tract (Michel *et al.*, 2002a). Alternatively, it is possible that the organism could be introduced via feed.

8

Epizootiology: Gram-negative bacteria

The value of survival experiments based on laboratory cultures is questionable. Cultures grown on laboratory media tend to be larger and less aggressive than their "natural" counterparts. So, when laboratory cultures are added to experimental microcosms, the outcome need not represent the true fate of the organism in the aquatic environment.

The source of many bacterial fish pathogens has not been considered. Details concerning the remainder are given below.

Aeromonadaceae representatives

Aeromonas hydrophila

The epizootiology of *Aer. hydrophila* has not been considered in any great detail, although it has been concluded that the organism is rife in freshwater (Heuschmann-Brunner, 1978; Allen *et al.*, 1983b), aquatic plants and fish (Trust and Sparrow, 1974; Ugajin, 1979) and fish eggs (Hansen and Olafsen, 1989), and may be associated with invertebrates, such as the ciliated protozoan *Tetrahymena pyriformis* (King and Shotts, 1988), from where it will be readily available for infection of fish. Some isolates have been demonstrated to exhibit chemotactic responses to the mucus of freshwater fish (Hazen *et al.*, 1982). The chemotaxic substance, which is heat-stable at 56°C, has been reported to have a molecular weight of 100 kDa.

The evidence points to a stress-mediated disease condition (Bullock *et al.*, 1971), in which mortalities, if indeed they occur at all (Huizinga *et al.*, 1979), are influenced by elevated water temperatures (Groberg *et al.*, 1978; Nieto *et al.*, 1985). Thus, Groberg and co-workers determined that deaths among fish, which were challenged via i.p. injection, occurred only at water temperatures in excess of 9.4°C. This implies that the disease is not associated with cold water. In addition to water temperatures, the presence of pollutants, notably nitrite at 6 mg/l, increased the susceptibility of

channel catfish to infection (Hanson and Grizzle, 1985). It is interesting to note that survivors possess high serum titres of an IgM-like antibody (Hazen *et al.*, 1981).

During an examination of likely portals of entry of the pathogen into walking catfish, Lio-Po *et al.* (1996) found evidence of localisation in the muscle.

Aeromonas salmonicida

Historical consideration

The origin of the pathogen will probably never be definitively resolved, and it remains uncertain whether the disease was spread by importation of fish stocks from Europe to North America or *vice versa*. Circumstantial evidence exists to support either interpretation. For instance, the observation that furunculosis was not confirmed in North America until 1902 (Marsh, 1902) compared with 1894 in Europe could indicate that the disease originated in Europe and was introduced into North America with imports of brown trout (*Salmo trutta*) (McCarthy, 1975a). An alternative suggestion has been that exports of sharta rainbow trout from North America to Europe were responsible for spreading the disease. In support of this contention is the fact that rainbow trout are relatively resistant to furunculosis, which is taken to imply that they were the original host, but had developed resistance to the disease (Fish, 1937). It appears that awareness of the pathogen was brought about through the initial reports, so that by the early 1900s, furunculosis was found to occur in a variety of European countries, notably Austria, Belgium, France, Great Britain, Ireland and Switzerland. It took until 1964 for the disease to be recognised in Norway; the spread being due to the importation of infected rainbow trout from Denmark. In 1985, furunculosis was recognised in Norwegian marine Atlantic salmon farms, the cause being infected smolts from Scotland (Johnsen and Jensen, 1994). Although at first it was believed that furunculosis was confined to hatcheries, this concept was dispelled when extensive studies by Plehn (1909, 1911) revealed the presence of the disease in wild trout taken from 25 rivers and streams in Bavaria. In Great Britain, the occurrence of furunculosis was first reported in the literature during 1909. The following discussion on the history of the disease in Great Britain is taken from Lund (1967), who noted that the first report of furunculosis did not attract significant attention, other than to cause an awareness of the disease among a few trout farm owners. However, in 1911 a conspicuously high mortality among salmonids in the rivers Wye, Exe, Teign and Dart occurred. This disease outbreak was investigated by Masterman and Arkwright (1911), who proved it to be furunculosis. By 1912 the disease had appeared in the rivers Test and Itchen, and eventually spread to other trout streams in the south of England. A host of reports followed, with the occurrence of furunculosis in the river Conway in 1917 or 1918, the river Kennett in 1924 and 1925, the Welsh rivers Teify, Towy, Usk and Elway in 1925, the river Coquet in Northumberland during 1926, and the Welsh river Dee in 1927. The disease also reached Scotland in 1926 where high mortalities were reported in salmon in the river Doon in southwest Scotland. By 1929, furunculosis had encroached upon the rivers of northern Scotland, and was confirmed in the Aberdeen Dee. As the seriousness of the situation became increasingly apparent, the first organized attempt to tackle the problem was

made by the Kennett Valley Fisheries Association in 1925, who raised a fund for studying the disease. The Fishery Board of Scotland followed suit in 1927, and in 1928 the efforts of the two groups were united. Thus, in 1929 the Furunculosis Committee was formed. This committee reviewed information about the spread of the disease, and compiled all the facts on the occurrence of furunculosis in other countries. In Great Britain, by the end of 1926 it was observed that furunculosis was proving to be less troublesome in some areas where the infection had initially been reported, but fresh outbreaks had been documented in other, additional salmon rivers. These new outbreaks also caused great consternation, due not only to a decline in fish numbers, especially valuable breeding stock, but also to loss of revenue from fishing rights (Lund, 1967). There seemed to be no doubt that the disease had become enzootic in Great Britain.

In North America the first report of furunculosis was made by Marsh (1902) who described an organism, which was named as *Bacillus truttae*. This caused an epizootic in hatchery fish in Michigan. Later, Belding (1927) reported the loss of an entire stock of brook trout due to furunculosis at the Massachusetts State Hatchery. Davis (1929) contended that serious mortality had been restricted to brown and brook trout in hatcheries and rearing ponds. However, the extent of the problem was reflected in the calculations of Smith (1942), who reckoned that the disease had occurred in more than 25% of the hatcheries in the U.S.A. However, as in Great Britain, furunculosis in North America was eventually shown to be not solely a hatchery disease; a study by Fish (1937) revealed the presence of *Aer. salmonicida* in wild stock of adult Loch Leven trout (*Salmo trutta levensis*) in Wyoming. In Canada, Duff and Stewart (1933) investigated disease outbreaks among salmonids in British Columbia. Several fish species, such as wild Rocky Mountain whitefish (*Prosopium williamsoni*), Dolly Varden (*Salvelinus marme* Walbaum) and cutthroat trout (*Salmo clarki*), were reported in 1929 and 1930 to be dying from a disease, a major symptom of which was the appearance of red "blisters" on the body. In 1931, *Bacillus salmonicida* was isolated from diseased specimens. Nevertheless, despite the occurrence of furunculosis in wild stock in North America, it does not appear to have assumed epizootic status in rivers.

At present, the geographical distribution of the pathogen is worldwide, including Australia (Trust *et al.*, 1980b) and the mainland of Asia, i.e. Korea (Fryer *et al.*, 1988) where it was formerly believed to be absent. The pattern formed by the locations from where the disease has occurred since the initial report of Emmerich and Weibel (1894) suggests that it spread from a focal point in Europe. However, according to McCarthy and Roberts (1980), this may reflect progress in bacteriological diagnostic facilities as well as an actual dissemination of the pathogen.

Investigations into the epizootiology of *Aer. salmonicida* have not provided unequivocal answers for several crucial questions concerning the factors which control or determine the dissemination of the pathogen. Although *Aer. salmonicida* has been recognised for ~100 years, the precise route of transmission has not been conclusively resolved. Some studies indicate that the pathogen is widespread among wild salmonids. For example, in a study using DNA probe technology of the blood from 61 fish caught from 3 rivers in Ireland, Mooney *et al.* (1995) reported

a widespread, low-level infection. Controversy persists as to whether or not the organism is capable of a free-living existence in the natural environment, away from the fish host. Certainly, infected fish are able to spread the pathogen to other aquatic invertebrates. Thus, when Arctic charr (*Salvelinus alpinus*) were challenged intraperitoneally and the fish began to die, the pathogen spread to and could be cultured from the freshwater bivalve *Amblema plicata*, which were placed in the experimental system. Fish added to the system with the infected *Amblema plicata* after 1 and 5, but not after 15, days succumbed to infection and died (Starliper, 2001a).

The site(s) of entry into the fish also remain uncertain. All of these questions must be answered before the gaps in the understanding of the epizootiology of diseases of *Aer. salmonicida* aetiology can be filled. Unfortunately, epizootiological studies have not been aided by modern scientific approaches, such as plasmid-profiling (Austin *et al.*, 1998) or ribotyping (Nielsen *et al.*, 1994).

The ecology of Aeromonas salmonicida

McCarthy (1980) performed detailed experiments concerned with the ecology of *Aer. salmonicida* and also reviewed the work carried out by others. According to his report, contact with infected fish or contaminated water and fish farm materials, and transovarian transmission have all been cited as probable routes of infection. Also, carrier fish, which show no overt signs of disease but harbour the pathogen in their tissues, appear to be implicated in horizontal or vertical transmission. Such carrier fish are presumed to provide a reservoir which retains the pathogen in fish populations. Sea lamprey have been found to harbour typical *Aer. salmonicida*, and it may well be that this fish species is a possible source of infection for salmonids (El Morabit *et al.*, 2004).

To understand how *Aer. salmonicida* is transmitted both among and within fish populations it is necessary to know the source of the pathogen and its capacity to survive in the environment. In fact, most of the work done on epizootiological aspects of fish diseases caused by *Aer. salmonicida* has focused on investigations of potential sources of infection. The role of water, mud and detritus, contaminated implements on fish farms, animals other than fish themselves, and, particularly, carrier fish (i.e. salmonids as well as non-salmonids) as potential sources of infection with *Aer. salmonicida* have been examined. The popular approach to the study of this subject has been to determine the presence of and survival capabilities of *Aer. salmonicida* in the variety of habitats listed above. Certainly, there is evidence that the pathogen can survive without a significant change in numbers in transport systems, such as containing Stuart's medium, at 18–20°C for 48 h (Cipriano and Bullock, 2001). This opens up the possibility of transporting samples from field to laboratory without greatly influencing the populations of *Aer. salmonicida.*

Aeromonas salmonicida—survival studies

The survival of *Aer. salmonicida* in water has been thoroughly examined by numerous investigators (Williamson, 1929; Smith, 1962; Lund, 1967; McCarthy, 1980; Sakai, 1986a, b; Rose *et al.*, 1990a, b; Morgan *et al.*, 1991; Effendi and Austin, 1991, 1994; Table 8.1). Unfortunately, caution must be used in the interpretation of some of the

data as many of the studies employed pre-sterilised water or types of water in which *Aer. salmonicida* would not normally be present, e.g. distilled or tap water. Thus, the information gleaned from such studies does not necessarily reflect the behaviour of the pathogen in the natural aquatic environment. However, enough work has been done to allow tentative conclusions to be drawn. Based on the survival data accumulated, it appears that *Aer. salmonicida* is capable of surviving for a prolonged period in fresh, brackish and seawater, although contradictory results as to the exact time interval involved abound. Thus, for unsterilised freshwater, including river water, recovery of the pathogen from as little as 24 h to as long as 19 days had been reported by different studies. Survival in unsterilised brackish water was between 16 and 25 days (Smith, 1962; McCarthy, 1980). In unsterilised seawater, the organism could be recovered from between 24 h and 8 days (McCarthy, 1980). If sterilised water samples were used, survival time in the absence of competing—antagonistic—organisms was invariably greatly increased. For example, survival times in freshwater of up to 63 days were reported (Cornick *et al.*, 1969), and in seawater up to 24 days (Lund, 1967). Using whole cells and DNA released into lake water micocosms, with media and PCR for detection, Deere *et al.* (1996a) cultured *Aer. salmonicida* for <4 weeks, but found the DNA remained intact for >13 weeks. This discrepancy between the results of culturing and other techniques opens a veritable Pandora's Box. Why should intact DNA be found 2+ months after culturing techniques indicated that the population of *Aer. salmonicida* had disappeared?

Using a laboratory-based microcosm and culturing, direct counts, respiratory activity (the reduction of tetrazoliums to coloured formazans; after Effendi and Austin, 1993), iFAT, epifluorescence microscopy and the direct viable count techniques (by incorporating yeast extract and nalidixic acid; after Kogure *et al.*, 1979), Effendi and Austin (1994) confirmed the emerging view that cells of *Aer. salmonicida* remained after plate counts reached zero. These workers determined that survival was maximal in brackish conditions, i.e. salinity = 25‰, notably on substrates—especially on wood but also in sediment—rather than in the water column. Similarly, using an oxidase-negative atypical isolate, sterilised microcosms and culturing techniques, Wiklund (1995a) deduced that survival was better at 4°C than 15°C in brackish rather than sea or freshwater, and in the presence of particulates, i.e. sand. The addition of nutrients did not resuscitate cells after colony counts declined to zero. This is important, insofar as workers have been generally unsuccessful at retrieving culturable cells after plate counts declined to zero, regardless of the method that indicated cells or cellular components remained.

McCarthy attributed the discrepancies among the various investigations to the technical difficulty of isolating *Aer. salmonicida* from mixed cultures. The temperature at which the experiments were run may well have also influenced the results. For instance, McCarthy (1980) conducted his experiments between 11 and 13°C, and reported longer survival times for the pathogen in fresh, brackish and seawater (17, 24 and 8 days, respectively), than had been recorded by most other investigators (Table 8.1). Also, the differences may reflect inherent variations between cultures.

In the majority of reports about the survival of *Aer. salmonicida*, a large initial inoculum of the bacterium, usually 10^6 to 10^7 cells/ml of sample, was used. It is

Table 8.1. Experimental data concerning the survival of *A. salmonicida* in water

Type of experimental system	Temperature (°C)	Survival time	Reference
Sterilised water			
Distilled water	20	35 days	Lund (1967)
Distilled water	20	< 7 days	Sakai (1986b)
Lake water	10	8 days	Morgan *et al.* (1991)
	10	21 days	Morgan *et al.* (1993)
	20	60 days	Deere *et al.* (1996a)
Physiological saline	20	< 7 days	Sakai (1986b)
Tap water	—	5 days	Arkwright (1912)
	—	3 days	Williamson (1929)
	20	12 h	Lund (1967)
Reservoir water, low inoculum of *A. salmonicida* (10^1–10^2 cells/ml), in mixed culture with other aquatic bacteria	15	*ca.* 3 days	Allen (1982)
Reservoir water, as above, supplemented with 0.005% (w/v) brain heart infusion	15	> 3 days	Allen (1982)
River water	—	5 days	Williamson (1929)
	20	8 days	Lund (1967)
	10	63 days	Cornick *et al.* (1969)
	20–25	28 days	Cornick *et al.* (1969)
Seawater		19 h	Arkwright (1912)
	—	3 days	Williamson (1929)
	20	24 days	Lund (1967)
	—	< 10 days	Rose *et al.* (1990b)
	20	> 24 days	Effendi and Austin (1991)
	5–25	≤28 days	Effendi and Austin (1994)
Unsterilised water			
Brackish water		16–25 days	Smith (1962)
	11–13	24 days	McCarthy (1980)
Distilled water	—	14 days	Horne (1928)
	—	4 days	Williamson (1929)
	—	7 days	Duncan (1932)
	20	9 days	Lund (1967)
	Fresh water	24–30 h	Duncan (1932)
	11–13	17 days	McCarthy (1980)
River water	—	2 days	Williamson (1929)
	—	7–19 days	Smith (1962)
	20	2 days	Lund (1967)
10^1–10^2 *A. salmonicida* cells/ml	15	*ca.* 3 days	Allen (1982)
10^5–10^6 *A. salmonicida* cells/ml	15	> 3 days	Allen (1982)

Type of experimental system	Temperature (°C)	Survival time	Reference
Seawater	—	2 days	Williamson (1929)
	—	24–30 h	Duncan (1932)
	20	5–6 days	Lund (1967)
	11–13	8 days	McCarthy (1980)
	—	< 10 days	Rose *et al.* (1990b)
	20	6 days	Effendi and Austin (1991)
Tap water	—	3–4 days	Horne (1928)
	—	3 days	Williamson (1929)
	—	4 days	Duncan (1932)
	20	3 days	Lund (1967)
Mixtures of water types			
50% seawater + 50% tap water	—	−19 h	Arkwright (1912)
25% seawater + 75% tap water	—	45–67 h	Arkwright (1912)

unlikely, however, that the pathogen would occur in these numbers even in the event of a free-living existence in the natural environment, except perhaps during epizootics where moribund and dead fish were releasing large numbers of *Aer. salmonicida* into the immediate vicinity. Therefore, studies were undertaken using a low number of cells, *ca.* 10^1 to 10^2/ml, as an inoculum (Allen, 1982). When the pathogen was placed in sterilised reservoir water in such minimal numbers, and incubated at 15°C, the organism underwent a severe reduction in numbers within 72 h, such as to be virtually unrecoverable by plating methods on solid non-selective medium. From these results, which are in contrast to other studies, it becomes apparent that several factors, including the size of the inoculum and the temperature at which the experiments are conducted, are crucial in determining the outcome of survival studies. In contrast, when an inoculum of approximately 10^5 to 10^6 cells/ml was placed into sterilised reservoir water, *Aer. salmonicida* survived and multiplied with up to 10^8 cells/ml in the system in 72 h. It could still be recovered in substantial numbers (10^7 cells/ml) at 55 days, when the experiment was concluded. In addition, nutrient conditions appeared to have an effect, as it was observed that supplementation with low concentrations of nutrient, e.g. 0.005% (w/v) brain heart infusion broth, caused an increase in the number of *Aer. salmonicida* cells within 24 h. This increase was maintained until the end of the sampling period. The addition of nutrient, moreover, caused the increase in numbers of the pathogen regardless of whether the initial inoculum of cells was large or small. Supplementation with nutrient was also reported to increase survival time by McCraw (1952), who observed that the addition of 0.1% (w/v) peptone to seawater enabled *Aer. salmonicida* to survive up to 80 days, a much longer time than recorded for unsupplemented seawater.

The ability of *Aer. salmonicida* to persist in mud (sediment) or detritus in the fish farm environment has also been examined. McCarthy (1980) demonstrated that the

pathogen was able to survive in numbers of *ca.* 10^5 viable cells in fish pond mud and detritus at least up to 29 days, and probably longer as the experiments had to be terminated prematurely due to decomposition of the dialysis bags containing the bacteria. He further pointed out that the 10^5 viable cells remaining after 29 days are significant, since from his studies it was shown that if this number of cells was released into freshwater, their survival time would be 14 days. Michel and Dubois-Darnaudpeys (1980) investigated the persistence of *Aer. salmonicida* in sediments and reported that the pathogen survived and grew in sterilised river sediments for over 10 months. However, pathogenicity of the two isolates tested was lost after 8 or 9 months. They concluded that in natural conditions such a length of time would enable the pathogen to be released from sediment into the water, and that the behaviour of bottom-feeding fishes would allow direct contamination of fish, possibly becoming carriers. A reduction in pathogenicity, subsequent to prolonged incubation in river sediments, was also noted by Sakai (1986a, b). He offered an explanation whereby avirulent cells (with a positive electrical charge), which originate from virulent cells (negatively charged) attached to sediment, spontaneously detach from the sediment particles (river sand in survival experiments), thus decreasing the number of virulent cells recovered. Michel and Dubois-Darnaudpeys (1980) conceded that competition of *Aer. salmonicida* with large numbers of other bacteria in streams, some with an ability to synthesise bacteriocins, may act as a regulatory mechanism and limit the proliferation of the pathogen. However, previous work by Dubois-Darnaudpeys (1977b) supports the concept that *Aer. salmonicida* is genuinely capable of survival and multiplication in natural sediments, hence providing a reservoir of infection, even though direct contact with diseased fish is likely to remain the primary route of transmission. In addition, the regular detection of bacteriophages specific for *Aer. salmonicida* in samples of river sediments was taken as an indirect demonstration that the pathogen was present throughout the year (Dubois-Darnaudpeys, 1977b). Sakai (1986b) reported extended survival times (>15 weeks) for virulent cultures of *Aer. salmonicida* if placed in the presence of dilute humic acid (10 μg/ml), tryptone (10 μg/ml) and cleaned river sand (100 g/100 ml of medium). Without the addition of the sand, detection of viable cells ceased within five weeks. However, avirulent strains of the pathogen did not survive more than two weeks regardless of whether or not sand was included in the experimental system. Sakai (1986b) determined that humic acid and amino acid–humic acid complexes were absorbed onto the sand, which led to a build-up of 30–50 times the environmental concentration of amino acids on the surface of the sand particles. This, in turn, allowed only colonisation of/attachment by bacterial cells with net negative electrical charges (virulent cells of *Aer. salmonicida* in this instance), which resulted in their enhanced survival in the presence of the sand. Thus, Sakai (1986b) concluded that the electrostatic interrelationship occurring among humic acid, river sand and the bacteria explain the ability of virulent *Aer. salmonicida* strains to survive for extended periods in river sediments.

McCarthy (1980) contended that during epizootics of furunculosis there existed a strong possibility that fish farm implements could become contaminated with *Aer. salmonicida*. In a study emphasising survival of *Aer. salmonicda* on fish nets which

would be used both to remove dead infected fish and to move healthy fish, it was established that the pathogen survived up to 6 days on both dry and wet contaminated nets. In addition, wet and dry contaminated netting was disinfected using three compounds, i.e. acriflavine, Teepol–sodium hyrdoxide and hypochlorite solutions. *Aer. salmonicida* was not recovered from either wet or dry netting disinfected with the acriflavine or Teepol–sodium hydroxide solutions, but the hypochlorite solution failed to disinfect dry nets. McCarthy (1980) concluded from these results that the use of contaminated and improperly disinfected nets is potentially dangerous to healthy stock as it is known that netting abrades fish to some extent, and such abrasions can facilitate bacterial invasion. In addition, *Aer. salmonicida* has been reported to attach in higher numbers to plastic rather than stainless steel surfaces, which opens up the possibility that the pathogen may have a preference for certain substrates/surfaces in the aquaculture environment (Carballo *et al.*, 2000).

More recently, it has been found that wrasse (these are small inshore benthic fish which have gained popularity as a means of controlling sea lice populations among infested Atlantic salmon) are also susceptible to furunculosis (Treasurer and Cox, 1991). These investigators reported the recovery of typical *Aer. salmonicida* from the liver and kidney, and the presence of skin lesions reminiscent of chronic furunculosis in golsinny, rock cook and cuckoo wrasse of fish farm origin. But, could salmon lice harbour and transmit *Aer. salmonicida*? By means of recovery techniques with immunomagnetic beads coated with monoclonal antibodies to LPS and culturing techniques, it was determined that *Aer. salmonicida* was recoverable from lice (~104 *Aer. salmonicida* cells/louse) and also marine plankton (600 *Aer. salmonicida* cells/g of homogenised plankton) (Nese and Enger, 1993). Perhaps more worrisome is the report by Frerichs *et al.* (1992) of the recovery of atypical *Aer. salmonicida* from apparently healthy wild wrasse captured in the open sea. Fortunately, in this instance the isolates were proven to be non-pathogenic to Atlantic salmon smolts. Consequently, both groups have cautioned against the arbitrary stocking of wrasse in fish farms without first checking for the possible presence of *Aer. salmonicida* in the fish. Furthermore, Treasurer and Cox (1991) recommended that wrasse should not be released back into the wild, or transferred between fish farms at the end of the production cycle.

Only a few investigators have examined animals, other than fish, as a potential source of infection. The extensive study carried out on this topic was that of Cornick *et al.* (1969); a total of 2,954 vertebrate and invertebrate specimens, collected from fish ponds during an epizootic of furunculosis, were examined for the presence of *Aer. salmonicida*. No isolates of the pathogen were recovered despite this heroic attempt. This study is cited time and time again as evidence against the likelihood of animals, other than fish, acting as reservoirs of infection. Williamson (1928) was also unsuccessful in isolating the pathogen from water snails under similar conditions. Allen (1982) examined macroscopic algae and zooplankton taken from fish tanks prior to, during and after a furunculosis epizootic at a fish-rearing unit in Essex, U.K., in an unsuccessful attempt to recover *Aer. salmonicida* from these organisms. In contrast, King and Shotts (1988) determined that viable cells of *Aer. salmonicida* survived and, indeed, multiplied (two-fold) within the digestive tract of the ciliated protozoan,

Tetrahymena pyriformis. It may be concluded from some of these studies that the pathogen may be found in association with other aquatic animals, but it is apparent that many of the existing methods available for detection of the pathogen are inadequate.

Data have pointed to the possibility that *Aer. salmonicida* may be disseminated in aerosols (Wooster and Bowser, 1996). In particular, experiments demonstrated that the pathogen travelled 104.1 cm (the limit of the test chamber), via the airborne route (Wooster and Bowser, 1996). Thus, another possible means of spreading the pathogen needs to be considered.

Difficulties in recovering Aeromonas salmonicida from the aquatic environment

It is relevant to digress, at this point, from the discussion of sources of infection, in order to comment upon the difficulties besetting the isolation of *Aer. salmonicida* from environmental samples, other than fish. A dependable isolation procedure for the pathogen is of critical importance to an understanding of the epizootiology of diseases caused by *Aer. salmonicida*. For example, if the pathogen is capable of a free-living existence outside a fish host, the prevention and control of diseases of *Aer. salmonicida* aetiology would be rendered much more difficult if not impossible. However, currently *Aer. salmonicida* is defined as an obligate fish pathogen not found in surface waters (Popoff, 1984). This definition has no doubt been formulated due to the paucity of conclusive evidence for a free-living existence of the pathogen. The organism, for instance, often cannot be isolated from water on fish farms even during an epizootic of the disease (Cornick *et al.*, 1969; Kimura, 1970; Allen, 1982). Several reasons have been put forward to explain this disconcerting phenomenon. One is that *Aer. salmonicida* is notoriously difficult to isolate from mixed microbial populations as it is quickly outcompeted in growth by most other commonly occurring aquatic bacteria. In addition, pigment production on agar plates, heavily relied upon as a first indication that *Aer. salmonicida* is present, is inhibited by the close proximity of colonies of other bacterial types. Therefore, there is the perceived problem of recognising *Aer. salmonicida* in large, mixed microbial communities. Many of the problems with habitat and survival studies on *Aer. salmonicida* are blamed on lack of adequate methodology. Both McCarthy (1980) and Michel and Dubois-Darnaudpeys (1980) stressed contamination difficulties when employing non-selective media, e.g. TSA, for isolation of the pathogen. Cornick *et al.* (1969) reported that *Aer. salmonicida* was isolated most frequently from environments containing few, if any, other bacterial species, particularly representatives of the genus *Pseudomonas*. Their preliminary experiments suggested that some pseudomonad taxa obtained from water and fish inhibited the growth of *Aer. salmonicida* in liquid and on solid culture media. Both cell-free filtrates and extracts of disrupted cells of the pseudomonads caused the inhibition, believed to be due to antimicrobial activity. Dubois-Darnaudpeys (1977a) also examined the effects of the bacterial flora commonly occurring in surface water, such as the *Pseudomonas–Achromobacter* and *Flavobacterium* groups, on the survival and growth of *Aer. salmonicida*. She found that the pathogen was inhibited

at all temperatures if the experiments were run in the presence of the other bacteria. The ability of other micro-organisms, e.g. *Acinetobacter*, *Aer. hydrophila*, *Chromobacterium*, *Esch. coli*, *Flavobacterium* and *Pseudomonas*, and their metabolites, to inhibit the survival of *Aer. salmonicida* in non-sterile seawater was also reported by Effendi and Austin (1991). Thus, because the isolation and study of the viability of the pathogen is complicated by competition and inhibition by other organisms, it is not surprising that investigations into survival of the pathogen, which could help to establish whether or not it is capable of a free-living existence outside of fish, have invariably retreated to laboratory-based experiments using sterilised water. A filtration method tested by Maheshkumar *et al.* (1990) attempted, with some degree of success, to overcome the difficulties of isolation of *Aer. salmonicida* posed by the presence of other bacteria, i.e. the possible overgrowth by small numbers of cells of the pathogen. In their studies, up to 5 l of hatchery water was seeded with *Aer. salmonicida* and passed through 1^{-MDS} electropositive filters. This technique was used in combination with removal of the filters after backwashing, soaking them in a small volume of 3% beef extract solution, and followed by the scraping of the filters to remove trapped bacteria. When all the eluates from the filters were combined, recovery of *Aer. salmonicida* was determined to be 35%. Thus, Maheshkumar *et al.* (1990) concluded that the filtration technique demonstrated greater sensitivity than the direct examination of water. Also, the enumeration of *Aer. salmonicida* was not overly affected by the presence of other bacteria because in the water samples the pathogen retained the ability to produce a brown pigment. However, these authors noted that biochemical/serological tests would be necessary for the detection of atypical non-pigmenting isolates. Nonetheless, the classic dilemma of many ecological studies persists, i.e. how does the response of an organism in laboratory-based experiments relate to its performance in the natural environment where it needs to interact in a diverse and heterogeneous community? It is a problem still in search of a reliable solution.

It has often been stated that ecological investigations of *Aer. salmonicida* are hampered by the lack of an effective selective isolation medium specifically formulated for the pathogen. It was, for example, the opinion of Cornick *et al.* (1969) that the development of a selective medium for *Aer. salmonicida* could quite possibly change the present views on the habitat and viability of the pathogen. McCarthy (1980) also believed a selective medium would greatly assist ecological work. No doubt such a medium would be extremely useful; however, it is unlikely that its existence alone would cause all the remaining difficulties concerning ecological work on *Aer. salmonicida* to evaporate. It should be noted, however, that, although a selective medium for *Aer. salmonicida* has not yet been formulated, CBB serves well as a differential growth medium, and is increasingly employed for this purpose. This medium is especially useful in the detection of *Aer. salmonicida* in fish tissues. For instance, using CBB, Cipriano *et al.* (1992) recovered the pathogen from 56% of mucus samples, but interestingly from only 6% of kidney material, taken from salmonids. This was reinforced by a later study which pointed to the presence of the organism in gills and well as mucus, i.e. external carriage (Cipriano *et al.*, 1996a, b). The data have been summarised as follows:

Number of samples revealing Aer. salmonicida

15/100	gill samples revealed the pathogen at	6.3×10^2–1×10^4/g
19/100	mucus samples revealed the pathogen at	9.1×10^2–1.7×10^4/g

Using CBB, Cipriano *et al.* (1996b) reported *Aer. salmonicida* in higher numbers from the mucus than kidney of six salmon. Thus, the populations of *Aer. salmonicida* were in the range of 1.1×10^3 to 1.8×10^7/g and 1.0×10^3 to 1.4×10^7/g for mucus and kidney, respectively. Hiney *et al.* (1994) reiterated the view that *Aer. salmonicida* may colonise mucus, gills and also fins. Also, these workers considered that the intestine may well be the primary location of *Aer. salmonicida* in Atlantic salmon with asymptomatic infections. In addition, there are other considerations that may contribute to the problems experienced. For instance, parallels might be drawn from studies on the isolation methods used for other micro-organisms, i.e. notably coliforms and enteric pathogens such as *Salmonella* spp., where it has been observed that a pre-enrichment step must be employed prior to use of selective media that impose too stringent conditions on these organisms which have been stressed, injured or are too sensitive to selective agents, and, thus, are rendered unrecoverable by selective methods alone (Geldreich, 1977; Kaper *et al.*, 1977; Olson, 1978). Alternatively, it can be postulated that *Aer. salmonicida* follows the pattern of certain other types of micro-organisms which are extremely difficult to detect in the natural environment by means of routine bacteriological procedures (i.e. plate counts) and, hence, have been assumed to be absent from these environments. Stevenson (1978) commented on adjustments made by bacteria, which enable the organisms to survive in the variable and often stressful conditions imposed upon them by existence in natural aquatic systems. He suggested that bacteria surmount changes in their environment, including varying degrees of solar input, temperature, availability of nitrogen and dissolved oxygen, by entering a state of dormancy defined as "any rest period or reversible interruption of the phenotypic development of an organism" (Sussman and Halvorson, 1966). Thus, the possibility that *Aer. salmonicida*, in the natural environment outside a fish host, assumes a physiological state such that it cannot be recovered on agar plates used for primary isolation should not be discounted. There is tentative evidence that the situation for *Aer. salmonicida* is similar to that of a related pathogen, *V. cholerae*. In the survival of *V. cholerae* in aquatic microcosms, Singleton *et al.* (1982a, b) reported that certain combinations of environmental parameters, i.e. suboptimal salinities and low nutrient concentrations, not only affected multiplication of *V. cholerae* populations but also recoverability of the cells. Thus, these investigators found that *V. cholerae* cells were observed using acridine orange staining in conjunction with epifluorescence microscopy when culturable cells were not detected. Further work on the theme of a "non-recoverable" stage of existence for bacterial populations which, however, remain viable was done by Xu *et al.* (1982), also for *V. cholerae*. They used direct viable-counting, a procedure allowing estimation of substrate responsiveness, i.e. viable cells, using microscopy. This method revealed that a significant proportion of the non-culturable cells were, in fact, viable.

Survival of Aeromonas salmonicida in the absence of culturing

The observations for *V. cholerae* were of especial interest because we found that cells of *Aer. salmonicida* could be enumerated by microscopic direct counts when no colony-forming units (CFUs) were recovered on agar plates, raising the possibility that problems concerning the recovery of *Aer. salmonicida* may bear similarities to *V. cholerae*. Our experiments had shown that if low numbers of *Aer. salmonicida* were inoculated into sterilised reservoir water the organism could not be recovered using plating methods after 72 h. However, when microscopic direct counts were done the number of bacteria underwent an initial increase 6 h post-inoculation, and maintained these numbers until the conclusion of the experiment. Unfortunately, it was not possible to know from these results whether the cells observed by microscopy were, indeed, viable. Thus, in a follow-up study, the hypothesis that *Aer. salmonicida* may enter a non-recoverable but viable state was tested. However, these data bore some similarity to survival studies with other pathogens, namely that there is often an initial increase in the number of cells as visualised by microscopic methods despite culturing indicating a progressive decline in numbers.

The non-culturable but viable (NCBV) state

It was confirmed by Allen-Austin *et al.* (1984) that small inocula of cells rapidly declined in filter-sterilised river water, when enumerated using total viable count procedures on agar plates, such that the system appeared to be totally devoid of viable cells by day 17. However, the microscopic procedures showed that, after an initial decrease, the number of *Aer. salmonicida* cells remained constant at approximately 8.0×10^2/ml. TSB to 0.01% (v/v) was added to the experimental system 7 days after the plate counts reached and remained at zero, and the sample was split into three equal volumes, incubated at 22 and 18°C in addition to 15°C. At 22°C, 150 CFU of *Aer. salmonicida*/ml of sample were recovered on TSA 6 h after supplementation with the nutrient. There was no apparent increase in the direct microscopic count at 6 h, but by 24 h the microscopic fields contained too many cells to count. This result demonstrated that 6 h after nutrient addition, a proportion of the cells had regained the ability to produce colonies on TSA, despite the fact that for the previous 7 days none had been capable of colony formation. At 18°C, the response to the added nutrient was much slower, insofar as colonies were not detected until after 4 days had elapsed. There was, however, a pronounced increase in the direct counts at 24 h after nutrient was added. This continued up to 4 days, when the first colonies were cultured on solid medium. Similarly, there was a lag of 5 days before colonies were recoverable at 15°C. This coincided with an increase in the direct count. In comparison, it is emphasized that plate counts remained at zero in unsupplemented river water at 15, 18 and 22°C. The factors involved in triggering the return of *Aer. salmonicida* to a culturable state need careful evaluation. As demonstrated in the experiment reported here, temperature and nutritional changes appear to be responsible for reactivating cells of *Aer. salmonicida* (Allen-Austin *et al.*, 1984).

Sakai (1986b) also proposed a mechanism for the long-term survival of *Aer. salmonicida* in the aquatic environment based on electrostatic charge differences on individual cells, with net negative charges reported on virulent, agglutinating cells,

and net positive charges on avirulent, non-agglutinating strains. He suggested that the negatively charged virulent form of *Aer. salmonicida* is able to persist, albeit under starvation conditions, retaining viability in river sediments. It was also proposed that the decline in negatively charged virulent cells in sediments over prolonged periods, also noted by other investigators (e.g. Michel and Dubois-Darnaudpeys, 1980), may be caused by the spontaneous occurrence of positively charged, avirulent, free-living cells of *Aer. salmonicida*. These cells originate from the virulent ones, attached to sediment particle surfaces, and subsequently detach from the sediment/sand particles. This free-living form could be considered to enter a dormant phase, according to Sakai (1986b), because the viability of these bacteria declines due to a lack of nutrients. It was further proposed that the free-living cells represent a transitional life stage of the pathogen which would ultimately lose viability (Sakai, 1986b).

Subsequently, Rose *et al.* (1990a) re-examined the possibility that *Aer. salmonicida* may enter a dormant state in water, using methods modified from the work of Allen-Austin *et al.* (1984), as described above. However, in their experiments the addition of 0.1% (w/v) TSB to aliquots withdrawn from microcosms after viable counts of *Aer. salmonicida* had reached zero, did not result in renewed growth of the organism. Thus, Rose *et al.* (1990a) concluded that the most probable explanation for the results obtained in the previous study (when there appeared to be resuscitation of dormant cells by added nutrients) was the presence of small numbers of viable, culturable cells, which were too few in quantity to be detected by the sampling protocol employed. This conclusion was based on the observation that the addition of 0.1% (w/v) TSB to microcosms after the viable count had reached zero resulted in the re-appearance of viable, culturable cells within 48 h of incubation at 22°C. However, in both studies bacteria enumerated by microscopic techniques remained at levels of approximately 10^4/ml in water samples retrieved from the experimental microcosms containing *Aer. salmonicida*, even after viable counts had apparently reached zero. It is curious that Rose *et al.* (1990a) proffered no explanation to account for the level of bacteria that were observed microscopically (were the cells alive or could they have been dead?). In a later study, which again addressed the issue of dormancy/NCBV for *Aer. salmonicida*, Morgan *et al.* (1991) assessed the survival of the pathogen in lake water, employing an extensive range of techniques, including epifluorescence microscopy, respiration, cell culture, cell revival, flow cytometry, plasmid maintenance and membrane fatty acid analysis. These workers found that *Aer. salmonicida* became unculturable in sterile lake water, but microscopic and flow-cytometric methods revealed the continued presence of cells. However, attempts to revive these cells by the addition of TBS were unsuccessful. Despite this, it was found that both genomic and plasmid DNA, and also RNA, were maintained in the cells, even though they could not be cultured on conventional media. Morgan *et al.* (1991) concluded that morphologically the cells remained intact, although their viability could not unfortunately be definitively demonstrated. In addition, they commented (and we strongly agree) that non-culturability of some bacteria from environmental samples maybe as much a function of the ignorance of the parameters necessary for their recovery, as the occurence of a truly non-culturable, specialised survival state. Subsequently, by means of flow cytometry with rhodamine-123, they established a

NCBV state in sterile lake water (Morgan *et al.*, 1993). However, flow cytometry indicated that cellular properties related with viability were lost shortly after culturability disappeared in distilled water, but not so in lake water (Deere *et al.*, 1996b). Additionally, these workers marked an isolate of *Aer. salmonicida* with the *xylE* gene, using the plasmid pLV1013. This isolate was culturable on TSA from normal (non-sterile) lake water for three weeks, after which non-culturability developed, with the NCBV cells retaining chromosomal and plasmid DNA. The NCBV state can be postponed (60 days was mentioned by Pickup *et al.*, 1996) by the addition of high levels of nutrient, especially 125 μM quantities of the amino acids arginine and methionine, to experimental microcosms. *Aer. salmonicida* decreased in size and became rounded, but were still culturable (Pickup *et al.*, 1996).

The development of a dormant, non-culturable state of *Aer. salmonicida* in seawater at 4°C was apparent from the work of Ferguson *et al.* (1995). These workers incorporated a luciferase gene, *luxAB*, from *V. fischeri* into *Aer. salmonicida* and followed the fate of the cells. As before, intact, non-culturable cells could not be resurrected.

There has always been a dilemma about the relevance of cells that cannot be cultured to fish. It is worth heeding the results of Stanley *et al.* (2002), who determined that only culturable cells in laboratory microcosms could induce furunculosis upon injection into fish, and not PCR- or ELISA-positive samples which could not be supported by culturing evidence.

Ecology of Aeromonas salmonicida—an explanation

To develop some previous points, there is tentative evidence to support the possibility that *Aer. salmonicida* undergoes sufficient modifications to its morphology in seawater so as to be only recoverable on specialised media. Thus, while conducting experiments on the survival of *Aer. salmonicida* in seawater, Effendi and Austin (1991) found that samples where the pathogen was believed to be absent (or unculturable) actually contained cells which passed through 0.22 and 0.45 μm pore size porosity filters. These isolates grew on specialised media designed for the recovery of L-forms, and showed agreement with the characteristics of *Aer. salmonicida* L-forms as reported by McIntosh and Austin (1988, 1990a, 1991b). Subsequently, *Aer. salmonicida* colonies developed on basal marine agar (BMA) plates inoculated with material from turbid L-form broth medium. On this basis, Effendi and Austin (1991) recorded populations of *ca.* 10^3 *Aer. salmonicida* cells/ml in the microcosms after corresponding enumeration of colonies on BMA had reached zero. Thus, they suggested that the existence of specialised forms, e.g. L-forms, of *Aer. salmonicida*, may be a factor in the difficulties previous researchers have experienced in attempts to recover the pathogen from environmental samples. Continuing this theme, Effendi and Austin (1995a) examined the characteristics of the so-called NCBV cells. Using a marine microcosm, it was observed that these NCBV cells became much smaller and coccoid while retaining respiratory activity as measured by the reduction of tetrazoliums to insoluble formazans. There was not any alteration in the LPS composition of the cells, but an alteration in the protein composition was recorded, with a reduction in some (15, 17, 22, 30 and 70 kDa proteins) and an increase in a 49 kDa

protein. This was accompanied by a loss in DNA. That these cells were still alive was indicated by the development of large, bizarre shapes following the addition of yeast extract and nalidixic acid (after Kogure *et al.*, 1979).

To summarise, the question as to whether *Aer. salmonicida* is able to persist in a free-living form outwith a fish host is still outstanding. An equally important corollary concerns the pathogenicity of such forms, assuming that they exist, i.e. can they retain the ability to infect fish? Traditionally, *Aer. salmonicida* has been defined as an obligate fish pathogen. However, there appears to be an increasing trend in ecological studies to at least consider the possibility that this definition may no longer hold true at all times. On the basis of the available data obtained from numerous survival studies, it may be stated that *Aer. salmonicida* has the ability to persist in the aquatic environment for protracted periods. It is the mechanism of this survival and its effects on the organism in the natural environment around which the debate now centres. It is possible that *Aer. salmonicida* could exist in so-called non-culturable stages (perhaps due to the presence of an altered morphological state, such as L-forms). After all, the reason for the occurrence of explosive outbreaks of furunculosis among fish populations, particularly salmonids, which have not been previously exposed to the disease, has yet to be explained. The answer to this important question of facultative versus obligate pathogen still awaits the development of methods for the more efficient and refined detection of the organism in the natural environment. As one astute scientist has said, "absence of evidence is not evidence of absence"; hence, further efforts to breach this gap in our understanding of *Aer. salmonicida* epizootiology are essential.

Aeromonas salmonicida—transmission by fish

Fish undoubtedly play a major role in the transmission of disease among themselves. One early study (Blake and Clark, 1931) stated that furunculosis was only spread by infected fish or by material which has come into contact with them. Fortunately, the recovery of *Aer. salmonicida* from fish tissues is generally less troublesome than seeking it in other environmental sources. Fish may act as a source of infection in two ways: those which have died or are ill with furunculosis are heavily contaminated with the pathogen; alternatively, they may be carriers which, although appearing healthy, harbour the pathogen in their organs where it can be released if the fish eventually succumb to the disease. Both these aspects have received attention. McCarthy (1980) established that material from a furuncle could contain up to 10^8 viable cells/ml of necrotic tissue, and was interested to assess the viability of *Aer. salmonicida* in dead fish and the degree to which they could contaminate pond water. He found that *Aer. salmonicida* remained viable in fish (muscle) tissue for 32 days, and for 40 days in the tank water where the dead fish had been kept, thus providing a possible source of infection for healthy fish. Another study showed that *Aer. salmonicida* remained viable in infected trout tissues and internal organs (heart, liver, spleen and kidney) for 49 days when the fish were stored at $-10^{\circ}C$ (Cornick *et al.*, 1969). However, the pathogen was isolated only from the kidney of infected trout held at $4^{\circ}C$ for 28 days. McCarthy (1980) pointed out that the prolonged survival of *Aer. salmonicida* in dead, diseased fish shows the risk of using scrap fish, which might

be infected with a chronic form of furunculosis, as food, particularly in view of the finding of Cornick *et al.* (1969) on the survival of the pathogen at low temperatures. Some investigations, which have examined the survival of *Aer. salmonicida* in water, have also commented on the potential of the non-culturable cells, detected microscopically in the experimental systems, to infect fish. However, these studies have generally concluded that *Aer. salmonicida* in this form does not appear to be pathogenic, as re-infection of fish does not occur (Rose *et al.*, 1990a; Morgan *et al.*, 1991).

Aeromonas salmonicida—L-forms

Another line of investigation examined L-forms (spherical, filterable cells) of *Aer. salmonicida*. The L-forms were induced experimentally, and were found to be unculturable by conventional plating methods. Subsequently, a stable L-form was induced with benzylpenicillin, and determined to contain more OMP of ~40 kDa molecular weight than its parental cell (Gibb *et al.* 1996). This stable L-form did not require specialised media, and could grow on BHIA at 0–5°C (Gibb and Austin, 1994). It could be argued that the development of an L-form state could enable it to persist in tissues of infected fish, although not causing clinical disease (McIntosh and Austin, 1991b). However, attempts to infect fish using L-forms did not result in recovery of the pathogen from fish, even after the administration of immunosuppressants. In addition, it was reported that small numbers of "natural" L-form colonies were observed, albeit infrequently, on specialised medium (containing horse serum and high quantities of sucrose), which had been inoculated with kidney and spleen samples taken from farmed Atlantic salmon suffering with furunculosis. This suggests that L-forms may have implications in the disease process. Such findings certainly merit further investigation to more conclusively establish the role of this form of *Aer. salmonicida* in disease processes. Obviously, if *Aer. salmonicida*, in a dormant or NCBV or indeed in any other altered state it may undergo outside a fish host, is genuinely unable to transmit infection, then control of the diseases is vastly simplified and rendered less difficult. It is not possible, on the basis of the limited data available, to draw grand, definitive conclusions about this crucially important aspect of the pathogen's behaviour. Further work will, hopefully, clarify the situation.

Aeromonas salmonicida—the role of carriers

The second mechanism by which fish may provide a source of infection is by becoming carriers of *Aer. salmonicida*. The existence of such fish and their role in transmission of the disease was recognised early in the history of furunculosis. In a study of furunculosis in trout in the river Kennett, Horne (1928) found that *Bacterium salmonicida* was recovered in the blood of 17% (3 out of 18) of trout examined, and it was concluded that carriers provided a source of infection in the river. He also sampled fish from fish farms and found them to be generally healthy and thus contended that these results indicated that trout farms were not harbouring the disease. Horne (1928) additionally commented that knowledge of carrier rates in fish populations before, during and after the furunculosis season would be of great epizootiological value.

Because of the obvious importance carrier fish have in the epizootiology of furunculosis, it is essential that the methods used to detect their presence are effective. However, research into the carrier state has been hampered by technical difficulties concerned with detecting such fish with certainty, as present culture isolation methods appear to be too insensitive (McCarthy, 1980). Blake and Clark (1931) reported that raising the temperature of the water in which suspected carriers (usually survivors which had been previously exposed to infection) were maintained from 5 to 18°C induced the disease. At present, a combination of increasing the water temperature to 18°C and the injection of corticosteroids is employed to activate the carrier state. This method is based on the work of Bullock and Stuckey (1975b), who tested corticosteroid injection and heat stress as means of producing overt furunculosis in carrier trout. They reported that, although heat stress alone produced mortality, the pathogen could not be recovered from the majority of test fish which died. Direct kidney cultures to isolate *Aer. salmonicida* were found to be ineffective for carrier detection. McCarthy (1980) reported success with the method of Bullock and Stuckey (1975b), although he stated that prednisolone acetate was the most effective corticosteroid of the several he tested for inducing furunculosis in fish. In further experiments, he assessed the prevalence of carriers in fish populations, examining 1-year-old brown trout from four different commercial fish farms. The fish had a high carrier rate of 40–80%; however, similar populations of rainbow trout tested had a very low incidence of <5% or were possibly free of carriers. The technique has also been applied by McCarthy (1975a) to non-salmonids, e.g. silver bream (*Blicca bjoerkna*), infected with a non-pigmented aberrant *Aer. salmonicida* strain.

The ELISA technique has also been assessed for its effectiveness in the detection of carrier fish. Rose *et al.* (1989) compared a commercial ELISA kit (obtained from Stirling Diagnostics Ltd.), the commonly used corticosteroid/heat protocol of McCarthy (1980) and plating of rectum and kidney samples from Atlantic salmon onto BHIA and TSA. The Atlantic salmon were obtained from a site where outbreaks of furunculosis had previously occurred. From the results, it was apparent that the ELISA was the most successful technique, with 56.17% (14/26) of the fish shown to be carriers. This compared with a carrier rate of 26.4% as determined by means of the corticosteroid/heat test. Yet, plating techniques failed to reveal *Aer. salmonicida* in any of the fish. However, some disadvantages of ELISA are apparent, namely the inability to distinguish living from dead cells, and, for that matter, to differentiate viable, pathogenic cells from those resulting from use of living, attenuated or dead, virulent cells from vaccines. Moreover, ELISA does not enable the provision of cultures which could be used for additional investigation, such as the determination of antibiogrammes. Notwithstanding such drawbacks, ELISA is a valuable tool for the rapid detection of *Aer. salmonicida* in clinically diseased and asymptomatic carrier fish.

It has not been conclusively established which organs of carrier fish serve as the site of carriage. From experiments using immunofluorescence techniques, Klontz (1968) concluded that the intestine is a primary site of infection, leading to the establishment of asymptomatic carriers. There is also some evidence to suggest that

the kidney is involved, but the intestinal tract has again been mentioned (McCarthy, 1980). This is a subject which requires further work to clarify it.

Another problematic aspect of the carrier state is that antibiotic therapy to control furunculosis outbreaks does not necessarily completely remove the bacterium from the tissues of fish, at least when sulphamerazine, furazolidone and potentiated sulphonamide are used. McCarthy (1980), however, reported that fish treated with tetracycline hydrochloride (i.m. injection of 50 mg/kg) survived attempts to induce the disease. On the basis of such findings, McCarthy (1980) warned that it must be anticipated that fish populations treated for furunculosis will remain carriers.

However. more recently other antibacterial compounds have been investigated for their ability to reduce or eliminate the carrier state of *Aer. salmonicida* in fish. Examples include erythromycin phosphate (Roberts, 1980), flumequine and an aryl-fluoroquinolone (Scallen and Smith, 1985) in combination with 0.01% Tween 80 to enhance the assimilation of the antimicrobial compound into fish (Markwardt *et al.*, 1989). The last-mentioned group of workers found that the aryl-fluoroquinolone/surfactant combination was effective in eliminating the asymptomatic carrier state of *Aer. salmonicida* within 48 h of treatment. Markwardt and Klontz (1989) also pointed out the advantages of the use of the aryl-fluoroquinolone, such as its broad spectrum activity, low *in vitro* minimal inhibitory concentration for many bacterial pathogens (Stamm *et al.*, 1986) and lack of occurrence of resistant strains (Fernandes *et al.*, 1987).

Aeromonas salmonicida—transmission; what does it all mean?

Several possible routes for the transmission of furunculosis have been propounded and investigated. It is commonly accepted that the disease is disseminated by lateral transmission of *Aer. salmonicida* which includes contact with contaminated water and infected fish in addition to possible infection via the gastro-intestinal tract. Also, vertical transmission has been considered in several investigations. A water-borne route where water contamination with *Aer. salmonicida* has occurred initially from moribund, infected fish or from overtly healthy carriers shedding the pathogen is favoured as the common means of transmission. Once released into the aquatic environment, the organism is then able to persist for a prolonged period of time and the disease spread in this way. Early studies demonstrated that trout placed into water that had contained diseased fish contracted the infection (Emmerich and Weibel, 1894; Horne, 1928; Blake and Clark, 1931). The Furunculosis Committee of the U.K. concluded, on the basis of available data, that both food and diseased fish could constitute sources of infection. McCarthy (1980) has examined in detail the likely transmission mechanisms, specifically with regard to the ability of *Aer. salmonicida* to penetrate fish tissues (invasiveness), a prerequisite for the occurrence of infection. Transmission by contaminated water was tested by McCarthy (1980) in laboratory-based experiments by seeding water in a tank containing six brown trout with a suspension of *Aer. salmonicida* to a final concentration of 10^6 cells/ml. Five of the six fish died of furunculosis, and the sixth succumbed when given an injection of prednisolone acetate. In a subsequent large-scale experiment using 50 brown trout placed in a pond on a fish farm experiencing a Summer epizootic of furunculosis,

41 fish had died within 28 days, and the remaining 9 succumbed after injection with corticosteroid. McCarthy (1980) concluded from these experiments that the disease is readily disseminated through water and also that brown trout surviving infection probably become carriers. However, both Blake and Clark (1931) and McCarthy (1980) reported failure in attempts to infect rainbow trout by co-habitation with infected brown trout or the addition of *Aer. salmonicida*, respectively. It is known that rainbow trout are more resistant to the disease than are brown trout. In experiments which examined different routes of exposure to *Aer. salmonicida* subsp. *salmonicida* in Atlantic salmon in seawater, Rose *et al.* (1989) noted that a minimum dose of 10^4 CFU/ml by bath was required to initiate infection.

Aeromonas salmonicida—uptake into fish

Another unresolved aspect of the transmission of furunculosis is the uptake of *Aer. salmonicida* into a fish host. It is possible that the pathogen may gain entry to a new host through the gills, lateral line, mouth, anus or a surface injury (e.g. Effendi and Austin, 1995b). McCarthy (1980) demonstrated that rainbow trout that resisted the disease subsequently died from furunculosis after their flanks had been abraded with sandpaper. Also, Lund (1967) found that infection was acquired by fish which had been scarified and experimentally challenged with the pathogen. However, these injuries were artificially induced. Effendi and Austin (1995b) evaluated many different routes for the possible uptake of *Aer. salmonicida* into fish. The data may be summarised as follows:

Route	Recovery of *Aer. salmonicida* from:
Gill	Blood and kidney
Oral	Blood
Lateral line	Blood and spleen (but not kidney)
Ventral surface	Blood and spleen (but not kidney)
Flank	Blood and spleen (but not kidney)
Anus	Blood (but not kidney or spleen)

Generally, *Aer. salmonicida* remained at the site of administration for 24 h. The most effective route of uptake leading to mortalities was the gill and anus. In contrast, fewer deaths resulted from challenge via the lateral line, flank or ventral surface (Effendi and Austin, 1995b). Yet, despite all this work, the natural mode of uptake remains unresolved.

In a study of uptake of *Aer. salmonicida* by rainbow trout, it was observed that the pathogen could be detected in the blood and kidney within 5 min of immersion in a suspension containing 10^5 cells/ml (Hodgkinson *et al.*, 1987). Interestingly, it was also found that uptake of the pathogen was enhanced by the addition of particulates, e.g. latex, to the bacterial suspension. If latex was indeed added, *Aer. salmonicida* was isolated from blood at 12 min, and from kidney, spleen and faeces at 4 h post-challenge. The organism was also cultured from the skin, gills, blood and faeces for up to 48 h. In the absence of latex, the pathogen could be again recovered at

12 min, but from a wider range of sites including kidney, spleen and the lower intestine. However, by 24 h the pathogen was no longer recovered from the fish. From culturing methods alone, it may not, however, be assumed that *Aer. salmonicida* had been totally removed from the animals. In fact, cultures of the pathogen were isolated from kidney, spleen and faeces within 1 to 4 h of immunosuppression of the fish at 7 days post-challenge. In addition, the method of challenge yielded different results. Thus, when the entire fish was immersed in the bacterial suspension, superior uptake occurred compared with exposing only the head or tail regions. The explanation of this phenomenon is unclear, but such results suggest that uptake may occur through several locations rather than a single site, e.g. mouth, nares, gill or anus. It is possible that the pathogen gains entry via all these sites or additionally through the lateral line and/or skin (Hodgkinson *et al.*, 1987). Perhaps, the most significant observation resulting from these experiments was the rapidity by which *Aer. salmonicida* entered the rainbow trout. Other investigators have not sampled so close to the initial time of challenge. McCarthy (1980) reported uptake to occur from the oral route within 5 h, with the organism found in the kidney. Tatner *et al.* (1984) first sampled the fish at 24 h post-challenge. However, all these studies indicate the localisation of *Aer. salmonicida* principally within the reticulo-endothelial systems of fish (Tatner *et al.*, 1984). Notwithstanding, evidence continues to be firmly pointed at the role of gills and skin/mucus in the uptake of *Aer. salmonicida* into fish (Ferguson *et al.*, 1998).

Another route of infection that has been proposed is via the gastro-intestinal tract, due to intake of contaminated food. However, there is disagreement as to whether or not this in fact occurs. Plehn (1911) and Blake and Clark (1931) reported success in experimentally infecting fish by feeding contaminated food. However, Krantz *et al.* (1964a, b) and McCarthy (1980) failed to infect brown trout by feeding with food containing the pathogen. Klontz and Wood (1972) reported clinical furunculosis in the sable fish apparently caused by ingestion of carrier coho salmon. Evidence has been published that shows *Aer. salmonicida* may translocate across the intestinal epithelia (Jutfelt *et al.*, 2006). Here, the authors exposed intestinal segments of rainbow trout for 90 minutes to isothiocyanate-labelled cells of virulent *Aer. salmonicida* and demonstrated translocation. In addition, in laboratory-based experiments that compared various methods designed to induce the carrier state of *Aer. salmonicida* in juvenile Spring chinook salmon, Markwardt and Klontz (1989) observed that gastric intubation (of *ca.* 1×10^8 bacteria) resulted in a 65% carrier state. This result was a significantly higher percentage than those recorded for exposure to a broth culture as a bath, ingestion of broth culture coated food, and exposure to intraperitoneally injected fish (40, 20 and 10% carrier rates, respectively). Markwardt and Klontz (1989) also commented that the exposure to clinically diseased fish and bathing in broth cultures most probably simulated the natural routes of infection (Paterson, 1982).

Aeromonas salmonicida—transmission; the role of eggs

Vertical or transovarian transmission of *Aer. salmonicida* as a possible route of infection has been widely studied with inconclusive results. Smith (1939) claimed

the pathogen could be carried on the egg surface, in contrast to Plehn (1911) and Mackie *et al.* (1930), who were of the opinion that the organism was unable to infect fish eggs. The possibility that the pathogen could be transmitted at fertilisation was examined by the Furunculosis Committee in the U.K. The experimental evidence gathered for their report indicated that furunculosis was not transmitted in such a fashion. Lund (1967), however, contended that since the conclusions were based on the results of a sole set of experiments, further work was necessary to confirm this point. In a detailed series of experiments aimed at clarifying the situation regarding transmission of *Aer. salmonicida* at fertilisation, Lund (1967) examined ovaries, testes and ova of experimentally infected fish for the presence of the pathogen. *Aer. salmonicida* was recovered in pure culture from the ovaries and testes of infected fish. Results of isolation of the pathogen from ova were decisive as, of 500 ova sampled, only 3 obtained from the same fish yielded the bacterium in pure culture from the interior of the ovum. In further experiments, using wild spawners, ova were contaminated with *Aer. salmonicida* on the external surface at the time fertilisation was effected, and the eggs then planted out in a river bed. It was observed that these ova died quickly and were subjected to *Saprolegnia ferax* infection. *Aer. salmonicida* was not isolated from dead or living ova, and Lund (1967) concluded that the experiment had been unsuccessful. Ova taken from parents experimentally infected with *Aer. salmonicida* also failed to yield the bacterium. Continuing the study of vertical transmission, McCarthy (1980) examined the fertilised ova of mature, broodstock brown trout taken from a known carrier population (5/8 proved to be carriers when challenged with prednisolone acetate). However, *Aer. salmonicida* was not recovered from the fertilised egg sample. When artificially infected broodstock were stripped as soon as signs of clinical furunculosis had developed, both fish organs and fertilised eggs were positive for *Aer. salmonicida*. However, the high numbers of viable cells initially present, i.e. 10^6 cells/ml of egg macerate, rapidly declined and could not be detected 5 days after incubation began. Based upon these experiments, McCarthy (1980) concluded that vertical transmission of *Aer. salmonicida* was not a significant means of disseminating the disease, and, moreover, in the improbable event that overtly infected fish were used for stripping, the pathogen was unlikely to survive to the eyed egg stage at which the eggs are marketed. Neither Lund (1967) nor McCarthy (1980) recovered *Aer. salmonicida* from fry derived from experimentally infected parents or of known carrier broodstock, respectively. However, both these authors pointed out the possibility that the negative results obtained may have been due to the inadequacy of techniques used for detection and isolation of the pathogen in the face of inhibition or overgrowth by commensal bacteria, or the presence of only small numbers of *Aer. salmonicida*.

Aeromonas salmonicida—transmission in seawater

A remaining aspect of the epizootiology of *Aer. salmonicida* diseases which requires consideration is the transmission of the infection in seawater. This is an important topic for the aquaculture industry, as salmonids are not infrequently placed in seawater for on-growing. In addition, early in the study of the pathogen, the possibility that migratory Salmonidae could spread the infection was considered. Lund (1967)

investigated the possibility that the disease could be carried by salmon or sea trout smolts (previously infected in freshwater) when they migrated to the sea. The Furunculosis Committee had not agreed with this theory because examination of large numbers of smolts taken from the river Coquet in 1928 and 1929 had given no evidence for the presence of *Aer. salmonicida*, although the reverse process, i.e. salmon or sea trout contracting the infection upon migration into rivers containing infected trout, had become generally accepted. In an examination of 234 smolts from the river Coquet, Lund (1967) isolated and confirmed *Aer. salmonicida* from four smolts (two salmon and two sea trout), and believed the findings to be significant as such fish would possibly develop the disease on exposure to suitable conditions or remain resistant, possibly transmitting the infection upon contact with healthy fish in sea or brackish waters. Lund (1967) could not offer a definitive reason for the results differing from those of Williamson and Anderson (see Mackie *et al.*, 1930), who examined 1,339 smolts taken from the Coquet without recovering any isolates of *Aer. salmonicida*. Certainly, mortalities attributed to *Aer. salmonicida* in anadromous fish in seawater and in trout grown on in seawater have been reported (Evelyn, 1971a; Håstein and Bullock, 1976; Novotny, 1978). However, it has not been determined whether the disease outbreaks resulted from stress experienced by fish carrying a latent infection initially contracted in freshwater, or whether they represented a case of lateral transmission of the pathogen via seawater. Smith (1962), for example, had established that *Aer. salmonicida* survived in seawater for a prolonged period of time. It had also been demonstrated that *Aer. salmonicida* is capable of infecting sea and brown trout by contact with infected fish in sea and brackish waters (Scott, 1968). She found that the infection was transmitted between salinities of 2.54 and 3.31% (w/v) at water temperatures ranging from 5.6 to 14.5°C. Smith *et al.* (1982) reported on mortalities of Atlantic salmon from two marine fish farms in Ireland, presenting evidence for the lateral transmission of *Aer. salmonicida* in seawater to a group of fish not known to be carriers. They also provided data suggesting that, subsequent to the stocking in Spring 1978 and removal of carrier fish in Summer 1979 at a marine fish farm, the pathogen became established and persisted in the fish farm environment for at least 6 months after the removal of the carrier fish. Thus, a carrier-free population placed on the site in the Spring of 1980 was infected. Unfortunately, it was not determined whether the pathogen persisted in feral fish outside the cages or in the sediments under the cages. To lend support to a seawater transmission of furunculosis, Evelyn (1971a) has documented isolation of *Aer. salmonicida* from a strictly marine host, the sable fish, although probably the route of infection was by ingestion of moribund or dead salmonid carrier fish (Klontz and Wood, 1972). Obviously, *Aer. salmonicida* has wider potential for causing disease problems than has been hitherto suspected. To study the epizootiology of *Aer. salmonicida* diseases in aquatic environments other than freshwater, again, as in so many other aspects of the pathogen, demands additional attention to unravel its complexities.

Aeromonas salmonicida—the summary

In summary, although substantial efforts have been made to reach an understanding of the natural disease cycle in the environment, there still remain many issues to be

resolved. Some of the data provide partial answers, some are contradictory. Lack of appropriate methodology appears to constitute a major hindrance to progress. In the face of the several unresolved questions concerning *Aer. salmonicida* epizootiology, McCarthy (1980) has valiantly put forth several hypotheses aimed at deriving an overall view of *Aer. salmonicida* epizootiology. The first of these is that furunculosis is introduced to a fish farm by importation of healthy carrier fish or that non-carrier resident fish may be infected from a water supply contaminated by wild or imported carrier fish. Accordingly, if contact with carriers of *Aer. salmonicida* can be prevented, furunculosis should not arise in the population. The second hypothesis McCarthy (1980) advanced is that the mechanisms by which carriers disseminate the disease to non-carriers is dependent upon environmental parameters at a given fish farm site. He postulated that if stressful conditions are absent, resident fish may become carriers without developing clinical signs of disease, and that under such conditions a high carrier rate could be maintained without clinical evidence of furunculosis. This, in the opinion of other workers, would be an arguable premise, as some believe all carriers will eventually succumb to the disease. It is reasonable to suppose that once carrier fish are present on a fish farm they are responsible for initiation of epizootics, although infection also occurs by the water-borne route. The third hypothesis is that the bacterium in the carrier state provides a measure of protection to the fish in return for shelter from the ravages of the aquatic environment. Were this the case, the observation by McCarthy (1980) that non-carrier and carrier fish exhibit marked differences in their susceptibility to furunculosis would be explained.

Finally, it is our opinion that, while a firm understanding of the interactions of the pathogen in its milieu and in its fish host is lacking, fisheries scientists will remain at a serious disadvantage when tackling the problems of infectious diseases of *Aer. salmonicida* aetiology that currently beset fish cultivation.

Aeromonas sobria

It may be assumed that the natural reservoir is eutrophic freshwater.

Alteromonadaceae representative

Shewanella putrefaciens

It is assumed that the organism was derived from the coastal marine environment. Possibly, the organism comprises part of the normal microflora of fish (see Lee *et al.*, 1977; Gillespie, 1981).

Enterobacteriaceae representatives

Citrobacter freundii

The organism is common in eutrophic freshwater, from which spread to fish is possible (Allen *et al.*, 1983b). In addition, the organism was widespread in the seawater in a Japanese aquarium (Sato *et al.*, 1982).

Edwardsiella ictaluri

The natural reservoir for infection has not yet been firmly established. It is known that the organism survives for limited periods, i.e. up to 8 days, in sterile pond water (Hawke, 1979). This suggests that *Edw. ictaluri* has only limited ability to survive in the aquatic environment. A carrier state in channel catfish has been documented (Klesius, 1992). Also, data have shown that cells can pass from dead fish to non-infected channel catfish (Klesius, 1994). Of course, the results of such laboratory-based survival experiments need to be treated cautiously. It is conceivable that the organism comprises part of the normal microflora of fish, lurking perhaps in the digestive tract.

Edwardsiella tarda

The precise source of infection is often unknown. However, between 2002 and 2004, *Edw. tarda* was recovered from seawater and the kidney and spleen of healthy Japanese flounder when disease was occurring in farmed fish populations. It was apparent that the antibody titre increased rapidly in the run-up to outbreaks of clinical disease. Interestingly, bacteriophage was found in the seawater at least one month before the onset of and during the period of the disease outbreaks, but not afterwards. It was speculated that the presence of bacteriophage could be used as an indicator for *Edw. tarda* cells (Matsuoka and Nakai, 2004). Also, there has been speculation that snakes or faecal contamination from humans or other animals may have been involved in the first documented outbreak (Meyer and Bullock, 1973). Certainly, experimentally infected fish (= Japanese flounder) were determined to shed the pathogen 1–6 days before death, with the number of bacterial cells shed from newly dead (and for several days afterwards) fish reaching 10^7–10^8 CFU/min. Of relevance, these cells were more virulent than their counterparts from TSA. Such bacteria, which are discharged from diseased/dead fish, may well have importance in the spread of disease among fish populations (Matsuoka, 2004). Environmental parameters, namely water temperature and the quantity of organic matter in the water, undoubtedly influence the severity of outbreaks. In particular, it is noteworthy that most disease outbreaks occur at high water temperature, i.e. 30°C, when high levels of organic matter are present. However, data showed that the incidence of disease in catfish ponds rarely exceeded 5%, except when infected fish were moved to holding tanks. During these periods, edwardsiellosis became rampant with total mortalities approaching 50% of the population (Meyer and Bullock, 1973).

There is some evidence that *Edw. tarda* occurs in sediment (Rashid *et al.*, 1994a, b) and water within the vicinity of fish farms (Minagawa *et al.*, 1983; Park *et al.*, 1983; Rashid *et al.*, 1994a, b). It has been reported to inhabit aquatic invertebrates, such as the freshwater aquarium snail (*Ampullaria* sp.; Bartlett and Trust, 1976) and sea-urchin (Sasaki and Aita, 1975), and has been associated with vertebrates, namely snakes (Iverson, 1971), frogs (Sharma *et al.*, 1974), turtles (Otis and Behler, 1973), gulls (Berg and Anderson, 1972) and human beings (Koshi and Lalitha, 1976). Any one of these hosts may serve as a reservoir for infections of fish. However, it is unclear whether *Edw. tarda* should be regarded as a primary or

opportunistic pathogen of fish. Indeed, it may comprise part of the normal microflora of fish surfaces, insofar as Wyatt *et al.* (1979) have indicated the widespread occurrence of the micro-organism in and around channel catfish. A suggestion has been made that the organism becomes non-culturable in the aquatic environment (Sakai *et al.*, 1994)

The presence of sub-lethal concentrations of copper (100–250 μg/l) in water leads to increased susceptibility of Japanese eels to infection (Mushiake *et al.*, 1984). This increased susceptibility seems likely to be attributed to a reduction in lymphocytes and granulocytes in the blood, leading to lowered phagocytosis (Mushiake *et al.*, 1985).

Pantoea agglomerans

The source of the pathogen was unknown (Hansen *et al.*, 1990).

Plesiomonas shigelloides

The outbreak in 1984 affected mostly 1–2-year-old fish, in which the total mortality approximated 40% of the population. There was a correlation with water temperature, which increased in June from 10 to 17°C. Moreover, there was a pronounced increase in organic matter from food within the water. These factors would undoubtedly contribute to stress in the fish. It has been noted that *P. shigelloides* may be normally resident in the gastro-intestinal tract of warm-water fish (Vandepitte *et al.*, 1980), from where it could serve as a reservoir of infection.

Providencia rettgeri

Inference was made that the source of infection was from poultry faeces, which were used extensively to fertilise the carp ponds (Bejerano *et al.*, 1979). In this connection, it is relevant to note that *Pr. rettgeri* has been associated with the digestive tract of poultry.

Salmonella enterica subsp. *arizonae*

Presumably, the pathogen had been derived from the aquarium water or from fish (carriers) (Kodama *et al.*, 1987).

Serratia liquefaciens

The natural reservoir of the organism was probably polluted waters (McIntosh and Austin, 1990b).

Serratia marcescens

Seemingly, the organism may be associated with fish, and possibly comprise part of the "normal" microflora of eutrophic waters (Baya *et al.*, 1992c).

Serratia plymuthica

Possibly, the pathogen comprises a component of the freshwater bacterial community, particularly where pollution with organic material is rife (Austin and Stobie, 1992b).

Yersinia intermedia

The organism has been associated with water and the intestines of apparently healthy fish (e.g. Kapperud, 1981; Shayegani *et al.*, 1986; Zamora and Enriquez, 1987), which are likely to be the source.

Yersinia ruckeri

In rainbow trout, ERM most commonly affects fish of approximately 7.5 cm in length. The disease is less severe but more chronic in larger fish, i.e. of 12.5 cm in length. Severity peaks with a water temperature of 15–18°C, and decreases when it drops to 10°C or below. Overly fat or debilitated (stressed) fish are thought to be more susceptible to severe epizootics (Rucker, 1966). Moreover, occurrence of ERM may be cyclic, suggesting the presence of asymptomatic carrier fish in the population (Busch and Lingg, 1975). Such fish would be capable of shedding the organism in the faeces. Alternatively, it may be argued that the pathogen is present normally in the water and/or sediment (Rucker, 1966). From the results of some fascinating experiments, Hunter *et al.* (1980) demonstrated that unstressed carrier fish did not transmit *Y. ruckeri* to recipient fish. In fact, it was necessary to stress these carriers, such as by heat (i.e. a water temperature of 25°C), in order to enable release and thus transmission of the pathogen to other fish. Even under these conditions, colonisation of the lower intestine took place in the recipient fish without any mortalities. However, the handling of fish, which may appear to be extremely healthy, and crowded conditions causing excess ammonia and metabolic waste products in the water and, thus, a decrease in oxygen levels, may precipitate outbreaks of clinical disease (Bullock and Snieszko, 1975). Nevertheless, outbreaks generally occur only after the fish have been exposed to large numbers of the pathogen (Ross *et al.*, 1966). Mortalities tend to start 5–19 days after exposure, depending on the size of the inoculum, and last for 30–60 days (Rucker, 1966; Busch, 1973). Cumulative losses due to ERM may account for as much as 30–35% of the rainbow trout population (Klontz and Huddleston, 1976).

There is controversy over some aspects of the ecology of *Y. ruckeri*, insofar as the organism has been alternatively considered as well-adapted as a normal aquatic saprophyte (McDaniel, 1972) and, second, as not capable of free-living for any extended period in water (Klontz and Huddleston, 1976). Nevertheless, these authors conceded that survival for up to 2 months was possible in mud. Further experiments indicated that the pathogen could survive for 4 months in unsupplemented water at a salinity of 0–20‰; survival being reduced in seawater (salinity = 35‰) (Thorsen *et al.*, 1992). Romalde *et al.* (1994) reported survival for 3 months. Therefore, the implication is that *Y. ruckeri* is capable of surviving in the (fresh) water column for a long

time after an outbreak of disease has occurred. Survival may be influenced by the ability of virulent cultures to adhere to surfaces, a characteristic linked to the presence of flagella. Interestingly, oxolinic acid was better able to inactivate planktonic rather than attached cells of the pathogen (Coquet *et al.*, 2002). Perhaps, the organism is a normal inhabitant of the digestive tract of fish or in contaminated waters. In this respect, *Y. ruckeri* has been isolated from sewage sludge (Dudley *et al.*, 1980) and a bacteriophage specific for *Y. ruckeri* found in sewage (Stevenson and Airdrie, 1984b). Moreover, aquatic invertebrates, notably crayfish, and even terrestrial mammals, namely muskrats (Stevenson and Daly, 1982), may harbour large numbers of the pathogen, thereby serving as a reservoir of infection. It is noteworthy that McArdle and Dooley-Martin (1985) recovered *Y. ruckeri* from the digestive tract of an apparently healthy goldfish following inspection of a consignment upon importation into Ireland. The isolate was subsequently demonstrated to be pathogenic for rainbow trout. Thus, it would appear that ornamental fish could pose a reservoir of infection into salmonids. If Dulin *et al.* (1976) are correct in asserting that *Y. ruckeri* is not ubiquitous in nature, it is puzzling why ERM should be found in remote fish stocks, without prior history or association with the disease (Janeke, pers. commun.). Under these conditions, it is doubtful if movement of infected fish could account for the spread of ERM. Yet, isolations have been made from wild salmonids. In one example, *Y. ruckeri* type I was recovered from a mature, wild Atlantic salmon in freshwater (in Scotland) (Petrie *et al.*, 1996). Whether or not this suggests a natural reservoir of the pathogen in wild fish or reflects a transfer from aquaculture remains to be established.

There is some evidence that *Y. ruckeri* may well be capable of surviving in the environment in a form (the so-called dormant or non-culturable state) that is not readily culturable on conventional media (Romalde *et al.*, 1994). These workers found that the number of culturable cells increased for the first 15 days after *Y. ruckeri* was seeded into an experimental system. Thereafter, a decline in numbers was recorded over a 100 day period. Culturable cells persisted in sediment more so than water at 6 and 18°C. Yet, by means of acridine orange staining and fluorescence microscopy, a so-called dormant state was considered to develop. There were slight changes in the LPS of the dormant cells, but not in membrane proteins or plasmid composition. Interestingly, virulence was maintained during the period of non-culturability. Unlike the situation with *Aer. salmonicida*, cells were resuscitated to a culturable state following the addition of nutrients (Romalde *et al.*, 1994).

A serotyping scheme, based on O-antigens, has been proposed (Davies, 1990), and may have value in epizootiological investigations.

Flavobacteriaceae representatives

The precise reservoir of most of these fish pathogens remains unclear. Yellow and orange-pigmented bacteria occur in large numbers in freshwater (Allen *et al.*, 1983b) and seawater (Austin, 1982a), and comprise part of the normal microflora of gills of healthy salmonids (Trust, 1975) and possibly even eggs (Hansen *et al.*, 1992). Therefore, it seems likely that the taxa comprising the fish pathogens occur naturally in the

aquatic environment. Rucker *et al.* (1953) isolated highly virulent strains of *Fla. columnare*, which were capable of killing fish within 24 h, from the water in the upper Columbia River basin. Furthermore, it has been demonstrated that large numbers of *Fla. columnare* cells are present in water during epizootics (McCarthy, 1975c), with good survival occurring over a wide range of pH and hardness values (Fijan, 1968). Moreover, in one study, Collins (1970) reported a relationship between eutrophication and the numbers of *Fla. columnare* cells in lake water. Although the precise reservoir of *Fla. psychrophilum* has not been established, we have recovered some organisms, with the key characteristics of *Fla. psychrophilum*, from freshwater in England. However, it is uncertain whether or not the organisms were native, or merely transient, in the aquatic environment. *Fla. psychrophilum* was isolated from the internal organs (spleen) and/or sexual products (gonad, but not the eggs, at the eyed stage) in 7/50 (= 14.0%) Baltic salmon (*Salmo salar*) brood fish sampled at capture and 63/272 (= 23.2%) of fish examined at stripping. Also, the pathogen was recovered from the spleen or gonads in 2/19 (= 10.5%) of the fish with abnormal wiggling behaviour. Overall, the possibility exists that brood fish could transfer the pathogen to their progeny (Ekman *et al.*, 1999). There is other evidence that *Fla. psychrophilum* may be transmitted within salmonid eggs (Brown *et al.*, 1997), possibly entering during water hardening (Kumagai *et al.*, 2000). This same team reported the presence of the organism on the surface of, but not inside, ayu eggs after surface sterilization with 5 mg/l of povidone–iodine for 10 min or 150 mg/l hydrogen peroxide for 30 min (Kumagai *et al.*, 2004a). Also, the organism has been found in wild fish, namely perch and roach, from the ovarian fluids and milt of rainbow trout broodstock, and from waters (in close proximity to farmed trout or their eggs) in rainbow trout farms in Denmark (Madetoja *et al.*, 2002; Madsen *et al.*, 2005), in the eggs, gill or kidney tissue of wild ayu and other feral fish in Japan (Amita *et al.*, 2000) and from water in Finnish trout farms as determined by nested PCR and iFAT, but not culturing which undoubtedly lacked sensitivity (Madetoja and Wiklund, 2002). More than likely, wild fish and broodstock are a possible source of infection.

Laboratory-based studies using sterilised freshwater have shown that *Fla. psychrophilum* has the capacity to survive for 300 days in freshwater, but in 30‰ sodium chloride culturability was <1 day (Madetoja *et al.*, 2003). However, in the absence of a normal microflora, the relevance of the data to explaining survival of the pathogen in the natural environment is debatable. Survival studies revealed that the organism stopped multiplying, but the numbers remained fairly constant, in stream water for 16 weeks, and then declined. The cells became smaller and more rounded. Culturable cells could not be recovered on *Cytophaga* agar after 19 weeks, but resuscitation in *Cytophaga* broth was possible for up to 36 weeks. In distilled water, culturability was lost after 1 h (Vatsos *et al.*, 2003). The pathogen has been detected on algae growing on the surface of stones in rivers that contain diseased (with *Fla. psychrophilum*) fish (Amita *et al.*, 2000).

Colonisation of fish may be a forerunner for the development of disease. For example, colonisation of eyed rainbow trout ova with *Fla. psychrophilum* has been considered to lead to the development of RTFS (Rangdale *et al.*, 1997b). Using a

modified Anacker and Ordal's medium, the pathogen was recovered from ovarian fluid of 2/15 fish egg surfaces 14 days after fertilisation, but not from milt (Rangdale *et al.*, 1996).

Ribotyping and plasmid-profiling appear to be useful for epizootiology. In particular, several ribotypes have been recognised among 85 isolates, and determined to be associated with the fish species from which the cultures were obtained (Chakroun *et al.*, 1998).

There is no dispute that these organisms are capable of causing severe losses to fish populations. Cold-water disease may cause losses of up to 50% of the fish population, as determined for coho salmon fry (Rucker *et al.*, 1953). This disease is especially troublesome at low water temperatures (Holt *et al.*, 1989), i.e. $\leq 15^{\circ}C$.

T. maritimum may also be responsible for heavy losses, i.e. 20–30% of the population, among juvenile red sea bream and black sea bream (Hikida *et al.*, 1979). In common with many organisms, extended survival of *T. maritimum* occurs in sterile, but not in natural, seawater (~5 days), i.e. with a resident microflora (Avendaño-Herrera *et al.*, 2006). *Fla. branchiophilum* may also be fatal to small fish following infection via water, i.e. adding a bacterial suspension to tank water (Wakabayashi *et al.*, 1980). Certainly, the pathogen is present in water at times of disease outbreaks (Heo *et al.*, 1990).

Concerning columnaris, the consensus of opinion is that the disease is problematic only in the warmer periods of the year. Generally, epizootics occur when the water temperature is in the region of 18–22°C, and the disease is rarely troublesome at $<15^{\circ}C$ (Amend, 1970). Thus, most outbreaks of columnaris occur between May and October (Bowser, 1973; Kuo *et al.*, 1981). To illustrate the dramatic effects of water temperature on the level of mortalities, the investigation of Holt *et al.* (1975) is especially relevant. This team challenged steelhead trout, chinook salmon and coho salmon with *Fla. columnare*, via the water-borne route. At a water temperature of 9.4°C there were no mortalities attributable to columnaris. By increasing the temperature to 12.2°C, 4–20% mortalities ensued; whereas at 20.5°C all the steelhead trout and coho salmon died, together with 70% of the chinook salmon.

The level of mortality may be extremely high, and figures of 60–90% are not uncommon. For example, columnaris was considered to be the most important contributing factor for 72.3–97.6% and 75.4–95.4% mortality among populations of adult sockeye salmon and adult chinook salmon, respectively (Fish and Hanavan, 1948). Similarly, Chen *et al.* (1982) reported 77% and 88.3% losses among groups of carp and goldfish, respectively. Certainly, there are good data demonstrating the seasonal effects of mortalities due to columnaris, insofar as Bowser (1973) reported 60% infection of bullheads in mid-May whereas only a few months later the incidence had dropped to only 10%.

In addition to water temperature, the severity of columnaris is influenced by a multiplicity of environmental (stress) and host-related factors. Chen *et al.* (1982) described the highest eel mortality levels to be associated with stagnant water, whereas the lowest losses occurred in running water. Interestingly, with aeration, the total losses fell between these two extremes. In this respect, the mortality rate has been inversely correlated with the level of dissolved oxygen in the water. Moreover,

with adequate dissolved oxygen, deaths increased with a concomitant rise in the level of ammonia.

The susceptibility of juvenile chinook salmon and rainbow trout has been correlated with the age of the fish and the level of crowding, as well as water temperature (Fujihara *et al.*, 1971). These workers concluded that rainbow trout (1 g average weight) and chinook salmon (average weight = 3 g) were less susceptible to the rigours of columnaris than smaller fish. Therefore, it was concluded that age is more important than weight in determining susceptibility to infection.

Chrys. scophthalmum was found in the water, from which it is surmised that spread to turbot occurred (Mudarris and Austin, 1989).

Halomonadaceae representative

Halomonas (= Deleya) cupida

H. cupida is a waterborne organism (Baumann *et al.*, 1972). Therefore, the source of infection was undoubtedly the marine environment.

Moraxellaceae representatives

Acinetobacter sp.

Unreactive Gram-negative rods that are difficult to identify and which resemble *Acinetobacter* are common inhabitants of freshwater (Allen *et al.*, 1983b) and marine ecosystems (Austin, 1982a). In particular, the organisms populate the skin and gills (Horsley, 1973) and digestive tract (Shewan, 1961; Trust and Sparrow, 1974; Roald, 1977) of salmonids. Therefore, a ready inoculum of cells is likely to be in continual contact with fish. Conceivably, any break in the integument of the host may lead to colonisation of the nutrient-rich tissues by components of the water-borne or, indeed, fish microflora. This may lead to the start of a disease cycle.

Moraxella sp.

It may be assumed that the organism constitutes part of the aquatic microflora.

Mycoplasmataceae representative

Mycoplasma mobile

Mycoplasma-like bacteria occur on fish (Kirchhoff and Rosengarten, 1984), from which infection probably occurs.

Oxalobacteraceae representative

Janthinobacterium lividum

Typical and atypical forms of *J. lividum* are regarded as part of the normal microflora of freshwater (Sneath, 1984) and soil (Moss and Ryall, 1980). Therefore, there would be a ready inoculum of the pathogen in the environment around fish.

Pasteurellaceae representative

Pasteurella skyensis

The source of the pathogen may well have been fish, e.g. mackerel (Birkbeck *et al.*, 2002).

Photobacteriaceae representatives

Photobacterium damselae subsp. *damselae*

The organism was associated initially with ulcerative lesions along the flank of blacksmith (*Chromis punctipinnis*), one of the damselfish. These ulcers were noted in Summer and Autumn among fish populations in the coastal waters of southern California. Surveys of wild fish populations led to a conclusion that the ulcers were restricted to species of damselfish. Additional information pointed to a role in human pathogenicity, insofar as the organism has been isolated from human wounds (Love *et al.*, 1981). Subsequent work demonstrated this organism in sharks (Grimes *et al.*, 1984a,b; Fujioka *et al.*, 1988), turbot (Fouz *et al.*, 1991, 1992), yellowtail (Sakata *et al.*, 1989) and nurse sharks and rainbow trout (in Denmark) (Pedersen *et al.*, 1997a).

Ulcerated fish, presumably the result of *Ph. damselae*, accounted for 10–70% of the population in King Harbor, Redondo Beach, California, during August to October, and at a second site (Ship Rock, Catalina Island) during June to October. This suggests a seasonal distribution in the incidence of disease, and possibly coincides with warmer water temperatures and lower resistance caused by physiological changes in the host during sexual maturity. Conceivably, *Ph. damselae* occurs normally in the marine environment, where it could pose a constant threat to susceptible fish species. Indeed, seawater is the likely mode of transmission of virulent cells of the pathogen, with cells adhering to and resisting the bacteriocidal effects of mucus. This suggests that skin is a site of entry into the host (Fouz *et al.*, 2000).

Photobacterium damselae subsp. *piscicida*

There is no doubt that pasteurellosis is a serious condition of both farmed and wild fish populations (Snieszko *et al.*, 1964a; Kusuda and Yamaoka, 1972; Ohnishi *et al.*, 1982; Yasunaga *et al.*, 1983). Heavy mortalities, in the range of 40–50% of the stock, have occurred during Summer months. Unfortunately, the reasons for these outbreaks are largely unknown. Likewise, the precise nature of the pathogenicity mechanism remains to be elucidated.

It is thought that infection takes place in seawater at temperatures of approximately 25°C (Yasunaga *et al.*, 1983). Toranzo *et al.* (1982) devised a series of survival experiments, and concluded that *Ph. damselae* subsp. *piscicida* was short-lived in freshwater and estuarine conditions. Thus, in freshwater the organism could not be cultured after 48 h at 20°C. Survival in estuarine water (salinity = 12‰) was slightly longer, i.e. 4–5 days. These results support the earlier findings of Janssen and Surgalla (1968) that the organism does not appear to survive well away from fish. However, there is some evidence that has demonstrated the discharge of viable cells from experimentally infected yellowtail for 1–2 days before death (Matsuoka and Kamada, 1995). The survival of starved cells in seawater needs to be clarified (Magariños *et al.*, 1997a). Furthermore, it was speculated that transmission of the disease is likely to be fish to fish. Although this reasoning is reminiscent of the debate about the spread of furunculosis, it should not be overlooked that *Ph. damselae* subsp. *piscicida* may survive in water, albeit in a non-culturable, dormant or altered form. Indeed, a view was expressed that the pathogen may well exhibit a dormant phase (Magariños *et al.*, 1994a). Plate count data revealed that *Ph. damselae* subsp. *piscicida* could survive in seawater and sediment for 6–12 days, with metabolism being reduced by 80%. Indeed, in terms of numbers, culturable cells persisted in sediment better than in seawater. However, when culture techniques inferred a reduction in bacterial numbers, microscopy using acridine orange suggested that the populations remain at 10^5 (Magariños *et al.*, 1994a).

A possibly useful tool for epizootiology is ribotyping, which has already successfully discriminated between European and Japanese isolates (Magariños *et al.*, 1997b).

Piscirickettsiaceae representative

Piscirickettsia salmonis

It is presumed that the pathogen is passed directly between fish, or via invertebrate vectors. Data have shown that physical contact may be necessary for horizontal transmission between salmonids (Almendras *et al.*, 1997). Certainly, the pathogen appears to survive extracellularly (Lannan and Fryer, 1994), and 16S rDNA product has been recovered from bacterioplankton DNA obtained from the coastal environment in the U.S.A. (Mauel and Fryer, 2001). Using competitive PCR, Heath *et al.* (2000) reported $3–4 \times 10^3$ cells or their DNA in surface seawater in a net pen in southern Chile.

Pseudomonadaceae representatives

Pseudomonas anguilliseptica

Red spot disease, also known as "Sekiten-byo", was first recognised in pond-cultured eels (*Anguilla japonica*) in Japan (Wakabayashi and Egusa, 1972). Since then, it has developed into one of the most destructive diseases of eels in Japan (Nakai and Muroga, 1979). During 1981, the disease was recognised in European eels

(*A. anguilla*) within Scotland (Nakai and Muroga, 1982; Ellis *et al.*, 1983; Stewart *et al.*, 1983). Conceivably, the disease is spreading, and this may reflect the natural migratory patterns of wild eel populations, or the rapid increase in intensive eel cultivation during the mid-1970s. A spread to other fish groups has now occurred. The pathogen was recovered from striped jack at water temperatures of 14–16°C during February to April, 1993 (Kusuda *et al.*, 1995), diseased rainbow trout, sea trout and whitefish (*Coregonus* sp.) in Finland during 1986–1991 (Wiklund and Lönnström, 1994), wild Baltic herring (*Clupea harengus membras*) (Lönnström *et al.*, 1994), sea bass, sea bream and turbot in France (Berthe *et al.*, 1995), and as a Winter disease outbreak in sea bream in Spain (Doménech *et al.*, 1997). However, in Finland there was some evidence also for the presence of *V. anguillarum* and *Aer. salmonicida.*

Very little has been published about the epizootiology of Sekiten-byo. The disease is recognised, however, to be prevalent in brackish water, when the water temperature is between 20 and 27°C (Muroga *et al.*, 1977b). Indeed, temperature is considered to be the major factor in influencing epizootics. All the available information points to the seriousness of outbreaks in terms of high mortalities. Thus, in one outbreak of the condition in Scotland, 67,000 elvers (96% of the total) plus 154 adult eels (3.9% of the total) died (Stewart *et al.*, 1983). These authors observed that generally the large adult eels fared worse than smaller adults. Nevertheless, the greatest losses occurred with elvers. It is interesting to note that the disease eliminated 14% of the total weight of the farm stock. This represents a significant investment; therefore, Sekiten-byo has the potential to be a severe economic problem.

Pseudomonas chlororaphis

Ps. chlororaphis occurs in water (Palleroni, 1984), which is assumed to be the reservoir of infection. No other information is available.

Pseudomonas fluorescens

In view of its widespread occurrence in the aquatic environment, *Ps. fluorescens* is likely to be spread through water, which will serve as the primary reservoir of infection. The disease is especially troublesome at low water temperatures, i.e. at approximately 1°C. In one experiment, Ahne *et al.* (1982) achieved 100% mortality in tench fry within 10 days at a water temperature of 10°C. Challenge was by i.p. injection of a bacterial suspension.

Ahne and co-workers noted that initial occurrence of disease, with 30% of the population developing skin haemorrhages, was 7 days after transfer of the tench to laboratory aquaria. Mortalities began on day 14, and by 2 weeks later 90% of the fish had died. It is noteworthy that the problem in silver carp and bighead developed after a stressful period during Winter, when the water temperature fluctuated around freezing. In this outbreak, the mortality rate was at 5% of the population per day (Csaba *et al.*, 1981b).

Pseudomonas plecoglossicida

Immersion leads to infection of ayu, with gills being the likely portal of entry as determined by real-time quantitative PCR. At 6 h after infection, the pathogen was located in the kidney, liver and spleen; septicaemia was apparent after 48 h when the organism was found in the blood (Sukenda and Wakabayashi, 2000).

Pseudomonas pseudoalcaligenes

Pseudomonads occur in polluted/eutrophic freshwater, which is considered to be the source of *Ps. pseudoalcaligenes* (Austin and Stobie, 1992b). Moreover, it was apparent that the fish farm waters received sewage from a neighbouring septic tank (B. Austin, unpublished data).

Vibrionaceae representatives

Vibrio alginolyticus

Vibrios abound in the marine and estuarine environments (see Kaneko and Colwell, 1974), and therefore present a constant threat for any susceptible host. In particular, *V. alginolyticus* has been recovered from the water in marine fish tanks (Gilmour, 1977).

Vibrio anguillarum

Vibriosis had gained considerable notoriety in mariculture, where it has become a major limiting factor in the successful rearing of salmonids (Mahnken, 1975). To cite one example, in Denmark the disease has resulted in cumulative losses of 30% among eel populations (Bruun and Heiburg, 1935). This represents a significant economic loss. An authoritative publication reported that vibriosis occurs in more than 14 countries, where it has ravaged approximately 48 species of marine fish. Vibriosis appears to have been confined initially in European waters. North America escaped the ravages of the disease until 1953 (Crosa *et al.*, 1977). Its arrival in Japan in 1975 may have resulted from the importation from France of contaminated eels (Muroga *et al.*, 1976a, b). Evidence is also accumulating that the disease may occur in freshwater conditions (Muroga, 1975; Ghittino and Andruetto, 1977). This suggests that vibriosis is an extremely widespread problem. Consequently, the literature abounds with reports of "new" isolations and titbits of gossip, which slowly contribute to an overall saga. However, it would appear that vibriosis is, in fact, a syndrome caused by a multiplicity of vibrios (see Schiewe, 1981). Here, emphasis will be placed on *V. anguillarum*.

The causal agent of "red-pest" in eels was first isolated by Canestrini (1893), who designated the organism as *Bacterium anguillarum*. A further case among eels in Sweden during 1907 was investigated by Bergman (1909), and it was directly attributable to this scientist that the name of *V. anguillarum* was coined.

V. anguillarum constitutes part of the normal microflora of the aquatic environment (e.g. West and Lee, 1982; Muroga *et al.*, 1986), particularly associated with

rotifers (Tatani *et al.*, 1985; Muroga and Yasunobu, 1987; Mizuki *et al.*, 2006), with maximal and minimal numbers in Summer and Winter, respectively (Larsen, 1982). Experiments have suggested that the pathogen survives for prolonged periods in seawater. Thus, Hoff (1989) reported survival for >50 months in a seawater microcosm. The organism may also constitute part of the normal microflora of marine fish (Oppenheimer, 1962; Mattheis, 1964). Some elegant work, albeit with only one isolate, has addressed the precise changes to the organism, i.e. starvation–stress responses, in the marine environment (Nelson *et al.*, 1997), where Na^+ is essential for starvation–survival (Fujiwara-Nagata and Eguchi, 2004). When starved of carbon, nitrogen and phosphorus, the number of CFUs (*note*: this is a dubious measure of viability) dropped rapidly over an initial 5–7 day period, and then gradually declined over 3–4 weeks. Some cells became small and spherical, corresponding to the notion of ultramicrobacteria (see Austin, 1988), whereas others elongated to short spirals. Protein synthesis, as measured by incorporation of [^{35}S]-methionine, declined during the first 6 h of starvation, and increased to >70% of the rate in exponentially growing cells by 5 days into the starvation regime (Nelson *et al.*, 1997).

The precise origin of an isolate has importance for epizootiology. In this respect, Olsen and Larsen (1990) detailed a seemingly useful method, namely restriction fragment length polymorphism of the 65–70 kb (kilobase) plasmid. This method should have value for epizootiological investigations.

The exact mode of infection is unclear, but undoubtedly involves colonisation of (attachment to) the host, and thence penetration of the tissues. Ransom (1978) postulated that infection probably begins with colonisation of the posterior gastro-intestinal tract and rectum. This conclusion resulted from the observation that *V. anguillarum* was seen initially in these sites. Horne and Baxendale (1983) reported adhesion of *V. anguillarum* to intestinal sections derived from rainbow trout. All regions of the intestine were colonised (approximately 10^3 cells/cm^2), with maximum attachment occuring within 100 min. The skin appears to become colonised within 12 h of immersion in a virulent culture (Kanno *et al.*, 1990). Then, invasion of the liver, spleen, muscle, gills and intestine follows (Muroga and De La Cruz, 1987).

It has been well documented that epizootics occur in the warm Summer months when water temperatures exceed 10°C, the water is depleted of dissolved oxygen, and the fish stressed by overcrowding and poor hygiene (Anderson and Conroy, 1970). There are exceptions to the norm insofar as outbreaks have been documented in freshwater (e.g. Rucker, 1959) and at low temperature, i.e. 1–4°C (Olafsen *et al.*, 1981). It is perhaps ironic that isolates recovered from rainbow trout in freshwater have an obvious salt requirement for growth (Rucker, 1959). Perhaps, the organisms were contained in a protected ecological niche, such as within the fish body, prior to the manifestation of the disease. However, it should be remembered that the pathogen has been recovered sporadically from freshwater (West and Lee, 1982). The determination of plasmid profiles may have value for epizootiological investigations (Wiik *et al.*, 1989).

The presence of heavy metals, notably copper and iron, contributes to an exacerbation of vibriosis. Yet, sublethal concentrations of chlorine do not appear to promote the development of infections (Hetrick *et al.*, 1984). Levels of only

30–60 μg copper/ml and 10 μg of iron/ml have caused severe problems (Rødsaether *et al.*, 1977; Nakai *et al.*, 1987). Further investigation demonstrated the susceptibility to vibriosis was dependent upon concentration and time of exposure to copper (Baker *et al.*, 1983). The debilitating effect has been attributed to coagulation in the mucus layer of the gills, and thus the inhibition of oxygen transport leading to respiratory stress (Westfall, 1945). The practical outcome from this information is that fish-holding facilities should not be coated with copper-containing, anti-fouling compounds, which could trigger vibriosis.

V. cholerae (non-O1)

V. cholerae survives in the aquatic environment. For example, at 25°C strain PS-7701 survived for 32 days in freshwater, saline Ringers buffer, and normal strength and diluted seawater. Survival was considerably reduced at 2°C (Yamanoi *et al.*, 1980), an observation which coincides with the findings of Singleton *et al.* (1982a, b). It is likely that infection occurs via the water-borne route, insofar as *V. cholerae* appears to inhabit the aquatic environment (Lee *et al.*, 1982; West and Lee, 1982).

Vibrio fischeri, V. furnissii, V. harveyi, V. ichthyoenteri and V. logei

It may be assumed that the source of the bacteria was seawater.

V. ordalii

V. ordalii appears to have a more restricted niche than *V. harveyi* and may be considered as a common water-borne organism. It has been postulated that infection (colonisation) begins in the rectum and posterior gastro-intestinal tract. Alternatively, its presence on skin suggests that entry may proceed by direct invasion of the integument (Ransom, 1978).

V. pelagius

This was not considered by Angulo *et al.* (1992).

V. salmonicida

It has been demonstrated that *V. salmonicida* survives for >14 months in laboratory-based experiments with seawater, when seeded at $\sim 10^6$ cells/ml (Hoff, 1989). Thus, there is the potential for long-term survival in the vicinity of fish farms, as confirmed by Husevåg *et al.* (1991). Moreover, the pathogen has been detected in the sediment (12–43 cells/ml) below fish farms, several months after an outbreak of Hitra disease. In addition, *V. salmonicida* has been detected in the sediments from fish farms which were not experiencing clinical disease (Enger *et al.*, 1989, 1991). Clearly, there will be a reservoir of the pathogen around farmed fish, from which further infections may occur.

V. splendidus

It seems likely that the organism is a component of the normal, aquatic, bacterial microflora, with survival of >114 days recorded (Lopez and Angulo, 1995).

V. vulnificus

V. vulnificus is ubiquitous in the coastal marine and estuarine environment, where it occurs routinely in low numbers (Oliver *et al.*, 1983), although serovar E (biotype 2) is regarded as being rare in natural waters, but extended survival occurs in sterile microcosms (Marco-Noales *et al.*, 2004). Populations of the pathogen are almost certainly controlled by grazing and microbial antagonism (Marco-Noales *et al.*, 2004). However, the reservoir is almost certainly the aquatic, especially seawater, environment (Høi *et al.*, 1998). It has been documented to survive in brackish water and on the surfaces of eels for 14 days (Amaro *et al.*, 1995). It is feasible that fish are constantly exposed to the potential vagaries of this organism. Moreover, it is capable of entering eels through the skin (Amaro *et al.*, 1995).

MISCELLANEOUS PATHOGEN

Causal agent of *Varracalbmi*

The source of the infection was unknown, but may well have been another cold-water marine fish (Valheim *et al.*, 2000).

9

Pathogenicity

Many publications about pathogenicity mechanisms have resulted from the examination of single isolates, often of questionable authenticity. The usefulness of such approaches to the understanding of pathogenicity of bacterial species is doubtful. Also, the value of studies involving bacterial subcellular components produced on agar plates or in broth cultures at explaining disease mechanisms *in situ* is unclear. Nevertheless, an interesting development concerns the potential role of quorum-sensing signal molecules (=acylated homoserine lactones [AHLs]) in the regulation of some virulence factors, with work revealing that AHLs are produced by some Gram-negative bacterial fish pathogens, notably *Aer. hydrophila*, *Aer. salmonicida*, *V. salmonicida*, *V. splendidus*, *V. vulnificus* and *Y. ruckeri*, but not in *Fla. psychrophilum*, *Moritella viscosa* or *Ph. damselae* (Bruhn *et al.*, 2005).

The pathogenicity of some bacterial fish pathogens has not been considered. Such organisms have not been included in this chapter.

ANAEROBES

Eubacteriaceae representative

Eubacterium tarantellae

Invasion of the body may occur through wounds or as a result of damage inflicted through parasites, weak pathogens or stress. Once inside the body tissues, further damage may be inflicted as a result of exo- or endotoxins. The organism produces haemolysins and lecithinase, which may harm the fish. Nevertheless, it should be emphasised that the precise pathogenicity mechanisms have yet to be elucidated (Udey *et al.*, 1976).

GRAM-POSITIVE BACTERIA—THE "LACTIC ACID" BACTERIA

Carnobacteriaceae representatives

Carnobacterium piscicola (and the lactobacilli)

Small-scale experiments with rainbow trout maintained in freshwater at 18°C have shown that death may result within 14 days of i.p. injection of 10^5 cells/fish. Dead and moribund fish had swollen kidneys, and ascitic fluid accumulated in the abdominal cavity. However, adverse effects were not recorded following injection of cell-free extracts. This suggests that exotoxins did not exert a significant role in pathogenicity. It remains for further work to elucidate the effect, if any, of endotoxins (Ross and Toth, 1974; Cone, 1982; Hiu *et al.*, 1984).

Enterococcaceae representatives

Laboratory infections with *Vag. salmoninarum* were achieved using a comparatively high dose of 1.8×10^6 cells/rainbow trout (Michel *et al.*, 1997).

Streptococcaceae representatives

Experimental infections with organisms likely to correspond with *Lactococcus garvieae* have been achieved by injection of 10^4 to 10^5 cells (Cook and Lofton, 1975), and by exposure of fish for 10 min to 10^6 bacteria (Robinson and Meyer, 1966). Thereafter, disease becomes established, and death ensues. Adherence of cells of *Lactococcus garvieae* to intestinal and brain gangliosides has been documented in yellowtail (Shima *et al.*, 2006). Some host specificity to Gram-positive cocci in chains exists, insofar as trout suffer heavy mortalities whereas Mozambique bream (*Sarotherodon mossambicus*), banded bream (*Tilapia sparramanii*), carp (*Cyprinus carpio*) and largemouth bass (*Microterus salmoides*) do not (Boomker *et al.*, 1979). It has been established that challenge with low-virulence isolates or low doses of high-virulence isolates together with cell-free culture supernatants are sufficient to establish infection (Kimura and Kusuda, 1979). The toxic activity of supernatants was further researched, and two fractions were demonstrated to have a significant effect on pathogenicity (Kimura and Kusuda, 1982). These were recovered in Todd–Hewitt broth after incubation at 30°C for 48 h. The fraction, although not toxic by oral administration (presumably the compounds were digested), produced damage, i.e. exophthalmia and petechial haemorrhages, following percutaneous injection of yellowtails. Co-infection of *Str. iniae* with aquabirnavirus has led to higher mortalities in Japanese flounder (Pakingking *et al.*, 2003).

Evidence has been presented that a cell capsule may be involved with the resistance of Gram-positive cocci in chains to opsonophagocytosis in yellowtail (Yoshida *et al.*, 1997). This view was reinforced by Miller and Neely (2005), who when using capsular mutants showed that the capsule was indeed important for the virulence of *Str. iniae*. Again, an effect on phagocytosis was reported. Similarly, capsules have been reported in *Lactococcus garvieae*, with encapsulated cultures being more

virulent (Barnes *et al.*, 2002) and less efficient at fixing complement compared with non-encapsulated isolates (Barnes and Ellis, 2004). Non-encapsulated cultures were more susceptible to normal rainbow trout serum than capsulated isolates (Barnes *et al.*, 2002). Two capsular types have been found among *Lactococcus garvieae*, one of which produces a well-developed capsule, whereas the second demonstrates a micro-capsule which contains fimbrial-type components projecting from the cell surface (Ooyama *et al.*, 2002). Also, polysaccharide capsules have been found on *Str. iniae* (Barnes *et al.*, 2003). The pathogen produces a cytolysin with haemolytic traits, which is a functional homologue of streptolysin S. Expression of this cytolysin is necessary for local tissue necrosis, but not to bacteraemia (Fuller *et al.*, 2002). When grown in serum, this streptococcus expresses surface factors that are capable of binding to trout immunoglobulin by the Fc region (=crystallisable fragment of the immuno-globulin) (Barnes *et al.*, 2003a). A range of isolates from fish, a dolphin and humans produced apoptosis and/or necrosis in tilapia non-specific cytotoxic cells and tilapia-continuous cell lines (Taylor *et al.*, 2001). Only serotype II strains entered, multiplied and survived in pronephros phagocytes (leading to apoptosis) for >48 h. This is relevant because it was estimated that ~70% of the bacteria contained in blood during sepsis were located within phagocytes, which suggests a preferred intracellular existence (Zlotkin *et al.*, 2003).

When administered i.m. at doses of just over 10^6 cells/fish, *Str. dysgalactiae* led to clinical disease resembling that of naturally infected fish (Nomoto *et al.*, 2004).

An isolate of *Str. milleri* (G3K) injected at 5×10^6 cells/fish caused 20% mortalities in Atlantic salmon. Interestingly, all the fish darkened, albeit with negligible signs of internal or external abnormalities. With rainbow trout, there was evidence of kidney liquefaction (Austin and Robertson, 1993).

Str. parauberis were examined for the presence of putative surface-associated virulence factors relevant to turbot for which the data indicated haemagglutination activity (against turbot erythrocytes), variable hydrophobicity due possibly to the presence of capsular material, and the ability to adhere to and invade cultured cells, e.g. CHSE-214 (Chinook salmon embryo) and SBL (striped bass larvae) cell lines (Romalde *et al.*, 2000).

AEROBIC GRAM-POSITIVE RODS AND COCCI

Renibacterium salmoninarum

Pathogenicity experiments have met with varying degrees of success. Mackie *et al.* (1933, 1935) succeeded in transmitting "Dee disease" to brown trout by subcutaneous and i.m. injections of emulsified spleen from Atlantic salmon. In these experiments, death followed in 5 weeks, although typical lesions, as found in field situations, did not occur. A similar observation was made by Belding and Merrill (1935) who injected, intramuscularly, brook trout with purulent material collected from kidney abscesses in the same species. Death followed in 18 to 25 days, but characteristic BKD lesions did not occur. This was, however, achieved by Earp (1950) following the

injection of chinook salmon with a pure culture of the BKD organism. Koch's postulates were finally satisfied by Ordal and Earp (1956) following the establishment of BKD in chinook salmon after i.p. injection of an organism obtained from sockeye salmon. Mortalities started after 12 days, and continued until day 23, when all the fish were dead. At this point, the organism was re-isolated. Sakai *et al.* (1989c) found mortalities began 17 days after rainbow trout were injected with 4×10^8 cells. In comparison, carp (*Cyprinus carpio*) were markedly resistant. Failure greeted the attempt by Snieszko and Griffin (1955) to transmit BKD to brook trout by co-habiting with diseased fish for 21 days, followed by feeding with infected viscera. However, using feeding, success was achieved by Wood and Wallis (1955) with 100% infection of 993 chinook salmon fingerlings. Later, Wolf and Dunbar (1959) achieved success by immersing experimentally wounded brook trout into a suspension of the pathogen. Murray *et al.* (1992) succeeded in inducing BKD in chinook salmon by immersion (10^4–10^6 cells/ml for 15–30 min) and co-habitation with other experimentally infected fish. However, the time to death was much longer than in most experimental models. By co-habitation and immersion, the average periods leading to mortalities were 145 and 203 days, respectively. Transmission from wild to cultured fish has been reported (Mitchum and Sherman, 1981) and *vice versa* (Frantsi *et al.*, 1975). Prior infection with *Ren. salmoninarum* may well contribute to the poor survival of coho salmon upon transfer from fresh to seawater (Moles, 1997).

Evidence has pointed to the ability of *Ren. salmoninarum* becoming internalised within non-phagocytic cells (González *et al.*, 1999) and macrophages in which putative virulence factors are produced (McIntosh *et al.*, 1997). Fish cell lines coupled with iFAT were used to study the internalisation of the pathogen with results revealing that *Ren. salmoninarum* became localised in the vacuoles of CHSE-214 and RTG-2 cells with some escape into the cytoplasm (González *et al.*, 1999). Within the phagocytic cells, *Renibacterium* exhibits a slow rate of division, and survives certainly for 10 or more days (Gutenberger *et al.*, 1997). Conversely, the macrophages may well inhibit the growth of and kill *Renibacterium* by the live bacterial cells generating respiratory burst products (Hardie *et al.*, 1996; Campos-Pérez *et al.*, 1997). With this scenario, exposure to *Ren. salmoninarum* would enhance the killing activity of the macrophages (Hardie *et al.*, 1996).

The hydrophobic, soluble cell surface p57 protein, which is released in large quantities as a monomer into the external environment from broth cutures and in infected fish (Wiens *et al.*, 1999), is responsible for cell agglutination, e.g. of salmonid leucocytes (Senson and Stevenson, 1999; Wiens *et al.*, 1999), and is encoded by *msa* (= major soluble antigen) genes—*msa1* and *msa2* and *msa3* (this is a duplicate of *msa1*, but is not present in all isolates of *Ren. salmoninarum*; Rhodes *et al.*, 2002, 2004), both *msa1* and *msa2* of which are needed for complete virulence (Coady *et al.*, 2006)—and is produced in comparatively large amounts and consequently has been a target for vaccine development. The role of p57 protein in the pathogenicity process has prompted some excellent research. Incubation of *Ren. salmoninarum* at 37°C for >4 h decreased cell surface hydrophobicity (this decrease was negated by pre-incubation in PMSF), as measured by salt aggregation, and decreased the quantity of cell-associated p57 protein (Piganelli *et al.*, 1999). Cell surface hydrophobicity was

re-instigated following incubation in ECP, reflecting re-association of the p57 protein onto the bacterial cell surface (Piganelli *et al.*, 1999). An attenuated culture, MT 239, differs from virulent isolates in expressing less p57 protein (O'Farrell and Strom, 1999). It has been demonstrated that a Norwegian isolate, strain 684, lacked a specific epitope (designated 4C11) and contained single alanine to glutamine substitution in the amino terminal region, which resulted in enhanced binding to leucocytes from Chinook salmon (Wiens *et al.*, 2002).

There is a divergent opinion as to the presence of biological activity in ECP of *Ren. salmoninarum*. One view is that the ECP is generally devoid of extracellular enzymes, haemolytic and cytolytic activity being absent (Bandín *et al.*, 1991a). Yet, in other investigations proteases (Sakai *et al.*, 1989c) and haemolysins (Grayson *et al.*, 1995a, 2001) have been detected. ECP at 0.1 mg/ml and 1.0 mg/ml inhibited respiratory burst, but not phagocytic activity in brook trout splenic phagocytes (Densmore *et al.*, 1998). Hydrophobicity, haemagglutination and haemolysin activity to rabbit and trout erythrocytes have been recorded from water-soluble extracts (proteins) (Bandín *et al.*, 1989; Daly and Stevenson, 1987, 1990; Evenden *et al.*, 1990). In particular, hydrophobicity and auto-aggregation have been linked with virulence (Bruno, 1988). *Ren. salmoninarum* has agglutinated spermatozoa from salmonids and goldfish (Daly and Stevenson, 1989). Shieh (pers. commun.) reported an unidentified toxin from *Renibacterium*, which was lethal to fingerling Atlantic salmon. Also, an iron acquisition mechanism has been found (Grayson *et al.*, 1995b).

There is some evidence that fish respond to infection with *Renibacterium* by the production of stress factors, including plasma cortisol and lactate, and reduced levels of plasma glucose (Mesa *et al.*, 1999). Thus, a 70 kDa stress protein (HSP70) was recognised in coho salmon with BKD (Forsyth *et al.*, 1997).

Bacillaceae representatives

Bacillus sp.

Oladosu *et al.* 1994) infected *Clarias gariepinus* via the oral and subcutaneous routes with a comparatively low dose of 0.5 ml, which contained 1.8×10^3 cells/ml. Thus, 60% and 30% mortalities were achieved over a 3 week period by oral and subcutaneous challenge, respectively.

Ferguson *et al.* (2001) reported that 2×10^7 cells of the putative *Bacillus* injected intraperitoneally led to clinical disease.

Bacillus mycoides

Injection of 1.6×10^4 cells intramuscularly led to lesions in channel catfish, as described in the original outbreak (Goodwin *et al.*, 1994). Intraperitoneal and subcutaneous injections did not lead to the development of any lesions in the infected fish.

Corynebacteriaceae representative

Corynebacterium aquaticum

The fish isolate, RB 968 BA, killed rainbow trout and striped bass, with LD_{50} doses calculated as 5.8×10^4 and 1.0×10^5, respectively (Baya *et al.*, 1992a). Experimentally infected fish developed haemorrhaging in the cranial cavity, but did not develop any external signs of disease. ECP, which contained caseinase and gelatinase activity, was harmful to fish, with an LD_{50} dose equivalent to 1.2 μg of protein/g of fish.

Coryneforms

As a result of pathogenicity experiments with rainbow trout (average weight = 8 g) maintained in freshwater at 18°C, it was established that 1.25×10^6 cells, administered by i.p. injection, were capable of killing fish within a few days (Austin *et al.*, 1985).

Micrococcaceae representative

Micrococcus luteus

Injection of 10^5 cells, via the i.m. and i.p. routes, led to 54% mortalities in rainbow trout fry within 14 days (Austin and Stobie, 1992a).

Mycobacteriaceae representatives

Mycobacterium spp.

At a water temperature of 12°C, experimental infections developed in rainbow trout which were injected, via the i.p. route, with approximately 10^7 cells of *Myc. chelonei* subsp. *piscarium*. Accumulative mortalities ranged from 20 to 52%. With juvenile chinook salmon, 98% mortalities were recorded within 10 days at a water temperature of 18°C (Arakawa and Fryer, 1984). Goldfish have been successfully infected within 8 weeks by i.p. injection with *Myc. fortuitum* and *Myc. smegmatis* ATCC 19420 at 10^7 CFU/fish and developed granulomatous lesions, typical of mycobacteriosis (Talaat *et al.*, 1999). Similarly, striped bass were infected using i.p. injections with $\sim 10^5$ cells of *Myc. gordonae*, *Myc. marinum* and *Myc. shottsii*. *Myc. marinum* caused peritonitis and the development of extensive granulomas particularly in the kidney, mesenteries and spleen, whereas the other two mycobacteria led to mild peritonitis, granulomas in the mesenteries which resolved with time, and persistent infections in the spleen (Gauthier *et al.*, 2003). Zebra fish were much more susceptible, with i.p. injection of $\sim 10^3$ cells of *Myc. marinum* leading to the development of granulomatous mycobacteriosis (Swaim *et al.*, 2006).

Evidence has indicated that a novel, plasmid-encoded, toxic macrolide, Mycolactone F (Ranger *et al.*, 2006) and ECP may well be involved with the pathogenic process (e.g. Chen *et al.*, 1997, 2001). Mycolactone F, being the smallest mycolactone recognised and having a molecular weight of 700, has been identified in *Myc.*

marinum and *Myc. pseudoshottsii* (Ranger *et al.*, 2006). Chen *et al.* (1997) determined the LD_{50} of ECP from *Mycobacterium* spp. as >400 μg of protein/fish to rainbow trout and Nile tilapia. Head kidney macrophages from rainbow trout demonstrated heightened macrophage activation when incubated with 1–100 μg/ml of ECP for 48 h (Chen *et al.*, 2001).

Nocardiaceae representatives

Nocardia spp.

Natural infections with *Noc. seriolae* have occurred in China when 15% losses were reported in seawater cages with large yellow croakers (*Larimichthys crocea*) during 2003 (Wang *et al.*, 2005). Yellowtail have been infected by i.p. and intradermal injection, immersion for 10 min and orally with LD_{50} values of 1.9×10^2, 4.3×10^6, 1.5×10^4, 1.7×10^7/ml, respectively (Itano *et al.*, 2006a). Co-habitation worked also in achieving infection (Itano *et al.*, 2006a). Experimental infections have been established in Formosa snakehead (*Chanos maculat*) and largemouth bass (*Micropterus salmoides*) (Chen, 1992). Thus, typical granulomatous lesions and mortality followed in 14 days of i.p. or i.m. injection of 8 mg of suspensions of *Noc. asteroides* (Chen, 1992).

Rhodococcus sp.

Intraperitoneal injection of Atlantic salmon smolts with a very high dose of 5×10^8 cells resulted in severe, peritoneal, granulomatous reactions, with a low accompanying mortality rate, within 21 days (Speare *et al.*, 1995). Unlike the natural disease where the most severe pathological changes occurred in the renal interstitium, experimental challenge resulted in damage in the direct vicinity of the injection site. Yet, the development of large bacterial colonies were common to both natural and artificial infections.

Rhodococcus erythropolis

Koch's postulates were eventually fulfilled using previously vaccinated fish which were challenged via i.p. injection with 2×10^5, 2×10^6 and 2×10^7 cells/fish (Olsen *et al.*, 2006a).

Planococcaceae representative

Planococcus sp.

Fish injected intraperitoneally with 10^5 cells displayed erratic swimming within 48 h. At this time, the gills were pale, the anus was protruded and abdomen was swollen. The intestine became swollen and haemorrhagic. Slight kidney liquefaction was noted. Approximately 30–40% of the infected fish died (Austin *et al.*, 1988; Austin and Stobie, 1992a).

Staphylococcaceae representatives

Staphylococcus warneri

Infectivity of brown trout was achieved, with an LD_{50} of 1.16×10^5 cells (Gil *et al.*, 2000).

Negligible information is available about the pathogenicity of other Gram-positive aerobic rods and cocci, as included in Table 1.1.

GRAM-NEGATIVE BACTERIA

Aeromonadaceae representatives

Aeromonas allosaccharophila

It was not concluded that the organisms were indeed pathogenic to fish. Yet, the recovery from diseased elvers suggests a pathogenic role for the organisms (Martinez-Murcia *et al.*, 1992).

Aeromonas hydrophila

Most of the information concerning pathogenicity mechanisms of *Aer. hydrophila* appertains to isolates of medical importance and will not be considered further here. The value of using cultures grown on nutrient-rich media has been cast into doubt following the observation that starved cells (NB: this is akin to the natural state of bacteria in the aquatic environment) are more virulent than their counterparts from nutrient-rich cultures (Rahman *et al.*, 1997). Nevertheless, as a general rule it is apparent that the pathogen has considerable exo-enzyme potential, including haemolysins, serine (=caseinase; 68 kDa)—and metallo-protease (=elastase; 31, 44 and 60 kDa) (Esteve and Birkbeck, 2004) some of which has relevance in fish pathology. The precise function of these "toxins", which number at least six (Bernheimer and Avigad, 1974; Donta and Haddow, 1978; Cumberbatch *et al.*, 1979), in fish pathology has yet to be fully elucidated. A 21 kb (kilobase) plasmid has been detected in pathogenic isolates associated with ulcerative disease syndrome, and correlated with antibiotic resistance. Curing the plasmid led to loss of virulence in Indian walking catfish (*Clarias batrachus*) whereas pathogenicity was restored when the plasmid was re-introduced into the bacterial cells (Majumdar *et al.*, 2006).

Surface structures

Recent studies have emphasised the surface structures of *Aer. hydrophila*, which appear to be involved in auto-aggregation/hydrophobicity and haemagglutination (e.g. Paula *et al.*, 1988). There is some evidence that a capsule may be produced *in vivo* (Mateos and Paniagua, 1995). The presence or absence of lateral flagella (as opposed to the more typical polar pattern) was demonstrated by electron microscopy on three isolates from catfish in Nigeria (Nzeako, 1991). Del Corral *et al.* (1990) demonstrated

the presence of pili/fimbriae, regardless of virulence. These workers considered that there was not a direct correlation between virulence and haemagglutination.

The surface array matrix, i.e. the S-layer, has been considered to influence the interaction between the bacterial cell and its environment (Esteve *et al.*, 2004). A major function is believed to be the provision of physical protection from lytic components, including serum proteins and bacteriophages (Dooley *et al.*, 1988). Work also links the presence of an S-layer with invasive disease in humans and mice (but not fish!) (Murray *et al.*, 1988). As a result of studying one isolate, i.e. TF7 (isolated from a lesion on trout in Quebec), it was determined that the S-layer did not confer any increase in surface hydrophobicity or any enhanced association with macrophages, and did not specifically bind porphyrin or immunoglobulin (Murray *et al.*, 1988). Nevertheless, in *Aer. salmonicida* the S-layer has indeed been shown to be a prerequisite for virulence, by increasing hydrophobicity and enhancing macrophage association (Murray *et al.*, 1988).

The detailed structure of the S-layer has been revealed in an excellent series of publications (Dooley *et al.*, 1986, 1988; Dooley and Trust, 1988). After studying eight isolates of a serogroup with a high virulence to fish, Dooley and Trust (1988) concluded that the S-layer was tetragonally arrayed. SDS–PAGE revealed a protein of 52 kDa molecular weight, which was the major surface (protein) antigen. This protein effectively masked the underlying OMP.

Ascencio *et al.* (1991) investigated extracellular matrix protein binding to *Aer hydrophila*. In particular, binding of ^{125}I-labelled collagen, fibronectin and laminin is common to isolates from diseased fish. Moreover, the binding property was specific, with cultural conditions influencing expression of the bacterial cell surface-binding structures. Experiments showed that calcium (in the growth medium) enhanced expression of the bacterial extracellular matrix protein surface receptors. The conclusion was reached that success in infecting/colonising a host depended on the ability of the pathogen to bind to specific cell surface receptors of the mucus layer, epithelial cells and subepithelial basement membranes.

"Adhesins"

It appears that the pathogen has the ability to attach to selected host cells, e.g. erythrocytes, and tissue proteins, i.e. collagen, fibronectin, serum proteins and glycoproteins, via the action of "adhesins" (Trust *et al.*, 1980a, b, c; Toranzo *et al.*, 1989; Ascencio *et al.*, 1991; Lee *et al.*, 1997; Fang *et al.*, 2004) and become internalised (Tan *et al.* 1998). The adhesins, of which a 43 kDa (AHA1) adhesin has been cloned and shown to have high homology to two OMPs (Fang *et al.*, 2004), appear to be extremely selective, recognising D-mannose and L-fucose side chains on polymers located on the surface of the eukaryotic cells. The specificity was further highlighted by the observation that human isolates of *Aer. hydrophila* failed to bind (or bound poorly) to fish tissue culture cells (Krovacek *et al.*, 1987). Indeed, using tissue culture cells from rainbow trout liver and chinook salmon embryo, Krovacek *et al.* (1987) demonstrated that some (~33%) isolates of *Aer. hydrophila* from fish adhered to the tissue culture cells and glass surfaces coated with rainbow trout mucus. Adhesion and

adsorption were time-dependent; and the activities were lost after treatment of the bacteria with heat, proteolytic enzymes or ultra-sound.

Invasion of fish cells

The 43 kDa protein has been regarded as important for the invasion of epithelial cells *in vitro* (Lee *et al.*, 1997; Fang *et al.*, 2004). Other workers have pointed to the relevance of capsular polysaccharides, which appear to enhance slightly adherence to fish cells, but contribute more significantly to cell invasion (Merino *et al.*, 1997a). A group II capsule gene cluster has been recognized, and the purified polysaccharide increased the ability of an avirulent culture to survive in (tilapia) serum and phagocytosis (Zhang *et al.*, 2003). With attachment, the host cell will be at the mercy of the pathogen. Although the precise mechanism of cell damage and tissue damage remains unproven, the available evidence points to the involvement of both endo- and exotoxins. Experiments with fish epidermal cells revealed that *Aer. hydrophila* could survive internally (Tan *et al.*, 1998). Here, a role for tyrosine phosphorylation in the internalisation process was suggested (Tan *et al.*, 1998).

Outer membrance proteins

Differences in the OMP according to incubation temperature have been documented, with a 40 kDa band produced following incubation at 17 and 25°C, which also coincided with the greatest virulence and least phagocytic activity by goldfish macrophages (Rahman *et al.*, 2001).

Extracellular products

In comparison with *Aer. salmonicida*, fish-pathogenic strains of *Aer. hydrophila* produce ECP, which contains considerable enzymatic activity (Shotts *et al.*, 1984; Santos *et al.*, 1987), including haemolysins and proteases (Angka *et al.*, 1995; Khalil and Mansour, 1997), and in particular a 64 kDa serine protease (Cascón *et al.*, 2000) with optimum production (of protease) at 27.6 ± 4.9°C (Uddin *et al.*, 1997). Interestingly, the highest mortalities were reported to occur in goldfish at 17 and 25°C (compared with 10 and 32°C) (Rahman *et al.*, 2001). The relevance of the ECP was highlighted by Allan and Stevenson (1981) and Stevenson and Allan (1981), who succeeded in causing a pathology in fish as a result of injection of the material. Yet, the role of ECP is debatable with contrasting views of the importance of "haemolysins" in virulence (Thune *et al.*, 1986; Toranzo *et al.*, 1989; Karunasagar *et al.*, 1990; Paniagua *et al.*, 1990). Stevenson and colleagues reported haemolytic (heat-labile) and proteolytic activity, the former of which was concluded to be of greater importance in pathogenesis. Kanai and Takagi (1986) recovered an a-type haemolysin which was deemed to be heat-stable at pH 4–1.2, but inactivated by EDTA, trypsin and papain. The crude preparation caused swelling and reddening of the body surface following injection into carp. Previously, Boulanger *et al.* (1977) isolated two types of haemolysins. The reasons then for the conclusion about the importance of haemolysins were based upon work with protease-deficient mutants, the ECP from which was more toxic to recipient fish than from wild-type cultures. Conversely, Thune *et al.* (1982a, b) obtained a fish-toxic fraction, which possessed proteolytic, but not haemo-

lytic, activity. Moreover, in a comparison of ECP from virulent and weakly virulent isolates, Lallier *et al.* (1984) noted that both were haemolytic, enterotoxigenic and dermonecrotic, but the weakly virulent isolate produced 20-fold more haemolysin than the virulent organism. Yet, only cell-free supernatants from virulent isolates produced toxic (oedematous) effects in fish. Following detailed chemical analyses, this heat-labile toxic factor was separated on Sephacryl S-200 from the haemolysin. These data suggest that factors other than haemolysins and proteases may be relevant in fish pathology. Indeed, after studying numerous isolates, Hsu *et al.* (1981, 1983), Shotts *et al.* (1985) and Paniagua *et al.* (1990) correlated virulence with extracellular proteolytic enzymes, notably caseinase and elastase. Santos *et al.* (1987) reported a relationship between virulence in fish and elastase and haemolysin (of human erythrocytes) production and fermentation of arabinose and sucrose. On this theme, Hsu *et al.* (1983) associated virulence with gas production from fructose, glucose, mannitol, mannose, salicin and trehalose, and the possession of resistance to colistin.

Extracellular metallo- and serine proteases of *Aer. hydrophila* (strain B5) have been characterised, and deemed to be heat- (to 56°C) (Leung and Stevenson, 1988) and cold-stable (to −20°C) (Nieto and Ellis, 1986). Most activity was inhibited by EDTA. Overall, there were many differences in the proteases (4 or 5 were present) described by Nieto and Ellis (1986) from the reports from other workers. This may be explained by the work of Leung and Stevenson (1988), who examined the proteases from 47 *Aer. hydrophila* isolates. Of these isolates, 27 produced both metallo- and serine proteases, 19 produced only metallo-proteases, and ATCC 7966 produced only a serine protease. The differences in these 47 isolates may well explain the apparent conflicting reports which result from the examination of only single isolates. Certainly, it seems that there are pronounced differences in the characteristics of the ECP and thus protease composition between strains.

It has been suggested that the proteases may be involved in protecting the pathogen against serum-bacteriocidal effects, by providing nutrients for growth following the destruction of host tissues, and by enhancing invasiveness (Leung and Stevenson, 1988). Also, proteases may be involved with the activation of haemolysin (Howard and Buckley, 1985).

A further study identified acetylcholinesterase (a 15.5 kDa polypeptide) in the ECP, and regarded the enzyme as a major lethal factor, possibly with neurotoxic activity (Nieto *et al.*, 1991; Rodriguez *et al.*, 1993a, b; Pérez *et al.*, 1998). The minimal lethal dose of the compound was given as 0.05 μg/g of fish.

Precipitation of Aeromonas hydrophila

The importance of precipitation after boiling is a debatable issue in screening of *Aer. hydrophila* isolates for virulence. Santos *et al.* (1988) considered that precipitation was not an important indicator, whereas Mittal *et al.* (1980) and Karunasagar *et al.* (1990) reported that settling after boiling was indeed an important measure of virulence.

Scavenging for iron

The ability of a potential pathogen to scavenge successfully for iron (in iron-limited conditions) will influence the outcome of the infection process. The haemolysins of *Aer. hydrophila* are iron-regulated, and access to iron in the haemolytic destruction of the host cells may be necessary (Massad *et al.*, 1991). The acquisition of iron from iron–transferrin in serum is dependent on the siderophore amonabactin. Many aeromonads use haem as a sole source of iron for growth. Some have evolved both siderophore-dependent (iron–transferrin) and -independent mechanisms (haem compounds) for the acquisition of iron from host tissues (Massad *et al.*, 1991).

Enterotoxigenicity

Strains have been attributed with enterotoxigenicity, as assessed by the rabbit ileal loop technique, and cytotoxicity (Boulanger *et al.*, 1977; Jiwa, 1983; Paniagua *et al.*, 1990), and correlated with lysine decarboxylase production (Santos *et al.*, 1987). Enterotoxigenic strains have been shown to produce two types of enterotoxins, which appear to be antigenically related, although the mode of action differs (Boulanger *et al.*, 1977). This was an interesting observation because de Meuron and Peduzzi (1979) isolated two types of antigen, of which the K-antigen (this was thermolabile at 100°C) was considered to represent a pathogenicity factor. Possibly, this corresponded to the enterotoxin or cytotoxin as described by Boulanger *et al.* (1977). However, the O (somatic) antigen, which was heat-stable, may have greater relevance, insofar as most virulent isolates share a common O-antigen (Mittal *et al.*, 1980). In an excellent study, Dooley *et al.* (1985) used SDS–PAGE to analyse LPS (considered to constitute an O-antigen) from virulent strains, which auto-agglutinated in static broth culture. The LPS contained O-polysaccharide chains of homogeneous chain length. Two strains produced a surface protein array, which was traversed by O-polysaccharide chains and thus exposed to the cell surface. Antigenic analysis revealed that the polysaccharide of the LPS carried three antigenic determinants.

Clearly, the evidence indicates the involvement of both endo- and exotoxins in the pathogenesis of *Aer. hydrophila* infections. It still remains for further work to elucidate the precise mechanism of action.

Evidence from molecular analyses

By comparing virulent and avirulent cultures, suppression subtractive hybridisation (SSH) was used to identify genetic differences, with the results highlighting 69 genomic regions absent from the latter (Zhang *et al.*, 2000). Genes considered to represent known virulence attributes included haemolysin, histone-like protein, oligoprotease A, OMP and multi-drug resistance protein. Other genes encoded synthesis of O-antigen (Zhang *et al.*, 2000).

Aeromonas jandaei

Esteve (1995) and Esteve *et al.* (1995b) reported a high LD_{50} dose of $\sim 10^6$ cells for eel. Possibly, the ECP activity, which was equated with production of caseinase,

collagenase, elastase, protease, lipase and haemolysin, caused pathogenicity (Esteve *et al.*, 1995b).

Aeromonas salmonicida

The spread of the pathogen

Historically, *Aer. salmonicida* was regarded as a risk primarily to salmonids (e.g. Mackie *et al.*, 1930, 1933, 1935). Then, cyprinids followed by other freshwater and marine fish became recognised to be vulnerable to infection (e.g. Herman, 1968; Austin *et al.*, 1998). Could farmed salmonids pose a realistic risk to native marine fish species? The data on this topic are confusing. Certainly, marine fish larvae have been infected with *Aer. salmonicida* subsp. *salmonicida*, with turbot regarded as being more susceptible than halibut (Bergh *et al.*, 1997). Using co-habitation and injection challenges, experiments suggested that *Aer. salmonicida* subsp. *salmonicida* could be transmitted rarely from Atlantic salmon to Atlantic cod, halibut and wrasse (Hjeltnes *et al.*, 1995). Could atypical isolates, which are appearing with increasing frequency in wild fish, pose a threat to cultured salmonids? Wiklund (1995) using an atypical isolate from ulcerated flounder concluded that there was not any risk to rainbow trout. What about the risk of transferring *Aer. salmonicida* from the freshwater to seawater stage of salmonids? Eggset *et al.* (1997) concluded that the susceptibility of Atlantic salmon to furunculosis in seawater possibly reflected the overall quality of the smolts.

Pathogenicity—historical aspects

Although the factors conferring pathogenicity on *Aer. salmonicida* strains have been the subject of speculation since early in the study of the pathogen, it is only relatively recently that the details concerning pathogenesis and virulence have begun to be elucidated. The initial investigations, carried out in the 1930s, resulted in several key observations, notably that prolonged laboratory maintenance of *Aer. salmonicida* isolates was frequently responsible for a loss of virulence, and that histopathological examinations of infected fish suggested the occurrence of leucopenia and proteolysis in certain tissues. Among the first studies concerned with virulence mechanisms of the organism was the extensive work of the Furunculosis Committee in the U.K. (Mackie *et al.*, 1930, 1933, 1935). This group did not detect any toxin production by *Aer. salmonicida* when either ultra-filtrates of broth cultures or diseased fish tissue was injected into healthy fish. Based on their failure to demonstrate toxin production, they hypothesised as a result of detailed clinical observations that the pathogenic processes caused by *Aer. salmonicida* could be explained by the prolific growth in the blood and tissues of its host which, in turn, interfered with blood supply resulting in anoxic cell necrosis and ultimately death. Additional evidence for a possible contribution to virulence, in the form of a leucocytolytic component, was provided by Blake (1935), who described the presence of "free" bacteria and little phagocytosis in the blood of diseased fish, with no definite leucocytic infiltration at the foci of infection. Mackie and Menzies (1938) confirmed the production of a leucocytolytic substance, as did Field *et al.* (1944), who determined the absence of leucocytosis by

performing repeated blood counts on experimentally infected carp. Perhaps, a more significant finding of their study, however, was the rapid decline in blood sugar levels resulting in hypoglycaemic shock, which was sufficient in some instances to cause acute mortalities. They suggested that the hypoglycaemic shock was the outcome of rapid utilisation of blood glucose by the multiplying pathogen. Regarding virulence mechanisms of *Aer. salmonicida*, Griffin (1953) theorised that leucocidin production *in vivo* by *Aer. salmonicida* would account for the observations by previous workers that marked cytolytic tissue necrosis did not seem to be accompanied by leucocytic infiltration. Another aspect of *Aer. salmonicida* pathogenicity, which eventually proved to be extremely important, was discussed by Duff (1937). He reported a loss in pathogenicity among strains after 6 or more months of maintenance on artificial culture in the laboratory. The loss was accompanied by a change in the appearance of colonies on nutrient agar from glistening, convex and translucent to strongly convex, distinctly opaque and cream-coloured. Because of such observations, Duff further investigated this phenomenon of dissociation into different colony types. Subsequently, he discovered that dissociation could be induced by culturing the pathogen in nutrient broth with the addition of either 0.25% lithium chloride or 0.1% phenol. Use of this procedure gave rise to several distinct colony forms. One of these resembled the original stock culture, a second corresponded to the "new" type and a third was intermediate between the other two forms. The colonies resembling those of the original stock culture were described as opaque, strongly convex, cream-coloured and friable, whereas the new colony form appeared translucent, slightly convex and a bluish-green in colour with a butyrous consistency. When the two different colony types were inoculated intraperitoneally into goldfish, the blue–green, translucent dissociant caused the deaths of the fish and was accompanied by lesions typical of the disease. In contrast, the original type of colony did not adversely affect fish, which survived for the 30-day duration of the experiment without any signs of illness. Thus, Duff concluded that the cream–opaque form which produced friable colonies was non-pathogenic, and more stable on prolonged storage. Duff designated this colony type as "rough". The "smooth" form (i.e. the blue–green–translucent dissociant, which produced butyrous colonies on agar media) was pathogenic, but less stable in prolonged storage. In the subsequent study, Duff (1939) also reported the presence of an extra antigen in the rough strains. Although Duff (1937) was the first worker to report the ability of *Aer. salmonicida* to dissociate into several distinct colony types with differences in pathogenicity, a phenomenon which is now widely accepted, it is curious that he ascribed pathogenicity to the smooth colony type. This is in contrast to the view currently held that the rough colony type is, in fact, virulent. Interestingly, the Furunculosis Committee had also reported a variation in colony morphology among isolates (it may be assumed that these corresponded to the rough and smooth variants), but contended that this phenomenon was not accompanied by a difference in virulence. It is regrettable that this initial confusion over dissociation occurred, preventing an earlier realisation of its significance. In fact, the relevance of dissociation of *Aer. salmonicida* colonies and the relationship to virulence was not made apparent until the work of Udey (1977), almost 40 years later. Early studies provided tentative evidence for a variety of possible pathogenic mechanisms, but

there is no doubt that progress in the understanding of *Aer. salmonicida* pathogenesis and virulence has been accelerated by rapid advances in the knowledge of cell biology and the development of sophisticated biochemical techniques. It is the application of such techniques that continues to yield considerable new information about the manner in which *Aer. salmonicida* may affect its disease processes in fish.

Pathogenicity—the value of intraperitoneal chambers

An intriguing and significant development concerned the description of intraperitoneal chambers, which could be implanted into fish (Garduño *et al.*, 1993a,b). These chambers could be filled with pathogens (or for that matter a range of other objects), implanted into fish, and measurements made with time. Garduño and colleagues placed *Aer. salmonicida* into a chamber, and studied its fate in the peritoneal cavity of rainbow trout. In one set of investigations, these workers oberved that when the pathogen was contained in the chamber killing occurred rapidly as a result of host-derived lytic activity (in the peritoneal fluid). In contrast, free cells had a better chance of survival (Garduño *et al.*, 1993a). Moreover, within the peritoneal chamber, *Aer. salmonicida* produced novel antigens, as determined by western blots (Thornton *et al.*, 1993). In another publication using the peritoneal chamber, evidence was presented that the capsular layer around *Aer. salmonicida* permitted the pathogen to resist host-mediated bacteriolysis, phagocytosis and oxidative killing (Garduño *et al.*, 1993b).

Pathogenicity—cell-associated versus extracellular components

A variety of pathogenicity mechanisms and virulence factors have been proposed for diseases caused by *Aer. salmonicida*, namely possession of an extracellular (A) layer (= the surface or "S" layer), a type III secretion system (e.g. Dacanay *et al.*, 2006) and the production of ECP, although there is confusion and even contradiction about the relative merits of the various components in pathogenicity (see Ellis *et al.*, 1988b). Yet, ironically, fish may mount an antibody response during infection (Hamilton *et al.*, 1986). Indeed, complement and non-a_2 *m*-antiprotease activity have been considered important host defence mechanisms against *Aer. salmonicida* (Marsden *et al.*, 1996c).

Munro (1984) has grouped the virulence/pathogenicity factors into cell-associated and extracellular components, a division which is convenient for the purpose of this narrative. The best-studied cell-associated factor is the additional layer, external to the cell wall, termed the A-layer.

The A-layer

The A-layer is now thought to be the product of a single chromosomal gene (Belland and Trust, 1985), is produced *in vivo* (Ellis *et al.*, 1997) and contributes to survival in macrophages (Daly *et al.*, 1996). The virulence array protein gene A (*vapA*), which encodes the A-protein has been sequenced, and differences noted in the amino acids between typical and atypical isolates, with homogeneity among the former, but heterogeneity with the latter. These differences undoubtedly lead to antigenic

differences among atypical isolates (Lund and Mikkelsen, 2004). First reported by Udey and Fryer (1978), and resulting from detailed electron-microscopic studies, the A-layer was determined to be correlated with virulence (e.g. Madetoja *et al.*, 2003a). Thus, it was observed that virulent strains possessed the A-layer, whereas avirulent isolates did not. In addition, the presence of the A-layer was found to correspond with strong auto-agglutinating properties of the organism, and to the adhesion to fish tissue culture cells. The auto-agglutination trait has been found to be influenced by temperature, with weak and strong auto-agglutination at 25 and 15–20°C, respectively (Moki *et al.*, 1995). The presence of the A-layer may confer protection against phagocytosis and thus destruction by macrophages (Olivier *et al.*, 1986; Graham *et al.*, 1988). Essentially, these workers noted that avirulent cells, i.e. those without an A-layer, were phagocytosed and destroyed when virulent cells with an A-layer were more resistant. Moreover, the bacteriocidal activity of macrophages was stimulated by prior exposure to low doses of *Ren. salmoninarum*, but inhibited by high amounts of living or dead renibacterial cells or the p57 antigen (Siegel and Congleton, 1997). Interestingly, it was deduced that living and formalised virulent cells, in the absence of serum, attracted macrophages more readily than avirulent cells after a period of 90 min (Weeks-Perkins and Ellis, 1995). The surface layer may inhibit growth at 30°C, enhance cell filamentation at 37°C, and enhance uptake of the hydrophobic antibiotics streptonigrin and chloramphenicol (Garduño *et al.*, 1994). Following the intravenous injection of purified A-layer protein into Atlantic salmon, the protein located to the epithelial cells in renal proximal tubules of the head kidney (Stensvåg *et al.*, 1999).

For its formation, Belland and Trust (1985) reasoned that the A-layer subunits pass though the periplasm and across the outer membrane for assembly on the cell surface. A requirement for the presence of O-polysaccharide chains, for which the AbcA protein is involved in biosynthesis (Noonan and Trust, 1995) on the LPS was reported as necessary for the assembly of A-layer (Dooley *et al.*, 1989). These virulent, auto-agglutinating forms produce characteristic deep-blue colonies on CBB agar (Wilson and Horne, 1986; Bernoth, 1990). Sakai (1986a, b) postulated that a possible mechanism for auto-agglutination and adhesion could be attributed to the presence of net negative electrical charge in the interiors or on the surfaces of cells. In particular, pathogenic cultures were highly adhesive (Sakai, 1987). It should be emphasised that Udey and Fryer (1978) determined that strains maintained for long periods in laboratory conditions were not auto-agglutinating, and demonstrated reduced virulence. Conversely, it was observed that fresh isolates, obtained from epizootics, were of the aggregating type. From the results of experiments, Udey and Fryer (1978) concluded that the presence of the A-layer was necessary for virulence. However, they contended that more work was needed to establish whether or not the A-layer alone could confer virulence. The discovery of the A-layer generated much interest, resulting in further study of its chemical composition and its specific role in fish pathology. Kay *et al.* (1981) succeeded in purifying the A-layer from virulent isolates, and concluded that it was composed of a surface-localised protein with a molecular weight of 49 kDa. Phipps *et al.* (1983) continued with work on purification and characterisation of the substance, determining that it

was hydrophobic in nature, present on the entire cell surface, did not possess any enzymic activity, but instead constituted a macromolecular, refractive protein barrier which was essential for virulence. Meanwhile, an independent parallel investigation of Evenberg *et al.* (1982) highlighted the relationship between auto-agglutination and the presence of the A-layer. This group examined the cell envelope protein patterns of a variety of isolates obtained from a wide range of geographical locations and different fish species (i.e. carp, minnow, goldfish and salmonids). These fish were suffering from either furunculosis, CE or ulcer disease. A major protein (molecular weight = 54 kDa) was found in all auto-aggregating strains, but little or no trace occurred in isolates which were not auto-agglutinating. When examinations for the presence of the protein were carried out after a change of growth medium, i.e. replacement of horse serum by synthetic sea salt, it was observed that an almost complete loss of the additional cell envelope and the auto-agglutinating ability of the isolate had occurred. Using gel immunoradio assays, it was also determined that the extra cell envelope proteins of all the isolates, irrespective of fish host, type of infection or geographical source, were immunologically related. Evenberg and Lugtenberg (1982) pursued this topic, and described the protein as water-insoluble with an amino acid composition similar to those of the additional surface layers of other bacteria, e.g. the adhesive K88 fimbriae of enteropathogenic strains of *Esch. coli*. It is particularly relevant that the findings of Evenberg *et al.* (1982), concerning the auto-agglutinating ability of "atypical" strains from cases of CE and ulcer disease, and the presence of the A-layer, were in excellent agreement with the work of Trust *et al.* (1980c) and Hamilton *et al.* (1981). These earlier studies deduced the presence of an outer layer protein, which was estimated to have a molecular weight of 50 kDa. Evidence was provided by Ishiguro *et al.* (1981) that loss of the A-layer and loss of auto-agglutinating properties resulted in decreased virulence. After examining the effects of temperatures on the growth of *Aer. salmonicida*, it was shown that in cells cultured at 30°C (the generally accepted upper limit for the organism) virulence was restricted to <10% of the population. The avirulent, attenuated cells that resulted from use of the higher growth temperature did not auto-agglutinate and, for that matter, did not possess the A-layer. It is interesting to note that higher maximum-growth temperatures were recorded for the attenuated strains, in comparison with their virulent counterparts. Perhaps, this is explained by their selection at high temperatures. Because of this observation, Ishiguro *et al.* (1981) hypothesised that the A-layer is important in determining the physical properties of the cell envelope, and that these properties undergo a change when the A-layer is lost, permitting growth at higher than normal temperatures. If the A-layer is a prerequisite for virulence, it may be assumed that its presence confers advantages on the bacterial cell in its role as a pathogen. Indeed, several prime functions for the A-layer have been proposed. Thus, evidence exists that the extracellular layer protects *Aer. salmonicida* cells from the action of protease (Kay and Trust, 1991) and bacteriophage, by shielding its phage receptors (Ishiguro *et al.*, 1981). In addition, the layer may protect the cell from serum complement, insofar as Munn and Trust (1984) demonstrated that virulent strains (with the A-layer) were resistant to complement bacteriocidal activity in the presence (and indeed absence) of specific antibody in

rainbow trout serum. Other investigations have revealed that hydrophobicity is conferred upon the bacterial surface by the A-layer (Trust *et al.*, 1983; Van Alstine *et al.*, 1986). These workers reported that the hydrophobic A-layer provided *Aer. salmonicida* cells with an affinity for fatty acid esters of polyethylene glycol and an enhanced ability to associate with rainbow trout and mouse phagocytic monocytes (macrophages), in the absence of opsonising antibody. Although Trust *et al.* (1983) conceded that the advantages to the pathogen of the increased association with macrophages remained to be determined, they suggested as a tentative explanation the possibility that *Aer. salmonicida* is a facultative intracellular pathogen able to survive within phagocytes. Indeed, Munn and Trust (1984) demonstrated that A-layer$^+$ bacteria (i.e. bacteria with an A-layer) were able to multiply within the principal phagocytic organs, e.g. the spleen, following experimental infection. Subsequently, it has become established that *Aer. salmonicida* is capable of replication in macrophages, where the pathogen is presumed to be able to resist reactive radicals (Garduño *et al.*, 1997). It has been argued that the surface layer constitutes the first line of defence for *Aer. salmonicida*, with an inducible catalase and manganese superoxide dismutase as second defensive systems against macrophage-mediated killing via reactive oxygen species.

The A-layer has also been implicated in a role concerning adhesion to fish tissues. By means of *in vitro* experiments, Parker and Munn (1985) examined the ability of avirulent (A-layer$^-$) cells to adhere to cells of baby hamster kidney and rainbow trout gonad in tissue culture. Attachment of A-layer$^+$ *Aer. salmonicida* to both types of cells was greater than for the A-layer$^-$ derivative. As a result, Parker and Munn (1985) proposed that since attachment to epithelial cells may be the primary step in the pathogenic process, their observations could account for the association of virulence with the presence of an extra outer membrane layer.

Another function of the A-layer is a possible interference with the antibacterial peptides, namely magainin, cecropins and defensins (Henry and Secombes, 2000).

To summarise, the accumulating body of evidence indicts the A-layer as a principal virulence determinant, even though its precise functions and the mechanism of action obviously require further clarification. However, blithe acceptance of an absolute relationship between virulence and possession of an A-layer must unfortunately be cautioned against. This is in view of reports by Johnson *et al.* (1985) and Ward *et al.* (1985) on the occurrence of virulent, auto-agglutinating strains that have no detectable A-layer. Conversely, Olivier (1990) recovered non-virulent A-layer$^+$ isolates. Thus, the association between presence of the extracellular layer and virulence, but not between auto-agglutination and virulence, appears to be open to question. It is important that the extent of this problem should be determined, particularly because the use of A-protein as an antigenic component of a potential vaccine for control of diseases caused by *Aer. salmonicida* has been advocated. This is due to the apparent immunological relatedness of the A-protein among isolates from different locations and a variety of fish hosts (Evenberg *et al.*, 1982). In view of the existence of virulent, auto-agglutinating *Aer. salmonicida* strains apparently lacking the A-layer, the effectiveness of such a vaccine would possibly be subject to severe limitations.

The type III secretion system

A type III secretion system, which utilises a 140 kbp plasmid and chromosome-encoded transmembrane injection device incorporating membrane proteins and a needle-like structure to translocate the effector protein AexT toxin from the cytosol into the host cell, has been linked to the virulence of *Aer. salmonicida* subsp. *salmonicida* and in particular the ability to achieve a systemic infection (Burr *et al.*, 2002; 2003a, b, 2005; Stuber *et al.*, 2003; Dacanay *et al.*, 2006; Ebanks *et al.*, 2006). In the case of one culture, A449, the expression of the type III secretion system was temperature-dependent, being active within 30 min at 28°C especially followed by exposure to low levels of calcium, but not 17°C, which is more usual for the outbreak of disease (Ebanks *et al.*, 2006). However, expression was induced at 16°C in the presence of 0.19 to 0.38 M NaCl. A second effector protein, AopP, has been identified, and found to inhibit the NF-κB pathway downstream of IκB kinase activation. The gene was found to be encoded on a small ~6.4 kb plasmid (Fehr *et al.*, 2006). The effector genes *aexT* (this codes for ADP [ribosyltransferase; Burr *et al.*, 2003a,b]), *oapH* and *aopO*, and *ascC* (which is the gene encoding the outer membrane pore of the secretion system) were inactivated by deletion and the effects examined in Atlantic salmon. The outcome was that the *ΔascC* mutant was not virulent. However, i.p. injection of *ΔaexT*, *ΔaopH* and *ΔaopO* resulted in disease, which was regarded as being indistinguishable from the parental wild-type culture. The conclusion was that, whereas the type III secretion system was essential for virulence, the individual effectors were less significant for virulence but were for colonisation (Dacanay *et al.*, 2006).

Type IV pilin

Type IV pili are regarded as important virulent determinants among Gram-negative, bacterial pathogens, participating as adhesins. A four-gene cluster, *tapABCD*, from a virulent *Aer. salmonicida* has been found to encode proteins with homology to those necessary for biogenesis of type IV pili (Masada *et al.*, 2002). *tapA*, which was regarded as ubiquitous among *Aer. salmonicida* isolates, encoded a protein with homology to type IV pilus subunits in common with other Gram-negative, bacterial pathogens, e.g. *Aer. hydrophila* and *V. vulnificus*. A mutant *Aer. salmonicida* defective in TapA was less pathogenic to rainbow trout following i.p. injection. TapB is part of the ABC-transporter family with nucleotide-binding regions; TapC homologues are cytoplasmic membrane proteins that exert a role in the anchoring and/or assembly of pili; TapD has homology with type IV prepilin leader peptidases (Masada *et al.*, 2002). TapD was capable of restoring type IV pilin assembly and type II extracellular protein secretion (albeit in *Ps. aeruginosa*) and was presumed to have a similar function in *Aer. salmonicida* (Masada *et al.*, 2002).

Outer membrane proteins

In many Gram-negative, bacterial pathogens, OMPs exert an important role in virulence. A proteomic analysis of *Aer. salmonicida* identified 76 unique proteins including the dominant S-layer Vap protein, >10 porins, phosphoglycerate kinase, enolase and receptors involved in nutrient acquisition (Ebanks *et al.*, 2005).

Capsules

Capsular polysaccharides have been found to develop around cells of *Aer. salmonicida* in the presence of glucose, phosphate, magnesium chloride and/or trace elements. Production of this material was improved in the presence of yeast extract (Bonet *et al.*, 1993). Interestingly, a striking difference in cells grown *in vitro* or *in vivo* reflected the presence or absence of capsules. Using intraperitoneal chambers, it was observed that *Aer. salmonicida* produced capsules with virulence functions (Garduño and Kay, 1995). Adherence to fish cell cultures was slightly higher in cultures of *Aer. salmonicida* grown in conditions to promote capsule formation. Also, invasion of fish cells was more pronounced in the capsulated cells (Merino *et al.*, 1996). Another role for the capsule concerns resistance to complement. Thus, it was recorded that, when grown under conditions promoting capsule development, *Aer. salmonicida* were partially resistant to complement (Merino *et al.*, 1997a,b).

Agglutination of fish cells

Another cell-associated factor, possibly relevant to virulence of *Aer. salmonicida*, is the ability of the pathogen to agglutinate trout and mammalian erythrocytes (Mellergaard and Larsen, 1981). The haemagglutination capability is purported to be related to the presence of adhesins, which are structures on the bacterial surface that mediate the attachment of the pathogen to the host's cell surface. Thus, the interest in haemagglutination is due primarily to its use to provide semi-quantitative information on the adhesive potential of a bacterial strain, while the sugar inhibition of haemagglutination has allowed adhesive specificity to be demonstrated (Duguid and Old, 1980). There is good agreement in the literature on the haemagglutinating ability of *Aer. salmonicida*. For instance, Jiwa (1983) reported mannose-resistant agglutination of bovine, chicken, human group A and guinea-pig erythrocytes by two *Aer. salmonicida* isolates which were recovered from diseased brown trout. It has also been demonstrated that smooth strains were unable to agglutinate erythrocytes, whereas rough strains showed a broad spectrum of haemagglutinating activity. Similarly, Parker and Munn (1985) observed that virulent, auto-agglutinating A-layer$^+$ cells agglutinated trout erythrocytes as well as a range of mammalian erythrocytes. They also noted that the process was not inhibited by specific sugars, and thus concluded that adhesion was a relatively non-specific process, attributable to the hydrophobic properties of the A-layer.

Extracellular products

Researchers interested in the biology of *Aer. salmonicida* have been aware for some time that extracellular substances (produced by the organism) presumably exerted a role in virulence. However, ECPs are not always harmful to fish. For example, Madetoja *et al.* (2003a) reported that ECPs, which lacked caseinase and gelatinase and had low cytotoxic activity in cell culture, from an atypical strain isolated from Arctic charr did not cause mortalities. Nevertheless, the ECPs have been the focus of numerous investigations. Unfortunately, the work has been rendered more difficult by the complexity of the substance(s), which, at present, is known to include an ADP-ribosyltransferase toxin (AexT) (Braun *et al.*, 2002), acetylcholinesterase (an

ichthyotoxin with neurotoxic activity; Pérez *et al.*, 1998), several proteases, namely two metallo-proteases [i.e. the 37 kDa leucine amino-peptidase and the 30 kDa metallo-protease 3 (Arnesen and Eggset, 1999)], P1, GCAT, AsaP1, P2 metallo-gelatinase and a serine caseinase (Gudmundsdóttir *et al.*, 1999), phospholipase, haemolysins and a leucocidin (Munro *et al.*, 1980; Sheeran and Smith, 1981; Shieh and MacLean, 1975; Titball and Munn, 1981; Cipriano *et al.*, 1981; Fuller *et al.*, 1977; Rockey *et al.*, 1988; Huntly *et al.*, 1992; Lygren *et al.*, 1998), as well as LPS. Work with monoclonal antibodies has shown heterogeneity in the LPS (Rockey *et al.*, 1991). Ellis *et al.* (1981) reported that ECP of the pathogen, prepared by a cellophane overlay method, reproduced the lesions normally associated with the chronic form of furunculosis, e.g. muscle necrosis and oedematous swelling at the site of injection. This suggests that the toxins and aggressins released by the bacteria *in vivo* are responsible for much of the pathology of the disease. In addition, when injected intraperitoneally into rainbow trout, the ECP proved to be fatal for the fish (Munro *et al.*, 1980). In preparations of fish cells, the ECP exhibited cytotoxic effects, and at higher concentrations was leucocytolytic and haemolytic. These investigators concluded that most of the virulence factors were produced extracellularly, with most strains of *Aer. salmonicida* producing similar compounds, although the quantities varied. However, a detailed chemical analysis was not carried out.

Other studies have also indicated that injection of ECP closely reproduces the pathological condition attributed to furunculosis (Sakai, 1977; Cipriano *et al.*, 1981). Cipriano *et al.* (1981) attempted to determine the role between ECP and virulence by extracting the compounds from culture supernatants. The ECP was resolved into four fractions by ion exchange chromatography. It was deduced that fraction II possessed leucocytolytic activity, although this fraction was not associated with virulence. Rather, a link between virulence and the toxicity of crude material, and fractions II and III, to cultured rainbow trout was observed. In this experiment, the extracted material from virulent isolates was more toxic to tissue culture cells than preparations derived from the avirulent strains. Fraction II also demonstrated proteolytic activity. Furthermore, results of *in vivo* toxicity studies revealed that three of the fractions were toxic to fish, although their activities varied according to the nature of the fish species used. Thus, mortalities, accompanied by haemorrhaging at the vent and fins, and inflammation at the site of injection, occurred in Atlantic salmon and brook trout that received fractions I and II. Fraction III also caused haemorrhaging at the base of the fins, injection site and in the mouth; however, the majority of fish administered with this fraction survived. In contrast, rainbow trout were relatively resistant to the effects of all four fractions, insofar as no mortalities resulted. However, administration of fraction II resulted in the development of characteristic furuncle-like lesions at the inoculation site. Fractions I, III and IV did not cause any obvious pathology. The results of Cipriano *et al.* (1981) supported the previous findings of Sakai (1977) who, on the basis of work with crude ECP preparations, considered that a protease was the most pathogenic substance produced by *Aer. salmonicida*. The effects ascribed to proteolytic activity by Sakai (1977) were analogous to those noted by Cipriano *et al.* (1981) for fraction II. In fact, the muscle necrosis and degeneration of connective tissue associated with furunculosis indicates proteolytic enzyme activity.

Yet, Fyfe *et al.* (1986) recorded that protease preparations were less effective than equivalent amounts of ECP (with similar amount of proteolytic activity) at causing lesions, i.e. furuncles, following i.m. injection of juvenile Atlantic salmon. This team identified three major components with molecular weights of 70 kDa (a serine protease; Ellis *et al.*, 1997), 56 kDa (a haemolysin) and 100 kDa (unidentified protein) in the ECP (Fyfe *et al.*, 1987a); the first mentioned of which was produced in greater quantities after incubation for 18 h at 25°C compared with 125 h at 10°C (Fyfe *et al.*, 1987b). Haemolysin production was similar at both temperatures, but 10-fold more of the 100 kDa protein was produced at the lower temperature.

Proteases, as prime candidates for exerting a significant role in disease pathogenesis, have aroused substantial interest as a research topic. Indeed, a variety of investigators have performed detailed analyses, and suggested heterogeneity among isolates. In particular, Gudmundsdóttir (1996) described 6 protease groups, but this information might have greater value for taxonomy than an understanding of pathogenicity. Thus, by examining 5 typical and 25 atypical isolates, it was determined that the proteases produced by the type strains of *Aer. salmonicida* subsp. *achromogenes* (this produced a metallo-caseinase = AsaP1) and *Aer. salmonicida* subsp. *salmonicida* were different from those of the fresh isolates. Moreover, all the typical isolates belonged to one protease group with proteolytic activities comparable with P1 and P2 proteases, whereas the atypical cultures were different. With the exception of three atypical oxidase-negative isolates, which secreted a protease reminiscent of P1, the others produced metallo-gelatinase. Ten of the atypical isolates produced AsaP1 (Gudmundsdóttir, 1996).

Shieh and MacLean (1975) purified a proteolytic enzyme, which was determined to have a molecular weight of 11 kDa, and an optimum pH range of 8–11. Because the enzyme was inhibited by PMSF, these workers concluded that it was a serine protease. Mellergaard (1983) also isolated and purified a proteolytic enzyme (molecular weight = 87.5 kDa; optimum pH of 9.0), as did Tajima *et al.* (1984), who reported the presence of an extracellular protease with a molecular weight of 71 kDa, and a pH range of 5–10. This enzyme was deduced to be an alkaline serine protease. Sheeran *et al.* (1984) described two extracellular proteolytic activities that differed in their susceptibiity to inhibitors and substrate specificity. One of the enzymes, designated P1, hydrolysed casein, elastin and gelatin, and showed a low, non-specific activity against collagen. The second enzyme (P2) hydrolysed collagen and gelatin, but not casein or elastin, a pattern that suggested it is a specific collagenase. Also, Rockey *et al.* (1988) described two proteases, coined P1 and P2, and a haemolysin (T-lysin) in the ECP. P1 and T-lysin were shown to work separately in the complete lysis of (rainbow trout) erythrocytes. T-lysin interacted with the outer membrane of the erythrocytes, whereas P1 destroyed the nuclear membrane. A role for P2 was not described. Hastings and Ellis (1985) recorded differences in the pattern of extracellular protein production, according to the origin of the bacterial isolates. For example, isolates from Iceland (achromogenic) and the U.S.A. lacked caseinase and gelatinase activity in the ECP. Indeed, an isolate of *Aer. salmonicida* subsp. *achromogenes* from Iceland has been credited with the production of a novel metallo-protease (Gudmundsdóttir *et al.*, 1990). For other strains, caseinase and

gelatinase activities were inhibited by PMSF and EDTA. These data suggested that both enzyme activities could be attributed to a single serine protease, which depends upon divalent cations for activity. An extracellular metallo-caseinase, AsaP1, has been linked with lethal toxicity of atypical *Aer. salmonicida* in Atlantic salmon, with furuncles being produced by ECP with AsaP1 (Gunnlaugsdóttir and Gudmundsdóttir, 1997).

Using the "P1" and "P2" terminology, Lygren *et al.* (1998) discussed differences in protease secretion according to the age of the culture of *Aer. salmonicida* subsp. *salmonicida*. Essentially, two different proteolytic activities were found in early and late exponential phases of growth. The P2 activity, found in culture supernatants in the early exponential phase, was described as a metallo-protease (molecular weight = 30–40 kDa) with activity against casein and gelatin. This caseinase activity was regarded as novel for metallo-proteases of *Aer. salmonicida* subsp. *salmonicida*. The second and major protease, P1, appeared in the culture supernatant during late exponential phase of growth. This protease was regarded as identical to the 70 kDa serine protease (Lygren *et al.*, 1998).

Intramuscular injection of the proteases into brown trout resulted in the development of gross symptoms similar to those occurring in natural outbreaks of furunculosis, i.e. muscle liquefaction along the flanks adjacent to the lesion and swelling at the site of injection (Sheeran *et al.*, 1984). From these data, Sheeran and co-workers concluded that their observations differed from those of other workers. They stated that the absence of significant mortalities, or haemorrhaging of the fins and anus in the experimentally infected fish, contrasted with the reports of others, suggesting that, in previous work, enzyme preparations may have contained toxic material other than proteases. Alternatively, it seems possible that Sheeran used insufficient quantities to achieve the pathological changes in question. However, the latter probability seems to be unlikely insofar as the levels of P1 enzyme injected by Sheeran *et al.* (1984) were equivalent to those of Sakai (1978), who reported furuncle formation, haemorrhaging and mortalities in kokanee salmon (*Oncorhynchus nerka*) following administration via i.m. injection. Further evidence for the role of protease production in the pathogenesis of furunculosis was provided by Sakai (1977), who reported a reduction in virulence for a proteolytically deficient, mutant strain of *Aer. salmonicida* compared with its isogenic wild type. In his later article, Sakai (1985) considered a role for proteases in reproduction of the pathogen by making available small peptides and amino acids from proteolysis. Of course, this would considerably benefit the nutrition of the pathogen. It should be emphasised that serum from salmonids is capable of neutralising lethal doses of proteases (Ellis *et al.*, 1981), possibly through the action of an a-migrating antiprotease (Grisley *et al.*, 1984). Nevertheless, it is apparent that proteases play an important role in the pathogenicity process. Indeed, a serine protease, which was reported to have a molecular weight of 64 kDa, suppressed the immune response of Atlantic salmon (Hussain *et al.*, 2000). A complication concerns the recovery of a pathogenic non-protease secreting strain (Tajima *et al.*, 1987b). Therefore, much work is still required to clarify the precise mode of action of proteases in the pathogenic process.

The greater susceptibility of brown trout (compared with rainbow trout) to furunculosis has long been recognised (e.g. McCarthy, 1975a, b). An explanation for this difference has been provided by Ellis and Stapleton (1988), who found that at low ratios of exotoxin to serum, brown trout serum considerably enhanced *Aer. salmonicida* proteolytic activity. Yet, at similar ratios rainbow trout serum demonstrated some inhibition of the bacterial protease activity. The interpretation of these data is that during the initial stages of infection, *Aer. salmonicida* would have greater potential to multiply in brown trout rather than rainbow trout. Furthermore, Rockey *et al.* (1988) determined that serum from rainbow trout protected the erythrocytes from the haemolysins of *Aer. salmonicida*.

A LPS-free phospholipase has also been recovered from the ECP, and demonstrated to cause disease signs, upon injection, into Atlantic salmon (Wong *et al.*, 1989; Huntly *et al.*, 1992). Death resulted overnight following injection of 10 μg/g body weight of fish. Disease signs included lethargy, melanosis and other defects characteristic of furunculosis. In addition, erythema was noted on the undersurfaces, particularly around the vent, at the bases of the pectoral and pelvic fins, and head (Huntly *et al.*, 1992). Erythrocyte membranes were degraded (= haemolytic activity). It was concluded that this phospholipase exhibited GCAT activity.

Production of haemolysins by *Aer. salmonicida* may also contribute to the pathogenesis of furunculosis, insofar as it has been established that ECP contain components with pronounced haemolytic activity for trout erythrocytes (Munro *et al.*, 1980). Titball and Munn (1981) carried out the first extensive study of haemolysin production by *Aer. salmonicida*. These authors reported the existence of two distinct haemolytic activities. Essentially, they determined that the supernatant from unshaken broth cultures contained haemolytic activity against erythrocytes from a diverse range of vertebrate species, with maximal activity against horse red blood cells. Titball and Munn termed this "H" activity. If cultures were shaken, however, the resulting supernatant yielded an activity against trout erythrocytes only (this was designated the "T" activity). Furthermore, the H-lysin was reported as unstable in culture supernatants, sensitive to heat after exposure to 56°C for 5 min, and became membrane-bound when solutions were filtered. In contrast, the T-lysin was stable in supernatants, and was inactivated by normal rainbow trout serum. Nomura and Saito (1982) also studied the extracellular haemolytic toxin which was recorded as cytotoxic for sheep and salmonid erythrocytes. These investigators observed that the production of haemolysin was stimulated by the addition of enzymic hydrolysates of protein, but suppressed by carbohydrates, such as glucose or sucrose. Moreover, bivalent metal ions, e.g. Ca^{2+}, Co^{2+} and Mn^{2+}, and phosphate ion ($[HPO_4]^{2-}$) were necessary for production of the haemolysin. The optimum pH range and optimum temperature for toxin production was 7.5–8.0 and 20°C, respectively. Nomura and Saito (1982) concluded that the haemolysin was produced during the stationary phase of the growth cycle, and was relatively heat-labile, being inactivated at 60°C. These observations coincided with those of Titball and Munn (1981).

In continued studies of the T- and H-lysins, Titball and Munn (1983, 1985a) purified the components, and examined properties of the haemolytic activity. Thus, the T-lysin activity was separated into two factors, namely a caseinase and another,

apparently membrane-associated (T_1) activity, which by itself caused only incomplete lysis. In fact, complete lysis of trout erythrocytes occurred only in the presence of both T_1 activity and the caseinase (also see Rockey *et al.*, 1988). Titball and Munn (1983) believed that this phenomenon was due to the co-operative effect of both activities on the red blood cell membrane, rather than the conversion of T_1 to T-lysin by caseinase. This opinion was reached because the inhibition of caseinase resulted in the loss of complete lytic potential from supernatant fluids containing T-lysin. Titball and Munn (1985a) regarded the H-lysin to be a proteinaceous substance, on the basis of results of the ultraviolet absorption spectrum. Additionally, they observed that the partially purified H-lysin contained detectable levels of GCAT. This enzyme possesses some similarities to the H-lysin, e.g. moleular weight of 23.2 and 25.9 kDa, respectively (Buckley *et al.*, 1982). Yet, the molecular weights were much smaller than the 200 kDa size of "salmolysin", the haemolytic toxin described by Nomura *et al.* (1988). The ionic strengths needed for the elution of GCAT and H-lysin from ion exchange gels were similar. These factors may complicate the isolation of pure H-lysin, assuming that GCAT and the haemolysins are separate entities, which appears to be the case. Thus, GCAT has not been reported to possess haemolytic activity, and is stable at room temperature (Buckley *et al.*, 1982). Membrane filtration of the preparation failed to remove GCAT, whereas H-lysin activity was lost after the procedure (Titball and Munn, 1981). Other observations of H-lysin activity have indicated that haemolysis of horse erythrocytes occurs in two steps, namely a first stage in which there is no detectable cell lysis (this was termed the pre-lytic stage), followed by a second phase involving haemoglobin release and disruption of the cell membrane. Binding of the H-lysin to the erythrocytes during the pre-lytic stage does not occur. Together with the observation of an optimum temperature of 25–33°C for lysis, this suggests that the H-lysin has enzymic action on the erythrocyte membrane. Nevertheless, fish injected with H-lysin appeared to be unaffected, despite an apparent toxicity to rainbow trout gonad tissue cell lines. Titball and Munn (1981) concluded that the failure of H-lysin to elicit a response in the fish experiments was explained by the use of an unsuitable route of administration or the injection of too low a quantity of the material. In addition, these authors argued that possibly H-lysin is non-toxic to fish, with no important role in the pathological process.

Several investigators have explained the relationship of haemolysins and proteases to virulence by using different strains of *Aer. salmonicida*. For example, Hackett *et al.* (1984) studied the possibility of a plasmid-encoded origin for these extracellular enzymes. Significantly, the team concluded that the loss of proteolytic and haemolytic activity in variants of wild-type *Aer. salmonicida*, obtained by treatment with ethidium bromide, did not correlate with loss of plasmid DNA. Moreover, there was no apparent change in the LD_{100} between the virulent wild-type strain and its protease haemolysin-deficient variant. This implied that the extracellular activities were not essential for virulence or, indeed, pathogenicity, at least with regard to the acute form of furunculosis in rainbow trout. Two clones derived from another virulent strain, one of which was negative for protease and haemolysin production whereas the second derivative was positive for these attributes, were avirulent (LD_{50} increased by greater than four orders of magnitude). This was an

important observation, denoting that attenuation of a virulent strain occurred without loss of the A-layer, plasmids or extracellular proteolytic and haemolytic activities. For this reason, Hackett *et al.* (1984) concluded that virulence was attributable to other, as yet unknown, factors. Titball and Munn (1985b) also studied the effects of quantitative differences in virulence on the production of potential toxins by *Aer. salmonicida*. The release of ECP, i.e. proteases and haemolysins, by virulent strains and their avirulent, attenuated derivatives (differing only in the presence or absence of the A-layer) could not be linked directly to virulence. Insofar as no appreciable differences were recorded between the levels of ECP from virulent (possessing an A-layer) and avirulent (no A-layer) cells, it appears that these compounds are not virulence determinants.

Hastings and Ellis (1985) reported that there was a marked variation in the production of haemolysins and proteases among different strains of *Aer. salmonicida*. In their study, four isolates, recovered from Atlantic salmon in Scotland, produced caseinase and gelatinase. Both of these enzymes were inhibited by PMSF (a serine protease inhibitor) and to a lesser extent by EDTA (a divalent metal ion chelator). This finding indicated that both enzyme activities could be attributed to a single serine protease, which was dependent upon divalent cations for activity. In contrast, an achromogenic isolate that was obtained from Iceland did not produce detectable quantities of haemolysin or gelatinase. It is noteworthy that the caseinase from this isolate differed from that of the Scottish strains, insofar as it resembled a metalloprotease. Hastings and Ellis (1985) noted that this enzyme appeared to be unique to fish pathogens. It is not known, however, if other achromogenic strains of *Aer. salmonicida* share similar properties regarding their ECPs. Nevertheless, it is relevant to note that yet another strain, recovered from the U.S.A., differed from the Scottish isolates insofar as it lacked both caseinase and gelatinase activity in the ECP. Moreover, its haemolysin production was notably lower. Hence, it seems that there is a marked variation in the nature of the precise components of the ECPs from different strains of the pathogen. Thus, there may be some variation in the modes of pathogenesis.

Yet another factor with a potential role in virulence and pathogenicity is the leucocytolytic component of the ECP. Although this component was recognised in the 1930s, many years passed before detailed study ensued. Thus, Klontz *et al.* (1966) reported leucopenia in rainbow trout, following injection of either viable cells or a saline-soluble extract of the culture. On the basis of these results it was hypothesised that a leucocytolytic compound was responsible for the limited leucocyte activity observed in infected fish. This group did not comment, however, on the biochemistry of the leucocytolytic compound. Nevertheless, this aspect was examined by Fuller *et al.* (1977), who deduced that the compound was a glycoprotein, which was distinct from the endotoxin, i.e. LPS, as previously studied by Ross (1966), Anderson (1973) and Paterson and Fryer (1974a,b). The leucocytolytic factor was present in the supernatants from broth cultures; moreover, virulent strains produced more than the avirulent counterparts. Furthermore, the glycoprotein was cytolytic for leucocytes *in vivo*, and produced a pronounced leucopenia when injected intravenously into adult rainbow trout. In addition, the factor appeared to enhance pathogenicity,

presumably by increasing susceptibility of the host. This opinion was reached after experiments in which small coho salmon were inoculated with the leucocytolytic compound in combination with live *Aer. salmonicida* (approximately a quantity sufficient to achieve a LD_{50}). The result was that 36/40 fish succumbed, in contrast to the death of only 14/40 animals injected with just the pathogen. So, the conclusion was reached that the glycoprotein constituted a virulence factor of *Aer. salmonicida.* However, the results of Cipriano *et al.* (1981) were not in accord with the findings of Fuller's team. Essentially, Cipriano and colleagues deduced that there was no correlation between virulence and the leucocytolytic properties. Therefore, according to Cipriano *et al.* (1981) leucocytolytic factors could not be considered as a principal virulence mechanism. Alternatively, the activity may not solely relate to the attack on leucocytes. Instead, Cipriano and colleagues suggested that the leucocytolytic factor, contained within fraction II of the ECP, contributed to virulence not only by way of its leucocytolytic properties, but also through its role as a generalised cytotoxin capable of generating pathological changes. Indeed, these investigators recorded that intramuscular injection of fraction II (produced by a virulent isolate) into brook trout caused haemorrhaging at the mouth, base of the fins and site of inoculation. Death occurred within 24 h. Such deleterious changes did not ensue in fish that received fraction II derived from an avirulent isolate.

It must be emphasised that the lack of a leucocytic response, apparent in the majority of salmonids with furunculosis, has not been substantiated in infections of coarse fish. In one example, a chronic leucocytosis was observed in goldfish (Mawdesley-Thomas, 1969).

Ellis *et al.* (1981) outlined a hypothesis for the role of ECP in pathology. Moreover, they highlighted some of the difficulties involved in reaching a complete understanding of the pathogenic process. Importantly, they emphasised that in many respects furunculosis is an inconsistent disease, insofar as a variety of lesions have been associated with invasion of the aetiological agent. Yet, virtually none of the symptoms may be considered as unique to the disease (Wolke, 1975). Consequently, it is hardly surprising that inconsistencies have resulted in conflicting opinions over the pathogenicity mechanisms. Ellis and co-workers formulated a tentative explanation for the lesions caused by *Aer. salmonicida.* Thus, they reported that nearly all of the lesions normally associated with the disease may be achieved by i.p. or i.m. injection of ECP. However, it would appear that artificially high doses are required to accomplish such lesions. Munro *et al.* (1980) suggested that the presence of an a-globulin in normal trout serum may have the ability to neutralise ECP activities. Indeed, other workers have confirmed such effects of fish serum on ECP. Rockey *et al.* (1989) published an article detailing the inhibition of haemolysin activity by salmonid serum. Sakai (1984) mentioned a decrease in, or absence of, mortality among rainbow trout that had received ECP first treated with large volumes of rainbow trout serum prior to injection. These results indicated involvement of complement in the detoxification of ECP. Continuing this theme, Grisley *et al.* (1984) reported the presence of an a-migrating protein (a possible homologue of mammalian a_2-macroglobulin) in normal rainbow trout serum. This protein apparently exerts a role in a non-specific defence function against microbial, proteolytic toxins. Ellis and

Grisley (1985) pursued the theme, concluding that normal trout serum inhibits ECP protease, but neutralisation is effected by different anti-proteases and less efficiently than trypsin. They contended that the data, to some extent, explained the potency of ECP in causing disease. Ellis *et al.* (1981) assumed that in natural infections lesions would be produced after the ECP had exhausted any inhibiting factors, either locally or systemically. They thought that the various symptoms of furunculosis were explained by the colonisation of different host tissues by the pathogen. It was concluded that the pathological effects resulting from infection by *Aer. salmonicida* were probably caused by the ECP released by the pathogen. Thus, the leucocytolytic component might act against leucocytes, eventually resulting in leucopenia, and preventing the destruction of the bacterial colonies, thus allowing microbes to be transmitted to other organs via the circulatory system where they may initiate the development of more colonies. It was further submitted by these authors that lesions and mortalities are due to the collagenolytic activity of the ECP (this is one of the notable features of furunculosis), with haemorrhaging resulting in the vicinity of bacterial colonisation. Generalised circulatory failure could ensue if the ECP subsequently entered the circulatory system.

Just when the role of ECP and proteases was becoming clarified, some elegant work with deletion mutants caused a fundamental re-think. It was obvious that to be sure of a specific component, eliminate the genes from the bacteria and determine the effect on the host. Using this approach, Vipond *et al.* (1998) confirmed that mutants lacking GCAT or serine protease (AspA) were not less virulent than the parental cell following i.p. or cohabitation challenge of Atlantic salmon.

Finally, it is appropriate to recall the words of Munn *et al.* (1982), who commented that the interrelationships of ECP suggest that the pathogen exerts its toxic effects *in vivo* by means of multiple factors that interact synergistically.

Scavenging for iron

A current theme, which has prompted some excellent work, concerns the ability of *Aer. salmonicida* cells to successfully scavenge for iron in iron-limited conditions. These conditions would be created in the host, and function as a defence mechanism against invasion by pathogens. Indeed, there is evidence that IROMP are produced *in vivo* (Ellis *et al.*, 1997). Thus, free iron would be bound to proteins, such as transferrin, resulting in iron-restricted conditions in the host. Initially, Chart and Trust (1983) demonstrated that typical strains of *Aer. salmonicida* were capable of sequestering iron. Then, Kay *et al.* (1985) determined that the A-layer was implicated as a component of an iron uptake mechanism. The conclusion was that the A-layer functioned as the initial stage of iron uptake, being a binding site for porphyrins, i.e. haemin and protoporphyrin. The difference between typical and atypical isolates was reinforced by the conclusion that there was a fundamental difference in the mechanism of utilisation of non-haem bound sources of iron. Hirst *et al.* (1991) and Hirst and Ellis (1996) described an inducible siderophore (these are soluble, low molecular weight iron chelators) dependent, iron-chelating system in typical strains and an unidentified siderophore-independent system in atypical *Aer.*

salmonicida. Among *Aer. salmonicida* subsp. *salmonicida* (17 isolates from Scotland and Spain were examined), the siderophore is regarded as homogeneous (Fernandez *et al.*, 1998).

In summary, a variety of cell-associated and extracellular factors have been investigated in order to determine their role in virulence and pathogenicity of diseases of *Aer. salmonicida* aetiology. Unfortunately, the overview that emerges for the current understanding of pathogenicity mechanisms is confused. Much of the evidence about the various factors suspected to be involved with virulence is contradictory, or is based solely upon *in vitro* studies. Thus, although the presence of an A-layer is firmly believed to be a primary determinant of virulence, reports of avirulent isolates with an A-layer (Udey, 1978) raises further questions. Conversely, the ECP contain such a diverse array of different factors implicated with virulence and pathogenicity that to pin-point the function of each *in vivo* has proved difficult. Hence, a definitive assessment of the role of the various haemolysins, proteases and leucocidins in the natural disease process still eludes us. However, it is conceded that substantial progress has been made in the isolation, purification and biochemical characterisation of the ECP. Moreover, strong evidence exists that the ECP are capable of eliciting a pathology reminiscent of the natural disease (Cipriano *et al.*, 1981; Ellis *et al.*, 1981). However, the interrelationships between the various sub-components remain unclear. Thus, Cipriano *et al.* (1981) believed that the leucocytolytic and proteolytic activities were dual expressions of a component, i.e. the chromatographic fraction II, a notion which requires more information for confirmation. This group opined that the generalised cytotoxicity for rainbow trout gonad cell lines by ECP was a better indicator of virulence. There was, however, agreement with the suggestion of Sakai (1977) that proteases constituted the most pathogenic element of the ECP. Yet, it is apt to recall the warnings of Sheeran *et al.* (1984), who emphasised that it is vital to establish the levels of the proteolytic enzymes in naturally infected fish tissues. Until this can by done even conclusions drawn from *in vivo* experiments remain speculative. Results from some investigations have demonstrated that there is some degree of variation in the quantities of potential virulence factors produced by different isolates of *Aer. salmonicida*. Thus, there may be some variation in the precise mode of pathogenesis (Hastings and Ellis, 1985). Yet, Titball and Munn (1985b) did not find any appreciable differences in the levels of ECP between virulent and avirulent isolates. Nevertheless, these authors admitted that this did not exclude a role for the substances as aggressins, although it was paradoxical that a delayed release of ECP by A-layer$^+$ strains was observed. As a possible explanation, the disadvantage of late release of toxic material may be counterbalanced by the role of the A-layer in conferring resistance to host defence mechanisms (Munn *et al.*, 1982). In yet another comment, Hackett *et al.* (1984) proposed that in peracute or acute forms of furunculosis, virulence is independent of the presence of protease and haemolysin. Accordingly, these workers suggested that death of the fish may result from organ dysfunction, due principally to massive growth of the pathogen. Alternatively, it was speculated that there may be involvement by an as yet unidentified component of the ECP.

The fate of Aeromonas salmonicida following infection

Workers have addressed the questions concerning the fate of *Aer. salmonicida* after infection by various routes. Some of this information is discussed elsewhere. Using radio-active methods, Svendsen *et al.* (1999) published evidence that, following infection by immersion, the pathogen could be readily found around surface wounds (the Atlantic salmon had been artificially wounded prior to use), the gills and hindgut (radioactivity increased here from 2 to 24 h). Two hours after challenge, bacteria were detected in the blood; at 24 h *Aer. salmonicida* was in the kidney, but not the blood (Svendsen *et al.*, 1999).

Aeromonas sobria

Cultures were pathogenic to rainbow trout, with the LD_{50} reported as 2×10^5 cells. Dead animals revealed the presence of haemorrhagic septicaemia. In addition, thermolabile ECP were cytotoxic and lethal (30 μg protein/fish) to rainbow trout. Further work on *Aer. sobria* by Paniagua *et al.* (1990) highlighted the role of caseinase, haemolysins and cytotoxins in the pathogenic process. Wahli *et al.* (2005) noted haemolytic activity on sheep and trout erythrocytes, and cytotoxicity to EPC cell lines.

Aeromonas veronii biovar sobria

The isolates produced adhesions, cytotoxin, haemagglutination (fish, human and rabbit blood) and haemolysin (Rahman *et al.*, 2002a).

Alteromonadaceae representatives

Pseudoalteromonas piscicida

Damsel fish eggs challenged with a culture resulted in enhanced mortalities, compared with those of uninfected controls (Nelson and Ghiorse, 1999).

Shewanella putrefaciens

Following i.p. injection, fish (average weight = 50 g) developed clinical disease, with 80% mortalities within 48 h. The organism was recovered from the kidney, liver and spleen of dead fish. Infection was not achieved following immersion in a dense suspension of the organism (Saeed *et al.*, 1987).

Campylobacteriaceae representative

Arcobacter cryaerophilus

Infections were achieved following i.m. injection with 5×10^5 cells/fish for 7–21 days (Aydin *et al.*, 2002).

Enterobacteriaceae representatives

Citrobacter freundii

The pathogenicity of isolates was not confirmed in laboratory experiments by Sato *et al.* (1982). Nevertheless, Baya *et al.* (1990a) and Karunasagar *et al.* (1992) demonstrated pathogenicity following i.p. injection of rainbow trout and carp, respectively. Here, the LD_{50} was in the range of 10^5–10^6 cells. Injection of cell-free extracts did not result in mortalities (Karunasagar *et al.*, 1992).

Edwardsiella ictaluri

Mortalities of up to 50% have been recorded. In one comparative study, an injection of 1.5×10^3 cells of the pathogen was sufficient to cause 100% mortality among a group of channel catfish; tilapia only demonstrated slight susceptibility, whereas golden shiner, bighead carp and largemouth bass were completely resistant (Plumb and Sanchez, 1983), with daily feeding (of channel catfish) leading to fewer mortalities than groups that were fed less often or starved (Lim and Klesius, 2003). Survival of natural outbreaks has led to high humoral antibody levels and protection from fresh onslaught with *Edw. ictaluri* (Vinitnantharat and Plumb, 1993). The aetiological agent has been also associated with disease outbreaks in non-ictalurid fish, e.g. danio (*Danio devario*) (Waltman *et al.*, 1985).

Little is known about the pathogenicity mechanisms of *Edw. ictaluri*. Extracellular products have been associated with virulence (Stanley *et al.*, 1994; Williams *et al.*, 2003). By comparing virulent with attenuated (these had been subcultured repeatedly in liquid medium) isolates, the latter lacked a 55 kDa OMP, showed markedly less haemolytic activity and had differences in the composition of core oligosaccharide sugars of the LPS compared with the former (Williams *et al.*, 2003). Lawrence *et al.* (2001) using transposon mutagenesis and O side chain mutants, deduced that the LPS O side chains were important for virulence. Rabbit antibodies to 3 (22, 31 and 59 kDa) of 4 major OMPs blocked invasion of cells from fathead minnow (*Pimephales promelas*) by *Edw. ictaluri* demonstrating that some if not all of these OMPs are involved in initial host–pathogen interactions (Skirpstunas and Baldwin, 2003). Saeed (1983) showed that cells are highly piliated, and inferred that the pili might be associated with virulence. By means of intragastric intubation and a comparatively high dose of 1×10^9 cells, *Edw. ictaluri* crossed the intestinal mucosa of channel catfish in 15 min (Baldwin and Newton, 1993). Using 1×10^6 cells/ml and an application directly into the olfactory organs of channel catfish, light and electron microscopy revealed damage after 1 h (Morrison and Plumb, 1994). Certainly, it has been firmly established that channel catfish are highly susceptible to the organism, with an injected dose of 1.5×10^3 cells capable of killing the host within 10 days at a water temperature of 26°C (Plumb and Sanchez, 1983). It does not appear that the organism produces abundant exo-enzymes, which would function as exotoxins in fish. It has been argued that both gut and nares are primary sites for the invasion of *Edw. ictaluri* in natural outbreaks of disease (Shotts *et al.*, 1986). Fluorescence microscopy evidence pointed to the localisation of the organism on the gill

within 5 min and within gill epithelia after 45 min and to the kidney within 4 h of a waterborne route (Nusbaum and Morrison, 2002); the outcome was a bacteraemia within 24 h (Wise *et al.*, 1997). By 72 h, the pathogen was recoverable from the blood. Then by 216 h, there was evidence of the pathogen clearing from the blood, with all survivors developing agglutinating antibodies to *Edw. ictaluri*. Entry, survival (many organisms were present in vacuoles) and replication in head kidney macrophages of channel catfish has been observed microscopically. Opsonisation with normal serum led to even greater internalisation of *Edw. ictaluri* at 0 h, but did not affect replication once internalised (Booth *et al.*, 2006). Uptake of the pathogen into host (epithelial) cells may well involve actin polymerisation and receptor-mediated endocytosis (Skirpstunas and Baldwin, 2002). The infection process was accompanied by shedding of the pathogen into the water, a process contributing to transmission of the disease (Wise *et al.*, 1997). It does not appear that the level of dietary iron affected antibody production and thereby influenced the course of an infection (Sealey *et al.*, 1997).

Edwardsiella tarda

To date, the disease has been recorded in a diverse array of fish species, including chinook salmon (Amandi *et al.*, 1982), channel catfish (Meyer and Bullock, 1973), mullet (Kusuda *et al.*, 1976b), carp (Sae-Oui *et al.*, 1984), eels (Wakabayashi and Egusa, 1973), tilapia (Kubota *et al.*, 1981), olive flounder (Han *et al.*, 2006) and flounder (Nakatsugawa, 1983; Mekuchi *et al.*, 1995a; Pakingking *et al.*, 2003). From laboratory-based experiments, pathogenicity has also been demonstrated in steelhead and rainbow trout (Amandi *et al.*, 1982), yellowtail (Nakatsugawa, 1983) and loach (Park *et al.*, 1983). Co-infection of *Edw. tarda* with aquabirnavirus has led to higher mortalities in Japanese flounder (Pakingking *et al.*, 2003).

There has been no difficulty achieving experimental infections of fish with *Edw. tarda*. Thus, using channel catfish, Meyer and Bullock (1973) established an infection by using the i.p. route of administration. At a water temperature of 27°C, deaths in 80% of the population of 5–10 cm long fingerling fish followed within 10 days of injecting an artificially high dose of 8.0×10^7 cells. Similar experiments, with 8×10^6 and 8×10^5 cells resulted in only 40% cumulative mortalities within 10 days. However, these workers pointed to the host specificity of *Edw. tarda*, insofar as brown trout, held at a water temperature of 13°C, did not show any mortalities following injection with the pathogen. Subsequent investigations, however, showed that *Edw. tarda* could indeed infect salmonids. Thus, Amandi *et al.* (1982) demonstrated that the LD_{50} for chinook salmon and steelhead trout, was 4.1×10^6 and 5.6×10^6 cells, respectively. It is worth emphasising that ictalurids were determined to be more sensitive than salmonids, insofar as these workers determined the LD_{50} for channel catfish to be only 4.0×10^5 cells. At lower water temperatures, e.g. 12°C, the LD_{50} was in the region of one order of magnitude higher. Ironically, a water-borne challenge proved to be a failure, although this may reflect the inability of cells grown in nutrient-rich media and therefore not typical of the natural physiological state to survive in the aquatic environment. Nevertheless, Song *et al.* (1982) deduced that of

110 isolates, the lowest LD_{50} (by water-borne challenge) was 3.1×10^7 cells/ml. Clearly, this is not conducive to the notion of a particularly virulent pathogen.

Using immersion, oral, and i.p. and i.m. routes, Mekuchi *et al.* (1995a) succeeded in infecting and killing Japanese flounder (by all routes). The LD_{50} dose was calculated at 7.1×10^1/fish (via i.m. injection), 1.2×10^2/fish (via the i.p. route), 3.6×10^6/fish (by immersion) and 1.3×10^6/fish (orally) (Mekuchi *et al.*, 1995a).

The pathogenic mechanisms were investigated by Ullah and Arai (1983a, b), who reported that, in contrast to *Edw. ictaluri*, pili were absent. Instead, cells were observed to be surrounded by a slime layer. This may help with the adhesion to host cells, and also protect the bacteria from host defences. Conversely, Sakai *et al.* (2004) pointed to a role for fimbriae on haemagglutination (this could also be induced or increased by 3% w/v NaCl; Yasunobu *et al.*, 2006). A relation between motility and virulence was indicated by Matsuyama *et al.* (2005), who observed that non-motile cells were pathogenic by i.p. injection to red sea bream (there was no difference between motile and non-motile cultures recorded in Japanese flounder or yellowtail). Following immersion challenge, Japanese flounder and red sea bream died as exposure to motile and non-motile cultures, respectively (Matsuyama *et al.*, 2005). Opsonised virulent, but not avirulent, cells adhered, survived and even replicated in phagocytes whereas non-opsonised avirulent cells could also replicate intracellularly in phagocytes (Rao *et al.*, 2001). Furthermore, only avirulent cells enabled higher production of ROS intermediates by phagocytes, which suggests that they are more likely to be susceptible to inactivation by this means (Rao *et al.*, 2001). A type III secretion system has been documented (Rao *et al.*, 2004), defective mutants having reduced virulence (Tan *et al.*, 2005). Variable OMP patterns, including some major (25–40 kDa) and many minor proteins (~10–120 kDa), have been identified in isolates when cultured at 25°C. Interestingly, salinity affected OMP composition in some cultures, suggesting heterogeneity in the taxon (Darwish *et al.*, 2001). Haemolysins and dermatotoxins, but not lipases or proteolytic enzymes, were produced *in vitro*, and it was postulated that these exo-enzymes may confer pathogenicity on *Edw. tarda* (Ullah and Arai, 1983a, b). Furthermore, the pathogenic role of dermatotoxins was highlighted in additional experiments (Ullah and Arai, 1983b). This work concluded that two high molecular weight, heat-sensitive dermatotoxins were produced, which in rabbits (not fish!) were found to have separate functions. Thus, one toxin caused erythema within 3–8 h of intracutaneous injection, whereas the second caused oedema followed by necrotic erythema in 5–7 days. Hopefully, at some point this work will be repeated in fish. Proteolytic toxins (molecular weight = 37 kDa), from ECP, have been purified from avirulent cultures (the toxin is not present in avirulent isolates) and the LD_{50} equated to 1.6 g of toxin/g of fish (Suprapto *et al.*, 1996). The organism has been associated with the development of liver hypertrophy following experimental infection of Japanese flounder (Miwa and Mano, 2000).

On iron-deficient medium, many *Edw. tarda* cultures, notably those associated with virulence, produced siderophores (Kokubo *et al.*, 1990; Mathew *et al.*, 2001; Igarishi *et al.*, 2002) and OMPs of which one was considered to be the receptor for the siderophore under iron-limited conditions. Such components permit the pathogen to scavenge for iron in the blood of the host. Certainly, it appears that the ability of

Edw. tarda to acquire iron is an important part of the infection process (Park, 1986; Iida and Wakabayashi, 1990), and it is relevant that an iron-regulated haemolysin gene has been reported (Hirono *et al.*, 1997a). The virulent strains are more resistant to the bacteriostasis of iron-chelating reagents than their avirulent counterparts.

Typical of many bacterial pathogens, *Edw. tarda* adheres to host cells before internalisation, which involves microfilaments and protein tyrosine kinase (Ling *et al.*, 2000). Using green fluorescent protein (GFP) tagged cells, the portals of entry after immersion challenge were identified as the digestive tract, i.e. anterior intestine, gills and body surface of blue gourami (Ling *et al.*, 2001). The bacteria were located in these sites and blood, heart, kidney, liver, muscles, posterior intestine and spleen after 3 days, but declined substantially by 7 days, with only substantial populations remaining in the intestine.

Escherichia vulneris

There is scant information about the pathogenicity of *Esch. vulneris*. It would appear that infection was achieved in rainbow trout of 120 g in weight with death ensuing in 175 h (Aydin *et al.*, 1997). Details about dosages were not included in the original publication.

Hafnia alvei

The culture caused clinical disease in laboratory experiments with rainbow trout. Injection (subcutaneous) of rainbow trout, weighing 150–200 g, and maintained at a water temperature of 4–6°C, resulted in clinical disease, with mortalities occurring between 3 and 10 days.

In the subsequent study by Teshima *et al.* (1992), it was reported that disease took 3 months to develop at 15°C following i.p. injection with 5×10^6 to 3×10^7 cells/ml. So, the inference was that the organism was not very aggressive. Yet, brown trout appeared to be more susceptible with LD_{50} doses of 21.5×10^4 (an isolate from human enteritis, U.K.) to 7.4×10^7 cells (an isolate from rainbow trout in Spain) depending on the culture (Acosta *et al.*, 2002).

Using 23 isolates that were at best of extremely low virulence to gilthead sea bream, pathogenicity was correlated with the bacteriocidal effect of serum. However, in the absence of any clinical signs of disease, the pathogen was capable of remaining viable within the gilthead sea bream for up to 3 months (Padilla *et al.*, 2005).

Klebsiella pneumoniae

Cultures could induce fin and tail rot by immersion for 5 min in 10^7 bacteria/ml, but only after prior abrading the surface of the fins. Disease signs became evident after 3 days, with mortalities being recorded after a further 2 days. Within 5 days of injecting 10^4 cells/fish, there was some reddening of the muscle in animals injected intramuscularly. Seven days after i.m. and i.p. injection, mortalities began, with disease signs including gastro-enteritis, liquefaction of the kidney and the presence

of ascitic fluid in the peritoneal cavity. All fish were dead within 12 days (Daskalov *et al.*, 1998).

Providencia rettgeri

Experimental infection of silver carp by i.m. injection of 5×10^2 bacteria, or by scarification of the fish surface and subsequent exposure to a broth culture, resulted in mortalities of 50% at a water temperature of 18–20°C. Experimentally infected fish showed lesions typical of the farmed stock (Bejerano *et al.*, 1979).

Salmonella enteritica subsp. *arizonae*

The pathogenicity of the single isolate was not confirmed (Kodama *et al.*, 1987).

Serratia liquefaciens

Injection of 10^3 cells killed Atlantic salmon within 72 h. Typically, there was pronounced muscle liquefaction within the vicinity of i.m. injections. ECP resulted in death within 48 h (McIntosh and Austin, 1990b).

Serratia marcescens

Laboratory-based studies confirmed pathogenicity for striped bass ($LD_{50} = 1 \times 10^5$ cells) (LD_{50} in rainbow trout $= 5 \times 10^3$ cells) with death occurring 1–3 or 1–7 days following administration of the cells via the i.m. or i.p. route, respectively (Baya *et al.*, 1992c). Experimentally infected fish displayed muscle necrosis and some signs of haemorrhagic septicaemia. Further work indicated a role for ECP, which possessed marked proteolytic and phospholipase activity. In fish, the ECP caused a cytotoxic response, with mortality occurring 24–48 h after administration. Here, the LD_{50} dose for rainbow trout and striped bass was 0.4 and 4.8 μg of protein/g of fish, respectively (Baya *et al.*, 1992c).

Serratia plymuthica

Laboratory experiments revealed that the LD_{50} dose for rainbow trout was 10^4–10^5 cells. Moreover, such infected fish displayed discoloration and abdominal swelling (Nieto *et al.*, 1990) and extensive surface lesions (Austin and Stobie, 1992b). Isolates were strongly hydrophobic (Rodriguez *et al.*, 1990); a property that may be involved in the adherence of the organism to surfaces.

Yersinia intermedia

It was considered that the organism was of endogenous origin, being a pathogen of cold-stressed (possibly immuno-compromised) fish (Carson and Schmidtke, 1993). Pathogenicity experiments have not been carried out.

Yersinia ruckeri

In terms of pathogenicity to rainbow trout, Type 1 (Hagerman) is the most virulent, followed by Type 2 (O'Leary) and then Type 3 (Australian) (Bullock *et al.*, 1983). The LD_{50} dose has been established to be 3.0×10^5 cells/ml for Type 1, 1.0×10^7 cells/ml for Type 2 and an as yet undetermined dose for Type 3. The degree of pathogenicity of serovars IV and V needs clarification. The new biogroup described by Austin *et al.* (2005a) killed rainbow trout within 4 days at a dose of 10^5 cells/fish. It should be emphasised that exposure to sublethal concentrations of copper, i.e. 7 µg/l for 96 h, rendered the fish more susceptible to infection by *Y. ruckeri* (Knittel, 1981). Moreover, the nature of the virulence factors is incompletely understood. The role of plasmids in virulence has been indicated insofar as the more pathogenic Type 1 isolates possess a large 40–50 mDa plasmid, and some contain a smaller 20–30 mDa plasmid. The larger plasmid is absent from Type 2 cultures (Cook and Gemski, 1982; De Grandis and Stevenson, 1985). Further work is necessary, however, to resolve the precise role of this large plasmid in pathogenicity, especially as it is not thought to carry virulence factors (Guilvout *et al.*, 1988).

The O-antigen, from the LPS of serogroup O1, has been determined to comprise a branched tetrasaccharide, with repeating units containing 2-acetamidino-2,6-dideoxy-L-galactose, 2-acetamido-2-deoxy-D-glucose and 7-acetamido-3,5,7,9-tetra-deoxy-5-(4-hydroxybutyramido)-D-glycero-L-galacto-nonulosonic acid (Beynon *et al.*, 1994).

ECP have been recovered, and demonstrated to have an LD_{50} of 2–9.12 µg of protein/g of fish. The ECP has been found to contain amylase, caseinase, gelatinase, haemolytic (salmon, sheep and trout erythrocytes) lipase and phospholipase activity. A novel 47 kDa azocasein hydrolysing protease, which was produced during the end of the exponential growth phase, was recovered from culture supernatants (Secades and Guijarro, 1999). Later, a serralysin metallo-protease (= metallo-endopeptidase), termed Yrp1, which hydrolyses actin, fibrinogen, gelatin, laminin and myson (but not Type II and Type IV collagen) has been linked to pathogenicity (Fernandez *et al.*, 2002; 2003). Also, aesculin has been attacked (Romalde and Toranzo, 1993). Adhesin activity, at least to the CHSE-214 cell culture, has been recorded (Romalde and Toranzo, 1993).

Evidence has been presented that *Y. ruckeri* may have a siderophore-mediated iron uptake system (Romalde *et al.*, 1991; Fernández *et al.*, 2004), in parallel to *Aer. salmonicida* and *V. anguillarum*. Romalde and colleagues revealed that >3 OMP were induced in iron-limiting conditions. Ruckerbactin, a catecholate siderophore iron acquisition system, has been described (Fernández *et al.*, 2004). As a result of a commendable study, Davies (1991) reported on the OMP of 135 *Y. ruckeri* isolates. Several 36.5–40.5 kDa peptidoglycan-associated proteins and a 36.5 or 38 kDa heat-modifiable protein were characterised. This 39.5 kDa peptidoglycan-associated protein was apparently not produced during logarithmic growth, but increased quantitatively in the stationary phase.

An example of the new approach in examining pathogenicity involved an inbred fish model, i.e. platyfish (*Xiphophorus maculatus*) and cultured fish cells. In particular,

infection with *Y. ruckeri* involved bathing in suspensions containing 10^6 to 10^8 cells (Kawula *et al.*, 1996). The result, in terms of invasion of key tissues, may be summarised as follows:

Cell type	% invasion
Rainbow trout gonad	2.6
Rainbow trout kidney	2.3
Minnow epithelial cells	10.2

Using a strain which had been genetically tagged with green fluorescent dye and by means of immersion and i.p. infectivity experiments, it was observed that the pathogen moved extracellularly and to a less extent intracellularly to the kidney, spleen and peripheral blood (Welch and Wiens, 2005).

Y. ruckeri may be able to outcompete other micro-organisms, which may be an advantage for any potential pathogen, by the production of water-soluble antimicrobial compounds (Michel and Faivre, 1987).

Flavobacteriaceae representatives

Little is known about the pathogenicity of the cytophagas, flavobacteria or flexibacters. Kimura *et al.* (1978a) established that fatal infections by *Fla. branchiophilum* were only induced following water-borne challenge in salmonids weighing ≤ 1.1 g each. Pathogenicity experiments with juvenile rainbow trout demonstrated that the organism occurred abundantly in the gills within 18–24 h after exposure to a dilute bacterial suspension, i.e. 10–20 ml of a 48 h broth culture in 2 l of freshwater. The gill lamellae became very swollen, but the reasons for subsequent fatalities remain unknown (Wakabayashi *et al.*, 1980). Initial attachment of the pathogen to host cells may be by means of pili (Heo *et al.*, 1990).

In laboratory-based experiments, black sea bream were more susceptible to *T. maritimum* than red sea bream following i.m. injection of 0.02 ml of culture per fish, or infection by bathing or direct application of cultures to the tail or mouth. Mortalities, of up to 10%, occurred in 3 days (Wakabayashi *et al.*, 1984). An infection model has also been established for turbot in which the fish were immersed for 18 h with the LD_{50} corresponding to containing 5×10^3 and 5×10^4 cells/ml for isolates from sole (serogroup O3) and turbot (serogroup O2), respectively (Avendaño-Herrera *et al.*, 2004a). In contrast, i.p. injection failed to establish an infection (Avendaño-Herrera *et al.*, 2004a). Other evidence has pointed to a role for the direct introduction of high numbers (4×10^{11} cells/fish) of bacteria onto gill abrasions for establishing infection (Powell *et al.*, 2004, 2005a). ECP, subcellular components (Wakabayashi *et al.*, 1984) and the ability to take up iron via siderophores and by utilization of haem groups (Avendaño-Herrera *et al.*, 2005) have been implicated with pathogenesis. Haemolysin (26.5 μg/fish) and ECP (25.5 μg/fish) killed black sea bream. Pathological signs included the presence of ascitic fluid, enlarged spleen, petechial haemorrhages in the visceral fat and intestine, and suppurating

(= pus-filled) liver. Also, mortalities ensued following i.p. injection of crude LPS with protease (but not when administered separately) (Wakabayashi *et al.*, 1984).

There is a marked variation in the virulence of isolates of *Fla. columnare*. Pacha and Ordal (1963) classified cultures into four grades of virulence, from high to low. Using a highly virulent culture, it was possible to achieve infection of chinook salmon and sockeye salmon following a 2 min dip in a diluted broth culture. Possibly, the organism entered the host through damaged areas of skin, especially if physically abraded or hot-branded (Bader *et al.*, 2003a, 2006), although it recognised that *Fla. columnare* attaches to the gill. Thus, using a common carp gill perfusion approach with bivalent ion-rich water, the presence of nitrite or organic material and high temperatures (= 28°C), it was noted that a highly virulent culture adhered more readily than a culture of low virulence (Decostere *et al.*, 1999a). The adherence receptor was considered to be composed at least partially of carbohydrate, with the adherence ability of *Fla. columnare* correlated with haemagglutination and the capsule (Decostere *et al.*, 1999b). Fujihara *et al.* (1971) achieved 100% mortality in chinook salmon following exposure for 25 min in a suspension containing 2.5×10^5 cells/ml. Deaths followed within 96 h and 8 h at water temperatures of 10 and 22°C, respectively. Adhesion and infection is inversely proportional to the salinity; specifically there was a decline in mortalities from freshwater (98% mortalities) to 3‰ (0% mortalities) for channel catfish (Altinok and Grizzle, 2001). However, the pathogenic mechanism is unclear, but recent work has pointed to an involvement of adhesion, principally insofar as an adhesion-defective mutant had reduced virulence (Bader *et al.*, 2005).

Extracellular proteases have been implicated with *Fla. columnare* (Newton *et al.*, 1997), including chondroitin lyase (Stringer-Roth *et al.*, 2002). Using cell-free extracts (culture supernatants) of *Fla. columnare*, Pacha (1961) obtained muscle damage following injection with the material. However, the result could not be substantiated by detailed, *in vitro*, chemical analyses of the supernatant. Later, two proteases of 53 and 58 kDa were isolated from *Fla. columnare* (Newton *et al.*, 1997), and we assume that these are responsible for tissue damage. Iron may influence the pathogenicity of *Fla. columnare*, insofar as the presence of 0.35–1.4 mg of iron/100 g of fish reduced the survival time following experimental challenge with the pathogen from 20 days to 1 day (Kuo *et al.*, 1981). In contrast, transferrin exerted a negligible effect. *Fla. johnsoniae* has infected barramundi and goldfish via water-borne challenge (Soltani *et al.*, 1994).

Fla. columnare cells have been detected in the gill, mucus and skin within 5 min of immersion challenge of previously abraded fish (15 min without abrading) (Bader *et al.*, 2003).

Fla. psychrophilum has been shown to attach to and even colonise the surface of fish (rainbow trout) eggs (Vatsos *et al.*, 2001, 2006), and is certainly capable of inducing infection of eggs (Atlantic salmon) (Cipriano, 2005). Exposure of disinfected eyed ova of rainbow trout with an extremely high dose of 10^{10} cells of *Fla. psychrophilum*/ml in PBS for 30 and 60 min at 10°C led to the development of clinical signs of RTFS (Rangdale *et al.*, 1997b). Nanoinjection of newly fertilised rainbow trout eggs with 10^1–10^3 CFU/egg led to higher mortalities (95–100% mortalities of the eggs)

than the uninfected controls (0% mortality) at 70 days post-hatch (Eckman *et al.*, 2003). Moreover, bathing rainbow trout fry in a logarithmic-phase culture, i.e. 24 h, led to higher mortalities than use of other phases of the culture growth cycle (Aoki *et al.*, 2005). Bathing (also with stress caused by treatment with formalin) and i.p. injection of 10^4 CFU/rainbow trout (average weight = 1 g) (10^7 cells were needed for larger fish) led to reproducible infections, with results reflecting the nature of the bacterial strain, the stocking density, and the source and weight of the fish (Madsen and Dalsgaard, 1999). Abrasion of skin and its mucus enhanced invasion of *Fla. psychrophilum* via immersion and cohabitation, with the shedding rate from infected fish reflecting water temperature, i.e. 15 rather than 4°C, and the presence of dead fish (Madetoja *et al.*, 2000). Highly virulent cultures are better capable of adhering to intestinal explants and gill tissue in gill perfusion models from rainbow trout than less virulent isolates (Nematollahi *et al.*, 2003, 2005a). The presence of 2 g/l of organic material or 5 mg/l of nitrite increased adhesion to gills (Nematollahi *et al.*, 2003). Adherence and temperature-mediated (greater at 5 than 15°C) agglutination of yeast cells and erythrocytes as a function of serology, i.e. serotype, has been documented, with haemagglutination inhibited by sialic acid, heating to 65°C or treatment with proteinase K (Møller *et al.*, 2003). Electron microscopy of haemagglutinating and non-haemagglutinating cultures revealed the presence of a thin capsule (in both types of cultures), an absence of pili, but the presence of long, tubular blebs particularly in iron-restricted media that released membrane vesicles into the supernatant. Only the membrane vesicles of haemagglutinating cultures had haemagglutinating activity. The conclusion reached by the workers (Møller *et al.*, 2005) was that via surface-blebbing the pathogen releases membrane vesicles with some proteolytic activity that may somehow impede the immune response of the host.

Following infection, the bacteria may be seen embedded in the mucus along the fins, which is where necrosis starts (Martínez *et al.*, 2004). Although the precise pathogenicity mechanism of the organism eluded scientists for many years, it is now appreciated that *Fla. psychrophilum* produces extracellular components, including the ability to degrade gelatin and type II, but not type I and IV, collagen and has zinc metallo-protease like activity (Ostland *et al.*, 2000), which in other organisms would be associated with virulence. Moreover, a psychrophilic metallo-protease, termed Fpp2 and with a molecular weight of ~62 kDa, has been described (Secades *et al.*, 2001, 2003). This protease is different from Fpp1, which is a 55 kDa metallo-protease, and cleaves actin and myosin, i.e. components of muscle (Secades *et al.*, 2003). An OMP of 18 kDa, termed P18, was described, and linked with the S-layer (Massius *et al.*, 2004). Once inside fish, *Fla. psychrophilum* appears to associate with kidney macrophages for which there is a role for sialic acid in the binding process (Wiklund and Dalsgaard, 2003). Moreover, the pathogen has been found to become internalised in spleen phagocytes of rainbow trout, with bacterial numbers increasing with time (Decostere *et al.*, 2001). Within macrophages, the pathogen is cytotoxic and resists ROS, thereby overcoming the bacteria-killing abilities of these cells. Spleen macrophages were found to have lower antibacterial activity compared with those from head kidney; therefore, it was reasoned that the former will be a safer location for *Fla. psychrophilum* to exist (Nematollahi *et al.*, 2005). Additional evidence has

been published that the addition of *Fla. psychrophilum* cells or their metabolites to head kidney phagocytes from rainbow trout lead to immediate oxidative activity as determined by chemiluminescence (Lammens *et al.*, 2000).

Also, research has indicated a link between the presence of oxidised lipids in diets with the development of RTFS (Daskalov *et al.*, 2000). In this work fish that were fed with diets containing oxidised lipids developed dystrophic changes in the kidney, liver and muscle.

Fla. hydatis, although not a proven fish pathogen, produces extracellular, thermostable, glucose-repressible collagenases, which could be involved in pathogenicity (Strohl, Gibb and Tait, pers. commun.).

Intraperitoneal injection of cell-free culture supernatants of *Chrys. scophthalmum* resulted in clinical disease in turbot, with swollen oedematous gill lamellae, and haemorrhaging in the stomach, gastro-intestinal tract, kidney and liver (Mudarris and Austin, 1989).

Francisellaceae representative

Francisella sp.

Kamaishi *et al.* (2005) succeeded in establishing an experimental challenge, and re-isolating the same organism from diseased fish.

Halomonadaceae representative

Halomonas cupida

Pathogenicity was confirmed in laboratory-based experiments involving water-borne challenge with 10^3–10^5 cells/ml. Approximately 75% mortalities were recorded within 4 days Kusuda *et al.* (1986).

Moraxellaceae representatives

Acinetobacter sp.

Nothing is known about the pathogenic mechanisms of this organism. Due to the comparative inactivity of cultures, it seems unlikely that exotoxins are implicated. This leaves a possible role for endotoxins. Nevertheless, it has been established that pure cultures will reproduce the disease condition. Thus, peptone water cultures, administered by i.m. injection into Atlantic salmon fingerlings, each of 15 g average weight, resulted in total mortalities of the population within 72 h at a water temperature of 12°C (Roald and Hastein, 1980).

Moraxella sp.

Laboratory-based experiments revealed that the organism was pathogenic to rainbow trout and striped bass, with an LD_{50} dose of 10^5–10^6 cells (Baya *et al.*, 1990b).

Moritellaceae representatives

Moritella marina

The LD_{50} dose to Atlantic salmon was $<3.5 \times 10^3$ cells (Benediktsdóttir *et al.*, 1998).

Moritella viscosa

The pathogen demonstrated the ability to adhere to mucus from Atlantic salmon epithelial surfaces, i.e. foregut, gills, hindgut, pyloric caeca and skin (Knudsen *et al.*, 1999). Experimental infections were achieved in juvenile cod (minimum lethal dose $= 4.0 \times 10^4$ CFU/fish by i.m. or i.p.) and to a lesser extent in halibut (minimum lethal dose $= 6.5 \times 10^4$ CFU/fish by i.m. or i.p). Cod was infected by immersion challenge in 2×10^7 CFU/ml for two separate periods of one hour with 37.5% mortalities ensuing within 22 days at $9 \pm 1°C$ (Gudmundsdóttir *et al.*, 2006).

Neisseriaceae representative

Aquaspirillum sp.

Slight lesions were reported to occur after 24 h of infection in experimental fish (Lio-Po *et al.*, 1998). This limited pathology casts doubt on the role of the organism as a fish pathogen. Perhaps, synergism with *Aer. hydrophila*, *Pseudomonas* sp. and *Streptococcus* may occur. Alternatively, *Aquaspirillum* may be an opportunistic invader or saprophyte living on diseased tissue.

Oxalobacteraceae representative

Janthinobacterium lividum

Injection of 5×10^2 cells/fish i.m. and i.p. resulted in 100% mortalities within 14 days. Generally, infected rainbow trout fry and fingerlings became lethargic within 2 days. Moribund and newly dead fish displayed pale (almost white) gills, elongated spleen, pale liver, swollen watery kidney, internal haemorrhaging, pronounced gastro-enteritis, and slight amounts of ascitic fluid in the peritoneal cavity (Austin *et al.*, 1992b).

Pasteurellaceae representative

Pasteurella skyensis

Intraperitoneal injection of $1–4 \times 10^6$ cells led to some mortalities among experimental groups of Atlantic salmon within 4 weeks at 15°C (Birkbeck *et al.*, 2002).

Photobacteriaceae representatives

Photobacterium damselae subsp. *damselae*

The pathogen has been implicated with disease in sharks, turbot and yellowtail (Fujioka *et al.*, 1988; Sakata *et al.*, 1989; Fouz *et al.*, 1991). Laboratory infections of *C. punctipinnis* have been established by removing 4 to 6 scales from the flank, scarifying the dermis, and swabbing the wound with 10^7–10^8 viable cells of *Ph. damselae*. At water temperatures of 16.0–16.5°C, the fish developed large ulcers in 3 days, with death following 24 h later. Similar data were recorded after experimental challenge of unscarified animals. However, in the initial study fish from other families appeared to be unaffected by *Ph. damselae*, pointing to host specificity of the pathogen. Thus, representatives of Atherinidae, Clinidae, Cottidae, Embiotocidae, Girellidae and Gobiidae resisted experimental challenge. This is interesting because representatives of these families co-habited the reefs with blacksmith (Love *et al.*, 1981). However, Grimes *et al.* (1984a,b) and Labella *et al.* (2006) successfully infected dogfish and red-banded sea bream ($LD_{50} = 3.9 \times 10^5$ CFU/g of fish) by i.p. injection with *Ph. damselae*. Death ensued in the dogfish within 18 h at an unspecified water temperature. Grimes *et al.* (1984a,b) reported that the organism was highly cytotoxic. A neurotoxic acetylcholinesterase has been described (Pérez *et al.*, 1998).

ECP have been implicated with disease (cytotoxic) processes, with the LD_{50} dose ranging from 0.02–0.43 μg of protein/g of fish with death occurring between 4 and 72 h after administration (Fouz *et al.*, 1993). The ECP were considered to have low proteolytic activity, without evidence of any caseinase, elastinase or gelatinase. In contrast, pronounced phospholipase and haemolytic activity was recorded for turbot (and human and sheep) erythrocytes. It was possible that LPS contributed to heat stability of the toxic fractions (Fouz *et al.*, 1993).

A siderophore-mediated iron-sequestering system has now been described, and almost certainly contributes to the pathogenicity of the organism (Fouz *et al.*, 1994, 1997).

Photobacterium damselae subsp. *piscicida*

Experimental infection may be achieved by i.m. injection, oral uptake or immersion, with maximum mortalities at 18 and 20, but fewer at 15°C (Magariños *et al.*, 2001). Medium composition, and in particular the presence of yeast extract and/or (fish) peptone, enhanced the toxicity of ECPs and the virulence of cells administered via immersion or i.p. injection (Bakopoulos *et al.*, 2002). The fate of the pathogen has been examined by FAT (Kawahara *et al.*, 1989). Thus, following i.m. injection, the pathogen became located initially in the kidney and spleen, before spreading to the gills, heart, intestine and pyloric caeca. Following oral uptake, the pathogen appeared in the stomach, before spreading to the internal organs. After immersion, *Ph. damselae* subsp. *piscicida* located in the gills, and then spread widely to the heart, kidney, liver, pyloric caeca and spleen (Kawahara *et al.*, 1989). Within the tissues of

infected fish, *Ph. damselae* subsp. *piscicida* was seen to accumulate and multiply in the macrophages (Nelson *et al.*, 1989; Elkamel *et al.*, 2003), perhaps after an initial cell adherence stage (Magariños *et al.*, 1996a, b), which appears to involve capsular polysaccharide (this is dependent on the presence of iron and younger, i.e. logarithmic, rather than lag phase cultures) (Magariños *et al.*, 1996b; do Vale *et al.*, 2001), which has a minor role in the binding of haemin (do Vale *et al.*, 2002). The surface-located 22–38 kDa sialic acid, which may inhibit the complement cascade and thus protect the pathogen from the host's antibodies, has a possible role in cell adhesion and survival in the host (Jung *et al.*, 2000). Another study reported that cells of the pathogen were killed by macrophages *in vitro* in 3–5 h (Skarmeta *et al.*, 1995). Yet, the ability to induce apoptosis and thence lysis of sea bass macrophages and neutrophiles has been linked to a plasmid-encoded 56 kDa protein, coined AIP56, which was secreted by virulent, but not avirulent, cultures (do Vale *et al.*, 2003, 2005). Interestingly, passive immunisation with rabbit antiserum against this protein led to protection (do Vale *et al.*, 2003). Using the EPC cell line, bacterial cells were seen to adhere to and become internalised by the cells within vacuoles possibly by endocytosis, remaining intracellular for 6–9 h. Intracellular multiplication was not recorded (López-Dóriga *et al.*, 2000). Increased catalase activity, which is inversely related to the quantity of iron, has been detected in virulent compared with non-virulent cultures leading to the thought that this enzyme may well be involved in survival within the host (Díaz-Rosales *et al.*, 2006).

Resistance to the pathogen may well reflect the size of the fish and the efficiency of the phagocytes (Noya *et al.*, 1995). There is a distinct role for the capsule to protect against phagocytosis (Arijo *et al.*, 1998) and, in immunised fish, protection against complement-mediated killing (Acosta *et al.*, 2006). Comparing five capsulated virulent cultures and one non-capsulated avirulent culture, Arijo *et al.* (1998) recorded significant differences in phagocytosis with the former resisting being engulfed and killed by the macrophages. In a separate development, it was considered that mucus from turbot—thought to contain a glycoprotein—inhibited *Ph. damselae* subsp. *piscicida*, but less so mucus from sea bass and sea bream (Magariños *et al.*, 1995). Perhaps, such observations explain the comparative sensitivity of some fish species, e.g. sea bass and sea bream, to the pathogen.

A siderophore-mediated iron-sequestering mechanism has been found in *Ph. damselae* subsp. *piscicida*, with IROMPs of 105, 118 and 145 kDa in size (Magariños *et al.*, 1994b). Some variability has been detected insofar as isolates expressed a 75 kDa IROMP. Also, others have reported different sizes for the novel proteins associated with iron limitation. Thus, European isolates were considered to express four novel proteins of 63 kDa and three at $\geq$200 kDa, whereas Japanese isolates did not form any different proteins (Bakopoulos *et al.*, 1997b). Bakopoulos *et al.* (2004) reported a novel >206 kDa protein associated with iron sequestration. Indeed, high levels of iron, i.e. 200 mg of iron/kg of feed and 2.5% carbonyl iron to separate groups, in the diet were found to adversely influence the pathogenicity of *Ph. damselae* subsp. *piscicida* in sea bass (Rodrigues and Pereira, 2004). In the carbonyl iron treated group, 64% of the fish died after challenge compared with only 9% of the controls.

Piscirickettsiaceae representative

Piscirickettsia salmonis

Coho salmon, Atlantic salmon and white sea bass were infected, and clinical disease with mortalities resulted after i.p. injection with cell lines of the rickettsia (Garcés *et al.*, 1991; Arkush *et al.*, 2005). Some differences have been detected in the comparative virulence of isolates to coho salmon from British Columbia, Chile and Norway (House *et al.*, 1999). For example, the LD_{50} dose for a Scottish isolate was calculated as $<2 \times 10^3$ cells (Birkbeck *et al.*, 2004). The nature of the antigens to which animals respond has been addressed by Kuzuk *et al.* (1996), who used rickettsias purified from CSE cells by differential and Percoll density gradient centrifugation and rabbit antiserum. The conclusion was that the rabbit antiserum reacted with four protein and two carbohydrate (core region of the LPS) surface-expressed antigens of 65, 60, 54, 51, 16 and ~11 kDa. However, a complication arises as a result of a subsequent study by Barnes *et al.* (1998), who concluded that the major antigens were of 56, 30 and 20 kDa. Nevertheless, by using rickettsial suspensions, attempts were made to infect coho salmon via the gills, intestine (by anal intubation) and skin, with the data revealing that use of all sites led to infection. However, intact skin (injury facilitated invasion) and gills were found to be the most effective entry sites, followed by intestine (Smith *et al.*, 1999, 2004). Isolations have been made from the brain (of coho salmon), and it is reasoned that this may well be an important location of the pathogen in the host (Skarmeta *et al.*, 2000). Experimental evidence has supported the possibility of vertical transmission when, after the examination of male and female broodstock, the pathogen was detected by immunofluorescence in milt and the coelomic fluid in 14/15 of the fish (Larenas *et al.*, 2003). Subsequently, the pathogen was detected in the resultant fry, albeit without evidence of clinical disease. After *in vitro* infection of ova, the organism was seen by SEM to be attached to the surface (of the ovum) by apparent membrane extensions; these were reasoned to allow the later entry into the ovum (Larenas *et al.*, 2003).

Pseudomonadaceae representatives

Pseudomonas anguilliseptica

Eels, challenged by i.p. injection with 3-day-old broth cultures, eventually displayed the same symptoms attributed to natural outbreaks of the disease. Thus, they became gradually inactive, and developed petechial haemorrhages prior to death, which usually occurred in 6 to 10 days (Wakabayashi and Egusa, 1972). This is a short period for death to ensue. Of course, this depends upon the number of cells in the initial challenge. We consider that this is indicative of the effect of exotoxins, probably exo-enzymes, although Dear (1985) could not obtain mortalities following injection of European eels with ECP. Of course, *Ps. anguilliseptica* is not metabolically very active, but most isolates attack proteins (gelatin) and lipids (Tween 80). Therefore, it is suggested that the pathogenicity mechanism involves proteases and lipases. There is no evidence for the presence of an extracellular layer in virulent

isolates as occurs in *Aer. salmonicida*. There is some evidence of species-based susceptibility to Sekiten-byo, with Japanese eels seemingly more prone to the disease than European eels.

It is speculative whether or not this infers that the organism may be more common in and around European eels (or may have originated with this species). A comparative observation is that brown trout are more susceptible than rainbow trout to furunculosis caused by *Aer. salmonicida*. Certainly, *Ps. anguilliseptica* is capable of infecting a greater range of species than represented by the genus *Anguilla*. Thus, experimental infections have been achieved in ayu, bluegill, carp, goldfish and loach (Muroga, *et al.*, 1975). The organism is only of low pathogenicity to rainbow trout (Lönnström *et al.*, 1994).

The presence of sublethal concentrations of copper (100–250 mg/l) in water exacerbates the disease (Mushiake *et al.*, 1984). Evidence points to a reduction in lymphocytes and granulocytes, which leads to lowered phagocytosis (Mushiake *et al.*, 1985).

Pseudomonas chlororaphis

The isolates from Amago trout and the neotype culture of *Ps. chlororaphis* were pathogenic to carp, eels and trout, following challenge by i.m. injection. Total mortalities occurred within 48 h at a water temperature of 22°C, with disease symptoms paralleling those on the naturally infected fish. However, the pathogenicity mechanism is unknown (Hatai *et al.*, 1975).

Pseudomonas fluorescens

Following invasion of the fish, extracellular proteases are probably responsible for the ensuing damage (Li and Fleming, 1967; Li and Jordan, 1968). Sakai *et al.* (1989a) reported the LD_{50} for rainbow trout as 4.2×10^5 cells at 18°C and 1.1×10^5 cells at 12°C.

Pseudomonas plecoglossicida

During surveys of dead ayu (with bloody ascites) in Japan during 1999 and 2001, with the exception of one isolate all the others were non-motile. Moreover, non-motile cells were injected intramuscularly into ayu leading to the recovery of both motile and non-motile cells from the kidney. However, motile cells were recovered after the injection of motile cultures (Park *et al.*, 2002). By use of GFP-labelled cells, the pathogen has been observed to adhere predominantly to the site of microscopic injuries in the fins and skin (Sukenda and Wakabayashi, 2001).

Pseudomonas pseudoalcaligenes

Injection of 10^5 cells by i.p. or i.m. injection into rainbow trout (average weight = 12 g), held at 15°C, resulted in total mortalities within 7 days. Moribund fish revealed the presence of haemorrhaging (internal and around the vent) and ascitic fluid in the peritoneal cavity (Austin and Stobie, 1992b).

Vibrionaceae representatives

Vibrio alginolyticus

Lee (1995) revealed that the organism produced ECP, which was lethal at 0.52 μg/g of fish. The ECP contained a 44 kDa toxic protease, for which the minimum lethal dose was 0.17 μg/g of fish. In comparison, the LD_{50} for ECP to silver sea bream was reported as 0.92 μg/g of fish, with haemolysins and proteases featuring in pathogenicity (Li *et al.*, 2003). Also, administration of ECP led to a reduction in one of the hepatic heat shock proteins (hsp90) during the latter stages of acute infection in silver sea bream (Deane *et al.*, 2004). The LD_{50} of the pathogen to silver sea bream was reported as 4.85×10^4, 5.01×10^5, 3.16×10^6 and $>2.5 \times 10^8$ CFU/ml for i.m., i.p., injury coupled with immersion and immersion, respectively (Li *et al.*, 2003). From, these data, the impact of injury leading to infection is apparent. Following infection of sea bream, there was a rapid and substantial decline in Na^+, K^+ and ATPase activity in the kidney, and a decreased expression of hsp70 in the kidney and liver (Deane and Woo, 2005).

Vibrio anguillarum

AHL signal molecules, which have been recognized in *V. anguillarum*, may well have a role in the expression of virulence factors, e.g. biofilm formation and protease production (Buchholtz *et al.*, 2006). Cultures produced two dominant molecules, i.e. *N*-(2-oxodecanoyl)-L-homoserine lactone and *N*-(3-hydroxy-hexanoyl)-L-homoserine lactone. Smaller amounts of other molecules were also present. Apart from production associated *in vitro* with laboratory cultures, there was evidence that infected fish produce the two dominant AHLs, although there may be some overall differences in balance between the molecules in *in vitro* and *in vivo* conditions (Buchholtz *et al.*, 2006).

The exact mode of infection is unclear, but undoubtedly involves colonisation of (attachment to) the host starting with the skin (Spanggaard *et al.*, 2000), and thence penetration of the tissues. It is regarded that chemotactic motility is necessary for virulence (O'Toole *et al.*, 1999; Larsen *et al.*, 2004), particularly invasion of the host. The pathogen is attracted to amino acids and carbohydrates particularly in intestinal and to a lesser extent to skin mucus (O'Toole *et al.*, 1999). Chemotaxis to serine—more so at higher, i.e. 25°C, than lower, namely 5 and 15°C, temperatures—has been documented (Larsen *et al.*, 2004). Also, chemotaxis was heightened when the bacterial cells were starved for 2 and 8 days (Larsen *et al.*, 2004). Evidence points to a 40.1 kDa flagellin A protein (encoded by the *flaA* gene) being essential for virulence (Milton *et al.*, 1996). Thus, loss of flagella by transposon mutagenesis led to a 500-fold reduction in virulence following an immersion challenge (O'Toole *et al.*, 1996). Flagellum production and virulence by the water-borne, but not i.p., route was correlated with RpoN (O'Toole *et al.*, 1997). As a cautionary note, it is possible that other changes to the bacterial cells may have occurred with the loss of flagella. Ransom (1978) postulated that infection probably begins with colonisation of the posterior gastro-intestinal tract and rectum. This conclusion resulted from the

observation that *V. anguillarum* was seen initially in these sites. Using GFP-labelled *V. anguillarum* cells and immersion challenge, the gastro-intestinal tract of zebrafish was the first site where the pathogen was observed, with chemotactic motility being regarded as essential for the association with the host surface (O'Toole *et al.*, 2004). Horne and Baxendale (1983) reported adhesion of *V. anguillarum* to intestinal sections derived from rainbow trout. All regions of the intestine were colonised (approximately 10^3 cells/cm^2), with maximum attachment occurring within 100 min. It is interesting to note that serogroup O1, but not O2, isolates demonstrated the ability to adhere to mucus from Atlantic salmon epithelial surfaces, i.e. foregut, gills, hindgut, pyloric caeca and skin (Knudsen *et al.*, 1999). Orally administered *V. anguillarum* survived in the stomach of juvenile turbot for several hours, persisted in the intestine and proliferated in faeces (Olsson *et al.*, 1998). This view has been reinforced by a study, which concluded that >50% of the spleens of turbot contained cells of *V. anguillarum* after infection via the oral and rectal routes (Olsson *et al.*, 1996). The skin appears to become colonised within 12 h of immersion in a virulent culture (Kanno *et al.*, 1990). Then, invasion of the liver, spleen, muscle, gills and intestine follows (Muroga and De La Cruz, 1987). Resistance to the potential debilitating effect of fish serum (Trust *et al.*, 1981) may hasten the invasion processes. Some degree of host specificity has been indicated, insofar as strains from rainbow trout were poorly pathogenic to saithe, and *vice versa* (Egidius and Andersen, 1978). This raises the question concerning the size of inoculum necessary to achieve clinical disease. Levine *et al.* (1972) reported lesions at the site of infection in winter flounder after exposure to only 640 cells. These were administered by intradermal injection. Much larger inocula resulted in sizeable mortalities. For example, Evelyn (1971b) determined that *Oncorhynchus keta* and *O. nerka* died within 48 h of receiving, by i.p. injection, 0.1 ml containing 10^7 viable cells of *V. anguillarum*. In a much more spectacular demonstration of virulence, Sawyer *et al.* (1979) established 80–100% mortality in a population of Atlantic salmon following exposure to $1–2.5 \times 10^5$ cells/ml as a bath for 1 h. In this demonstration, the fish were maintained at a water temperature of 10–15°C. However, temperature shocking does exacerbate mortality. Thus, in one series of experiments using rainbow trout, the temperature was decreased from 23 to 10°C resulting in a significantly increased level of mortality, an increase which was not correlated with an impairment in immune parameters (Aoshima *et al.*, 2005).

Turbot larvae have been successfully challenged with *V. anguillarum* orally via live feed (Grisez *et al.*, 1996; Planas *et al.*, 2005). Using 10^3 *Artemia* nauplii/ml and 10^9 *V. anguillarum* cells/ml, the recipient fish died within 4 days (Grisez *et al.*, 1996). Similarly, feeding with rotifers containing *V. anguillarum* cells led to a successful infection of turbot larvae (Planas *et al.*, 2005).

The precise nature of the virulence mechanism of *V. anguillarum* has prompted some excellent work. With the advent of random genome-sequencing, a strain (H775-3) was examined and 40 genes which may well be related to virulence identified, of which 36 genes were considered to be novel to *V. anguillarum*, and included genes for capsule biosynthesis, enterobactin, haemolysin, flagella, LPS biosynthesis, pilus and protease (Rodkhum *et al.*, 2006). The highlight of the early studies was the

discovery that *bona fide* strains of serogroup O1 of the pathogen contained a virulence plasmid, which was associated with an iron uptake system expressed under iron-limited conditions (Crosa *et al.*, 1977, 1980; Crosa, 1980; Wolf and Crosa, 1986; Chen , 1996). This plasmid, designated pJM1 and of 67 kb, has been fully sequenced (Di Lorenzo *et al.*, 2003), and is always present in virulent isolates (Pedersen *et al.*, 1996b,c, 1997b) and may be included on a transposon-like structure (Tolmasky and Crosa, 1995), but absent from those of low virulence. Conversely, the pJM1 plasmid has been found in some avirulent isolates (Pedersen *et al.*, 1997b). Yet, virulence may be attenuated by curing this plasmid (Crosa *et al.*, 1980) or by deleting three plasmid-encoded gene products (Singer *et al.*, 1991). The role for pJM1 concerns specifying an iron-sequestering mechanism, i.e. the low molecular weight siderophore anguibactin for which the precursor is chromosome-mediated 2,3-dihydroxybenzoic acid (Chen *et al.*, 1994), and specific iron transport proteins, of which the angR protein (this is regulated by the regulatory gene *angR* [Salinas and Crosa, 1995] which has been reported as similar to bacteriophage P22 [Farrell *et al.*, 1990]) acts as a positive regulator of anguibactin biosynthesis and the transcription of the iron transport genes *FatA* and *FatB* (Actis *et al.*, 1995; Chen *et al.*, 1996). Also, *V. anguillarum* has a plasmid-encoded histamine decarboxylase gene *angH*, which is essential for the biosynthesis of anguibactin (Barancin *et al.*, 1998). The overall effect is that the system enables the bacterial cell to compete for available iron in the fish tissues. Two OMP have been designated as OM2 (molecular weight = 86 kDa) and OM3 (molecular weight = 79 kDa). The siderophore and OM2 are coded by plasmid pJM1, whereas OM3 is a function of chromosomal involvement. The basic mechanism involves diffusion of the siderophore into the environment, and the formation of iron complexes that attach to OM2, presumably leading to transport of the iron into the bacterial cell (Crosa and Hodges, 1981; Crosa *et al.*, 1983; Tolmasky and Crosa, 1984; Actis *et al.*, 1985; Mackie and Birkbeck, 1992). Thus, invading bacteria may multiply in the host by scavenging successfully for the iron that is bound by high-affinity iron-binding proteins, such as transferrin, lactoferrin and ferritin. These are present in the serum, secretions and tissues, respectively (Bullen *et al.*, 1978). Toranzo *et al.* (1983a) complicated the issue by publishing data that showed that virulent strains, obtained from striped bass, did not contain plasmids. Yet, all the isolates grew in iron-limiting conditions, during which new OMP and a siderophore were found. Chromosomal DNA sequences, which hybridised with pJM1, were present. Thus, it seems likely that the plasmid DNA had become integrated into the bacterial chromosome.

A pJM1-like plasmid, pEIB1, has been sequenced, and determined to comprise 66,164 bp encoding 44 ORFs (>400 bp), containing genes for biosynthesis and regulation of anguibactin, transport of ferric–anguibactin complexes and DNA replication (Wu *et al.*, 2004).

Work proved that a separate iron uptake system was contained on the chromosome. This differed from the plasmid-mediated system, insofar as the diffusible siderophore is not utilised as an external siderophore, and different OMP are synthesised (Lemos *et al.*, 1988; Conchas *et al.*, 1991; Mackie and Birkbeck, 1992). Siderophores of the phenolate class, possibly related to enterobactin, have been

found in New Zealand isolates (Pybus *et al.*, 1994). Interestingly, iron uptake mechanisms have been reported in non-pathogenic cultures (Lemos *et al.*, 1991), casting some doubt on the precise relevance of the mechanism to pathogenicity.

But what about the other serogroups? The virulence plasmid, pJM1, has not been found in representatives of any other serogroup (Austin *et al.*, 1995a). Yet, we regard other serogroups, especially serogroup O2 which produces a 50 kDa porin (Davey *et al.*, 1998), as more aggressive to fish than serogroup O1.

The presence of common antigens between *V. anguillarum* and other taxa would explain the cross-protection observed with fish vaccines (e.g. Nakai *et al.*, 1989b). What is the nature of these cross-protecting antigens? From capsular antigens (Rasmussen and Larsen, 1987), attention focused on the OMP (Chart and Trust, 1984)—a porin of ~40 kDa molecular weight, which is now regarded as a common antigen (Simón *et al.*, 1996). This porin, Om1, was examined by immunoblotting, ELISA and dot blot; antiserum to Om1 of serogroup 01 cross-reacted with *Vibrio* spp., but not with other genera—except *Plesiomonas shigelloides* (Simón *et al.*, 1998). Detailed investigation described a 38 kDa OMP, which was considered to be involved in environmental adaptation and resistance to bile (Wang *et al.*, 2003).

Debate has centred over the possible interaction of exotoxins and/or endotoxins (Bullock and Conroy, 1971; Abe, 1972; Umbreit and Ordal, 1972; Grischkowsky, 1973; Inamura *et al.*, 1984; De La Cruz and Muroga, 1989). Umbreit and Ordal (1972) reported mortalities in goldfish following injection of filter-sterilised supernatant derived from 24 h broth cultures. Thus, the debilitating effect of bacterial ECP was suggested (but not proven!) at this stage, but later held responsible for virulence (a neurotoxic acetylcholinestase has been isolated from the ECP; Pérez *et al.*, 1998) (Lamas *et al.*, 1994a, b). This experiment was repeated, and resulted in >70% mortalities among a group of goldfish (Umbreit and Tripp, 1975). Unfortunately, the 3-year interval between publications did not achieve any significant improvement in knowledge. A similar basic theme was used by Abe (1972), who injected "endotoxins" into chinook salmon and recorded the presence of haemorrhaging lesions at the point of injection. A significant development stems from the work of Wolke (1975) and Roberts (1976), who suggested that "haemolytic toxins" might be responsible for the anaemic response in infected fish. Subsequent efforts by Munn (1978, 1980) demonstrated conclusively that haemolysins were involved. These were described as thermolabile enzymes (activity was lost by heating to 50°C for 10 min) with optimum pH of 7.2–7.4, which were inactivated by gangliosides. The molecular weight of one of these enzymes was estimated as 191 kDa (Munn, 1980). In the ensuing experiments, haemolytic activity was first detectable in cultures (filtrates) after 19 h incubation at 20°C. Production peaked at 39 h, and then declined. One explanation for this result is that haemolysin production only occurs during the stationary phase of growth. Another possibility is that production of the enzyme occurs intracellularly, with release into the environment taking place at a later time, perhaps during autolysis of the bacterial cells. Munn postulated that inactive haemolysins may also be secreted by the bacteria. These enzymes could then be re-activated by as yet unknown phenomena at a later period. More recently, proteases of 36 kDa molecular weight have also been implicated with virulence (Inamura *et al.*, 1984,

1985; Kodama *et al.*, 1984; Kanemori *et al.*, 1987). In particular, a zinc metallo-protease has been associated with invasion processes (Norqvist *et al.*, 1990). Crude ECP has led to the development of an inflammatory response, including leucopenia, in rainbow trout (Lamas *et al.*, 1994a, b). No doubt the debate will continue unabated for some considerable period, but the ultimate result should be a better understanding of the pathogenicity mechanisms. Basically, at present the scenario involves uptake and penetration of the host tissues, scavenging for iron as a result of a plasmid/chromosomal-mediated trait, and damage to the fish by means of haemolysins and proteases. It is relevant to note that an *empA* zinc metallo-protease, which is secreted as a ~48 kDa proenzyme followed by extracellular activation involving removal of ~10 kDa peptide, was detected during the stationary phase in strains which had been incubated in Atlantic salmon gastro-intestinal mucus (Denkin and Nelson, 2004; Staroscik *et al.*, 2005). Is there a role for quorum-sensing in controlling the expression of virulence genes? Homologues of four quorum-sensing genes, coined *VanT* (this was expressed at low cell density), *VanMN*, *VanPQ* and *VanOU*, have been identified in *V. anguillarum*, and influence expression of the *empA* metallo-protease (Croxatto *et al.*, 2004).

In a rather elegant set of experiments, Harbell *et al.* (1979) catalogued the precise changes in the blood following infection with the pathogen. As anaemia is one of the signs of vibriosis, it seems hardly surprising that the haemoglobin level decreases concomitant with an increase in the erythrocyte osmotic fragility, and a decline in the number of leucocytes. Reductions were also recorded in osmolarity, and in the amounts of plasma protein, albumin, chloride, sodium and alkaline phosphate. In contrast, levels of plasma glucose, lactate dehydrogenase, and glutamic oxaloacetic transaminase increased.

Vibrio cholerae (non-O1)

The evidence suggests that *V. cholerae* is a highly virulent fish pathogen, insofar as ayu and eels may be infected following immersion in only 1.26×10^4 cells/ml and 1.26×10^2 cells/ml, respectively. Yamanoi's team noted that with ayu, mortalities began in 2 to 7 days at water temperatures of 21 and 26°C, but no deaths occurred if the water temperature was at 16°C. In comparison, an eel population suffered 10% mortalities within 5 days at a water temperature of 21°C, and 30% deaths in 3–7 days at 26°C. Clearly, this information suggests that *V. cholerae* is likely to be troublesome only in higher water temperatures.

Vibrio harveyi

Infected (i.p. injection) dogfish (*Squalus acanthias*) died within 18 h. Lemon sharks were more resistant to infection, although internal damage followed the injection of 5×10^7 cells. Yii *et al.* (1997) reported an LD_{50} dose for grouper of 2.53×10^7 CFU/g of body weight, with disease signs reminiscent of the natural infection, i.e. swollen intestine full of a yellowish fluid. This was higher than the LD_{50} value of 1.5×10^5 to 1.6×10^6 CFU/fish reported for sea bass (Pujalte *et al.*, 2003). Although the pathogenic mechanism remains to be fully elucidated, it is relevant to note that the

organism is slightly cytotoxic and produces ECPs (Zhang and Austin, 2000; Zorrilla *et al.*, 2003) containing haemolysins (Zhang and Austin, 2000; Zhu *et al.*, 2006), caseinaase, gelatinase, lipase and phospholipase (Zhang and Austin, 2000). Of relevance, Zhang *et al.* (2001) correlated virulence to salmonids with the possession of duplicate haemolysin genes, termed *vhhA* and *vhhB*. The VHH haemolysin protein demonstrated homology to the lecithinase of other vibrios, namely *V. mimicus* and *V. cholerae*. *vhhA* was over-expressed in *Escherichia coli*, and the purified protein was characterised, and determined to be cytotoxic to flounder gill cells and lethal to flounder with an LD_{50} dose of 18.4 μg of protein/fish (Zhong *et al.*, 2006). Also, siderophores are produced (Owens *et al.*, 1996). More recently, research has highlighted a role for bacteriophage (Oakey and Owens, 2000; Austin *et al.*, 2003) and bacteriocin-like substances (BLIS; Prasad *et al.*, 2005). The *Vibrio harveyi* myovirus like (VHML) phage enhanced virulence of *V. harveyi* to Atlantic salmon and enhanced haemolytic activity (Austin *et al.*, 2003). Using a culture that was pathogenic for salmonids, it was revealed that BLIS was inhibitory to four other isolates of the same taxon and to representatives of other vibrios, including *V. parahaemolyticus*. The BLIS was extracted from cell-free supernatants, and determined to be an unique protein of ~32 kDa molecular weight (Prasad *et al.*, 2005).

Comparatively high doses of *V. trachuri* were required to cause disease in Japanese horse mackerel. Thus, using 36.8 g fish at a water temperature of 26°C, 1.1×10^8 cells/fish caused 100% mortalities within 24 h of i.p. injection. A dose of 1.1×10^7 cells/ml led to 50% mortalities within 4 days. By immersion in 3.6×10^7 cells/ml for 2 min, 100% mortalities ensued within 3 days (Iwamoto *et al.*, 1995). The disease signs mimicked those on naturally infected fish, i.e. erratic swimming and melanosis. Incidentally, the organism failed to infect red seabream.

Vibrio ordalii

The virulence plasmid, pJM1, has not been detected in *V. ordalii* (Crosa, 1980). However, a 30 kb cryptic plasmid, designated pMJ101 and which replicates in the absence of DNA polymerase I without generating single-stranded intermediates, has been found in all isolates of *V. ordalii* (Bidinost *et al.*, 1994; 1999). Moreover, haemolysins and proteases have not been found (Kodama *et al.*, 1984).

Vibrio pelagius

Infectivity experiments with rainbow trout (10 g) and turbot (5 g in size) confirmed virulence, and an LD_{50} of 1.9×10^5 cells/fish and 9.5×10^4 cells/fish, respectively (Angulo *et al.*, 1992). Subsequently, Villamil *et al.* (2003) published the LD_{50} for larval and post-larval turbot as <5 bacteria/ml and 3.9×10^5 bacteria/ml, respectively. The profound virulence for larvae is clearly demonstrated. The administration of bacterial cells to head kidney macrophages resulted in a marked inhibition of the chemiluminescence response when compared with controls, i.e. untreated macrophages, but an increase in the nitric oxide production. Additionally, in turbot larvae, the application of live cells of the pathogen via i.p. injection led to a dramatic inhibition of the chemiluminescence response in one day (Villamil *et al.*, 2003a).

Vibrio salmonicida

Intraperitoneal injection and immersion of Atlantic salmon and rainbow trout with broth cultures led to clinical disease. Atlantic salmon were more susceptible than rainbow trout (Egidius *et al.*, 1986; Hjeltnes *et al.*, 1987). The LD_{50} dose ranges from 4×10^6–1×10^8 cells/fish (Wiik *et al.*, 1989). The presence of other acute diseases, such as infectious pancreatic necrosis, has exacerbated infections in Atlantic salmon caused by *V. salmonicida* (Johansen and Sommer, 2001). In addition, *V. salmonicida* has caused mortalities in cod (Jørgensen *et al.*, 1989). The plasmids, which are regarded as being present in all strains, do not appear to be related to virulence (Valla *et al.*, 1992). Using isolated macrophages from Atlantic salmon and rainbow trout with immunofluorescence techniques, the pathogen has been observed to be internalised (Brattgjerd *et al.*, 1995).

The pathogen demonstrated the ability to adhere to mucus from Atlantic salmon epithelial surfaces, i.e. foregut, gills, hindgut, pyloric caeca and skin (Knudsen *et al.*, 1999).

What about the risk of disease after transferring salmon from fresh to seawater? Eggset *et al.* (1997) concluded that the susceptibility of Atlantic salmon to Hitra disease in seawater possibly reflected the overall quality of the smolts.

Vibrio splendidus

The bacterium was pathogenic to rainbow trout and turbot, with an LD_{50} of 2.2×10^4 cells and 1.2×10^4 cells, respectively (Angulo *et al.*, 1994).

Vibrio vulnificus

The source of infection would seem to be water, with gills as a principal portal of entry into the eel (Marco-Noales *et al.*, 2001). In other work, intramuscular injection of eels with large numbers of bacterial cells, i.e. 4.85×10^8, resulted in 80% mortality in the population within 7 days, at a water temperature of 25°C. This confirms the pathogenicity, albeit weak, of *V. vulnificus*. The pathogenicity mechanisms contained in ECP which are lethal to fish (Lee *et al.*, 1997) included haemolysins, lipases, phospholipases and proteases (Amaro *et al.*, 1992). Evidence has been presented which attributed virulence to the LPS O side chain (Amaro *et al.*, 1997). Interestingly, cultures from diseased European eel produced opaque, translucent colonies, and the cells possessed a capsule, which was not essential for the development of disease (Biosca *et al.*, 1993).

MISCELLANEOUS PATHOGENS

"*Candidatus* Arthromitus"

It was considered that an unspecified toxin may be involved in the pathogenic process (Michel *et al.*, 2002a).

UNKNOWN GRAM-NEGATIVE ROD

According to Palmer *et al.* (1994) infectivity was achieved by injecting cells into Atlantic salmon, which became moribund after 6–10 days, and died within 30 days. Moribund fish displayed haemorrhagic areas in the jaw, cranium and at the base of the fins.

The organism considered to be the cause of *Varracalbmi* was challenged i.p. into groups of Atlantic salmon smolts (average weight = ~37 g) with the outcome that total mortalitiy resulted with a dose of 4×10^7 cells (51% mortality with a dose of 4×10^4 cells) (Valheim *et al.*, 2000).

10

Control

It is worth remembering the age-old adage that "prevention is better than cure", and certainly it is possible to devote more attention to preventing the occurrence of disease in fish. This is especially true for farmed fish, which tend to be at the mercy of all the extremes which their owners are capable of devising. Principally, in the industrialised nations farmed fish are subjected to questionable water quality and high stocking regimes. These are among the known prerequisites for the onset of disease cycles. Yet, the owners are among the first to seek help if anything adverse happens to the valuable stock. Fish may be reared under ideal conditions, in which case, the stock are inevitably in excellent condition without signs of disease. Such sites, for example located in Venezuela and the former Yugoslavia, are usually supplied by fast-flowing, clear river water. Careful feeding regimes are adopted, and the stocking levels are comparatively low. The latter point would make the enterprise unacceptable in the more industrialised nations of Western Europe. Therefore, much attention has been devoted to control measures. These have been categorised in Table 10.1. Although most emphasis has been placed on aquaculture, some effort has gone towards considering disease in wild fish stocks.

WILD FISH STOCKS

It is questionable what, if anything, can be done to control disease in wild fish stocks. Perhaps the first step should be to determine the precise extent of disease among wild fish populations. Surveys have been carried out with a view to assessing the incidence of "abnormalities" in marine fish. Indeed, some workers have attempted to correlate the incidence of disease with pollution (see Chapter 11). It is suggested that attention be focused on archive material, collected at or before the turn of the century. Such material is housed in some museums, e.g. in Liverpool, U.K., and access is usually granted to interested individuals. A detailed study would soon demonstrate whether

Table 10.1. Methods of controlling bacterial fish diseases

Classification of fish stocks	Disease control measures
Wild	Control of pollutants (water quality)
Farmed	1. Adequate husbandry practices 2. Use of genetically resistant fish strains 3. Adequate diet or, where appropriate, use of dietary supplements 4. Use of vaccines 5. Use of non-specific immunostimulants 6. Use of antimicrobial compounds 7. Water treatments 8. Preventing the movement of infected stock 9. Probiotics/Biological control

or not "abnormalities" in fish are a new or old phenomenon. Surely, this information could then be correlated with the changes in pollution of the aquatic environment. It is our contention that dense populations of fish have always maintained a given level of diseased individuals, regardless of whether the populations are shoals in the sea or aquacultural stocks. Therefore, it is possible that reducing pollution will not noticeably alter the health index of wild fish. Nevertheless, by using a circuitous route, it may be possible to ensure that wild fish stocks are not likely to be exposed to pathogens, and therefore be at less risk of disease. Theoretically, this could be achieved by controlling outbreaks of disease in farmed fish and thereby reducing the possibility of pathogens escaping into the environment. It should be emphasised, however, that there is a dearth of information, which suggests that disease may be transferred from farmed to wild fish stocks. At worst, there is a perceived problem, and this could easily escalate into adverse propaganda for the aquaculture industry. It is essential that consideration should be urgently given to control measures, which will reduce any possible risk of pathogens escaping into the natural environment.

FARMED FISH

There are many approaches which need to be adopted in order to control bacterial disease in farmed fish (Table 10.1). These will be explained separately below.

Husbandry

To reiterate a previous point, it is a common problem that, under severe economic pressures, the aquaculturist is tempted to produce the maximum yield of fish in a finite volume of water. Sympathy must be addressed towards the fish farmer, especially in the U.K., insofar as the prices of the principal products, i.e. Atlantic salmon and rainbow trout, have not kept pace with inflation. Smaller profit margins

(if any!) have resulted, which may be offset only by increased production levels. Quite simply, within the intensive cultivation systems, the fish may be "stressed" beyond the limit commensurate with the production of healthy specimens. The stocking density may be too high, and it has been suggested that reducing the stocking density when water temperatures are high may well prevent some diseases, such as columnaris outbreaks in rainbow trout (Suomalainen *et al.*, 2005a). Stress may be compounded by other inappropriate management practices, in which aeration and water flow are insufficient, overfeeding occurs, and hygiene declines below the threshold at which disease is more likely to ensue. It may need only one individual to act as a reservoir of infection to the rest of the stock. Unsatisfactory occurrences, which are readily controlled, include:

- the accumulation of organic matter, namely faecal material and uneaten fish food, within the fish-holding facilities;
- the presence of dead fish for prolonged periods (bad sanitation);
- the accumulation of a biofouling community, i.e. algae and slime, in the fish tanks;
- the depletion of the oxygen content of the water with a concomitant increase in nitrogen levels, especially as ammonium salts;
- the lack of proper disinfection for items entering the fish-holding facilities. Reference is made here to nets, protective footwear and size-grading machinery.

Good basic hygiene (water quality) and farm husbandry practices may successfully alleviate many of the problems attributed to vibriosis. In the case of eels, Kocylowski (1963) recommended transfer to cold, well-aerated water, i.e. to conditions less conducive to the pathogen. Perhaps, it is appropriate to cite the old adage that "cleanliness is next to godliness". Control of Sekiten-byo is possible by means of raising the water temperature in the fish-holding areas to 26–27°C. By keeping the water at this temperature for 2 weeks, followed by reducing it to approximately 21°C, there was no further outbreak of the disease for 5 months (Wakabayashi and Egusa, 1972). On a parallel theme, as columnaris is most severe at high water temperatures, it has been suggested that control may be exercised by keeping the water as cool as possible. However, it is recognised that, in the fish farm environment, this method of control is likely to be virtually impossible.

Genetically resistant stock

This is a topic worthy of greater attention, insofar as there are numerous observations which point to the value of genetically resistant strains or selective breeding for reducing the problems of disease. As a word of caution, however, comparative studies need to be carefully controlled so that meaningful results are obtained. In any comparison, the age, size and relative condition of the animals need to be standardised. Nevertheless, there has been prolonged interest in breeding disease-resistant fish. The starting point was the work of Embody and Hayford (1925) who increased resistance in brook trout to furunculosis by selective breeding. Subsequently, Wolf

(1954) reported the start of an investigation aimed at developing ulcer disease and furunculosis-resistant strains of brook trout and brown trout. Five years later, Snieszko *et al.* (1959) concluded that disease was, indeed, genetically determined. Ehlinger (1964, 1977) echoed this opinion by determining resistance to furunculosis in the progeny of brook trout. Thereafter, a substantial leap forward in knowledge occurred following a publication by Cipriano (1982), who reported varying degrees of resistance to furunculosis among 11 different strains of rainbow trout, and correlated this with the serum neutralisation titre. Cipriano determined that the McConnaughy strain was the most susceptible, with 83% of the animals dying within 14 days of challenge with 1.2×10^9 cells administered in a 1 min bath. The serum neutralisation titre was 1:80 against one of the extracellular fractions of *Aer. salmonicida*. In contrast, there was no mortality among the Wytheville strain, which demonstrated a serum neutralisation titre of 1:2,560. Cipriano (1983) concluded that serum from rainbow trout (which are naturally resistant to furunculosis) could protect passively immunised brook trout from challenge with a virulent culture. In contrast, the administration of serum from susceptible Atlantic salmon was unsuccessful in conferring resistance upon brook trout. The protective effect of rainbow trout serum was believed to be attributed to the neutralisation of toxic components produced by the pathogen. Recent unpublished data have pointed to the ability of certain strains of rainbow trout to tolerate the rigours of furunculosis. Genetic variation in susceptibility of Atlantic salmon has been examined in the study of one-year-old fish (Gjedrem and Gjoen, 1995).

Do resistant fish strains exist against other diseases? An affirmative response must be given as work has pointed to some strains of fish which are resistant to BKD (Suzumoto *et al.*, 1977; Winter *et al.*, 1979; Withler and Evelyn, 1990) and selective breeding enhances resistance to ERM and RTFS (Henryon *et al.*, 2005). Studying comparative resistance to BKD in three juvenile coho salmon and steelhead trout strains (transferrin genotypes AA, AC and CC), it was found that the AA genotype was the most susceptible to BKD, whereas the CC genotype was the most resistant. Withler and Evelyn (1990) found a variation in resistance to BKD in two strains of coho salmon from British Columbia, Canada. In particular, survival was greater and the time to death was longer in juvenile animals from the Kitimat River strain than from the Robertson Creek strain. A similar approach has been used to investigate the control of *Edw. ictaluri* infections in channel catfish, with data pointing to a difference in susceptibility according to genetic factors (Camp *et al.*, 2000). Whereas both sensitive and resistant fish produced antibodies to *Edw. ictaluri*, the latter produced more T-lymphocytes in peripheral blood and more macrophage aggregations in the posterior kidney and spleen (Camp *et al.*, 2000).

Amphidromous stock of ayu challenged with *Fla. psychrophilum* experienced fewer mortalities after challenge than domesticated, hybridised or landlocked fish (Nagai *et al.*, 2004; Nagai and Sakamoto, 2006). The comparative resistance of the amphidromous stock could not be correlated with innate immune parameters, for example respiratory burst, serum-killing and phagocytic activity of leucocytes.

There is contradictory evidence about the role of genetically resistant fish strains at conferring resistance to vibriosis. Winter *et al.* (1979) determined that there was no

variation in resistance to vibriosis among different transferrin genotypes of coho salmon and steelhead trout. We support this observation with regards to rainbow trout. However, in contrast, Pratschner (1978) reported the presence of differential resistance to vibriosis between transferrin genotypes of coho salmon. Similarly, Gjedrem and Aulstad (1974) noted significant variation in resistance—also to *V. salmonicida*—among strains of Atlantic salmon.

It is obvious that the breeding of disease-resistant fish may be a valuable addition in the armoury of disease control in aquaculture. However, in fish farming where more than one disease is prevalent, it is not necessarily the case that a fish strain which is resistant to one disease would be similarly tolerant of others. Nevertheless, we consider that disease-resistant strains of fish have potential for areas in which diseases are enzootic. Further effort is clearly required to bring the concept to fruition.

Adequate diets/dietary supplements

An area of comparatively recent interest is that of dietary influence on fish health. The precise nutritional value of commercial feeds is largely unknown. Could essential nutrients be lacking, or other compounds be present in dangerous excess? The answers are largely unknown, although it has been established that some dietary supplements may be beneficial for maintaining the health of fish. For example, Ketola (1983) highlighted a requirement for arginine and lysine by rainbow trout fry, with fin erosion resulting from a deficiency of lysine. Furthermore, enriched—i.e. 2% (w/v)—levels of dietary lysine fed to demand over two weeks led to resistance of channel catfish to *Edw. ictaluri* (Alejandro-Buentello and Gatlin, 2001). Eya and Lovell (1998) reported the beneficial effect of dietary phosphorus at enhancing resistance of channel catfish to infection by *Edw. ictaluri*. Paterson *et al.* (1981) discussed the importance of nutrition in the manifestation of BKD in Atlantic salmon. These workers noted that infected fish had lower serum levels of vitamin A, zinc and iron than uninfected animals. Subsequent experimentation showed that the level of BKD could be reduced by feeding with high levels of trace elements, notably cobalt, copper, iodine, iron, fluorine and manganese, and reducing the quantity of calcium. In further experiments, Lall *et al.* (1985) concluded that high levels of iodine and fluorine, each dosed at 45 mg/kg of food, reduced the occurrence of natural infections of BKD to 3% and 5%, respectively, as compared with 95% and 38% infection in Atlantic salmon fed with commercial diets. Earlier, Woodall and Laroche (1964) demonstrated a reduction in BKD infections by feeding chinook salmon with high levels of iodine (i.e. 10.1 μg/g). This theme was continued by Bell *et al.* (1984), who investigated the effects of sodium-L-ascorbate, zinc, iron and manganese as dietary supplements on the manifestation of BKD. They noted that survival time was inversely related to dietary ascorbate levels when the food was otherwise low in zinc and manganese.

Vitamins—i.e. A, C and E—in diets are of value for controlling infections by a range of bacterial pathogens (Navarre and Halver, 1989; Hardie *et al.*, 1990), including *Aer. hydrophila* (Sobhana *et al.*, 2002), *Edw. tarda* (Durve and Lovell, 1982), *Edw. ictaluri* (Lim *et al.*, 2000) and *Y. ruckeri* (Vigneulle and Gérard, 1986), but not *Str. iniae* (Sealey and Gatlin, 2002). For example, Vigneulle and Gérard (1986) reported

that 48,000 IU/kg, 8,650 mg/kg and 500 mg/kg of vitamin A, C and E for 5 days respectively enhanced resistance to ERM. Clearly, field trials are warranted.

Incorporated into a purified basal medium (Table 10.2), vitamin C (dosed at 150 mg/kg of food) dramatically increased the resistance of catfish to *Edw. tarda* infections. This was carried out at a water temperature of 23°C, but curiously this observation was not confirmed at higher temperatures, e.g. 33°C, when less resistance to infection was noted (Durve and Lovell, 1982). Control groups of fish held at 23°C and fed with diets devoid of vitamin C all died within 96 h of infection with 10^3 cells administered via the i.p. route. With only 30 mg of vitamin C/kg of diet, 85% mortality resulted in the recipient fish after challenge. These mortalities were reduced to 60% and 20% following administration of 60 mg and 150 mg of vitamin C/kg of diet, respectively. However, it is relevant to enquire whether or not the effective dose should be higher. Studies with channel catfish have shown than only 30 mg of vitamin C/kg of diet was sufficient to prevent vitamin deficiencies, which manifest themselves as scoliosis and lordosis. Doubling the dose to 60 mg of vitamin C/kg of diet enabled the maximum rate of wound repair in channel catfish (Lim and Lovell, 1978). As might be expected, there is some variation in the precise levels of vitamin C needed for nutritional requirement among various species of fish. Among the salmonids,

Table 10.2. Composition of the purified basal medium to which different concentrations of vitamin C at 0–150 mg/kg were added[a]

Ingredient	% composition
Carboxymethyl-cellulose	3.0
Cellulose	10.0
Cod liver oil (contains 850 IU of vitamin A and 85 IU of vitamin D/g)	3.0
Dextrin	33.05
Gelatin	9.4
Mineral mix of Williams and Briggs (1963) supplemented with cobalt chloride (1 mg/kg of diet), aluminium potassium sulphate (0.7 mg/kg of diet) and sodium selenite (0.08 mg/kg of diet)	4.0
Soybean oil	4.0
Vitamin-free casein	32.6
Vitamin mix[b] (minus vitamin C)	0.95

[a] From Durve and Lovell (1982).

[b] This contains thiamin (10 mg/kg of diet), riboflavin (20 mg/kg of diet), pyridoxine (10 mg/kg of diet), folic acid (5 mg/kg of diet), calcium pantothenate (40 mg/kg of diet), choline chloride (3,000 mg/kg of diet), niacin (150 mg/kg of diet), vitamin B12 (0.6 mg/kg of diet), retinyl acetate (5,000,000 IU/kg of diet), α-tocopherol (50 mg/kg of diet), cholecalciferol (1,000,000 IU/g) (6 mg/kg of diet), menadione sodium bisulphite (80 mg/kg of diet), inositol (400 mg/kg of diet), biotin (2 mg/kg of diet) and ethoxyquin (200 mg/kg of diet).

rainbow trout needed 100 mg of vitamin C/kg of diet for normal growth, but a 10-fold increase enabled maximum wound repair to occur (Halver *et al.*, 1969). The precise effect of vitamin C on retarding bacterial infections remains largely unknown, although several mechanisms have been postulated. Perhaps, leucocytic or phagocytic activity is stimulated, or synthesis and release of gluco-corticoids enhanced (Durve and Lovell, 1982). Evidence has been presented which pointed to the role of vitamin C in stimulating the humoral and cell-mediated immune response in fish vaccinated with *Aer. hydrophila* vaccines (Anbarasu and Chandran, 2001). The potential benefit of vitamin B6 as a nutritional supplement for Atlantic salmon has been examined, albeit without success (Albrektsen *et al.*, 1995). Thus, fish of 14 g weight were fed with diets supplemented with 0–160 mg of vitamin B_6/kg of feed for 20 weeks. However, challenge with *Aer. salmonicida* revealed that increased dietary levels of vitamin B_6 did not increase resistance to furunculosis.

β-Glucans enhance the non-specific resistance to disease, including *Aer hydrophila* infection (Selvaraj *et al.*, 2005; Kumari and Sahoo, 2006), pasteurellosis (Couso *et al.*, 2003), ERM, Hitra disease and vibriosis, by immunostimulation (Robertsen *et al.*, 1990; Kumari and Sahoo, 2006), In one study, the administration of glucan to carp led to significantly increased leucocyte populations, enhanced proportions of neutrophils and monocytes, and elevated superoxide anion production by kidney macrophages (Selvaraj *et al.*, 2005). Moreover, spray-dried, heterotrophically grown preparations of the unicellular alga *Tetraselmis suecica* have been accredited with antimicrobial activity and possibly immunostimulatory activity when used as dietary supplements (Austin *et al.*, 1992a). In contrast, the presence of heavy metals, principally copper, has been implicated as an initiating factor of vibriosis in eels (Rødsaether *et al.*, 1977). Heavy metals may be exposed to the fish in diets (such as via fish meal), or by way of environmental pollution. It seems likely that the emergence and spread of some diseases, such as RTFS, may be aided by or result from the use of inadequately prepared or stored diets. It is not difficult to see that a poorly nourished fish would be more prone to disease, but greater research effort is needed if this area is to be better understood.

Yeasts have also furnished nucleotides, which have shown promise with controlling infections caused by *Str. iniae*. Using a commercial product, Ascogen, which comprises oligonucleotides from brewer's yeast, feeding trials were carried out for 7–8 weeks with hybrid striped bass followed by bath challenge with *Str. iniae*, with the result that experimental groups showed a higher level of protection when compared with the controls (Li *et al.*, 2004).

Aloe has been found to increase resistance to *V. alginolyticus* infections in rockfish when fed at 5 g aloe/kg of diet for 6 weeks (Kim *et al.*, 1999).

Yeast RNA incorporated in diets at 0.4% (w/v) and fed for 60 days reduced mortalities caused by *Aer. hydrophila* in rohu (*Labeo rohita*) and enhanced the phagocyte respiratory burst activity (Choudhury *et al.*, 2005).

There is a growing interest in the use of whole plant extracts for disease control. As an example, rosemary (*Rosmarinus officinalis*) was used as dried and powdered leaves and as ethyl acetate extracts in feeds in a ratio of 1 : 17 and 1 : 24, respectively, to tilapia for 5 days followed by infection with *Str. iniae* and feeding with the rosemary

and its extract for a further 10 days with a resulting marked reduction in mortalities (Abutbul *et al.*, 2004). The Indian medicinal herb, *Azardirachta indica*, demonstrated marked *in vitro* inhibitory activity against *Aer. hydrophila*. Furthermore, aqueous and ethanol extracts of equi-mixtures of *Azardirachta indica*, *Curcuma longa* and *Ocimum sanctum* had demonstrable *in vitro* inhibitory activity (Harikrishnan and Balasundaram, 2005).

Synergism between low levels of iron and high amounts of long-chain polyunsaturated fatty acids led to a RPS of 70 and 96% after challenge with *Aer. salmonicida* and *V. salmonicida*, respectively (Rørvik *et al.*, 2003).

VACCINES

The rationale for the development of fish vaccines parallels that of other aspects of veterinary and human medicine, i.e. a Utopian desire to rid fish stocks of disease coupled with a healthy regard for profit. In practical terms, the aquaculturist needs to control specific diseases which may be financially crippling in terms of high mortalities. From the opposite viewpoint, the vaccine manufacturer needs substantial (perhaps even multinational) markets in order to ensure profitability of the products. A complicating factor concerns cost of the vaccines to the user. Generally, fish farmers who produce fish for human consumption demand inexpensive, easy-to-use, reliable products; whereas the vaccine supplier needs to charge high fees, which are sufficient to recoup developing and licensing costs, pay current expenses and invest for the future. This difference in opinion between user and supplier may lead to difficulty. Moreover, with a comparatively small aquaculture industry, private vaccine manufacturers are likely to invest resources only in developing vaccines against diseases which are prevalent in many countries, rather than those restricted to small geographical areas or representing novel and emerging conditions. This attitude undermines the whole basis of prophylaxis. No easy solution is envisaged unless research costs are supported by public monies or even from the aquaculture industry itself, as already happens in Scotland with the salmon growers.

Historically, the first serious attempt to develop a bacterial fish vaccine may be traced to the work of Duff (1942), who used chloroform-inactivated cells to protect cutthroat trout (*Salmo clarki*) against furunculosis. Since then, vaccines have been formulated against approximately half of the total number of bacterial fish pathogens. From these endeavours, vaccines to protect against edwardsiellosis, ERM, furunculosis, Hitra disease and vibriosis have reached large-scale commercial production. This is hardly encouraging for a primary prophylactic tool. It is noteworthy that the simplistic approach of using formalin-inactivated whole cells, which works well with edwardsiellosis, ERM, Hitra disease and vibriosis, has met with conflicting results with furunculosis. However, more sophisticated approaches, such as involving genetic engineering techniques, offer hope for the future.

Composition of bacterial fish vaccines

The composition of bacterial fish vaccines may be categorised as follows:

- Chemically or heat-inactivated whole cells. These vaccines may be mono- or polyvalent. Essentially, these are the simplest, crudest and cheapest forms of fish vaccines.
- Inactivated soluble cell extracts, i.e. toxoids.
- Cell lysates.
- Attenuated live vaccines, possibly genetically engineered cells. These would be unacceptable to some regulatory authorities because of the perceived risk that the vaccine strain may revert to a pathogenic mode.
- Subunit vaccines, e.g. the gene product of the *tapA* gene for the control of *Aer. salmonicida* infections (Nilsson *et al.*, 2006).
- DNA vaccines (e.g. Pasnik and Smith, 2006).
- Purified sub-cellular components, e.g. OMP and LPS. These vaccines require a detailed understanding of microbial chemistry, aspects of which are deficient for many of the bacterial fish pathogens.
- Serum for passive immunisation (e.g. Shelby *et al.*, 2002). This is largely of academic interest only, insofar as it is difficult to envisage use of the technique in the fish farm environment. A possible exception is for brood stock or pet fish.
- Mixtures of the components detailed above.

It is difficult to identify any particular type of preparation which excels in terms of protection. Generally, the simplest approach of using inactivated whole cells has received greatest attention. This technique has been successful with a wide assortment of pathogens, including *Aer. hydrophila*, *Edw. ictaluri*, *Fla. columnare*, *Ph. damselae* subsp. *piscicida*, *V. anguillarum*, *V. ordalii*, *V. salmonicida* and *Y. ruckeri*. Indeed, with these pathogens (except *V. anguillarum* and *V. ordalii*) whole-cell vaccines gave superior results to other more complex forms of vaccines (Austin, 1984b). However, even the best vaccines do not completely prevent the occurrence of disease, necessitating the use of costly drugs to combat low levels of infection. Clearly, more research is needed, particularly in determining the precise nature of the protective antibody and of the important antigens. With this information, it may be possible to synthesise the antigens or use genetic-engineering techniques to create vaccine strains suitable for inactivation in straightforward ways.

Methods of vaccine inactivation

Attention has focused on seven methods for inactivating bacterial cells for incorporation into fish vaccines (Austin, 1984b). These are the use of chemicals, namely 3% (v/v) chloroform, 0.3–0.5% (v/v) formalin and 0.5–3.0% (v/v) phenol, heat (e.g. 56°C or 100°C for 30 or 60 min), sonication, and lysis with sodium hydroxide at pH 9.5 or with SDS. Commercially, most interest has centred on the use of formalin, which has given encouraging results with *Aer. hydrophila*, *Edw. ictaluri*, *Ph. damselae* subsp.

piscicida, *Ps. anguilliseptica*, *V. anguillarum*, *V. ordalii* and *V. salmonicida*. However, it is unfortunate that only a few studies have been carried out to compare different inactivated preparations.

Methods of administering vaccines to fish

A number of methods of administering vaccines to fish have been tried with varying degrees of success (see Austin, 1984b), and include:

- Injection, with or without the presence of adjuvant, such as FCA/FIA. This technique is slow, and will inevitably require prior anaesthesia of the animals. Injection is only feasible for valuable fish, brood stock or pet fish. Fortunately, mass injection techniques are available.
- Oral uptake, via food. This should be the method of choice insofar as fish could be fed and vaccinated simultaneously. However, there may be problems with the degradation of the vaccine in the gastro-intestinal tract, although this is being overcome by new oralising compounds.
- Immersion in a solution/suspension of the vaccine. This is quick (i.e. taking 30–120 sec to perform) and easy, permitting large numbers of fish to be readily vaccinated. However, there could be problems regarding disposal of the spent vaccine. Thus, it is debatable whether or not disposal should take place in the fish farm effluent.
- Bathing in a very dilute preparation of the vaccine for prolonged periods, i.e. several hours. This is obviously very economic in the use of vaccine. It is feasible that the technique could be carried out during routine periods of confinement, such as during transportation of the stock between sites. However, with immersion, careful thought needs to be given to the question of disposal.
- Spraying or showering the vaccine onto fish. This can be automated, such that fish are vaccinated on conveyor belts during routine grading.
- Hyperosmotic infiltration. This involves a brief immersion (30–60 sec) in a strong salt solution, i.e. 3–8% (w/v) sodium chloride, followed by dipping for 30–60 sec into the vaccine. This method is very stressful to fish, and its use has been consequently reduced.
- Anal/oral intubation. In particular, anal intubation offers possibilities for by-passing the deleterious effects of the stomach and intestine. The technique is, however, cumbersome and requires further development.
- Ultrasonics (Zhou *et al.*, 2002).

It is often difficult to determine which is the most effective method of vaccine application. The method of choice often reflects the whims of the user as much as scientific reasoning. The available evidence suggests that oral administration fares least well, although it offers some potential, especially as booster doses, for *Aer. hydrophila*, *Aer. salmonicida*, *Fla. columnare*, *Ph. damselae* subsp. *piscicida*, *V. anguillarum*, *V. ordalii* and *Y. ruckeri* vaccines; injection is better that oral uptake in terms of the resultant humoral antibody titre and protection (Austin, 1984b). However,

with *Edw. ictaluri*, *Edw. tarda*, *V. anguillarum* and *V. ordalii* preparations, immersion has been demonstrated as superior to injection. Similarly, with the "vibrio" vaccines the shower method exceeds injection in terms of resulting protection. Only with a *Y. ruckeri* product has injection been determined to be better than immersion vaccination (Austin, 1984b). It is apparent that further, detailed, comparative work is required to emphasise the most appropriate methods for vaccinating fish. However, it must be accepted that vaccines will be the prime prophylactic measure of the future.

VACCINE DEVELOPMENT PROGRAMMES: GRAM-POSITIVE BACTERIA

Streptococcaceae representatives

A formalised suspension of β-haemolytic *Streptococcus* was successful when applied to rainbow trout by immersion or by injection with or without FCA (Sakai *et al.*, 1987, 1989d). An RPS of 70% was achieved, which was superior to the results of Iida *et al.* (1982). Yet, only low titres of agglutinating antibody occurred in fish vaccinated by injection. Conversely, antibodies were not detected in trout, which were vaccinated by immersion (Sakai *et al.*, 1987, 1989d). Using formalin-inactivated capsulated and uncapsulated cells of *Lactococcus garvieae*, which were applied to yellowtail by i.p. injection, long-term protection resulted with challenge with a capsulated culture (Ooyama *et al.*, 1999). Because of differences in antibody response (to uncapsulated, but not capsulated cultures), these workers concluded that the capsule affected immunogenicity, and the protective antigens were most likely to be on the surface of uncapsulated cells and not in the capsule. It was noted that capsulated cells became phagocytosed, and fimbrial-like appendages were seen in the cells after treatment with immune serum (Ooyama *et al.*, 1999).

A vaccine was applied orally to Nile tilapia for 5 days, and following challenge 23 days after the conclusion of vaccination achieved an RPS of 63%, thus demonstrating the feasibility and usefulness of the oral approach (Shoemaker *et al.*, 2006).

A toxoid-enriched whole-cell vaccine, administered to turbot by i.p injection and immersion, gave long-term protection, with RPS of 89–100% and 67–86% recorded for 45 g and 150 g fish, respectively (Toranzo *et al.*, 1995).

Formalin-killed cells of *Str. difficilis* and a culture extract containing 50% protein conjugated to alum, administered intraperitoneally, protected tilapia against challenge with a virulent strain (Eldar *et al.*, 1995c). Protection was correlated by the presence of humoral antibodies. Of relevance for vaccine development, western blots indicated that only a few proteins were actually protective (Eldar *et al.*, 1995c). Using ECPs and encapsulated formalin-inactivated cells, which were administered to Nile tilapia by i.p. injection, Pasnik *et al.* (2005) reported good protection even after 6 months when challenged with a pathogen labelled as *Str. agalactiae*. Also, there were demonstrable antibodies produced in the vaccinates, with a 55 kDa ECP antigen being implicated in vaccine efficacy (Pasnik *et al.*, 2005a). Bath vaccination was less

successful, with an RPS of 34% compared with 80% after administration intraperitoneally (Evans *et al.*, 2004).

VACCINE DEVELOPMENT PROGRAMMES: AEROBIC GRAM-POSITIVE RODS AND COCCI

Renibacterium salmoninarum

There is evidence that under some conditions, renibacteria elicit a humoral and innate immune response in fish (Sanders *et al.*, 1978; Young and Chapman, 1978; Bruno, 1987; Jansson and Ljungberg, 1998; Jansson *et al.*, 2003), for example directed to metallo-protease and haemolysin (Grayson *et al.*, 2001). However, it is apparent that early exposure to the p57 antigen can lead to long-term immunosuppression (Brown *et al.*, 1996). Conversely, removal of the p57 antigen from the surface of renibacterial cells has led to enhanced immunogenicity (Wood and Kaattari, 1996). Administration of experimental vaccines prepared in FCA resulted in the development of humoral antibody (Evelyn, 1971c; Baudin-Laurençin *et al.*, 1977). Evelyn (1971c) detected antibodies in immature sockeye salmon at least 16 months after an i.p. injection with a heat-killed suspension in adjuvant. A second injection after 13 months resulted in a sharp increase in antibody titre from 1:2,560 (after the first injection) to 1:10,247. The protective ability of vaccines is, however, questionable (Sakai *et al.*, 1989e, 1993b). Sakai *et al.* (1993b) compared formalised (RPS = 10–23.8%), heat-killed, pH-lysed (RPS = 35–36%) and UV-killed (RPS = 25%) cells of *Ren. salmoninarum* and streptococci, and concluded that protection of rainbow trout did not develop. Also, Baudin-Laurençin *et al.* (1977) found no protective effect after injection of coho salmon with cells contained in FCA. Paterson *et al.* (1981), using a similar vaccine in Atlantic salmon, reported high agglutination titres and a reduced incidence of BKD lesions after one year, but FAT revealed the same number of bacteria in both vaccinated and unvaccinated (control) fish (Paterson *et al.*, 1981). Although McCarthy *et al.* (1984) reported optimistically that their vaccine worked in fish, close scrutiny of the data suggests success comparable with that of Paterson *et al.* (1981). McCarthy and co-workers used a number of vaccine formulations without adjuvants, including a formalised (0.3% v/v formaldehyde) suspension of cells grown in KDM2, a lysed cell suspension (this was lysed at pH 9.5 by the addition of 10 N sodium hydroxide for 1 h, after which the pH was re-adjusted to 7.2 with 10 N hydrochloric acid), and 50% concentrates of the vaccine. Juvenile rainbow trout were vaccinated by i.p. injection, hyperosmotic infiltration and by 2 min immersion. Vaccinated fish were maintained for 6 weeks at 11°C and then challenged by i.p. injection with living cells of the homologous organism. Best success occurred with the lysed preparation, administered by i.p. injection, although failure greeted attempts to vaccinate fish by immersion or hyperosmotic infiltration. When $\geq$80% of the unvaccinated controls were infected, $\leq$10% of the vaccinated fish were affected. This seems encouraging until it is realised that the workers measured the presence of infection by the presence of macroscopic lesions and the occurrence of Gram-positive bacteria in

the anterior part of the kidney. The occurrence of carriers could not be assessed, because the Gram-staining method is not the most sensitive technique for ascertaining the presence of renibacteria. Attenuated cells of *Ren. salmoninarum* or *Arthrobacter davidanieli* (Salonius *et al.*, 2005) (a commercially available live vaccine named Renogen) gave limited protection, but addition of purified *Ren. salmoninarum* genomic DNA or synthetic oligodeoxynucleotides did not improve protection of chinook salmon following i.p. challenge with a virulent culture (Rhodes *et al.*, 2004a). More recently, a comparison was made between inactivated whole cells of two cultures including the type strain without or with prior heating at 37°C for 48 h that destroys the p57 antigen, a recombinant product based on the p57 antigen in FIA, Renogen and PBS with or without FIA. Following i.p. injection vaccination, the chinook salmon were co-habited with mortalities recorded up to 285 days with the result that protective immunity was not demonstrated in any group (Alcorn *et al.*, 2005).

Although renibacterium is normally regarded as being nutritionally fastidious, two "strains" were isolated from colonies on KDM2 that could grow on regular laboratory media, i.e. TSA and BHIA, and were non-pathogenic when injected i.p. into Atlantic salmon at a dose of 5×10^6 (Daly *et al.*, 2001). When evaluated as live vaccines, the culture which grew on TSA (= Rs TSA1) led to an RPS of 50 and 74% at 74 and 60 days after challenge (Daly *et al.*, 2001).

Mycobacteriaceae representatives

Although there are no vaccines commercially available against fish-pathogenic mycobacteria, it is recognised that there is a cell-mediated response in fish, i.e. rainbow trout (Bartos and Sommer, 1981). Immunisation with *Myc. salmoniphilum* mixed with Freund's adjuvant resulted in delayed hypersensitivity reactions. A DNA vaccine involving *Ag85A* gene-encoding for one of the major secreted fibronectin-binding proteins of *Myc. marinum* and cloned in a eukaryotic expression vector stimulated a protective (120 days after vaccination) humoral immune response, but macrophage phagocytosis or respiratory burst activities, in hybrid striped bass when administered i.m. (RPS = 80% and 90% for 25 μg and 50 mg doses of vaccine, respectively) and to some extent by i.p. (RPS = 20% for the 25 μg dose) (Pasnik and Smith, 2005, 2006). This would indicate the feasibility of eliciting protection in fish against some of the fish-pathogenic mycobacteria. Therefore, there is potential for the development of vaccines.

Nocardiaceae representatives

Initial research was not promising (Kusuda and Nakagawa, 1978; Shimahara *et al.*, 2005), but subsequent research directed at controlling *Noc. seriolae* infection in yellowtail by using live cells of low-virulent isolates of the same taxon (dose = 3.1×10^4 or 10^5 CFU/fish) and other nocardial species (dose = $1.1–1.5 \times 10^8$ CFU/fish), i.e. *Noc. soli*, *Noc. fluminea* and *Noc. uniformis*, which were administered intraperitoneally, led to some benefit. This was the case with *Noc. soli*

(RPS = ~65%) and *Noc. fluminea*, and more so with the low-virulent *Noc. seriolae*. The survivors were completely resistant to *Noc. seriolae* (Itano *et al.*, 2006b).

VACCINE DEVELOPMENT PROGRAMMES: GRAM-NEGATIVE BACTERIA

Aeromonadaceae representatives

Aeromonas hydrophila

Some attention has been devoted to developing vaccines, although commercial products are still not available. Simple preparations of inactivated whole cells, ECPs or OMPs, which may be administered by immersion, injection or via the oral route, appear to work quite well (Schachte, 1978; Acuigrup, 1980b; Lamers and de Haas, 1983; Ruangpan *et al.*, 1986; Rahman and Kawai, 2000; Chandran *et al.*, 2002) with the host response including the production of superoxide anion by the head kidney leucocytes (Basheera John *et al.*, 2002). In this connection, Schachte (1978) recorded that the most convincing immune response, measured in terms of antibody titre, was achieved after using injection techniques. Using formalised whole cells applied by i.p. injection, Ruangpan *et al.* (1986) recorded complete protection in Nile tilapia within only two weeks. Some protection, i.e. 53–61%, occurred only one week after vaccination. Incorporating purified 43 kDa OMP of *Aer. hydrophila* in FCA and a booster 3 weeks later (without FCA) led to a demonstrable immune response and protection in blue gourami (*Trichogaster trichopterus*) (Fang *et al.*, 2000). The next most promising method of application was immersion vaccination, and thence oral methods of administration, which were used successfully by Yasumoto *et al.* (2006), who entrapped *Aer. hydrophila* antigens (protein concentration = 33 μg/ml) in liposomes. The vaccine was fed to carp at doses of 30 μl/fish/day over 3 days leading to detectable humoral antibodies after 2 and 3 weeks (there was a decline in titre at 4 weeks) and protection (at 22 days) after subcutaneous injection with *Aer. hydrophila* at 3.0×10^5 (RPS = 63.6%) or 1.0×10^6 (RPS = 55%) CFU/fish (Yasumoto *et al.*, 2006).

Concerning the method of vaccine inactivation, Lamers and de Haas (1983) deduced that heat-inactivated vaccines (60°C/1 h) gave superior results to formalised products (0.3% formalin). However, it was apparent that concentration of the vaccine, in terms of the numbers of cells, was very important in eliciting an immune response. Thus, using carp as the experimental animal, Lamers and de Haas (1983) concluded that 10^7–10^9 cells generated a distinct agglutinating response whereas 10^5 cells did not. Moreover, secondary doses of vaccine were shown to be beneficial. Nevertheless, single doses of a formalin-inactivated vaccine (containing 10^7–10^9 cells), administered via i.m. injection, were capable of eliciting an immune response which was maintained for 360 days. This demonstrates that fish have immunological memory (Lamers *et al.*, 1985a). Continuing the work, Lamers *et al.* (1985b) vaccinated carp by bathing. Although a single immersion did not result in significant serum antibody levels, secondary vaccination after 1, 3 or 8 months gave rise to a

dramatic immune response. In particular, the highest response resulted from using booster doses at 3 months. However, at 12 months there was no response.

The ability of *Aer. hydrophila* to develop biofilms on surfaces has been exploited, and a study with walking catfish (*Clarias batrachus*) demonstrated that cells from biofilms on chitin flakes gave a higher RPS (=91–100%) and serum antibody titre when administered orally for 20 days compared with preparations derived from suspensions in TSB (RPS = 29–42%) (Nayak *et al.*, 2004). Similarly, Azad *et al.* (1999) used an oral biofilm vaccine (dose = 10^{10} and 10^{13} CFU/g; the bacterial cells were grown on chitin flakes) for 15 days in carp and demonstrated high humoral antibody titres and protection. The question concerning what is so special about biofilms needs to be addressed.

Subcellular components, particularly LPS, offer promise as components of vaccines. Indeed, evidence has been presented that LPS induces cell-mediated protection (= regulates a T-cell like macrophage system) in carp (Baba *et al.*, 1988). Loghothetis and Austin (1996) echoed this view about the immunogenicity of LPS, but also emphasised that rainbow trout responded to exopolysaccharide. Using the modern approach to vaccine development, a live *aroA* vaccine has been evaluated in rainbow trout with success. Interestingly, the growth medium was shown to have marked effect on immunogenicity, with cultures prepared in glucose-containing media—i.e. brain heart infusion (BHI), Luria broth with 0.25% (w/v) glucose and TSB—leading to a reduction in complement consumption and reduced serum susceptibility compared with BHI and Luria broth grown cells which were suspended in PBS. Indeed, these preparations led to higher and longer-lasting serum antibody titres than cells cultured in TSB (Vivas *et al.*, 2005). Another live genetically modified auxotrophic mutant of *Aer. hydrophila* has been evaluated, and environmental concerns addressed in work which determined that the cells disappeared within 15 days, but may well enter an NCBV state (Vivas *et al.*, 2004). In short, there is every possibility that vaccines against *Aer. hydrophila* should work.

Aeromonas salmonicida

The development of an effective vaccine against the rigours of *Aer. salmonicida* infections remains one of the great challenges to researchers. Interest in vaccine development may be traced to the pioneering work of Duff (1942), who produced an orally administered, chloroform-inactivated whole-cell preparation. It is enigmatic that his reported success has not been surpassed, and indeed often not equalled, by subsequent workers. Unfortunately, efforts concerning vaccine development languished as chemotherapy became established as the principal means of disease control. Eventually, however, aforementioned resistance problems with chemotherapeutants led researchers to recognise the need for alternative control measures, and, thus, a resurgence of interest in vaccines ensued. However, it now appears that *Aer. salmonicida* is an inefficient antigen, in terms of its overall capability of stimulating a protective immune response (Tatner, 1989). There is some controversy over the effectiveness of formulations based on ECP. Some studies indicate that they may well be immunosuppressive (Sövényi *et al.*, 1990), whereas others describe their

benefit in terms of immunogenicity (Kawahara *et al.*, 1990). Notwithstanding, modern molecular techniques, principally the PCR, have demonstrated that vaccine antigens do get taken up into the body of fish, namely the head kidney and spleen (Høie *et al.*, 1996).

Some of the problems associated with vaccine development have been summarised below. Essentially, the problems reflect economics, i.e. the perceived need for low-cost products on the part of the fish farmer, versus the desire for substantial profit margins on the part of the vaccine manufacturer/suppliers. Scientific problems exist due to an incomplete understanding of the biology of *Aer. salmonicida*. Specifically, progress has been hindered by the uncertainty surrounding the nature of the antigenic components of *Aer. salmonicida*, the effect of strain differences (NB: Gudmundsdóttir and Gudmundsdóttir, 1997 while examining the cross-protection of vaccines against typical and atypical isolates of *Aer. salmonicida*, concluded that the best protection resulted with autogenous products), and the lack of a consistent and reliable challenge method, although the latter has been improved by the development of effective co-habitation and bath methods (e.g. Bricknell, 1995; Nordmo *et al.*, 1998). Injectable vaccines based on micro-encapsulation with *V. anguillarum* LPS led to significantly higher oxygen consumption, lysozyme activity, specific growth rates and antibody titre to *Aer. salmonicida* in rainbow trout than fish which received inactivated whole cells with or without levamisole or emulsified oil as adjuvants, or micro-encapsulated with or without muramyl dipeptide or β-1,3-glucan (Ackerman *et al.*, 2000). Severe side-effects have resulted from the i.p. injection of oil-adjuvanted vaccines, with the ECP component contributing to inflammation (Mutoloki *et al.*, 2006). Intra-abdominal adhesions have been reported in Atlantic salmon following the i.p. injection of oil-adjuvanted vaccines (Gudmundsdóttir *et al.*, 2003a). Also, there is evidence of temporary immunosuppression following the administration of some vaccines (Inglis *et al.*, 1996). One solution to this problem has been the use of antibiotics, namely amoxycillin dosed at 0.1 ml containing 150 mg/fish, which are administered by injection with the vaccine (Inglis *et al.*, 1996).

The precise composition of the vaccine is of critical importance. To date, scientists have evaluated inactivated whole cells (including those based on IROMP), inactivated L-forms, soluble extracts, attenuated live cells (such as those lacking an A-layer and O-antigen; Thornton *et al.*, 1994), inactivated cells supplemented with toxoids and/or purified sub-cellular components, immune serum (for passive immunisation) and polyvalent preparations, usually including inactivated whole cells of *Aer. salmonicida* and *Vibrio* spp. (e.g. Hoel *et al.*, 1997). Most of the early formulations yielded poor or equivocal results (Table 10.3). The notable exceptions are passive immunisation and the use of attenuated live vaccines (Cipriano and Starliper, 1982; Ellis *et al.*, 1988a, b; Vaughan *et al.*, 1993). The latter was particularly effective in Atlantic salmon, in which experimental use resulted in 12.5% mortalities in the vaccinated group compared with 87.5% mortality among control fish, after challenge with a virulent culture of *Aer. salmonicida*. A live aromatic-dependent *Aer. salmonicida* vaccine, *aroA*, was administered intraperitoneally at 2×10^6 to 2×10^9 live bacteria/fish, resulting in a 253-fold increase in LD_{50} (Vaughan *et al.*, 1993). This live vaccine stimulated T-cells rather than B-cell responses in rainbow trout (Marsden

et al., 1996a). But how long did this live vaccine remain in fish tissues? The evidence revealed that, following i.p. injection, the live vaccine became widely distributed throughout fish (in this case rainbow trout) tissues, with clearance taking 7–9 days at 16°C. Lower temperatures led to more prolonged retention of the bacterial cells within the vaccinated fish (Marsden *et al.*, 1996b).

The novel approaches of using IROMP and inactivated L-forms have met with success (Durbin *et al.*, 1999; McIntosh and Austin, 1993). Using formalin-inactivated cells of *Aer. salmonicida* subsp. *salmonicida* grown in iron-depleted conditions administered to rainbow trout intraperitoneally followed by an oral boost, antibodies were produced against OMP (maximum titre = 1:2,560 at day 105) and IROMPs (maximum titre = 1:12,800 at day 105) and conferred protection (RPS $\geq$ 80%; Durbin *et al.*, 1999). A complex formulation of atypical *Aer. salmonicida* cells (with, an A-layer) grown in iron-depleted and iron-supplemented conditions plus cells of *Aer. bestiarum* in TSB successfully protected goldfish against ulcer disease (RPS $\geq$ 90%) when administered by immersion ($\sim 5 \times 10^7$ cells/ml for 60 sec) followed by oral boosting over 7 days after 28 days (5×10^7 cells/g of feed) (Robertson *et al.*, 2005). Avirulent cells, with altered A-layer, have also been proposed as candidates for live vaccines (Thornton *et al.*, 1991). However, a complication to the various developmental studies comes from the fascinating work of Norqvist *et al.* (1989), who used live attenuated cells of a different bacterial taxon, namely *V. salmonicida*, and reported their effectiveness at controlling infections by *Aer. salmonicida*.

A detailed study revealed that a 28 kDa outer-membrane pore-forming protein (= porin) from *Aer. salmonicida* led to the development of protective immunity in rainbow trout (Lutwyche *et al.*, 1995).

The commercial interest in polyvalent vaccines has resulted in several products, which are regularly used in Europe and elsewhere. The benefit of this approach to controlling furunculosis may be illustrated by the observation that *Vibrio* antigens, particularly *V. salmonicida*, appear to enhance the humoral immune response to *Aer. salmonicida* (Hoel *et al.*, 1997). Moreover, vaccination with *V. salmonicida* antigens led to protection against *Aer. salmonicida* following challenge by co-habitation (Hoel *et al.*, 1998a). This approach could well overcome the perceived problem that *Aer. salmonicida* is a weak antigen (Tatner, 1989). Also, this cross-protection may explain the often superior protection afforded by polyvalent vaccines (Hoel *et al.*, 1998a).

Concerning the use of rough and smooth strains for vaccine preparation, discrepancies are apparent among results obtained by different groups of investigators. Michel (1979) reported that there was no difference in the effectiveness of vaccines prepared with either rough or smooth cultures, when administered orally or via i.p. injection to rainbow trout. In fact, neither type of vaccine was protective. Yet, circulating antibodies were present in fish which received the vaccines via injection. Cipriano (1982a), examining the effectiveness of vaccines prepared from virulent and avirulent cultures, determined an equal level of protection from passive immunisation of brook trout. Similar agglutinin titres, i.e. 1:512, were found in both groups of vaccinated fish. He concluded, therefore, that protective immunogens were common to both virulent and avirulent cultures. In contrast, McCarthy *et al.* (1983) reported

Table 10.3. Vaccines for *A. salmonicida*

A. salmonicida strain used for vaccine and/or challenge	Nature of vaccine	Method of adminis-tration	Nature of challenge	Type of fish used	Water temp. (°C)	Ability of vaccine to protect fish	Ability of vaccine to induce antibody response	Reference
WHOLE CELL								
Virulent	Chloroform-inactivated	Oral	Immersion/i.p./ co-habitation	Cutthroat trout (1–2 yr)	19	+	+(av. 1:80)	Duff (1942)
NG	Heat-inactivated	Oral	i.p.	Brook trout	—	–	–	Snieszko and Friddle (1949)
NG	Heat-inactivated	Oral	Natural	As above	—	+	–	As above
Ex-Brook trout (vaccine)	Formalised	i.p.	i.p.	Brook and Brown trout (0 to 2+ yr)	11	–	+(1:160)	Krantz *et al.* (1964b)
Ex-Brook trout (vaccine)	Formalised + adjuvant	i.p.	i.p.	As above	11	+	+(1:10,520)	Krantz *et al.* (1964b)
ATCC 14174+6 strains from hatcheries	Formalised	Oral	Water-borne	Coho salmon (0 to 1+ yr)	13	–	–	Spence *et al.* (1965)
As above	Formalised + FCA	i.p.	NG	Rainbow trout (2 to 4+ yr)	12	ND	+(1:5,120)	Spence *et al.* (1965)
AS 67	Formalised + FCA	i.p.	ND	Coho salmon (1+ yr)	13	ND	+(1:20,480)	Cisar and Fryer (1974)
Ex-salmon	Formalised + FCA	i.p.	i.p.	Coho salmon (juveniles)	12	+	+(1:40960)	Paterson and Fryer (1974a)

SS-70 (virulent)	Formalised	Oral	Natural	Coho salmon (juveniles)	NG	–	–	Udey and Fryer (1978)
SS-70 (virulent)	Formalised + $Al(OH)_3$	Oral	Natural	Coho salmon (juveniles)	NG	–	–	Udey and Fryer (1978)
S-70 (virulent)	Formalised + FCA	i.p.	Natural	Coho salmon (juveniles)	NG	+	+(NG)	Udey and Fryer (1978)
36/75	Formalised	Oral	i.m.	Rainbow trout	15	–	–	Michel (1979)
36/75	Formalised	i.p.	i.m.	Rainbow trout	15	–	+(1:80,000)	Michel (1979)
FD-2-75	Formalised + FCA	i.p.	ND	Atlantic salmon (1+ yr)	12–15	ND	+(1:0–1:640)	Weber and Zwicker (1979)
A47R	Formalised + FCA	i.p.	Natural	Atlantic salmon (1+ yr)	ND	–	+(1:32)	Palmer and Smith (1980)
A47R	As above	h.i.	Natural	As above	ND	–	+(1:16)	As above
A	Formalised	Oral	Natural	Brown trout (0+ yr)	Ambient	+	+(1:40)	Smith *et al.* (1980)
Virulent	Formalised	Oral	Natural	Brown trout (0+ yr)	Ambient	+	+(NG)	Austin and Rodgers (1981)
Virulent	Unwashed cells, Formalised	i.p.	Water-borne	Brook trout (0+ yr)	12.5	–	+ (1:205)	Cipriano (1982a)
Virulent	washed cells, Formalised	i.p.	Water-borne	Brook trout (0+ yr)	12.5	–	+(1:14)	Cipriano (1982a)
Avirulent	Attenuated, live	Immersion/ dip	Water-borne	Brook trout, Atlantic salmon	12.5	+	+(NG)	Cipriano and Starliper (1982)

(*continued*)

Table 10.3 (*cont.*)

A. salmonicida strain used for vaccine and/or challenge	Nature of vaccine	Method of administration	Nature of challenge	Type of fish used	Water temp. (°C)	Ability of vaccine to protect fish	Ability of vaccine to induce antibody response	Reference
WHOLE CELL (*cont.*)								
Virulent	Formalised + FCA+$Al(OH)_3$	i.p.	Immersion, injection, or co-habitation	Salmon	10–12	+	ND	McCarthy *et al.* (1983)
Avirulent	As above	i.p.	As above	Salmon	10–12	–	ND	As above
Virulent	Formalised	Immersion	Immersion	Chinook and Coho salmon	8-18	+	ND	Johnson and Amend (1984)
Virulent	Formalised	i.p.	i.p.	Coho salmon	13–15 (0+ yr)	+	+(1:5,120)	Olivier *et al.* (1985a)
Avirulent	Formalised	i.p.	i.p.	As above	13–15	+/–	+(1:2,560)	As above
Virulent (MT004; =A-layer$^-$)	Formalised + FCA or FIA	i.p.	Water-borne	Atlantic salmon, rainbow trout	NG	–	ND	Adams *et al.* (1988)
Virulent	Attenuated live cells of *V. anguillarum*	Immersion	i.p.	Rainbow trout	18	+	ND	Norqvist *et al.* (1989)
Virulent (TG 36-75)	Culture supernatant + purified antigen	i.p.	i.m.	Rainbow trout	15	–	+(1:~844)	Michel *et al.* (1990)

MT 423	Formalised + mineral oil adjuvant	i.p.	i.p.	Atlantic salmon parr	9–15	+	+	Inglis *et al.* (1996)
S24-92, V341-95	Formalised autogenous + mineral oil adjuvant	i.p.	i.m.	Atlantic salmon fingerlings	10	+	ND	Gudmundsdóttir and Gudmundsdóttir (1997)
AL2017 (with A-Layer)	Formalised adjuvanted with Montanide	i.p.	Co-habitation	Atlantic salmon	12	+	+	Lund *et al.* (2003)
Atypical	Cell fractions + oil adjuvant	i.p.	i.p.	Spotted wolffish	12	+	?	Lund *et al.* (2003a)
Aer. hydrophila aroA	Live, mutant	i.p.	i.p.	Rainbow trout	16	+	+	Vivas *et al.* (2004a)
ORN2; ORN6	Formalised; IROMP + iron-supplemented	Immersion + Oral boost	i.m.	Goldfish	17	+	+(1:39 =1:396)	Robertson *et al.* (2005)
Atypical	Contained in liposomes	Oral	Immersion	Carp	23	+	+	Irie *et al.* (2005)
Commercial, and autogenous	Killed	i.p.	Immersion	Turbot	15	+	+	Santos *et al.* (2005)

(*continued*)

Table 10.3 (*cont.*)

A. salmonicida strain used for vaccine and/or challenge	Nature of vaccine	Method of administration	Nature of challenge	Type of fish used	Water temp. (°C)	Ability of vaccine to protect fish	Ability of vaccine to induce antibody response	Reference
DISRUPTED CELLS/SUB-CELLULAR COMPONENTS								
Virulent	Water-soluble extract, toxoided with alum	Oral	Natural	Coho salmon (juveniles)	Ambient	–	ND	Klontz and Anderson (1970)
Virulent (AS-Sil 67 to AS SS 70)	LPS endotoxin	i.p.	ND	Coho salmon (juveniles)	7–18	ND	+(1:10,000)	Paterson and Fryer (1974b)
NG	Toxoid	Oral/i.p.	Natural	Coho salmon (juveniles)	Ambient	+/–	+(1:16–1:2048)	Udey and Fryer (1978)
B	Disrupted with SDS	h.i.	Natural	Brown trout	Ambient	+/–	+(1:40)	Smith *et al.* (1980)
B	Disrupted with ultra-sonication	h.i.	Natural	Brown trout	Ambient	+/–	ND	Smith *et al.* (1980)
Virulent	Toxoid, formalised	Oral	Natural	Brown trout (0+ yr)	Ambient	–	–	Austin and Rodgers (1981)
Virulent	ECP, precipitated $(NH_4)_2SO_4$ + ethanol	i.p.	Water-borne	Brook trout (juveniles)	12.5	+	+ (1:122)	Cipriano (1982a)

Virulent/avirulent	Lysed broth cultures + FCA + $Al(OH)_3$	i.p.	Immersion	Salmon	10–18	+	ND	McCarthy *et al.* (1983)
Virulent	Toxoid, formalised and with chloroform	Oral	Natural	Rainbow trout	Ambient	–	ND	Rodgers and Austin (1985)
Virulent	ECP, precipitated with $(NH_4)_2SO_4$	i.p.	i.p.	Coho salmon (juveniles)	13–15	+/–	+(1:1,280)	Olivier *et al.* (1985a)
Avirulent	As above	i.p.	i.p.	As above	13–15	–	+(1:1,280)	As above
Avirulent	Protease	i.m.	i.m.	Atlantic salmon (0+ yr)	NG	+	NG	Shieh (1985)
Virulent (MT004, = A-layer$^-$)	ECP, toxoided	Immersion	Water-borne	Atlantic salmon, rainbow trout	NG	+/–	ND	Adams *et al.* (1988)
265-87, M108-91, S24-92	Formalised ECP + FIA	i.p.	i.m.	Atlantic salmon fingerlings	10	+	+(1:102,400)	Gudmundsdóttir *et al.* (1997)
Various	Heated or formalised + adjuvant	i.p.	Immersion (MT16)	Rainbow trout	13	NG	NG	Lutwyche *et al.* (1995)
Mixed	Polyvalent, formalised + adjuvant	i.p.	Water-borne	Atlantic salmon	7–9	+	+	Hoel *et al.* (1997)
VI 88/09/03175	Formalised + adjuvant	i.p.	Co-habitation	Atlantic salmon	11	+	+ (1:20)	Hoel *et al.* (1998a)

(*continued*)

Table 10.3 (*cont.*)

A. salmonicida strain used for vaccine and/or challenge	Nature of vaccine	Method of adminis-tration	Nature of challenge	Type of fish used	Water temp. (°C)	Ability of vaccine to protect fish	Ability of vaccine to induce antibody response	Reference
DISRUPTED CELLS/SUB-CELLULAR COMPONENTS (*cont.*)								
Avirulent	Formalised	i.p.	Immersion (MT 26)	Rainbow trout	13	+	+(1:128)	Thornton *et al.* (1994)
Virulent	Monovalent + trivalent + adjuvant	i.p., oral + immersion	Co-habitation	Atlantic salmon pre-smolts	10	+	NG	Midtlyng *et al.* (1996)
NG	Formalised + mineral oil adjuvant	i.p.	Natural	Atlantic salmon pre-smolts	6–9	+	NG	Midtlyng *et al.* (1996)
Virulent (Linne, LL, S24, 256/91)	Formalised L-forms	Immersion	i.p.	Atlantic salmon	NG	+(varying)	+(0–1:256)	McIntosh and Austin (1993)
Avirulent (AS 14)	—	—	—	Rainbow trout	—	—	—	—
COMPOUND VACCINES/APPROACHES								
Avirulent	Whole cells, chloroform-inactivated + supernatant	Immersion	Natural	Brown trout	NG	+	ND	Cipriano (1983)
Virulent	Polyvalent whole cells, formalised + toxoid,	Oral	Natural	Rainbow trout (0+ yr)	Ambient	–	ND	Rodgers and Austin (1985)

Virulent	formalin + chloroform-inactivated whole cells, Formalised with A-layer + toxoid, formalin, chloroform + lysine-inactivated	Oral	Natural	Rainbow trout (0+ yr)	Ambient	+	ND	Rodgers and Austin (1985)
Virulent	whole cells, Formalised + A-layer	Oral	Natural	Rainbow trout (0+ yr)	Ambient	+/-	ND	Rodgers and Austin (1985)
Virulent, 3SA	Whole cells, formalised + toxoid + LPS +/− liposomes	Immersion	Natural	Rainbow trout, fry	Ambient	+	ND	Rodgers (1990)
Derived from 644RB (Brivax II)	Formalised or sonicated	i.p.	—	Rainbow trout	16	+	+(1:162,755)	Marsden *et al.* (1996a)
Various virulent	Cloned aromatic dependent mutant	i.p.	i.m.	Rainbow trout, Atlantic salmon	5–14	+	NG	Vaughan *et al.* (1993)
Virulent	Formalised + therapy with oxolinic acid	i.p.	Natural	Atlantic salmon	NG	+	NG	Ford *et al.* (1998)

(*continued*)

Table 10.3 (*cont.*)

A. salmonicida strain used for vaccine and/or challenge	Nature of vaccine	Method of adminis-tration	Nature of challenge	Type of fish used	Water temp. (°C)	Ability of vaccine to protect fish	Ability of vaccine to induce antibody response	Reference
PASSIVE IMMUNISATION								
Virulent	Immune serum	i.p.	Scarification/ water-borne	Coho salmon (0+ yr)	NG	ND	NG	Spence *et al.* (1965)
Virulent	Immune serum (Rainbow trout)	i.p.	Water-borne	Brook trout	12.5	+	ND	Cipriano (1983)
Virulent	Immune serum (Atlantic salmon)	i.p.	Water-borne	Brook trout	12.5	–	ND	Cipriano (1983)
R, virulent strain	Immune serum (rainbow trout)	i.p.	Water-borne	Sockeye salmon	10–18	+	+(1:2,048)	McCarthy *et al.* (1983)
R, virulent (boiled)	As above	i.p.	Water-borne	As above	10–18	–	+ (1:2048)	As above
Virulent	As above	i.p.	Water-borne	As above	10–18	–	+(1:512)	As above
Virulent	Immune serum (rabbit)	i.p.	i.p.	Coho salmon	NG	+	ND	Olivier *et al.* (1985a)
Virulent (MT028, MT048)	Immune serum (rabbit)	i.p.	i.p.	Rainbow trout	11–14	+	ND	Ellis *et al.* (1988a)

NG = not given
ND = not done
h.i. = hyperosmotic infiltration

that, in general, only rough variants conferred protective immunity. A parallel result emanated from the work of Olivier *et al.* (1985a), who ascertained that avirulent cells were less effective immunogens than their virulent counterparts. Both of these groups regarded the A-layer protein as the antigen which probably conferred a protective response by the fish. In another development, Hastings and Ellis (1988) recorded that rainbow trout responded to A-protein and LPS O-antigen and some of the components of the ECP (including proteases; Ellis *et al.*, 1988b). So, it is not surprising that Shieh (1985) demonstrated protection with protease fractions. Others have also demonstrated that the A-layer protein is an important protective antigen in non-oily, Montanide-adjuvanted, injectable, whole-cell inactivated vaccines (RPS = 51–78%), with preparations without an A-layer lacking efficacy in Atlantic salmon, as did those with purified LPS. Again, there was no correlation between protection and antibody production (Lund *et al.*, 2003). Striving to protect spotted wolffish against atypical *Aer. salmonicida*, Lund *et al.* (2003a) confirmed the need for an A-layer in vaccine preparations, but highlighted the necessity of incorporating atypical rather than typical cells (RPS = 82–95%) . The explanation given was that atypical *Aer. salmonicida* had genetically (by AFLP) a serologically different A-layer than their typical counterparts (Lund *et al.*, 2003a). Unfortunately, the desired immersion vaccination strategy did not work insofar as high levels of mortalities resulted after challenge, even when adopting an immersion boost (Grøntvedt *et al.*, 2004).

Researchers should consider the interesting work of Olivier *et al.* (1985b), who noted protection to *Aer. salmonicida* in coho salmon after i.p. injection of formalised cells as well as after injection with FCA. Undoubtedly, the use of adjuvant stimulated non-specific immunity, probably involving macrophage activity. Certainly, i.p. injection has led to activation of leucocytes (Köllner and Kotterba, 2002). From the study of Norqvist *et al.* (1989), it is necessary to question the need for incorporation of *Aer. salmonicida* cells or their cellular components into furunculosis vaccines.

Injection techniques appear to be the most efficacious, whereas the oral route is least promising (Midtlyng *et al.*, 1996). The use of adjuvants, especially mineral oil, in injectable vaccines is clearly beneficial (Midtlyng, 1996) with 16S rRNA and LPS being detected in the head kidney and spleen at two weeks (and in the head kidney at 12 weeks) after injection with a commercial, oil-adjuvanted, formalin-inactivated vaccine (Grove *et al.*, 2003). Indeed, Midtlyng (1996) determined from a field study in Norway that i.p. administration of furunculosis vaccine in a mineral oil adjuvant gave the best protection in Atlantic salmon. Apart from the obvious benefits of FCA (Olivier *et al.*, 1985b), the use of β-1,3 glucan (Vita-Stim-Taito), lentinan and formalin-killed cells of *Ren. salmoninarum* have enhanced the effectiveness of vaccines based on formalised *Aer. salmonicida* cells (Nikl *et al.*, 1991). Possibly, with oral uptake degradation of the vaccine in the gastro-intestinal tract may occur. In this case, there may be potential for the use of micro-encapsulation techniques to avoid such pitfalls. One interesting and relevant approach, which is reminiscent of the probiotic saga, involved the i.p. administration of 10^7 cells/fish of a live auxotrophic *aroA* mutant of *Aer. hydrophila* that protected rainbow trout 30 days later against furunculosis (RPS $\geq$ 60%) and stimulated the humoral and cellular immune response (Vivas *et al.*, 2004a).

Immersion techniques have generated much useful data. Rodgers (1990) reported the benefits of using inactivated whole cells, toxoided ECP and LPS for the protection of juvenile salmonids. Moreover, the vaccinated animals grew better than the controls. Work has indicated that the duration of the immersion vaccination process does not affect the uptake of the vaccine, providing that the antigens are not in low concentrations (Tatner, 1987). Therefore, there appears to be some promise for the widely used immersion vaccination technique with furunculosis vaccines.

Traditionally, oral vaccines were considered to be the least successful insofar as it was reasoned that the antigens became degraded during passage through the stomach and possibly there were issues regarding access to the antibody-producing sites. Liposome-entrapped antigens of atypical *Aer. salmonicida* were fed to carp with the result that there was a stimulation of the immune response, specifically the presence of antibodies in bile, intestinal mucus and serum, and greater protection (fewer mortalities) and a reduction in ulceration compared with the controls (Irie *et al.*, 2005). Gradually, however, oral vaccines have attained favour, and commercial products are now available.

Some of the difficulties with ascertaining the efficacy of vaccines have been ascribed to methods of experimental challenge. Indeed, it is not unusual for vaccines to appear to work in laboratory conditions but to fail dismally in field trials. Under such circumstances, it is questionable whether or not meaningful challenge techniques have been used. For example, the precise dosage of cells to be employed remains undetermined. Apparently, there is substantial variation in virulence among strains. In addition, the most effective means of administering the challenges remains to be elucidated. In this respect, Michel (1980) and Cipriano (1982b) suggested standardised methods of challenge. However, the effectiveness of these techniques awaits clarification.

It is readily admitted that much effort has been expended on the development of furunculosis vaccines. Yet, after 40 years the quest continues. Most studies, to date, have measured effectiveness in terms of the humoral antibody response (e.g. Michel *et al.*, 1990). Unfortunately, there is now some doubt as to whether the presence of humoral agglutinins actually correlates with protection. Maybe, it would be preferable to emphasise other aspects of fish immunology, such as cell-mediated immunity, a notion which has been suggested by McCarthy and Roberts (1980).

Ford *et al.* (1998) treated sea run salmon brood stock with oxolinic acid and vaccinated with a formalised whole-cell vaccine in an attempt to reduce the impact of furunculosis. Encouraging results were obtained insofar as of 2,552 fish captured from the rivers Connecticut and Merrimack and treated in 1986–1992 only 362 died, of which 65 (18%) were diagnosed with furunculosis. In comparison, 206 fish served as untreated controls, with just over half, i.e. 109, dying, of which 63 (= 58%) had furunculosis.

There is ongoing concern about the value of those furunculosis vaccines developed for use in salmonids containing antigens of *Aer. salmonicida* subsp. *salmonicida*, for application in other groups of fish, which may be affected by atypical isolates of the pathogen. For example, a commercial polyvalent product for salmon failed to protect turbot from experimental challenge with *Aer. salmonicida* subsp. *achromo-*

genes (Björnsdóttir *et al.*, 2005). However, Santos *et al.* (2005) appear to have experienced better success with turbot, although the specific pathogen was not equated with subsp. *achromogenes*. Nevertheless, the commercial vaccine, Furovac 5 and an autogenous vaccine resulted in RPS of 72–99% when challenged 120 days after administration intraperitoneally. Even after 6 months, there was still reasonable protection (RPS = 50–52%). In contrast, vaccination by immersion did not lead to significant protection. Interestingly, an oral booster dose did not improve protection (Santos *et al.*, 2005).

Alteromonadaceae representative

Shewanella putrefaciens

A formalin-killed suspension showed promise at controlling mortalities when applied (twice) by i.p. injection (Saeed *et al.*, 1987). Thus, two injections resulted in 40% less mortality than the unvaccinated controls. Vaccination by immersion was unsuccessful.

Enterobacteriaceae representatives

Edwardsiella ictaluri

Studies have been carried out to demonstrate the feasibility of developing a vaccine against *Edw. ictaluri* (Plumb, 1984). Fortunately, the organism is highly immunogenic, with agglutination titres of 1 : 10,000 found in the serum of channel catfish after receiving only single injections of *Edw. ictaluri* cells mixed in Freund's adjuvant (cited in Plumb, 1984). Furthermore, Saeed (1983) and Saeed and Plumb (1987), using an LPS extract, demonstrated protection following i.p. injection. In these experiments, 0.2 mg of LPS injected into channel catfish (individual weight = 60 g) induced agglutination titres of >1 : 500, which was sufficient to confer ≥80% survival of the population. This compared with <30% survival of the unvaccinated controls. Antigenicity of the LPS extract was enhanced in FCA, with protection conferred by single or multiple injections. Similarly, it should be emphasised that an inactivated whole-cell vaccine administered with FCA also showed promise. Immersion and oral boosting of channel catfish fry with a commercial vaccine was successful at controlling mortalities (Plumb and Vinitnantharat, 1993). With this study, mortalities were as follows:

Controls	96.7% mortalities
Immersion-vaccinated group	6.7% mortalities
Immersion-vaccinated + oral-boosted group	3.3% mortalities

In a comparison of techniques (immersion, immersion and oral in combination, and injection), injection led to the highest antibody titre after 10 weeks in channel catfish

fry (Thune *et al.*, 1997). However, a booster by immersion after 25 weeks gave a significant increase in titre. A modern approach reflected the development of an adenine-auxotrophic strain, the virulence of which was attenuated (Lawrence *et al.*, 1997). Following injection, fish were protected against challenge with a virulent culture. An *aroA* mutant achieved success as a live vaccine (Thune *et al.*, 1999). Also, an isogenic transposon-generated O-polysaccharide mutant strain was evaluated as a vaccine administered intraperitoneally and immersion to channel catfish, and achieved useful protection (Lawrence and Banes, 2005).

Edwardsiella tarda

Prophylaxis by vaccination has shown some signs of success (e.g. Guttierez and Miyazaki, 1994; Kwon *et al.*, 2006), with oral administration immunostimulants, namely β-glucan, levamisole and vitamins C and E heightening protection further, especially if harmful (aflatoxin B1 was identified) conditions prevailed (Sahoo and Mukherjee, 2002). Eels responded to the administration of heat- or formalin-killed cells (preferably by injection) by producing humoral antibodies with titres of up to 1:4,096 (Song and Kou, 1981). Song *et al.* (1982) vaccinated eels by immersion for periods of 20 sec to 3 min in suspensions containing 10^5 to 10^8 bacterial cells/ml. Following the i.m. injection of 10 mg amounts of formalised cells/100 g of Japanese eels and a booster after 7 days, an oral challenge resulted in 60–87.5% mortalities in the vaccinates, compared with 80–100% deaths among controls (Gutierrez and Miyazaki, 1994). Rather better protection resulted from use of 1 mg of LPS/100 g body weight of Japanese eels. With this vaccine, 40–57% mortalities were recorded compared with 80–90% of the controls (Gutierrez and Miyazaki, 1994). Unfortunately, Mekuchi *et al.* (1995b) did not find any clear sign of protection in Japanese flounder that had been vaccinated with formalised cells via the i.m., oral or immersion routes. Other work has doubted the importance of cell-mediated immunity in the protection of fish against disease (Miyazaki and Egusa, 1976). This is perhaps surprising in view of the current opinions concerning fish immunology.

Intramuscular injection of eels and red sea bream with LPS resulted in protection from challenge with a virulent culture of *Edw. tarda* (Salati *et al.*, 1987a, b). Moreover, there was a demonstrable humoral immune response (titre = 1:2,048) and phagocytosis by T-lymphocytes. Phagocytic activity in the eels peaked three weeks after vaccination (Kusuda and Taira, 1990). Indeed, the evidence showed that LPS was much more successful as an immunogen than vaccination with a formalised culture (Salati *et al.*, 1987a, b).

Igarashi and Iida (2002) used live-attenuated and formalin-inactivated cells of a mutant, SPM31, constructed with transposon Tn5 with reduced siderophore producing capability. Tilapia were vaccinated intraperitoneally (0.1 mg of vaccine/100 g of fish) whereupon antibodies were produced and protection recorded after challenge for the live (0% mortality), but not the formalin-inactivated (mortality = 80–100%),

preparation (Igarashi and Iida, 2002). Also, Kwon *et al.* (2006) used ghost cells, which were generated by gene *E*-mediated lysis, in tilapia, and demonstrated high protection.

Serratia liquefaciens

Whole-cell formalised vaccines and toxoid preparations were effective for prophylaxis in laboratory-based experiments with Atlantic salmon (McIntosh and Austin, 1990b).

Yersinia ruckeri

The development of vaccines is a story of success until the advent of the new biogroup (Austin *et al.*, 2005a), with commercially available products being marketed for use in aquaculture. Ironically, it is still unknown how these vaccines are taken up by the fish, or for that matter the exact mechanism of their action.

The initial attempts to produce vaccines for ERM may be traced back to the work of Ross and Klontz (1965). These workers used a phenol-inactivated vaccine, which was administered orally, via the food. Success was most encouraging, insofar as 90% of the vaccinated fish survived subsequent infection with *Y. ruckeri*. A comparison of different methods of bacterial inactivation convinced Anderson and Ross (1972) that 3% chloroform was better than sonication, 1% formalin, or 0.5% or 3% phenol. Passive immunisation confirmed that the fish produced humoral antibodies to chloroform-inactivated cells (Busch, 1978). Commercial interest in *Y. ruckeri* vaccines grew with the involvement of the now defunct Tavolek Company. Amend *et al.* (1983), examining factors affecting the potency of preparations, reported that potency was not affected by pH values of 6.5 to 7.7, or by cultivation for up to 96 h in TSB at room temperature. This team concluded that inactivation, whether by formalin or chloroform, did not matter. However, there was good evidence that protection was enhanced by culturing the cells for 48 h at pH 7.2, lysing them at pH 9.8 for 1–2 hours and then adding 0.3% (w/v) formalin. A live auxotrophic *aroA* mutant was evaluated by i.p. injection in rainbow trout, and an RPS of 90% recorded after challenge (Temprano *et al.*, 2005).

Application of these vaccine formulations may be by the oral route, i.e. on food (Klontz, 1963; Ross and Klontz, 1965; Anderson and Nelson, 1974), by injection (Anderson and Nelson, 1974; Cossarini-Dunier, 1986), by immersion, shower or spray (Johnson and Amend, 1983a, b), or by anal intubation (Johnson and Amend, 1983b). Problems have been recorded with the oral method, insofar as protection is short-lived. Thus, in a comparison of injection and oral methods of uptake with a chloroform-inactivated vaccine, Anderson and Nelson (1974) did not find any antibody in fish fed with a vaccine for 7 days; whereas a low titre of 1:16 and 1:32 resulted in trout which were injected. Moreover, injected fish were protected for 12 weeks compared with only 6 weeks in the group which received the oral preparation. Similarly, a comparison of injection, immersion, shower and spray methods

showed that injection offered the best protection against artificial challenge with *Y. ruckeri* (Johnson and Amend, 1983a). In a comparison of the efficacy of injection, oral uptake and anal intubation, Vigneulle (1990) favoured the first-mentioned in terms of protection. Interestingly, antibodies were found in the serum of rainbow trout vaccinated by injection and anal intubation, but not by the oral route. Unfortunately, it must be emphasised that injection is only feasible for large and/or valuable fish and not for the millions of fry/fingerlings which abound on the typical fish farm. However, ERM vaccines have been used successfully on fish farms when administered by bathing (Tebbit *et al.*, 1981). In one investigation, 22,959,239 rainbow trout were vaccinated by a 90 sec dip in a commercial vaccine preparation. The results were very encouraging with significantly reduced losses attributable to ERM. In this particular trial, the vaccine effected an 84% reduction in mortalities due to ERM (Tebbit *et al.*, 1981). Of additional benefit, there was a concomitant decrease in the use of medication by 77%, and an increase in food conversion of 13.7%. Analogous findings have been reported by Amend and Eschenour (1980) and Newman and Majnarich (1982).

Addressing the question of the interval necessary for the onset and duration of immunity to develop, Johnson *et al.* (1982a) reported that a 5 sec immersion in a vaccine suspension was sufficient to induce protection within 5 days at 18°C, or 10 days at 10°C. The minimum size of salmonids necessary for maximal protection was estimated to be in the range of 1.0–2.5 g. In fact, these authors concluded that protection was correlated with size of the fish, and not their age. Thus, with 1.0, 2.0 and 4.0 g fish, immunity lasted for approximately 4, 6 and 12 months, respectively (Johnson and Amend, 1983b). There was some variation in results between species, with coho salmon and sockeye salmon retaining immunity for longer than pink salmon. Lamers and Muiswinkel (1984) concluded that a secondary immune response occurred as long as 7 months after primary contact with the antigen, indicating the presence of a fairly long-lived memory. Cossarini-Dunier (1986) found protection lasted for 445 days after intraperitoneal injection of a formalised culture which was suspended in saline or oily adjuvant. Thus, after challenge, 88.5% of the controls died, but only a few vaccinates.

The commercial immersion vaccines were less successful at controlling the new biogroup (RPS = 47% compared with 95% for the Hagerman strain). This prompted an approach to supplement a current commercial vaccine with formalised whole cells of the new biogroup (RPS = 56% compared with 97% for the Hagerman strain) or by using an autologous vaccine comprised exclusively of formalin-inactivated cells of the new biogroup (RPS = 76% compared with 58% for the Hagerman strain) (Austin *et al.*, 2005). So, there is the potential to increase protection against the new biogroup but at the expense of the more traditional Hagerman strain.

Flavobacteriaceae representatives

Vaccines have been developed only for *Fla. columnare*, although a commercial product is not available. Fujihara and Nakatani (1971) experimented with heat-killed cells, which were administered via food to juvenile coho salmon. The fish responded

with the production of antibody (titre = 1:5,120). Schachte and Mora (1973) concurred with the general view by demonstrating agglutinating antibody in channel catfish. Survivors of infection experiments resisted re-infection, suggesting the presence of a protective immune response (Fujihara *et al.*, 1971). Formalised cells of *Fla. columnare* were administered to eel by immersion and injection, resulting in an immune response (in the skin) two weeks later, and survival of 60% and 20%, respectively (Mano *et al.*, 1996). Interestingly, 14 days following vaccination by immersion and injection of eel with formalin-killed cells of *Fla. columnare*, agglutinating antibody could not be detected in the serum or mucus. Instead, there was inhibition of bacterial adhesion in the skin of the immersion-vaccinated eel (Mano *et al.*, 1996). Formalin-inactivated sonicated and whole-cell preparations of *Fla. columnare* were applied intraperitoneally and by immersion to tilapia with booster doses after 4 weeks (Grabowski *et al.*, 2004). The data revealed that use of formalised sonicated cells in FCA injected by i.p. led to a significant humoral immune response (titre = 1:11,200 by ELISA after 2 weeks; titre = 1:30,600 after boosting). However, there was no information about protection.

Fla. psychrophilum has been investigated as a vaccine candidate: by passive immunisation (LaFrentz *et al.*, 2003); with formalin-inactivated cells administered orally at 0.1–0.2 g/kg body weight of fish for 2 weeks or on 5 days over 2 weeks which led to good protection of ayu after immersion challenge (Kondo *et al.*, 2003); formalin-inactivated cells with water-soluble adjuvant, i.e. Montanide IMS1312 administered i.p. to ayu which led to an RPS of 33% and 39.6% (Nagai *et al.*, 2003); by use of surface antigens (Dumetz *et al.*, 2006); and by use of an auxotroph, i.e. an *aroA* mutant (Thune *et al.*, 2003). Administration of a formalin-killed whole vaccine in FCA intraperitoneally led to high serum and mucosal antibody titres in 9 weeks, and commendable protection (RPS = 83%) (LaFrentz *et al.*, 2002). In parallel, formalin- and heat-inactivated whole-cell preparations of two serotypes in oily adjuvant led to high antibody titres, but not in the skin mucus, and protection (Madetoja *et al.*, 2006). Similarly, use of OMP administered intraperitoneally led to a demonstrable immune response and protection of ayu (RPS = 64 and 71%) and rainbow trout (RPS = 93 and 95%) (Rahman *et al.*, 2002). A surface protein, coined P18, was purified and the responsible gene identified which encoded a 166 amino acid OmpH-like protein. In vaccine trials, using rainbow trout and intraperitoneal administration with FCA, a high antibody titre developed and protection ensued (RPS = 88%) (Dumetz *et al.*, 2006). The auxotroph, which has a mutation in the shikimate pathway, was used successfully by injection and immersion with hybrid striped bass (RPS = 85%) (Thune *et al.*, 2003). Similarly, the use of subcellular components, specifically fractions of 18–28, 41–49 and 70–100 kDa were identified by western blotting in rainbow trout immune serum, and adjuvanted in FCA. Commendable protection was reported after i.p. injection of rainbow trout fry for the 41–49 (RPS = 58%) and 70–100 kDa (O-proteins and O-polysaccharide) fractions with an RPS (for the latter fraction) of 94% (LaFrentz *et al.*, 2004). Certainly, there is evidence that fish respond by producing antibodies to LPS and a ~20 kDa surface protein; the latter of which could be considered for any future vaccine development (Crump *et al.*, 2001, 2005).

Moritellaceae representative

Moritella viscosa

Atlantic salmon, which were vaccinated intraperitoneally with an adjuvanted, whole-cell, formalised suspension containing *Moritella viscosa*, were protected against subsequent challenge, achieving an RPS of 97% (Greger and Goodrich, 1999). A multivalent (containing antigens to five pathogens), oil-adjuvanted vaccine, which contained *Moritella viscosa* antigens, did not induce protection in turbot, but did lead to some intra-abdominal adhesions (Björnsdóttir *et al.*, 2004).

Photobacteriaceae representative

Photobacterium damselae subsp. *piscicida*

Much effort has been expended on vaccine development, with recent research highlighting major antigenic proteins of 7 kDa and 45 kDa (Hirono *et al.*, 1997b). Programmes have included the use of passive immunisation (Fukuda and Kusuda, 1981a), which is of dubious practical value, the more conventional approach of using formalin-inactivated whole-cell preparations (Kusuda and Fukuda, 1980; Fukuda and Kusuda, 1981b; Afonso *et al.*, 2005) and the more modern approaches of genetic engineering. Of relevance, the salinity of the growth medium composition appears to have an effect on the subsequent immune response after vaccination, with 2.5% (w/v) rather than 0.5% NaCl being the more effective (Nitzan *et al.*, 2004). Bacteriological media containing peptones, yeast extract and salt led to the synthesis of a wider range of cellular components, including novel compounds of ~14 and ~21.3 kDa, than those produced in more *in vivo*-type conditions. These compounds were recognised by post-disease sea bass serum (Bakopoulos *et al.*, 2003a). It was demonstrated that administration of a formalin-inactivated preparation in Freund's complete adjuvant by i.p. injection induced agglutinating antibodies in yellowtail. Thus, titres of 1:256–1:2,048 were achieved 5 weeks after vaccination (Kusuda and Fukuda, 1980). Vaccination enhances the nitric oxide response, i.e the production of reactive nitrogen intermediates with their antimicrobial activities, to infection with the pathogen, and is correlated with the level of protection (Acosta *et al.*, 2005). Further work, using a variety of vaccines and application methods, demonstrated conclusively that fish could be protected against subsequent infection by *Ph. damselae* subsp. *piscicida*, although this has been refuted by some workers (e.g. Hamaguchi and Kusuda, 1989). Toxoid-enriched whole cells applied by immersion led to a low-antibody response and a RPS of 37–41% in sea bream (Magariños *et al.*, 1994c). An improved RPS of >60% after 35 days resulted from use of an LPS-mixed, chloroform-killed, whole-cell vaccine (Kawakami *et al.*, 1997). Using formalin-inactivated cells with or without FCA and a range of application methods—namely i.p. injection, 5–7 sec spray, hyperosmotic infiltration and oral uptake via food—Fukuda and Kusuda (1981b) reported encouraging results within 21 days following artificial challenge with *Ph. damselae* subsp. *piscicida*. The best results, conferring 100% protection to the fish, were obtained by use of i.p. injection or by spraying. The titre of agglutinating

antibodies was measured at between 1:4 and 1:128. A subsequent study by these authors has pointed to the value of vaccinating with sub-cellular components, notably bacterial LPS (Fukuda and Kusuda, 1982). In this connection, a whole-cell vaccine in combination with ECPs was used more successfully than a commercial product by immersion for 1 h and i.p. injection in sea bass (Bakopoulos *et al.*, 2003). However, formalin-inactivated whole cells administered intraperitoneally achieved an RPS of 96% in sea bream (Hanif *et al.*, 2005). The question about the nature of the immune response after i.p. vaccination with or without a booster after 4 weeks with an FIA-adjuvanted, inactivated, whole-cell vaccine was addressed by Arijo *et al.* (2004), who demonstrated a humoral response to ECPs, OMP, outer (extremely immunogenic) and cytoplasmic membranes, LPS, and O-antigen.

A bivalent vaccine (with *V. harveyi*) based on formalised cells and ECP administered to sole by immersion with booster or by i.p. injection led to high levels of protection (RPS = ~82%) for 4 months after which the benefit declined (Arijo *et al.*, 2005).

A ribosomal vaccine has been evaluated following administration by i.p. injection into yellowtail. Certainly, the initial evidence pointed to success with ribosomal antigen P (Kusuda *et al.*, 1988; Ninomiya *et al.*, 1989). In a further development, this group experimented with a potassium thiocyanate extract and acetic acid treated "naked cells" obtained from a virulent culture (Muraoka *et al.*, 1991). Yellowtail were vaccinated twice i.p., at one week intervals with the extract (with or without the naked cells), and were challenged two weeks after the second injection. Results indicated partial success for the extract when used alone. However, the extract used in conjunction with naked cells led to good protection (RPS = 36.5). Yet, the corresponding antibody levels were low, suggesting to the researchers that humoral antibodies did not play an important role in protection (Muraoka *et al.*, 1991).

Piscirickettsiaceae representative

Piscirickettsia salmonis

Attempts have been made to develop a vaccine. In one study, formalised cells ($10^{6.7}$ $TCID_{50}$/ml) administered i.p. led to the development of good protection in a field trial with coho salmon (Smith *et al.*, 1995). Heat-inactivated (100°C for 30 min) and formalised whole-cell suspensions containing 10^9 cells/ml gave commendable protection with RPS of 71 and 50%, respectively, when applied intraperitoneally in adjuvant to Atlantic salmon (Birkbeck *et al.*, 2004).

Pseudomonadaceae representatives

Pseudomonas anguilliseptica

Attempts have been made to develop formalin-inactivated vaccines. It is encouraging that fish are capable of eliciting an immune response against *Ps. anguilliseptica*, insofar as experimentally vaccinated eels developed agglutinating antibody within

2 weeks at water temperatures of between 15 and 28°C. The maximum titre recorded was 1:256, which was reached during the 7-week period that an immune response could be detected. Although injection in Freund's adjuvant produced the highest immune response in terms of agglutinating antibodies (titre = 1:4,096), all the commonly used vaccination techniques protected the recipient fish against experimental challenge with virulent cells (Nakai and Muroga, 1979). In field trials with batches of eels, each comprising 2,000 animals, Nakai *et al.*, (1982b) confirmed the efficacy of injectable heat-killed vaccine.

Pseudomonas plecoglossicida

Formalin-inactivated cells administered in oily adjuvant, i.e. Montanide-ISA711 or Montanide-ISA763A, or saline to ayu followed by challenge after 22 and 52 days led to reasonable to excellent protection. Thus, the RPS for the Montanide-ISA711, Montanide-ISA763A and saline vaccines were 17–58%, 57–92% and 65–86%, respectively (Ninomiya and Yamamoto, 2001). An acetone-killed (37°C for 2 h), dehydrated, oral whole-cell vaccine was developed and fed to ayu at two week intervals before challenge (RPS = 40–79%) (Kintsuji *et al.*, 2006).

Vibrionaceae representatives

Vibrio anguillarum

V. anguillarum has been one of the few successful candidates for vaccine development. Commercial formalin-inactivated vaccines are available, which have gained widespread use in mariculture. The benefit of these products is attested by their success in Atlantic halibut (Bricknell *et al.*, 2000; Bowden *et al.*, 2002), African catfish (Vervarcke *et al.*, 2004) and sea bass (Angelidis *et al.*, 2006) when, after application by bathing and following challenge, complete protection was recorded (RPS = 100%). Ironically, the reasons for the success of these products are often obscure, although there is evidence that one commercial, formalin-inactivated whole-cell vaccine induces *Mx* gene (these are inducible by Type I interferons and have a role in antiviral activity) expression in Atlantic salmon after administration intraperitoneally (Acosta *et al.*, 2004). Moreover, there is some evidence to suggest that vaccinated fish generally fare much better, i.e. they exhibit better all-round health and growth characteristics, than the unvaccinated counterparts. Moreover, immunostimulants, e.g. levamisole, further enhance protection (Kajita *et al.*, 1990).

The immunogenicity appears to be a reflection on the presence of heat-stable (to 100–121°C) LPS in the cell wall (Salati *et al.*, 1989b; Kawai and Kusuda, 1995), which may be released in the culture supernatant (Chart and Trust, 1984; Evelyn, 1984), and OMP (Boesen *et al.*, 1997). It has been postulated that a probable mechanism of protection concerns the inhibition of bacterial attachment by unknown factors in the skin mucus (Kawai and Kusuda, 1995). That supernatants are also among the most immunogenic parts of *V. anguillarum* vaccines was verified following the anal uptake of different vaccine fractions in carp and rainbow trout (Joosten *et al.*, 1996). The

large molecular weight LPS, i.e. 100 kDa (Evelyn and Ketcheson, 1980), are considered to confer protection to the recipient host. Moreover, the compounds are able to withstand severe extraction methods. Also, Chart and Trust (1984) isolated, from the outer membrane, two minor proteins with molecular weights of 49–51 kDa, which were potent antigens. A weakly antigenic protein, with a molecular weight of ~40 kDa was also present. Perhaps, these are heat-labile and explain the reasons for the greater protection achieved with formalin-inactivated vaccines compared with heat-killed products (Kusuda *et al.*, 1978c; Itami and Kusuda, 1980). The potential of LPS as an immunogen was clearly demonstrated by Salati *et al.* (1989b). These workers injected i.p. crude LPS (0.05–0.5 mg) into ayu. Following challenge, mortalities among the vaccinates and controls were 0% and 86.7%, respectively. Similarly, O-antigen preparations induced an immune response following injection in a wide range of fish species, including ayu, carp, Japanese eel, Japanese flounder, rainbow trout and red sea bream (Nakamura *et al.*, 1990). Incorporating purified 43 kDa OMP of *Aer. hydrophila* in FCA and a booster 3 weeks later (without FCA) led to a demonstrable immune response and protection against challenge by *V. anguillarum* in blue gourami (*Trichogaster trichopterus*) (Fang *et al.*, 2000). Development of live attenuated vaccines have been tried, with some success (Norquist *et al.*, 1989; 1994). A field trial with a live attenuated *V. anguillarum* vaccine (VAN1000) involved bathing 10 g rainbow trout in a dose equivalent to 1×10^6 cells/ml for 60 min at 9°C in brackish water. Following a natural challenge, 68% of the unvaccinated controls succumbed compared with 14% of the vaccinates (Norquist *et al.*, 1994). Interestingly, these workers considered that the live vaccine protected against both furunculosis and vibriosis. However, there may be problems with regulatory authorities regarding licensing for fisheries use.

To date, most of the vaccine development programmes have concentrated on bivalent products, containing cells of *V. anguillarum* and *V. ordalii* (e.g. Nakai *et al.*, 1989b). At various times, these have been applied to fish by injection (of dubious practicality for masses of fish), on food (oral administration), by bathing/immersion, by spraying, and by anal and oral intubation. The evidence has shown that oral application, perhaps the most convenient method, fares least successfully. Indeed, comparative vaccine trials have produced a wealth of information. For example, Baudin-Laurençin and Tangtrongpiros (1980) reported cumulative percentage mortalities among experimental groups of fish as follows:

Unvaccinated controls	33.8%
Oral-vaccinated fish	31.7%
Immersion-vaccinated group	2.1%
Group vaccinated by injection	1.4%

Similar findings, although generally more favourable for orally administered vaccines, were published by Amend and Johnson (1981) and Horne *et al.* (1982). Thus, Amend and Johnson (1981) revealed the following mortalities in vaccinated salmonids:

Unvaccinated controls	52%
Oral-vaccinated fish	27%
Immersion-vaccinated group	4%
Spray-vaccinated fish	1%
Group vaccinated by injection	0%

This compares with the work of Horne *et al.* (1982), who reported mortalities of:

Unvaccinated controls	100%
Oral-vaccinated fish	94%
Immersion-vaccinated group	53%
Group vaccinated by injection	7%

In a detailed examination of the effects of oral administration of formalin-inactivated vaccines in chinook salmon, Fryer *et al.* (1978) noted that maximal protection followed the feeding of 2 mg of dried vaccine/g of food for 15 days at temperatures even as low as 3.9°C. An important corollary was the observation that longer feeding regimes did not result in enhanced protection. This should be considered if prolonged durations of vaccination, via the oral route, are advocated. The reason for the apparently discouraging results with oral vaccination regimes may reflect the breakdown of vaccine inside the digestive tract (Johnson and Amend, 1983b). To resolve this problem, Johnson and Amend (1983b) incorporated a vaccine into gelatin, and applied it orally and anally in attempts to overcome digestion in the stomach and intestine. Encouraging results for application anally were obtained, in which mortalities following challenge were:

Unvaccinated controls	97%
Vaccine (minus gelatin) applied orally	35%
Vaccine (with gelatin) applied orally	69%
Vaccine (minus gelatin) applied anally	37%
Vaccine (with gelatin) applied anally	7%

Similar encouraging data were published by Dec *et al.* (1990). These workers used a commercial vaccine (produced by Rhône-Merieux), which was administered orally to turbot and sea bass. Following challenge 28 days later, the following mortalities were reported:

Oral-vaccinated sea bass	11.3%
Unvaccinated sea bream	40.9%
Oral-vaccinated turbot	19.2%
Unvaccinated turbot	65.4%

Incorporation of vaccine with natural food, i.e. plankton, has shown promise with ayu (Kawai *et al.*, 1989). Thus, in one set of experiments 7.6% of the vaccinates died, compared with 35.8% of the controls.

Noting that a pJM1 plasmid free culture was comparatively attenuated, Shao *et al.* (2005) used a plasmid-free culture, coined MVAV6201, as a live vaccine to deliver two recombinant proteins, GFP-HlyAs (HlyA = *Esch. coli* [-haemolysin]) and AngE-HlyAs, which were fused with the α-haemolysin secretion signal and expressed from the secretion vector pMOhlyl. Almost 70% and ~300 μg/l of GFP-HlyAs and AngE-HlyAs were secreted into the culture supernatant, respectively (Shao *et al.*, 2005).

Bypassing the potential deleterious effects of the stomach and upper regions of the gastro-intestinal tract enables effective vaccination to proceed. This suggests that micro-encapsulation techniques may be important for the development of successful oral vaccines. In this respect, the use of alginate microparticles has given promising results with an orally administered *V. anguillarum* vaccine (Joosten *et al.*, 1997). An interesting point is the implication that the posterior region of the gastro-intestinal tract is involved with the correct functioning of oral vaccines. This region has also been determined to be one of the initial sites of attachment of the pathogen. Therefore, it may be inferred that the best protection stems from methods paralleling those of the natural infection cycle.

In contrast to oral methods, injection has proved to be excellent as a means of vaccinating fish against vibriosis, with the development of high levels of immunity (Antipa, 1976; Antipa and Amend, 1977; Sawyer and Strout, 1977; Harrell, 1978; Evelyn and Ketcheson, 1980). Evidence suggests that 24 h and up to 14 days (but not 21 days) after i.p. injection with formalin-killed whole cells, the bacteria migrate to the spleen (particularly around small blood vessels when applied in FCA), heart, kidney and peritoneum of Atlantic cod (Arnesen *et al.*, 2002). Unfortunately, the injection technique is slow, and seems feasible only for large and/or valuable fish. Nevertheless, several types of preparations, including heat-killed and formalised vaccines, have been evaluated by injection. In addition, passive immunisation (by injection) has demonstrated the transfer of immunity between fish. In one comparison, it was clearly demonstrated that heat-killed preparations were more successful than products treated with formalin, when administered by injection. Reference is made to the work of Antipa (1976), who injected chinook salmon with vaccines and, following challenge with the pathogen, reported cumulative mortalities of:

Unvaccinated controls	85.4%
Formalised vaccine	37.8%
Heat-killed vaccine	22.3%

Sonicated heat-killed vaccines, administered in adjuvant, also stimulate elevated levels of antibody in the skin and mucus (Harrell *et al.*, 1976; Evelyn, 1984). At least these studies indicate the presence of heat-stable antigen, which features significantly in the establishment of protective immunity.

Anal intubation, but not i.p. injection, of African catfish (*Clarias gariepinus*) with a whole-cell vaccine of *V. anguillarum* O2 led to increased antibody levels after 14 days in the bile and skin mucus as detected by ELISA (Vervarcke *et al.*, 2005).

Antibodies in a group vaccinated by oral intubation were lower, but still higher than the i.p.-vaccinated group (Vervarcke *et al.*, 2005).

Immersion techniques are most suited for the vaccination of animals in the fish farm environment. Formerly, considerable attention was focused on hyperosmotic infiltration, involving use of a strong salt solution prior to immersion in a vaccine suspension (Croy and Amend, 1977; Aoki and Kitao, 1978; Nakajima and Chikahata, 1979; Antipa *et al.*, 1980; Giorgetti *et al.*, 1981). However, it is now appreciated that the technique is extremely stressful to fish (Busch *et al.*, 1978), and the level of protection achieved is only comparable with the much simpler direct immersion method (Antipa *et al.*, 1980), which is consequently favoured. Indeed, many articles have been published about the benefit of immersion vaccination (Håstein *et al.*, 1980; Song *et al.*, 1982; Amend and Johnson, 1981; Giorgetti *et al.*, 1981; Horne *et al.*, 1982; Johnson *et al.*, 1982a, b; Kawai and Kusuda, 1995) and the longer, i.e. 2 h, "bath" technique (Egidius and Andersen, 1979).

A further refinement involves use of low-pressure sprays, which are easy to use, and apparently economic in the quantity of vaccine administered (Gould *et al.*, 1978). The success was illustrated by 0% mortalities in a group of fish spray-vaccinated compared with 80% mortalities among unvaccinated controls after challenge (Gould *et al.*, 1978).

All of the aforementioned methods enable fish to develop an immune response to the pathogen. This aspect has been discussed comprehensively, as regards chinook salmon, by Fryer *et al.* (1972). It is thought that the maximum agglutination titre is in the region of 1:8,192, depending on the fish species used (Groberg, 1982). The development of immunity is clearly a function of water temperature, and generally humoral antibodies are formed more rapidly at high rather than low temperatures. For example, in coho salmon, humoral antibodies appeared in 25 days and 10 days at water temperatures of 6°C and 18°C, respectively (Groberg, 1982). The poor relative performance of orally administered vaccines has been partially attributed to an inability of the fish to develop humoral antibodies (Fryer *et al.*, 1978; Gould *et al.*, 1978; Kusuda *et al.*, 1978c; Groberg, 1982). However, the role of these antibodies in protection against disease is unclear.

Vibrio harveyi

Vaccine development programmes aimed at *V. harveyi* have not been especially successful, although this situation appears to be slowly changing. A whole-cell preparation, which was applied to barramundi (*Lates calcarifer*) by i.p. injection, anal intubation and immersion, led to antibody production, thereby demonstrating that fish could respond to vaccination (Crosbie and Nowak, 2004). By expressing the *HL1* gene, which encodes the haemolysin from *V. harveyi*, in yeast (*Saccharomyces cerevisiae*), the protein (= haemolysin) was expressed on the cell surface and was active against flounder erythrocytes. Moreover, serum from flounder that had received the live modified yeast cells by i.p. injection revealed haemolytic activity. Challenge experiments demonstrated that flounder and turbot were protected soon after

administration of yeast and then exposure to a virulent culture of *V. harveyi* (Zhu *et al.*, 2006).

A bivalent vaccine (with *Ph. damselae* subsp. *piscicida*) based on formalised cells and ECP administered to sole by immersion with booster or by i.p. injection led to high levels of protection (RPS = ~88%) for 4 months, after which the benefit declined (Arijo *et al.*, 2005).

Vibrio ordalii

The methods discussed for *V. anguillarum* apply. Likewise with *V. anguillarum*, the immunogenicity of LPS has been demonstrated (Velji *et al.*, 1990, 1991, 1992).

Vibrio salmonicida

There has been success with formalised vaccines for the prophylaxis of Hitra disease. Immersion of Atlantic salmon in these vaccines resulted in protection, even after 6 months (Holm and Jørgensen, 1987). It has emerged that *V. salmonicida* vaccines exert adjuvant activities on T-dependent and T-independent antigens in salmonids, namely rainbow trout. Essentially, vaccine preparations enhance antibody responses, notably to LPS (Steine *et al.*, 2001). Thus, the inclusion of inactivated *V. salmonicida* antigens in vaccine preparations may have an overall beneficial effect on the recipient fish (Hoel *et al.*, 1998b). The incubation temperature used to culture *V. salmonicida* is an important aspect of vaccine production, with 10°C (this coincides with the upper range of water temperatures at which cold-water vibriosis is most likely to occur) rather than 15°C giving a higher yield of cells in broth media (Colquhoun *et al.*, 2002).

At least one vaccine has been commercialised in a polyvalent form.

Vibrio vulnificus

A vaccine, coined Vulnivaccine which contains capsular antigens and toxoids (being the best of several alternatives; Collado *et al.*, 2000) of serovar E, and was administered by immersion for 1 h in three doses at 12 day intervals, has been evaluated in eels with the result that protection (RPS = 60–90%) was correlated with serum and local (mucus) antibody levels (Esteve-Gassent *et al.*, 2003), with the eels responding to 70–80 kDa OMP, protease and LPS (Esteve-Gassent and Amaro, 2004). During field trials by prolonged immersion and boostering after 14 and 24–28 days of 9.5 million glass eels in Spain and parallel experiments in Denmark, Vulnivaccine achieved RPS of 62–86% (Fouz *et al.*, 2001). With the appearance of a second serotype, i.e. A, a bivalent vaccine was constructed, and verified to be effective in terms of protection and humoral and local immunity following application orally, by anal and oral intubation, and by i.p. injection (RPS = 80–100%) (Esteve-Gassent *et al.*, 2004).

NON-SPECIFIC IMMUNOSTIMULANTS

A potential success story concerns the use of immunostimulatory compounds in fish (see Sakai, 1999). Such compounds, which have often been applied by i.p. injection, include Baypamum, chitin, dimerised lysozyme, β-1,3 glucans, killed cells of mycobacteria, laminaran, sulphated laminaran, lactoferrin, levamisole, LPS, oligosaccharides, prolactin and synthetic peptides (Dalmo and Seljelid, 1995; Yoshida *et al.*, 1995; Ortega *et al.*, 1996; Siwicki *et al.*, 1998; Sakai, 1999). Initially, Olivier *et al.* (1985a,b) observed that administration of killed cells of mycobacteria enhanced resistance in coho salmon to various bacteria. Then, Kitao and Yoshida (1986) found that synthetic peptides could enhance resistance of rainbow trout to *Aer. salmonicida*. The use of bovine lactoferrin, dosed orally at 100 mg/kg for 3 days enhanced the resistance of rainbow trout to subsequent challenge by streptococci and *V. anguillarum* (Sakai *et al.*, 1993a). Administration of Baypamum to rainbow trout led to a reduction in symptoms and mortalities attributed to furunculosis (Ortega *et al.*, 1996). Dimerised lysozyme, which is regarded as less toxic than the monomer, was injected into rainbow trout at a dose of 10 or 100 μg/kg, and stimulated cellular and humoral mechanisms giving protection against furunculosis (Siwicki *et al.*, 1998). One and three injections of lysozyme led to 45% and 25% mortalities following challenge with *Aer. salmonicida*. This compares with 85% mortality among the untreated controls (Siwicki *et al.*, 1998). Some immunomodulatory compounds, e.g. laminaran, accumulate in the kidney and spleen of Atlantic salmon (Dalmo *et al.*, 1995).

The greatest interest has been towards the potential for β-1,3 glucans. Certainly, a rapidly growing literature points to the success of glucans in preventing disease (Yano *et al.*, 1991; Raa *et al.*, 1990; Robertsen *et al.*, 1990; Nikl *et al.*, 1991; Matsuyama *et al.*, 1992; Chen and Ainsworth, 1992). For example, Yano *et al.* (1991) published data that showed β-1,3 glucans, when applied by i.p. injection at 2–10 mg/kg of fish, enhanced resistance to infection by *Edw. tarda*. This effect was measured by heightened phagocytic activity. Use of β-1,3-glucan and chitosan for 30 min immersion in 100 μg/ml or as single i.p. injections with 100 μg led to protection in brook trout against *Aer. salmonicida* from 1 to 3 days after administration (Anderson and Siwicki, 1994). Generally, injection was superior to immersion (Anderson and Siwicki, 1994). Others have found oral administration to be superior to immersion (Nikl *et al.*, 1993). Nikl *et al.* (1991) reported success at preventing infection by *Ren. salmoninarum*. Similarly, Matsuyama *et al.* (1992) used the glucans schizophyllan and scleroglucan to protect against streptococci. Thus, 2–10 mg of glucans/kg of fish, when administered by i.p. injection, enhanced resistance of yellowtail to streptococcicosis. In particular, there was an elevation of serum complement and lysozyme, and an increase in phagocytic activity of pronephros cells. Initially, success only appeared to result from injection of the glucans into fish. Yet, claims have now been made that application via food also meets with success (Onarheim, 1992). Also, resistance to streptococcicosis and vibriosis has been enhanced following the oral administration of peptidoglycan from *Bifidobacterium* (Itami *et al.*, 1996) and *Cl. butyricum* (Sakai *et al.*, 1995), respectively.

Peptidoglycan, derived from *Bifidobacterium thermophilus*, was administered in

feed (fed at 3% of body weight daily) at 0.2 and 2 mg/kg to rainbow trout of 0.12 g average weight for 56 days (Matsuo and Miyazono, 1993). These doses were the equivalent of 6 or 60 μg of peptidoglycan/kg body weight of fish/day. Sub-groups of the fish were challenged on day 26 and 56 by immersion in *V. anguillarum*, with mortalities monitored over a 21 day period. At the half-way point of the feeding trial, survival following challenge with *V. anguillarum* was markedly higher than among the controls. Yet, at day 56 there was not any apparent difference in survival between the experimental groups and controls. So, it would appear that the benefits of this approach were short-lived, and in the long term were not beneficial (Matsuo and Miyazono, 1993).

Vitamin E and iron sulphate, dosed at 2,500 mg/kg and 60 mg/kg, respectively, have been reported to be beneficial in enhancing the immune response of channel catfish, especially by improved phagocytosis, to *Edw. ictaluri* (Wise *et al.*, 1993; Lim *et al.*, 1996; Sealey *et al.*, 1997). Certainly, this aspect of research looks promising, and it is envisaged that other immunostimulatory compounds will be identified in the future.

Feeding with 3,3′,5-triiodo-L-thyronine at 5 mg/kg of feed for 60 days to rohu (*Labeo rohita*) led to enhanced growth, serum protein and globulin levels, superoxide production of the neutrophils and antibodies against *Aer. hydrophila*. Moreover, there was a reduction in mortalities after challenge with *Aer. hydrophila* compared with the controls (Sahoo, 2003).

Injection of 0.25 or 0.5 μg/fish of synthetic cytidine-phosphate-guanosine (CpG) oligodeoxynucleotide (ODN) with olive flounder led to higher chemiluminescence by phagocytes; supernatants from leucocytes, which received CpG ODN as a pulse, induced much higher respiratory burst activity after 3–7 days. Additionally, the fish which received CpG ODN were better protected against challenge with *Edw. tarda* (mortality = 17%) compared with the controls (mortality = 92%) (Lee *et al.*, 2003).

ANTIMICROBIAL COMPOUNDS

Use of antimicrobial compounds in fisheries is a highly emotive issue in which the possibility of tissue residues and the development of bacterial resistance feature prominently in any list of complaints. It is astounding that so many compounds (these have been reviewed by Snieszko, 1978; Herwig, 1979; Austin, 1984a) have found use in aquaculture. The complete list reads like an inventory from any well-equipped pharmacy. Antibiotics, many of which are important in human medicine, appear side by side with compounds used almost exclusively in fisheries. In many instances, the introduction of a compound into fisheries use has followed closely after the initial use in human medicine. Perhaps, in retrospect it is surprising that there has not been any significant furore from the medical profession about what could be perceived as misuse of pharmaceutical compounds. Unfortunately, any backlash may come in the foreseeable future; therefore, it is in the interest of aquaculture that antimicrobial compounds should be carefully used.

The use of antimicrobial compounds in fisheries essentially started with the work of Gutsell (1946), who recognised the potential of sulphonamides for combating furunculosis. Indeed, it may be argued that the effectiveness of sulphonamides led to a temporary decline of interest in vaccine development. This was the era when antimicrobial compounds were starting to have a profound and beneficial effect on human and animal health. In fact, the eventual emergence of antibiotic-resistant strains of fish-pathogenic bacteria led to renewed interest in vaccines. However, during the years following the Second World War sulphonamides appeared to be the mystical saviour of fish farming. Important developments included the work of Rucker *et al.* (1951), who identified sulphadiazine as an effective chemotherapeutant for BKD. This claim was subsequently refuted by Austin (1985). The next substantial improvement with sulphonamides resulted from potentiation, i.e. the use of mixtures of trimethoprim and sulphonamide. These have proved to be extremely useful for the treatment of furunculosis. Indeed, formulations are currently licensed for fisheries use in Great Britain.

Following the introduction of sulphonamides, the range of antimicrobial compounds in aquaculture rapidly expanded to encompass chloramphenicol (Wold, 1950), oxytetracycline (Snieszko and Griffin, 1951), kanamycin (Conroy, 1961), nifurprazine (Shiraki *et al.*, 1970), oxolinic acid (Endo *et al.*, 1973), sodium nifurstyrenate (Kashiwagi *et al.*, 1977a,b), flumequine (Michel *et al.*, 1980) and Baytril (Bragg and Todd, 1988). Unfortunately, detailed comparative studies of the various antimicrobial compounds are rare; consequently, it is often difficult to assess the value of one drug (=any medicinal compound; Sykes, 1976) over another. Nevertheless, a pattern has emerged which points to the benefits of quinolines for controlling diseases caused by a wide range of Gram-negative bacteria. Currently, there is extensive use of oxolinic acid and flumequine in Europe. Newer quinolones offer hope for the future, although some as yet unpublished evidence points to possible problems with this class of molecules.

Whatever the range of compounds available, their effectiveness is a function of the method of administration to fish (and in the way in which it is carried out). We have listed seven basic approaches to the administration of antimicrobial compounds to fish (Table 10.4). These are the oral route via medicated food and bioencapsulation, bath, dip and flush treatments, injection, and topical application. With the oral method drugs are mixed with food and then fed to the fish. Usually, the treatment regime leads to the administration of a unit weight of drug to a standard weight of fish per day for a predetermined period. Examples of commonly used antimicrobial compounds have been included in Table 10.5. Fortunately, medicated food appears to be quite stable (McCracken and Fidgeon, 1977). Moreover, this method is advantageous insofar as the quantities of compound fed to the fish are carefully controlled, and if sensible feeding regimes are adopted, only minimal quantities would reach the waterways. Three provisos exist, namely that:

- the fish are capable of feeding;
- the drug is palatable;
- the drug is capable of absorption intact through the gut.

Table 10.4. Methods for application of antimicrobial compounds to fish

Method of application	Comments
Oral route (on food)	Need palatable components; minimal risk of environmental pollution
Bioencapsulation	Need palatable compounds; minimal risk of environmental pollution
Bath	Need for fairly lengthy exposure to compound, which must be soluble or capable of being adequately dispersed; problem of disposal of spent drug
Dip	Brief immersion in compound, which must be soluble or capable of being adequately dispersed; problem of disposal of dilute compound
Flush	Compound added to fish holding facility for brief exposure to fish; must be soluble or capable of being adequately dispersed; poses problem of environmental pollution
Injection	Feasible for only large and/or valuable fish; usually requires prior anaesthesia; slow; negligible risk of environmental pollution
Topical application	Feasible for treatment of ulcers on valuable/pet fish

A more recent approach has involved bioencapsulation, principally of quinolones (Duis *et al.*, 1995). This theme was expanded with some excellent work which examined the potential for *Artemia* nauplii to serve as carriers to sulphamethoxazole and trimethoprim for the chemotherapy of diseased marine fish fry (Touraki *et al.*, 1996). Both these compounds accumulated in the nauplii, with maximal levels recorded after 8 h. In a trial with sea bass larvae challenged with *V. anguillarum*, an improvement in survival followed use of the medicated nauplii (Touraki *et al.*, 1996). Whether or not the fish will feed is largely a function of the nature and severity of the disease. Often in advanced cases of disease the fish will not feed. Therefore, it is vitally important that treatment begins as soon as possible after diagnosis has been established. The aquaculturist will need to seek specialist advice as soon as any abnormal behaviour or unhealthy condition is noted. This means that good management practices need to be routinely adopted.

The palatability of fisheries antimicrobial compounds receives only scant attention. Whereas it is accepted that little can be done to improve the palatability of the active ingredient, effort could be directed towards improving binders and bulking agents, which are commonly contained in proprietary mixes. Perhaps, consideration could be given to using chemical attractants.

Application by the water-borne route becomes necessary if the fish refuse to eat, and, therefore, would be unlikely to consume any medicated food. With these methods, the fish are exposed to solutions/suspensions of the drug for a

Table 10.5. Methods of administering commonly used antimicrobial compounds to fish

Antimicrobial compound	Diseases controlled	Method(s) of administration
Acriflavine, neutral	Columnaris	5–10 mg/l in water for several hours to several days
Amoxycillin	Furunculosis, gill disease	60–80 mg/kg body weight of fish/day for 10 days
Benzalkonium chloride	Fin rot, gill disease	1–2 mg/l of water for 1 h; 100 mg/l of water for 2 min
Chloramine B or T	Fin rot, gill disease mycobacteriosis	18–20 mg/l of water at pH 7.5–8.0, treat for 2–3 days
Chloramphenicol	CE, columnaris, ERM, fin rot, furunculosis, haemorrhagic septicaemia, pasteurellosis, ulcer disease, vibriosis	(a) 50–70 mg/kg of food/day for 5–10 days; (b) 10–50 mg/l of water, as a bath
Difloxacin	Furunculosis	5 mg/kg body weight for 5 or 10 days
Doxycycline	Streptococcicosis	20 mg/kg body weight of fish/day for an unspecified duration
Enrofloxacin (= Baytril)	BKD, furunculosis	10 or 20 mg/kg body weight/day for 10 days
Erythromycin	BKD, streptococcicosis	25–100 mg/kg of fish/day for 4–21 days; 20 mg of erythromycin/kg of broodstock as an injection
Florfenicol,	Furunculosis, vibriosis	10 mg/kg body weight of fish/day for 10 days
Flumequine	Furunculosis, ERM, vibriosis	6 mg/kg of fish/day for 6 days
Formalin	Fin/tail rot	2–5 mg/l for 15–60 min
Fosfomycin	Pasteurellosis	40 mg/kg body weight of fish/day for 5 days

Furanace	Coldwater disease, columnaris, fin rot, gill disease, haemorrhagic septicaemia, vibriosis	(a) 2–4 mg/kg of fish/day for 3–5 days; (b) 0.5–1 mg/l of water for 5–10 min, as a bath
Furazolidone	CE, furunculosis, vibriosis	25–75 mg/kg of fish/day for up to 20 days
Iodophors	*Acinetobacter* disease, BKD, flavobacteriosis, furunculosis, haemorrhagic septicaemia, mycobacteriosis	50–200 mg of available iodine/l of water for 10–15 min
Josamycin	Streptococcicosis	30 mg/kg body weight of fish/day for 3 days (or dosed at 20 mg/kg body weight of fish/day for 5 days)
Kanamycin	Fin rot, haemorrhagic septicaemia, mycobacteriosis, vibriosis	50 mg/kg of fish/day for 7 days
Malachite green	Columnaris, fin rot, gill disease	1–3 mg/l of water for up to 1 h
Methylene blue	Salmonid blood spot	1–3 mg/l of water for 3–5 days
Nifurprazine hydrochloride	Furunculosis, vibriosis	(a) 10 mg/kg of food, fed for 3–6 days (b) 0.01–0.1 mg/l of water, as a bath for an undefined period
Nitrofurantoin	Vibriosis	50 mg/l of water, as a bath for 1 h
Oxolinic acid	Columnaris, ERM, furunculosis, haemorrhagic septicaemia, vibriosis	(a) 10 mg/kg of fish/day for 10 days (b) 1 mg/l of water, as a bath for 24 h (recommended for columnaris)
Oxytetracycline	*Acinetobacter* disease, CE, coldwater disease, columnaris, edwardsiellosis, emphysematous putrefactive disease, ERM, enteric septicaemia, fin rot, furunculosis, gill disease, haemorrhagic septicaemia, redpest, salmonid blood spot, saltwater columnaris, streptococcicosis, ulcer disease	50–75 mg/kg of fish/day for 10 days (doses of 300 mg/kg of fish/day for indefinite periods are used to treat RTFS)

(*continued*)

Table 10.5 (*cont.*)

Antimicrobial compound	Diseases controlled	Method(s) of administration
Penicillin G	BKD	75–100 mg/kg of fish/ day for 10–21 days
Polymyxin B nonapeptide	Furunculosis	8–20 mg/kg of fish/day for 1–2 days
Potentiated sulphonamide	ERM, furunculosis, haemorrhagic septicaemia, vibriosis	30 mg/kg of fish/day for 10 days
Sodium nifurstyrenate	Streptococcicosis	50 mg/kg of fish/day for 3–5 days
Streptomycin	Haemorrhagic septicaemia, mycobacteriosis	50–75 mg/kg of fish/day for 5–10 days
Sulphonamides (sulphisoxazole, sulphamerazine, sulphamethazine)	BKD, coldwater disease, columnaris, ERM, furunculosis, haemorrhagic septicaemia, mycobacteriosis, nocardiosis, vibriosis	100–200 mg/kg of fish/day for 10–20 days
Tetracycline	CE, columnaris, furunculosis, streptococcicosis	75–100 mg/kg of fish/day for 10–14 days
Tiamulin	ERM	5 mg/kg of fish/day for 14 days

predetermined period. This may be only briefly, i.e. a few seconds duration ("dip"), or for many minutes to several hours ("bath"). It is essential that the compounds are soluble or, if insoluble, are dispersed evenly in the water by means of surfactants or other dispersants (Austin *et al.*, 1981). Also, seawater cations may well antagonise antimicrobial compounds in seawater (Barnes *et al.*, 1995). One major drawback, however, concerns the disposal of the spent compound. Ideally, it should not be released into the aquatic environment, particularly if there are any abstraction points for potable water supply systems in the vicinity. Neglect of this point could lead to legal repercussions.

Flush treatments, e.g. with malachite green, also involve the addition of drugs, albeit at high concentrations, to the water in stock-holding areas. After addition, the drug is flushed through the system by normal water flow. Flushing inevitably results in only a brief exposure to the inhibitory compound; therefore, quick-acting agents are absolutely necessary. As before, the major problem is adequate disposal of the spent drug.

Injection of drug solutions is feasible for valuable stock, such as brood fish and ornamental/pet fish. However, the technique is slow and will undoubtedly require prior anaesthesia of the animals.

The topical application of antimicrobial compounds is worthy of consideration for valuable and/or pet fish. In the case of ulcers, we recommend that the animal should be gently removed from the water, and the antimicrobial compound (preferably as a powder) applied to the lesion, which is then sealed with a waterproof covering, e.g. with dental paste. The lesions tend to heal quickly, with only limited evidence of scarring.

Whatever the chosen method of application, drugs may be used for prevention, i.e. prophylaxis, or treatment, i.e. chemotherapy, of fish diseases. Certainly, it is comforting to note that there are treatments available for the majority of the bacterial fish pathogens. Providing that drugs are used prudently and correctly, they will continue to offer relief from the rigours of disease for the foreseeable future.

CHEMOTHERAPY DEVELOPMENT PROGRAMMES: ANAEROBES

Eubacteriaceae representative

Eubacterium tarantellae

Udey *et al.* (1977) reported that isolates were sensitive to chloramphenicol, erythromycin, novobiocin, penicillin and tetracycline when examined by *in vitro* methods. It is possible that one or more of these compounds may be useful for chemoprophylaxis, but the value of any of these antimicrobial agents for chemotherapy is dubious. However, it seems likely that, once the pathogen has entered brain tissue, antimicrobial compounds would probably not be able to reach the site of infection.

CHEMOTHERAPY DEVELOPMENT PROGRAMMES: GRAM-POSITIVE BACTERIA

Carnobacteriaceae representatives

Carnobacterium piscicola (and the lactobacilli)

In vitro methods have shown that isolates are sensitive to ampicillin, cephaloridine, chloramphenicol, furazolidone and tetracycline, but not to erythromycin, novobiocin, streptomycin, sulphamerazine or sulphamethoxazole (Michel *et al.*, 1986b; B. Austin, unpublished data). Unfortunately, these antimicrobial compounds were not evaluated in experiments with fish.

Enterococcaceae representatives

***Enterococcus*-like bacteria**

The benefits of ionophores, namely lasalocid, monensin, narasin and salinomycin, were reported for an *Enterococcus*-like pathogen of rainbow trout in Australia. Following a comparison of 40 isolates, it was noted that the MIC of the ionophores was markedly less than erythromycin (0.1–0.8 μg/ml). Thus, the MIC for lasalocid, monensin, narasin and salinomycin were 0.8 μg/ml, 0.4–1.5 μg/ml, 0.2–04 μg/ml and 0.4–0.8 μg/ml, respectively. Perhaps, there are future opportunities for the use of some of these compounds in aquaculture.

Streptococcaceae representatives

Lactococcus garvieae (and the streptococci)

Both *Str. difficilis* and *Str. shiloi* (= *Str. iniae*) revealed identical antimicrobial susceptibility patterns, with susceptibility to ampicillin, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, erythromycin, fusidic acid, methicillin, mezlocillin, nitrofurantoin, penicillin, potentiated sulphonamide, tetracycline and vancomycin, but resistance to amikacin, colistin, gentamicin and nalidixic acid. Erythromycin, dosed at 25 mg/kg body weight of fish/day for 4 to 7 days, controlled streptococcicosis in yellowtail (Kitao, 1982b), and worked better than oxytetracycline or ampicillin (Shiomitsu *et al.*, 1980). Doxycycline, at 20 mg/kg body weight of fish/day for an unspecified duration (Nakamura, 1982), and josamycin, dosed at 30 mg/kg body weight of fish/day for 3 days (or dosed at 20 mg/kg body weight of fish/day for 5 days) (Kusuda and Takemaru, 1987; Takemaru and Kusuda, 1988) have also been advocated. It is of particular interest that a novel fisheries therapeutant, namely sodium nifurstyrenate dosed at 50 mg/kg body weight of fish/day for 3–5 days (Kashiwagi *et al.*, 1977a, b), has found use for streptococcicosis. This drug appears to be particularly effective, and should not have problems with plasmid-mediated resistance. However, resistance to erythromycin, lincomycin and oxytetracycline has been reported among Japanese isolates of *Lactococcus garvieae*, with the problem recognised for well over a decade (Kawanishi *et al.*, 2005).

Streptococcus iniae

Success has been reported with the fluoroquinolone compound, enrofloxacin, dosed at 5 or 10 mg/kg body weight of fish/day for 10 days (Stoffregen *et al.*, 1996). Mortalities declined rapidly upon instigating treatment, such that by the end of treatment a total of 11% and 17% of the treated fish has died compared with 55% and 40% of the untreated controls, respectively. Also, laboratory studies have indicated the vale of oxytetracycline (Darwish *et al.*, 2002) and amoxycillin (Darwish and Ismaiel, 2003) for the control of infection in blue tilapia and sunshine bass, respectively. Experimental evidence has pointed to the efficacy of using amoxycillin for controlling *Str. iniae* infection, especially when dosed at 80 mg/kg of fish/day for 12 days when mortalities dropped from 96% in the controls to 6% in the treated blue tilapia (Darwish and Hobbs, 2005). Moreover, the treated survivors did not carry the pathogen.

Streptococcus milleri

The two isolates were sensitive to tetracycline, which might be of value for chemotherapy (Austin and Robertson, 1993).

Streptococcus parauberis

It was reported that isolates were resistant to flumequine, oxolinic acid and streptomycin, moderately susceptible to oxytetracycline, tetracycline and sulphamethoxazole-trimethoprim, and highly sensitive to ampicillin, chloramphenicol, erythromycin, nitrofurantoin and penicillin (Doménech *et al.*, 1996).

CHEMOTHERAPY DEVELOPMENT PROGRAMMES: AEROBIC GRAM-POSITIVE RODS AND COCCI

Renibacterium salmoninarum

Chemotherapy offers some promise of success (Bandín *et al.*, 1991b). Although BKD has become regarded as one of the most difficult bacterial fish diseases to treat (Bullock *et al.*, 1975; Fryer and Sanders, 1981), some success at chemotherapy has been reported with erythromycin (Wolf and Dunbar, 1959), sulphonamides (Rucker *et al.*, 1951), chloramphenicol (Rucker *et al.*, 1953; Wood and Wallis, 1955; Millan, 1977), penicillin (Decew, 1972), clindamycin, kitasamycin and spiramycin (Austin, 1985), and enrofloxacin (Baytril) (Hsu *et al.*, 1994). An MIC of 0.25–0.5 μg of enrofloxacin/ml was calculated (Hsu *et al.*, 1994). Furthermore, some beneficial effects have been indicated from trials using a dose of 20 mg of enrofloxacin/kg body weight/day for 10 days when there was a reduction in mortalities compared with controls. Over two trials, the deaths in the treated groups and the controls were 43% and 72%, and 93% and 100%, respectively (Hsu *et al.*, 1994). In addition, cephradine, lincomycin and rifampicin were found to be effective for prophylaxis of BKD,

although they were of no use for therapeutic purposes (Austin, 1985). Undoubtedly, many of the problems with control measures revolve around the intracellular nature of the organism (Young and Chapman, 1978). Quite simply, many of the drugs probably do not reach the actual foci of infection. Nevertheless, experiments with liposomes, which target drugs to given organs, proved to be disastrous, insofar as BKD was exacerbated (Austin, 1985). Perhaps, the value of micro-encapsulation techniques should be assessed.

The pioneering work with drugs for the control of BKD was undertaken by Rucker *et al.* (1951). They reported a decrease in the level of mortalities following the administration of sulphadiazine, via the oral route, at 250 mg of drug/kg body weight of fish/day for 15 days. This was confirmed by Earp *et al.* (1953) and Allison (1958). However, the drug failed to eliminate the pathogen from the fish. Subsequently, Wolf and Dunbar (1959), in a comparison of 34 compounds, concluded that erythromycin, dosed at 100 mg of drug/kg body weight of fish/day for 21 days, gave the best result. The value of erythromycin at this concentration was confirmed by Austin (1985), although it was suggested that treatment need only be continued for 10 days. Erythromycin has also been reported to prevent vertical transmission of renibacteria (Evelyn *et al.*, 1986a,b; Brown *et al.*, 1990). Also, an injection of 20 mg of erythromycin/kg of brood stock fish is useful in preventing vertical transmission (Lee and Evelyn, 1994).

Bacillaceae representatives

Bacillus sp.

It was reported that *Bacillus* sp. was sensitive to tetracycline, but not penicillin (Oladosu *et al.* (1994).

Bacillus mycoides

Sensitivity was reported to erythromycin, nalidixic acid, nitrofurazone, novobiocin and oxytetracycline, but not to Romet (= orthometoprim-sulphadimethoxine) (Goodwin *et al.*, 1994).

Corynebacteriaceae representative

Corynebacterium aquaticum

The organism was sensitive to ampicillin, erythromycin, oxytetracycline and potentiated sulphonamide, one or more of which may be useful for chemotherapy (Baya *et al.*, 1992b).

Micrococcaceae representative

Micrococcus luteus

Sensitivity was recorded to chloramphenicol, streptomycin, potentiated sulphonamides and tetracycline. These compounds may be useful at controlling the progress of infections in rainbow trout fry (Austin and Stobie, 1992a).

Mycobacteriaceae representatives

Mycobacterium spp.

Some workers advocate that clinically diseased fish should be destroyed, by incineration or burying in quick lime, because of the necessity for prolonged use of chemotherapeutic agents, and the potential hazard to human health (Dulin, 1979; Van Duijn, 1981). Other scientists have described treatments with chloramine B or T, cycloserine, doxycycline, erythromycin, ethambutol, ethionamide, isoniazid, kanamycin, minocycline, penicillin, rifampicin, streptomycin, sulphonamides and tetracycline. Of these, the most economical treatment is with chloramine B or T at 10 mg/l of tank water for an exposure period of 24 h, after which the water should be changed (Van Duijn, 1981). Erythromycin, rifampicin and streptomycin appear to be highly effective against some isolates (Kawakami and Kusuda, 1989, 1990).

Nocardiaceae representatives

Nocardia spp.

As with mycobacteria, it has been argued that infected fish should be destroyed so as to prevent any human health hazard. However, some success may result with chemotherapy, in particular with sulphonamides, e.g. sulphisoxazole at 2 mg/g of food (Van Duijn, 1981). An improvement in the fish becomes apparent within 10 days of the commencement of treatment, but it is advisable to continue chemotherapy for 21 days. This method may be suitable for the treatment of pet fish. Isolates of *Noc. seriolae* recovered from Japan during 1999 to 2001 were susceptible to kanamycin but uniformly resistant to fosfomycin and oxolinic acid. Some isolates displayed resistance to erythromycin, kitasamycin and spiramycin. Of concern, multiple antibiotic resistance was observed in some cultures (Itano and Kawakami, 2002).

Planococcaceae representative

Planococcus sp.

The organism was sensitive to carbenicillin, erythromycin, penicillin G and tetracycline, which may be effective for chemotherapy (Austin *et al.*, 1988; Austin and Stobie, 1992a).

Staphylococcaceae representatives

Staphylococcus epidermidis

This was not discussed in the original paper, but Wang *et al.* (1996) reported success with erythromycin, dosed at 20 mg/kg body weight of fish/day for 10 days.

Staphylococcus warneri

Sensitivity was recorded to amoxicillin, erythromycin and trimethoprim-sulphamethoxazole (Gil *et al.*, 2000), which may be worthy of examination in *in vivo* experiments.

CHEMOTHERAPY DEVELOPMENT PROGRAMMES: GRAM-NEGATIVE BACTERIA

Aeromonadaceae representatives

Aeromonas hydrophila

Chemotherapy of *Aer. hydrophila* infections corresponds closely to that of *Aer. salmonicida*. For example, the relevance of oxytetracycline has been well documented (Meyer, 1964). Unfortunately, plasmid-mediated resistance by means of 20–30 mDa plasmids is similarly widespread in fish farms, e.g. eel ponds (Aoki, 1988), and may negate the potential benefit of some antimicrobial compounds (Aoki and Egusa, 1971; Toranzo *et al.*, 1983a). It is alarming that R-plasmids with common sequence DNA structures have now been found in several unrelated species of fish pathogens, including *Aer. hydrophila*, *Aer. salmonicida* and *Edw. tarda* (Aoki, 1988). Resistance in *Aer. hydrophila* has been recorded to a wide range of antimicrobial compounds, including ampicillin, chloramphenicol, erythromycin, nitrofurantoin, novobiocin, streptomycin, sulphonamides and tetracycline (Aoki, 1988; De Paola *et al.*, 1988). Indeed, it has been estimated that as many as 38% of the *Aer. hydrophila* isolates from diseased catfish are resistant to oxytetracycline (De Paola *et al.*, 1988). For the future, new antimicrobial compounds, such as enrofloxacin, offer promise. This compound is antibacterial even at low dosages, i.e. with an MIC reported as 0.002 μg/ml (Bragg and Todd, 1988).

Aeromonas salmonicida

Over the past few decades, a variety of inhibitory agents have been applied with varying degrees of success to the treatment of furunculosis. Early studies established that sulphonamides, notably sulphamerazine, were successful in controlling furunculosis when administered orally with food at a dose of 22 g of drug/100 kg of fish/day (Gutsell, 1946; Snieszko, 1958a). Among the antibiotics, Snieszko (1958a) showed the usefulness of chloramphenicol and oxytetracycline when dosed at 5–7 g/100 kg of

fish/day. Furazolidone was also briefly mentioned as having promise. More recently, polymyxin B nonapeptide has been found to inhibit *Aer. salmonicida*, probably by disrupting the A-layer (McCashion and Lynch, 1987).

Curiously, the use of some compounds follows a geographical pattern, e.g. in France flumequine (a quinolone) is favoured (Michel *et al.*, 1980), whereas in England and Japan oxolinic acid has been used extensively (see Endo *et al.*, 1973; Austin *et al.*, 1983b). It is debatable as to whether flumequine or oxolinic acid has been more successful at combating furunculosis (Barnes *et al.*, 1991a). Additionally, in England potentiated sulphonamides have been used widely (McCarthy *et al.*, 1974). Within the U.K., four compounds, namely amoxycillin, oxolinic acid, oxytetracycline and potentiated sulphonamide, are currently licensed for fisheries use in the treatment of furunculosis. For other diseases caused by *Aer. salmonicida*, i.e. CE and goldfish ulcer disease, less information is available. It appears, however, that potentiated sulphonamide and oxytetracycline are generally effective against the pathogen, regardless of the disease manifestation and assuming that treatment begins at an early stage in the disease cycle (Gayer *et al.*, 1980). Unfortunately, despite its comparatively recent arrival in the armoury of fisheries chemotherapeutants, resistance to amoxycillin has been documented in Scotland (Barnes *et al.*, 1994). For the future, florfenicol, dosed at 10 mg/kg body weight of fish/day for 10 days, offers promise, insofar as it has already been used with some success against furunculosis in Norway (Nordmo *et al.*, 1994; Samuelsen *et al.*, 1998).

Since the initial work with oxolinic acid (Endo *et al.*, 1973; Austin *et al.*, 1983b), it is apparent that substantial quantities have been used in aquaculture within many countries. Consequently, it is hardly surprising that resistant strains of *Aer. salmonicida* have emerged (Hastings and McKay, 1987; Tsoumas *et al.*, 1989; Barnes *et al.*, 1990a). Yet, the widespread usefulness of the compound at controlling furunculosis has prompted a search for other related compounds. The fruits of this research may be illustrated by the apparent success of 4-quinolones/fluoroquinolones at inhibiting the pathogen (Barnes *et al.*, 1990b; 1991b; Bowser and House, 1990; Lewin and Hastings, 1990; Martinsen *et al.*, 1991; Inglis and Richards, 1991; Stoffregen *et al.*, 1993; Elston *et al.*, 1995). Thus, enrofloxacin and sarofloxacin have been found to be more effective than oxolinic acid, in terms of MIC, at inactivating *Aer. salmonicida*. The effectiveness of enrofloxacin at 10 mg/kg body weight of fish/day for 10 days has been attested by field trials with lake trout (*Salvelinus namaycush*) (Hsu *et al.*, 1995). In addition, enrofloxacin has been effective in controlling atypical *Aer. salmonicida* in tom cod, insofar as a single injection with 5 mg of enrofloxacin/kg of fish stopped furunculosis (Williams *et al.*, 1997). Difloxacin, dosed at 5 or 10 days at 5 mg/kg body weight, and 10 days at 1.25 or 2.5 mg/kg body weight resulted in significantly lowered mortalities compared with controls following i.m. injection with a virulent culture of *Aer. salmonicida*. Of relevance, there was little difference in the results between 5 and 10 day treatment regimes with 5 mg/kg body weight (Elston *et al.*, 1995).

With the realisation that *Aer. salmonicida* occurs on the external surfaces, i.e. gills and mucus, attempts have been made at disinfection. Cipriano *et al.* (1996c, d) evaluated chloramine T, dosed at 15 mg/l for 60 min on three consecutive days, but the infection was not controlled. Success occurred with 77 mg of oxytetracycline/kg

body weight of fish/day for 10 days with mortalities stopping within 4 days of starting treatment (Cipriano *et al.*, 1996c).

There can be no dispute that chemotherapeutic agents are (and will continue to be) invaluable for preventing heavy mortalities during outbreaks of furunculosis. Nonetheless, there are substantial reasons for avoiding total reliance upon such compounds. For instance, the development of resistance by the pathogens to some widely used drugs is cause for serious concern (Wood *et al.*, 1986). Thus, it is disquieting that plasmids carrying antibiotic resistance factors (R-factors) have been isolated from *Aer. salmonicida* strains (Aoki *et al.*, 1971). Indeed, a strain resistant to sulphathiazole and tetracycline was recovered as early as 1959. Moreover, Snieszko and Bullock (1957) reported the occurrence of cultures which were resistant to sulphonamides, although at the time the mechanism of resistance was not known. Now, it seems that resistance may reflect alterations in the outer membrane of *Aer. salmonicida* (Barnes *et al.*, 1992). Aoki *et al.* (1983) examined 175 isolates, which had been isolated from cultured and wild salmonids in Japan, for susceptibility to a wide range of antimicrobial agents. They noted that 96% of the isolates from cultured fish were resistant to at least one, and up to six, of the drugs, particularly nalidixic acid and nitrofuran derivatives. In addition, transferable R-plasmids, coding for resistance to chloramphenicol, streptomycin and sulphonamides, and non-transferrable plasmids conveying resistance against tetracycline were found in several strains. It was concluded from these results that drug-resistant strains of *Aer. salmonicida* had increased in direct proportion to the enhanced use of antimicrobial compounds in fish culture. This was particularly evident in view of the observation that few isolates recovered from wild salmonids exhibited drug resistance. Toranzo *et al.* (1983a, b) characterised the plasmids, determining that the organism frequently possessed more than one plasmid. In particular, five strains possessed six plasmids of varying molecular weights. However, these workers did not observe any correlation between loss of plasmids, or changes in plasmid mobilities, and the loss of resistance to sulphadiazine, in the case of one strain which was studied in detail. Nevertheless, the molecular genetic studies of Mitoma *et al.* (1984) identified gene sequences in R-plasmids coding for either chloramphenicol or tetracycline resistance. Hedges *et al.* (1985), using aeromonad isolates obtained from France, Ireland, Japan and the U.K., determined that plasmids from *Aer. hydrophila* and *Aer. salmonicida* were similar. These workers reported that some plasmids were transmissible to *Esch. coli*, whereas others were unstable in this recipient organism. The R-plasmids of *Aer. salmonicida* were considered to confer upon the pathogen the potential to withstand the onslaught of a wide variety of inhibitory agents, thus diminishing the effectiveness of chemotherapy. It may be hoped that the development of new compounds, particularly synthetic or semi-synthetic molecules, and the strict rotation in the use of currently available drugs may assist with the problem of resistance in the pathogen. Obviously, a constant awareness of the problem must be maintained.

Another aspect of chemotherapy concerns the presence and retention of the compounds in fish tissues. In fact, difficulties of this nature were recognised as early as 1951 with a report by Snieszko and Friddle who expressed concern with tissue levels of sulphamerazine in trout. McCarthy and Roberts (1980) pointed out that in

some countries, such as the U.S.A., there existed legislation restricting the number of antimicrobial compounds which may be used on fish destined for human consumption. In many countries, drugs may only be obtained on veterinary prescription. The caveat to the use of chemotherapeutants is that a suitable period of time must lapse following the conclusion of treatment, before the fish may be sold for human consumption. This should allow for the purging from the fish of all traces of the active compound and the metabolites. It is worth remembering the opinions of Snieszko (1958a), who cautioned that drug therapy should only be considered as a stopgap measure until the sources of infection by *Aer. salmonicida* could be eliminated, or disease-resistant strains of fish introduced.

Campylobacteriaceae representative

Arcobacter cryaerophila

Sensitivity was recorded to formalin and enrofloxacin, with bathing in the former followed by oral application of the latter controlling natural infection (Aydin *et al.*, 2000).

Enterobacteriaceae representatives

Citrobacter freundii

According to Sato *et al.* (1982) and Baya *et al.* (1990a), isolates were resistant to chloramphenicol, potentiated sulphonamides and tetracycline. Indeed, chemotherapy was unsuccessful at controlling the disease in a Japanese aquarium.

Edwardsiella ictaluri

Clinical cases of disease have been greatly reduced, although not completely eliminated, using oxytetracycline, dosed at 2.5 g of drug/45.4 kg body weight of fish/day for 4–5 days (Hawke, 1979). Certainly, isolates have been found to be susceptible to a wide range of inhibitory compounds, including cefaperazone, cinoxacin, florfenicol, kanamycin, moxalactam, neomycin, nitrofurantoin, oxolinic acid, streptomycin, ticarcillin and trimethoprim (Waltman and Shotts, 1986a; Gaunt *et al.*, 2003). Yet, plasmid-mediated resistance to antimicrobial compounds (including tetracycline) has been detected (Waltman *et al.*, 1989). Therefore, problems with chemotherapy may be envisaged in the future.

Edwardsiella tarda

It was reported that isolates were highly susceptible to cinoxacin, nitrofurantoin, oxolinic acid, kanamycin, moxalactam, trimethoprim, piperacillin, potentiated sulphonamide, neomycin, mezlocillin and streptomycin, but not to colistin, cloxacillin, clindamycin, bacitracin, erythromycin, lincomycin, methicillin, penicillin G, novobiocin or spectinomycin (Waltman and Shotts, 1986b). Chemotherapy by means of oxytetracycline, dosed at 55 mg of drug/kg body weight of fish/day for 10 days, has

been recommended (Meyer and Bullock, 1973). Assuming that infected fish consume the medicated diet, mortalities apparently dwindle away within 48–72 h of initiating treatment. However, recovery is slow, and the survivors may exhibit scar tissue. A complication with chemotherapy concerns R-plasmids which have been demonstrated in cultures of *Edw. tarda* isolated from eels (Aoki *et al.*, 1977). Conceivably, this may cause problems for chemotherapy in the future.

Pantoea agglomerans

The pathogen was sensitive to ampicillin, chloramphenicol, streptomycin and tetracycline, but not to novobiocin or penicillin (Hansen *et al.*, 1990). Based on these data, it would be prudent to evaluate tetracycline as a chemotherapeutant on fish farms, if the disease recurs.

Plesiomonas shigelloides

A 10-day treatment regime with potentiated sulphonamide (sulphadiazine at 200 mg/kg of body weight of fish/day and trimethoprim at 50 mg/kg body weight of fish/day) was effective in reducing mortality levels (Cruz *et al.*, 1986).

Salmonella enterica subsp. *arizonae*

Although control regimes were not adopted, the isolate was sensitive to chloramphenicol, fradiomycin, gentamicin, kanamycin, nalidixic acid, oxytetracycline, streptomycin and tetracycline, but resistant to erythromycin, spiramycin and sulphadimethoxine (Kodama *et al.*, 1987).

Serratia liquefaciens

There was contradictory evidence regarding the value of chemotherapy with oxytetracycline. Nevertheless, the disease could be controlled with oxolinic acid (McIntosh and Austin, 1990b).

Serratia marcescens

Isolates were sensitive to flumequine, oxolinic acid and potentiated sulphonamide (Baya *et al.*, 1992c).

Serratia plymuthica

Isolates were sensitive to chloramphenicol, flumequine, oxolinic acid, oxytetracycline, potentiated sulphonamide and streptomycin, but not to nitrofurantoin or sulphadiazine (Nieto *et al.*, 1990; Austin and Stobie, 1992b). Presumably, effective chemotherapy could be achieved with one or more of these compounds.

Yersinia ruckeri

Control of clinical cases of ERM may be mediated by means of antimicrobial compounds, including sulphamerazine and oxytetracycline (Rucker, 1966), methylene blue and oxytetracycline (Llewellyn, 1980), potentiated sulphonamides (Bullock *et al.*, 1983), tiamulin (Bosse and Post, 1983) and oxolinic acid (Rodgers and Austin, 1982). Early success was obtained by administering medicated diet containing sulphamerazine (200 mg/kg body weight of fish/day for 3 days; Rucker, 1966; Klontz and Huddleston, 1976), followed by oxytetracycline (50 mg/kg body weight of fish/day for 3 days). A parallel therapy worked against salmonid blood spot (Llewellyn, 1980). This involved treatment with methylene blue dosed at 1 g of dye/kg of food for 5 days), followed by oxytetracycline (66 mg/kg body weight of fish/day for 10 days) and then a repeat dose of methylene blue. Llewellyn (1980) reported that in hatchery conditions the disease cleared up in 10 to 14 days.

Two groups of workers demonstrated success with potentiated sulphonamides. Bullock *et al.* (1983) described the beneficial effects of a mixture of sulphadimethoxime and ormetroprim, dosed at 50 mg/kg body weight of fish/day for 5 days, whereas Bosse and Post (1983) discussed the usefulness of a combination of sulphadiazine and trimethoprim at the very low dose of 1 mg/kg body weight of fish/day for 14 days. This latter group also emphasised the benefit of using tiamulin at 5 mg/kg body weight of fish/day for 14 days. Finally, on the basis of laboratory experiments, Rodgers and Austin (1982) reported the effectiveness of oxolinic acid (10 mg/kg body weight of fish/day for 10 days). Unfortunately, the presence of R-plasmids may reduce the effect of some antibiotics (De Grandis and Stevenson, 1985).

Flavobacteriaceae representatives

Chryseobacterium scophthalmum

Furazolidone, used by i.p. injection (50 mg/kg body weight of fish) or by immersion (50 mg/l for 30 min daily for 10 days) was effective at controlling mortalities caused by the organism (Mudarris and Austin, 1989).

Flavobacterium spp.

Apparently, some level of control was exercised with oxytetracycline (Acuigrup, 1980a). *In vitro* experiments have also pointed to the value of oxytetracycline and other drugs commonly used in aquaculture (Farkas, 1985).

Flavobacterium columnare

Farkas and Oláh (1980) suggested the use of a salt (sodium chloride) bath for controlling infections. We agree with this suggestion, insofar as a 30 sec dip in 8% (w/v) sodium chloride cleared up an infection in rainbow trout fingerlings within a few days. Other remedies which have met with varying degrees of success include:

- arsenic, cadmium, copper, lead and selenium mixture, dosed at 1–3 μg/l for 1 day (MacFarlane *et al.*, 1986);
- copper sulphate, used at a dilution of 1:2,000, for a 1–2 min dip (Snieszko, 1958b); malachite green, used at a dilution of 1:15,000, for a 1–30 sec dip (Snieszko, 1958b);
- pyridylmercuric acetate, used at 2 mg/l for 1 h (Snieszko, 1958b);
- diquat, used at 1–2 mg/l for 30–60 min (McCarthy, 1975c);
- quaternary ammonium compounds, used at 2 mg/l for 1 h (McCarthy, 1975c);
- oxytetracycline, used at 50–100 mg/kg body weight of fish/day for 10 days (Snieszko, 1964; Ferguson, 1977). For external infections, oxytetracycline or chlortetracycline may also be used as a bath, i.e. 26–60 mg/l for 1 h (Snieszko and Hoffman, 1963; Wood, 1968);
- oxolinic acid, used at 10 mg/kg of body weight/day for 10 days (Soltani *et al.*, 1995);
- chloramphenicol, used at 5–10 mg/l, has been suggested for aquarium fish (Snieszko, 1958b);
- sulphadiazine, sulphamerazine or sulphamethiazone, used at 220 mg/kg body weight of fish/day for 10 days (Snieszko, 1954; Wolf and Snieszko, 1963). Sulphamerazine has been used successfully to treat rainbow trout, but not chinook salmon (Johnson and Brice, 1952).

Flavobacterium hydatis

Antibiogrammes confirmed sensitivity to chloramphenicol (30 μg), erythromycin (15 μg), nalidixic acid (30 μg), streptomycin (10 μg), sulphathiazole (1 mg) and tetracycline (30 μg), but not to lincomycin (2 μg), methicillin (5 μg), penicillin (10 IU) or sulphadiazine (300 μg) (Strohl and Tait, 1978). From the uniform sensitivity to nalidixic acid, sulpha drugs and tetracycline, these could be considered for chemotherapeutic use, should the need arise.

Flavobacterium johnsoniae

Acriflavine and oxolinic acid were considered to be effective treatments (Carson *et al.*, 1993).

Flavobacterium psychrophilum

Chemotherapy with oxytetracycline (Winton *et al.*, 1983), sulphonamides (Amend *et al.*, 1965) and furanace (Holt *et al.*, 1975) has been advocated. In particular, with infected fry, furanace dosed at 0.5 μg/ml for 1 h on every third day has been useful. From a comparison of 48 isolates from RTFS, sensitivity was recoded to doxycycline, enrofloxacin, florfenicol and sarafloxacin (Rangdale *et al.*, 1997a).

Tenacibaculum maritimum

From an examination of 75 isolates, it was determined that *T. maritimum* was highly susceptible to ampicillin, erythromycin, josamycin, nifurpirinol, penicillin G and

sodium nifurstyrenate, moderately sensitive to chloramphenicol, doxycycline, oleandomycin, oxytetracycline, sulphamonomethoxine and thiamphenicol, weakly sensitive to nalidixic acid, oxolinic acid, spiramycin and sulphisoxazole, and resistant to colistin and streptomycin (Baxa *et al.*, 1988b). In *in vivo* experiments, the efficacy of sodium nifurstyrenate was confirmed for chemotherapy. Thus, immersion (0.5 μg/μl for 1 h) or oral administration (30 mg/kg body weight of fish/day for 4 days) reduced mortalities in yellowtail to 60.5%, compared with 100% among the untreated controls (Baxa *et al.*, 1988b). Trimethoprim and amoxycillin (dosed at 80 mg/kg body weight of fish) have been recommended in Australia (Soltani *et al.*, 1995).

Sporocytophaga sp.

Data have indicated that control may be exercised by use of pyridylmercuric acetate, ethylmercuric phosphate, oxytetracycline or chlortetracycline, at 1 mg/l for 1 h (Wood, 1968), or a 2 min dip in copper sulphate (repeated on three consecutive days) at 1:2,000 (Anderson and Conroy, 1969).

Moraxellaceae representatives

Acinetobacter sp.

Oxytetracycline, at a single dose of 100 mg/fish, appeared to be effective for chemotherapy when administered by intramuscular injection.

Moraxella sp.

The pathogen was susceptible to chloramphenicol, nitrofurantoin, oxolinic acid, penicillin and tetracycline (Baya *et al.*, 1990b).

Moritellaceae representative

Moritella viscosa

Florfenicol should work, with *in vitro* and *in vivo* experiments being seemingly successful. Plasma concentration of 3.0 ± 1.8 mg/ml were recorded in Atlantic salmon after the administration of suitably medicated feed. However, mortality patterns after infection were less convincing (Coyne *et al.*, 2006).

Oxalobacteraceae representative

Janthinobacterium lividum

Cultures were sensitive to furazolidone, oxolinic acid, oxytetracycline and potentiated sulphonamides (Austin *et al.*, 1992b). Therefore, it is surmised that one or more of these compounds would be useful in chemotherapy.

Photobacteriaceae representative

Photobacterium damselae subsp. *piscicida*

Little is known about the value of chemotherapeutants. An *in vitro* study highlighted the value of ampicillin (Kusuda and Inoue, 1976), but field trials were not carried out. In a further study, Kusuda *et al.* (1988) reported marked sensitivity to ampicillin and oxolinic acid, moderate sensitivity to nalidixic acid and sodium nifurstyrenate, but resistance to chloramphenicol, chlortetracycline, oxytetracycline and tetracycline. Again, field evidence was not supplied. Sano *et al.* (1994) noted the value of fosfomycin (MIC = 1.56–3.13 μg/ml) at controlling laboratory infections. An effective dose was 40 mg of fosfomycin/kg body weight of fish/day for 5 days, albeit administered only one hour after infection. This dose reduced mortalities by *Ph. damselae* subsp. *piscicida* to 0% (Sano *et al.*, 1994). R-plasmids have been identified among isolates, conferring resistance to chloramphenicol, kanamycin, sulphamonomethoxine and tetracycline (Aoki and Kitao, 1985), and florfenicol (Kim *et al.*, 1993). Although the isolates described by Aoki and Kitao (1985) were confined to one locality in Japan, there is the likelihood that the resistance will spread quickly to other sites.

Piscirickettsiaceae representative

Piscirickettsia salmonis

Sensitivity was recorded to clarithromycin, chloramphenicol, erythromycin, gentamicin, oxytetracycline, sarafloxin, streptomycin and tetracycline, but not to penicillin or spectinomycin (Cvitanich *et al.*, 1991).

Rickettsia-like organisms

Treatment with oxytetracycline was reported to be successful (Chern and Chao, 1994).

Pseudomonadaceae representatives

Pseudomonas anguilliseptica

Jo (1978) discussed the usefulness of nalidixic acid, oxolinic acid and piromidic acid for chemotherapy.

Pseudomonas fluorescens

Bath treatments with benzalkonium chloride (1–2 mg/l of water/1 h), furanace (0.5–1 mg/l of water/5–10 min) or malachite green (1–5 mg/l of water/1 h) may help control early clinical cases of disease (Austin, 1984b). In one study, isolates showed susceptibility to kanamycin, nalidixic acid and tetracycline (Sakai *et al.*, 1989a). A second investigation reported widespread susceptibility to gentamicin, kanamycin and neomycin, less to amikacin and oxytetracycline, and total resistance to chlor-

amphenicol, erythromycin, penicillin and potentiated sulphonamide (Markovic *et al.*, 1996).

Pseudomonas pseudoalcaligenes

Antibiogrammes revealed sensitivity to oxytetracycline, oxolinic acid and potentiated sulphonamides (Austin and Stobie, 1992b).

Vibrionaceae representatives

Vibrio alginolyticus

Colorni *et al.* (1981) achieved success with chloramphenicol, dosed at 50 mg of drug/kg body weight of fish/day for an unspecified period, and nitrofurantoin (50 mg/l of water/1 h), both of which alleviated mortalities. However, we would caution against the use of chloramphenicol in fisheries, in view of the report of the Swann Committee (Report, 1969). In essence, chloramphenicol should be restricted to use in human beings.

Vibrio anguillarum

Antimicrobial compounds, including florfenicol (Seljestokken *et al.*, 2006), have proved to be very useful in controlling vibriosis. It is perhaps ironic that emphasis has been placed on using drugs as food additives, because vibriosis is typified by inappetence. Consequently, antimicrobial compounds need to be administered (by food) very early in the disease cycle, if success is to be achieved. Workers have indicated the value of many compounds, including chloramphenicol, flumequine (Vik-Mo *et al.*, 2005), furanace, nitrofurazone, oxolinic acid, oxytetracycline and sulphamerazine. As a general comment, we advise upon caution when contemplating the need for pharmaceuticals, particularly antibiotics, because of the potential risk of resistance which may be attributed to plasmids, i.e. R-factors (Aoki, 1988). Aoki *et al.* (1974) reported that 65/68 *V. anguillarum* isolates carried R-factors, conveying resistance to chloramphenicol, streptomycin, sulphonamides and tetracycline. Therefore, if R-factors abound, it is unlikely that the common antibiotics will do much to retard the disease cycle.

Another approach has involved the use of antimicrobial peptides, namely a cecropin–melittin hybrid peptide and pleurocidin amide, which is a C-terminally amidated form of a natural flounder peptide. These were applied continuously at a rate of 200 μg/day and 250 μg/for for cecropin–melittin hybrid peptide and pleurocidin amide, respectively, by mini-osmotic pumps installed in the peritoneal cavity of coho salmon with the result that fewer mortalities occurred compared with the controls. In the case of pleurocidin amide, 5% mortalities were recorded compared with 67–75% of the controls (Jia *et al.*, 2000).

Vibrio fischeri

Chemotherapy was ineffective at reducing mortalities (Lamas *et al.*, 1990).

Vibrio harveyi

Kraxberger-Beatty *et al.* (1990) reported success with Prefuran (Argent) dosed at 0.1 mg/l for an unspecified period. Saeed (1995) found success with oxytetracycline as a food additive. Yii *et al.* (1997) determined susceptibility to a wide range of inhibitory compounds, including chloramphenicol, doxycycline, nalidixic acid, oxolinic acid, oxytetracycline and sulphonamide, but not ampicillin or penicillin G.

Vibrio pelagius

Treatment with oxytetracycline was effective at stopping mortalities (Angulo *et al.*, 1992). Also, it was considered that potentiated sulphonamides and flumequine would be successful.

Vibrio salmonicida

Oxolinic acid controls mortalities in Atlantic salmon (B. Austin, unpublished data).

Vibrio splendidus

Susceptibility was recorded to chloramphenicol, flumequine, nitrofurantoin, nifurpirinol, oxolinic acid and potentiated sulphonamide, but not to ampicillin, oxytetracycline or streptomycin. Treatment with oxolinic acid was partially successful. However, the presence of virus undoubtedly complicated the chemotherapeutic regime (Lupiani *et al.*, 1989). In a subsequent study, Angulo *et al.* (1994) reported success with flumequine as a feed additive.

Vibrio tapetis

Sensitivity was recorded to flumequine, oxolinic acid and oxytetracycline (Jensen *et al.*, 2003).

Vibrio vulnificus

Although this aspect has not been addressed, it seems likely that infections will respond to broad-spectrum antimicrobial compounds, such as flumequine, oxolinic acid, oxytetracycline and potentiated sulphonamides (Muroga *et al.*, 1976a, b; Nishibuchi and Muroga, 1977, 1980; Nishibuchi *et al.*, 1979, 1980).

MISCELLANEOUS PATHOGENS

Unknown Gram-negative rod

According to Palmer *et al.* (1994), the pathogen was susceptible to amoxycillin and penicillin G, but resistant to cotrimoxazole and oxytetracycline.

DISINFECTION/WATER TREATMENTS

Apart from the use of antibiotics and related compounds, the application of other chemicals to water as disinfectants is effective for disease control Such chemicals include benzalkonium chloride, chloramine B and T, chlorine, formalin, iodophors, malachite green and methylene blue. For example, Sato *et al.* (1982) used chlorine to disinfect water, and thus control infection by *Cit. freundii.*

Another approach is to alter (increase or decrease, according to the season) the temperature of water within fish-holding facilities. We have noticed that this approach appears to be effective at reducing the incidence of diseases such as columnaris. Specifically:

Aeromonas salmonicida

Adequate husbandry practices—such as maintenance of good water quality, disinfection of fish farm equipment and utensils—especially when disease outbreaks occur, and routine disinfection policies for eggs upon arrival at receiving sites (Herman, 1972; McCarthy and Roberts, 1980).

Citrobacter freundii

Outbreaks of the disease abated following adoption of water disinfection (with chlorine) practices (Sato *et al.*, 1982).

Clostridium botulinum

As there is no effective chemotherapy for botulism in fish, disinfection of the ponds has been advocated. Success has resulted from moving the stock to clean areas, draining the contaminated ponds, and removing surface mud and detritus before disinfecting with slaked lime at $1.6\,kg/m^2$. The disinfectant should be worked well into the layers at the bottom of the ponds, left for a period of not less than 7 days, and the pond then returned to use (Huss *et al.*, 1974; Cann and Taylor, 1982). To remove clostridia from the gastro-intestinal tract of trout, it has been recommended that the fish should be starved for 5 days (Wenzel *et al.*, 1971). However, Huss *et al.* (1974) showed that, whereas starvation does indeed reduce contamination with *C. botulinum*, the overall rate of success depends on other factors, including the nature of the water supply. It should be emphasised that clostridia may be normal inhabitants of the fish intestine.

Flavobacterium columnare

Diquat was effective as a bath treatment for channel catfish, effectively stopping mortalities caused by the pathogen. Also, there was some reduction in mortalities following bathing in chloramine T and potassium permanganate, but not so with copper sulphate and hydrogen peroxide (Thomas-Jinu and Goodwin, 2004). Experiments with rainbow trout fingerlings have demonstrated a potential for the use of

sodium chloride baths in reducing mortalities caused by columnaris following waterborne challenge (Suomalainen *et al.*, 2005).

Mycobacterium marinum

Ethyl alcohol (50 and 70%), 1% benzyl-4-chlorophenol/phenylphenol and sodium chlorite (1:5:1 or 1:18:1 in the ratio of base:water:activator) were most effective at reducing or eliminating *Myc. marinum* within 1 min. Sodium hypochlorite (50,000 mg/l) was less effective, and needed 10 min contact time to reduce bacterial numbers. Ethyl alcohol (30%), 1:256 *N*-alkyl-dimethyl-benzyl ammonium chloride and 1% potassium peroxymonosulphate–NaCl were generally ineffective even after 1 h (Mainous and Smith, 2005).

Renibacterium salmoninarum

Disinfection of egg surfaces has also been utilised to control BKD. Iodophors, at 25–100 mg/l for 5 min, have proved beneficial at reducing transmission of the disease (Amend and Pietsch, 1972; Ross and Smith, 1972; Bullock *et al.*, 1978b), although they will not eliminate the pathogen from inside eggs (Evelyn *et al.*, 1984). The use of erythromycin phosphate, at 1–2 mg/l for 30 min, has been advocated as an additive for water-hardening of eggs (Klontz, 1978). However, it is debatable whether or not it is wise to use antibiotics in this way.

Another approach has been to disinfect the water in fish farms. In particular, a level of only 0.05 mg of free chlorine/l was sufficient to inactivate cells of the pathogen in 18 sec (Pascho *et al.*, 1995). With such rapid inactivation, there must surely be a use for the technique in hatcheries.

Staphylococcus aureus

A bath of potassium permanganate (1 ml/l) for 5–10 min, together with treating the pond water with 250 mg/l of lime and 1 mg/l of potassium permanganate every fourth day, was considered effective at halting mortalities, except with advanced cases of the disease (Shah and Tyagi, 1986).

PREVENTING THE MOVEMENT AND/OR SLAUGHTERING OF INFECTED STOCK

Some diseases, e.g. BKD, ERM and furunculosis, are suspected to be spread through the movement of infected stock. Therefore, it is sensible to apply movement restrictions or even adopt a slaughter policy to diseased stock, as a means of disease control. This may prevent the spread of disease to both farmed and wild fish. Of course, the issue of movement restrictions is highly emotive among fish farmers. However, the procedure may be beneficial to the industry when viewed as a whole. Evidence from Iceland has revealed that the culling of infected Atlantic salmon brood stock led to a reduction in the incidence of BKD (Gudmundsdóttir *et al.*, 2000). At the start of the

programme the incidence of infection was reported as ~35% of the brood stock on two ranch sites, but after a few years of adopting the programme of culling the incidence fell to <2% (Gudmundsdóttir *et al.*, 2000). Certainly, the concept of movement restrictions usually involves legislative machinery, of which the Diseases of Fish Act (1983) in Great Britain is a prime example. To work effectively, there is a requirement for both the efficient monitoring of all stock at risk to disease, and the dissemination of the information to all interested parties. However, we believe that in any allegedly democratic society where such measures are adopted, there should be adequate compensation to the fish farmer for loss of revenue.

PROBIOTICS/BIOLOGICAL CONTROL

What is the difference between a probiotic, an immunostimulant (administered orally) and an oral vaccine? The answer to this rhetorical question has not been fully considered, but there is likely to be considerable overlap between all three. Certainly, there is increasing evidence that members of the natural aquatic microflora are effective at inhibiting fish pathogens, inevitably by competitive exclusion which may involve the production of antibiotics or low molecular weight inhibitors. Dopazo *et al.* (1988) discovered the presence in the marine environment of antibiotic-producing bacteria, which inhibited a range of bacterial fish pathogens, including *Aer. hydrophila*. These inhibitors produced low molecular weight (<10 kDa) anionic, thermolabile antibiotics. Subsequently, Chowdhury and Wakabayashi (1989), Austin and Billaud (1990) and Westerdahl *et al.* (1991) reported the presence of microbial inhibitors of *Fla. columnare*, *Ser. liquefaciens* and *V. anguillarum*. Smith and Davey (1993) identified a fluorescent pseudomonad which antagonised *Aer. salmonicida*. "Good" bacteria have been described for the control of numerous diseases, and there is a tendency that the probiotic works faster than an oral vaccine (see Irianto and Austin, 2002). The assumption that probiotics must be live preparations was dashed when it was demonstrated that formalised suspensions of cells were effective at controlling atypical *Aer. salmonicida* infection in goldfish (Irianto *et al.*, 2003) and furunculosis in rainbow trout (Irianto and Austin, 2003), when applied as feed additives. Whereas in terrestrial animals probiotics are considered to work by means of competitive exclusion, in fish stimulation of the innate immune response (Irianto and Austin, 2003; Kim and Austin, 2006) and interference with adhesion to intestinal mucosal surfaces (Chabrillón *et al.*, 2005) have been demonstrated. The progress of infection of *Fla. columnare* was mediated by *Aer. hydrophila* and *Cit. freundii* (Chowdhury and Wakabayashi, 1989). Sugita *et al.* (1997) isolated a *Vibrio*, coined strain NM10, from ponyfish (*Leiognathus nuchalis*) intestines, and determined antagonism of *Ph. damselae* subsp. *piscicida* by a heat-labile proteinaceous compound of 5 kDa. Putative *Aeromonas* and *Vibrio*, from halibut, have been found to inhibit the growth of fish-pathogenic *Vibrio* (Bergh, 1995). Moreover, an isolate of *V. alginolyticus*, previously used as a probiotic in Ecuadorian shrimp hatcheries, has been effective at controlling diseases caused by *Aer. salmonicida*, *V. anguillarum* and *V. ordalii* (Austin *et al.*, 1995b). The relative incidence of microbial antagonists is

indicated from a study of >400 bacterial isolates from the gastro-intestinal tract and surface of turbot, fish food and water, in which 28% (mostly from the intestinal mucus) were inhibitory to *V. anguillarum* (Westerdahl *et al.*, 1991). Lactococcosis and streptococcicosis, which are difficult to control by other means, have been conducive to probiotics, with *Aer. sobria* GC2 (recovered from the digestive tract of ghost carp) dosed at 5×10^7 cells/g of feed and fed over 14 days, stimulating the innate immune response (increase in leucocytes, phagocytosis and respiratory burst activity) and conferring excellent protection against challenge (Brunt and Austin, 2005). Consequently, it is apparent that the native aquatic microflora may be important as a natural means of disease control. In addition, there is a report of the benefits for disease control of using the biopesticide, *Bacillus thuringiensis* (Meshram *et al.*, 1998).

Interest has been expressed in the possible use of bacteriophage for moderating fish disease. Of relevance, two lytic bacteriophages—Podoviridae PPp-W4 and Myoviridae PppW-3—which were recovered from diseased ayu and pond water used to rear fish (Park *et al.*, 2000) have been considered for use against *Ps. plecoglossicida* in ayu (Park and Nakai, 2003). In *in vitro* work, PPp-W4 was the more successful at inhibiting the pathogen. In fish experiments involving challenge with feed supplemented with *Ps. plecoglossicida* at 10^7 CFU/fish, followed by use of feed containing bacteriophage (10^7 PFU/fish), the resulting mortalities were greatly reduced. Thus, by use of groups with PPp-W3, PPp-W4 and a mixture of both, mortalities of 53, 40 and 20% were recorded compared with 93% mortalities among the controls (Park and Nakai, 2003). Field trials were equally successful, and there was no evidence for the development of bacteriophage resistance by *Ps. plecoglossicida* (Park and Nakai, 2003). There are promising signs that bacteriophage administered in feed may be able to moderate infections by *Lactococcus garvieae* in yellowtail (Nakai *et al.*, 1999). Certainly, these data deserve further attention.

INHIBITORS OF QUORUM-SENSING

Some fish pathogens, e.g. *V. anguillarum*, produce quorum-sensing molecules, which may well be involved with the regulation of virulence. If these molecules could be blocked using quorum-sensing inhibitors, then virulence could be reduced or even annulled. Funanone C-30, dosed at 0.01 or 0.1 μM, was determined to be beneficial at reducing mortalities caused by co-habitation with *V. anguillarum* infected rainbow trout from 80–100% in the controls to 4–40% in treated groups (Rasch *et al.*, 2004).

11

Conclusions

It should be abundantly clear that comparatively few aspects of the biology of bacterial fish pathogens have been studied in detail. In particular, it has been fashionable to describe the pathogenicity mechanisms and control measures (by vaccines, non-specific immunostimulants and probiotics). However, even these areas are incomplete. The outlook for the future is nevertheless encouraging, and it seems likely that there will be increasing interest in the study of fish diseases. To some extent, this will be justified by the continued expansion of aquaculture and the expected increase in outbreaks of disease. It seems likely that future developments will encompass the following.

RECOGNITION OF EMERGING CONDITIONS

Any systematic study of fish populations will undoubtedly reveal the presence of novel disease conditions. Of course, whether or not such conditions will be truly emerging or merely ignored in the past is the subject of conjecture. New pathogens may well appear to take the place of some of the existing problems. During the preparation of this book, we have noted the spread of red mark syndrome (Figures 11.1–11.5 [see colour section]), from its initial focal point in Scotland. Now, the condition is widespread throughout the U.K. What will be next?

TAXONOMY AND DIAGNOSIS

As the rules governing bacterial taxonomy have been considerably improved, it is anticipated that the descriptions of novel pathogens will be much better than published in some of the earlier reports. However, there is an annoying tendency of naming new taxa after studying only a few (perhaps even one) isolates. We argue

that this practice is not reflective of good science. It is to be hoped that more reliable identification methods will emerge, i.e. identification should lose some of the subjectivity, although the current descriptions of new taxa lack sufficient information to enable diagnosis of fresh isolates. It would be nice to think that diagnosticians may even use systems designed for fish pathogens, rather than relying on kits developed for or adapted from medical microbiology. The rapid developments in molecular biology are already impacting on diagnosis, although current methods are expensive and slower than serology. Other methods, such as pyrolysis techniques, may offer excellent opportunities for the detection of pathogens and the identification of pure cultures (see Gutteridge and Norris, 1979). Perhaps, future developments will lead to molecular techniques that can be used by diagnosticians outwith specialised laboratories, and preferably in field conditions.

ISOLATION AND SELECTIVE ISOLATION OF PATHOGENS

Since 1980, there have been many improvements with isolation procedures for some fish pathogens. For example, selective media for *Ren. salmoninarum* (Austin *et al.*, 1983a) and *Y. ruckeri* (Waltman and Shotts, 1984) have been formulated. This impetus should continue, particularly for some of the difficult-to-isolate pathogens, such as *Mycobacterium.*

ECOLOGY (EPIZOOTIOLOGY)

Overall and with few exceptions, the ecology of fish pathogens has been neglected. Consequently, it is often unclear whether a pathogen is a representative of the natural aquatic microflora or is restricted to fish. Nevertheless, a wealth of information has been gathered on a few organisms, including *Aer. salmonicida*, although it could be argued that the true reservoir of this pathogen remains to be discovered. True, there is an interest in ecological matters, but progress seems to reflect changing attitudes—sometimes ecology is fashionable, but not so at other times. We argue that an improvement in epizootiology will be invaluable in the design of disease control measures, particularly those involving the legislative machinery for movement restriction policies.

PATHOGENICITY MECHANISMS

There has been commendable progress with some pathogens, notably *Aer. salmonicida*, *Ren. salmoninarum* and *V. anguillarum.* However, the research input needs to continue with attention being diverted to other pathogens. Information about pathogenicity mechanisms is especially relevant for vaccine development programmes.

CONTROL MEASURES

Although conventional vaccine development programmes seem likely to continue, further progress on difficult candidates, such as *Rickettsia* and *Ren. salmoninarum*, necessitate other approaches, such as offered by genetic engineering. Also, the current interest in non-specific immunostimulants, probiotics and medicinal plants/herbs seems likely to continue for the foreseeable future. Antimicrobial compounds continue to be used extensively in many parts of the world, but there is concern about tissue residues and the development and spread of resistance. Therefore, curbs on the non-medical use of antimicrobial compounds are likely to be imposed in some countries. It is to be envisaged that there will be ever-tighter regulations on the use of antimicrobial compounds in aquaculture. Attention should also be focused on stock improvement programmes, which may highlight genetically determined disease-resistant fish strains.

THE EFFECTS OF POLLUTION

There is increasing concern about the possible role of pollution in disease, particularly of wild fish stocks (see also Pippy and Hare, 1969; Mahoney *et al.*, 1973; Robohm *et al.*, 1979). As this is a politically emotive issue, there is likely to be an increase in monies available and, thus, a stimulation of research interest. At present, there is considerable confusion over the precise role of pollution and fish health (Bucke, 1991, 1997). Nevertheless, there are good data that long-term exposure to pollutants has adversely affected the health of fish, especially in the North Sea and Great Lakes. However, mortalities among fish populations do not necessarily imply disease. Furthermore, disease may develop long after the pollutant has been effectively removed from the aquatic environment. Much of the work attempting to correlate fish disease with aquatic pollution has resulted from surveys, many of which have been carried out in the North Sea (e.g. Dethlefsen and Watermann, 1980; Dethlefsen *et al.*, 1987, 2000; McVicar *et al.*, 1988; Vethaak and ap Rheinallt, 1992). Briefly, fish are caught with nets, and the relative incidence of disease determined. One conclusion from these surveys is that larger numbers of diseased fish occur generally in the polluted compared with clean/unpolluted locations (Dethlefsen *et al.*, 2000). However, the distinction between polluted and clean sites is imprecise. Therefore, there would be some uncertainty as to what comprises a truly polluted or clean site. Moreover, it is uncertain from surveys how long fish might have been in a polluted environment prior to capture. Thus, the effects of fish migration on the incidence of disease needs to be considered (Vethaak *et al.*, 1992; Bucke *et al.*, 1992; Jacquez *et al.*, 1994).

Pollution has been associated with some bacterial diseases, namely fin and tail rot (Vethaak, 1992; Vethaak *et al.* 1996), gill disease/hyperplasia (Kirk and Lewis, 1993), and skin disease/ulceration (Vethaak, 1992; Vethaak and Jol, 1996). The trigger has been attributed to contaminated diets (Landsberg, 1995), heavy metals, e.g. chromium (Rødsaether *et al.*, 1977; Prabakaran *et al.*, 2006), hydrocarbons (Khan,

1987), nitrogenous compounds, i.e. ammonia (Kirk and Lewis, 1993) and nitrites (Hanson and Grizzle, 1985), pesticides (e.g. Voigt, 1994), polychlorinated biphenyls (Ekman *et al.*, 2004), sewage (e.g. Austin and Stobie, 1992b), organic pollutants (Grawinski and Antychowicz, 2001) and unspecified pollutants (e.g. Vethaak and Jol, 1996). In one example, organic pollution has been attributed to the high occurrence of *Ser. plymuthica* infections in salmonid farms in Poland since 1996 (Grawinski and Antychowicz, 2001). Generally, the reasons for the association between pollution and disease need to be better researched. However, proof of correlation between the occurrence of specific pollutants and disease has seldom been documented. Surveys, which have pointed to a correlation between pollution and disease, have generally not considered the nature or concentration of the pollutant(s).

An association has been made between fish diseases and unknown components of sewage dumping (Siddall *et al.*, 1994). For example in a survey of 16 sites in the Dutch Wadden Sea, a higher incidence of skin ulcers and fin rot was noted in fish caught near freshwater drainage sluices than elsewhere (Vethaak, 1992). Pollution by domestic sewage, i.e. leakage from a septic tank, was attributed to a new skin disease, which was characterised by the presence of extensive skin lesions and muscle necrosis, in rainbow trout (otherwise infected with ERM for which there might also be a link with sewage sludge; Dudley *et al.*, 1980) in Scotland during 1992 (Austin and Stobie, 1992b). Interestingly, the skin lesions—but not ERM—declined substantially after the leaking septic tank was repaired.

There is accumulating evidence that contaminated waters lead to immunosuppression. One recent example describes the increased susceptibility of chinook salmon from a contaminated estuary to *V. anguillarum* (Arkoosh *et al.*, 1998). Undoubtedly, the future will bring further examples.

ZOONOSES

There is a growing awareness that some bacterial fish pathogens may also infect human beings. Although the number of cases is mercifully small, some have attracted media attention. Generally, official statistics are absent, and, therefore, only an educated guess can be taken as to the types and severity of risks which may exist for humans. Some publications—e.g. Austin (1981), Grimes (1986) and Austin and Austin (1989)—have begun to address the problem. The range of fish-associated bacteria (pathogens and commensals) that may infect humans include:

- *Aer. hydrophila* (causing diarrhoea and septicaemias);
- *Campylobacter jejuni* (gastro-enteritis);
- *Cl. botulinum* type E (botulism);
- *Cl. perfringens* (food-poisoning);
- *Edw. tarda* (diarrhoea);
- *Erysipelothrix rhusiopathiae* (fish rose);
- *Legionella pneumoniae* (atypical pneumonia);
- *Leptospira interrogans* (leptospirosis, Weil's disease);

- *Myc. fortuitum* (mycobacteriosis; fish tank granuloma);
- *Myc. marinum* (mycobacteriosis; fish tank granuloma);
- *Ph. damselae* (necrotising fasciitis, bacteraemia);
- *Plesiomonas shigelloides* (gastro-enteritis);
- *Ps. aeruginosa* (wound infections);
- *Ps. fluorescens* (wound infections);
- *Salmonella* spp. (food-poisoning);
- *Sta. aureus* (food-poisoning);
- *Str. iniae* ("mad fish disease");
- *V. cholerae* (cholera);
- *V. parahaemolyticus* (food-poisoning);
- *V. vulnificus* (wound infections) (Novotny *et al.*, 2004).

The source of some of these organisms may well be the waters in which the fish are to be found. Others occur in the tissues and the digestive tract of apparently healthy fish. For example, *Plesiomonas shigelloides* and *V. vulnificus* have been cultured from the digestive tract of pike (Gonzalez *et al.*, 1999) and numerous fish from the U.S. Gulf Coast (DePaola *et al.*, 1994), respectively. According to DePaola *et al.* (1994), a seasonal fluctuation was recorded with minimum and maximum numbers occurring in Winter and April to October, respectively. The highest populations of *V. vulnificus* (10^8 bacteria/100 g) were associated with the gut contents of bottom-feeding fish, especially those that consumed molluscs and crustacea. In comparison, plankton-feeding fish contained 10^5 cells of *V. vulnificus*/100 g. Overall, the incidence of *V. vulnificus* was comparatively uncommon in offshore fish, instead being restricted to specimens from estuaries. Also, *V. cholerae* was recovered from sharks (Grimes *et al.*, 1993). *Sta. aureus* has been isolated repeatedly from striped bass reared in flow-through and recirculating systems (Nedoluha and Westhoff, 1995). The transfer to humans will probably reflect the handling of diseased specimens. Uptake into humans may be via cuts or grazes, or, less likely, via the digestive tract.

Aeromonas hydrophila

Aer. hydrophila has been implicated as an opportunistic and occasionally as a primary pathogen of humans. In one example, a fish farm worker developed a bacterial septicaemia, which was attributed to *Aer. hydrophila*, and presumed to have resulted from exposure in the fish farm (Austin, 1981).

Edwardsiella tarda

Enterics have occasionally spread from fish to humans, and caused gastro-enteritis/diarrhoea. In one documented case, *Edw. tarda* from tropical ornamental fish, *Pterophyllum scalare*, caused diarrhoea in an infant (Vandepitte *et al.*, 1983).

Leptospira interrogans

During 1980 and 1981, three cases of leptospirosis attributed to serogroup icterohaemorrhagiae, of which one was a fatality, occurred in fish farm workers in the U.K. Workers on one of the sites, which harboured rats, possessed antibodies to the pathogen, which indicated a risk of infection (Robertson *et al.*, 1991). Previously, only one other case of leptospirosis in a fish farm worker had been reported in the U.K., i.e. in 1968 (Gill *et al.*, 1983). Elsewhere, the disease had been found among prawn farmers in Hawaii (Anderson *et al.*, 1982). Subsequently, an examination of blood from >200 fish farm workers from 82 fish farms in the U.K. revealed the presence of antibodies to serogroup icterohaemorrhagiae in three of the samples (Gill *et al.*, 1983). Consequently, it would appear that there is minimal risk to leptospirosis among fish farm workers.

Mycobacterium marinum

Myc. marinum, and occasionally *Myc. fortuitum*, may be transmitted from diseased fish to humans (especially aquarists), where the organisms cause localised superficial (papulopustular) lesions on the hands and arms (referred to as fish tank granuloma; Bruckner-Tuderman and Blank, 1985; Gray *et al.*, 1990; Boyce, 1997). Although not fatal, the condition may need months or occasionally years of chemotherapy (Black and Eykyn, 1977; Austin, 1981). Within the U.K., there appears to be a steady, low number of infections each year (e.g. Ryan and Bryant, 1997; Boyce, 1997). Typically, patients, who are usually in middle age, have handled ornamental fish or the tanks/tank water prior to infection (Ryan and Bryant, 1997).

Streptococcus iniae

During 1995–1996, *Str. iniae* became associated with a cluster of four cases, totaling nine patients, of invasive disease (including endocarditis and cellulitis of the hand), coined by the press as "mad fish disease" because of the neurological symptoms in fish, in females of Asian origin in the Toronto area of Canada (Centers for Disease Control, 1996; Weinstein *et al.*, 1997). Anecdotal evidence suggested that the women purchased clinically diseased tilapia and following handling/preparation of the whole fish became infected. By PFGE, all the isolates from the infected women and some from diseased fish were indistinguishable. The conclusion was reached that the fish pathogen *Str. iniae* could cause human disease. As streptococcal fish infections inevitably produce clearly visible lesions, it is curious why individuals would purchase something that did not look in prime condition. Also, it is relevant to enquire why a fish farmer would sell diseased stock for human consumption. In addition to the North American experience, *Str. iniae* has been recovered in Asia from two cases of septic arthritis and bacteraemic cellulitis. The patients had been handling fresh fish (Lau *et al.*, 2006).

Vibrio vulnificus

In Amsterdam, The Netherlands, a 63-year-old man was hospitalised because of severe pain in the right arm (Veenstra *et al.*, 1992). The arm became progressively swollen, and was painful. Also, it was noted that there were small wounds on the hands. In hospital, the patient's condition deteriorated with skin and muscle necrosis, necessitating surgery to remove the diseased tissue, before eventual recovery. Interestingly, the day before the onset of symptoms, he had cleaned eels purchased from a local market. Bacteriology revealed the presence of *V. vulnificus* from blood. Indeed, this isolate was considered to resemble one recovered previously from eels in a Dutch eel farm (Veenstra *et al.*, 1992). It was presumed that the man became infected from the eels through open wounds on his hand. Interestingly, we determined that the isolate caused disease in laboratory–infectivity experiments with Atlantic salmon.

Clearly, there should not be complacency in the handling of diseased fish; otherwise the infection may well spread to the handlers. This is certainly an area deserving of further consideration.

Bibliography

Abe, P.M. (1972) Certain chemical and immunological properties of the endotoxin from *Vibrio anguillarum*. M.S. thesis, Oregon State University, Corvallis.

Abutbul, S., Golan-Goldhirsh, A., Barazani, O. and Zilberb, D. (2004) Use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* sp.). *Aquaculture* **238**, 97–105.

Ackerman, P.A., Iwama, G.K. and Thornton, J.C. (2000) Physiological and immunological effects of adjuvanted *Aeromonas salmonicida* vaccines on juvenile rainbow trout. *Journal of Aquatic Animal Health* **12**, 157–164.

Acosta, F., Real, F., Caballero, M.J., Sieiro, C., Fernández, A. and Rodríguez, L.A. (2002) Evaluation of immunohistochemical and microbiological methods for the diagnosis of brown trout infected with *Hafnia alvei*. *Journal of Aquatic Animal Health* **14**, 77–83.

Acosta, F., Lockhart, K., Gahlawat, S.K., Real, F. and Ellis, A.E. (2004) Mx expression in Atlantic salmon (*Salmo salar* L.) parr in response to *Listonella anguillarum* bacterin, lipopolysaccharide and chromosomal DNA. *Fish & Shellfish Immunology* **17**, 255–263.

Acosta, F., Real, F., Ellis, A.E., Tabraue, C., Padilla, D. and Ruiz de Galarreta, C.M. (2005) Influence of vaccination on the nitric oxide response of gilthead seabream following infection with *Photobacterium damselae* subsp. *piscicida*. *Fish & Shellfish Immunology* **18**, 31–38.

Acosta, F., Ellis, A.E., Vivas, J., Padilla, D., Acosta, B., Déniz, S., Bravo, J. and Real, F. (2006) Complement consumtion by *Photobacterium damselae* subsp. *piscicida* in seabream, red porgy and seabass normal and immune serum. Effect of the capsule on the bactericidal effect. *Fish & Shellfish Immunology* **20**, 709–717.

Actis, L.A., Potter, S.A. and Crosa, J.W. (1985) Iron-regulated outer membrane protein OM2 of *Vibrio anguillarum* is encoded by virulence plasmid pJM1. *Journal of Bacteriology* **161**, 736–742.

Actis, L.A., Tolmasky, M.E., Crosa, L.M. and Crosa, J.H. (1995) Characterization of the expression of FATB, an iron transport protein encoded by the pJM1 virulence plasmid. *Molecular Miocrobiology* **17**, 197–204.

Acuigrup (1980a) Flavobacteriosis in coho salmon (*Oncorhynchus kisutch*). In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 212–217.

Acuigrup (1980b) Trial vaccination of rainbow trout against *Aeromonas liquefaciens*. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 206–211.

Adams, A., Auchinachie, N., Bundy, A., Tatner, M.F. and Horne, M.T. (1988) The potency of adjuvanted injected vaccines in rainbow trout (*Salmo gairdneri* Richardson) and bath vaccines in Atlantic salmon (*Salmo salar* L.) against furunculosis. *Aquaculture* **69**, 15–26.

Adams, A., Thompson, K.D., Morris, D., Farias, C. and Chen, S.C. (1995) Development and use of monoclonal antibody probes for immunohistochemistry, ELISA and IFAT to detect bacterial and parasitic fish pathogens. *Fish & Shellfish Immunology* **5**, 537–547.

Afonso, A., Gomes, S., da Silva, J., Marques, F. and Henrique, M. (2005) Side effects in sea bass (*Dicentrarchus labrax* L.) due to intraperitoneal vaccination against vibriosis and pasteurellosis. *Fish & Shellfish Immunology* **19**, 1–16.

Aguayo, J., Miquel, A., Aranki, N., Jamett, A., Valenzuela, P.D.T. and Burzio, L.O. (2002) Detection of *Piscirickettsia salmonis* in fish tissues by an enzyme-linked immunosorbent assay using specific monoclonal antibiodies. *Diseases of Aquatic Organisms* **49**, 33–38.

Ahne, W. (1981) Serological techniques currently used in fish virology. *Developments in Biological Standardization* **49**, 3–27.

Ahne, W., Popp, W. and Hoffmann, R. (1982) *Pseudomonas fluorescens* as a pathogen of tench (*Tinca tinca*). *Bulletin of the European Association of Fish Pathologists* **4**, 56–57.

Ainsworth, A.J., Capley, G., Waterstreet, P. and Munson, D. (1986) Use of monoclonal antibodies in the indirect fluorescent antibody technique (IFA) for the diagnosis of *Edwardsiella ictaluri*. *Journal of Fish Disease* **9**, 439–444.

Ajmal, M. and Hobbs, B.C. (1967) Species of *Corynebacterium* and *Pasteurella* isolated from diseased salmon, trout and rudd. *Nature (London)* **215**, 142–143.

Akazaka, H. (1968) Bacterial disease of marine fishes. *Bulletin of the Japanese Society of Scientific Fisheries* **34**, 271–272.

Albrektsen, S., Snadnes, K., Glette, J. and Waagbo, R. (1995). The influence of dietary vitamin B_6 on tissue vitamin B_6 contents and immunity in Atlantic salmon, *Salmo salar* L. *Aquaculture Research* **26**, 331–339.

Alcaide, E., Herraiz, S. and Esteve, C. (2006) Occurrence of *Edwardsiella tarda* in wild European eels *Anguilla anguilla* from Mediterranean Spain. *Diseases of Aquatic Organisms* **73**, 77–81.

Alcorn, S., Murray, A.L., Pascho, R.J. and Varney, J. (2005). A cohabitation challenge to compare the efficacies of vaccines for bacterial kidney disease (BKD) in Chinook salmon *Onchorhynchus tshawytscha*. *Diseases of Aquatic Organisms* **63**, 151–160.

Al-Harbi, A.H. (1994) First isolation of *Streptococcus* sp. from hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) in Saudi Arabia. *Aquaculture* **128**, 195–201.

Al-Marzouk, A.E. (1999) Association of *Pseudomonas anguilliseptica* with mortalities in cultured marine orange-spotted grouper *Epinephelus coioides* in Kuwait. *Fish Pathology* **34**, 167–168.

Alejandro-Buentello, J. and Gatlin III, D.M. (2001) Effects of elevated dietary arginine on resistance of channel catfish to exposure to *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* **13**, 194–201.

Alexander, D.M. (1913) A review of piscine tubercles, with a description of an acid-fast bacillus found in the cod. *Report of the Lancashire Sea Fish Laboratory* **21**, 43–49.

Ali, A., Carnahan, A.M., Altwegg, M., Lüthy-Hotenstein, J. and Joseph, S.W. (1996) *Aeromonas bestiarum* sp. nov. (formerly genomospecies DNA group 2 *A. hydrophila*), a new species isolated from non-human sources. *Medical Microbiology Letters* **5**, 156–165.

Allan, B.J. and Stevenson, R.M.W. (1981) Extracellular virulence factors of *Aeromonas hydrophila* in fish infections. *Canadian Journal of Microbiology* **27**, 1114–1122.

Allen, D.A. (1982) Bacteria associated with freshwater fish farming, with emphasis on the fish pathogen, *Aeromonas salmonicida*. Ph.D. thesis, University of Maryland.

Allen, D.A., Austin, B. and Colwell, R.R. (1983a) *Aeromonas media*, a new species isolated from river water. *International Journal of Systematic Bacteriology* **33**, 599–604.

Allen, D.A., Austin, B. and Colwell, R.R. (1983b) Numerical taxonomy of bacterial isolates associated with a freshwater fishery. *Journal of General Microbiology* **129**, 2043–2062.

Allen-Austin, D., Austin, B. and Colwell, R.R. (1984) Survival of *Aeromonas salmonicida* in river water. *FEMS Microbiology Letters* **21**, 143–146.

Allison, L.N. (1958) Multiple sulfa therapy of kidney disease among brook trout. *Progressive Fish Culturist* **20**, 66–68.

Almendras, F.E., Fuentealba, I.C., Jones, S.R.M., Markham, F. and Spangler, E. (1997) Experimental infection and horizontal transmission of *Piscirickettsia salmonis* in freshwater-raised Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **20**, 409–418.

Alsina, M. and Blanch, A.R. (1994a) A set of keys for biochemical identification of environmental *Vibrio* species. *Journal of Applied Bacteriology* **76**, 79–85.

Alsina, M. and Blanch, A.R. (1994b) Improvement and update of a set of keys for biochemical identification of *Vibrio* species. *Journal of Applied Bacteriology* **77**, 719–721.

Alsina, M., Martínez-Picardo, J., Jofre, J. and Blanch, A.R. (1994) A medium for the presumptive identification of *Vibrio anguillarum*. *Applied and Environmental Microbiology* **60**, 1681–1683.

Altinok, I. and Grizzle, J.M. (2001) Effects of low salinity on *Flavobacterium columnare* infection of euryhaline and freshwater stenohaline fish. *Journal of Fish Diseases* **24**, 361–367.

Altinok, I. and Grizzle, J.M. (2001a) Effects of salinity on *Yersinia ruckeri* infection of rainbow trout and brown trout. *Journal of Aquatic Animal Health* **13, 334–339.**

Altinok, I., Grizzle, J.M. and Liu, Z. (2001) Detection of *Yersinia ruckeri* in rainbow trout blood by the polymerase chain reaction. *Diseases of Aquatic Organisms* **44**, 29–34.

Altinok, I., Kayis, S. and Capkin, E. (2006) *Pseudomonas putida* infection in rainbow trout. *Aquaculture* **261**, 850–855.

Amandi, A., Hiu, S.F., Rohovec, J.S. and Fryer, J.L. (1982) Isolation and characterization of *Edwardsiella tarda* from chinook salmon (*Oncorhynchus tshawytscha*). *Applied and Environmental Microbiology* **43**, 1380–1384.

Amaro, C., Biosca, E.G., Esteve, C., Fouz, B. and Toranzo, A.E. (1992) Comparative study of phenotypic and virulence properties in *Vibrio vulnificus* biotypes 1 and 2 obtained from a European eel farm experiencing mortalities. *Diseases of Aquatic Organisms* **13**, 29–35.

Amaro, C., Biosca, E.G., Fouz, B., Alcaide, E. and Esteve, C. (1995) Evidence that water transmits *Vibrio vulnificus* biotype 2 infections to eels. *Applied and Environmental Microbiology* **61**, 1133–1137.

Amaro, C., Fouz, B., Biosca, E.G., Marco-Noales, E. and Collado, R. (1997) The lipopolysaccharide O side chain of *Vibrio vulnificus* serogroup E is the virulence determinant for eels. *Infection and Immunity* **65**, 2475–2479.

Amend, D.F. (1970) Myxobacterial infections of salmonids: Prevention and treatment. In: Snieszko, S.F. (ed.), *A Symposium on Diseases of Fishes and Shellfishes*. Washington, D.C., American Fisheries Society Special Publication No. 5, pp. 258–265.

Amend, D.R. and Eschenour, R.W. (1980) Development and use of commercial fish vaccines. *Salmonid* (March/April), 8–12.

Amend, D.F. and Johnson, K.A. (1981) Current status and future needs of *Vibrio anguillarum* bacterins. *Developments in Biological Standardization* **49**, 403–417.

Amend, D.F. and Pietsch, J.P. (1972) Virucidal activity of two iodophors to salmonid viruses. *Journal of the Fisheries Research Board of Canada* **29**, 61–65.

Amend, D.F., Fryer, J.L. and Pilcher, K.S. (1965) Production trials utilizing sulfonamide drugs for the control of "cold-water" disease in juvenile coho salmon. *Research Briefs, Fish Commission of Oregon* **11**, 14–17.

Amend, D.F., Johnson, K.A., Croy, T.R. and McCarthy, D.H. (1983) Some factors affecting the potency of *Yersinia ruckeri* bacterins. *Journal of Fish Diseases* **6**, 337–344.

Amita, K., Hoshino, M., Honma, T. and Wakabayashi, H. (2000) An investigation on the distribution of *Flavobacterium psychrophilum* in the Umikawa River. *Fish Pathology* **35**, 193–197.

Amlacher, E. (1961) *Taschenbuch der Fischkrankheiten.* Jena, Gustav Fisher Verlag, 286 pp.

Anacker, R.L. and Ordal, E.J. (1959) Studies on the myxobacterium *Chondrococcus columnaris*. 1. Serological typing. *Journal of Bacteriology* **78**, 25–32.

Anbarasu, K. and Chandran, M.R. (2001) Effect of ascorbic acid on the immune response of the catfish, *Mystus gulio* (Hamilton), to different bacterins of *Aeromonas hydrophila*. *Fish & Shellfish Immunology* **11**, 347–355,

Anderson, B.S., Brock, J.A., Higa, H.H., Gooch, J.M., Wiebenga, N.H., Palumbo, N.E., Perri, S. and Sato, V.T. (1982) *Epidemiology of Leptospirosis on Aquaculture Farms in Hawaii.* State of Hawaii, Department of Health Publication.

Anderson, D.P. (1973) Investigations of the lipopolysaccharide fractions from *Aeromonas salmonicida* smooth and rough forms. In: *Symposium on the Major Communicable Fish Diseases in Europe and their Control.* Rome, United Nations Food and Agricultural Organization, pp. 175–179.

Anderson, D.P. and Nelson, J.R. (1974) Comparison of protection in rainbow trout (*Salmo gairdneri*) inoculated with and fed Hagerman redmouth bacterins. *Journal of the Fisheries Research Board of Canada* **31**, 214–216.

Anderson, D.P. and Ross, A.J. (1972) Comparative study of Hagerman redmouth disease oral bacterins. *Progressive Fish Culturist* **34**, 226–228.

Anderson, D.P. and Siwicki, A.K. (1994) Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection or immersion. *Progressive Fish Culturist* **56**, 258–261.

Anderson, J.W. and Conroy, D.A. (1969) Myxobacteria as fish pathogens. *Journal of Applied Bacteriology* **32**, 30–39.

Anderson, J.W. and Conroy, D.A. (1970) *Vibrio* diseases in fishes. In: Snieszko, S.F. (ed.), *A Symposium on Diseases of Fishes and Shellfishes.* Washington, D.C., American Fisheries Society, Special Publication No. 5, pp. 266–272.

Angelidis, P., Karagiannis, D. and Crump, E.M. (2006) Efficacy of a *Listonella anguillarum* (syn. *Vibrio anguillarum*) vaccine for juvenile sea bass *Dicentrarchus labrax*. *Diseases of Aquatic Organisms* **71**, 19–24.

Angka, S.L., Lam, T.J. and Sin, Y.M. (1995) Some virulence characteristics of *Aeromonas hydrophila* in walking catfish (*Clarias gariepinus*). *Aquaculture* **130**, 103–112.

Angulo, L., Lopez, J.E., Lema, C. and Vicente, J.A. (1992) *Vibrio pelagius* associated with mortalities in farmed turbot, *Scophthalmus maximus*. Thalassas **10**, 129–133.

Angulo, L., Lopez, J.E., Vicente, J.A. and Saborido, A.M. (1994) Haemorrhagic areas in the mouth of farmed turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases* **17**, 163–169.

Antipa, R. (1976) Field testing of injected *Vibrio anguillarum* bacterins in pen-reared Pacific salmon. *Journal of the Fisheries Research Board of Canada* **33**, 1291–1296.

Antipa, R. and Amend, D.P. (1977) Immunization of Pacific salmon: Comparison of intraperitoneal injection and hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* bacterins. *Journal of the Fisheries Research Board of Canada* **34**, 203–208.

Antipa, R., Gould, R. and Amend, D.P. (1980) *Vibrio anguillarum* vaccination of sockeye salmon (*Oncorhynchus nerka*) by direct immersion and hyperosmotic immersion. *Journal of Fish Diseases* **3**, 161–165.

Antychowicz, J., Lipiec, M. and Matusiewicz, J. (2003) Infection of African catfish (*Clarias gariepinus*) in an intensive culture facility *Mycobacterium marinum*. *Bulletin of the European Association of Fish Pathologists* **23**, 60–66.

Aoki, T. (1988) Drug-resistant plasmids from fish pathogens. *Microbiological Sciences* **5**, 219–223.

Aoki, T. and Egusa, S. (1971) Drug sensitivity of *Aeromonas liquefaciens* isolated from freshwater fishes. *Bulletin of the Japanese Society of Scientific Fisheries* **37**, 176–185.

Aoki, T. and Kitao, T. (1978) Vibriosis in ayu. *Fish Pathology* **13**, 19–24.

Aoki, T. and Kitao, T. (1985) Detection of transferable R plasmids in strains of the fish-pathogenic bacterium *Pasteurella piscicida*. *Journal of Fish Diseases* **8**, 345–350.

Aoki, T., Egusa, S., Kimura, T. and Watanabe, T. (1971) Detection of R factors in naturally occurring *Aeromonas salmonicida* strains. *Applied Microbiology* **22**, 716–717.

Aoki, T., Egusa, S. and Arai, T. (1974) Detection of R factor in naturally occurring *Vibrio anguillarum* strains. *Antimicrobial Agents and Chemotherapy* **6**, 534–538.

Aoki, T., Arai, T. and Egusa, S. (1977) Detection of R plasmids in naturally occurring fish-pathogenic bacteria, *Edwardsiella tarda*. *Microbiologia et Immunologia (Scandinavia)* **21**, 77–83.

Aoki, T., Kitao, T., Itabashi, T., Wada, Y. and Sakai, M. (1981) Proteins and lipopolysaccharides in the membrane of *Vibrio anguillarum*. *Developments in Biological Standardization* **49**, 225–232.

Aoki, T., Kitao, T., Iemura, N., Mitoma, Y. and Nomura, T. (1983) The susceptibility of *Aeromonas salmonicida* strains isolated in cultured and wild salmonids to various chemotherapeutants. *Bulletin of the Japanese Society of Scientific Fisheries* **49**, 17–22.

Aoki, T., Ikeda, D., Katagiri, T. and Hirono, I. (1997) Rapid detection of the fish-pathogenic bacterium *Pasteurella piscicida* by polymerase chain reaction targetting nucleotide sequences of the species-specific plasmid pZP1. *Fish Pathology* **32**, 143–151.

Aoki, T., Park, C.-I., Yamashita, H. and Hirono, I. (2000) Species-specific polymerase chain reaction primers for *Lactococcus garvieae*. *Journal of Fish Diseases* **23**, 1–6.

Aoki, T., Kondo, M., Kawai, K. and Oskhima, S.-I. (2005) Experimental bath infection with *Flavobacterium psychrophilum*, inducing typical signs of rainbow trout *Oncorhynchus mykiss* fry syndrome. *Diseases of Aquatic Organisms* **67**, 73–79.

Aoshima, S., Ishitake, Y. and Okamoto, N. (2005) Discrepancy between *in vitro* non-specific defense activities of peripheral blood phagocytes and resistance against vibriosis in rainbow trout under temperature stress. *Fish Pathology* **40**, 47–51.

Arakawa, C.K. and Fryer, J.L. (1984) Isolation and characterization of a new subspecies of *Mycobacterium chelonei* infections for salmonid fish. *Helgoländer Meeresuntersuchungen* **37**, 329–342.

Arakawa, C.K., Sanders, J.E. and Fryer, J.L. (1987) Production of monoclonal antibodies against *Renibacterium salmoninarum*. *Journal of Fish Diseases* **10**, 249–253.

Argenton, F., De Mas, S., Malocco, C., Dalla Valle, L., Giorgetti, G. and Colombo, L. (1996) Use of random DNA amplification to generate specific molecular probes for hybridization tests and PCR-based diagnosis of *Yersinia ruckeri*. *Diseases of Aquatic Organisms* **24**, 121–127.

Arias, C.R., Verdonck, L., Swings, J., Garay, E. and Aznar, R. (1997a) Intraspecific differentiation of *Vibrio vulnificus* biotypes by amplified fragment length polymorphism and ribotyping. *Applied and Environmental Microbiology* **63**, 2600–2606.

Arias, C.R., Verdonck, L., Swings, J., Aznar, R. and Garay, E. (1997b) A polyphasic approach to study the intraspecific diversity amongst *Vibrio vulnificus* isolates. *Systematic and Applied Microbiology* **20**, 622–633.

Arias, C.R., Welker, T.L., Shoemaker, C.A., Abernathy, J.W. and Klesius, P.H. (2004) Genetic fingerprinting of *Flavobacterium columnare* isolates from cultured fish. *Journal of Applied Microbiology* **97**, 421–428.

Arijo, S., Borrego, J.J., Zorrilla, I., Balebona, M.C. and Moriñigo, M.A. (1998) Role of the capsule of *Photobacterium damselae* subsp. *piscicida* in protection against phagocyctosis and killing by gilt-head seabream (*Sparus aurata* L.) macrophages. *Fish & Shellfish Immunology* **8**, 63–72.

Arijo, S., Balebona, C., Martinez-Manzanares, E. and Moriñigo, M.A. (2004) Immune respone of gilt-head seabream (*Sparus aurata*) to antigens from *Photobacterium damselae* subsp. *piscicida*. *Fish & Shellfish Immunology* **16**, 65–70.

Arijo, S., Rico, R., Chabrillon, M., Diaz-Rosales, P., Martínez-Manzanares, E., Balebona, M.C., Toranzo, A.E. and Moriñigo, M.A. (2005) Effectiveness of a divalent vaccine for sole, *Solea senegalensis* (Kaup), against *Vibrio harveyi* and *Photobacterium damselae* subsp. *piscicida*. *Journal of Fish Diseases* **28**, 33–38.

Arkoosh, M.R., Casillas, E., Huffman, P., Clemons, E., Evered, J., Stein, J.E. and Varanasi, U. (1998) Increased susceptibility of juvenile chinook salmon from a contaminated estuary to *Vibrio anguillarum*. *Transactions of the American Fisheries Sociaty* **127**, 360–374.

Arkush, K.D., Mcbride, A.M., Mendonca, H.L., Okihiro, M.S., Andree, K.B., Marshall, S., Henriquez, V. and Hedrick, R.P. (2005) Genetic characterization and experimental pathogenesis of *Piscirickettsia salmonis* isolated from white seabass *Atractascion nobilis*. *Diseases of Aquatic Organisms* **63**, 139–149.

Arkwright, J.A. (1912) An epidemic disease affecting salmon and trout in England during the summer of 1911. *Journal of Hygiene, Cambridge* **12**, 391–413.

Armstrong, R.D., Martin, S.W., Evelyn, T.P.T., Hicks, B., Dorward, W.J. and Ferguson, H.W. (1989) A field evaluation of the indirect fluorescent antibody-based broodstock screening test used to evaluate the vertical transmission of *Renibacterium salmoninarum* in chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Veterinary Research* **53**, 385–389.

Arnesen, J.A. and Eggset, G. (1999) Isolation and characterisation of two extracellular metalloproteases from *Aeromonas salmonicida* subsp. *salmonicida*. *Journal of Fish Diseases* **22**, 35–43.

Arnesen, S.M., Schrøder, M.B., Dalmo, R.A. and Bøgwald, J. (2002) Antigen uptake and immunoglobulin production in Atlantic cod (*Gadus morhua* L.) after intraperitoneal injection of *Vibrio anguillarum*. *Fish & Shellfish Immunology* **13**, 159–170.

Aronson, J.D. (1926) Spontaneous tuberculosis in salt water fish. *Journal of Infectious Diseases* **39**, 315–320.

Ascencio, F., Ljungh, Å. and Wadstrom, T. (1991) Comparative study of extracellular matrix protein binding to *Aeromonas hydrophila* isolated from diseased fish and human infection. *Microbios* **65**, 135–146.

Ashburner, L.D. (1978) Management and diseases of hatchery fish. *Proceedings of the Fauna Course for Veterinarians, Taronga Zoo, Mosman, Australia* **36**, 387–449.

Atsuta, S., Yoshimoto, J., Sakai, M. and Kobayashi, M. (1990) Streptococcicosis occurred in the pen-cultured coho salmon *Oncorhynchus kisutch*. *Suisanzoshoku* **38**, 215–219.

Austin, B. (1981) Human diseases associated with fish culture. *Proceedings of the Institute of Fisheries Management*, pp. 170–175.

Austin, B. (1982a) Taxonomy of bacteria isolated from a coastal, marine fish-rearing unit. *Journal of Applied Bacteriology* **53**, 253–268.

Austin, B. (1982b) Close-up of a killer. *Fish Farmer* **5**, 30–31.

Austin, B. (1984a) The control of bacterial fish diseases by antimicrobial compounds. In: Woodbine, M. (ed.), *Antibiotics and Agriculture, Benefits and Malefits*. Sevenoaks, U.K., Butterworths, pp. 255–268.

Austin, B. (1984b) The future of bacterial fish vaccines. *Vaccine* **2**, 249–254.

Austin, B. (1985) Evaluation of antimicrobial compounds for the control of bacterial kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **8**, 209–220.

Austin, B. (1986) Ecology of *Renibacterium salmoninarum*, the causal agent of bacterial kidney disease in salmonids. *Proceedings of the 4th International Symposium on Microbial Ecology, Ljublana*, pp. 650–654.

Austin, B. (1988) *Marine Microbiology*. Cambridge, U.K., Cambridge University Press.

Austin, B. (1993) Recovery of "atypical" isolates of *Aeromonas salmonicida*, which grow at 37°C, from ulcerated non-salmonids in England. *Journal of Fish Diseases* **16**, 165–168.

Austin, B. (1999) The effect of pollution on fish health. *Journal of Applied Microbiology* **28**, 234S–242S.

Austin, B. (2006) The bacterial microflora of fish, revised. *The Scientific World Journal* **6**, 931–934.

Austin, B. and Austin, D.A. (1989) 1. General introduction. In: Austin, B. and Austin, D.A. (eds), *Methods for the Microbiological Examination of Fish and Shellfish*. Chichester, U.K., Ellis Horwood, pp. 1–24.

Austin, B. and Billaud, A.–C. (1990) Inhibition of the fish pathogen, *Serratia liquefaciens*, by an antibiotic-producing isolate of *Planococcus* recovered from sea water. *Journal of Fish Diseases* **13**, 553–556.

Austin, B. and Rayment, J. (1985) Epizootiology of *Renibacterium salmoninarum*, the causal agent of bacterial kidney disease in salmonid fish. *Journal of Fish Diseases* **8**, 505–509.

Austin, B. and Robertson, P.A.W. (1993) Recovery of *Streptococcus milleri* from ulcerated koi carp (*Cyprinus carpio* L.) in the U.K. *Bulletin of the European Association of Fish Pathologists* **13**, 207–209.

Austin, B. and Rodgers, C.J. (1980) Diversity among strains causing bacterial kidney disease in salmonid fish. *Current Microbiology* **3**, 231–235.

Austin, B. and Rodgers, C.J. (1981) Preliminary observations on *Aeromonas salmonicida* vaccines. *Development in Biological Standardization* **49**, 387–393.

Austin, B. and Stobie, M. (1992a) Recovery of *Micrococcus luteus* and presumptive *Planococcus* from moribund fish during outbreaks of rainbow trout (*Oncorhynchus mykiss* Walbaum) fry syndrome (RTFS) in England. *Journal of Fish Diseases* **15**, 203–206.

Austin, B. and Stobie, M. (1992b) Recovery of *Serratia plymuthica* and presumptive *Pseudomonas pseudoalcaligenes* from skin lesions in rainbow trout, *Oncorhynchus mykiss* (Walbaum), otherwise infected with enteric redmouth. *Journal of Fish Diseases* **15**, 541–543.

Austin, B., Allen, D.A., Zachary, A., Belas, M.R. and Colwell, R.R. (1979) Ecology and taxonomy of bacteria attaching to wood surfaces in a tropical harbor. *Canadian Journal of Microbiology* **25**, 447–461.

Austin, B., Morgan, D.A. and Alderman, D.J. (1981) Comparison of antimicrobial agents for the control of vibriosis in marine fish. *Aquaculture* **26**, 1–12.

Austin, B., Green, M. and Rodgers, C.J. (1982) Morphological diversity among strains of *Yersinia ruckeri*. *Aquaculture* **27**, 73–78.

Austin, B., Embley, T.M. and Goodfellow, M. (1983a) Selective isolation of *Renibacterium salmoninarum*. *FEMS Microbiology Letters* **17**, 111–114.

Austin, B., Rayment, J. and Alderman, D.J. (1983b) Control of furunculosis by oxolinic acid. *Aquaculture* **31**, 101–108.

Austin, B., Bucke, D., Feist, S. and Rayment, J. (1985) A false positive reaction in the indirect fluorescent antibody test for *Renibacterium salmoninarum* with a "coryneform" organism. *Bulletin of the European Association of Fish Pathologists* **5**, 8–9.

Austin, B., Bishop, I., Gray, C., Watt, B. and Dawes, J. (1986). Monoclonal antibody-based enzyme-linked immunosorbent assays for the rapid diagnosis of clinical cases of enteric redmouth and furunculosis in fish farms. *Journal of Fish Diseases* **9**, 469–474.

Austin, B., McIntosh, D. and Murray, K.R. (1988) Infection of Atlantic salmon (*Salmo salar*) with *Planococcus*. *Bulletin of the European Association of Fish Pathologists* **8**, 21–22.

Austin, D.A., McIntosh, D. and Austin, B. (1989) Taxonomy of fish associated *Aeromonas* spp., with the description of *Aeromonas salmonicida* subsp. *smithia* subsp. nov. *Systematic and Applied Microbiology* **11**, 277–290.

Austin, B., Baudet, E. and Stobie, M. (1992a) Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. *Journal of Fish Diseases* **15**, 55–61.

Austin, B., Gonzalez, C.J., Stobie, M., Curry, J.I. and McLoughlin, M.F. (1992b) Recovery of *Janthinobacterium lividum* from diseased rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Northern Ireland and Scotland. *Journal of Fish Diseases* **15**, 357–359.

Austin, B., Stobie, M., Robertson, P.A.W., Glass, H.G., Stark, J.R. and Mudarris, M. (1993) *Vibrio alginolyticus*: The cause of gill disease leading to progressive low-level mortalities among juvenile turbot, *Scophthalmus maximus* L., in a Scottish aquarium. *Journal of Fish Diseases* **16**, 277–280.

Austin, B., Alsina, M., Austin, D.A., Blanch, A.R., Grimont, F., Grimont, P.A.D., Jofre, J., Koblavi, S., Larsen, J.L., Pedersen, K., Tiainen, T., Verdonck, L. and Swings, J. (1995a) Identification and typing of *Vibrio anguillarum*: A comparison of different methods. *Systematic and Applied Microbiology* **18**, 285–302.

Austin, B., Stuckey, L.F., Robertson, P.A.W., Effendi, I. and Griffith, D.R.W. (1995b) A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii*. *Journal of Fish Diseases* **18**, 93–96.

Austin, B., Austin, D.A., Falconer, V.M., Pedersen, K., Larsen, J.L., Swings, J. and Verdonck, L. (1996) Dissociation of *Vibrio anguillarum* and *V. ordalii* cultures into two or three distinct colony types. *Bulletin of the European Association of Fish Pathologists* **16**, 101–103.

Austin, B., Austin, D.A., Blanch, A.R., Cerdà, M., Grimont, F., Grimont, P.A.D., Jofre, J., Koblavi, S., Larsen, J.L., Pedersen, K. *et al.* (1997) A comparison of methods for the typing of fish-pathogenic *Vibrio* spp. Systematic and *Applied Microbiology* **20**, 89–101.

Austin, B., Austin, D.A., Dalsgaard, I., Gudmundsdóttir, B.K., Høie, S., Thornton, J.M., Larsen, J.L., O'Hici, B. and Powell, R. (1998) Characterization of atypical *Aeromonas salmonicida* by different methods. *Systematic and Applied Microbiology* **21**, 50–64.

Austin, B., Pride, A.C. and Rhodie, G.A. (2003a) Association of a bacteriophage with virulence in *Vibrio harveyi*. *Journal of Fish Diseases* **26**, 55–58.

Austin, D.A, Jordan, E.M. and Austin, B. (2003b) Recovery of an unusual Gram-negative bacterium from ulcerated rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Scotland. *Journal of Fish Diseases* **26**, 247–249.

Austin, B., Austin, D., Sutherland, R., Thompson, F. and Swings, J. (2005) Pathogenicity of vibrios to rainbow trout (*Oncorhynchus mykiss*, Walbaum) and *Artemia* nauplii. *Environmental Microbiology* **7**, 1488–1495.

Austin, D.A., Robertson, P.A.W. and Austin, B. (2005a) Recovery of a new biogroup of *Yersinia ruckeri* from diseased rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Systematic and Applied Microbiology* **26**, 127–131.

Avendaño-Herrera, R., Magariños, B., Toranzo, A.E., Beaz, R. and Romalde, J.L. (2004) Species-specific polymerase chain reaction primner sets for the diadnosis of *Tenacibaculum maritimum* infection. *Diseases of Aquatic Organisms* **62**, 75–83.

Avendaño-Herrera, R., Toranzo, A.E. and Magariños, B. (2004a) A challenge model for *Tenacibaculum maritimum* infection in turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases* **29**, 371–374.

Avendaño-Herrera, R., Rodríquez, J., Magariños, B., Romalde, J.L. and Toranzo, A.E. (2004b) Intraspecific diversity of the marine fish pathogen *Tenacibaculum maritimum* as determined by randomly amplified polymorphic DNA-PCR. *Journal of Applied Microbiology* **96**, 871–877.

Avendaño-Herrera, R., Magariños, B., López–Romalde, S., Romalde, J.L. and Toranzo, A.E. (2004c) Phenotyphic characterization and description of two major O-serotypes in *Tenacibaculum maritimum* strains from marine fishes. *Diseases in Aquatic Organisms* **58**, 1–8.

Avendaño-Herrera, R., Núñez, S., Magariños, B. and Toranzo, A.E. (2004d) A non-destructive method for rapid detection of *Tenacibaculum maritimum* in farmed fish using nested PCR amplification. *Bulletin of the European Association of Fish Pathologists* **24**, 280–286.

Avendaño-Herrera, R., Toranzo, A.E., Romalde, J.L., Lemos, M.L. and Magariños, B. (2005) Iron uptake mechanisms in the fish pathogen *Tenacibaculum maritimum*. *Applied and Environmental Microbiology* **71**, 6947–6953.

Avendaño-Herrera, R., Magariños, B., Moriñigo, M.A., Romalde, J.L. and Toranzo, A.E. (2005a) A novel O-serotype in *Tenacibaculum maritimum* strains isolated from cultured sole (*Solea senegalensis*). *Bulletin of the European Association of Fish Pathologists* **25**, 70–74.

Avendaño-Herrera, R., Irgang, R., Magariños, B., Romalde, J.L. and Toranzo, A.E. (2006) Use of microcosms to determine the survival of the fish pathogen *Tenacibaculum maritimum* in seawater. *Environmental Microbiology* **8**, 921–928.

Aydin, S., Çelebi, S. and Akyurt, I. (1997) Clinical, haematological and pathological investigations of *Escherichia vulneris* in rainbow trout (*Oncorhynchus mykiss*). *Fish Pathology* **32**, 29–34.

Aydin, S., Gültepe, N. and Yildiz, H. (2000) Natural and experimental infections with *Campylobacter cryoarophilus* in rainbow trout: Gross pathology, bacteriology, clinical pathology, and chemotherapy. *Fish Pathology* **35**, 117–123.

Aydin, S., Engin, M. and Bircan, R. (2002) A comparative investigation of *Arcobacter cryaerophilus* infection among Albino crosses and high- and low-body-weight rainbow trout. *Journal of Aquatic Animal Health* **14**, 39–44.

Azad, I.S., Shankar, K.M., Mohan, C.V. and Kalita, B. (1999) Biofilm vaccine of *Aeromonas hydrophila*: Standardization of dose and duration for oral vaccination of carps. *Fish & Shellfish Immunoloogy* **9**, 519–528.

Baba, T., Imamura, J., Izawa, K. and Ikeda, K. (1988) Cell-mediated protection in carp, *Cyprinus carpio* L., against *Aeromonas hydrophila*. *Journal of Fish Diseases* **11**, 171–178.

Bach, R., Wenzel, S., Müller-Prasuhn, G. and Gläsker, M. (1971) Farmed trout as a carrier of *Clostridium botulinum* and a cause of botulism. III. Evidence of *Clostridium botulinum* type E on a fish farm with processing station and in fresh and smoked trout from different sources. *Archives für Lebensmittelhygiene* **22**, 107.

Bachrach, G., Zlotkin, A., Hurvitz, A., Evans, D.L. and Eldar, L. (2001) Recovery of *Streptococcus iniae* from diseased fish previously vaccinated with a *Streptococcus* vaccine. *Applied and Environmental Microbiology* **67**, 3756–3758.

Backman, S., Ferguson, H.W., Prescott, J.F. and Wilcock, B.P. (1990) Progressive panophthalmitis in chinook salmon, *Oncorhynchus tshawytscha* (Walbaum): A case report. *Journal of Fish Diseases* **13**, 345–353.

Bader, J.A., Shoemaker, C.A., Klesius, P.H., Connolly, M.A. and Barbaree, J.M. (1998) Genomic subtyping of *Edwardsiella ictaluri* isolated from diseased channel catfish by arbitrary primed polymerase chain reaction. *Journal of Aquatic Animal Health* **10**, 22–27.

Bader, J.A., Shoemaker, C.A. and Klesius, P.H. (2003) Rapid detection of columnaris disease in channel catfish (*Ictalurus punctatus*) with a new species-specific 16-S rRNA gene-based PCR primer for *Flavobacterium columnare*. *Journal of Microbiological methods* **52**, 209–220.

Bader, J.A., Nusbaum, K.E. and Shoemaker, C.A. (2003a) Comparative challenge model of *Flavobacterium columnare* using abraded and unabraded channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* **26**, 461–467.

Bader, J.A., Shoemaker, C.A.. and Klesius, P.H. (2005) Production, characterization and evaluation of virulence of an adhesion defective mutant of *Flavobacterium columnare* produced by β–lactam selection. *Letters in Applied Microbiology* **40**, 123–127.

Bader, J.A., Moore, S.A. and Nusbaum, K.E. (2006) The effect of cutaneous injury on a reproducible immersion challenge model for *Flavobacterium columnare* infection in channel catfish (*Ictalurus punctatus*). *Aquaculture* **253**, 1–9.

Bagge, J. and Bagge, O. (1956) *Vibrio anguillarum* som arsag til ulcussygdom hos torsk (*Gadus callaris* Linné). *Nordisk Veterinarmedicin* **8**, 481–292.

Baird-Parker, A.C. (1963) A classification of micrococci and staphylococci based on physiological and biochemical tests. *Journal of General Microbiology* **30**, 409–427.

Baird-Parker, A.C. (1965) The classification of staphylococci and micrococci from worldwide sources. *Journal of General Microbiology* **39**, 363–387.

Baker, K.J., Knittel, M.D. and Fryer, J.L. (1983) Susceptibility of chinook salmon, *Oncorhynchus tshawytsha* (Walbaum), and rainbow trout, *Salmo gairdneri* Richardson, to infection with *Vibrio anguillarum* following sublethal copper exposure. *Journal of Fish Diseases* **6**, 267–275.

Bakopoulos, V., Volpatti, D., Papapanagiotou, E., Richards, R., Galleoti, M. and Adams, A. (1997a) Development of an ELISA to detect *Pasteurella piscicida* in culture and in "spiked" fish tissue. *Aquaculture* **156**, 359–366.

Bakopoulos, V., Adams, A. and Richards, R.H. (1997b) The effect of iron limitation growth conditions on the cell and extracellular components of the fish pathogen *Pasteurella piscicida*. *Journal of Fish Diseases* **20**, 297–305.

Bakopoulos, V., Poulos, K., Adams, A., Galeotti, M. and Dimitriadis, G.J. (2002) The effect of novel growth media on the virulence and toxicity of cellular and extracellular components of the fish pathogen *Photobacterium damselae* subsp. *piscicida*. *Bulletin of the European Association of Fish Pathologists* **22**, 272–279.

Bakopoulos, V., Volpatti, D., Gusmani, L., Galeotti, M., Adams, A. and Dimitriadis, G.J. (2003) Vaccination of sea bass, *Dicentrachus labrax* (L.), against *Photobacterium damsela* subsp. *piscicida* using novel vaccine mixtures. *Journal of Fish Diseases* **26**, 77–90.

Bakopoulos, V., Pearson, M., Volpatti, D., Gousmani, L., Adams, A., Galeotti, M. and Dimitriadis, G.J. (2003a) Investigation of media formulations promoting differential antigen expression by *Photobacterium damsela* subsp. *piscicida* and recognition by sea bass, *Dicentrarchus labrax* (L.), immune sera. *Journal of Fish Diseases* **26**, 1–13.

Bakopoulos, V., Hanif, A., Poulos, K., Galeotti, M., Adams, A. and Dimitriadis, G.J. (2004) The effect of *in vivo* growth on the cellular and extracellular components of the marine bacterial pathogen *Photobacterium damsela* subsp. *piscicida*. *Journal of Fish Diseases* **27**, 1–13.

Baldacci, E., Farina, G. and Locci, R. (1966) Emendation of the genus *Streptoverticillium* Baldacci (1958) and revision of some species. *Giornale Microbiologica* **14**, 153–171.

Baldwin, T.J. and Newton, J.C. (1993) Pathogenesis of enteric septicemia of channel catfish, caused by *Edwardsiella ictaluri*: Bacteriologic and light and electron microscopic findings. *Journal of Aquatic Animal Health* **5**, 189–198.

Balebona, M.C., Zorrilla, I., Moriñigo, M.A. and Borrego, J.J. (1998) Survey of bacterial pathogens affecting farmed gilt-head sea bream (*Sparus aurata* L.) in southwestern Spain from 1990 to 1996. *Aquaculture* **166**, 19–35.

Balfry, S.K., Albright, L.J. and Evelyn, T.P.T. (1996) Horizontal transfer of *Renibacterium salmoninarum* among farmed salmonids via the faecal–oral route. *Diseases of Aquatic Organisms* **25**, 63–69.

Balfry, S.K., Shariff, M. and Iwama, G.K. (1997) Strain differences in non-specific immunity of tilapia *Oreochromis niloticus* following challenge with *Vibrio parahaemolyticus*. *Diseases of Aquatic Organisms* **30**, 77–80.

Baliarda, A., Faure, D. and Urdaci, M.C. (2002) Development and application of a nested PCR to monitor brood stock salmonid ovarian fluid and spleen for detection of the fish pathogen *Flavobacterium psychrophilum*. *Journal of Applied Microbiology* **92**, 510–516.

Bandín, I., Santos, Y., Barja, J.L. and Toranzo, A.E. (1989) Influence of the growth conditions on the hydrophobicity of *Renibacterium salmoninarum* evaluated by different methods. *FEMS Microbiology Letters* **60**, 71–78.

Bandín, I., Santos, Y., Bruno, D.W., Raynard, R.S., Toranzo, A.E. and Barja, J.L. (1991a) Lack of biological activities in the extracellular products of *Renibacterium salmoninarum*. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 421–425.

Bandín, I., Santos, Y., Toranzo, A.E. and Barja, J.L. (1991b) MICs and MBCs of chemotherapeutic agents against *Renibacterium salmoninarum*. *Antimicrobial Agents and Chemotherapy* **35**, 1011–1013.

Bandín, I., Santos, Y., Magariños, B., Barja, J.L. and Toranzo, A.E. (1992) The detection of two antigenic groups among *Renibacterium salmoninarum* isolates. *FEMS Microbiology Letters* **94**, 105–110.

Bandín, I., Santos, Y., Barja, J.L. and Toranzo, A.E. (1993) Detection of a common antigen among *Renibacterium salmoninarum*, *Corynebacterium aquaticum* and *Carnobacterium piscicola* by the Western Blot Technique. *Journal of Aquatic Animal Health* **5**, 172–176.

Bandín, I., Heinen, P., Brown, L.L. and Toranzo, A.E. (1996) Comparison of different ELISA kits for detecting *Renibacterium salmoninarum*. *Bulletin of the European Association of Fish Pathologists* **16**, 19–22.

Banner, C.R., Long, J.J., Fryer, J.L. and Rohovec, J.S. (1986) Occurrence of salmonid fish infected with *Renibacterium salmoninarum* in the Pacific Ocean. *Journal of Fish Diseases* **9**, 273–275.

Banner, C.R., Rohovec, J.S. and Fryer, J.L. (1991) A new value for mol percent guanine + cytosine of DNA for the salmonid pathogen *Renibacterium salmoninarum*. *FEMS Microbiology Letters* **79**, 57–60.

Barancin, C.E., Smoot, J.C., Findlay, R.H. and Actis, L.A. (1998) Plasmid-mediated histamine biosynthesis in the bacterial fish pathogen *Vibrio anguillarum*. *Plasmid* **39**, 235–244.

Barham, W.T., Schoonbee, H. and Smit, G.L. (1979) The occurrence of *Aeromonas* and *Streptococcus* in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology* **15**, 457–460.

Barnes, A.C., Lewin, C.S., Hastings, T.S. and Amyes, S.G.B. (1990a) Cross resistance between oxytetracycline and oxolinic acid in *Aeromonas salmonicida* associated with alterations in outer membrane proteins. *FEMS Microbiology Letters* **72**, 337–340.

Barnes, A.C., Lewin, C.S., Hastings, T.S. and Amyes, S.G.B. (1990b) *In vitro* activities of 4-quinolones against the fish pathogen *Aeromonas salmonicida*. *Antimicrobial Agents and Chemotherapy* **34**, 1819–1820.

Barnes, A.C., Lewin, C.S., Hastings, T.S. and Amyes, S.G.B. (1991a) *In vitro* susceptibility of the fish pathogen *Aeromonas salmonicida* to flumequine. *Antimicrobial Agents and Chemotherapy* **35**, 2634–2635.

Barnes, A.C., Amyes, S.G.B., Hastings, T.S. and Lewin, C.S. (1991b) Fluoroquinolones display rapid bactericidal activity and low mutation frequencies against *Aeromonas salmonicida*. *Journal of Fish Diseases* **14**, 661–667.

Barnes, A.C., Lewin, C.S., Hastings, T.S. and Amyes, S.G.B. (1992) Alterations in outer membrane proteins identified in a clinical isolate of *Aeromonas salmonicida* subsp. *salmonicida*. *Journal of Fish Diseases* **15**, 279–282.

Barnes, A.C., Hastings, T.S. and Amyes, S.G. (1994) Amoxycillin resistance in Scottish isolates of *Aeromonas salmonicida*. *Journal of Fish Diseases* **17**, 357–363.

Barnes, A.C., Hastings, T.S. and Amyes, S.G.B. (1995) Aquaculture antibacterials are antagonized by seawater cations. *Journal of Fish Diseases* **18**, 463–465.

Barnes, A.C., Guyot, C., Hansen, B.G., Mackenzie, K., Horne, M.T. and Ellis, A.E. (2002) Resistance to serum killing may contribute to differences in the abilities of capsulate and non-capsulated isolates of *Lactococcus garvieae* to cause disease in rainbow trout (*Oncorhynchus mykiss* L.). *Fish & Shellfish Immunology* **12**, 155–168.

Barnes, A.C., Young, F.M., Horne, M.T. and Ellis, A.E. (2003) *Streptococcus iniae*: Serological differences, presence of capsule and resistance to immune serum killing. *Diseases of Aquatic Organisms* **53**, 241–247.

Barnes, A.C., Horne, M.T. and Ellis, A.E. (2003a) *Streptococcus iniae* expresses a cell surface non-immune trout immunoglobulin-binding factor when grown in normal trout serum. *Fish & Shellfish Immunology* **15**, 425–431.

Barnes, A.C. and Ellis, A.E. (2004) Role of capsule in serotypic differences and complement fixation by *Lactococcus garvieae*. *Fish & Shellfish Immunology* **16**, 207–214.

Barnes, M.N., Landolt, M.L., Powell, D.B. and Winton, J.R. (1998) Purification of *Piscirickettsia salmonis* and partial characterization of antigens. *Diseases of Aquatic Organisms* **33**, 33–41.

Barry, T., Powell, R. and Gannon, F. (1990) A general method to generate DNA probes for microorganisms. *Biotechnology* **8**, 233–236.

Bartlett, K.H. and Trust, T.J. (1976) Isolation of salmonellae and other potential pathogens from the freshwater aquarium snail, *Ampullaria*. *Applied and Environmental Microbiology* **31**, 635–639.

Bartos, J.M. and Sommer, C.V. (1981) *In vitro* cell mediated immune response to *M. tuberculosis* and *M. salmoniphilum* in rainbow trout (*Salmo gairdneri*). *Developmental and Comparative Immunology* **5**, 75–83.

Basheera John, M., Chandran, M.R., Aruna, B.V. and Anbarasu, K. (2002) Production of superoxide anion by head-kidney leucocytes of Indian major carps immunised with bacterins of *Aeromonas hydrophila*. *Fish & Shellfish Immunology* **12**, 201–207.

Bast, L., Daly, J.G., DeGrandis, S.A. and Stevenson, R.M.W. (1988) Evaluation of profiles of *Aeromonas salmonicida* as epidemiological markers of furunculosis infections in fish. *Journal of Fish Diseases* **11**, 133–145.

Bataillon, E., Dubard, and Terre, L. (1897) Un nouveau type de tuberculose. *Comptes rendus des Scéances de la Société Biologie* **49**, 446–449.

Baudin-Laurençin, F. and Tangtrongpiros, J. (1980) Some results of vaccination against vibriosis in Brittany. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 60–68.

Baudin-Laurençin, F., Vigneulle, M. and Mevel, M. (1977) Premieres observations sur la Corynebacterioses des salmonides en Brétagne. *Bulletin de l'Office International des Epizooties* **87**, 505–507.

Baudin-Laurençin, F., Castric, J.-C., Vigneulle, M. and Tixerant, G. (1989) La myxobacteriose viscérale de la truite arc-en-ciel *Salmo gairdneri* R.: une forme nouvelle de la maladie de l'eau froide à *Cytophaga psychrophila*. *Bulletin d'Academie de Véterinaire de France* **62**, 147–157.

Bauer, O.N., Musselius, V.A. and Strelkov, Yu. A. (1973) *Diseases of Pond Fishes*. Jerusalem, Keter Press, pp. 39–40.

Baumann, L., Baumann, P., Mandel, M. and Allen, R.D. (1972) Taxonomy of aerobic marine eubacteria. *Journal of Bacteriology* **110**, 402–429.

Baumann, P., Bang, S.S. and Baumann, L. (1978) Phenotypic characterization of *Beneckea anguillara* biotypes I and II. *Current Microbiology* **1**, 85–88.

Baumann, L., Bowditch, R.D. and Baumann, P. (1983) Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus* and *Pseudomonas marina*. *International Journal of Systematic Bacteriology* **33**, 793–802.

Baumann, P., Furniss, A.L. and Lee, J.V. (1984) Genus I: *Vibrio* Pacini 1854, 411[AL]. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 518–538.

Baxa, D.V., Kawai, K. and Kusuda, R. (1988a) Detection of *Flexibacter maritimus* by fluorescent antibody technique in experimentally infected black sea bream fry. *Fish Pathology* **23**, 29–32.

Baxa, D.V., Kawai, K. and Kusuda, R. (1988b) Chemotherapy of *Flexibacter maritimus* infection. *Reports of the Usa Marine Biological Institute, Kochi University* **10**, 9–14.

Baya, A.M., Lupiani, B., Hetrick, F.M. and Toranzo, A.E. (1990a) Increasing importance of *Citrobacter freundii* as a fish pathogen. *Fish Health Section/American Fisheries Society Newsletter* **18**, 4.

Baya, A., Toranzo, A.E., Núñez, S., Barja, J.L. and Hetrick, F.M. (1990b) Association of a *Moraxella* sp. and a reo-like virus with mortalities of striped bass, *Morone saxatilis*. *Pathology in Marine Science*, 91–99.

Baya, A.M., Lupiani, B., Hetrick, F.M., Roberson, B.S., Lukacovic, R., May, E. and Poukish, C. (1990c) Association of *Streptococcus* sp. with fish mortalities in the Chesapeake Bay and its tributaries. *Journal of Fish Diseases* **13**, 251–253.

Baya, A.M., Toranzo, A.E., Lupiani, B., Li, T., Roberson, B.S. and Hetrick, F.M. (1991) Biochemical and serological characterization of *Carnobacterium* spp. isolated from farmed and natural populations of striped bass and catfish. *Applied and Environmental Microbiology* **57**, 3114–3120.

Baya, A.M., Lupiani, B. and Hetrick, F. (1992a) *Bacillus cereus*, a pathogen for striped bass. *Eastern Fish Health and American Fisheries Society Fish Health Section Workshop, 16–19 June 1992, Auburn University, Auburn, Alabama*, pp. 67.

Baya, A.M., Lupiani, B., Bandín, I., Hetrick, F.M., Figueras, A., Carnahan, A., May, E.M. and Toranzo, A.E. (1992b) Phenotypic and pathobiological properties of *Corynebacterium aquaticum* isolated from diseased striped bass. *Diseases of Aquatic Organisms* **14**, 115–126.

Baya, A.M., Toranzo, A.E., Lupiani, B., Santos , Y. and Hetrick F.M. (1992c) *Serratia marcescens*: A potential pathogen for fish. *Journal of Fish Diseases* **15**, 15–26.

Becker, C.D. and Fujiwaha, M.P. (1978) *The Bacterial Pathogen Flexibacter columnaris and Its Epizootiology among Columbia River Fish*, Monograph No. 2. Washington, D.C., American Fisheries Society.

Bein, S.J. (1954) A study of certain chromogenic bacteria isolated from "red-tide" water with a description of a new species. *Bulletin of Marine Science in the Gulf and Caribbean* **4**, 110–119.

Bejerano, Y., Sarig, S., Horne, M.T. and Roberts, R.J. (1979) Mass mortalities in silver carp *Hypophthalmichthys molitrix* (Valenciennes) associated with bacterial infection following handling. *Journal of Fish Diseases* **2**, 49–56.

Belding, D.L. (1927) Fish disease epidemics. *Transactions of the American Fisheries Society* **57**, 217–221.

Belding, D.L. and Merrill, B. (1935) A preliminary report upon a hatchery disease of the Salmonidae. *Transactions of the American Fisheries Society* **65**, 76–84.

Bell, G.R. (1961) Two epidemics of apparent kidney disease in cultured pink salmon (*Oncorhynchus gorbuscha*). *Journal of the Fisheries Research Board of Canada* **18**, 559–562.

Bell, G.R., Higgs, D.A. and Traxler, G.S. (1984) The effect of dietary ascorbate, zinc, and manganese on the development of experimentally induced bacterial kidney disease in sockeye salmon (*Oncorhynchus nerka*). *Aquaculture* **36**, 293–311.

Belland, R.J. and Trust, T.J. (1985) Synthesis, export, and assembly of *Aeromonas salmonicida* A-layer analyzed by transposon mutagenesis. *Journal of Bacteriology* **163**, 877–881.

Belland, R.J. and Trust, T.J. (1988) DNA:DNA reassociation analysis of *Aeromonas salmonicida*. *Journal of General Microbiology* **134**, 307–315.

Belland, R.J. and Trust, T.J. (1989) *Aeromonas salmonicida* plasmids: Plasmid-directed synthesis of proteins *in vitro* and in *Escherichia coli* minicells. *Journal of General Microbiology* **135**, 513–524.

Benediktsdóttir, E., Helgason, S. and Gudmundsdóttir, S. (1991) Incubation time for the cultivation of *Renibacterium salmoninarum* from Atlantic salmon, *Salmo salar* L., broodfish. *Journal of Fish Diseases* **14**, 97–102.

Benediktsdóttir, E., Helgason, S. and Sigurjónsdóttir, H. (1998) *Vibrio* spp. isolated from salmonids with shallow skin lesions and reared at low tempterature. *Journal of Fish Diseases* **21**, 19–28.

Benediktsdóttir, E., Verdonck, L., Spröer, C., Helgason, S. and Swings, J. (2000) Characterization of *Vibrio viscosus* and *Vibrio wodanis* isolated from different geographical locations: A proposal for re-classification of *Vibrio viscosus* as *Moritella viscosa* comb. nov. *International Journal of Systematic and Evolutionary Microbiology* **50**, 479–488.

Beran, V., Matlova, L., Dvorska, L., Svastova, P. and Pavlik, I. (2006) Distribution of mycobacteria in clinically healthy ornamental fish and their aquatic environment. *Journal of Fish Diseases* **29**, 383–393.

Bercovier, H. and Mollaret, H.H. (1984) Genus XIV: *Yersinia* van Loghem 1944, 15^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. I. Baltimore, MD, Williams & Wilkins, pp. 489–506.

Berg, R.W. and Anderson, A.W. (1972) Salmonellae and *Edwardsiella tarda* in gull feces: A source of contamination in fish processing plants. *Applied Microbiology* **24**, 501–503.

Bergh, Ø. (1995) Bacteria associated with early life stages of halibut, *Hippoglossus hippoglossus* L., inhibit growth of a pathogenic *Vibrio* sp. *Journal of Fish Diseases* **18**, 31–40.

Bergh, Ø., Hjeltnes, B. and Skiftesvik, A.B. (1997) Experimental infection of turbot *Scophthalmus maximus* and halibut *Hippoglossus hippoglossus* yolk sac larvae with *Aeromonas salmonicida* subsp. *salmonicida*. *Diseases of Aquatic Organisms* **29**, 13–20.

Bergman, A.M. (1909) Die rote Beulenkrankheit des Aals. *Bericht aus der Königlichen Bayerischen Versuchsstation* **2**, 10–54.

Bernardet, J.-F. (1989) "*Flexibacter columnaris*": First description in France and comparison with bacterial strains from other origins. *Diseases of Aquatic Organisms* **6**, 37–44.

Bernardet, J.-F. and Grimont, P.A.D. (1989) Deoxyribonucleic acid relatedness and phenotypic characterization of *Flexibacter columnaris* sp. nov., nom. rev., *Flexibacter psychrophilus* sp. nov., nom. rev., and *Flexibacter maritimus* Wakabayashi, Hikida and Masumura 1986. *International Journal of Systematic Bacteriology* **39**, 346–354.

Bernardet, J.-F. and Kerouault, B. (1989) Phenotypic and genomic studies of "*Cytophaga psychrophila*" isolated from diseased rainbow trout (*Oncorhynchus mykiss*) in France. *Applied and Environmental Microbiology* **55**, 1796–1800.

Bernardet, J.-F., Campbell, A.C. and Buswell, J.A. (1990) *Flexibacter maritimus* is the agent of "black patch necrosis" in Dover sole in Scotland. *Diseases of Aquatic Organisms* **8**, 233–237.

Bernardet, J.-F., Kerouault, B. and Michel, C. (1994) Comparative study on *Flexibacter maritimus* strains isolated from farmed sea bass (*Dicentrarchus labrax*) in France. *Fish Pathology* **29**, 105–111.

Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. and Vandamme, P. (1996) Cutting a Gordian know: emended classification and description of the family Flavobacteriaceae, and proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *International Journal of Systematic Bacteriology* **46**, 128–148.

Bernheimer, A.W. and Avigad, L.S. (1974) Partial characterization of aerolysin, a lytic exotoxin from *Aeromonas hydrophila*. *Infection and Immunity* **9**, 1016–1021.

Bernoth, E.-M. (1990) Autoagglutination, growth on tryptone–soy–Coomassie–agar, outer membrane protein patterns and virulence of *Aeromonas salmonicida* strains. *Journal of Fish Diseases* **13**, 145–155.

Bernoth, E.-M., Ellis, A.E., Midtlyng, P.J., Olivier, G. and Smith, P. (1997) *Furunculosis Multidisciplinary Fish Disease Research*. San Diego, CA, Academic Press.

Berthe, F.C.J., Michel, C. and Bernardet, J.-F. (1995) Identification of *Pseudomonas anguilliseptica* isolated from several fish species in France. *Diseases of Aquatic Organisms* **21**, 151–155.

Bertone, S., Gili, C., Moizo, A. and Calegari, L. (1996) *Vibrio carchariae* associated with a chronic skin ulcer on a shark, *Carcharhinus plumbeus* (Nardo). *Journal of Fish Diseases* **19**, 429–434.

Beynon, L.M., Richards, J.C. and Perry, M.B. (1994) The structure of the lipopolysaccharide O antigen from *Yersinia ruckeri* serotype O1. *Carbohydrate Research* **256**, 303–317.

Bidinost, C., Crosa, J.H. and Actis, L.A. (1994) Localization of the replication region of the pMJ101 plasmid from *Vibrio ordalii*. *Plasmid* **31**, 242–250.

Bidinost, C., Wilderman, P.J., Dorsey, C.W. and Actis, L.A. (1999) Analysis of the replication elements of the pMJ101 plasmid from the fish pathogen *Vibrio ordalii*. *Plasmid* **42**, 20–30.

Bilodeau, A.L., Waldbieser, G.C., Terhune, J.S., Wise, D.J. and Wolters, W.R. (2003) A real-time polymerase chain reaction assay of the bacterium *Edwardsiella ictaluri* in channel catfish. *Journal of Aquatic Animal Health* **15**, 80–86.

Biosca, E.G., Amaro, C., Esteve, C., Alcaide, E. and Garay, E. (1991) First record of *Vibrio vulnificus* biotype 2 from diseased European eel, *Anguilla anguilla* L. *Journal of Fish Diseases* **14**, 103–109.

Biosca, E.G., Llorens, H., Garay, E. and Amaro, C. (1993) Presence of a capsule in *Vibrio vulnificus* biotype 2 and its relationship to virulence for eels. *Infection and Immunity* **61**, 1611–1618.

Biosca, E.G., Amaro, C., Larsen, J.L. and Pedersen, K. (1997a) Phenotypic and genotypic characterization of *Vibrio vulnificus*: Proposal for the substitution of the subspecific taxon biotype for serovar. *Applied and Environmental Microbiology* **63**, 1460–1466.

Biosca, E.G., Marco-Noales, E., Amaro, C. and Alcaide, E. (1997b) An enzyme-linked immunosorbent assay for detection of *Vibrio vulnificus* biotype 2: Development and field studies. *Applied and Environmental Microbiology* **63**, 537–542.

Birkbeck, T.H., Laidler, L.A., Grant, A.N. and Cox, D.I. (2002) *Pasteurella skyensis* sp. nov., isolated from Atlantic salmon (*Salmo salar* L.). *International Journal of Systematic and Evolutionary Microbiology* **52**, 699–704.

Birkbeck, T.H., Rennie, S., Hunter, D., Laidler, L.A. and Wadsworth, S. (2004) Infectivity of a Scottish isolate of *Piscirickettsia salmonis* for Atlantic salmon *Salmo salar* and immune response of salmon to this agent. *Diseases of Aquatic Organisms* **60**, 97–103.

Björnsdóttir, B., Gudmundsdóttir, S., Bambir, S.H., Magnadóttir, B. and Gudmundsdóttir, B.K. (2004) Experimental infection of turbot, *Scopthalmus maximus* (L.), by *Moritella viscosa*, vaccination effort and vaccine-induced side-effects. *Journal of Fish Diseases* **27**, 645–655.

Björnsdóttir, B., Gudmundsdóttir, S., Bambir, S.H. and Gudmundsdóttir, B.K. (2005) Experimental infection of turbot, *Scophthalmus maximus* (L.), by *Aeromonas salmonicida* subsp. *achromogenes* and evaluation of cross protection induced by a furunculosis vaccine. *Journal of Fish Diseases* **28**, 181–188.

Black, M.M. and Eykyn, S.J. (1977) The successful treatment of tropical fish tank granuloma (*Mycobacterium marinum*) with co-trimoxazole. *British Journal of Dermatology* **97**, 689–692.

Blake, I. (1935) Some observations on other bacterial diseases of fish, with particular reference to the vascular and tissue reactions in these infections. In: Adamson, W. and Baxton, N. (eds), *Final Report of the Furunculosis Committee*. Edinburgh, H.M.S.O., pp. 1–13.

Blake, I. and Anderson, E.J.M. (1930) The identification of *Bacillus salmonicida* by the complement fixation test: A further contribution to the study of furunculosis of the Salmonidae. *Fisheries Board of Scotland, Salmon Fisheries No.* **1**. Edinburgh, H.M.S.O.

Blake, I. and Clark, J.C. (1931) Observations on experimental infection of trout by *B. salmonicida*, with particular reference to "carriers" of furunculosis and to certain factors influencing susceptibility. *Fisheries Board of Scotland, Salmon Fisheries No.* **7**. Edinburgh, H.M.S.O., pp. 1–13.

Blanco, M.M., Gibello, A., Vela, A.I., Moreno, M.A., Domínguez, L. and Fernández-Garayzábal, J.F. (2002) PCR detection and PFGE macrorestriction analyses of clinical isolates of *Pseudomonas anguilliseptica* from winter disease outbreaks in sea bream *Sparus aurata*. *Diseases of Aquatic Organisms* **50**, 19–27.

Boesen, H.T., Pedersen, K., Koch, C. and Larsen, J.L. (1997) Immune response of rainbow trout (*Oncorhynchus mykiss*) to antigenic preparations from *Vibrio anguillarum* serogroup O1. *Fish & Shellfish Immunology* **7**, 543–553.

Bolinches, J., Romalde, J.L. and Toranzo, A.E. (1988) Evaluation of selective media for isolation and enumeration of vibrios from estuarine waters. *Journal of Microbiological Methods* **8**, 151–160.

Bolinches, J., Lemos, M.L., Fouz, B., Cambra, M., Larsen, J.L. and Toranzo, A.E. (1990) Serological relationships among *Vibrio anguillarum* strains. *Journal of Aquatic Animal Health* **2**, 21–29.

Bonaveri, G.F. (1761) Quoted by Drouin de Bouville (1907).

Bonet, R., Simon-Pujol, M.D. and Congregado, F. (1993) Effects of nutrients on exopolysaccharide production and surface properties of *Aeromonas salmonicida*. *Applied and Environmental Microbiology* **59**, 2437–2441.

Boomker, J., Imes, G.D., Cameron, C.M., Naudé, T.W. and Schoonbee, H.J. (1979) Trout mortalities as a result of *Streptococcus* infection. *Onderstepoort Journal of Veterinary Research* **46**, 71–77.

Booth, N.J., Elkamel, A. and Thune, R.L. (2006) Intracellular replication of *Edwardsiella ictaluri* in channel catfish macrophages. *Journal of Aquatic Animal Health* **18**, 101–108.

Bootsma, R. and Clerx, J.P.M. (1976) Columnaris disease of cultured carp *Cyprinus carpio* L.: Characteristics of the causative agent. *Aquaculture* **7**, 371–384.

Bootsma, R., Fijan, N. and Blommaert, J. (1977) Isolation and identification of the causative agent of carp erythrodermatitis. *Veterinarski Archiv* **47**, 291–302.

Borg, A.F. (1948) Studies on myxobacteria associated with diseases of salmonid fishes. Ph.D. thesis, University of Washington, Seattle.

Borg, A.F. (1960) Studies on myxobacteria associated with diseases in salmonid fishes. *Journal of Wildlife Diseases* **8**, 1–85.

Bosse, M.P. and Post, G. (1983) Tribrissen and tiamulin for control of enteric redmouth disease. *Journal of Fish Diseases* **6**, 27–32.

Bott, T.L., Johnson, J., Foster, E.M. and Sugiyama, H. (1968) Possible origin of the high incidence of *Clostridium botulinum* type E in an inland bay (Green Bay of Lake Michigan). *Journal of Bacteriology* **95**, 1542–1547.

Boulanger, Y., Lallier, R. and Cousineau, G. (1977) Isolation of enterotoxigenic *Aeromonas* from fish. *Canadian Journal of Microbiology* **23**, 1161–1164.

Bøvre, K. (1984) Genus II: *Moraxella* Lwoff 1939, 173 emend. Henriksen & Bøvre 1968, 391^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Baltimore, MD, Williams & Wilkins, pp. 296–303.

Bowden, T.J., Menoyo-Luque, D., Bricknell, I.R. and Wegeland, H. (2002) Efficacy of different administration routes for vaccination against *Vibrio anguillarum* in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Fish & Shellfish Immunology* **12**, 283–285.

Bowser, P.R. (1973) Seasonal prevalence of *Chondrococcus columnaris* infection in black bullheads from Lake, Iowa. *Journal of Wildlife Diseases* **9**, 115–119.

Bowser, P.R. and House, M. (1990) *In vitro* sensitivity of some fish pathogens to the quinolones nalidixic acid and oxolinic acid and the fluoroquinolone enrofloxacin. *Bulletin of the European Association of Fish Pathologists* **10**, 48–49.

Boyce, S.H. (1997) Fish tank granuloma: An unusual cause of skin infection. *Journal of Accident and Emergency Medicine* **14**, 400.

Bragg, R.R. and Todd, J.M. (1988) *In vitro* sensitivity to Baytril of some bacteria pathogenic to fish. *Bulletin of the European Association of Fish Pathologists* **8**, 5.

Bransden, M.P., Carson, J., Munday, B.L., Handlinger, J.H., Carter, C.G. and Nowak, B.F. (2000) Nocardiosis in tank-reared Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **23**, 83–85.

Branson, E.J. and Diaz-Munoz, D.N. (1991) Description of a new disease condition occurring in farmed coho salmon, *Oncorhynchus kisutch* (Walbaum), in South America. *Journal of Fish Diseases* **14**, 147–156.

Brattgjerd, S., Evensen, Ø., Speilberg, L. and Lauve, A. (1995) Internalization of *Vibrio salmonicida* in isolated macrophages from Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) evaluated by a paired immunofluorescence technique. *Fish & Shellfish Immunology* **5**, 121–135.

Braun, M., Stuber, K., Schlatter, Y., Wahli, T., Kuhnert, P. and Frey, J. (2002) Characterization of an ADP-ribosyltransferase toxin (AexT) from *Aeromonas salmonicida* subsp. *salmonicida*. *Journal of Bacteriology* **184**, 1851–1858.

Bravo, S. (1994) Piscirickettsiosis in freshwater. *Bulletin of the European Association of Fish Pathologists* **14**, 137–138.

Bravo, S. and Kojagura, V. (2004) First isolation of *Yersinia ruckeri* from rainbow trout (*Oncorhynchus mykiss*) in Peru. *Bulletin of the European Association of Fish Pathologists* **24**, 104–108.

Brenner, D.J. (1984) Family 1. Enterobacteriaceae Rahn 1937, Nom. fam. cons. Opin. 15, Jud. Comm. 1958, 73; Ewing, Farmer and Breener 1980, 674; Judicial Commission 1981, 104. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 408–420.

Brenner, D.J., McWhorter, A.C., Knutson, J.K.L. and Steigerwalt, A.G. (1982) *Escherichia vulneris*: A new species of Enterobacteriaceae associated with human wounds. *Journal of Clinical Microbiology* **15**, 1133–1140.

Bricknell, I.R. (1995) A reliable method for the induction of experimental furunculosis. *Journal of Fish Diseases* **18**, 127–133.

Bricknell, I.R., Bowden, T.J., Verner-Jeffreys, D.W., Bruno, D.W., Shields, R.J. and Ellis, A.E. (2000) Susceptibility of juvenile and sub–adult Atlantic halibut (*Hippoglossus hippoglossus* L.) to infection by *Vibrio anguillarum* and efficacy of protection induced by vaccination. *Fish & Shellfish Immunology* **10**, 319–327.

Bridge, P.D. and Sneath, P.H.A. (1983) Numerical taxonomy of *Streptococcus*. *Journal of General Microbiology* **129**, 565–597.

Brisou, J., Tysset, C. and Vacher, B. (1964) Recherches sur les Pseudomonadaceae. Etude de deux souches de *Flavobacterium* isolées des poissons d'eau douce. *Annals Institut Pasteur* **74**, 633–638.

Brocklebank, J.R. (1998) Ulcerative dermatitis caused by *Aeromonas salmonicida* spp. *salmonicida* in farmed Atlantic salmon in British Columbia. *Revue Vetérinaire Canadienne* **39**, 110.

Bromage, E.S., Thomas, A. and Owens, L. (1999) *Streptococcus iniae*, a bacterial infection in barramundi *Lates calcarifer*. *Diseases of Aquatic Organisms* **36**, 177–181.

Broom, A.K. and Sneath, P.H.A. (1981) Numerical taxonomy of *Haemophilus*. *Journal of General Microbiology* **126**, 123–149.

Brown, L.L., Albright, L.J. and Evelyn, T.P.T. (1990) Control of vertical transmision of *Renibacterium salmoninarum* by injection of antibiotics into maturing female coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* **9**, 127–131.

Brown, L.L., Iwana, G.K., Evelyn, T.P.T., Nelson, W.S. and Levine, R.P. (1994) Use of the polymerase chain reaction (PCR) to detect DNA from *Renibacterium salmoninarum* within individual salmonid eggs. *Diseases of Aquatic Organisms* **18**, 165–171.

Brown, L.L., Evelyn, T.P.T., Iwama, G.K., Nelson, W.S. and Levine, R.P. (1995) Bacterial species other than *Renibacterium salmoninarum* cross react with antisera against *Renibacterium salmoninarum* but are negative for the p57 gene of *Renibacterium salmoninarum* as detected by the polymerase chain reaction (PCR). *Diseases of Aquatic Organisms* **21**, 227–231.

Brown, L.L., Iwama, G.K. and Evelyn, T.P.T. (1996) The effect of early exposure of coho salmon (*Oncorhynchus kisutch*) eggs to the p57 protein of *Renibacterium salmoninarum* on the development of immunity to the pathogen. *Fish & Shellfish Immunology* **6**, 149–165.

Brown, L.L., Cox, W.T. and Levine, R.P. (1997) Evidence that the causal agent of bacterial cold-water disease *Flavobacterium psychrophilum* is transmitted within salmonid eggs. *Diseases of Aquatic Organisms* **29**, 213–218.

Bruckner-Tuderman, L. and Blank, A.A. (1985) Unusual cutaneous dissemination of a tropical fish tank granuloma. *Cutis* **36**, 405–406.

Bruhn, J.B., Dalsgaard, I., Nielsen, K.F., Buchholtz, C., Larsen, J.L. and Gram, L. (2005) Quorum sensing signal molecules (acylated homoserine lactones) in Gram-negative fish pathogenic bacteria. *Diseases in Aquatic Organisms* **65**, 43–52.

Bruno, D.W. (1987) Serum agglutinating titres against *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease, in rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* **30**, 327–334.

Bruno, D.W. (1988) The relationship between auto-agglutination, cell surface hydrophobicity and virulence of the fish pathogen *Renibacterium salmoninarum*. *FEMS Microbiology Letters* **51**, 135–140.

Bruno, D.W. and Brown, L.L. (1999) The occurrence of *Renibacterium salmoninarum* within vaccine adhesion components from Atlanic salmon, *Salmo salar* L., and coho salmon, *Oncorhynchus kisutch* Walbaum. *Aquaculture* **170**, 1–5.

Bruno, D.W. and Munro, A.L.S. (1992) Detection of the causative agent of bacterial kidney disease. *Bulletin of the European Association of Fish Pathologists* **2**, 10–12.

Bruno, D.W., Hastings, T.S., Ellis, A.E. and Wootten, R. (1985) Outbreak of a cold-water vibriosis in Atlantic salmon in Scotland. *Bulletin of the European Association of Fish Pathologists* **5**, 62–63.

Bruno, D.W., Griffiths, J., Mitchell, C.G., Wood, B.P., Fletcher, Z.J., Drobniewski, F.A. and Hastings, T.S. (1998) Pathology attributed to *Mycobacterium chelonae* infection among farmed and laboratory-infected Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* **33**, 101–109,

Brunt, J. and Austin, B. (2005) Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **28**, 693–702.

Bryant, T.N. and Smith, K.R. (1991) *Bacterial Identifier*, Version M1.0. Oxford, U.K., Blackwell.

Bruun, A.F. and Heiberg, B. (1935) Weitere Untersuchungen über die Rotseuche des Aales in den dänischen Gewässern. *Zeitshcrift für Fischerei und deren Hilfwissenschaften* **33**, 379–382.

Buchholtz, C. Nielsen, K.F., Milton, D.L., Larsen, J.L. and Gram, L. (2006) Profiling of acylated homoserine lactones of *Vibrio anguillarum in vitro* and *in vivo*: Influence of growth conditions and serotype. *Systematic and Applied Microbiology* **29**, 433–445.

Bucke, D. (1978) Bacterial kidney disease (BKD) of fish. *Fisheries Notice*. Lowestoft, U.K., M.A.F.F., **60**, 1–4.

Bucke, D. (1979) Investigation into the cause of an epizootic in perch (*Perca fluviatilis* L.). In: O'Hara, K. (ed.), *Proceedings of the First British Freshwater Fish Conference*. London, University of Liverpool, Janssen Services, p. 45.

Bucke, D. (1991) Current approaches to the study of pollution-related diseases of fish. *Bulletin of the European Association of Fish Pathologists* **11**, 46–53.

Bucke, D. (1997) Facts and myths regarding pollution and fish health. *Bulletin of the European Association of Fish Pathologists* **17**, 191–196.

Bucke, D., Vethaak, A.D. and Lang, T. (1992) Quantitative assessment of melanomacrophage centres (MMCs) in dab *Limanda limanda* as indicators of pollution effects on the non-specific immune system. *Marine Progress Series* **91**, 193–196.

Buckley, J.T., Halasa, L.N. and MacIntyre, S. (1982) Purification and partial characterization of a bacterial phospholipid:cholesterol acyltransferase. *Journal of Biological Chemistry* **257**, 3320–3325.

Bullen, J.J., Roger, H.J. and Griffiths, E. (1978) Role of iron in bacterial infections. *Current Topics in Microbiology and Immunology* **80**, 1–35.

Bullock, G.L. (1965) Characteristics and pathogenicity of a capsulated *Pseudomonas* isolated from goldfish. *Applied Microbiology* **13**, 89–92.

Bullock, G.L. (1966) Precipitins and agglutinin reactions of aeromonads isolated from fish and other sources. *Bulletin de l'Office International des Epizooties* **65**, 805–824.

Bullock, G.L. (1981) Streptococcal infections of fishes. *United States Department of the Interior, Fish and Wildlife Service, Fish Disease Leaflet* **63**, 1–7.

Bullock, G.L. and Anderson, D.P. (1984) Immunization against *Yersinia ruckeri*, cause of enteric redmouth disease. In: De Kinkelin, P. (ed.), *Symposium on Fish Vaccination; Theoretical Background and Practical Results on Immunization against Infectious Diseases*. Paris, Office International des Epizooties, pp. 151–166.

Bullock, G.L. and Conroy, D.A. (1971) Vibrio diseases. In: Snieszko, S.F. and Axelrod, H.R. (eds), *Diseases of Fishes, 2A. Bacterial Diseases of Fishes*. Jersey City, NY, T.F.H. Publications, pp. 42–50.

Bullock, G.L. and McLaughlin, J.J.A. (1970) Advances in knowledge concerning bacteria pathogenic to fishes (1954–1968). In: Snieszko, S.F. (ed.), *A Symposium on Diseases of Fishes and Shellfishes*. Washington, D.C., American Fisheries Society, Special Publication No. 5, pp. 231–242.

Bullock, G.L. and Snieszko, S.F. (1970) Fin rot, coldwater disease, and peduncle disease of salmonid fish. *United States Department of the Interior, Fish and Wildlife Service, Fish Disease Leaflet* **25**, 1–3.

Bullock, G.L. and Snieszko, S.F. (1975) Hagerman redmouth, a disease of salmonids caused by a member of the Enterobacteriaceae. *United States Department of the Interior, Fish and Wildlife Service, Fish Disease Leaflet* **42**, 1–5.

Bullock, G.L. and Stuckey, H.M. (1975a) *Aeromonas salmonicida*: Detection of asymptomatically infected trout. *Progressive Fish Culturist* **37**, 237–239.

Bullock, G.L. and Stuckey, H.M. (1975b) Fluorescent antibody identification and detection of the *Corynebacterium* causing kidney disease of salmonids. *Journal of the Fisheries Research Board of Canada* **32**, 2224–2227.

Bullock, G.L., Conroy, D.A. and Snieszko, S.F. (1971) Bacterial diseases of fishes. In: Snieszko, S.F. and Axelrod, H.R. (eds), *Diseases of Fishes, Book 2A*. Neptune, NJ, T.F.H. Publications, 151 pp.

Bullock, G.L., Stuckey, H.M. and Chen, P.K. (1974) Corynebacterial kidney disease of salmonids: Growth and serological studies on the causative bacterium. *Applied Microbiology* **28**, 811–814.

Bullock, G.L., Stuckey, H.M. and Wolf, K. (1975) Bacterial kidney disease of salmonid fishes. *United States Department of the Interior, Fish and Wildlife Service, Fish Disease Leaflet* **41**.

Bullock, G.L., Stuckey, H.M. and Shotts, E.B. (1978a) Enteric redmouth bacterium: Comparison of isolates from different geographical areas. *Journal of Fish Diseases* **1**, 351–354.

Bullock, G.L., Stuckey, H.M. and Mulcahy, D. (1978b) Corynebacterial kidney disease: Egg transmission following iodophore disinfection. *Fish Health News* **76**, 51–52.

Bullock, G.L., Griffin, B.R. and Stuckey, H.M. (1980). Detection of *Corynebacterium salmoninus* by direct fluorescent antibody test. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 719–721.

Bullock, G.L., Maestrone, G., Starliper, C. and Schill, B. (1983) Potentiated sulfonamide therapy of enteric redmouth disease. *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 101–102.

Bullock, G.L., Hsu, T.C. and Shotts, E.B. (1986) *Columnaris Disease of Fishes*. Fish disease leaflet No. 72, U.S. Department of the Interior, Fish and Wildlife Service, Division of Fisheries and Wetlands Research, Washington, DC, 9 pp.

Bullock, G.L., Cipriano, R.C. and Schill, W.B. (1997) Culture and serodiagnostic detection of *Aeromonas salmonicida* from covertly-infected rainbow trout given the stress-induced furunculosis test. *Biomedical Letters* **55**, 169–177.

Burke, J. and Rodgers, L. (1981) Identification of pathogenic bacteria associated with the occurrence of "red spot" in sea mullet, *Mugil cephalus* L., in south-eastern Queensland. *Journal of Fish Diseases* **3**, 153–159.

Burr, S.E., Stuber, K., Wahli, T. and Frey, J. (2002) Evidence for a type III secretion system in *Aeromonas salmonicida* subsp. *salmonicida*. *Journal of Bacteriology* **184**, 5966–5970.

Burr, S.E., Stuber, K. and Frey, J. (2003a) The ADP-ribosylating toxin AexT from *Aeromonas salmonicida* subsp. *salmonicida* is translocated via a type III secretion pathway. *Journal of Bacteriology* **185**, 6583–6591.

Burr, S.E., Wahli, T., Segner, H., Pugovkin, D. and Frey, J. (2003b) Association of type III secretion genes with virulence of *Aeromonas salmonicida* subsp. *salmonicida*. *Diseases of Aquatic Organisms* **57**, 167–171.

Burr, S.E., Pugovkin, D., Wahli, T., Segner, H. and Frey, J. (2005) Attenuated virulence of an *Aeromonas salmonicida* subsp. *salmonicida* type III secretion mutant in a rainbow trout model. *Microbiology* **151**, 2111–2118.

Busch, R.A. (1973) The serological surveillance of salmonid populations for presumptive evidence of specific disease association. Ph.D. Dissertation, University of Idaho.

Busch, R.A. (1978) Enteric redmouth disease (Hagerman) strain. *Marine Fisheries Review* **40**, 42–51.

Busch, R.A. (1981) The current status of diagnostic serology for the major bacterial disease of fish. *Developments in Biological Standardization* **49**, 85–96.

Busch, R.A. and Lingg, A. (1975) Establishment of an asymptomatic carrier state infection of enteric redmouth disease in rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **32**, 2429–2432.

Busch, R.A., Burmeister, N.E. and Scott, A.L. (1978) Field and laboratory evaluation of a commercial enteric redmouth disease vaccine for rainbow trout. *Proceedings of the Joint 3rd Biennial Fish Health Section and 9th Annual Midwest Fish Disease Workshop*, p. 67.

Bustos, P.A., Calbuyahue, J., Montaña, J., Opazo, B., Entrala, P. and Solervicens, R. (1995) First isolation of *Flexibacter psychrophilus*, as causative agent of rainbow trout fry syndrome (RTFS), producing rainbow trout mortality in Chile. *Bulletin of the European Association of Fish Pathologists* **15**, 162–164.

Byers, H.K., Gudkovs, N. and Crane, M.St.J. (2002a) PCR-based assays for the fish pathogen *Aeromonas salmonicida*. 1. Evaluation of three PCR primer sets for detection and identification. *Diseases of Aquatic Organisms* **49**, 129–138.

Byers, H.K., Cipriano, R.C., Gudkovs, N. and Crane, M.St.J. (2002b) PCR-based assays for the fish pathogen *Aeromonas salmonicida*. II. Further evaluation and validation of three PCR primer sets with infected fish. *Diseases of Aquatic Organisms* **49**, 139–144.

Camp, K.L., Wolters, W.R. and Rice, C.D. (2000) Survivability and immune responses after challenge with *Edwardsiella ictaluri* in susceptible and resistant families of channel catfish, *Ictalurus punctatus*. *Fish & Shellfish Immunology* **10**, 475–487.

Campbell, E.J.M., Scadding, J.G. and Roberts, M.S. (1979) The concept of disease. *British Medical Journal* **2**, 757–762.

Campbell, G. and MacKelvie, R.M. (1968) Infection of brook trout (*Salvelinus fontinalis*) by nocardiae. *Journal of the Fisheries Research Board of Canada* **25**, 423–425.

Campos-Pérez, J.J., Ellis, A.E. and Secombes, C.J. (1997) Investigation of factors influencing the ability of *Renibacterium salmoninarum* to stimulate rainbow trout macrophage respiratory burst activity. *Fish & Shellfish Immunology* **7**, 555–566.

Candan, A., Küçüker, M.Ang. and Karatas, S. (1995) Motile aeromonad septicaemia in *Salmo salar* cultured in the Black Sea in Turkey. *Bulletin of the European Association of Fish Pathologists* **15**, 195–196.

Canestrini, G. (1893) La malattia dominate delle anguille. *Atti Institute Veneto Service* **7**, 809–814.

Cann, D.C. and Taylor, L.Y. (1982) An outbreak of botulism in rainbow trout, *Salmo gairdneri* Richardson, farmed in Britain. *Journal of Fish Diseases* **5**, 393–399.

Cann, D.C. and Taylor, L.Y. (1984) An evaluation of residual contamination by *Clostridium botulinum* in a trout farm following an outbreak of botulism in the fish stock. *Journal of Fish Diseases* **7**, 391–396.

Cann, D.C., Wilson, B.B., Hobbs, G. and Shewan, J.M. (1965a) The growth and toxin production of *Clostridium botulinum* type E in certain vacuum packed fish. *Journal of Applied Bacteriology* **28**, 431–436.

Cann, D.C., Wilson, B.B., Hobbs, G., Shewan, J.M. and Johannsen, A. (1965b) The incidence of *Clostridium botulinum* type E in fish and bottom deposits in the North Sea and off the coast of Scandinavia. *Journal of Applied Bacteriology* **28**, 426–430.

Cann, D.C., Taylor, L.Y. and Hobbs, G. (1975) The incidence of *Cl. botulinum* in farmed trout raised in Great Britain. *Journal of Applied Bacteriology* **39**, 321–326.

Carballo, J., Seoane, R.M. and Nieto, T.P. (2000) Adhesion of *Aeromonas salmonicida* to materials used in aquaculture. *Bulletin of the European Association of Fish Pathologists* **20**, 77–82.

Carlson, R.V. and Pacha, R.E. (1968) Procedure for the isolation and enumeration of myxobacteria from aquatic habitats. *Applied Microbiology* **16**, 795–796.

Carnahan, A.M. and Altwegg, M. (1996) 1. Taxonomy. In: *The Genus Aeromonas* (edited by B. Austin, M. Altwegg, P.J. Gosling and S. Joseph), pp. 1–38. Chichester, U.K., John Wiley & Sons.

Carson, J. and Munday, B. (1990) Streptococcosis: An emerging disease in aquaculture. *Australasian Aquaculture* **5**, 32–33.

Carson, J. and Schmidtke, L.M. (1993) Opportunistic infection by psychrotrophic bacteria of cold-compromised Atlantic salmon. *Bulletin of the European Association of Fish Pathologists* **13**, 49–52.

Carson, J. and Statham, P. (1993) The inhibition by ionophores *in vitro* of an *Enterococcus*-like pathogen of rainbow trout, *Oncorhynchus mykiss*. *Veterinary Microbiology* **36**, 253–259.

Carson, J., Schmidtke, L.M. and Munday, B.L. (1993) *Cytophaga johnsonae*: A putative skin pathogen of juvenile farmed barramundi, *Lates calcarifer* Bloch. *Journal of Fish Diseases* **16**, 209–218.

Casanova, A., Obreque, J.R., Gaggero, A., Landskron, E., Sandino, A.M. and Jashés M. (2003) Electrophoretic analysis of ITS from *Piscirickettsia salmonis* Chilean isolates. *FEMS Microbiology Letters* **225**, 173–176.

Cascón, A., Fregeneda, J., Aller, M., Yugueros, J., Temprano, A., Hernanz, C., Sanchez, M., Rodríguez-Aparicio, L. and Naharro, G. (2000) Cloning, characterization, and insertional inactivation of a major extracellular serine protease gene with elastolytic activity from *Aeromonas hydrophila*. *Journal of Fish Diseases* **23**, 49–59.

Castro, N., Toranzo, A.E., Barja, J.L., Núñez, S. and Magariños, B. (2006) Characterization of *Edwardsiella tarda* strains isolated from turbot, *Psetta maxima* (L.). *Journal of Fish Diseases* **29**, 541–547.

Cato, E.P., George, W.L. and Finegold, S.M. (1986) Genus: *Clostridium* Prazmowski 1880, 23^{AL}. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 2. Baltimore, MD, Williams & Wilkins, pp. 1141–1200.

Centers for Disease Control (1996) Invasive infection with *Streptococcus iniae*—Ontario, 1995–1996. *Morbidity and Mortality Weekly Report* **45**, 650–653.

Cepeda, C., García-Márquez, S. and Santos, Y. (2003) Detection of *Flexibacter maritimus* in fish tissue using nested PCR amplification. *Journal of Fish Diseases* **26**, 65–70.

Cepeda, C., García-Márquez, S. and Santos, Y. (2004) Improved growth of *Flavobacterium sychrophilum* using a new culture medium. *Aquaculture* **238**, 75–82.

Ceschia, G., Giorgetti, G., Giavenni, R. and Sarti, M. (1992) A new problem for Italian trout farms: Streptococcosis in rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the European Association of Fish Pathologists* **12**, 1–72.

Chabrillón, M., Rico, R.M., Balebona, M.C. and Moriñigo, M.A. (2005) Adhesion to sole, *Solea senegalensis* Kaup, mucus of microorganisms isolated from farmed fish, and their interactions with *Photobacterium damselae* subsp. *piscicida*. *Journal of Fish Diseases* **28**, 229–238.

Chakroun, C., Grimont, F., Urdaci, M.C. and Bernardet, J.-F. (1998) Fingerprinting of *Flavobacterium psychrophilum* isolates by ribotyping and plasmid profiling. *Diseases of Aquatic Organisms* **33**, 167–177.

Chambers, E. and Barker, G. (2006) Comparison of culture media for the isolation of *Renibacterium salmoninarum* from naturally infected rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the European Association of Fish Pathologists* **26**, 137–142.

Chandran, M.R., Aruna, B.V., Logambal, S.M. and Michael, R.D. (2002) Immunisation of Indian major carps against *Aeromonas hydrophila* by intraperitoneal injection. *Fish & Shellfish Immunology* **13**, 1–9.

Chang, T.-C., Hsieh, C.-Y., Chang, C.-D., Shen, Y.-L., Huang, K.-C., Tu, C., Chen, L.-C., Wu, Z.-B. and Tsai, S.-S. (2006) Pathological and molecular studies on mycobacteriosis of milkfish *Chanos chanos* in Taiwan. *Diseases of Aquatic Organisms* **72**, 147–151.

Chart, H. and Trust, T.J. (1983) Acquisition of iron by *Aeromonas salmonicida*. *Journal of Bacteriology* **156**, 758–764.

Chart, H. and Trust, T.J. (1984) Characterization of the surface antigens of the marine fish pathogens, *Vibrio anguillarum* and *Vibrio ordalii*. *Canadian Journal of Microbiology* **30**, 703–710.

Chen, C.-R.L., Chung, Y.-Y. and Kuo, G.-H. (1982) Studies on the pathogenicity of *Flexibacter columnaris*—1. Effect of dissolved oxygen and ammonia on the pathogenicity of *Flexibacter columnaris* to eel (*Anguilla japonica*). *CAPD Fisheries Series No. 8, Reports on Fish Disease Research* **4**, 57–61.

Chen, D. and Ainsworth, A.J. (1992) Glucan administration potentiates immune defence mechanisms of channel catfish, *Ictalurus punctatus* Rafinesque. *Journal of Fish Diseases* **15**, 295–304.

Chen, M.F., Yun, S., Marty, G.D., McDowell, T.S., House, M.L., Appersen, J.A., Guenther, T.A., Arkush, K.D. and Hedrick, R.P. (2000) A *Piscirickettsia salmonis*-like bacterium associated with mortalities of white seabass *Atraroscion nobilis*. *Diseases of Aquatic Organisms* **43**, 117–126.

Chen, P.K., Bullock, G.L., Stuckey, H.M. and Bullock, A.C. (1974) Serological diagnosis of corynebacterial kidney disease of salmonids. *Journal of the Fisheries Research Board of Canada* **31**, 1939–1940.

Chen, S.-C. (1992) Study on the pathogenicity of *Nocardia asteroides* to the Formosa snakehead (*Channa maculata* Lacepède). *Journal of Fish Diseases* **15**, 47–53.

Chen, S.-C., Tung, M.-C., Chen, S.-P., Tsai, J.-F., Wang, P.-C., Chen, R.-S., Lin, S.-C. and Adams, A. (1994) Systematic granulomas caused by a *Rickettsia*-like organism in Nile tilapia, *Oreochromus niloticus* (L.), from southern Taiwan. *Journal of Fish Diseases* **17**, 591–599.

Chen, S.-C., Adams, A. and Richards, R.H. (1997) Extracellular products from *Mycobacterium* spp. in fish. *Journal of Fish Diseases* **20**, 19–25.

Chen, S.-C., Lee, J.-L., Lai, C.-C., Gu, Y.-W., Wang, C.-T., Chang, H.-Y. and Tsai, K.-H. (2000) Nocardiosis in sea bass, *Lateolabrax japonicus* in Taiwan. *Journal of Fish Diseases* **23**, 299–307.

Chen, S.-C., Wang, P.-C., Tung, M.-C., Thompson, K.D. and Adams, A. (2000a) A *Piscirickettsia salmonis*-like organism in grouper, *Epinephelus melanostigma*, in Taiwan. *Journal of Fish Diseases* **23**, 415–418.

Chen, S.-C., Thompson, K.D., Adams, A. and Richards, R.H. (2001) The production of a lymphokine (macrophage activating factor) by rainbow trout, *Oncorhynchus mykiss* (Walbaum), leucocytes stimulated with the extracellular products of *Mycobacterium* sp. *Journal of Fish Diseases* **24**, 217–223.

Chen, S.-C., Liaw, L.-L., Su, H.-Y., Ko, S.-C., Wu, C.-Y., Chaung, H.-C., Tsai, Y.-H., Yang, K.-L., Chen, Y.-C., Chen, T.-H. *et al.* (2002) *Lactococcus garvieae*, a cause of disease in grey mullet, *Mugil cephalus* L., in Taiwan. *Journal of Fish Diseases* **25**, 727–732.

Chen, Q., Actis, L.A., Tolmasky, M.E. and Crosa, J.H. (1994) Chromosome-mediated 2,3-dihydroxybenzoic acid is a precursor in the biosynthesis of the plasmid-mediated siderophore anguibactin in *Vibrio anguillarum*. *Journal of Bacteriology* **176**, 4226–4234.

Chen, Q., Wertheimer, A.M., Tolmasky, M.E. and Crosa, J.H. (1996) The AngR protein and the siderophore anguibactin positively regulate the expression of iron-transport genes in *Vibrio anguillarum*. *Molecular Microbiology* **22**, 127–143.

Chern, R.S. and Chao, C.B. (1994) Outbreaks of disease caused by *Rickettsia*-like organism in cultured tilapias in Taiwan. *Fish Pathology* **29**, 61–71.

Chester, F.D. (1897) A preliminary arrangement of the species of the genus *Bacterium*. Contributions to determinative bacteriology. Part 1. *Ninth Annual Report, Delaware College Agricultural Experimental Station*, 92 pp.

Chomarat, M., Guerin-Faublee, V., Kodjo, A., Breysse, F. and Flandrois, J.P. (1998) Molecular analysis of the fish pathogen *Aeromonas salmonicida* subsp. *salmonicida* by pulsed field gel electrophoresis. *Revue de Medecine Vetérinaire* **149**, 245–250.

Choudhury, D., Pal, A.K., Sahu, N.P., Kumar, S., Das, S.S. and Mukherjee, S.C. (2005) Dietary yeast RNA supplementation reduces mortality by *Aeromonas hydrophila* in rohu (*Labeo rohita* L.) juveniles. *Fish & Shellfish Immunology* **19**, 281–291.

Chowdbury, M.B.R. and Wakabayashi, H. (1989) Effects of competitive bacteria on the survival and infectivity of *Flexibacter columnaris*. *Fish Pathology* **24**, 9–15.

Christensen, P.J. (1977) The history, biology and taxonomy of the *Cytophaga* group. *Canadian Journal of Microbiology* **23**, 1599–1653.

Chu, W.-H. And Lu, C.-P. (2005) Multiplex PCR assay for the detection of pathogenic *Aeromonas hydrophila*. *Journal of Fish Diseases* **28**, 437–442.

Chun, S.-K. (1975) The pathogenicity of myxobacteria isolated from infected fish. *Bulletin of the National Fisheries University, Busan* **15**, 31–42.

Cipriano, R.C. (1982a) Immunization of brook trout (*Salvelinus fontinalis*) against *Aeromonas salmonicida*: Immunogenicity of virulent and avirulent isolates and protective ability of different antigens. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 218–221.

Cipriano, R.C. (1982b) Furunculosis in brook trout: Infection by contact exposure. *Progressive Fish Culturist* **44**, 12–14.

Cipriano, R.C. (1982c) Resistance of salmonids to *Aeromonas salmonicida*: Relation between agglutinins and neutralizing activities. *Transactions of the American Fisheries Society* **112**, 95–99.

Cipriano, R.C. (1983) Resistance of salmonids to *Aeromonas salmonicida*: Relation between agglutinins and neutralizing activities. *Transactions of the American Fisheries Society* **112**, 95–99.

Cipriano, R.C. (2005) Intraovum infection caused by *Flavobacterium psychrophilum* among eggs from captive Atlantic salmon broodfish. *Journal of Aquatic Animal Health* **17**, 275–283.

Cipriano, R.C. and Bullock, G.L. (2001) Evaluation of commercially prepared transport systems for nonlethal detection of *Aeromonas salmonicida* in salmonid fish. *Journal of Aquatic Animal Health* **13**, 96–104.

Cipriano, R.C. and Starliper, C.E. (1982) Immersion and injection vaccination of salmonids against furunculosis with an avirulent strain of *Aeromonas salmonicida*. *Progressive Fish Culturist* **44**, 167–169.

Cipriano, R.C., Griffin, B.R. and Lidgerding, B.C. (1981) *Aeromonas salmonicida*: Relationship between extracellular growth products and isolate virulence. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1322–1326.

Cipriano, R.C., Ford, L.A., Teska, J.D. and Hale, L.E. (1992) Detection of *Aeromonas salmonicida* in the mucus of salmonid fishes. *Journal of Aquatic Animal Health* **4**, 14–118.

Cipriano, R.C., Bullock, G.L. and Noble, A. (1996a) Nature of *Aeromonas salmoncida* carriage on asymptomatic rainbow trout maintained in a culture system with recirculating water and fluidized sand biofilters. *Journal of Aquatic Animal Health* **8**, 47–51.

Cipriano, R.C., Ford, L.A., Teska, J.D., Schachte, J.H., Petrie, C., Novak, B.M. and Flint, D.E. (1996b). Use of non-lethal procedures to detect and monitor *Aeromonas salmonicida* in potentially endangered or threatened populations of migrating and post-spawning salmon. *Diseases of Aquatic Organisms* **27**, 233–236.

Cipriano, R.C., Ford, L.A., Starliper, C.E., Teska, J.D., Nelson, J.T. and Jensen, B.N. (1996c) Control of external *Aeromonas salmonicida*: Topical disinfection of salmonids with chloramine T. *Journal of Aquatic Animal Health* **8**, 52–57.

Cipriano, R.C., Ford, L.A., Nelson, J.T. and Jenson, B.N. (1996d) Monitoring for early detection of *Aeromonas salmonicida* to enhance antibiotic therapy and control furunculosis in Atlantic salmon. *Progressive Fish Culturist* **58**, 203–208.

Cipriano, R.C., Ford, L.A., Smith, D.R., Schachte, J.H. and Petrie, C.J. (1997) Differences in detection of *Aeromonas salmonicida* in covertly infected salmonid fishes by the stress-inducible furunculosis test and culture-based assays. *Journal of Aquatic Animal Health* **9**, 108–113.

Cisar, J.O. and Fryer, J.L. (1974) Characterization of anti-*Aeromonas salmonicida* antibodies from coho salmon. *Infection and Immunity* **9**, 236–243.

Citarella, R.V. and Colwell, R.R. (1970) Polyphasic taxonomy of the genus *Vibrio*: Polynucleotide sequence relationships among selected *Vibrio* species. *Journal of Bacteriology* **104**, 434–442.

Clausen, H.J. and Duran-Reynals, F. (1937) Studies on the experimental infection of some reptiles, amphibia and fish with *Serratia anolium*. *American Journal of Pathology* **13**, 441–541.

Claveau, R. (1991) Néphrite granulomateuse à *Rhodococcus* spp dans un élevage de saumons de l'Atlantique (*Salmo salar*). *Le Médecin Vétérinaire du Québec* **21**, 160–161.

Coady, A.M., Murray, A.L., Elliott, D.G. and Rhodes, L.D. (2006) Both msa genes in *Renibacterium salmoninarum* are needed for full virulence in bacterial kidney disease. *Applied and Environmental Microbiology* **72**, 2672–2678.

Collado, R., Fouz, B., Sanjuán, E. and Amaro, C. (2000) Effectiveness of different vaccine formulations against vibriosis caused by *Vibrio vulnificus* serovar E (biotype 2) in European eels *Anguilla anguilla*. *Diseases of Aquatic Organisms* **43**, 91–101.

Collins, M.D., Farrow, J.A.E., Phillips, B.A., Ferusu, S. and Jones, D. (1987) Classification of *Lactobacillus divergens*, *Lactobacillus piscicola*, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, *Carnobacterium*. *International Journal of Systematic Bacteriology* **37**, 310–316.

Collins, V.G. (1970) Recent studies on bacterial pathogens of freshwater fish. *Water Treatment and Examination* **19**, 3–31.

Colorni, A., Paperna, I. and Gordin, H. (1981) Bacterial infections in gilthead sea bream *Sparus aurata* cultured in Elat. *Aquaculture* **23**, 257–267.

Colorni, A., Diamant, A., Eldar, A., Kvitt, H. and Zlotkin, A. (2002) *Streptococcus iniae* infections in Red Sea cage-cultured and wild fishes. *Diseases of Aquatic Organisms* **49**, 165–170.

Colorni, A., Ravelo, C., Romalde, J.L., Toranzo, A.E. and Diamant, A. (2003) *Lactococcus garvieae* in wild Red Sea wrasse *Coris aygula* (Labridae). *Diseases of Aquatic Organisms* **56**, 275–278.

Colquhoun, D.J., Alvheim, K., Dommarsnes, K., Syvertsen, C. and Sørum, H. (2002) Relevance of incubation temperature for *Vibrio salmonicida* vaccine production. *Journal of Applied Microbiology* **92**, 1087–1096.

Colquhoun, D.J., Hovland, H., Hellberg, H., Haug, T. and Nilsen, H. (2004) *Moritella viscosa* isolated from farmed Atlantic cod (*Gadus morhua*). *Bulletin of the European Association of Fish Pathologists* **24**, 109–114.

Colwell, R.R. and Grimes, D.J. (1984) *Vibrio* diseases of marine fish populations. *Helgoländer Meeresuntersuchungen* **37**, 265–287.

Colwell, R.R., MacDonell, M.T. and De Ley, J. (1986) Proposal to recognize the family Aeromonadaceae fam. nov. *International Journal of Systematic Bacteriology* **36**, 473–477.

Comps, M., Raymond, J.C. and Plassiart, G.N. (1996) *Rickettsia*-like organism infecting juvenile sea-bass *Dicentrarchus labrax*. *Bulletin of the European Association of Fish Pathologists* **16**, 30–33.

Conchas, R.F., Lemos, M.L., Barja, J.L. and Toranzo, A.E. (1991) Distribution of plasmid- and chromosome-mediated iron uptake systems in *Vibrio anguillarum* strains of different origins. *Applied and Environmental Microbiology* **57**, 2956–2962.

Cone, D.K. (1982) A *Lactobacillus* sp. from diseased female rainbow trout, *Salmo gairdneri* Richardson, in Newfoundland, Canada. *Journal of Fish Diseases* **5**, 479–485.

Conroy, D.A. (1961) Estudio in vitro de la acción de la kanamicina sobre bacterias patogenas para los peces. *Microbiología Española* **14**, 147–155.

Conroy, D.A. (1964) Notes on the incidence of piscine tuberculosis in Argentina. *Progressive Fish Culturist* **26**, 89–90.

Conroy, D.A. (1966) Observaciones sobre casos espontáneos de tuberculosis ictica. *Microbiología Española* **19**, 93–113.

Conroy, D.A. (1986) Agents: Bacteria. In: Kinne, O. (ed.), *Diseases of Marine Animals*, Vol. IV. Helgoland, Germany, Biologische Anstalt.

Conroy, D.A. and Valdez, I.E. (1962) Un casos de tuberculosis en peces tropicales. *Revista Latinoamericana de Microbiología* **5**, 9–16.

Cook, D.W. and Lofton, S.R. (1975) Pathogenicity studies with a *Streptococcus* sp. isolated from fishes in an Alabama–Florida fish kill. *Transactions of the American Fisheries Society* **104**, 286–288.

Cook, M. and Lynch, W.H. (1999) Sensitive nested reverse transcriptase PCR assay to detect viable cells of the fish pathogern *Renibacterium salmoninarum* in Atlantic salmon (*Salmo salar* L.). *Applied and Environmental Microbiology* **65**, 3042–3047.

Cook, T.M. and Gemski, P. (1982) Studies of plasmids in the fish pathogen, *Yersinia ruckeri*. *Proceedings of the International Congress of Microbiologists* **13**, 97.

Coquet, L., Cosette, P., Quillet, L., Petit, F., Junter, G.-A. and Jouenne, T. (2002) Occurrence and phenotypic characterization of *Yersinia ruckeri* strains with biofilm-forming capacity in a rainbow trout farm. *Applied and Environmental Microbiology* **68**, 470–475.

Corbeil, S., McColl, K.A. and Crane, M.St.J. (2003) Development of a Taqman quantitative PCR assay for the identification of *Piscirickettsia salmonis*. *Bulletin of the European Association of Fish Pathologists* **23**, 95–101.

Corbeil, S., Hyatt, A.D. and Crane, M.St.J. (2005) Characterisation of an emerging *Rickettsia*-like organism in Tasmanian farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* **64**, 37–44.

Cornick, J.W., Chudyk, R.V. and McDermott, L.A. (1969) Habitat and viability studies on *Aeromonas salmonicida*, causative agent of furunculosis. *Progressive Fish Culturist* **31**, 90–93.

Cossarini-Dunier, M. (1985) Indirect enzyme-linked immunosorbent assay (ELISA) to titrate rainbow trout serum antibodies against two pathogens: *Yersinia ruckeri* and Egtved virus. *Aquaculture* **49**, *197–208*.

Cossarini-Dunier, M. (1986) Protection against enteric redmouth disease in rainbow trout, *Salmo gairdneri* Richardson, after vaccination with *Yersinia ruckeri* bacterin. *Journal of Fish Diseases* **9**, 27–33.

Couso, N., Castro, R., Magariños, B., Obach, A. and Lamas, J. (2003) Effect of oral administration of glucans on the resistance of gilthead seabream to pasteurellosis. *Aquaculture* **219**, 99–109.

Cowan, S.T. (1974) *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge, U.K., Cambridge University Press.

Coyne, R., Smith, P., Dalsgaard, I., Nilsen, H., Kongshaug, H., Bergh, Ø. and Samuelsen, O. (2006) Winter ulcer disease of post-smolt Atlantic salmon: An unsuitable case for treatment? *Aquaculture* **253**, 171–178.

Crosa, J.H. (1980) A plasmid associated with virulence in the marine fish pathogen *Vibrio anguillarum* specifies an iron-sequestering system. *Nature (London)* **283**, 566–568.

Crosa, J.H. and Hodges, L.L. (1981) Outer membrane proteins induced under conditions of iron limitation in the marine fish pathogen *Vibrio anguillarum*. *Infection and Immunity* **31**, 223–227.

Crosa, J.H., Schiewe, M.H. and Falkow, S. (1977) Evidence for plasmid contribution to the virulence of the fish pathogen *Vibrio anguillarum*. *Infection and Immunity* **18**, 509–513.

Crosa, J.H., Hodges, L.L. and Schiewe, M.H. (1980) Curing of a plasmid is correlated with an attenuation of virulence in the marine fish pathogen *Vibrio anguillarum*. *Infection and Immunity* **27**, 897–902.

Crosa, J.H., Walter, M.A. and Potter, S.A. (1983) The genetics of plasmid-mediated virulence in the marine fish pathogen *Vibrio anguillarum*. In: Crosa, J.H. (ed.), *Bacteria and Viral Diseases of Fish, Molecular Studies*. Seattle, University of Washington, pp. 21–30.

Crosbie, P.B.B. and Nowak, B.F. (2004) Immune responses of barramundi, *Lates calcarifer* (Bloch), after administration of an experimental *Vibrio harveyi* bacterin by intraperitoneal injection, anal intubation and immersion. *Journal of Fish Diseases* **27**, 623–632.

Croxatto, A., Pride, J., Hardman, A., Williams, P., Cámara, M. and Milton, D.L. (2004) A distinctive dual-channel quorum-sensing system operates in *Vibrio anguillarum*. *Molecular Microbiology* **52**, 1677–1689.

Croy, T.R. and Amend, D.F. (1977) Immunization of sockeye salmon (*Oncorhynchus nerka*) against vibriosis using the hyperosmotic infiltration technique. *Aquaculture* **12**, 317–325.

Crumlish, M., Dung, T.T., Turnbull, J.F., Ngoc, N.T.N. and Ferguson, H.W. (2002) Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. *Journal of Fish Diseases* **25**, 733–736.

Crump, E.M., Perry, M.B., Clouthier, S.C. and Kay, W.W. (2001) Antigenic characterization of the fish pathogen *Flavobacterium psychrophilum*. *Applied and Environmental Microbiology* **67**, 750–759.

Crump, E.M., Burian, J., Allen, P.D. and Kay, W.W. (2005) Identification and expression of a host-recognized antigen, FspA, from *Flavobacterium psychrophilum*. *Microbiology* **151**, 3127–3135.

Cruz, J.M., Saraiva, A., Eiras, J.C., Branco, R. and Sousa, J.C. (1986) An outbreak of *Plesiomonas shigelloides* in farmed rainbow trout, *Salmo gairdneri* Richardson, in Portugal. *Bulletin of the European Association of Fish Pathologists* **6**, 20–22.

Csaba, Gy., Körmendy, B. and Békési, L. (1981a) Observations on the causative agent of carp erythrodermatitis in Hungary. *Proceedings of an International Seminar on Fish, Pathogens and Environment in European Polyculture, Szarvas, Hungary*, pp. 95–110.

Csaba, Gy., Prigli, M., Békési, L., Kovács-Gayer, E., Bajmócy, E. and Fazekas, B. (1981b) Septicaemia in silver carp (*Hypophthalmichthys molitrix*, Val.) and bighead (*Aristichthys nobilis* Rich.) caused by *Pseudomonas fluorescens*. In: Oláh, J., Molnár, K. and Jeney, S. (eds), *Fish, Pathogens and Environment in European Polyculture*. Szarvas, Hungary, F. Muller (Fisheries Research Institute), pp. 111–123.

Cumberbatch, N., Gurwith, M.J., Langston, C., Sack, R.B. and Brunton, J.L. (1979) Cytotoxic enterotoxin produced by *Aeromonas hydrophila*: Relationship of toxigenic isolates to diarrheal disease. *Infection and Immunity* **23**, 829–837.

Currás, M., Magariños, B., Toranzo, A.E. and Romalde, J.L. (2002) Dormancy as a survival strategy of the fish pathogen *Streptococcus parauberis* in the marine environment. *Diseases of Aquatic Organisms* **52**, 129–136.

Cusack, R.R., Groman, D.B. and Jones, S.R.M. (2002) Rickettsial infection in farmed Atlantic salmon in eastern Canada. *Canadian Veterinary Journal—Revue Veterinaire Canadienne* **43**, 435–440.

Cvitanich, J.D. (1994) Improvements in the direct fluorescent antibody technique for the detection, identification, and quantification of *Renibacterium salmoninarum* in salmonid kidney smears. *Journal of Aquatic Animal Health* **6**, 1–12.

Cvitanich, J.D. (2004) *Renibacterium salmoninarum* bar forms: Characterization, occurrence, and evidence of a host response to a *R. salmoninarum* infection. *Journal of Fish Diseases* **27**, 193–211.

Cvitanich, J., Garate, O. and Smith, C.E. (1991) The isolation of a *Rickettsia*-like organism causing disease and mortality in Chilean salmonids and its confirmation by Koch's postulate. *Journal of Fish Diseases* **14**, 121–145.

Dacanay, A., Knickle, L., Solanky, K.S., Boyd, J.M., Walter, J.A., Brown, L.L., Johnson, S.C. and Reith, M. (2006) Contribution of the type III secretion system (TTSS) to virulence of *Aeromonas salmonicida* subsp. *salmonicida*. *Microbiology* **152**, 1847–1856.

Dalmo, R.A. and Seljelid, R. (1995) The immunomodulatory effect of LPS, laminaran and sulphated laminaran [β(1,3)–D–glucan] on Atlantic salmon, *Salmo salar* L., macrophages *in vitro*. *Journal of Fish Diseases* **18**, 175–185.

Dalmo, R.A., Ingebrigtsen, K., Bøgwald, J., Horsberg, T.E. and Seljelid, R. (1995) Accumulation of immunomodulatory laminaran (β(1,3)-D-glucan) in the spleen and kidney of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **18**, 545–553.

Dalsgaard, I., From, J. and Hørlyck, V. (1984) First observation of *Yersinia ruckeri* in Denmark. *Bulletin of the European Association of Fish Pathologists* **4**, 10.

Dalsgaard, I., Nielsen, B. and Larsen, J.L. (1994) Characterization of *Aeromonas salmonicida* subsp. *salmonicida*: A comparative study of strains of different geographic origin. *Journal of Applied Bacteriology* **77**, 21–30.

Dalsgaard, I., Gudmundsdóttir, B.K., Helgason, S., Høie, S., Thoresen, O.F., Wichardt, U.-P. and Wiklund, T. (1998) Identification of atypical *Aeromonas salmonicida*: Inter-laboratory evaluation and harmonization of methods. *Journal of Applied Microbiology* **84**, 999–1006.

Dalsgaard, I., Høi, L., Siebeling, R.J. and Dalsgaard, A. (1999) Indole-positive *Vibrio vulnificus* isolated from disease outbreaks on a Danish eel farm. *Diseases of Aquatic Organisms* **35**, 187–194.

Daly, J.G. and Stevenson, R.M.W. (1985a) Charcoal agar, a new growth medium for the fish disease bacterium *Renibacterium salmoninarum*. *Applied and Environmental Microbiology* **50**, 868–871.

Daly, J.G. and Stevenson, R.M.W. (1985b) Importance of culturing several organs to detect *Aeromonas salmonicida* in salmonid fish. *Transactions of the American Fisheries Society* 114, 909–910.

Daly, J.G. and Stevenson, R.M.W. (1987) Hydrophobic and haemagglutinating properties of *Renibacterium salmoninarum*. *Journal of General Microbiology* **133**, 3575–3580.

Daly, J.G. and Stevenson, R.M.W. (1989) Agglutination of salmonid spermatozoa by *Renibacterium salmoninarum*. *Fish Health News*.

Daly, J.G. and Stevenson, R.M.W. (1990) Characterization of the *Renibacterium salmoninarum* haemagglutinins. *Journal of General Microbiology* **136**, 949–953.

Daly, J.G., Kew, A.K., Moore, A.R. and Olivier, G. (1996) The cell surface of *Aeromonas salmonicida* determines *in vitro* survival in cultivated brook trout (*Salvelinus fontinalis*) peritoneal macrophages. *Microbial Pathogenesis* **21**, 447–461.

Daly, J.G., Griffiths, S.G., Kew, A.K., Moore, A.R. and Olivier, G. (2001) Characterization of attenuated *Renibacterium salmoninarum* strains and their use as live vaccines. *Diseases of Aquatic Organisms* **44**, 121–126.

Danley, M.L., Goodwin, A.E. and Killian, H.S. (1999) Epizootics of farm-raised channel catfish, *Ictalurus punctatus* (Rafinesque), caused by the enteric redmouth bacterium *Yersinia ruckeri*. *Journal of Fish Diseases* **22**, 451–456.

Darwish, A.M. and Hobbs, M.S. (2005) Laboratory efficacy of amoxicillin for the control of *Streptoococcus iniae* infection in blue tilapia. *Journal of Aquatic Animal Health* **17**, 197–202.

Darwish, A.M. and Ismaiel, A.A. (2003) Laboratory efficacy of amoxicillin for the control of *Streptococccus iniae* infection in sunshine bass. *Journal of Aquatic Animal Health* **15**, 209–214.

Darwish, A.M., Newton, J.C. and Plumb, J.A. (2001) Effect of incubation teperature and salinity on expression of the outer membrane protein profile of *Edwardsiella tarda*. *Journal of Aquatic Animal Health* **13**, 269–275.

Darwish, A.M., Rawles, S.D. and Griffin, B.R. (2002) Laboratory efficacy of oxytetracycline or the control of *Streptococcus iniae* infection in blue tilapia. *Journal of Aquatic Animal Health* **14**, 184–190.

Daskalov, H., Stobie, M. and Austin, B. (1998) *Klebsiella pneumoniae*: A pathogen of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Bulletin of the European Association of Fish Pathologists* **18**, 26–28.

Daskalov, H., Austin, D.A. and Austin, B. (1999) An improved growth medium for *Flavobacterium psychrophilum*. *Letters in Applied Microbiology* **28**, 297–299.

Daskalov, H., Robertson, P.A.W. and Austin, B. (2000) Influence of oxidised lipids in diets on the development of rainbow trout fry syndrome. *Journal of Fish Diseases* **23**, 7–14.

Davey, M.L., Hancock, R.E.W. and Mutharia, L.M. (1998) Influence of culture conditions on expression of the 40-kilodalton porin protein of *Vibrio anguillarum* serotype O2. *Applied and Environmental Microbiology* **64**, 138–146.

David, H. (1927) Über eine durch cholera ähnliche Vibrionen hervorgerufene Fischseuche. *Zentralblatt für Bakteriologie und Parasitenkunde*, Abteilung 1, Originale 102, 46–60.

Davies, J. A. (1969) Isolation and identification of clostridia from North Sea sediments. *Journal of Applied Bacteriology* **32**, 164–169.

Davies, R.L. (1990) O-serotyping of *Yersinia ruckeri* with special emphasis on European isolates. *Veterinary Microbiology* **22**, 299–307.

Davies, R.L. (1991) Outer membrane protein profiles of *Yersinia ruckeri*. *Veterinary Microbiology* **26**, 125–140.

Davis, H.S. (1922) A new bacterial disease of freshwater fishes. *United States Bureau of Fisheries Bulletin* **38**, 261–280.

Davis, H.S. (1929) Care and diseases of trout. *United States Department of Commerce, Bureau of Fisheries Document* **1061**, 155.

Davis, H.S. (1946) Care and diseases of trout. *Research Report, United States Fisheries and Wildlife Service* **12**, 98.

Deane, E.E, and Woo, N.Y.S. (2005) Evidence of disruption of Na^+–K^+–ATPase and hsp70 during vibriosis of sea bream, *Sparus (= Rhabdosargus) sarba* Forsskål. *Journal of Fish Diseases* **28**, 239–251.

Deane, E.E., Li, J. and Woo, N.Y.S. (2004) Modulated heat shock protein expression during pathogenic *Vibrio alginolyticus* stress of sea bream. *Diseases of Aquatic Organisms* **62**, 205–215.

Dear, G. (1985) Studies on the biology, metabolism and pathogenicity of *Pseudomonas anguilliseptica*. Ph.D thesis, Heriot-Watt University.

Dec, C., Angelidus, P. and Baudin-Laurençin, F. (1990) Effects of oral vaccination against vibriosis in turbot, *Scophthalmus maximus* (L.), and sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* **13**, 369–376.

Decew, M.G. (1972) Antibiotic toxicity, efficacy and teratogenicity in adult spring chinook salmon (*Oncorhynchus tschawytscha*). *Journal of the Fisheries Research Board of Canada* **29**, 1513–1517.

Decostere, A., Haesebrouck, F., Turnbull, J.F. and Charlier, G. (1999a) Influence of water quality and temperature on adhesion of high and low virulence *Flavobacterium columnare* strains to isolated gill arches. *Journal of Fish Diseases* **22**, 1–11.

Decostere, A., Haesebrouck, F., Van Driessche, E., Charlier, G. and Ducatelle, R. (1999b) Characterization of the adhesion of *Flavobactium columnare* (*Flexibacter columnaris*) to gill tissue. *Journal of Fish Diseases* **22**, 465–474.

Decostere, A., D'Haese, E., Lammens, M., Nelis, H. and Haesebrouck, F. (2001) *In vivo* study of phagocytosis, intracellular survival and multiplication of *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum) spleen phagocytes. *Journal of Fish Diseases* **24**, 481–487.

Deere, D., Porter, J., Pickup, R.W. and Edwards, C. (1996a) Survival of cells and DNA of *Aeromonas salmonicida* released into aquatic microcosms. *Journal of Applied Bacteriology* **81**, 309–318.

Deere, D., Porter, J., Pickup, R. and Edwards, C. (1996b) Direct analysis of starved *Aeromonas salmonicida*. *Journal of Fish Diseases* **19**, 459–467.

De Grandis, S.A. and Stevenson, R.M.W. (1985) Antimicrobial susceptibility patterns and R plasmid-mediated resistance of the fish pathogen *Yersinia ruckeri*. *Antimicrobial Agents and Chemotherapy* **27**, 938–942.

De Grandis, S.A., Krell, P.J., Flett, D.E. and Stevenson, R.M.W. (1988) Deoxyribonucleic acid relatedness of serovars of *Yersinia ruckeri*, the enteric redmouth bacterium. *International Journal of Systematic Bacteriology* **38**, 49–55.

De La Cruz, M. and Muroga, K. (1989) The effects of *Vibrio anguillarum* extracellular products on Japanese eels. *Aquaculture* **80**, 201–210.

Del Cerro, A., Mendoza, M.C. and Guijarro, J.A. (2002) Usefulness of a TaqMan-based polymerase chain reaction assay for the detection of the fish pathogen *Flavobacterium psychrophilum*. *Journal of Applied Microbioogy* **93**, 149–156.

Del Cerro, Al., Marquez, I. and Guijarro, J.A. (2002a) Simultaneous detection of *Aeromonas salmonicida*, *Flavobacterium psychrophilum* and *Yersinia ruckeri*, three major fish pathogens, by multiplex PCR. *Applied and Environmental Microbiology* **68**, 5177–5180.

Del Corral, F., Shotts, E.B. and Brown, J. (1990) Adherence, haemagglutination and cell surface characteristics of motile aeromonads virulent for fish. *Journal of Fish Diseases* **13**, 255–268.

De Meuron, P.-A. and Peduzzi, R. (1979) Caractérisation de souches du genre *Aeromonas* isolées chez de poissons d'eau douce et quelques reptiles. *Zentralblatt für Veterinaermedizin Reihe* **B26**, 153–167.

Denkin, S.M. and Nelson, D.R. (2004) Regulation of *Vibrio anguillarum* empA metalloprotease expression and its role in virulence. *Applied and Environmental Microbiology* **70**, 4193–4204.

Densmore, C.L., Smith, S.A. and Holladay, S.D. (1998) *In vitro* effects of the extracellular protein of *Renibacterium salmoninarum* of phagocyte function in brook trout (*Salvelinus fontinalis*). *Veterinary Immunology and Immunopathology* **62**, 349–357.

De Paola, A., Flynn, P.A., McPhearson, R.M. and Levy, S.B. (1988) Phenotypic and genotypic characterization of tetracycline- and oxytetracycline-resistant *Aeromonas hydrophila* from cultured channel catfish (*Ictalurus punctatus*) and their environment. *Applied and Environmental Microbiology* **54**, 1861–1863.

De Paola, A., Capers, G.M. and Alexander, D. (1994) Densities of *Vibrio vulnificus* in the intestines of fish from the U.S. Gulf-Coast. *Applied and Environmental Microbiology* **60**, 984988.

Dethlefsen, V. and Watermann, B. (1980) Epidermal papilloma of North Sea dab, *Limanda limanda*: Histology, epidemiology and relation to dumping from TiO_2 industry. *ICES Special Meeting on Diseases of Commercially Important Marine Fish and Shellfish* **8**, 1–30.

Dethlefsen, V., Watermann, B. and Hoppenheit, M. (1987) Diseases of North Sea dab (*Limanda limanda* L.) in relation to biological and chemical parameters. *Archiv für Fishereiwissenschaft* **37**, 101–237.

Dethlefsen, V., Lang, T. and Koves, P. (2000) Regional patterns in prevalence of principal external diseases of dab *Limanda limanda* in the North Sea and adjacent areas 1992–1997. *Diseases of Aquatic Organisms* **42**, 119–132.

Di Lorenzo, M., Stork, M., Tolmasky, M.E., Actis, L.A., Farrell, D., Welch, T.J., Crosa, L.M., Wertheimer, A.M., Chen, Q., Salinas, P. *et al.* (2003) Complete sequence of virulence

plasmid pJM1 from the marine fish pathogen *Vibrio anguillarum* strain 775. *Journal of Bacteriology* **185**, 5822–5830.

Díaz-Rosales, P., Chabrillón, M., Arijo, S., Martinez-Manzanares, E., Moriñigo, M.A. and Balebona, M.C. (2006) Superoxide dismutase and catalase activities in *Photobacterium damselae* subsp. *piscicida*. *Journal of Fish Diseases* **29**, 355–364.

Diggles, B.K., Carson, J., Hine, P.M., Hickman, R.W. and Tait, M.J. (2000) *Vibrio* species associated with mortalities in hatchery-reared turbot (*Colistium nudipinnis*) and brill (*C. guntheri*) in New Zealand. *Aquaculture* **183**, 1–12.

do Vale, A., Ellis, A.E. and Silva, M.T. (2001) Electron microscopic evidence that expression of capsular polysaccharide by *Photobacterium damselae* subsp. *piscicida* is dependent on iron availability and growth phase. *Diseases of Aquatic Organisms* **44**, 237–240.

do Vale, A., Magariños, B., Romalde, J.L., Lemos, M.L., Ellis, A.E. and Toranzo, A.E. (2002) Binding of haemin by the fish pathogen *Photobacterium damselae* subsp. *piscicida*. *Diseases of Aquatic Organisms* **48**, 109–115.

do Vale, A., Silva, M.T., dos Santos, N.M.S., Nascimento, D.S., Reis-Rodriques, P., Costa-Ramos, C., Ellis, A.E. and Azevedo, J.E. (2005) AIP56, a novel plasmid-encoded virulence factor of *Photobacterium damselae* subsp. *piscicida* with apoptogenic activity against sea bass macrophages and neutrophils. *Molecular Microbiology* **58**, 1025–1038.

Dobson, S.J. and Franzmann, P.D. (1996) Unification of the genera *Deleya* (Baumann et al 1983), *Halomonas* (Vreeland *et al.* 1980) and *Halovibrio* (Robinson and Gibbons, 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family Halomonadaceae. *International Journal of Systematic Bacteriology* **46**, 550–558.

Dodson, S.V., Maurer, J.J. and Shotts, E.C. (1999) Biochemical and molecular typing of *Strepococcus iniae* isolated from fish and human cases. *Journal of Fish Diseases* **22**, 331–336.

Doetsch, R.N. (1981) 3. Determinative method of light microscopy. In: Gerhardt, P. (ed.), *Manual of Methods for General Bacteriology*. Washington, D.C., American Society of Microbiology, pp. 21–23.

Doménech, A., Fernández-Garayzábal, J.F., Pascual, C., García, J.A., Cutuli, M.T., Moreno, M.A., Collins, M.D. and Dominguez, L. (1996) Streptococcosis in cultured turbot, *Scophthalmus maximus* (L.), associated with *Streptococcus parauberis*. *Journal of Fish Diseases* **19**, 33–38.

Doménech, A., Fernández-Garayzábal, J.F., Lawson, P., García, J.A., Cutuli, M.T., Blanco, M., Gibello, A., Moreno, M.A., Collins, M.D. and Domínguez, L. (1997) Winter disease outbreak in sea-bream (*Sparus aurata*) associated with *Pseudomonas anguilliseptica* infection. *Aquaculture* **156**, 317–326.

Doménech, A., Fernández-Garayzábal, J.F., García, J.A., Cutuli, M.T., Blanco, M., Gibello, A., Moreno, M.A. and Domínguez, L. (1999) Association of *Pseudomonas anguilliseptica* infection with "winter disease" in sea bream, *Sparus aurata* L. *Journal of Fish Diseases* **22**, 69–71.

Donlon, J., McGettigan, S., O'Brien, P. and O'Carra, P. (1983) Reappraisal of the nature of the pigment produced by *Aeromonas salmonicida*. *FEMS Microbiology Letters* **19**, 285–290.

Donta, S.T. and Haddow, A.D. (1978) Cytotoxic activity of *Aeromonas hydrophila*. *Infection and Immunity* **21**, 989–993.

Dooley, J.S.G. and Trust, T.J. (1988) Surface protein composition of *Aeromonas hydrophila* strains virulent for fish: Identification of a surface array protein. *Journal of Bacteriology* **170**, 499–506.

Dooley, J.S.G., Lallier, R., Shaw, D.H. and Trust, T.J. (1985) Electrophoretic and immunochemical analyses of the lipopolysaccharides from various strains of *Aeromonas hydrophila*. *Journal of Bacteriology* **164**, 262–269.

Dooley, J.S.G., Lallier, R. and Trust, T.J. (1986) Surface antigens of virulent strains of *Aeromonas hydrophila*. *Veterinary Immunology and Immunopathology* **12**, 339–344.

Dooley, J.S.G., McCubbin, W.D., Kay, C.M. and Trust, T.J. (1988) Isolation and biochemical characterization of the S-layer protein from a pathogenic *Aeromonas hydrophila* strain. *Journal of Bacteriology* **170**, 2631–2638.

Dooley, J.S.G., Engelhardt, H., Baumeister, W., Kay, W.W. and Trust, T.J. (1989) Three-dimensional structure of an open form of the surface layer from the fish pathogen *Aeromonas salmonicida*. *Journal of Bacteriology* **171**, 190–197.

Dopazo, C.P., Lemos, M.L., Lodeiros, C., Bolinches, J., Barja, J.L. and Toranzo, A.E. (1988) Inhibitory activity of antibiotic-producing marine bacteria against fish pathogens. *Journal of Applied Bacteriology* **65**, 97–101.

Dos Santos, N.M.S., do Vale, A., Sousa, M.J. and Silva, M.T. (2002) Mycobacterial infection in farmed turbot *Scophthalmus maximus*. *Diseases in Aquatic Organisms* **52**, 87–91.

do Vale, A., Marques, F. and Silva, M.T. (2003) Apoptosis of sea bass (*Dicentrarchus labrax* L.) neutrophils and macrophages induced by experimental infection with *Photobacterium damselae* subsp. *piscicida*. *Fish & Shellfish Immunology* **15**, 129–144.

Dror, M., Sinyakov, M.S., Okun, E., Dym, M., Sredni, B. and Avtalion, R.R. (2006) Experimental handling stress as infection-facilitating factor for the goldfish ulcerative disease. *Veterinary Immunology and Immunopathology* **109**, 279–287.

Drouin de Bouville, R. de (1907) Les maladies des poissons d'eau donce d'Europe. *Annales des Sciences Agronomique* **1**, 120–250.

Dubois-Darnaudpeys, A. (1977a) Epidémiologie de la furonculose des salmonides. II. Etude expérimentale de divers facteurs microbiotiques de l'environment. *Bulletin Français de Pisciculture* **49**, 128–133.

Dubois-Darnaudpeys, A. (1977b) Epidémiologie de la furonculose des salmonides. III. Ecologie de *Aeromonas salmonicida* proposition d'un modèle epidémiologique. *Bulletin Français de Pisciculture* **50**, 21–32.

Dudley, D.J., Guentzel, M.N., Ibarra, M.J., Moore, B.E. and Sagik, B.P. (1980) Enumeration of potentially pathogenic bacteria from sewage sludges. *Applied and Environmental Microbiology* **39**, 118–126.

Duff, D.C.B. (1937) Dissociation in *Bacillus salmonicida*, with special reference to the appearance of the G form of culture. *Journal of Bacteriology* **34**, 49–67.

Duff, D.C.B. (1939) Some serological relationships of the S, R and G phases of *Bacillus salmonicida*. *Journal of Bacteriology* **38**, 91–103.

Duff, D.C.B. (1942) The oral immunization of trout against *Bacterium salmonicida*. *Journal of Immunology* **44**, 87–94.

Duff, D.C.B. and Stewart, B. (1933) Studies on furunculosis of fish in British Columbia. *Contributions of Canadian Biology and Fisheries* **8**, No. 8, Series A, General Number 35, 103.

Duguid, J.P. and Old, D.C. (1980) Adhesive properties of Enterobacteriaceae. In: Beachey, E.H. (ed.), *Receptors and Recognition*, Series B, Vol. 6. London, Chapman & Hall, pp. 185–217.

Duis, K., Hammer, C., Beveridge, M.C.M., Inglis, V. and Braum, E. (1995) Delivery of quinolone antibacterials to turbot, *Scophthalmus maximus* (L.), via bioencapsulation: Quantification and efficacy trial. *Journal of Fish Diseases* **18**, 229–238.

Dulin, M.P. (1979) A review of tuberculosis (mycobacteriosis) in fish. *Veterinary Medicine/Small Animal Clinician* May 1979, 735–737.

Dulin, M.P., Huddleston, T.R., Larson, R. and Klontz, G. (1976) Enteric redmouth disease. *Forest Wildlife Range Experimental Station, University of Idaho Bulletin* **8**, 1–15.

Dumetz, F., Duchaud, E., LaPatra, S.E., Le Marrec, C., Claverol, S., Urdaci, M.-C. and Le Hénaff, M. (2006) A protective immune response is generated in rainbow trout by an OmpH-like surface antigen (P18) of *Flavobacterium psychrophilum*. *Applied and Environmental Microbiology* **72**, 4845–4852.

Duncan (1932) Cited by McCraw (1952).

Durbin, M., McIntosh, D., Smith, P.D., Wardle, R. and Austin, B. (1999) Immunization against furunculosis in rainbow trout with iron-regulated outer membrane protein vaccines: Relative efficacy of immersion, oral and injection delivery. *Journal of Aquatic Animal Health* **11**, 68–75.

Duremdez, R., Al-Marzouk, A., Qasem, J.A., Al-Harbi, A. and Gharabally, H. (2004) Isolation of *Streptococcus agalactiae* from cultured silver pomfet, *Pampus argenteus* (Euphrasen) in Kuwait. *Journal of Fish Diseases* **27**, 307–310.

Durve, V.S. and Lovell, T.T. (1982) Vitamin C and disease resistance in channel catfish (*Ictalurus punctatus*). *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 948–951.

Earlix, D., Plumb, J.A. and Rogers, W.A. (1996) Isolation of *Edwardsiella ictaluri* from channel catfish by tissue homogenization, filtration and enzyme linked immunosorbent assay. *Diseases of Aquatic Organisms* **27**, 19–24.

Earp, B.J. (1950) Kidney disease in young salmon. M.S. thesis, University of Washington.

Earp, B.J., Ellis, C.H. and Ordal, E.J. (1953) Kidney disease in young salmon. *Washington, Department of Fisheries, Special Report* **1**, 1–74.

Ebanks, E.O., Goguen, M., McKinnon, S., Pinto, D.M. and Ross, N.W. (2005) Identification of the major outer membrane proteins of *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* **68**, 29–38.

Ebanks, R.O., Knickle, L.C., Goguen, M., Boyd, J.M., Pinto, D.M., Reith, M. and Ross, N.W. (2006) Expression of and secretion through the *Aeromonas salmonicida* type III secretion system. *Microbiology* **152**, 1275–1286.

Eckman, E., Åkerman, G., Balk, L. and Norrgren, L. (2003) Nanoinjection as a tool to mimic vertical transmission of *Flavobacterium psychrophilum* in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **55**, 93–99.

Eddy, B.P. (1960) Cephalotrichous, fermentative Gram-negative bacteria: The genus *Aeromonas*. *Journal of Applied Bacteriology* **23**, 216–248.

Eddy, B.P. (1962) Further studies on *Aeromonas*. I. Additional strains and supplementary biochemical tests. *Journal of Applied Bacteriology* **25**, 137–146.

Eddy, B.P. and Carpenter, K.P. (1964) Further studies on *Aeromonas*. II. Taxonomy of *Aeromonas* and C27 strains. *Journal of Applied Bacteriology* **27**, 96–100.

Effendi, I. and Austin, B. (1991) Survival of the fish pathogen *Aeromonas salmonicida* in seawater. *FEMS Microbiology Letters* **84**, 103–106.

Effendi, I. and Austin, B. (1993) A rapid method for the determination of viability of *Aeromonas salmonicida* in seawater. *Bulletin of the European Association of Fish Pathologists* **13**, 171–173.

Effendi, I. and Austin, B. (1994) Survival of the fish pathogen *Aeromonas salmonicida* in the marine environment. *Journal of Fish Diseases* **17**, 375–385.

Effendi, I. and Austin, B. (1995a) Dormant/unculturable cells of the fish pathogen *Aeromonas salmonicida*. *Microbial Ecology* **30**, 183–192.

Effendi, I. and Austin, B. (1995b) Uptake of *Aeromonas salmonicida* by Atlantic salmon (*Salmo salar* L.). *Bulletin of the European Association of Fish Pathologists* **15**, 115–118.

Eggset, G., Mortensen, A., Johansen, L.-H. and Sommer, A.-I. (1997) Susceptibility to furunculosis, cold water vibriosis, and infectious pancreatic necrosis (IPN) in post-smolt Atlantic salmon (*Salmo salar* L.) as a function of smolt status by seawater transfer. *Aquaculture* **158**, 179–191.

Egidius, E. and Andersen, K. (1978) Host-specific pathogenicity of strains of *Vibrio anguillarum* isolated from rainbow trout *Salmo gairdneri* Richardson and saithe *Pollachius virens* (L.). *Journal of Fish Diseases* **1**, 45–50.

Egidius, E. and Andersen, K. (1979) Bath immunization: A practical and non-stressing method of vaccinating farmed sea rainbow trout *Salmo gairdneri* Richardson against vibriosis. *Journal of Fish Diseases* **2**, 405–410.

Egidius, E., Andersen, K., Causen, E. and Raa, J. (1981) Cold water vibriosis or "Hitra disease" in Norwegian salmonid farming. *Journal of Fish Diseases* **4**, 353–354.

Egidius, E., Wiik, R., Andersen, K., Hoff, K.A. and Hjeltnes, B. (1986) *Vibrio salmonicida* sp. nov., a new fish pathogen. *International Journal of Systematic Bacteriology* **36**, 518–520.

Egusa, S. (1983) Disease problems in Japanese yellowtail, *Seriola quinqueradiata*, culture: A review. In: Stewart, J.E. (ed.), *Diseases of Commercially Important Marine Fish and Shellfish*. Conseil International pour l'Exploration de la Mer, Copenhagen, pp. 10–18.

Ehlinger, N.F. (1964) Selective breeding of trout for resistance to furunculosis. *New York Fish and Game Journal* **11**, 78–90.

Ehlinger, N.F. (1977) Selective breeding of trout for resistance to furunculosis. *New York Fish and Game Journal* **24**, 25–36.

Eklund, M. W., Peterson, M. E., Poysky, F. T., Peck, L. W. and Conrad, J. F (1982) Botulism in juvenile coho salmon (*Oncorhynchus kisutch*) in the United States. *Aquaculture* **27**, 1–11.

Ekman, E., Börjeson, H. and Johanson, N. (1999) *Flavobacterium psychrophilum* in Baltic salmon *Salmo salar* brood fish and their offspring. *Diseases of Aquatic Organisms* **37**, 159–163.

Ekman, E., Åkerman, G., Balk, L. and Norrgren, L. (2004) Impact of PCB on resistance to *Flavobacterium psychrophilum* after experimental infection of rainbow trout *Oncorhynchus mykiss* eggs by nanoinjection. *Diseases of Aquatic Organisms* **60**, 31–39.

El Morabit, A., García-Márquez, S. and Santos, Y. (2004) Is sea lamprey a potential source of infection with *Aeromonas salmonicida* for wild and farmed fish? *Bulletin of the European Association of Fish Pathologists* **24**, 100–103.

Eldar, A. and Ghittino, C. (1999) *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: Similar, but different diseases. *Disease of Aquatic Organisms* **36**, 227–231.

Eldar, A., Bejerano, Y. and Bercovier, H. (1994) *Streptococcus shiloi* and *Streptococcus difficile*: Two new streptococcal species causing a meningoencephalitis in fish. *Current Microbiology* **28**, 139–143.

Eldar, A., Frelier, P.F., Assenta, L., Varner, P.W., Lawhon, S. and Bercovier, H. (1995a) *Streptococcus shiloi*, the name for an agent causing septicemia infection in fish, is a junior synonym of *Streptococcus iniae*. *International Journal of Systematic Bacteriology* **45**, 840–842.

Eldar, A., Bejerano, Y., Livoff, A., Horovitcz, A. and Bercovier, H. (1995b) Experimental streptococcal meningo-encephalitis in cultured fish. *Veterinary Microbiology* **43**, 33–40.

Eldar, A., Shapiro, O., Bejerano, Y. and Bercovier, H. (1995c) Vaccination with whole-cell vaccine and bacterial protein extract protects tilapia against *Streptococcus difficile* meningoencephalitis. *Vaccine* **13**, 867–870.

Eldar, A., Ghittino, C., Asanta, L., Bozzetta, E., Goria, M., Prearo, M. and Bercovier, H. (1996) *Enterococcus seriolicida* is a junior synonym of *Lactococcus garvieae*, a causative agent of septicemia and meningoencephalitis in fish. *Current Microbiology* **32**, 85–88.

Elkamel, A.A., Hawke, J.P., Henk, W.G. and Thune, R.L. (2003) *Photobacterium damselae* subsp. *piscicida* is capable of replicating in hybrid striped bass macrophages. *Journal of Aquattic Animal Health* **15**, 175–183.

Elliott, D.G. and McKubben, C.L. (1997) Comparison of two fluorescent antibody techniques (FATs) for detection and quantification of *Renibacterium salmoninarum* coelomic fluid of spawning chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Oganisms* **30**, 37–43.

Elliott, D.G. and Pascho, R.J. (2001) Evidence that coded-wire-tagging procedures can enhance transmission of *Renibacterium salmoninarum* in chinook salmon. *Journal of Aquatic Animal Health* **13**, 181–193.

Elliott, D.G. and Shotts, E.B. (1980) Aetiology of an ulcerative disease in goldfish *Carassius auratus* (L.): Microbiological examination of diseased fish from seven locations. *Journal of Fish Diseases* **3**, 133–143.

Ellis, A.E. and Grisley, M.S. (1985) Serum antiproteases of salmonids: Studies on the inhibition of trypsin and the proteolytic activity of *Aeromonas salmonicida* extracellular products. In: Ellis, A.E. (ed.), *Fish and Shellfish Pathology*. London, Academic Press, pp. 85–96.

Ellis, A.E. and Stapleton, K.J. (1988) Differential susceptibility of salmonid fishes to furunculosis correlates with differential serum enhancement of *Aeromonas salmonicida* extracellular protease activity. *Microbial Pathogenesis* **4**, 299–304.

Ellis, A.E., Hastings, T.S. and Munro, A.L.S. (1981) The role of *Aeromonas salmonicida* extracellular products in the pathology of furunculosis. *Journal of Fish Diseases* **4**, 41–51.

Ellis, A.E., Dear, G. and Stewart, D.J. (1983) Histopathology of Sekiten-byo caused by *Pseudomonas anguilliseptica* in the European eel, *Anguilla anguilla* L., in Scotland. *Journal of Fish Diseases* **6**, 77–79.

Ellis, A.E., Burrows, A.S. and Stapleton, K.J. (1988a) Lack of relationship between virulence of *Aeromonas salmonicida* and the putative virulence factors: A-layer, extracellular proteases and extracellular haemolysins. *Journal of Fish Diseases* **11**, 309–323.

Ellis, A.E., Burrows, A.S., Hastings, T.S. and Stapleton, K.J. (1988b) Identification of *Aeromonas salmonicida* extracellular proteases as a protective antigen against furunculosis by passive immunization. *Aquaculture* **70**, 207–218.

Ellis, A.E., doVale, A., Bowden, T.J., Thompson, K. and Hastings, T.S. (1997) *In vivo* production of A-protein, lipopolysaccharide, iron-regulated outer membrane proteins and 70-kDa serine protease by *Aeromonas salmonicida* subsp. *salmonicida*. *FEMS Microbiology Letters* **149**, 157–163.

Ellis, R.W., Novotny, A.J. and Harrell, L.W. (1978) Case report of kidney disease in a wild chinook salmon, *Oncorhynchus tshawytscha* in the sea. *Journal of Wildlife Diseases* **14**, 120–123.

Elsayed, E.E., Eissa, A.E. and Faisal, M. (2006) Isolation of *Flavobacterium psychrophilum* from sea lamprey, *Petromyzon marinus* (L.), with skin lesions in Lake Ontario. *Journal of Fish Diseases* **29**, 629–632.

Elston, R., Drum, A.S. and Bunnell, P.R. (1995) Efficacy of orally administered difloxacin for the treatment of Atlantic salmon held in seawater. *Journal of Aquatic Animal Health* **7**, 22–28.

Embley, T.M. (1983) Aspects of the biology of *Renibacterium salmoninarum*. Ph.D. thesis, University of Newcastle-upon-Tyne.

Embley, T.M., Goodfellow, M. and Austin, B. (1982) A semi-defined growth medium for *Renibacterium salmoninarum*. *FEMS Microbiology Letters* **14**, 299–301.

Embley, T.M., Goodfellow, M., Minnikin, D.E. and Austin, B. (1983) Fatty acid, isoprenoid quinone and polar lipid composition in the classification of *Renibacterium salmoninarum*. *Journal of Applied Bacteriology* **55**, 31–37.

Embody, G.C. and Hayford, C.O. (1925) The advantage of rearing brook trout fingerlings from selected breeders. *Transactions of the American Fisheries Society* **55**, 135–142.

Emmerich, R. and Weibel, E. (1894) Über eine durch Bakterien erzengte Seuche unter den Forellen. *Archiv für Hygiene und Bakteriologie* **21**, 1–21.

Endo, T., Ogishima, K., Hayasaki, H., Kaneko, S. and Ohshima, S. (1973) Application of oxolinic acid as a chemotherapeutic agent for treating infectious diseases in fish. I. Antibacterial activity, chemotherapeutic effect and pharmacokinetic effect of oxolinic acid in fish. *Bulletin of the Japanese Society of Scientific Fisheries* **3**, 165–171.

Enger, Ø., Husevåg, B. and Goksøyr, J. (1989) Presence of the fish pathogen *Vibrio salmonicida* in fish farm sediments. *Applied and Environmental Microbiology* **55**, 2815–2818.

Enger, Ø., Husevåg, B. and Goksøyr, J. (1991) Seasonal variation in presence of *Vibrio salmonicida* and total bacterial counts in Norwegian fish farm water. *Canadian Journal of Microbiology* **37**, 618–623.

Esteve, C. (1995) Numerical taxonomy of Aeromonadaceae and Vibrionaceae associated with reared fish and surrounding fresh and brackish water. *Systematic and Applied Microbiology* **18**, 391–402.

Esteve, C. and Birkbeck, T.H. (2004) Secretion of haemolysins and proteases by *Aeromonas hydrophila* EO63: Separation and characterization of the serine protease (caseinase) and the metalloprotease (elastase). *Journal of Applied Microbiology* **96**, 994–1001.

Esteve, C., Biosca, E.G. and Amaro, C. (1993) Virulence of *Aeromonas hydrophila* and some other bacteria isolated from European eels *Anguilla anguilla* reared in fresh water. *Diseases of Aquatic Organisms* **16**, 15–20.

Esteve, C., Amaro, C. and Toranzo, A.E. (1994) O-serotyping and surface components of *Aeromonas hydrophila* and *Aeromonas jandaei* pathogenic for eels. *FEMS Microbiology Letters* **117**, 85–90.

Esteve, C., Gutiérrez, M.C. and Ventosa, A. (1995a) DNA relatedness among *Aeromonas allosaccharophila* strains and DNA hybridization groups of the genus *Aeromonas*. *International Journal of Systematic Bacteriology* **45**, 390–391.

Esteve, C., Amaro, C., Garay, E., Santos, Y. and Toranzo, A.E. (1995b) Pathogenicity of live bacteria and extracellular products of motile *Aeromonas* isolated from eels. *Journal of Applied Bacteriology* **78**, 555–562.

Esteve, C., Alcaide, E., Canals, R., Merino, S., Blasco, D., Figueras, M.J. and Tomás, J.M. (2004) Pathogenic *Aeromonas hydrophila* serogroup O:14 and O:81 strains with an S layer. *Applied and Environmental Microbiology* **70**, 5898–5904.

Esteve-Gassent, M.D. and Amaro, C. (2004) Immunogenic antigens of the eel pathogen *Vibrio vulnificus* serovar E. *Fish & Shellfish Immunology* **17**, 277–291.

Esteve-Gassent, M.D., Nielsen, M.E. and Amaro, C. (2003) The kinetics of antibody production in mucus and serum of European eel (*Anguilla anguilla* L.) after vaccination against *Vibrio vulnificus*: Development of a new method for antibody quantification in skin mucus. *Fish & Shellfish Immunology* **15**, 51–61.

Esteve-Gassent, M.D., Fouz, B. and Amaro, C. (2004) Efficacy of a bivalent vaccine against eel diseases caused by *Vibrio vulnificus* after its administration by four different routes. *Fish & Shellfish Immunology* **16**, 93–105.

Etchegaray, J.P., Martínez, M.A., Krauskopf, M. and León, G. (1991) Molecular cloning of *Renibacterium salmoninarum* DNA fragments. *FEMS Microbiology Letters* **79**, 61–64.

Eurell, T.E., Lewis, D.H. and Grumbles, L.C. (1978) Comparison of selected diagnostic tests for detection of motile *Aeromonas* septicaemia in fish. *American Journal of Veterinary Research* **39**, 1384–1386.

Euzéby, J.P. (1998) Taxonomic note: Necessary correction of specific and subspecific epithets according to Rules 12c and 13b of the International Code of Nomenclature of Bacteria (1990 Revision). *International Journal of Systematic Bacteriology* **48**, 1073–1075.

Evans, J.J., Klesius, P.H., Gilbert, P.M., Shoemaker, C.A., Al Sarawi, M.A., Landsberg, J., Duremdez, R., Al Marzouk, A. and Al Zenki, S. (2002) Characterization of β-haemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. *Journal of Fish Diseases* **25**, 505–513.

Evans, J.J., Klesius, P.H. and Shoemaker, C.A. (2004). Efficacy of *Streptococcus agalactiae* (group B) vaccine in tilapia (*Oreochromis niloticus*) by intraperitoneal and bath immersion administration. *Vaccine* **22**, 3769–3773.

Evelyn, T.P.T. (1971a) An aberrant strain of the bacterial fish pathogen *Aeromonas salmonicida* isolated from a marine host, the sablefish (*Anoplopoma fimbria*) and from two species of cultured Pacific salmon. *Journal of the Fisheries Research Board of Canada* **28**, 1629–1634.

Evelyn, T.P.T. (1971b) First records of vibriosis in Pacific salmon cultured in Canada, and taxonomic studies of the responsible bacterium, *Vibrio anguillarum*. *Journal of the Fisheries Research Board of Canada* **28**, 517–525.

Evelyn, T.P.T. (1971c) The agglutinin response in sockeye salmon vaccinated intraperitoneally with a heat-killed preparation of the bacterium responsible for salmonid kidney disease. *Journal of Wildlife Diseases* **7**, 328–335.

Evelyn, T.P.T. (1977) An improved growth medium for the kidney bacterium and some notes on using the medium. *Bulletin de l'Office International des Epizooties* **87**, 511–513.

Evelyn, T.P.T. (1978) Sensitivities of bacterial kidney disease detection methods with special remarks on the culture method. *Proceedings of the Joint 3rd Biennial Fish Health Section, American Fisheries Society and 9th Annual Midwest Fish Disease Workshops, Kansas City*, pp. 1–2.

Evelyn, T.P.T. (1984) Immunization against pathogenic vibrios. In: De Kinkelin, P. (ed.), *Symposium on Fish Vaccination*. Paris, Office International des Epizooties, pp. 121–150.

Evelyn, T.P.T. and Ketcheson, J.E. (1980) Laboratory and field observations on antivibriosis vaccines. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 45–54.

Evelyn, T.P.T. and Prosperi-Porta, L. (1989) Inconsistent performance of KDM2, a culture medium for the kidney disease bacterium *Renibacterium salmoninarum*, due to variation in the composition of its peptone ingredient. *Diseases of Aquatic Organisms* **7**, 227–229.

Evelyn, T.P.T., Hoskins, G.E. and Bell, G.R. (1973) First record of bacterial kidney disease in an apparently wild salmonid in British Columbia. *Journal of the Fisheries Research Board of Canada* **30**, 1578–15870.

Evelyn, T.P.T., Ketcheson, J.E. and Prosperi-Porta, L. (1981) The clinical significance of immunofluorescence-based diagnosis of the bacterial kidney disease carrier. *Fish Pathology* **15**, 293–300.

Evelyn, T.P.T., Ketcheson, J.E. and Prosperi-Porta, L. (1984) Further evidence for the presence of *Renibacterium salmoninarum* in salmonid eggs and for the failure of povidine–iodine to reduce the intra-ovum infection in water-hardened eggs. *Journal of Fish Diseases* **7**, 173–182.

Evelyn, T.P.T., Prosperi-Porta, L. and Ketcheson, J.E. (1986a) Experimental intra-ovum infection of salmonid eggs with *Renibacterium salmoninarum* and vertical transmission of the pathogen with such eggs despite their treatment with erythromycin. *Diseases in Aquatic Organisms* **1**, 197–202.

Evelyn, T.P.T., Ketcheson, J.E. and Prosperi-Porta, L. (1986b) Use of erythromycin as a means of preventing vertical transmission of *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* **2**, 7–11.

Evelyn, T.P.T., Bell, G.R., Prosperi-Porta, L. and Ketcheson, J.E. (1989) A simple technique for accelerating the growth of the kidney disease bacterium *Renibacterium salmoninarum* on a commonly used culture medium (KDM2). *Diseases of Aquatic Organisms* **7**, 231–234.

Evelyn, T.P.T., Prosperi-Porta, L. and Ketcheson, J.E. (1990) Two new techniques for obtaining consistent results when growing *Renibacterium salmoninarum* on KDM2 medium. *Diseases of Aquatic Organisms* **9**, 209–212.

Evenberg, D. and Lugtenberg, B. (1982) Cell surface of the fish pathogenic bacterium *Aeromonas salmonicida*. II. Purification and characterization of a major cell envelope protein related to autoagglutination. *Biochemica et Biophysica Acta* **684**, 249–254.

Evenberg, D., van Boxtel, R., Lugtenberg, B., Frank, S., Blommaert, J. and Bootsma, R. (1982) Cell surface of the fish pathogenic bacterium *Aeromonas salmonicida*. 1. Relationship between autoagglutination and the presence of a major cell envelope protein. *Biochemica et Biophysica Acta* **684**, 241–248.

Evenden, A.J., Gilpin, M.L. and Munn, C.B. (1990) The cloning and expression of a gene encoding haemolytic activity from the fish pathogen *Renibacterium salmoninarum*. *FEMS Microbiology Letters* **71**, 31–34.

Evensen, Ø., Espelid, S. and Håstein, T. (1991) Immunohistochemical identification of *Vibrio salmonicida* in stored tissues of Atlantic salmon *Salmo salar* from the first known outbreaks of coldwater vibriosis ("Hitra disease"). *Diseases of Aquatic Organisms* **10**, 185–189.

Ewing, W.H., Hugh, R. and Johnson, J.G. (1961) Studies on the *Aeromonas* group. *United States Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center*, 37 pp.

Ewing, W.H., MacWhorter, A.C., Escobar, M.R. and Lubin, A.H. (1965) *Edwardsiella*, a new genus of Enterobacteriaceae based on a new species, *E. tarda*. *International Bulletin of Bacterioloigcal Nomenclature and Taxonomy* **15**, 33–38.

Ewing, W.H., Ross, A.J., Brenner, D.J. and Fanning, G.R. (1978) *Yersinia ruckeri* sp. nov., the redmouth (RM) bacterium. *International Journal of Systematic Bacteriology* **28**, 37–44.

Eya, J.C. and Lovell, R.T. (1998) Effects of dietary phosphorus on resistance of channel catfish to *Edwardsiella ictaluri* challenge. *Journal of Aquatic Animal Health* **10**, 28–34.

Eyngor, M., Zlotkin, A., Ghittino, C., Prearo, M., Douet, D.-G., Chilmonczyk, S. and Eldar, A. (2004) Clonality and diversity of the fish pathogen *Lactococcus garvieae* in Mediterranean countries. *Applied and Environmental Microbiology* **70**, 5132–5137.

Ezura, Y., Tajima, K., Yoshimizu, M. and Kimura, T. (1980) Studies on the taxonomy and serology of causative organisms of fish vibriosis. *Fish Pathology* **14**, 167–179.

Fabre-Domerque, J.P. (1890) On a disease of fish. *Comptes Rendues Memoranda du Société Biologique, Paris* **42**, 127–129.

Facklam, R. and Moody, M. (1970) Presumptive identification of group D streptococci: The bile–esculin test. *Applied Microbiology* **20**, 245–250.

Fang, H.-M., Ling, K.C., Ge, R. and Sin, Y.M. (2000) Enhancment of protective imunity in blue gourami, *Trichogaster trichopterus* (Pallas), against *Aeromonas hydrophila* and *Vibrio anguillarum* by *A. hydrophila* major adhesin. *Journal of Fish Diseases* **23**, 137–145.

Fang, H.-M., Ge, R. and Sin, Y.M. (2004) Cloning, characterisation and expression of *Aeromonas hydrophila* major adhesin. *Fish & Shellfish Immunology* **16**, 645–658.

Farkas, J. (1985) Filamentous *Flavobacterium* sp. isolated from fish with gill diseases in cold water. *Aquaculture* **44**, 1–10.

Farkas, J. and Oláh, J. (1980) Characterization and antibiotic resistance of a "gliding bacterium" isolated from sheatfish fry (*Silurus glanis* L.). *Aquacultura Hungarica (Szarvas)* **11**, 131–138.

Farmer, J.J. and Kelly, M.T. (1991) Enterobacteriaceae. In: *Manual of Clinical Microbiology*, 5th edn. (edited by Balows, A., Hausler, W.J., Herman, K.L., Isenberg, H.D. and Shadomy, S.). Washington, D.C., American Society for Microbiology, pp. 360–383.

Farmer, J.J. and McWhorter, A.L. (1984) Genus X: *Edwardsiella* Ewing & McWhorter 1965, 37^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. I, Baltimore, MD, Williams & Wilkins, pp. 486–491.

Farmer III, J.J., Janda, M., Brenner, F.W., Cameron, D.N. and Birkhead, K.M. (2005) Genus I: *Vibrio* Pacini 1854, 411^{AL}. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 494–546.

Farrell, D., Mikesell, P., Actis, L.A. and Crosa, J.H. (1990) A regulatory gene, *angR*, of the iron uptake system of *Vibrio anguillarum*: Similarity with phage P22 *cro* and regulation by iron. *Gene* **86**, 45–51.

Farto, R., Armada, S.P., Montes, M., Perez, M.J. and Nieto, T.P. (2006) Presence of a lethal protease in the extracellular products of *Vibrio splendidus–Vibrio lentus* related strains. *Journal of Fish Diseases* **29**, 701–707.

Fehr, D., Casanova, C., Liverman, A., Blazkova, H., Orth, K., Dobbelaere, D., Frey, J. and Burr, S.E. (2006) AopP, a type III effector protein of *Aeromonas salmonicida*, inhibits the NF-αB signalling pathway. *Microbiology* **152**, 2809–2818.

Ferguson, H.W. (1977) Columnaris disease in rainbow trout (*Salmo gairdneri*) in Northern Ireland. *Veterinary Record* **101**, 55–56.

Ferguson, H.W., Collins, R.O., Moore, M., Coles, M. and MacPhee, D.D. (2004) *Pseudomonas anguilliseptica* infection in farmed cod, *Gadus morhua* L. *Journal of Fish Diseases* **27**, 249–254.

Ferguson, H.W., Turnbull, J.F., Shinn, A., Thompson, K., Dung, T.T. and Crumlish, M. (2001) Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. *Journal of Fish Diseases* **24**, 509–513.

Ferguson, Y., Glover, L.A., McGillivray, D.M. and Prosser, J.I. (1995) Survival and activity of lux-marked *Aeromonas salmonicida* in seawater. *Applied and Environmental Microbiology* **61**, 3494–3498.

Ferguson, Y., Bricknell, I.R., Glover, L.A., MacGregor, D.M. and Prosser, J.I. (1998) Colonisation and transmission of lux-marked and wild type *Aeromonas salmonicida* strains in Atlantic salmon (*Salmo salar* L.). *FEMS Microbiology Ecology* **27**, 251–260.

Fernandes, P.B., Hanson, C.W., Stamm, J.M., Chu, D.T.W., Bailer, R. and Vojtko, C. (1987) The frequency of *in vitro* resistance development to fluoroquinolones and the use of a murine pyelonephritis model to demonstrate selection of resistance *in vivo*. *Journal of Antimicrobial Chemotherapy* **19**, 446–449.

Fernandez, A.I.G., Fernández, A.F., Pérez, M.J., Nieto, T.P. and Ellis, A.E. (1998) Siderophore production by *Aeromonas salmonicida* subsp. *salmonicida*. Lack of strain specificity. *Diseases of Aquatic Organisms* **33**, 87–92.

Fernández, L., Secades, P., Lopez, J.R., Márquez, I. and Guijarro, J.A. (2002) Isolation and analysis of a protease gene with an ABC transport system in the fish pathogen *Yersinia*

ruckeri: Insertional mutagenesis and involvement in virulence. *Microbiology* **148**, 2233–2243.

Fernández, L., Lopez, J.R., Secades, P., Menendez, A., Marquez, I. and Guijarro, J.A. (2003) In vitro and in vivo studies of the Yrp1 protease from *Yersinia ruckeri* and its role in protective immunity against enteric redmouth disease of salmonids. *Applied and Environmental Microbiology* **69**, 7328–7335.

Fernández, L., Márquez, I. and Guijarrro, J.A. (2004) Identification of specific in vivo-induced (*ivi*) genes in *Yersinia ruckeri* and analysis of ruckerbacterin, a catecholate siderophore iron acquisition system. *Applied and Environmental Microbiology* **70**, 5199–5207.

Fiedler, F. and Draxl, R. (1986) Biochemical and immunochemical properties of the cell surface of *Renibacterium salmoninarum*. *Journal of Bacteriology* **168**, 799–804.

Field, J.B., Gee, L.L., Elvehjem, C.A. and Juday, C. (1944) The blood picture in furunculosis induced by *Bacterium salmonicida* in fish. *Archives of Biochemistry* **3**, 277–284.

Fijan, N.N. (1968) The survival of *Chondrococcus columnaris* in waters of different quality. *Bulletin de l'Office International des Epizooties* **69**, 1158–1166.

Fijan, N.N. (1969) Antibiotic additives for the isolation of *Chondrococcus columnaris* from fish. *Applied Microbiology* **17**, 333–334.

Fijan, N.N. (1972) Infectious dropsy of carp: A disease complex. *Proceedings of the Symposia of the Zoological Society of London* **30**, 39–57.

Fischel, D. and Enoch, H.P. (1892) Cited by Wiliamson (1929).

Fish, F.F. (1937) Furunculosis in wild trout. *Copeia* **37**, 40.

Fish, F.F. and Hanavan, M.G. (1948) A report upon the Grand Coulee Fish-Maintenance Project of 1939–1947. *United States Department of the Interior, Fish and Wildlife Service, Special Scientific Report* **55**, 1–63.

Ford, L.A. (1994) Detection of *Aeromonas salmonicida* from water using a filtration method. *Aquaculture* **122**, 1–7.

Ford, L.A., Barbash, P.A. and Cipriano, R.C. (1998) Control of furunculosis and enteric redmouth disease in sea run salmon broodstock in the Connecticut and Merrimack rivers. *Progressive Fish Culturist* **60**, 88–94.

Forel, F. (1868) Red disease of trout. *Bulletin de la Société Vandoise des Sciences Naturelles, Lausanne* **9**, 599–608.

Forsyth, R.B., Candido, E.P.M., Babich, S.L. and Iwama, G.K. (1997) Stress protein expression in coho salmon with bacterial kidney disease. *Journal of Aquatic Animal Health* **9**, 18–25.

Fouz, B.I., Larsen, J.L. and Toranzo, A.E. (1991) *Vibrio damsela* as a pathogenic agent causing mortalities in cultured turbot (*Scophthalmus maximus*). *Bulletin of the European Association of Fish Pathologists* **11**, 80–81.

Fouz, B., Larsen, J.L., Nielsen, B., Barja, J.L. and Toranzo, A.E. (1992) Characterization of *Vibrio damsela* strains isolated from turbot, *Scophthalmus maximus* in Spain. *Diseases of Aquatic Organisms* **12**, 155–166.

Fouz, B., Barja, J.L., Amaro, C., Rivas, C. and Toranzo, A.E. (1993) Toxicity of the extracellular products of *Vibrio damsela* isolated from diseased fish. *Current Microbiology* **27**, 341–347.

Fouz, B., Toranzo, A.E., Biosca, E.G., Mazoy, R. and Amaro, C. (1994) Role of iron in the pathogenicity of *Vibrio damsela* for fish and mammals. *FEMS Microbiology Letters* **121**, 181.

Fouz, B., Biosca, E.G. and Amaro, C. (1997) High affinity iron-uptake systems in *Vibrio damsela*: Role in the acquisition of iron from transferrin. *Journal of Applied Microbiology* **82**, 157–167.

Fouz, B., Toranzo, A.E., Milán, M. and Amaro, C. (2000) Evidence that water transmits the disease caused by the fish pathogen *Photobacterium damselae* subsp. *damselae*. *Journal of Applied Microbiology* **88**, 531–535.

Fouz, B., Esteve-Gassent, M.D., Barrera, R., Larsen, J.L., Nielsen, M.E. and Amaro, C. (2001) Field testing of a vaccine against eel diseases caused by *Vibrio vulnificus*. *Diseases of Aquatic Organisms* **45**, 183–189.

Fouz, B., Larsen, J.L. and Amaro, C. (2006) *Vibrio vulnificus* serovar A: An emerging pathogen in European anguilliculture. *Journal of Fish Diseases* **29**, 285–291.

Fouz, B., Zarza, C. and Amaro, C. (2006a) First description of non-motile *Yersinia ruckeri* serovar I strains causing disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), cultured in Spain. *Journal of Fish Diseases* **29**, 339–346.

Frantsi, C., Flewelling, T.C. and Tidswell, K.G. (1975) Investigations on corynebacterial kidney disease and *Diplostomulum* sp. (eye-fluke) at Margaree Hatchery, 1972–73. *Technical Report Series*, MAR/T–75–9: 30pp. Resource Development Branch of the Fisheries and Marine Service, Canadian Department of the Interior.

Frederiksen, W. (2005) Genus X: *Citrobacter* Werkmand & Gillen 1932, 173^{AL}. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 651–656.

Frerichs, G.N., Millar, S.D. and McManus, C. (1992) Atypical *Aeromonas salmonicida* isolated from health wrasse (*Ctenolabrus rupestris*). *Bulletin of the European Association of Fish Pathologists* **12**, 48–49.

Fryer, J.L. and Sanders, J.E. (1981) Bacterial kidney disease of salmonid fish. *Annual Review of Microbiology* **35**, 273–298.

Fryer, J.L., Nelson, J.S. and Garrison, R.L. (1972) Vibriosis in fish. In: Moore, R.W. (ed.), *Progress in Fishery and Food Science*. Seattle, University of Washington, Publications in Fisheries, pp. 129–133.

Fryer, J.L., Rohovec, J.S. and Garrison, R.L. (1978) Immunization of salmonids for control of vibriosis. *Marine Fisheries Review* **40**, 20–23.

Fryer, J.L., Hedrick, R.P., Park, J.W. and Hah, Y.C. (1988) Isolation of *Aeromonas salmonicida* from masu salmon in the Republic of Korea. *Journal of Wildlife Diseases* **24**, 364–365.

Fryer, J.L., Lannan, C.N., Garcés, L.H., Larenas, J.J. and Smith, P.A. (1990) Isolation of a rickettsiales-like organism from diseased coho salmon (*Oncorhynchus kisutch*) in Chile. *Fish Pathology* **25**, 107–114.

Fryer, J.L., Lannan, C.N., Giovannoni, S.J. and Wood, N.D. (1992) *Piscirickettsia salmonis* gen. nov., the causative agent of an epizootic disease in salmonid fishes. *International Journal of Systematic Bacteriology* **42**, 120–126.

Fuhrmann, H., Böhm, K.H. and Schlotfeldt, H.-J. (1983) An outbreak of enteric redmouth disease in West Germany. *Journal of Fish Disease* **6**, 309–311.

Fujihara, M.P. and Nakatani, R.E. (1971) Antibody production and immune responses of rainbow trout and coho salmon to *Chondrococcus columnaris*. *Journal of the Fisheries Research Board of Canada* **28**, 1253–1258.

Fujihara, M.P., Olson, P.A. and Nakatani, R.E. (1971) Some factors in susceptibility of juvenile rainbow trout and chinook salmon to *Chondrococcus columnaris*. *Journal of the Fisheries Research Board of Canada* **28**, 1739–1743.

Fujioka, R.S., Greco, S.B., Cates, M.B. and Schroeder, J.P. (1988) *Vibrio damsela* from wounds in bottlenose dolphins, *Tursiops truncatus*. *Diseases of Aquatic Organisms* **4**, 1–8.

Fujiwara-Nagata, E. and Eguchi, M. (2004) Significance of Na^+ in the fish pathogen, *Vibrio anguillarum*, under energy depleted condition. *FEMS Microbiology Letters* **234**, 163–167.

Fukuda, Y. and Kusuda, R. (1981a) Passive immunization of cultured yellowtail against pseudotuberculosis. *Fish Pathology* **16**, 85–89.

Fukuda, Y. and Kusuda, R. (1981b) Efficacy of vaccination for pseudotuberculosis in cultured yellowtail by various routes of administration. *Bulletin of the Japanese Society of Scientific Fisheries* **47**, 147–150.

Fukuda, Y. and Kusuda, R. (1982) Detection and characterization of precipitating antibody in the serum of immature yellowtail immunized with *Pasteurella piscicida* cells. *Fish Pathology* **17**, 125–127.

Fuller, D.W., Pilcher, K.S. and Fryer, J.L. (1977) A leucocytolytic factor isolated from cultures of *Aeromonas salmonicida*. *Journal of the Fisheies Research Board of Canada* **34**, 1118–1125.

Fuller, J.D., Camus, A.C., Duncan, C.L., Nizet, V., Bast, D.J., Thune, R.L., Low, D.E. and de Azavedo, J.C.S. (2002) Identification of a streptolysin S-associated gene cluster and its role in the pathogenesis of *Streptococcus iniae* disease. *Infection and Immunity* **70**, 5730–5739.

Ferguson, H.W., Turnbull, J.F., Shinn, A., Thompson, K., Dung, T.T. and Crumlish, M. (2001) Bacillary necrosis in farmed *Pangasius hypopththalmus* (Sauvage) from the Mekong Delta, Vietnam. *Journal of Fish Diseases* **24**, 509–513.

Furones, M.D., Gilpin, M.L. and Munn, C.B. (1993) Culture media for the differentiation of isolates of *Yersinia ruckeri*, based on detection of a virulence factor. *Journal of Applied Bacteriology* **74**, 360–366.

Fyfe, L., Finley, A., Coleman, G. and Munro, A.L.S. (1986) A study of the pathological effect of isolated *Aeromonas salmonicida* extracellular protease on Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **9**, 403–409.

Fyfe, L., Coleman, G. and Munro, A.L.S. (1987a) Identification of major common extracellular proteins secreted by *Aeromonas salmonicida* strains isolated from diseased fish. *Applied and Environmental Microbiology* **53**, 722–726.

Fyfe, L., Coleman, G. and Munro, A.L.S. (1987b) A comparative study of the formation of extracellular proteins by *Aeromonas salmonicida* at two different temperatures. *Journal of Applied Bacteriology* **62**, 367–370.

Gaggero, A., Castro, H. and Sandino, A.M. (1995) First isolation of *Piscirickettsia salmonis* from coho salmon, *Oncorhynchus kisutch* (Walbaum), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), during the freshwater stage of their life cycle. *Journal of Fish Diseases* **18**, 277–279.

Garcés, L.H., Larenas, J.J., Smith, P.A., Sandino, S., Lannan, C.N. and Fryer, J.L. (1991) Infectivity of a rickettsia isolated from coho salmon (*Oncorhynchus kisutch*). *Diseases of Aquatic Organisms* **11**, 93–97.

Garduño, R.A. and Kay, W.W. (1995) Capsulated cells of *Aeromonas salmonicida* grown *in vitro* have different functional properties than capsulated cells grown *in vivo*. *Canadian Journal of Microbiology* **41**, 941–945.

Garduño, R.A., Thornton, J.C. and Kay, W.W. (1993a) Fate of the fish pathogen *Aeromonas salmonicida* in the peritoneal cavity of rainobw trout. *Canadian Journal of Microbiology* **39**, 1051–1058.

Garduño, R.A., Thornton, J.C. and Kay, W.W. (1993b) *Aeromonas salmonicida* grown *in vivo*. *Infection and Immunity* **61**, 3854–3862.

Garduño, R.A., Phipps, B.M. and Kay, W.W. (1994) Physiological consequences of the S-layer of *Aeromonas salmonicida* in relation to growth, temperature, and outer mebrane permeation. *Canadian Journal of Microbiology* **40**, 622–629.

Garduño, R.A., Kuzyk, M.A. and Kay, W.W. (1997) Structural and physiological determinants of resistance of *Aeromonas salmonicida* to reactive radicals. *Canadian Journal of Microbiology* **43**, 1044–1053.

Garnjobst, L. (1945) *Cytophaga columnaris* (Davis) in pure culture: A myxobacterium pathogenic to fish. *Journal of Bacteriology* **49**, 113–128.

Gauger, E. and Gómez-Chiarri, M. (2002) 16S ribosomal DNA sequncing confirms the synonymy of *Vibrio harveyi* and *V. carchariae*. *Diseases of Aquatic Organisms* **52**, 39–46.

Gauger, E., Smolowitz, R., Uhlinger, K., Casey, J. and Gómez-Chiarri, M. (2006) *Vibrio harveyi* and other bacterial pathogens in cultured summer flounder, *Paralichthys dentatus*. *Aquaculture* **260**, 10–20.

Gaunt, P., Endris, R., Khoo, L., Leard, A.T., Jack, S., Santucci, T., Katz, T., Radecki, S.V. and Simmons, R. (2003) Preliminary assessment of the tolerance and efficacy of florfenicol to *Edwardsiella ictaluri* administered in feed to channel catfish. *Journal of Aquatic Animal Health* **15**, 239–247.

Gauthier, D.T., Rhodes, M.W., Vogelbein, W.K., Kator, H. and Ottinger, C.A. (2003) Experimental mycobacteriosis in striped bass *Morone saxatilis*. *Diseases in Aquatic Organisms* **54**, 105–117.

Gauthier, D.T., Vogelbein, W.K. and Ottinger, C.A. (2004) Ultrastructure of *Mycobacterium marinum* granuloma in striped bass *Morone saxatilis*. *Diseases in Aquatic Organisms* **62**, 121–132.

Gauthier, G., Lafay, B., Ruimy, R., Breittmayer, V., Nicolas, J.L., Gauthier, M. and Christen, R. (1995) Small-subunit rRNA sequences and whole DNA relatedness concur for the reassignment of *Pasteurella piscicida* (Snieszko *et al.*) Janssen and Surgalla to the genus *Photobacterium* as *Photobacterium damsela* subsp. *piscicida* comb. nov. *International Journal of Systematic Bacteriology* **45**, 139–144.

Gayer, E.K., Bekesi, L. and Csaba, G. (1980) Some aspects of the histopathology of carp erythrodermatitis (CE). In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 127–136.

Geck, P. (1971) India ink immuno-reaction for the rapid detection of enteric pathogens. *Acta Microbiologica Academicae Scientiarum Hungaricae* **18**, 191–196.

Geldreich, E.E. (1977) Microbiology of water. *Journal of the Water Pollution Control Federation* **69**, 122–144.

Gelev, I. and Gelev, E. (1988) A new species of fish-pathogenic bacterium antigenically related to classical *Brucellae*. *Zentralblatt für Bakteriologie und Hygiene, Reihe A* **269**, 1–6.

Gelev, I., Gelev, E., Steigerwalt, A.G., Carter, G.P. and Brenner, D.J. (1990) Identification of the bacterium associated with haemorrhagic septicaemia in rainbow trout as *Hafnia alvei*. *Research in Microbiology, Institut Pasteur* **141**, 573–576.

Getchell, R.G., Rohovec, J.S. and Fryer, J.L. (1985) Comparison of *Renibacterium salmoninarum* isolates by antigenic analysis. *Fish Pathology* **20**, 149–159.

Ghittino, C. and Prearo, M. (1992) Segnalazione di streptococcosi nella trota iridea (*Oncorhynchus mykiss*) in Italia: Nota preliminare. *Boll. Soc. It. Patol. Ittica* **8**, 4–9.

Ghittino, C. and Prearo, M. (1993) Comparazione di alcuni ceppi isolati da trote iridea affette da streptococcosi. *Boll. Soc. It. Patol. Ittica* **11**, 30–43.

Ghittino, P. (1967) Eziologia e lesioni anatomo-patologiche della malattia branchiale (MB) delle trotelline in Italie. *Rivista Italiane di Piscicultura e Ittiopatologia* **A11**, 24–29.

Ghittino, P. and Penna, P. (1968) Recherches microbiologiques sur la nocardiose de la truite arc-en-ciel. *Bulletin de l'Office International des Epizooties* **69**, 1045–1056.

Ghittino, P. and Andruetto, S. (1977) Fish vibriosis in fresh and salt waters of Italy. *Bulletin de l'Office International des Epizooties* **87**, 483–485.

Gibb, A. and Austin, B. (1994) A stable L-form of the fish pathogen *Aeromonas salmonicida*. *Letters in Applied Microbiology* **19**, 473–476.

Gibb, A., McIntosh, D. and Austin, B. (1996) Characterization of a stable L-form of the fish pathogen *Aeromonas salmonicida*. *Letters in Applied Microbiology* **23**, 445–447.

Gibello, A., Blanco, M.M., Moreno, M.A., Cutuli, M.T., Doménech, A., Dominguez, L. and Fernández-Garayzábal, J.F. (1999) Development of PCR assay for detection of *Yersinia ruckeri* in tissues of inoculated and naturally infected trout. *Applied and Environmental Microbiology* **65**, 346–350.

Gil, P., Vivas, J., Gallardo, C.S. and Rodríguez, L.A. (2000) First isolation of *Staphylococcus warneri*, from diseased rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Northwest Spain. *Journal of Fish Diseases* **23**, 295–298.

Giles, J.S., Hariharan, H. and Heaney, S.B. (1995) The plasmid profiles of fish pathogenic isolates of *Aeromonas salmonicida*, *Vibrio anguillarum*, and *Vibrio ordalii* from the Atlantic and Pacific coasts of Canada. *Canadian Journal of Microbiology* **41**, 209–216.

Gill, O.N., Coghlan, J.D. and Calder, I.M. (1983) The risk of leptospirosis in United Kingdom fish farm workers: Results from a 1981 serological survey. *Journal of Hygiene, Cambridge* **94**, 81–86.

Gillespie, N.C. (1981) A numerical taxonomic study of *Pseudomonas*-like bacteria isolated from fish in southeastern Queensland and their association with spoilage. *Journal of Applied Bacteriology* **50**, 29–44.

Gilmour, A. (1977) Characteristics of marine vibrios isolated from fish farm tanks. *Aquaculture* **11**, 51–62.

Giorgetti, G., Tomasin, A.B. and Ceschia, G. (1981) First Italian anti-vibriosis vaccination experiments of freshwater farmed rainbow trout. *Developments in Biological Standardization* **49**, 455–459.

Gjedrem, T. and Aulstad, D. (1974) Selection experiments with salmonids. 1. Differences in resistance to vibrio disease of salmon parr (*Salmo salar*). *Aquaculture* **3**, 51–59.

Gjedrem, T. and Gjoen, H.M. (1995) Genetic variation in susceptibility of Atlantic salmon, *Salmo salar* L., to furunculosis, BKD and cold water vibriosis. *Aquaculture Research* **26**, 129–134.

Goerlich, R., Schlüsener, H.J., Lehmann, J. and Greuel, E. (1984) The application of monoclonal antibodies to diagnosis of *Aeromonas salmonicida* infections in fishes. *Bulletin of the European Association of Fish Pathologists* **4**, 66.

Gomez, S., Bernabe, A., Gomez, M.A., Navarro, J.A. and Sanchez, J. (1993) Fish mycobacteriosis: Morphological and immunocytochemical aspects. *Journal of Fish Diseases* **16**, 137–141.

Gomez-Gil, B., Soto-Rodriguez, S., Garcia-Gasca, A., Roque, A., Vazquez-Juarez, R., Thompson, F.J. and Swings, J. (2004) Molecular identification of *Vibrio harveyi*-related isolates associated with diseased aquatic organisms. *Microbiology* **150**, 1769–1777.

Gonzalez, C.J., Lopez-Diaz, T.M., Garcia-Lopez, M.L., Prieto, M. and Otero, A. (1999) Bacterial microflora of wild brown trout (*Salmo trutta*), wild pike (*Esox lucius*), and aquacultured rainbow trout (*Oncorhynchus mykiss*). *J. Food Protection* **62**, 1270–1277.

González, M., Sánchez, F., Concha, M.I., Figueroa, J., Montecinos, M.I. and León, G. (1999) Evaluation of the internalization process of the fish pathogen *Renibacterium salmoninarum* in cultured fish cells. *Journal of Fish Diseases* **22**, 231–235.

González, S.F., Osorio, C.R. and Santos, Y. (2003) Development of a PCR-based method for the detection of *Listonella anguillarum* in fish tissues and blood samples. *Diseases of Aquatic Organism* **55**, 109–115.

González, S.F., Krug, M.J., Nielsen, M.E., Santos, Y. and Call, D.R. (2004) Simultaneous detection of marine fish pathogens by using multiplex PCR and a DNA microarray. *Journal of Clinical Microbiology* **42**, 1414–1419.

Goodfellow, M. (1971) Numerical taxonomy of some nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.

Goodfellow, M., Embley, T.M. and Austin, B. (1985) Numerical taxonomy and emended description of *Renibacterium salmoninarum*. *Journal of General Microbiology* **131**, 2739–2752.

Goodwin, A.E., Spencer Roy, Jr, J., Grizzle, J.M. and Terrell Goldsby Jr, M. (1994) *Bacillus mycoides*: A bacterial pathogen of channel catfish. *Diseases of Aquatic Organisms* **18**, 173–179.

Gordon, R.E. and Mihm, J.M. (1959) A comparison of four species of mycobacteria. *Journal of General Microbiology* **21**, 736–748.

Gould, R.W., O'Leary, P.J., Garrison, R.L., Rohovec, J.S. and Fryer, J.L. (1978) Spray vaccination: A method for the immunization of fish. *Fish Pathology* **13**, 63–68.

Grabowski, L.D., LaPatra, S.E. and Cain, K.D. (2004) Systemic and mucosal antibody response in tilapia, *Oreochromis niloticus* (L.), following immunization with *Flavobacterium columnare*. *Journal of Fish Diseases* **27**, 573–581.

Graham, S., Jeffries, A.H. and Secombes, C.J. (1988) A novel assay to detect macrophage bacterial activity in fish: Factors influencing the killing of *Aeromonas salmonicida*. *Journal of Fish Diseases* **11**, 389–396.

Grawinski, E. and Antychowicz, J. (2001) The pathogenicity of *Serratia plymuthica* for salmonid fish. *Medycyna Weterynaryjna* **57**, 187–189.

Gray, S.F., Smith, R.S., Reynolds, N.J. and Williams, E.W. (1990) Fish tank granuloma. *British Medical Journal* **300**, 1069–1070.

Grayson, T.H., Evenden, A.J., Gilpin, M.L. and Munn, C.B. (1995a) Production of a *Renibacterium salmoninarum* hemolysin fusion protein in *Escherichia coli* K12. *Diseases of Aquatic Organisms* **22**, 153–156.

Grayson, T.H., Bruno, D.W., Evenden, A.J., Gilpin, M.L. and Munn, C.B. (1995b) Iron acquisition by *Renibacterium salmoninarum*: Contribution of iron reductase. *Diseases of Aquatic Organisms* **22**, 157–162.

Grayson, T.H., Cooper, L.F., Atienzar, F.A., Knowles, M.R. and Gilpin, M.L. (1999) Molecular differentiation of *Renibacterium salmoninarum* isolates from worldwide locations. *Applied and Environmental Microbiology* **65**, 961–968.

Grayson, T.H., Gilpin, M.L., Evenden, A.J., and Munn, C.B. (2001) Evidence for the immune recognition of two haemolysins of *Renibacterium salmoninarum* by fish displaying clinical symptoms of bacterial kidney disease (BKD). *Fish & Shellfish Immunology* **11**, 367–370.

Green, M. and Austin, B. (1982) The identification of *Yersinia ruckeri* and its relationship to other representatives of the Enterobacteriaceae. *Aquaculture* **34**, 185–192.

Greger, E. and Goodrich, T. (1999) Vaccine development for winter ulcer disease, *Vibrio viscosus*, in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **22**, 193–199.

Griffin, P.J. (1953) The nature of bacteria pathogenic to fish. *Transactions of the American Fisheries Society* **83**, 241–253.

Griffin, P.J., Snieszko, S.F. and Friddle, S.B. (1953a) A more comprehensive description of *Bacterium salmonicida*. *Transactions of the American Fisheries Society* **82**, 129–138.

Griffin, P.J., Snieszko, S.F. and Friddle, S.B. (1953b) Pigment formation by *Bacterium salmonicida*. *Journal of Bacteriology* **65**, 652–659.

Griffiths, S.G., Olivier, G., Fildes, J. and Lynch, W.H. (1991) Comparison of western blot, direct fluorescent antibody and drop-plate culture methods for the detection of *Renibacterium salmoninarum* in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **97**, 117–129.

Griffiths, S.G., Liska, K. and Lynch, W.H. (1996) Comparison of kidney tissue and ovarian fluid from broodstock Atlantic salmon for detection of *Renibacterium salmoninarum*, and

use of SKDM broth culture with Western blotting to increase detection in ovarian fluid. *Diseases of Aquatic Organisms* **24**, 3–9.

Grimes, D.J. (1986) Human pathogens associated with elasmobranchs and other marine animals. In: Megusar, F. and Gantar, M. (eds), *Perspectives in Microbial Ecology*. Ljubljana, Slovenia, Slovene Society for Microbiology, pp. 417–421.

Grimes, D.J., Stemmler, J., Hada, H., May, E.B., Maneval, D., Hetrick, F.M., Jones, R.T., Stoskopf, M. and Colwell, R.R. (1984a) *Vibrio* species associated with mortality of sharks held in captivity. *Microbial Ecology* **10**, 271–282.

Grimes, D.J., Colwell, R.R., Stemmler, J., Hada, H., Maneval, D., Hetrick, F.M., May, E.B., Jones, R.T. and Stoskopf, M. (1984b) *Vibrio* species as agents of elasmobranch disease. *Helgoländer Meeresuntersuchungen* **37**, 309–315.

Grimes, D.J., Gruber, S.H. and May, E.B. (1985) Experimental infection of lemon sharks, *Negaprion brevirostris* (Poey), with *Vibrio* species. *Journal of Fish Diseases* **8**, 173–180.

Grimes, D.J, Jacobs, D., Swartz, D.G., Brayton, P.R., and Colwell, R.R. (1993) Numerical taxonomy of Gram-negative, oxidase-positive rods from carcharhinid sharks. *International Journal of Systematic Bacterioliology* **43**, 88–98.

Grimont, F. and Grimont, P.A.D. (2005) Genus XXIII: *Pantoea* Gavini, Mergaert, Beji, Meilcarek, Izard, Kersters & De Ley 1989b, 343VP, emend. Mergaerts, Verdonck and Kersters 1993, 171. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 713–720.

Grimont, F. and Grimont, P.A.D. (2005a) Genus XXXIV: *Serratia* Bizio 1823, 288^{AL}. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*, New York, Springer-Verlag, pp. 799–811.

Grimont, P.A.D. and Grimont, F. (1984) Genus VIII: *Serratia* Bizio 1823, 288^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. I., Baltimore, MD, Williams & Wilkins, pp. 477–484.

Grischkowsky, R.S. (1973) Studies of the nature of Pacific oyster (*Crassostrea gigas* Thunberg) mortalities: I. Implications of bacterial pathogenicity and II. Pathogenicity testing of vibrios on chinook salmon (*Oncorhynchus tshawytscha* Walbaum) and Pacific oysters. Ph.D. dissertation, University of Washington, Seattle.

Grisez, L. and Ollevier, F. (1995) Comparative serology of the marine fish pathogen *Vibrio anguillarum*. *Applied and Environmental Microbiology* **61**, 4367–4373.

Grisez, L., Chair, M., Sorgeloos, P. and Ollevier, F. (1996) Mode of infection and spread of *Vibrio anguillarum* in turbot *Scophthalmus maximus* larvae after oral challenge through live feed. *Diseases of Aquatic Organisms* **26**, 181–187.

Grisley, M.S., Ellis, A.E., Hastings, T.S. and Munro, A.L.S. (1984) An alpha-migrating antiprotease in normal salmonid plasma and its relationship to the neutralization of *Aeromonas salmonicida* toxins. In: Acuigrup (ed.), *Fish Diseases, Fourth COPRAQ Session. Madrid, Editora ATP, pp. 77–82.*

Groberg, W.J. (1982) Infection and the immune response induced by *Vibrio anguillarum* in juvenile coho salmon (*Oncorhynchus kisutch*). Ph.D. thesis, Oregon State University, Corvallis.

Groberg, W.J., McCoy, R.H., Pilcher, K.S. and Fryer, J.L. (1978) Relation of water temperature to infections of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), and steelhead trout (*Salmo gairdneri*) with *Aeromonas salmonicida* and *A. hydrophila*. *Journal of the Fisheries Research Board of Canada* **35**, 1–7.

Grøntvedt, R.N., Lund, V. and Espelid, S. (2004) Atypical furunculosis in spotted wolffish (*Anarhichas minor* O.) juveniles: Bath vaccination and challenge. *Aquaculture* **232**, 69–80.

Grove, S., Høie, S. and Evensen, Ø. (2003) Distribution and retention of antigens of *Aeromonas salmonicida* in Atlantic salmon (*Salmo salar* L.) vaccinated with a *ΔaroA* mutant or formalin-inactivated bacteria in oil–adjuvant. *Fish & Shellfish Immunology* **15**, 349–358.

Gudmundsdóttir, B.K. (1996) Comparison of extracellular proteases by *Aeromonas salmonicida* strains, isolated from various fish species. *Journal of Applied Bacteriology* **80**, 105–113.

Gudmundsdóttir, B.K. and Gudmundsdóttir, S. (1997) Evaluation of cross protection by vaccines against atypical and typical furunculosis in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **20**, 343–350.

Gudmundsdóttir, B.K., Hasting, T.S. and Ellis, A.E. (1990) Isolation of a new toxic protease from a strain of *Aeromonas salmonicida* subspecies *achromogenes*. *Diseases of Aquatic Organisms* **9**, 199–208.

Gudmundsdóttir, B.K., Jónsdóttir, H., Steinthorsdóttir, V., Magnadóttir, B. and Gudmundsdóttir, S. (1997) Survival and humoral antibody response of Atlantic salmon (*Salmo salar* L.) vaccinated against *Aeromonas salmonicida* subsp. *achromogenes*. *Journal of Fish Diseases* **20**, 351–360.

Gudmundsdóttir, B.K., Hvanndal, Í, Björnsdóttir, B. and Wagner, U. (2003) Analysis of exotoxins produced by atypical isolates of *Aeromonas salmonicida*, by enzymatic and serological methods. *Journal of Fish Diseases* **26**, 15–29.

Gudmundsdóttir, B.K., Björnsdóttir, B., Gudmundsdóttir, S. and Bambir, S.H. (2006) A comparative study of susceptibility and induced pathology of cod, *Gadus morhua* (L.), and halibut, *Hippoglossus hippoglossus* (L.), following experimental infection with *Moritella viscosa*. *Journal of Fish Diseases* **29**, 481–487.

Gudmundsdóttir, S., Helgason, S. and Bendiktsdóttir, E. (1991) Comparison of the effectiveness of three different growth media for primary isolation of *Renibacterium salmoninarum* from Atlantic salmon, *Salmo salar* L., broodfish. *Journal of Fish Diseases* **14**, 89–96.

Gudmundsdóttir, S., Benediktsdóttir, E. and Helgason, S. (1993) Detection of *Renibacterium salmoninarum* in salmonid kidney samples: A comparison of results using double sandwich ELISA and isolation on selective medium. *Journal of Fish Diseases* **16**, 185–195.

Gudmundsdóttir, S., Helgason, S., Sigurjónsdóttir, H., Matthíadóttir, S., Jónsdóttir, H., Laxdal, B. and Benediktsdóttir, E. (2000) Measures applied to control *Renibacterium salmoninarum* infection in Atlantic salmon: A retrospective study of two sea ranches in Iceland. *Aquaculture* **186**, 193–203.

Gudmunsdóttir, S., Lange, S., Magnadóttir, B. and Gudmundsdóttir, B.K. (2003) Protection against atypical furunculosis in Atlantic halibut, *Hippoglossus hippoglossus* (L.): A comparison of a commercial furunculosis vaccine and an autogenous vaccine. *Journal of Fish Diseases* **26**, 331–338.

Guilvout, I., Quilici, M.L., Rabot, S., Lesel, R. and Mazigh, D. (1988) BamHI restriction endonuclease analysis of *Yersinia ruckeri* plasmids and their relatedness to the genus *Yersinia* 42- to 47-megadalton plasmid. *Applied and Environmental Microbiology* **54**, 2594–2597.

Gunn, B.A., Singleton, F.L., Peele, E.R. and Colwell, R.R. (1982) A note on the isolation and enumeration of Gram-positive cocci from marine and estuarine waters. *Journal of Applied Bacteriology* **53**, 127–129.

Gunnlaugsdóttir, B. and Gudmundsdóttir, B.K. (1997) Pathogenicity of atypical *Aeromonas salmonicida* in Atlantic salmon compared with protease production. *Journal of Applied Microbiology* **83**, 542–551.

Gutenberger, S.K., Giovannoni, S.J., Field, K.G., Fryer, J.L. and Rohovec, J.S. (1991) A phylogenetic comparison of the 16S rRNA sequence of the fish pathogen, *Renibacterium salmoninarum*, to Gram-positive bacteria. *FEMS Microbiology Letters* **77**, 151–156.

Gutenberger, S.K., Duimstra, J.R., Rohovec, J.S. and Fryer, J.L. (1997) Intracellular survival of *Renibacterium salmoninarum* in trout mononuclear macrophages. *Diseases of Aquatic Organisms* **28**, 93–106.

Gutierrez, M.A. and Miyazaki, T. (1994) Responses of Japanese eels to oral challenge with *Edwardsiella tarda* after vaccination with formalin-killed cells or lipopolysaccharide of the bacterium. *Journal of Aquatic Animal Health* **6**, 110–117.

Gutsell, J. (1946) Sulfa drugs and the treatment of furunculosis in trout. *Science* **104**, 85–86.

Gutteridge, C.S. and Norris, J.R. (1979) The application of pyrolysis techniques to the identification of micro-organisms. *Journal of Applied Bacteriology* **47**, 5–43.

Hackett, J.L., Lynch, W.H., Paterson, W.D. and Coombs, D.H. (1984) Extracellular protease, extracellular hemolysis, and virulence in *Aeromonas salmonicida*. *Canadian Journal of Fisheries and Aquatic Sciences* **41**, 1354–1360.

Hahnel, G.B., Gould, R.W. and Boatman, E.S. (1983) Serological comparison of selected strains of *Aeromonas salmonicida* subsp. *salmonicida*. *Journal of Fish Diseases* **6**, 1–11.

Haley, R., Davis, S.P. and Hyde, J.M. (1967) Environmental stress and *Aeromonas liquefaciens* in American and threadfin shad mortalities. *Progressive Fish Culturist* **29**, 193.

Hall, J.D. (1963) An ecological study of the chestnut lamprey, *Ichthyomyzon castaneus* Girard, in the Manistee River, Michigan. Ph.D. thesis, University of Michigan, Ann Arbor, p. 106.

Halver, J.E., Ashley, L.M. and Smith, R.E. (1969) Ascorbic acid requirements of coho salmon and rainbow trout. *Transactions of the American Fisheries Society* **98**, 762–772.

Hamaguchi, M. and Kusuda, R. (1989) Field testing of *Pasteurella piscicida* formalin killed bacterin against pseudotuberculosis in cultured yellowtail, *Seriola quinqueradiata*. *Bulletin of Marine Science and Fisheries, Kochi University* **11**, 11–16.

Hamilton, A.J., Fallon, M.J.M., Alexander, J. and Canning, E.U. (1986) A modified enzyme linked immunosorbent assay (ELISA) for monitoring antibody production during experimental *Aeromonas salmonicida* infection in rainbow trout (*Salmo gairdneri*). *Developmental and Comparative Immunology* **10**, 443–448.

Hamilton, R.C., Kalnins, H., Ackland, N.R. and Ashburner, L.D. (1981) An extra layer in the surface layers of an atypical *Aeromonas salmonicida* isolated from Australian goldfish. *Journal of General Microbiology* **122**, 363–366.

Han, H.-J., Kim, D.-H., Lee, D.-C., Kim, S.-M. and Park, S.-I. (2006) Pathogenicity of *Edwardsiella tarda* to olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel). *Journal of Fish Diseases* **29**, 601–609.

Handlinger, J., Soltani, M. and Percival, S. (1997) The pathology of *Flexibacter maritimus* in aquaculture species in Tasmania, Australia. *Journal of Fish Diseases* **20**, 159–168.

Hanif, A., Bakopoulos, V., Leonardos, I. and Dimitriadis, G.J. (2005) The effect of sea bream (*Sparus aurata*) broodstock and larval vaccination on the susceptibility by *Photobacterium damsela* subsp. *piscicida* and on the humoral immune parameters. *Fish & Shellfish Immunology* **19**, 345–361.

Hänninen, M.-L. and Hirvelä-Koski, V. (1997) Molecular and phenotypic methods for the characterization of atypical *Aeromonas salmonicida*. *Veterinary Microbiology* **56**, 147–158.

Hänninen, M.-L., Ridell, J. and Hirvelä-Koski, V. (1995) RAPD analysis of *Aeromonas salmonicida–Aeromonas hydrophila*. *Journal of Applied Bacteriology* **79**, 181–185.

Hansen, C.B. and Lingg, A.J. (1976) Inert particle agglutination tests for detection of antibody to enteric redmouth bacterium. *Journal of the Fisheries Research Board of Canada* **33**, 2857–2860.

Hansen, G.H. and Olafsen, J.A. (1989) Bacterial colonization of cod (*Gadus morhua* L.) and halibut (*Hippoglossus hippoglossus*) eggs in marine aquaculture. *Applied and Environmental Microbiology* **55**, 1435–1446.

Hansen, G.H., Raa, J. and Olafsen, J.A. (1990) Isolation of *Enterobacter agglomerans* from dolphin fish, *Coryphaena hippurus* L. *Journal of Fish Diseases* **13**, 93–96.

Hansen, G.H., Bergh, Ø., Michaelsen, J. and Knappskog, D. (1992) *Flexibacter ovolyticus* sp. nov., a pathogen of eggs and larvae of Atlantic halibut, *Hippoglossus hippoglossus* L. *International Journal of Systematic Bacteriology* **42**, 451–458.

Hanson, L.A. and Grizzle, J.M. (1985) Nitrite-induced predisposition of channel catfish to bacterial diseases. *Progressive Fish Culturist* **47**, 98–101.

Hao, M.V. and Komagata, K. (1985) A new species of *Planococcus*, *P. kocurii* isolated from fish, frozen foods, and fish curing brine. *Journal of General and Applied Microbiology* **31**, 441–455.

Harbell, S.O., Hodgins, H.O. and Schiewe, M.H. (1979) Studies on the pathology of vibriosis in coho salmon (*Oncorhynchus kisutch*). *Journal of Fish Diseases* **2**, 527–535.

Hardie, L.J., Fletcher, T.C. and Secombes, C.J. (1990) The effect of vitamin E on the immune response of the Atlantic salmon (*Salmo salar* L.). *Aquaculture* **87**, 1–13.

Hardie, L.J., Ellis, A.E. and Secombes, C.J. (1996) *In vitro* activation of rainbow trout macrophages stimulates inhibition of *Renibacterium salmoninarum* growth concomitant with augmented generation of respiratory burst products. *Diseases of Aquatic Organisms* **25**, 175–183.

Hariharan, H., Qian, B., Despres, B., Kibenge, F.S., Heaney, S.B. and Rainnie, D.J. (1995) Development of a specific biotinylated DNA probe for the detection of *Renibacterium salmoninarum*. *Canadian Journal of Veterinary Research* **59**, 306–310.

Harikrishnan, R. and Balasundaram, C. (2005) Antimicrobial activity of medicinal herbs *in vitro* against fish pathogen, *Aeromonas hydrophila*. *Fish Pathology* **40**, 187–189.

Harrell, L.W. (1978) Vibriosis and current vaccination procedures in Puget Sound, Washington. *Marine Fisheries Review* **40**, 24–25.

Harrell, L.W., Etlinger, H.M. and Hodgins, H.O. (1976) Humoral factors important in resistance of salmonid fish to bacterial disease. II. Anti *Vibrio anguillarum* activity in mucus and observations on complement. *Aquaculture* **7**, 363–370.

Harrison, F.C. and Sadler, W. (1929) Discoloration of halibut. *Biological Board of Canada, Bulletin* **12**, 1–18.

Hashimoto, S., Muraoka, A. and Kusuda, R. (1989) Effects of carbohydrates, amino acids, vitamins, inorganic salts and peptones on the growth of *Pasteurella piscicida*. *Nippon Suisan Gakkaishi* **55**, 1791–1797.

Håstein, T. and Bullock, A.M. (1976) An acute septicaemic disease of brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) caused by a *Pasteurella*-like organism. *Journal of Fish Biology 8*, 23–26.

Håstein, T. and Smith, J.E. (1977) A study of *Vibrio anguillarum* from farms and wild fish using principal components analysis. *Journal of Fish Biology* **11**, 69–75.

Håstein, T., Hallingstad, F., Refsti, T. and Roald, S.O. (1980) Recent experience of field vaccination trials against vibriosis in rainbow trout (*Salmo gairdneri*). In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 53–59.

Hastings, T.S. and Bruno, D.W. (1985) Enteric redmouth disease: Survey in Scotland and evaluation of a new medium, Shotts–Waltman, for differentiating *Yersinia ruckeri*. *Bulletin of the European Association of Fish Pathologists* **5**, 32–35.

Hastings, T.S. and Ellis, A.E. (1985) Differences in the production of haemolytic and proteolytic activities by various isolates of *Aeromonas salmonicida*. In: Ellis, A.E. (ed.), *Fish and Shellfish Pathology*. London, Academic Press, pp. 69–77.

Hastings, T.S. and Ellis, A.E. (1988) The humoral immune response of rainbow trout, *Salmo gairdneri* Richardson, and rabbits to *Aeromonas salmonicida* extracellular products. *Journal of Fish Diseases* **11**, 147–160.

Hastings, T.S. and McKay, A. (1987) Resistance of *Aeromonas salmonicida* to oxolinic acid. *Aquaculture* **61**, 165–171.

Hatai, K., Egusa, S., Nakajima, M. and Chikahata, H. (1975) *Pseudomonas chlororaphis* as a fish pathogen. *Bulletin of the Japanese Society of Scientific Fisheries* **41**, 1203.

Hatai, K., Lawhavinit, O., Toda, K. and Sugou, Y. (1993) *Mycobacterium* infection in pejerrey, *Odonthestes bonariensis* Cuvier & Valenciennes. *Journal of Fish Diseases* **16**, 397–402.

Hawke, J.P. (1979) A bacterium associated with disease of pond cultured channel catfish, *Ictalurus punctatus*. *Journal of the Fisheries Research Board of Canada* **36**, 1508–1512.

Hawke, J.P., McWhorter, A.C., Steigerwalt, A.G. and Brenner, D.J. (1981) *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicaemia of catfish. *International Journal of Systematic Bacteriology* **31**, 396–400.

Hawke, J.P., Thune, R.L., Cooper, R.K., Judice, E. and Kelly-Smith, M. (2003) Molecular and phenotypic characterization of strains of *Photobacterium damselae* subsp. *piscicida* isolated from hybrid striped bass cultured in Louisiana USA. *Journal of Aquatic Animal Health* **15**, 189–201.

Hazen, T.C., Raker, M.L., Esch, G.W. and Fliermans, C.B. (1978) Ultrastructure of red-sore lesions on largemouth bass (*Micropterus salmoides*): Association of the ciliate *Epistylis* sp. and the bacterium *Aeromonas hydrophila*. *Journal of Protozoology* **25**, 351–355.

Hazen, T.C., Esch, G.W. and Raker, M.L. (1981) Agglutinating antibody to *Aeromonas hydrophila* in wild largemouth bass. *Transactions of the American Fisheries Society* **110**, 514–518.

Hazen, T.C., Esch, G.W., Dimock, R.V. and Mansfield, A. (1982) Chemotaxis of *Aeromonas hydrophila* to the surface mucus of fish. *Current Microbiology* **7**, 371–375.

Heath, S., Pak, S., Marshall, S., Prager, E.M. and Orrego, C. (2000) Monitoring *Piscirickettsia salmonis* by denaturant gel electrophoresis and competitive PCR. *Diseases of Aquatic Organisms* **41**, 19–29.

Heckert, R.A., Elankumaran, S., Milani, A. and Baya, A. (2001) Detection of a new *Mycobacterium* species in wild striped bass in the Chesapeake Bay. *Journal of Clinical Microbiology* **39**, 710–715.

Hedges, R.W., Smith, P. and Brazil, G. (1985) Resistance plasmids of aeromonads. *Journal of General Microbiology* **131**, 2091–2095.

Hellberg, H., Moksness, E. and Høie, S. (1996) Infection with atypical *Aeromonas salmonicida* in farmed common wolffish, *Anarhichas lupus*. *Journal of Fish Diseases* **19**, 329–332.

Henley, M. W. and Lewis, D. L. (1976) Anaerobic bacteria associated with epizootics in grey mullet (*Mugil cephalus*) and redfish (*Sciaenops acellata*) along the Texas coast. *Journal of Wildlife Diseases* **12**, 448–453.

Hennigan, M., Vaughan, L.M., Foster, T.J., Smith, P. and Gannon, F. (1989) Characterization of *Aeromonas salmonicida* strains using DNA probe technology. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 877–879.

Henríquez, V., Rojas, M.V. and Marshall, S.H. (2003) An alternative efficient procedure for purification of the obligate intracellular fish bacterial pathogen *Piscirickettsia salmonis*. *Applied and Environmental Microbiology* **69**, 6268–6271.

Henry, M.A. and Secombes, C.J. (2000) The A-layer influences the susceptibility of *Aeromonas salmonicida* to antibacterial peptides. *Fish & Shellfish Immunology* **10**, 637–642.

Henryon, M., Berg, P., Olesen, N.J., Kjaer, T.E., Slierendrecht, W.J., Jokumsen, A. and Lund, I. (2005) Selective breeding provides an approach to increase resistance of rainbow trout *Oncorhynchus mykiss*) to the diseases, enteric redmouth disease, rainbow trout fry syndrome, and viral haemorrhagic septicaemia. *Aquaculture* **250**, 621–636.

Heo, G.-J., Kawai, K. and Wakabayashi, H. (1990) Occurrence of *Flavobacterium branchiophila* associated with bacterial gill disease at a trout hatchery. *Fish Pathology* **25**, 99–105.

Herman, R.L. (1968) Fish furunculosis 1952–1966. *Transactions of the American Fisheries* **97**, 221–230.

Herman, R.L. (1972) A review of the prevention and treatment of furunculosis. *FI: EIFAC 72/SC II Symposium* **19**, 1–6.

Herwig, N. (1979) *Handbook of Drugs and Chemicals Used in the Treatment of Fish Diseases: A Manual of Fish Pharmacology and Material Medica*. Springfield, IL, Charles C. Thomas.

Hester, F.E. (1973) Fish health: A nationwide survey of problems and needs. *Progressive Fish Culturist* **35**, 11–18.

Hetrick, F.M., Hall, L.W., Wolski, S., Graves, W.C., Robertson, B.S. and Burton, D.T. (1984) Influence of chlorine on the susceptibility of striped bass (*Morone saxatilis*) to *Vibrio anguillarum*. *Canadian Journal of Fisheries and Aquatic Sciences* **9**, 1375–1380.

Hettiarachchi, D.C. and Cheong, C.H. (1994) Some characteristics of *Aeromonas hydrophila* and *Vibrio* species isolated from bacterial disease outbreaks in ornamental fish culture in Sri Lanka. *Journal of the National Science Council of Sri Lanka* **22**, 261–269.

Heuschmann-Brunner, G. (1965a) Nocardiose bei Fischen des Süsswassers und des Meeres. *Berliner und Munchener Tierärzliche Wochenschrift* **78**, 95–97.

Heuschmann-Brunner, G. (1965b) Ein Beitrag zur Erregerfrage der Infektiösen Bauchwassersucht des Karpfens. *Allgemeine Fischerei Zeitung* **90**, 41–49.

Heuschmann-Brunner, G. (1978) Aeromonads of the "*Hydrophila punctata* group" in freshwater fishes. *Archiv für Hydrobiologie* **83**, 99–125.

Hickman-Brenner, F.W., MacDonald, K.L., Steigerwalt, A.G., Fanning, G.R., Brenner, D.J. and Farmer, J.J. (1987) *Aeromonas veronii*, a new ornithine decarboxylase-positive species that may cause diarrhea. *Journal of Clinical Microbiology* **25**, 900–906.

Hikada, T., Yanohara, Y. and Shibata, T. (1983) On the causative bacteria of head ulcer disease in cultured eels. *Memoirs of the Faculty of Fisheries, Kagoshima University* **32**, 147–165.

Hikida, M., Wakabayashi, H., Egusa, S. and Masumura, K. (1979) *Flexibacter* sp., a gliding bacterium pathogenic to some marine fishes in Japan. *Bulletin of the Japanese Society of Scientific Fisheries* **45**, 421–428.

Hiney, M.P. and Smith, P.R. (1998) Validation of polymerase chain reaction-based techniques for proxy detection of bacterial fish pathogens: Framework, problems and possible solutions for environmental applications. *Aquaculture* **162**, 41–68.

Hiney, M., Dawson, M.T., Heery, D.M., Smith, P.R., Gannon, F. and Powell, R. (1992) DNA probe for *Aeromonas salmonicida*. *Applied and Enviromental Microbiology* **58**, 1039–1042.

Hiney, M.P., Kilmartin, J.J. and Smith, P.R. (1994) Detection of *Aeromonas salmonicida* in Atlantic salmon with asymptomatic furunculosis infections. *Diseases of Aquatic Organisms* **19**, 161–167.

Hirono, I., Tange, N. and Aoki, T. (1997a) Iron-regulated haemolysin gene from *Edwardsiella tarda*. *Molecular Microbiology* **24**, 851–856.

Hirono, I., Kato, M. and Aoki, T. (1997b) Identification of major antigenic proteins of *Pasteurella piscicida*. *Microbial Pathogenesis* **23**, 371–380.

Hirst, I.D. and Ellis, A.E. (1996) Utilization of transferrin and salmon serum as sources of iron by typical and atypical strains of *Aeromonas salmonicida*. *Microbiology* **142**, 1543–1550.

Hirst, I.D., Hastings, T.S. and Ellis, A.E. (1991) Siderophore production by *Aeromonas salmonicida*. *Journal of General Microbiology* **137**, 1185–1192.

Hirvelä-Koski, V. (2004) *Renibacterium salmoninarum*: Effect of hypochlorite treatment, and survival in water. *Diseases of Aquatic Organisms* **59**, 27–33.

Hirvelä-Koski, V., Koski, P. and Niiranen, H. (1994) Biochemical properties and drug resistance of *Aeromonas salmonicida* in Finland. *Diseases of Aquatic Organisms* **20**, 191–196.

Hirvelä-Koski, V., Pohjanvirta, T., Koski, P. and Sukura, A. (2006). Atypical growth of *Renibacterium salmoninarum* in subclinical infections. *Journal of Fish Diseases* **29**, 21–29.

Hispano, C., Nebra, Y. and Blanch, A.R. (1997) Isolation of *Vibrio harveyi* from an ocular lesion in the short sunfish (*Mola mola*). *Bulletin of the European Association of Fish Pathologists* **17**, 104–107.

Hiu, S.F., Holt, R.A., Sriranganathan, N., Seidler, R.J. and Fryer, J.L. (1984) *Lactobacillus piscicola*, a new species from salmonid fish. *International Journal of Systematic Bacteriology* **34**, 393–400.

Hjeltnes, B., Andersen, K., Ellingsen, H.-M. and Egidius, E. (1987) Experimental studies on the pathogenicity of a *Vibrio* sp. isolated from Atlantic salmon, *Salmo salar* L., suffering from Hitra disease. *Journal of Fish Diseases* **10**, 21–27.

Hjeltnes, B., Bergh, Ø., Wergeland, H. and Holm, J.C. (1995) Susceptibility of Atlantic cod *Gadus morhua*, halibut *Hippoglossus hippoglossus* and wrasse (Labridae) to *Aeromonas salmonicida* subsp. *salmonicida* and the possibility of transmission of furunculosis from farmed salmon *Salmo salar* to marine fish. *Diseases of Aquatic Organisms* **23**, 25–31.

Hodgkinson, J.L., Bucke, D. and Austin, B. (1987) Uptake of the fish pathogen, *Aeromonas salmonicida*, by rainbow trout (Salmo gairdneri L.). *FEMS Microbiology Letters* **40**, 207–210.

Hoel, K., Salonius, K. and Lillehaug, A. (1997) *Vibrio* antigens of polyvalent vaccines enhance the humoral immune response to *Aeromonas salmonicida* antigens in Atlantic salmon (*Salmo salar* L.). *Fish & Shellfish Immunology* **7**, 71–80.

Hoel, K., Reitan, L.J. and Lillehaug, A. (1998a) Immunological cross reactions between *Aeromonas salmonicida* and *Vibrio salmonicida* in Atlantic salmon (*Salmo salar* L.) and rabbit. *Fish & Shellfish Immunology* **8**, 171–182.

Hoel, K., Holstad, G. and Lillehaug, A. (1998b) Adjuvant activities of a *Vibrio salmonicida* bacterin on T-dependent and T-independent antigens in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology* **8**, 287–293.

Hoff, K.A. (1989) Survival of *Vibrio anguillarum* and *Vibrio salmonicida* at different salinities. *Applied and Environmental Microbiology* **55**, 1775–1786.

Hoffman, R., Popp, W. and Van der Graaff, S. (1984) Atypical BKD predominantly causing ocular and skin lesions. *Bulletin of the European Association of Fish Pathologists* **4**, 7–9.

Hoffman, R.W., Bell, G.R., Pfeil-Putzien, C. and Ogawa, M. (1989) Detection of *Renibacterium salmoninarum* in tissue sections by different methods: A comparative study with special regard to the indirect immunohistochemical peroxidase technique. *Fish Pathology* **24**, 101–104.

Høi, L., Larsen, J.L., Dalsgaard, I. and Dalsgaard, A. (1998) Occurrence of *Vibrio vulnificus* biotypes in Danish marine environments. *Applied and Environmental Microbiology* **64**, 7–13.

Høie, S., Heum, M. and Thoresen, O.F. (1996) Detection of *Aeromonas salmonicida* by polymerase chain reaction in Atlantic salmon vaccinated against furunculosis. *Fish & Shellfish Immunology* **6**, 199–206.

Høie, S., Heum, M. and Thoresen, O.F. (1997) Evaluation of a polymerase chain reaction-based assay for the detection of *Aeromonas salmonicida* subsp. *salmonicida* in Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* **30**, 27–35.

Høie, S., Dalsgaard, I., Aase, I.L., Heum, M., Thornton, J.M. and Powell, R. (1999) Polymerase chain reaction (PCR) based typing analysis of atypical isolates of the fish pathogen *Aeromonas salmonicida*. *Systematic and Applied Microbiology* **22**, 403–411.

Holder-Franklin, M.A., Thorpe, A. and Cormier, C.J. (1981) Comparison of numerical taxonomy and DNA–DNA hybridization in diurnal studies of river bacteria. *Canadian Journal of Microbiology* **27**, 1165–1184.

Hollis, D.G., Weaver, R.E., Steigerwalt, A.G., Wenger, J.D., Moss, C.W. and Brenner, D.J. (1989) *Francisella philomiragia* comb. nov. (formerly *Yersinia philomiragia*) and *Francisella tularensis* biovar *novicida* (formerly *Francisella novicida*) associated with human disease. *Journal of Clinical Microbiology* **27**, 1601–1608.

Holm, K.O. and Jørgensen, T. (1987) A successful vaccination of Atlantic salmon, *Salmo salar* L., against "Hitra disease" or coldwater vibriosis. *Journal of Fish Diseases* **10**, 85–90.

Holm, K.O., Strøm, E., Stemsvåg, K., Raa, J. and Jørgensen, T. (1985) Characteristics of a *Vibrio* sp. associated with the "Hitra Disease" of Atlantic salmon in Norwegian fish farms. *Fish Pathology* **20**, 125–129.

Holmes, B. (1992) Synonymy of *Flexibacter maritimus* Wakabayashi, Hikida & Masumura 1986 and *Cytophaga marina* Reichenbach 1989. *International Journal of Systematic Bacteriology* **42**, 185.

Holmes, B., Owen, R.J. and McMeekin, T.A. (1984) Genus: *Flavobacterium* Bergey, Harrison, Breed, Hammer & Huntoon 1923, 97^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 353–361.

Holmes, B., Pinning, C.A. and Dawson, C.A. (1986) A probabilistic matrix for the identification of Gram-negative, aerobic, non-fermentative bacteria that grow on nutrient agar. *Journal of General Microbiology* **132**, 1827–1842.

Holt, R.A., Conrad, J.F. and Fryer, J.L. (1975) Furanace for control of *Cytophaga psychrophila* the causal agent of cold-water disease in coho salmon. *Progressive Fish Culturist* **37**, 137–139.

Holt, R.A., Amandi, A., Rohovec, J.S. and Fryer, J.L. (1989) Relation of water temperature to bacterial cold-water disease in coho salmon, chinook salmon and rainbow trout. *Journal of Aquatic Animal Health* **1**, 94–101.

Horiuchi, M., Sato, T., Takagi, H. and Tozuka, K. (1980) Studies on rapid diagnosis system of main bacterial diseases of pond cultured eels in Japan—1. Basic investigations on the diagnosis of edwardsiellosis by direct immunofluorescence. *Fish Pathlology* **15**, 49–55.

Horne, J.H. (1928) Furunculosis in trout and the importance of carriers in the spread of disease. *Journal of Hygiene, Cambridge* **28**, 67–78.

Horne, M.T. and Baxendale, A. (1983) The adhesion of *Vibrio anguillarum* to host tissues and its role in pathogenesis. *Journal of Fish Diseases* **6**, 461–471.

Horne, M.T., Tatner, M., McDerment, S. and Agius, C. (1982) Vaccination of rainbow trout, *Salmo gairdneri* Richardson, at low temperatures and the long-term persistence of protection. *Journal of Fish Diseases* **5**, 343–345.

Horsley, R.W. (1973) The bacterial flora of the Atlantic salmon (*Salmo salar* L.) in relation to its environment. *Journal of Applied Bacteriology* **36**, 337–386.

Hoshina, T. (1956) An epidemic disease affecting rainbow trout in Japan. *Journal of Tokyo University, Fisheries* **42**, 15–16.

Hoshina, T. (1962) On a new bacterium *Paracolobactrum anguillimortiferum*. *Bulletin of the Japanese Society of Scientific Fisheries* **28**, 162–164.

Hoshina, T., Sano, T. and Morimoto, Y. (1958) A *Streptococcus* pathogenic to fish. *Journal of Tokyo University Fisheries* **44**, 57–58.

House, M.L., Bartholomew, J.L., Winton, J.R. and Fryer, J.L. (1999) Relative virulence of three isolates of *Piscirickettsia salmonis* for coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* **35**, 107–113.

Howard, S.P. and Buckley, J.T. (1985) Activation of the hole-forming toxin aerolysin by extracellular processing. *Journal of Bacteriology* **163**, 336–340.

Hsu, H.-M., Bowser, P.R. and Schachte, J.H. (1991) Development and evaluation of a monoclonal antibody-based enzyme-linked immunosorbant assay for the diagnosis of *Renibacterium salmoninarum* infection. *Journal of Aquatic Animal Health* **3**, 168–175.

Hsu, H.-M., Wooster, G.A. and Bowser, P.R. (1994) Efficacy of enrofloxacin for the treatment of salmonids with bacterial kidney disease, caused by *Renibacterium salmoninarum*. *Journal of Aquatic Animal Health* **3**, 220–223.

Hsu, H.-M., Bowser, P.R., Schachte Jr, J.H., Scarlett, J.M. and Babish, J.G. (1995) Winter field trials of enrofloxacin for the control of *Aeromonas salmonicida* infection in salmonids. *Journal of the World Aquaculture Society* **26**, 307–314.

Hsu, T.C., Waltman, W.D. and Shotts, E.B. (1981) Correlation of extracellular enzymatic activity and biochemical characteristics with regard to virulence of *Aeromonas hydrophila*. *Developments in Biological Standardization* **49**, 101–111.

Hsu, T.C., Shotts, E.B. and Waltman, W.D. (1983) A selective medium for the isolation of yellow pigmented bacteria associated with fish disease. *Newsletter for the Flavobacterium–Cytophaga Group* **3**, 29–30.

Hsu, T.C., Shotts, E.B. and Waltman, W.D. (1983) Quantitation of biochemical and enzymatic characteristics with pathogenicity of *Aeromonas hydrophila* complexes in fish. *Proceedings of the Republic of China–Japan Symposium on Fish Diseases*.

Hugh, R. and Leifson, E. (1953) The taxonomic significance of fermentative versus oxidative metabolism of carbohydrate by various Gram-negative bacteria. *Journal of Bacteriology* **66**, 24–26.

Huh, G.-J. and Wakabayashi, H. (1989) Serological characteristics of *Flavobacterium branchiophila* isolated from gill diseases of freshwater fishes in Japan, USA and Hungary. *Journal of Aquatic Animal Health* **1**, 142–147.

Huizinga, H.W., Esch, G.W. and Hazen, T.C. (1979) Histopathology of red-sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterus salmoides* (Lacépède). *Journal of Fish Diseases* **2**, 263–277.

Hunter, V.A., Knittel, M.D. and Fryer, J.L. (1980) Stress-induced transmission of *Yersinia ruckeri* infection from carriers to recipient steelhead trout *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **3**, 467–472.

Huntly, P.J., Coleman, G. and Munro, A.L.S. (1992) The nature of the lethal effect on Atlantic salmon, *Salmo salar* L., of a lipopolysaccharide-free phospholipase activity isolated from the extracellular products of *Aeromonas salmonicida*. *Journal of Fish Diseases* **15**, 99–102.

Husevåg, B., Lunestad, B.T., Johannessen, P.J., Enger, Ø. and Samuelsen, O.B. (1991) Simultaneous occurrence of *Vibrio salmonicida* and antibiotic-resistant bacteria in sediments at abandoned aquaculture sites. *Journal of Fish Diseases* **14**, 631–640.

Huss, H.H. and Eskildsen, U. (1974) Botulism in farmed trout caused by *Clostridium botulinum* type E. *Nordic Journal of Veterinary Medicine* **26**, 733–738.

Huss, H.H., Pedersen, A. and Cann, D.C. (1974a) The incidence of *Clostridium botulinum* in Danish trout farms. 1. Distribution in fish and their environment. *Journal of Food Technology* **9**, 445–450.

Huss, H.H., Pedersen, A. and Cann, D.C. (1974b) The incidence of *Clostridium botulinum* in Danish trout farms. 2. Measures to reduce contamination of the fish. *Journal of Food Technology* **9**, 451–458.

Hussain, I., Mackie, C., Cox, D., Alderson, R. and Birkbeck, T.H. (2000) Suppression of the humoral immune response of Atlantic salmon, *Salmo salar* L. by the 64 kDa serine protease of *Aeromonas salmonicida*. *Fish & Shellfish Immunology* **10**, 359–373.

Huys, G., Coopman, R., Janssen, P. and Kersters, K. (1996) High-resolution genotypic analysis of the genus *Aeromonas* by AFLP fingerprinting. *International Journal of Systematic Bacteriology* **46**, 572–580.

Huys, G., Kämpfer, P. and Swings, J. (2001) New DNA–DNA hybridization and phenotypic data on the species *Aeromonas ichthiosmia* and *Aeromonas allosaccharophila*: *A. ichthiosmia* is a later synonym of *A. veronii* Hickman-Brenner *et al.* 1987. *Systematic and Applied Microbiology* **24**, 177–182.

Huys, G., Kämpfer, P., Albert, M.J., Kúhn, I., Denys, R. and Swings, J. (2002) *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov., isolated from children with diarrhoea in Bangladesh, and extended description of *Aeromonas hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (Approved Lists 1980). *International Journal of Systematic and Evolutionary Microbiology* **52**, 705–712.

Ibrahim, A., Goebel, B.M., Liesack, W., Griffiths, M. and Stackebrandt, E. (1993) The phylogeny of the genus *Yersinia* based on 16S rDNA sequences. *FEMS Microbiology Letters* **114**, 173–178.

Igarishi, A. and Iida, T. (2002) A vaccinatioin trial using live cells of *Edwardsiella tarda* in tilapia. *Fish Pathology* **37**, 145–148.

Igarishi, A., Iida, T. and Crosa, J.H. (2002) Iron-acquisition ability of *Edwardsiella tarda* with involvement in its virulence. *Fish Pathology* **37**, 53–57.

Iida, T. and Wakabayashi, H. (1990) Relationship between iron acquisition ability and virulence of *Edwardsiella tarda*, the etiological agent of Paracolo disease in Japanese eel *Anguilla japonica*. In: Hirano, R. and Hanyu, I. (eds), *The Second Asian Fisheries Forum*. Manila, Asian Fisheries Society, pp. 667–670.

Iida, T., Wakabayashi, H. and Egusa, S. (1982) Vaccination for control of streptococcal disease in cultured yellowtail. *Fish Pathology* **16**, 201–206.

Iida, T., Furukawa, K., Sakai, M. and Wakabayashi, H. (1986) Non-haemolytic *Streptococcus* isolated from the brain of the vertebral deformed yellowtail. *Fish Pathology* **21**, 33–38.

Iida, T. and Sorimachi, M. (1994) Cultural characteristics of the bacterium causing jaundice of yellowtail, *Seriola quinqueradiata*. *Fish Pathology* **29**, 25–28.

Iida, T., Sakata, C., Kawatsu, H. and Kukuda, Y. (1997) Atypical *Aeromonas salmonicida* infection in cultured marine fish. *Fish Pathology* **32**, 65–66.

Inamura, H., Muroga, K. and Nakai, T. (1984) Toxicity of extracellular products of *Vibrio anguillarum*. *Fish Pathology* **19**, 89–96.

Inglis, V. and Richards, R.H. (1991) The *in vitro* susceptibility of *Aeromonas salmonicida* and other fish-pathogenic bacteria to 29 antimicrobial agents. *Journal of Fish Diseases* **14**, 641–650.

Inglis, V., Robertson, D., Miller, K., Thompson, K.D. and Richards, R.H. (1996) Antibiotic protection against recrudescence of latent *Aeromonas salmonicida* during furunculosis vaccination. *Journal of Fish Diseases* **19**, 341–348.

Irianto, A. and Austin, B. (2002) Probiotics in aquaculture. *Journal of Fish Diseases* **25**, 633–642.

Irianto, A. and Austin, B. (2003) Use of dead probiotic cells to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **26**, 59–62.

Irianto, A., Robertson, P.A.W. and Austin, B. (2003) Oral administration of formalin–inactivated cells of *Aeromonas hydrophila* A3-51 controls infection by atypical *Aeromonas salmonicida* in goldfish, *Carassius auratus* (L.). *Journal of Fish Diseases* **26**, 117–120.

Irie, T., Watarai, S., Iwasaki, T. and Kodama, H. (2005) Protection against experimental *Aeromonas salmonicida* infection in carp by oral immunisation with bacterial antigen entrapped liposomes. *Fish & Shellfish Immunology* **18**, 235–242.

Ishiguro, E.E., Kay, W.W., Ainsworth, T., Chamberlain, J.B., Austen, R.A., Buckley, J.T. and Trust, T.J. (1981) Loss of virulence during culture of *Aeromonas salmonicida* at high temperature. *Journal of Bacteriology* **148**, 333–340.

Ishimaru, K. and Muroga, K. (1997) Taxonomical re-evaluation of two pathogenic *Vibrio* species isolated from milkfish and swimming crab. *Fish Pathology* **32**, 59–64.

Ishimaru, K., Akagawa-Matsushita, M. and Muroga, K. (1996) *Vibrio ichthyoenteri* sp. nov., a pathogen of Japaneses flounder (*Paralichthys olivaceus*). *International Journal of Systematic Bacteriology* **46**, 155–159.

Isik, K., Chun, J., Hah, Y.C. and Goodfellow, M. (1999) *Nocardia salmonicida* nom. rev., a fish pathogen. *International Journal of Systematic Bacteriology* **49**, 833–837.

Itami, T. and Kusuda, R. (1980) Studies on spray vaccination against vibriosis in cultured ayu. II. Duration of vaccination efficacy and effect of different vaccine preparation. *Bulletin of the Japanese Society of Scientific Fisheries* **46**, 699–703.

Itami, T., Kondo, M., Uozu, M., Suganuma, A., Abe, T., Nakagawa, A., Suzuki, N. and Takahashi, Y. (1996) Enhancement of resistance against *Enterococcus serioloicida* infection in yellowtail, *Seriola quinqueradiata* (Temminck & Schlegel), by oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. *Journal of Fish Diseases* **19**, 185–187.

Itano, T. and Kawakami, H. (2002) Drug susceptibility of recent isolates of *Nocardia seriolae* from cultured fish. *Fish Pathology* **37**, 152–153.

Itano, T., Kawakami, H., Kono, T. and Sakai, M. (2006) Detection of fish nocardiosis by loop-mediated isothermal amplification. *Journal of Applied Microbiology* **100**, 1381–1387.

Itano, T., Kawakami, H., Kono, T. and Sakai, M. (2006a) Experimental induction of nocardiosis in yellowtail, *Seriola quinqueradiata* Temminck & Schlegel by artificial challenge. *Journal of Fish Diseases* **29**, 529–534.

Itano, T., Kawakami, H., Kono, T. and Sakai, M. (2006b) Live vaccine trials against nocardiosis in yellowtail *Seriola quinqueradiata*. *Aquaculture* **261**, 1175–1180.

Iverson, J.B. (1971) Strontium chloride B and E enrichment broth media for the isolation of *Edwardsiella*, *Salmonella* and *Arizona* species from tiger snakes. *Journal of Hygiene, Cambridge* **69**, 323–330.

Iwamoto, Y., Suzuki, Y., Kurita, A., Watanabe, Y., Shimizu, T., Ohgami, H. and Yanagihara, Y. (1995) *Vibrio trachuri* sp. nov., a new species isolated from diseased Japanese horse mackerel. *Microbiology and Immunology* **39**, 831–837.

Iwanowicz, L.R., Griffin, A.R., Cartwright, D.D. and Blazer, V.S. (2006) Mortality and pathology in brown bullheads *Amieurus nebulosus* associated with a spontaneous *Edwardsiella ictaluri* outbreak under tank culture conditions. *Diseases of Aquatic Organisms* **70**, 219–225.

Izumi, S. and Aranishi, F. (2004) Plasmid profiling of Japanese *Flavobacterium psychrophilum* isolates. *Journal of Aquatic Animal Health* **16**, 99–103.

Izumi, S. and Wakabayashi, H. (1997) Use of PCR to detect *Cytophaga psychrophila* from apparently healthy juvenile ayu and coho salmon eggs. *Fish Patholology* **32**, 169–173.

Izumi, S., Liu, H., Aranishi, F. and Wakabayashi, H. (2003) A novel serotype of *Flavobacterium psychrophilum* detected using antiserum against an isolate from amago, *Oncorhynchus masou rhodurus* Jordan & Gilbert in Japan. *Journal of Fish Diseases* **26**, 677–680.

Izumi, S., Aranishi, F. and Wakabayashi, H. (2003a) Genotyping of *Flavobacterium psychrophilum* using PCR-RFLP analysis. *Diseases of Aquatic Organisms* **56**, 207–214,

Izumi, S., Fujii, H. and Aranishi, F. (2005) Detection and identification of *Flavobacterium psychrophilum* from gill washings and benthic diatoms by PCR-based sequencing analysis. *Journal of Fish Diseases* **28**, 559–564.

Jacquez, G.M., Ziskowski, J. and Rolfe, F.J. (1994) Criteria for the evaluation of alternative environmental monitoring variables: Theory and an application using winter flounder (*Pleuronectes americanus*) and Dover sole (*Microstomus pacificus*). *Environmental Monitoring and Assessment* **30**, 275–290.

Janssen, W.A. and Surgalla, M.J. (1968) Morphology, physiology and serology of a *Pasteurella* species pathogenic for white perch (*Roccus americanus*). *Journal of Bacteriology* **96**, 1606–1610.

Jansson, E. and Ljungberg, O. (1998) Detection of humoral antibodies to *Renibacterium salmoninarum* in rainbow trout *Oncorhynchus mykiss* and Atlantic salmon *Salmo salar* challenged by immersion and in naturally infected populations. *Diseases of Aquatic Organisms* **33**, 93–99.

Jansson, E., Hongslo, T., Lindberg, R., Ljungberg, O. and Svensson, B.-M. (1991) Detection of *Renibacterium salmoninarum* and *Yersinia ruckeri* by the peroxidase–antiperoxidase immunohistochemical technique in melanin-containing cells of fish tissue. *Journal of Fish Diseases* **14**, 689–692.

Jansson, E., Hongslo, T., Höglund, J. and Ljungberg, O. (1996) Comparative evaluation of bacterial culture and two ELISA techniques for the detection of *Renibacterium salmoninarum* antigens in salmonid kidney tissues. *Diseases of Aquatic Organisms* **27**, 197–206.

Jansson, E., Hongslo, T., Johannisson, A., Pilström, L., Timmusk, S. and Norrgren, L. (2003) Bacterial kidney disease as a model for studies of cell mediated immunity in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology* **14**, 347–362.

Jensen, S., Samuelsen, O.B., Andersen, K., Torkildsen, L., Lambert, C., Choquet, G., Paillard, C. and Bergh, Ø. (2003) Characterization of strains of *Vibrio splendidus* and *V. tapetis* isolated from corkwing wrasse *Symphodus melops* suffering vibriosis. *Diseases of Aquatic Organisms* **53**, 25–31.

Jensen, W.I., Owen, C.R. and Jellison, W.J. (1969) *Yersinia philomiragia* sp. n., a new member of the *Pasteurella* group of bacteria, naturally pathogenic for the muskrat (*Ondatra zibethica*). *Journal of Bacteriology* **100**, 1237–1241.

Jia, X., Patrzykat, A., Devlin, R.H., Ackerman, P.A., Iwama, G.K. and Hancock, R.E.W. (2000) Antimicrobial peptides protect coho salmon from *Vibrio anguillarum* infections. *Applied and Environmental Microbiology* **66**, 1928–1932.

Jiwa, S.F.H. (1983) Enterotoxigenicity, haemagglutination and cell-surface hydrophobicity in *Aeromonas hydrophila*, *A. sobria* and *A. salmonicida*. *Veterinary Microbiology* **8**, 17–34.

Jo, Y. (1978) Therapeutic experiments on red spot disease. *Fish Pathology* **13**, 41–42.

Johansen, L.-H. and Sommer, A.-I. (2001) Infectious pancreatic necrosis virus infection in Atlantic salmon *Salmo salar* post-smolts affects the outcome of secondary infections with infectious salmon anaemia virus and *Vibrio salmonicida*. *Diseases of Aquatic Organisms* **47**, 109–117.

Johnsen, B.O. and Jensen, A.J. (1994) The spread of furunculosis in salmonids in Norwegian rivers. *Journal of Fish Biology* **45**, 47–55.

Johnson, C.M., Tatner, M.F. and Horne, M.T. (1985) Comparison of the surface properties of seven strains of a fish pathogen, *Aeromonas salmonicida*. *Journal of Fish Biology* **27**, 445–458.

Johnson, G.R., Wobeser, G. and Rouse, B.T. (1974) Indirect fluorescent antibody technique for detection of RM bacterium of rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **31**, 1957–1959.

Johnson, G.S. (1977) Immunological studies on *Vibrio anguillarum*. *Aquaculture* **10**, 221–230.

Johnson, H.E. and Brice, R.F. (1952) Observations on columnaris in salmon and trout. *Progressive Fish Culturist* **14**, 104–109.

Johnson, K.A. and Amend, D.F. (1983a) Comparison of efficacy of several delivery methods using *Yersinia ruckeri* bacterin on rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **6**, 331–336.

Johnson, K.A. and Amend, D.F. (1983b) Efficacy of *Vibrio anguillarum* and *Yersinia ruckeri* bacterins applied by oral and anal intubation of salmonids. *Journal of Fish Diseases* **6**, 473–476.

Johnson, K.A. and Amend, D.F. (1984) Potential for immersion vaccination against *Aeromonas salmonicida*. *Journal of Fish Diseases* **7**, 101–105.

Johnson, K.A., Flynn, J.K. and Amend, D.F. (1982a) Onset of immunity in salmonid fry vaccinated by direct immersion in *Vibrio anguillarum* and *Yersinia ruckeri* bacterins. *Journal of Fish Diseases* **5**, 197–205.

Johnson, K.A., Flynn, J.K. and Amend, D.F. (1982b) Duration of immunity in salmonid fry vaccinated by direct immersion with *Yersinia ruckeri* and *Vibrio anguillarum* bacterins. *Journal of Fish Diseases* **5**, 207–213.

Johnson, R., Colwell, R.R., Sakazaki, R. and Tamura, K. (1975) Numerical taxonomy study of the Enterobacteriaceae. *International Journal of Systematic Bacteriology* **25**, 12–37.

Jones, G.L., Hebert, G.A. and Cherry, W.B. (1978) Fluorescent antibody techniques and bacterial application. *United States Department of Health, Education and Welfare Publication Number (CDC) 78–83 64*, 118 pp.

Jones, M.W. and Cox, D.I. (1999) Clinical disease in sea farmed Atlantic salmon (*Salmo salar*) associated with a member of the family Pasteurellaceae: A case history. *Bulletin of the European Association of Fish Pathologists* **19**, 75–78.

Jones, S.R.M., Markham, R.J.F., Groman, D.B. and Cusack, R.R. (1998) Virulence and antigenic characteristics of a cultured Rickettsiales-like organism isolated from farmed Atlantic salmon *Salmo salar* in eastern Canada. *Diseases of Aquatic Organisms* **33**, 25–31.

Jónsdóttir, H., Malmquist, H.J., Snorrason, S.S., Gudbergsson, G. and Gudmundsdóttir, S. (1998) Epidemiology of *Renibacterium salmoninarum* in wild Arctic charr and brown trout in Iceland. *Journal of Fish Biology* **53**, 322–339.

Joosten, P.H.M., Kruijer, W.J. and Rombout, J.H.W.M. (1996) Anal immunisation of carp and rainbow trout with different fractions of *Vibrio anguillarum* bacterin. *Fish & Shellfish Immunology* **6**, 541–551.

Joosten, P.H.M., Tiemersma, E., Threels, A., Caumartin-Dhieux, C. and Rombout, J.H.W.M. (1997) Oral vaccination of fish against *Vibrio anguillarum* using alginate microparticles. *Fish & Shellfish Immunology* **7**, 471–485.

Jørgensen, T., Midling, K., Espelid, S., Nilsen, R. and Stensvåg, K. (1989) *Vibrio salmonicida*, a pathogen in salmonids, also causes mortality in net-pen captured cod (*Gadus morhua*). *Bulletin of the European Association of Fish Pathologists* **9**, 42–44.

Juíz-Río, S., Osorio, C.R., de Lorenzo, V. and Lemos, M.L. (2005) Subtractive hybridization reveals a high genetic diversity in the fish pathogen *Photobacterium damselae* subsp. *piscicida*: Evidence of a SXT-like element. *Microbiology* **151**, 2659–2669.

Jung, T.S., Thompson, K. and Adams, A. (2000) Identification of sialic acid on *Photobacterium damsela* subspecies *piscicida*: Possible role in cell adhesion and survival in the fish host. *Fish & Shellfish Immunology* **10**, 285.

Juni, E. (1984) Genus III: *Acinetobacter* Brisou & Prevot 1954, 727^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 303–307.

Juni, E. (2005) Genus II: *Acinetobacter* Brisou & Prévot 1954, 727^{AL}. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 425–437.

Juni, E. and Bøvre, K. (2005) Genus I: *Moraxella* Lwoff 1939, 173, emend. Henriksen & Bøvre 1968, 391^{AL}. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 417–425.

Jutfelt, F., Olsen, R.E., Glette, J., Ringø, E. and Sundell, K. (2006) Translocation of viable *Aeromonas salmonicida* across the intestine of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **29**, 255–262.

Kaattari, I.M., Rhodes, M.W., Kator, H. and Kaattari, S.L. (2005) Comparative analysis of mycobacterial infections in wild striped bass *Morone saxatilis* from Chesapeake Bay. *Diseases of Aquatic Organisms* **67**, 125–132.

Kaattari, I.M., Rhodes, M.W., Kaattari, S.L. and Shotts, E.B. (2006) The evolving story of *Mycobacterium* tuberculosis clade members detected in fish. *Journal of Fish Diseases* **29**, 509–520.

Kajita, Y., Sakai, M., Atsuta, S. and Kobayashi, M. (1990) The immunomodulatory effects of levamisole on rainbow trout, *Oncorhynchus mykiss*. *Fish Pathology* **25**, 93–98.

Kaku, Y., Yamada, Y. and Wakabayashi, H. (1999) Characterization of atypical *Aeromonas salmonicida* isolated from an epizootic ulcerative disease in carp (*Cyprinus carpio*). *Fish Pathology* **34**, 155–162.

Kamaishi, T., Fukuda, Y., Nishiyama, M., Kawakami, H., Matsuyama, T., Yoshinaga, T. and Oseko, N. (2005) Identification and pathogenicity of intracellular *Francisella* bacterium in three-line grunt *Parapristipoma trilineatum*. *Fish Pathology* **40**, 67–71.

Kanai, K. and Takagi, Y. (1986) Alpha-hemolytic toxins of *Aeromonas hydrophila* produced *in vitro*. *Fish Pathology* 21, 245–250.

Kanai, K., Notohara, M., Kato, T., Shutou, K. and Yoshikoshi, K. (2006) Serological characterization of *Streptococcus iniae* strains isolated from cultured fish in Japan. *Fish Pathology* **41**, 57–66.

Kaneko, T. and Colwell, R.R. (1974) Distribution of *Vibrio parahaemolyticus* and related organisms in the Atlantic Ocean off South Carolina and Georgia. *Applied Microbiology* **28**, 1009–1017.

Kanemori, Y., Nakai, T. and Muroga, K. (1987) The role of extracellular protease produced by *Vibrio anguillarum*. *Fish Pathology* **22**, 153–158.

Kanetsuna, F. and Bartoli, A. (1972) A simple chemical method to differentiate *Mycobacterium* from *Nocardia*. *Journal of General Microbiology* **70**, 209–212.

Kanno, T., Nakai, T. and Muroga, K. (1990) Scanning electron microscopy on the skin surface of ayu *Plecoglossus altivelis* infected with *Vibrio anguillarum*. *Diseases of Aquatic Organisms* **8**, 73–75.

Kaper, J.B., Sayler, G.S., Baldini, M.M. and Colwell, R.R. (1977) Ambient-temperature primary nonselective enrichment for isolation of *Salmonella* spp. from an estuarine environment. *Applied and Environmental Microbiology* **33**, 829–835.

Kaper, J.B., Seidler, R.J., Lockman, H. and Colwell, R.R. (1979) A medium for the presumptive identification of *Aeromonas hydrophila* and Enterobacteriaceae. *Applied and Environmental Microbiology* **38**, 1023–1026.

Kaper, J.B., Lockman, H., Remmers, E.F., Kristensen, K. and Colwell, R.R. (1983) Numerical taxonomy of vibrios isolated from estuarine environments. *International Journal of Systematic Bacteriology* **33**, 229–255.

Kapperud, G. (1981) Survey on the reservoirs of *Yersinia enterocolitica* and *Yersinia enterocolitica*-like bacteria in Scandinavia. *Acta Pathologica et Microbiologica, Scandinavia Section B* **89**, 29–35.

Kariya, T., Kubota, S., Nakamura, Y. and Kira, K. (1968) Nocardial infection in cultured yellowtail *Seriola quinqueradiata* and *S. purpurascens*. 1. Bacteriological study. *Fish Pathology* **3**, 16–23.

Karunasagar, I., Segar, K., Karunasagar, I., Ali, P.K.M.M. and Jeyasekaran, G. (1990) Virulence of *Aeromonas hydrophila* strains from fish ponds and infected fishes. In: Chang, S.-T., Chan, K.-Y. and Norman, Y.S.W. (eds), *Recent Advances in Biotechnology and Applied Biology*. Hong Kong, Chinese University Press.

Karunasagar, I., Karunasagar, I. and Pai, R. (1992) Systemic *Citrobacter freundii* infection in common carp, *Cyprinus carpio* L., fingerlings. *Journal of Fish Diseases* **15**, 95–98.

Karlsson, K.A. (1962) An investigation of the *Aeromonas salmonicida* haemolysin. *Ninth Nordic Veterinary Cogress, Kobenhavn.*

Karlsson, K.A. (1964) Serological studies of *Aeromonas salmonicida. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* **194**, 73–80.

Kashiwagi, S., Sugimoto, N., Watanabe, K., Ohta, S. and Kusuda, R. (1977a) Chemotherapeutic studies on sodium nifurstyrenate against *Streptococcus* infection in cultured yellowtails—I. *In vitro* studies on sensitivity and bacteriocidal effect. *Fish Pathology* **12**, 11–14.

Kashiwagi, S., Sugimoto, N., Ohta, S. and Kusuda, R. (1977b) Chemotherapeutical studies on sodium nifurstyrenate against*Streptococcus* infection in cultured yellowtail—II. Effect of sodium nifurstyrenate against experimental streptococcal infection. *Fish Pathology* **12**, 157–162.

Kawahara, E. and Kusuda, R (1987). Direct fluorescent antibody technique for diagnosis of bacterial disease in eel. *Nippon Suisan Gakkaishi* **53**, 395–399.

Kawahara, E., Kawai, K. and Kusuda, R. (1989) Invation of *Pasteurella piscicida* in tissues of experimentally infected yellowtail *Seriola quinqueradiata. Nippon Suisan Gakkaishi* **55**, 499–501.

Kawahara, E., Oshima, S. and Nomura, S. (1990) Toxicity and immunogenicity of *Aeromonas salmonicida* extracellular products to salmonids. *Journal of Fish Diseases* **13**, 495–503.

Kawai, K. and Kusuda, R. (1995) A review: *Listonella anguillarum* infection in ayu, *Plecoglossus altivelis*, and its prevention by vaccination. *Israeli Journal of Aquaculture Bamidgeh* **47**, 173–177.

Kawai, K., Yamamoto, S. and Kusuda, R. (1989) Plankton-mediated oral delivery of *Vibrio anguillarum* vaccine to juvenile ayu. *Nippon Suisan Gakkaishi* **55**, 35–40.

Kawakami, H., Shinohara, N., Fukuda, Y., Yamashita, H., Kihara, H. and Sakai, M. (1997) The efficacy of lipopolysaccharide mixed chloroform-killed cell (LPS-CKC) bacterin of *Pasteurella piscicida* on yellowtail, *Seriola quinqueradiata. Aquaculture* **154**, 95–105.

Kawakami, K. and Kusuda, R. (1989) *In vitro* effect of some chemotherapeutics on the causative of *Mycobacterium* infection in yellowtail. *Nippon Suisan Gakkaishi* **55**, 2111–2114.

Kawakami, K. and Kusuda, R. (1990) Efficacy of rifampicin, streptomycin and erythromycin against experimental *Mycobacterium* infection in cultured yellowtail. *Nippon Suisan Gakkaishi* **56**, 51–53.

Kawanishi, M., Kojima, A., Ishihara, K., Esaki, H., Kijima, M., Takahashi, T., Suzuki, S. and Tamura, Y. (2005) Drug resistance and pulsed-field gel electrophoresis patterns of *Lactococcus garvieae* isolates from cultured *Seriola* (yellowtail, amberjack and kingfish) in Japan. *Letters in Applied Microbiology* **40**, 322–328.

Kawanishi, M., Yoshida, T., Yagashiro, S., Kijima, M., Yagyu, K., Nakai, T., Murakami, M., Morita, H. and Suzuki, S. (2006) Differences between *Lactococcus garvieae* isolated from the genus *Seriola* in Japan and those isolated from other animals (trout, terrestrial animals from Europe) with regard to pathogenicity, phage susceptibility and genetic characterization. *Journal of Applied Microbiology* **101**, 496–504.

Kawula, T.H., Lelivelt, M.J. and Orndorff, P.E. (1996) Using a new inbred fish model and cultured fish tissue cells to study *Aeromonas hydrophila* and *Yersinia ruckeri* pathogenesis. *Microbial Pathogenesis* **20**, 119–125.

Kay, W.W. and Trust, T.J. (1991) Form and functions of the regular surface array (S-layer) of *Aeromonas salmonicida*. *Experientia* **47**, 412–414.

Kay, W.W., Buckley, J.T., Ishiguro, E.E., Phipps, B.M., Monette, J.P.L. and Trust, T.J. (1981) Purification and disposition of a surface protein associated with virulence of *Aeromonas salmonicida*. *Journal of Bacteriology* **147**, 1077–1084.

Kay, W.W., Phipps, B.M., Ishiguro, E.E. and Trust, T.J. (1985) Porphyrin binding by the surface array virulence protein of *Aeromonas salmonicida*. *Journal of Bacteriology* **164**, 1332–1336.

Kent, M.L. (1982) Characteristics and identification of *Pasteurella* and *Vibrio* species pathogenic to fishes using API-20E (Analytab Products) multitube test strips. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 1725–1729.

Kent, M.L., Dungan, C.F., Elston, R.A. and Holt, R.A. (1988) *Cytophaga* sp. (Cytophagales) infection in seawater pen-reared Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* **4**, 173–179.

Kent, M.L., Groff, J.M., Morrison, J.K., Yasutake, W.T. and Holt, R.A. (1989) Spiral swimming behavior due to cranial and vertebral lesions associated with *Cytophaga psychrophila* infections in salmonid fishes. *Diseases of Aquatic Organisms* **5**, 11–16.

Kersters, K. and De Ley, J. (1984) Genus: *Alcaligenes* Castellani & Chalmers 1919, 936^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. I. Baltimore, MD, Williams & Wilkins, pp. 361–373.

Ketola, H.G. (1983) Requirement for dietary lysine and arginine by fry of rainbow trout. *Journal of Animal Science* **56**, 101–107.

Khalil, A.H. and Mansour, E.H. (1997) Toxicity of crude extracellular products of *Aeromonas hydrophila* in tilapia, *Tilapia nilotica*. *Letters in Applied Microbiology* **25**, 269–273.

Khan, R.A. (1987) Crude oil and parasites of fish. *Parasitology Today* **3**, 99–100.

Khoo, L., Dennis, P.M. and Lewbat, G.A. (1995) Rickettsia-like organisms in the blue-eyed plecostomus, *Panaque suttoni* (Eigenmann & Eigenmann). *Journal of Fish Diseases* **18**, 157–163.

Kiehn, E.D. and Pacha, R.E. (1969) Characterization and relatedness of marine vibrios pathogenic to fish: Deoxyribonucleic acid homology and base composition. *Journal of Bacteriology* **100**, 1248–1255.

Kiiyukia, C., Nakajima, A., Nakai,, T., Muroga, K., Kawakami, H. and Hashimoto, H. (1992) *Vibrio cholerae* non-01 isolated from ayu fish (*Plecoglossus altivelis*) in Japan. *Applied and Environmental Microbiology* **58**, 3078–3082.

Kilian, M. (1976) A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. *Journal of General Microbiology* **93**, 9–62.

Kim, D.-H. and Austin, B. (2006) Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. *Fish & Shellfish Immunology* **21**, 513–524.

Kim, D.-H., Han, H.-J., Kim, S.-M., Lee, D.-C. and Park, S.-I. (2004) Bacterial enteritis and the development of the larval digestive tract in olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel). *Journal of Fish Diseases* **27**, 497–505.

Kim, E.-H., Yoshida, T. and Aoki, T. (1993) Detection of R plasmid encoded with resistance to florfenicol in *Pasteurella piscicida*. *Fish Pathology* **28**, 165–170.

Kim, K.H., Hwang, Y.J. and Bai, S.C. (1999) Resistance to *Vibrio alginolyticus* in juvenile rockfish (*Sebastes schlegeli*) fed diets containing difference doses of aloe. *Aquaculture* **180**, 13–21.

Kimura, H. and Kusuda, R. (1979) Studies on the pathogenesis of streptococcal infection in cultured yellowtails *Seriola* spp.: Effect of the cell free culture on experimental streptococcal infection. *Journal of Fish Disease* **2**, 501–510.

Kimura, H. and Kusuda, R. (1982) Studies on the pathogenesis of streptococcal infection in cultured yellowtails *Seriola* spp.: Effect of crude exotoxin fractions from cell free culture on experimental streptococcal infection. *Journal of Fish Diseases* **5**, 471–478.

Kimura, T. (1969a) A new subspecies of *Aeromonas salmonicida* as an etiological agent of furunculosis on "Sakuramasu" (*Oncorhynchus masou*) and pink salmon (*O. gorbuscha*) rearing for maturity. Part 1. On the serological properties. *Fish Pathology* **3**, 34–44.

Kimura, T. (1969b) A new subspecies of *Aeromonas salmonicida* as an etiological agent of furunculosis on "Sakuramasu" (*Oncorhynchus masou*) and pink salmon (*O. gorbuscha*) rearing for maturity. Part 2. On the morphological and physiological properties. *Fish Pathology* **3**, 45–52.

Kimura, T. (1970) Studies on a bacterial disease of adult "Sakuramasu" (*Oncorhynchus masou*) and pink salmon (*O. gorbuscha*) reared for maturity. *Scientific Report of the Hokkaido Salmon Hatchery* **24**, 9–100.

Kimura, T. and Yoshimizu, M. (1981) A coagglutination test with antibody-sensitized staphylococci for rapid and simple diagnosis of bacterial kidney disease (BKD). *Developments in Biological Standardization* **49**, 135–148.

Kimura, T. and Yoshimizu, M. (1983) Coagglutination test with antibody-sensitized staphylococci for rapid and simple serological diagnosis of fish furunculosis. *Fish Pathology* **17**, 259–262.

Kimura, T. and Yoshimizu, M. (1984) Coagglutination test with antibody-sensitized staphylococci for rapid serological identification of rough strains of *Aeromonas salmonicida*. *Bulletin of the Japanese Society of Scientific Fisheries* **50**, 439–442.

Kimura, T., Wakabayashi, H. and Kudo, S. (1978a) Studies on bacterial gill disease in salmonids. 1. Selection of bacterium transmitting gill disease. *Fish Pathology* **12**, 233–242.

Kimura, T., Ezura, K., Tajima, K. and Yoshimizu, M. (1978b) Serological diagnosis of bacterial kidney disease (BKD): Immunodiffusion test by heat stable antigen extracted from infected kidney. *Fish Pathology* **13**, 103–108.

King, C.H. and Shotts, E.B. (1988) Enhancement of *Edwardsiella tarda* and *Aeromonas salmonicida* through ingestion by the ciliated protozoan *Tetrahymena pyriformis*. *FEMS Microbiology Letters* **51**, 95–100.

King, E.O., Ward, M.K. and Raney, D.E. (1954) Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine* **44**, 301–307.

Kinne, O. (1980) *Diseases of Marine Animals*, Vol. 1. *General Aspects, Protozoa to Gastropoda*. Chichester, U.K., John Wiley & Sons.

Kintsuji, H., Ninomiya, K. and Yamamoto, M. (2006) Vaccination with acetone-dry bacterin against bacterial hemorrhagic ascites of ayu *Plecoglossus altivelis*. *Fish Pathology* **41**, 121–122.

Kirchhoff, H. and Rosengarten, R. (1984) Isolation of a motile mycoplasma from fish. *Journal of General Microbiology* **130**, 2439–2445.

Kirchhoff, H., Beyenne, P., Fischer, M., Flossdorf, J., Heitmann, J., Khattab, B., Lopatta, D., Rosengarten, R., Seidel, G. and Yousef, C. (1987) *Mycoplasma mobile* sp. nov., a new species from fish. *International Journal of Systematic Bacteriology* **37**, 192–197.

Kirk, R.S. and Lewis, J.W. (1993) An evaluation of pollutant induced changes in the gills of rainbow trout using scanning electron microscopy. *Environmental Technology* **14**, 577–585.

Kitao, T. (1982a) The methods for detection of *Streptococcus* sp. causative bacteria of streptococcal disease of cultured yellowtail (*Seriola quinqueradiata*). *Fish Pathology* **17**, 17–26.

Kitao, T. (1982b) Erythromycin: The application to streptococcal infection in yellowtails. *Fish Pathology* **17**, 77–85.

Kitao, T. and Yoshida, Y. (1986) Effect of an immunopotentiator on *Aeromonas salmonicida* infection in rainbow trout (*Salmo gairdneri*). *Veterinary Immunology and Immunopathology* **12**, 287–296.

Kitao, T., Aoki, T. and Iwata, K. (1979) Epidemiological study on streptococcicosis of cultured yellowtail (*Seriola quinqueradiata*)—I. Distribution of *Streptococcus* sp. in sea water and muds around yellowtail farms. *Bulletin of the Japanese Society of Scientific Fisheries* **45**, 567–572.

Kitao, T., Aoki, T. and Sakoh, R. (1981) Epizootics caused by β-haemolytic *Streptococcus* species in cultured freshwater fish. *Fish Pathology* **15**, 301–307.

Kitao, T., Aoki, T., Fukodome, M., Kawano, K., Wada, Y. and Mizuno, Y. (1983) Serotyping of *Vibrio anguillarum* isolated from diseased freshwater fish in Japan. *Journal of Fish Diseases* **6**, 175–181.

Kitao, T., Yoshida, T., Aoki, T. and Fukudome, M. (1984) Atypical *Aeromonas salmonicida*, the causative agent of an ulcer disease of eel occurred in Kagoshima Prefecture. *Fish Pathology* **19**, 113–117.

Klein, B.U., Kleingeld, D.W. and Böhm, K.H. (1993) First isolations of *Plesiomonas shigelloides* from samples of cultured fish in Germany. *Bulletin of the European Association of Fish Pathologists* **13**, 70–72.

Klein, B.U., Kleingeld, D.W. and Böhm, K.H. (1994) First isolation of a non-motile/Tween 80 negative *Yersinia ruckeri* strain in Germany. *Bulletin of the European Association of Fish Pathologists* **14**, 165–166.

Klesius, P.H. (1992) Carrier state of channel catfish infected with *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* **4**, 220–230.

Klesius, P. (1994) Transmission of *Edwardsiella ictaluri* from infected, dead to non-infected channel catfish. *Journal of Aquatic Animal Health* **6**, 1–12.

Klesius, P., Evans, J., Shoemaker, C., Yeh, H., Goodwin, A.E., Adams, A. and Thompson, K. (2006) Rapid detection and identification of *Streptococcus iniae* using a monoclonal antibody-based indirect fluorescent antibody technique. *Aquaculture* **258**, 180–186.

Klontz, G.W. (1963) Oral immunization of rainbow trout against redmouth. In: *Proceedings of the Northwest Fish Culture Conference, Olympia, Washington, 5–6 December 1963*, pp. 121.

Klontz, G.W. (1968) Oral immunization of coho salmon against furunculosis. In: *Progress in Sport Fishery Research. B.S.R.W. Resource Publication* **39**, 81–82.

Klontz, G. (1978) Prevention of bacterial kidney disease in adult and juvenile salmonids. *Proceedings of the Joint 3rd Biennial Fish Health Section and 9th Annual Midwest Fish Disease Workshops, Kansas City*, pp. 11–14.

Klontz, G.W. and Anderson, D.P. (1968) Fluorescent antibody studies of isolates of *Aeromonas salmonicida*. *Bulletin de l'Office International des Epizooties* **69**, 1149–1157.

Klontz, G.W. and Anderson, D.P. (1970) Oral immunization of salmonids: A review. In: Snieszko, S.F. (ed.), *Symposium on Diseses of Fishes and Shellfishes*. Washington, D.C., American Fisheries Society, Special Publication No. 5, pp. 16–20.

Klontz, G.W. and Huddleston, T.R. (1976) *Control of Enteric Redmouth Disease. A Twelve Month Activity Report on Contract 10690004 DEMO–1014*. Moscow, ID, University of Idaho.

Klontz, G.W. and Wood, J.W. (1972) Observations on the epidemiology of furunculosis disease in juvenile coho salmon (*Oncorhynchus kisutch*). *FI:EIFAC 72/SC II Symposium* **27**, 1–8.

Klontz, G.W., Yasutake, W.T. and Ross, A.J. (1966) Bacterial disease of the Salmonidae in the western United States: Pathogenesis of furunculosis in rainbow trout. *American Journal of Veterinary Resarch* **27**, 1455–1460.

Kloos, W.E. and Schliefer, K.H. (1986) Genus IV: *Staphylococcus* Rosenbach 1884, 18^{AL}, Nom. Cons. Opin. 17 Jud. Comm. 1858, 153. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 2. Baltimore, MD, Williams & Wilkins, pp. 1013–1035.

Kluyver, A.J. and Van Niel, C.B. (1936) Prospects for a natural system of classification of bacteria. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Originale, 2 Abteilung* **94**, 369–403.

Knibb, W., Colorni, A., Ankaoua, M., Lindell, D., Diamant, A. and Gordin, H. (1993) Detection and identification of a pathogenic marine mycobacterium from the European seabass *Dicentrarchus labrax* using polymerase chain reaction and direct sequencing of 16S rRNA sequences. *Molecular Marine Biology and Biotechnology* **2**, 225–232.

Knittel, M.D. (1981) Susceptibility of steelhead trout *Salmo gairdneri* Richardson to redmouth infection by *Yersinia ruckeri* following exposure to copper. *Journal of Fish Diseases* **4**, 33–40.

Knudsen, G., Sørum, H., Press, C.McL. and Olafsen, J.A. (1999) *In situ* adherence of *Vibrio* spp. to cryosections of Atlantic salmon, *Salmo salar* L., tissue. *Journal of Fish Diseases* **22**, 409–418.

Ko, Y.-M. and Heo, G.-J. (1997) Characteristics of *Flavobacterium branchiophilum* isolated from rainbow trout in Korea. *Fish Pathology* **32**, 97–102.

Kobayashi, T., Imai, M., Ishitaka, Y. and Kawaguchi, Y. (2004) Histopathological studies of bacterial haemorrhagic ascites of ayu, *Plecoglossus altivelis* (Temminck & Schlegel). *Journal of Fish Diseases* **27**, 451–457.

Kobayashi, T., Imai, M. and Kawaguchi, Y. (2005) A Gram-negative bacillus infection causing gill lesions in cultured rockfish *Sebastes schlegeli*. *Fish Pathology* **40**, 143–145.

Kocur, M. (1986) Genus I: *Micrococcus* Cohn 1872, 151^{AL}. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 2. Baltimore, MD, Williams & Wilkins, pp. 1004–1008.

Kocylowski, B. (1963) Etat actuel des maladies des poissons. Organisation de l'inspection des poissons et de leurs de consommation en Pologne. *Bulletin de l'Office International des Epizooties* **59**, 89–109.

Kodama, H., Moustafa, M., Ishiguro, S., Mikami, T. and Izawa, H. (1984) Extracellular virulence factors of fish *Vibrio*: Relationships between toxic material, hemolysin, and proteolytic enzyme. *American Journal of Veterinary Research* **45**, 2203–2207.

Kodama, H., Nakanishi, Y., Yamamoto, F., Mikami, T., Izawa, H., Imagawa, T., Hashimoto, Y. and Kudo, N. (1987) *Salmonella arizonae* isolated from a pirarucu, *Arapaima gigas* Cuvier, with septicaemia. *Journal of Fish Diseases* **10**, 509–512.

Kogure, K., Simidu, U. and Taga, N. (1979) Tentative direct microscopic method for counting living marine bacteria. *Canadian Journal of Microbiology* **25**, 415–420.

Kokubo, T., Iida, T. and Wakabayashi, H. (1990) Production of siderophore by *Edwardsiella tarda*. *Fish Pathology* **25**, 237–241.

Köllner, B. and Kotterba, G. (2002) Temperature dependent activation of leucocyte populations of rainbow trout, *Oncorhynchus mykiss*, after intraperitoneal immunisation with *Aeromonas salmonicida*. *Fish & Shellfish Immunology* **12**, 35–48.

Kondo, M., Kawai, K., Okabe, M., Nakano, N. and Oshima, S.-I. (2003) Efficacy of oral vaccine against bacterial coldwater disease in ayu *Plecoglossus altivelis*. *Diseases of Aquatic Organisms* **55**, 261–264.

Koppang, E.O., Fjølstad, M., Melgård, B., Vigerust, M. and Sørum, H. (2000) Non-pigment-producing isolates of *Aeromonas salmonicida* subspecies *salmonicida*: Isolation, identification, transmission and pathogenicity in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **23**, 39–48.

Koshi, G. and Lalitha, M.K. (1976) *Edwardsiella tarda* in a variety of human infections.*Indian Journal of Medical Research* **64**, 1753–1759.

Koski, P., Hirvelä-Koski, V. and Bernardet, J.F. (1993) *Flexibacter columnaris* infection in Arctic char (*Salvelinus alpinus* L.): First isolation in Finland. *Bulletin of the European Association of Fish Pathologists* **13**, 66–69.

Kou, G.H. (1981) Some bacterial diseases of eel in Taiwan. *Proceedings of the Republic of China/U.S. Cooperative Science Seminar on Fish Diseases, National Science Council Series* **3**, 11–20.

Krantz, G.E., Reddecliff, J.M. and Heist, C.E. (1964a) Immune response of trout to *Aeromonas salmonicida*. Part 1. Development of agglutinating antibodies and protective immunity. *Progressive Fish Culturist* **26**, 3–10.

Krantz, G.E., Reddecliff, J.M. and Heist, C.E. (1964b) Immune response of trout to *Aeromonas salmonicida*. Part 2. Evaluation of feeding techniques. *Progressive Fish Culturist* **26**, 65–69.

Kraxberger-Beatty, T., McGarey, D.J., Grier, H.J. and Lim, D.V. (1990) *Vibrio harveyi*, an opportunistic pathogen of common snook, *Centropomus undecimalis* (Block), held in captivity. *Journal of Fish Diseases* **13**, 557–560.

Krovacek, K., Faris, A., Ahne, W. and Månsson, I. (1987) Adhesion of *Aeromonas hydrophila* and *Vibrio anguillarum* to fish cells and to mucus-coated glass slides. *FEMS Microbiology Letters* **42**, 85–89.

Kubilay, A. and Uloköy, G. (2004) First isolation of *Staphylococcus epidermidis* from cultured gilthead sea bream (*Sparus aurata*)in Turkey. *Bulletin of the European Association of Fish Pathologists* **24**, 137–143.

Kubota, S.S. and Takakuwa, M. (1963) Studies on the disease of marine culture fishes. 1. General description and preliminary discussion of fish diseases at Mie Prefecture. *Journal of the Faculty of Fisheries of the Prefectural University of Mie* **6**, 107–124.

Kubota, S., Kariya, T., Nakamura, Y. and Kira, K. (1968) Nocardial infection in cultured yellowtails (*Seriola quinqueradiata* and *S. purpurascens*). II. Histological study. *Fish Pathology* **3**, 24–33.

Kubota, S.S., Kaige, N., Miyazaki, T. and Miyashita, T. (1981) Histopathological studies on edwardsiellosis of Tilapia—1. Natural infection. *Bulletin of the Faculty of Fisheries, Mie University* **9**, 155–165.

Kudo, S. and Kimura, N. (1983a) Transmission electron microscopic studies on bacterial gill disease in rainbow trout fingerlings. *Japanese Journal of Ichthyology* **30**, 247–260.

Kudo, S. and Kimura, N. (1983b) Scanning electron microscopic studies on bacterial gill disease in rainbow trout fingerlings. *Japanese Journal of Ichthyology* **30**, 393–403.

Kudo, T., Hatai, K. and Seino, A. (1988) *Nocardia seriolae* sp. nov. causing nocardiosis of cultured fish. *International Journal of Systematic Bacteriology* **38**, 173–178.

Kumagai, A., Yamaoki, S. Takahashi, K., Fukuda, H. and Wakabayashi, H. (2000) Waterborne transmission of *Flavobacterium psychrophilum* in coho salmon eggs. *Fish Pathology* **35**, 25–28.

Kumagai, A., Nakayasu, C. and Oseko, N. (2004) Effect of tobramycin supplementation to medium on isolation of *Flavobacterium psychrophilum* from ayu *Plecoglossus altivelis*. *Fish Pathology* **39**, 75–78.

Kumagai, A., Nakayasu, C. and Oseko, N. (2004a) No evidence for the presence of *Flavobacterium psychrophilum* within ayu eggs. *Fish Pathlogy* **39**, 183–187.

Kumagai, A., Sugimoto, K., Itou, D., Kamaishi, T., Miwa, S. and Iida, T. (2006) Atypical *Aeromonas salmonicida* in cultured marbled sole *Pleuronectes yokohamae*. *Fish Pathology* **41**, 7–12.

Kumari, J. and Sahoo, P.K. (2006) Dietary immunostimulants influence specific immune response and resistance of healthy and immunocompromised Asian catfish *Clarias batrachus* to *Aeromonas hydrophila* infection. *Diseases of Aquatic Organisms* **70**, 63–70.

Kumon, M., Iida, T., Fukuda, Y., Arimoto, M. and Shimizu, K. (2002) Blood fluke promotes mortality of yellowtail caused by *Lactococcus garvieae*. *Fish Pathology* **37**, 201–203.

Kuo, S.C., Chung, H.Y. and Kou, G.H. (1981) Studies on artificial infection of the gliding bacteria in cultured fishes. *Fish Pathology* **15**, 309–314.

Kusser, W. and Fiedler, F. (1983) Murein type and polysaccharide composition of cell walls from *Renibacterium salmoninarum*. *FEMS Microbiology Letters* **20**, 391–394.

Kusuda, R. and Fukuda, Y. (1980) Agglutinating antibody titers and serum protein changes of yellowtail after immunization with *Pasteurella piscicida* cells. *Bulletin of the Japanese Society of Scientific Fisheries* **46**, 801–807.

Kusuda, R. and Inoue, K. (1976) Studies on the application of ampicillin for pseudotuberculosis of cultured yellowtail—1. *In vitro* studies on sensitivity, development of drug-resistance and reversion of acquired drug-resistance characteristics of *Pasteurella piscicida*. *Bulletin of the Japanese Society of Scientific Fisheries* **42**, 969–973.

Kusuda, R. and Komatsu, I. (1978) A comparative study of fish pathogenic *Streptococcus* isolated from saltwater and freshwater fishes. *Bulletin of the Japanese Society of Scientific Fisheries* **44**, 1073–1078.

Kusuda, R. and Nakagawa, A. (1978) Nocardia infection of cultured yellowtail. *Fish Pathology* **13**, 25–31.

Kusuda, R. and Sugiyama, A. (1981) Studies on the characters of *Staphylococcus epidermidis* isolated from diseased fishes—1. On the morphological, biological and biochemical properties. *Fish Pathology* **16**, 15–24.

Kusuda, R. and Taira, T. (1990) Change of biological activities of phagocytes from the eel immunized with *Edwardsiella tarda*. *Fish Pathology* **25**, 53–58.

Kusuda, R. and Takemaru, I. (1987) Efficacy of josamycin against experimental streptococcal infection in cultured yellowtail. *Nippon Suisan Gakkaishi* **53**, 1519–1523.

Kusuda, R. and Yamaoka, M. (1972) Etiological studies on bacterial pseudotuberculosis in cultured yellowtail with *Pasteurella piscicida* as the causative agent—1. On the morphological and biochemical properties. *Bulletin of the Japanese Society of Scientific Fisheries* **38**, 1325–1332.

Kusuda, R., Taki, H. and Takeuchi, T. (1974) Studies on a nocardia infection of cultured yellowtail. II. Characteristics of *Nocardia kampachi* isolated from a gill-tuberculosis of yellowtail. *Bulletin of the Japanese Society of Scientific Fisheries* **40**, 369–373.

Kusuda, R., Kawai, T., Toyoshima, T. and Komatsu, I. (1976a) A new pathogenic bacterium belonging to the genus *Streptococcus*, isolated from an epizootic of cultured yellowtail. *Bulletin of the Japanese Society of Scientific Fisheries* **42**, 1345–1352.

Kusuda, R., Toyoshima, T., Iwamura, Y. and Sano, H. (1976b) *Edwardsiella tarda* from an epizootic of mullets (*Mugil cephalus*) in Okitsu Bay. *Bulletin of the Japanese Society of Scientific Fisheries* **42**, 271–275.

Kusuda, R., Kawai, K. and Masui, T. (1978a) Etiological studies on bacterial pseudotuberculosis in cultured yellowtail with *Pasteurella piscicida* as the causative agent—II. On the serological properties. *Fish Pathology* **13**, 79–83.

Kusuda, R., Komatsu, I. and Kawai, K. (1978b) *Streptococcus* sp. isolated from an epizootic of cultured eels. *Bulletin of the Japanese Society of Scientific Fisheries* **44**, 295.

Kusuda, R., Kawai, K., Jo, Y., Akizuki, T., Fukunaga, M. and Kotake, N. (1978c) Efficacy of oral vaccination for vibriosis in cultured ayu. *Bulletin of the Japanese Society of Scientific Fisheries* **44**, 21–25.

Kusuda, R., Sako, H. and Kawai, K. (1979) Classification of vibrios isolated from diseased fishes—1. On the morphological, biological and biochemical properties. *Fish Pathology* **13**, 123–137.

Kusuda, R., Kawai, K. and Shirakawa, T. (1982) Serological study of *Streptococcus* sp. pathogenic to cultured yellowtail. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 1731–1738.

Kusuda, R., Yokoyama, J. and Kawai, K. (1986) Bacteriological study on cause of mass mortalities in cultured black sea bream fry. *Bulletin of the Japanese Society of Scientific Fisheries* **52**, 1745–1751.

Kusuda, R., Kawakami, K. and Kawai, K. (1987) A fish-pathogenic *Mycobacterium* sp isolated from an epizootic of culture yellowtail. *Nippon Suisan Gakkaishi* **53**, 1797–1804.

Kusuda, R., Itaoka, M. and Kawai, K. (1988) Drug sensitivity of *Pasteurella piscicida* strains isolated from cultured yellowtail from 1984 to 1985. *Nippon Suisan Gakkaishi* **54**, 1521–1526.

Kusuda, R., Kawai, K., Salati, F., Banner, C.R. and Fryer, J.L. (1991) *Enterococcus seriolicida* sp. nov., a fish pathogen. *International Journal of Systematic Bacteriology* **41**, 406–409.

Kusuda, R., Dohata, N., Fukuda, Y. and Kawai, K. (1995) *Pseudomonas anguilliseptica* infection of striped jack. *Fish Pathology* **30**, 121–122.

Kuzuk, M.A., Thornton, J.C. and Kay, W.W. (1996) Antigenic characterization of the salmonid pathogen *Piscirickettsia salmonis*. *Infection and Immunity* **64**, 5205–5210.

Kvitt, H. and Colorni, A. (2004) Strain variation and geographic endemism in *Streptococcus iniae*. *Diseases of Aquatic Organisms* **61**, 67–73.

Kvitt, H., Ucko, M., Colorni, A., Batargias, C., Zlotkin, A. and Knibb, W. (2002) *Photobacterium damselae* subsp. *piscicida*: Detection by direct amplification of 16S rRNA gene sequences and genotypic variation as determined by amplified fragment length polymporphism (AFLP). *Diseases of Aquatic Organisms* **48**, 187–195.

Kwon, M.G., Lee, J.-Y., Park, S., Iida, T., Hirono, I. and Aoki, T. (1997) RAPD analysis of atypical *Aeromonas salmonicida* isolated in Japan. *Fish Pathology* **32**, 109–115.

Kwon, S.R., Nam, Y.K., Kim, S.K. and Kim, K.H. (2006) Protection of tilapia (*Oreochromis mossambicus*) from edwardsiellosis by vaccination with *Edwardsiella tarda* ghosts. *Fish & Shellfish Immunology* **20**, 621–626.

Labella, A., Vida, M., Alonso, M.C., Infante, C., Cardenas, S., Lopez-Romalde, S., Manchado, M. and Borrego, J.J. (2006) First isolation of *Photobacterium damselae* subsp. *damselae* from cultured redbanded seabream, *Parus auriga* Valenciennes in Spain. *Journal of Fish Diseases* **29**, 175–179.

LaFrentz, B.R., LaPatra, S.E., Jones, G.R., Congleton, J.L., Sun, B. and Cain, K.D. (2002) Characterization of the serum and mucosal antibody responses and relative per cent survival in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following immunization and challenge with *Flavobacterium psychrophilum*. *Journal of Fish Diseases* **25**, 703–713.

LaFrentz, B.R., LaPatra, S.E., Jones, G.R. and Cain, K.D. (2003) Passive immunization of rainbow trout, *Oncorhynchus mykiss* (Walbaum), against *Flavobacterium psychrophilum*, the causative agent of bacterial coldwater disease and rainbow trout fry syndrome. *Journal of Fish Diseases* **26**, 377–384.

LaFrentz, B.R., LaPatra, S.E., Jones, G.R. and Cain, K.D. (2004) Protective immunity in rainbow trout *Oncorhynchus mykiss* following immunization with distinct molecular mass fractions isolated from *Flavobacterium psychrophilum*. *Diseases of Aquatic Organisms* **59**, 17–26.

Lahav, D., Eyngor, M., Hurvitz, A., Ghittino, C., Lublin, A. and Eldar, A. (2004) *Streptococcus iniae* type II infections in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **62**, 177–180.

Laidler, L.A. (1980) Detection and identification of the bacterial kidney disease (BKD) organism by the indirect fluorescent antibody technique. *Journal of Fish Diseases* **3**, 67–69.

Laidler, L.A., Treasurer, J.W., Grant, A.N. and Cox, D.I. (1999) Atypical *Aeromonas salmonicida* infection in wrasse (Labridae) used as cleaner fish of farmed Atlantic salmon, *Salmo salar* L., in Scotland. *Journal of Fish Diseases* **22**, 209–213.

Lall, S.P., Paterson, W.D., Hines, J.A. and Adams, N.J. (1985) Control of bacterial kidney in Atlantic salmon, *Salmo salar* L., by dietary modification. *Journal of Fish Diseases* **8**, 113–124.

Lallier, R., Bernard, F. and Lalonde, G. (1984) Difference in the extracellular products of two strains of *Aeromonas hydrophila* virulent and weakly virulent for fish. *Canadian Journal of Microbiology* **30**, 900–904.

Lamas, J., Anadon, R., Devesa, S. and Toranzo, A.E. (1990) Visceral neoplasia and epidermal papillomas in cultured turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* **8**, 179–187.

Lamas, J., Santos, Y., Bruno, D., Toranzo, A.E. and Anadon, R. (1994a) A comparison of pathological changes caused by *Vibrio anguillarum* and its extracellular products in rainbow trout (*Oncorhynchus mykiss*). *Fish Pathology* **29**, 79–89.

Lamas, J., Santos, Y., Bruno, D.W., Toranzo, A.E. and Anadón, R. (1994b) Non-specific cellular responses of rainbow trout to *Vibrio anguillarum* and its extracellular products (ECPs). *Journal of Fish Biology* **45**, 839–854.

Lamers, C.H.L. and de Haas, M.J.M. (1983) The development of immunological memory in carp (*Cyprinus carpio* L.) to a bacterial antigen. *Developmental and Comparative Immunology* **7**, 713–714.

Lamers, C.H.J. and Muiswinkel, W.B. (1984) Primary and secondary immune responses in carp (*Cyprinus carpio*) after administration of *Yersinia ruckeri* O-antigen. In: Acuigrup (ed.), *Fish Diseases*. Madrid, Editora ATP, pp. 119–127.

Lamers, C.H.J., de Haas, M.J.M. and van Muiswinkel, W.B. (1985a) Humoral response and memory formation in carp after injection of *Aeromonas hydrophila* bacterin. *Developmental and Comparative Immunology* **9**, 65–75.

Lamers, C.H.J., de Haas, M.J.M. and van Muiswinkel, W.B. (1985b) The reaction of the immune system of fish to vaccination: Development of immunological memory in carp, *Cyprinus carpio* L., following direct immersion in *Aeromonas hydrophila* bacterin. *Journal of Fish Diseases* **8**, 253–262.

Lammens, M., Decostere, A. and Haesebrouck, F. (2000) Effect of *Flavobacterium psychrophilum* strains and their metabolites on the oxidative activity of rainbow trout *Oncorhynchus mykiss* phagocytes. *Diseases of Aquatic Organisms* **41**, 173–179.

Landsberg, J.H. (1995) Tropical reef-fish disease outbreaks and mass mortalities in Florida, USA: what is the role of dietary biological toxins? *Diseases of Aquatic Organisms* **22**, 83–100.

Lannan, C.N. and Fryer, J.L. (1991) Recommended methods for inspection of fish for the salmonid rickettsia. *Bulletin of the European Association of Fish Pathologists* **11**, 135–136.

Lannan, C.N. and Fryer, J.L. (1994) Extracellular survival of *Piscirickettia salmonis*. *Journal of Fish Diseases* **17**, 545–548.

Lannan, C.N., Ewing, S.A. and Fryer, J.L. (1991) A fluorescent antibody test for detection of the rickettsia causing disease in Chilean salmonids. *Journal of Aquatic Animal Health* **3**, 229–234.

Lansdell, W., Dixon, B., Smithin, N. and Benjamin, L. (1993) Isolation of several *Mycobacterium* species from fish. *Journal of Aquatic Animal Health* **5**, 73–76.

Larenas, J., Astorga, C., Contreras, J., Garcés, H., Fryer, J. and Smith, P. (1996) Rapid detection of *Piscirickettsia salmonis* using microwave irradiation. *Fish Pathology* **31**, 231–232.

Larenas, J.J., Batholomew, J., Troncoso, O., Fernández, S., Ledezina, H., Sandoval, N., Vera, P., Contreras, J. and Smith, P. (2003) Experimental vertical transmission of *Piscirickettsia salmonis* and *in vitro* study of attachment and mode of entrance into the fish ovum. *Diseases of Aquatic Organisms* **56**, 25–30.

Larsen, J.L. (1982) *Vibrio anguillarum*: Prevalence in three carbohydrate loaded marine recipients and a control. *Zentralblatt für Bakteriologie und Hygiene, 1, Abteilung Originale C* **3**, 519–530.

Larsen, J.L. and Jensen, N.J. (1977) An *Aeromonas* species implicated in ulcer-disease of the cod (*Gadus morhua*). *Nordisk Veterinaermedicin* **29**, 199–211.

Larsen, J.L. and Olsen, J.E. (1991) Occurrence of plasmids in Danish isolates of *Vibrio anguillarum* serovars O1 and O2 and association of plasmids with phenotypic characteristics. *Applied and Environmental Microbiology* **57**, 2158–2163.

Larsen, M.H., Blackburn, N., Larsen, J.L. and Olsen, J.E. (2004) Influences of temperature, salinity and starvation on the motility and chemotactic response of *Vibrio anguillarum*. *Microbiology* **150**, 1283–1290.

Lau, S.K.P., Woo, P.C.Y., Luk, W.-K., Fung, A.M.Y., Hui, W.-T., Fong, A.H.C., Chow, C.-W., Wong, S.S.Y. and Yuen, K.-Y. (2006) Clinical isolates of *Streptococcus iniae* from Asia are more mucoid and β-hemolytic than those from North America. *Diagnostic Microbiology and Infectious Diseases* **54**, 177–181.

Lawrence, M.L. and Banes, M.M. (2005) Tissue and vaccine efficacy of an O polysaccharide mutant strain of *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* **17**, 228–232.

Lawrence, M.L., Cooper, R.K. and Thune, R.L. (1997) Attenuation, persistence, and vaccine potenital of an *Edwardsiella ictaluri purA* mutant. *Infection and Immunity* **65**, 4642–4651.

Lawrence, M.L., Banes, M.M. and Williams, M.L. (2001) Phenotype and virulence of a transposon-derived lipopolysaccharide O side-chain mutant strain of *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* **13**, 291–299.

Leadbetter, E.R. (1974) Genus II: *Flexibacter* Soriano 1945, 92, Lewin 1969, 192 emend. mut. char. In: Buchanan, R.E. and Gibbons, N.E. (eds), *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore, MD, Williams & Wilkins, pp. 105–107.

Leblanc, D., Mittal, K.R., Olivier, G. and Lallier, R. (1981) Serogrouping of motile *Aeromonas* species isolated from healthy and moribund fish. *Applied and Environmental Microbiology* **42**, 56–60.

Lee, C.H., Jeong, H.D., Chung, J.K., Lee, H.H. and Kim, K.H. (2003) CpG motif synthetic ODN primes respiratory burst of live olive flounder *Paralichthys olivaceus* phagocytes and enhances protection against *Edwardsiella tarda*. *Diseases of Aquatic Organisms* **56**, 43–48.

Lee, E.G.-H. and Evelyn, T.P.T. (1989) Effect of *Renibacterium salmoninarum* levels in the ovarian fluid of spawning chinook salmon on the prevalence of the pathogen in their eggs and progeny. *Diseases of Aquatic Organisms* **7**, 179–184.

Lee, E.G.-H. and Evelyn, T.P.T. (1994) Prevention of vertical transmission of the bacterial kidney disease agent *Renibacterium salmoninarum* by broodstock injection with erythromycin. *Diseases of Aquatic Organisms* **18**, 1–4.

Lee, E.G.-H. and Gordon, M.R. (1987) Immunofluorescence screening of *Renibacterium salmoninarum* in the tissues and eggs of farmed chinook salmon spawners. *Aquaculture* **65**, 7–14.

Lee, J.V., Gibson, D.M. and Shewan, J.M. (1977) A numerical taxonomic study of some *Pseudomonas*-like marine bacteria. *Journal of General Microbiology* **98**, 439–451.

Lee, J.V., Shread, P., Furniss, A.L. and Bryant, T.N. (1981) Taxonomy and description of *Vibrio fluvialis* sp. nov. (Synonym Group F Vibrios, Group EF6). *Journal of Applied Bacteriology* **50**, 73–94.

Lee, J.V., Bashford, D.J., Donovan, T.J., Furniss, A.L. and West, P.A. (1982) The incidence of *Vibrio cholerae* in water, animals and birds in Kent, England. *Journal of Applied Bacteriology* **52**, 281–291.

Lee, K.-K. (1995) Pathogenesis studies on *Vibrio alginolyticus* in the grouper, *Epinephelus malabaricus*, Bloch & Schneider. *Microbial Pathogenesis* **19**, 39–48.

Lee, K.-K., Chiang, H.T., Yii, K.C., Su, W.M. and Liu, P.C. (1997) Effects of extracellular products of *Vibrio vulnificus* on *Acanthopagrus schlegeli* serum components *in vitro* and *in vivo*. *Microbios* **92**, 209–217.

Lee, S.Y., Yin, Z., Ge, R. and Sin, Y.M. (1997) Isolation and characterization of fish *Aeromonas hydrophila* adhesins important for *in vitro* epithelial cell invasion. *Journal of Fish Diseases* **20**, 169–175.

Lehmann, J., Mock, D., Stürenberg, F.-J. and Bernardet, J.-F. (1991) First isolation of *Cytophaga psychrophila* from a systemic disease in eel and cyprinids. *Diseases of Aquatic Organisms* **10**, 217–220.

Lehmann, K.B. and Neumann, R. (1896) *Atlas und Grundress der Bakteriologie*. München, J.F. Lehmann.

Lei, Q., Yin-Geng, W., Zheng, Z. and Shao-Li, Y. (2006) The first report on fin rot disease of cultured turbot *Scophthalmus maximus* in China. *Journal of Aquatic Animal Health* **18**, 83–89.

Leifson, E. (1963) Determination of carbohydrate metabolism of marine bacteria. *Journal of Bacteriology* **85**, 1183–1184.

Le Minor, L. (1984) Genus III: *Salmonella* Lignières 1900, 389[AL]. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. I. Baltimore, MD, Williams & Wilkins, pp. 427–458.

Lemos, M.L., Salinas, P., Toranzo, A.E., Barja, J.L. and Crosa, J.H. (1988) Chromosome-mediated iron uptake system in pathogenic strains of *Vibrio anguillarum*. *Journal of Bacteriology* **170**, 1920–1925.

Lemos, M.L., Mazoy, R., Conchas, R.F. and Toranzo, A.E. (1991) Presence of iron uptake mechanisms in environmental non-pathogenic strains of *Vibrio anguillarum*. *Bulletin of the European Association of Fish Pathologists* **11**, 150–152.

León, G., Maulén, N., Figueroa, J., Villanueva, J., Rodríguez, C., Vera, M.I. and Krauskopf, M. (1994a) A PCR based assay for the identification of the fish pathogen *Renibacterium salmoninarum*. *FEMS Microbiology Letters* **115**, 131–136.

León, G., Martinez, M.A., Etchegaray, J.P., Vera, M.I., Figueroa, J. and Krauskopf, M. (1994b) Specific DNA probes for the identification of the fish pathogen, *Renibacterium salmoninarum*. *World Journal of Microbiology & Biotechnology* **10**, 149–153.

Lesel, R., Lesel, M., Gavini, F. and Vuillaume, A. (1983) Outbreak of enteric redmouth disease in rainbow trout, *Salmo gairdneri* Richardson in France. *Journal of Fish Diseases* **6**, 385–387.

Leung, K.-Y. and Stevenson, R.M.W. (1988) Characteristics and distribution of extracellular proteases from *Aeromonas hydrophila*. *Journal of General Microbiology* **134**, 151–160.

Levi, M.H., Bartell, J., Gandolfo, L., Smole, S.C., Costa, S.F., Weiss, L.M., Johnson, L.K., Osterhout, G. and Herbst, L.H. (2003) Characterization of *Mycobacterium montefiorense* sp. nov., a novel pathogenic mycobacterium from moray eels that is related to *Mycobacterium triplex*. *Journal of Clinical Microbiology* **41**, 2147–2152.

Levine, M.A., Wolke, R.E. and Cabelli, V.J. (1972) *Vibrio anguillarum* as a cause of disease in winter flounder (*Pseudopleuronectes americanus*). *Canadian Journal of Microbiology* **18**, 1585–1892.

Lewin, C.S. and Hastings, T.S. (1990) *In vitro* activities of oxolinic acid, ciprofloxacin and norfloxacin against *Aeromonas salmonicida*. *Journal of Fish Diseases* **13**, 377–384.

Lewis, D.H., Grumbles, L.C., McConnell, S. and Flowers, A.I. (1970) *Pasteurella*-like bacteria from a epizootic in menhaden and mullet in Galveston Bay. *Journal of Wildlife Diseases* **6**, 160–162.

Li, G., Zhao, D., Huang, L., Sun, J., Gao, D., Wang, H., Tan, Y. and Liang, L. (2006) Identification and phylogenetic analysis of *Vibrio vulnificus* isolated from diseased *Trachinotus ovatus* in cage mariculture. *Aquaculture* **261**, 17–25.

Li, J., Zhou, L. and Woo, N.Y.S. (2003) Invasion routes and pathogenicity mechanisms of *Vibrio alginolyticus* to silver sea bream *Sparus sarba*. *Journal of Aquatic Animal Health* **15**, 302–313.

Li, M.F. and Flemming, C. (1967) A proteolytic pseudomonad from skin lesions of rainbow trout (*Salmo gairdneri*). I. Characteristics of the pathogenic effects and the extracellular proteinase. *Canadian Journal of Microbiology* **13**, 405–416.

Li, M.F. and Jordan, C. (1968) A proteolytic pseudomonad from skin lesions of rainbow trout (*Salmo gairdneri*). II. Some properties of the proteinase. *Canadian Journal of Microbiology* **14**, 875–880.

Li, M.F. and Traxler, G.S. (1971) A proteolytic pseudomonad from skin lesions of rainbow trout (*Salmo gairdneri*). III. Morphological studies. *Journal of the Fisheries Research Board of Canada* **28**, 104–105.

Li, P., Lewis, D.H. and Gatlin III, D.M. (2004) Dietary oligonucleotides from yeast RNA immune responses and resistance of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) to *Streptococcus iniae* infection. *Fish & Shellfish Immunology* **16**, 561–569.

Lim, C. and Klesius, P.H. (2003) Influence of feed deprivation on haematology, macrophage chemotaxis, and resistance to *Edwardsiella ictaluri* challenge of channel cafish. *Journal of Aquatic Animal Health* **15**, 13–20.

Lim, C. and Lovell, R.T. (1978) Pathology of vitamin C deficiency syndrome in channel catfish. *Journal of Nutrition* **108**, 1137–1141.

Lim, C., Sealey, W.M. and Klesius, P.H. (1996) Iron methionine and iron sulfate as sources of dietary iron for channel catfish *Ictalurus punctatus*. *Journal of the World Aquaculture Society* **27**, 290–296.

Lim, C., Klesius, P.H., Li, M.H. and Robinson, E.H. (2000) Interaction between dietary levels of iron and vitamin C on growth, haematology, immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture* **185**, 313–327.

Ling, S.H.M., Wang, X.H., Xie, L., Lim, T.M. and Leung, K.Y. (2000) Use of green fluorescent protein (GFP) to study the invasion pathways of *Edwardsiella tarda* in *in vivo* and *in vitro* fish models. *Microbiology* **146**, 7–19.

Ling, S.H.M., Wang, X.H., Lim, T.M. and Leung, K.Y. (2001) Green fluorescent protein-tagged *Edwardsiella tarda* reveals portal of entry in fish. *FEMS Microbiology Letters* **194**, 239–243.

Lio-Po, G.D., Albright, L.J., Michel, C. and Leano, E.M. (1998) Experimental induction of lesions in snakeheads (*Ophicephalus striatus*) and catfish (*Clarias batrachus*) with *Aeromonas hydrophila*, *Aquaspirillum* sp., *Pseudomonas* sp. and *Streptococcus* sp. *Journal of Applied Ichthyology* **14**, 75–79.

Liu, H., Izumi, S. and Wakabayashi, H. (2001) Detection of *Flavobacterium psychrophilum* in various organs of ayu *Plecoglossus altivelis* by *in situ* hybridization. *Fish Pathology* **36**, 7–11.

Liu, P.-C., Chuang, W.-H. and Lee, K.-K. (2003) Infectious gastroenteritis caused by *Vibrio harveyi* (*V. carchariae*) in cultured red drum (*Sciaenops ocellatus*). *Journal of Applied Ichthyology* **19**, 59–61.

Liu, P.V. (1961) Observations on the specificities of extra-cellular antigens of the genera *Aeromonas* and *Serratia*. *Journal of General Microbiology* **24**, 145–153.

Livesley, M.A., Smith, S.N., Armstrong, R.A. and Barker, G.A. (1997) Analysis of plasmid profile of *Aeromonas salmonicida* isolates by pulsed field gel electrophoresis. *FEMS Microbiology Letters* **146**, 297–301.

Llewellyn, L.C. (1980) A bacterium with similarities to the redmouth bacterium and *Serratia liquefaciens* (Grimes & Hennerty) causing mortalities in hatchery reared salmonids in Australia. *Journal of Fish Diseases* **3**, 29–39.

Llobrera, A.T. and Gacutan, R,Q, (1987) *Aeromonas hydrophila* associated with ulcerative disease epizootic in Laguna de Bay, Philippines. *Aquaculture* **67**, 273–278.

Lobb, C.J. and Rhoades, M. (1987) Rapid plasmid analysis for identification of *Edwardsiella ictaluri* from infected channel catfish (*Ictalurus punctatus*). *Applied and Environmental Microbiology* **53**, 1267–1272.

Lobb, C.J., Ghaffari, S.H., Hayman, J.R. and Thompson, D.T. (1993) Plasmid and serological differences between *Edwardsiella ictaluri* strains. *Applied and Environmental Microbiology* **59**, 2830–2836.

Locci, R., Baldacci, E. and Petrolini-Baldacci, B. (1969) The genus *Streptoverticillium*, a taxonomic study. *Giornale Microbiologica* **17**, 1–60.

Logan, N.A. (1989) Numerical taxonomy of violet-pigmented, Gram-negative bacteria and description of *Iodobacter fluviatile* gen. nov., comb. nov. *International Journal of Systematic Bacteriology* **39**, 450–456.

Loghothetis, P.N. and Austin, B. (1996) Antibody responses of rainbow trout *Oncorhynchus mykiss*, Walbaum to live *Aeromonas hydrophila* as assessed by various antigen preparations. *Fish & Shellfish Immunology* **6**, 455–464.

Loi-Po, G.D., Albright, L.J., Michel, C. and Leaño, E.M. (1998) Experimental induction of lesions snakeheads (*Ophiocephalus striatus*) and catfish (*Clarias batrachus*) with *Aeromonas hydrophila*, *Aquaspirillum* sp., *Pseudomonas* sp. and *Streptococcus* sp. *Journal of Applied Ichthyology* **14**, 75–79.

Lönnström, L., Wiklund, T. and Bylund, G. (1994) *Pseudomonas anguilliseptica* isolated from Baltic herring *Clupea harengus membras* with eye lesions. *Diseases of Aquatic Organisms* **18**, 143–147.

Lopez, J.E. and Angulo, L. (1995) Survival of *Vibrio splendidus* biotype I in seawater. *Bulletin of the European Association of Fish Pathologists* **15**, 70–72.

López-Dóriga, M.V., Barnes, A.C., dos Santos, N.M.S. and Ellis, A.E. (2000) Invasion of fish epithelial cells by *Photobacterium damselae* subsp. *piscicida*: Evidence for receptor specificity, and effect on capsule and serum. *Microbiology* **146** 21–30.

López-Romalde, S., Magariños, B., Núñez, S., Toranzo, A.E. and Romalde, J.L. (2003) Phenotypic and genotypic characterization of *Pseudomonas anguilliseptica* from fish. *Journal of Aquatic Animal Health* **15**, 39–47.

López-Romalde, S., Núñez, S., Toranzo, A.E. and Romalde, J.L. (2003a) Black spot seabream (*Pagellus bogaraveo*), a new susceptible host for *Pseudomonas anguilliseptica*. *Bulletin of the European Association of Fish Pathologists* **23**, 258–264.

Lorenzen, E. (1993) The importance of the brand of the beef extract in relation to the growth of *Flexibacter psychrophilus* in Anacker and Ordals medium. *Bulletin of the European Associaiton of Fish Pathologists* **13**, 64–65.

Lorenzen, E. and Karas, N. (1992) Detection of *Flexibacter psychrophilus* by immunofluorescence in fish suffering from fry mortality syndrome: A rapid diagnostic method. *Diseases of Aquatic Organisms* **13**, 231–234.

Lorenzen, E. and Olesen, N.J. (1997) Characterization of isolates of *Flavobacterium psychrophilum* associated with coldwater disease or rainbow trout fry syndrome. II. Serological studies. *Diseases of Aquatic Organisms* **31**, 209–220.

Lorenzen, E., Dalsgaard, I., From, J., Hansen, E.M., Hørlyck, V, Korsholm, H., Mellergaard, S. and Olesen, N.J. (1991) Preliminary investigations of fry mortality syndrome in rainbow trout. *Bulletin of the European Association of Fish Pathology* **11**, 77–79.

Lorenzen, E., Olesen, N.J., Korsholm, H., Heuer, O.E. and Evensen, Ø. (1996) First demonstration of *Renibacterium salmoninarum* BKD in Denmark. *Bulletin of the European Association of Fish Pathologists* **17**, 140–144.

Lorenzen, E., Dalsgaard, I. and Bernardet, J.-F. (1997) Characterization of isolates of *Flavobacterium psychrophilum* associated with coldwater disease or rainbow trout fry syndrome. I. Phenotypic and genotypic studies. *Diseases of Aquatic Organisms* **31**, 197–208.

Love, M., Teebken-Fisher, D., Hose, J.E., Farmer III, J.J., Hickman, F.W. and Fanning, G.R. (1981) *Vibrio damsela*, a marine bacterium, causes skin ulcers on the damselfish *Chromis punctipinnis*. *Science (New York)* **214**, 1139–1140.

Lovely, J.E., Cabo, C., Griffiths, S.G. and Lynch, W.H. (1994) Detection of *Renibacterium salmoninarum* in asymptomatic Atlantic salmon. *Journal of Aquatic Animal Health* **6**, 126–132.

Lowe, G.H. (1962) The rapid detection of lactose fermentation in paracolon organisms by the demonstration of β-galactosidase. *Journal of Medical Laboratory Technology* **19**, 21–31.

Lund, M. (1967) A study of the biology of *Aeromonas salmonicida* (Lehmann & Neumann 1896) Griffin 1954. M.Sc. thesis, University of Newcastle-upon-Tyne, U.K.

Lund, V. and Mikkelsen, H. (2004) Genetic diversity among A-proteins of atypical strains of *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* **61**, 257–262.

Lund, V., Jenssen, L.M. and Wesmajervi, M.S. (2002) Assessment of genetic variability and relatedness among atypical *Aeromonas salmonicida* from marine fishes, using AFLP fingerprinting. *Diseases of Aquatic Organisms* **50**, 119–126.

Lund, V., Arnesen, J.A., Coucheron, D., Modalsli, K. and Syvertsen, C. (2003) The *Aeromonas salmonicida* A-layer protein is an important protective antigen in oil-adjuvanted vaccines. *Fish & Shellfish Immunology* **15**, 367–372.

Lund, V., Espelid, S. and Mikkelsen, H. (2003a) Vaccine efficacy in spotted wolffish *Anarhichas minor*: Relationship to molecular variation in A-layer protein of atypical *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* **56**, 31–42.

Lunder, T., Evensen, Ø., Holstad, G. and Håstein, T. (1995) "Winter ulcer" in the Atlantic salmon *Salmo salar*. Pathological and bacteriological investigations and transmission experiments. *Diseases of Aquatic Organisms* **23**, 39–49.

Lunder, T., Sørum, H., Holstad, G., Steigerwalt, A.G., Mowinckel, P. and Brenner, D.J. (2000) Phenotypic and genotypic characterization of *Vibrio viscocus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with "winter ulcer". *International Journal of Systematic and Evolutionary Microbiology* **50**, 427–450.

Lupiani, B., Dopazo, C.P., Ledo, A., Fouz, B., Barja, J.L., Hetrick, F.M. and Toranzo, A.E. (1989) New syndrome of mixed bacterial and viral etiology in cultured turbot *Scophthalmus maximus*. *Journal of Aquatic Animal Health* **1**, 197–204.

Lutwyche, P., Exner, M.M., Hancock, R.E.W. and Trust, T.J. (1995) A conserved *Aeromonas salmonicida* porin provides protective immunity to rainbow trout. *Infection and Immunity* **63**, 3137–3142.

Lygren, B., Fausa Pettersen, E., Wergeland, H.I. and Endresen, C. (1998) An extracellular low molecular weight protease with activity against gelatin and casein produced earlier during growth of *Aeromonas salmonicida* subsp. *salmonicida* than the serine protease. *Bulletin of the European Association of Fish Pathologists* **18**, 96–101.

MacDonell, M.T. and Colwell, R.R. (1985) Phylogeny of the Vibrionaceae and recommendations for two new genera, *Listonella* and *Shewanella*. *Systematic and Applied Microbiology* **6**, 171–182.

MacDonell, M.T., Swartz, D.G., Ortiz-Conde, B.A., Last, G.A. and Colwell, R.R. (1986) Ribosomal RNA phylogenies for the vibrio-enteric group of eubacteria. *Microbiological Sciences* **3**, 172–179.

MacFarlane, R.D., Bullock, G.L. and McLaughlin, J.J.A. (1986) Effects of five metals on susceptibility of striped bass to *Flexibacter columnaris*. *Transactions of the American Fisheries Society* **115**, 227–231.

Mackie, C. and Birkbeck, T.H. (1992) Siderophores produced by *Vibrio anguillarum in vitro* and in infected rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **15**, 37–45.

Mackie, T.J. and Menzies, W.J.M. (1938) Investigations in Great Britain of furunculosis of the Salmonidae. *Journal of Comparative Pathology and Therapeutics* **51**, 25.

Mackie, T.J., Arkwright, J.A., Pryce-Tannatt, T.E., Motram, J.C. and Johnstone, W.R. (1930, 1933 and 1935) *Interim, Second and Final Reports of the Furunculosis Committee*. Edinburgh, H.M.S.O.

MacInnes, J.I., Trust, T.J. and Crosa, J.H. (1979) Deoxyribonucleic acid relationships among members of the genus *Aeromonas*. *Canadian Journal of Microbiology* **25**, 579–586.

MacLean, D.G. and Yoder, W.G. (1970) Kidney disease among Michigan salmon in 1967. *Progressive Fish Culturist* **32**, 26–30.

MacPhee, D.D., Ostland, V.E., Lumsden, J.S. and Ferguson, H.W. (1995) Development of an enzyme-linked immunosorbent assay (ELISA) to estimate the quantity of *Flavobacterium branchiophilum* on the gills of rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **21**, 13–23.

Madetoja, J. and Wiklund, T. (2002) Detection of the fish pathogen *Flavobacterium psychrophilum* in water from fish farms. *Systematic and Applied Microbiology* **25**, 259–266.

Madetoja, J., Nyman, P. and Wiklund, T. (2000) *Flavobacterium psychrophilum*, invasion into and shedding by rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **43**, 27–38.

Madetoja, J., Hänninen, M.-L., Hirvelä-Koski, V., Dalsgaard, I. and Wiklund, T. (2001) Phenotypic and genotypic characterization of *Flavobacterium psychrophilum* from Finnish fish farms. *Journal of Fish Diseases* **24**, 469–479.

Madetoja, J., Dalsgaard, I. and Wiklund, T. (2002) Occurrence of *Flavobacterium psychrophilum* in fish-farming environments. *Diseases of Aquatic Organisms* **52**, 109–118.

Madetoja, J., Nystedt, S. and Wiklund, T. (2003) Survival and virulence of *Flavobacterium psychrophilum* in water microcosms. *FEMS Microbiology Ecology* **43**, 217–223.

Madetoja, J., Pylkkö, P., Pohjanvirta, T., Schildt, L. and Pelkonen, S. (2003a) Putative virulence factors of atypical *Aeromonas salmonicida* isolated from Arctic charr, *Salvelinus alpinus* (L.), and European grayling, *Thymallus thymallus* (L.). *Journal of Fish Diseases* **26**, 349–360.

Madetoja, J., Lönnström, L.-G., Björkblom, C., Uluköy, G., Bylund, G., Syvertsen, C., Gravningen, K., Norderhus, E.-A. and Wiklund, T. (2006) Efficacy of injection vaccines against *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **29**, 9–20.

Madsen, L. and Dalsgaard, I. (1999) Reproducible methods for experimental infection with *Flavobacterium psychrophilum* in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **36**, 169–176.

Madsen, L. and Dalsgaard, I. (2000) Comparative studies of Danish *Flavobacterium psychrophilum* isolates: Ribotypes, plasmid profiles, serotypes and virulence. *Journal of Fish Diseases* **23**, 211–218.

Madsen, L., Møller, J.D. and Dalsgaard, I. (2005) *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum), hatcheries: Studies on broodstock, eggs, fry and environment. *Journal of Fish Diseases* **28**, 39–47.

Magariños, B., Romalde, J.L., Bandín, I., Fouz, B. and Toranzo, A.E. (1992) Phenotypic, antigenic, and molecular characterization of *Pasteurella piscicida* strains isolated from fish. *Applied and Environmental Microbiology* **58**, 3316–3322.

Magariños, B., Romalde, J.L., Barja, J.L. and Toranzo, A.E. (1994a) Evidence of a dormant but infective state of the fish pathogen *Pasteurella piscicida* in seawater and sediment. *Applied and Environmental Microbiology* **60**, 180–186.

Magariños, B., Romalde, J.L., Lemos, M.L., Barja, J.L. and Toranzo, A.E. (1994b) Iron uptake by *Pasteurella piscicida* and its role in pathogenicity for fish. *Applied and Environmental Microbiology* **60**, 2990–2998.

Magariños, B., Romalde, J.L., Santos, Y., Casal, J.F., Barja, J.L. and Toranzo, A.E. (1994c) Vaccination trials on gilthead sea bream (*Sparus aurata*) against *Pasteurella piscicida*. *Aquaculture* **120**, 201–208.

Magariños, B., Pazos, F., Santos, Y., Romalde, J.L. and Toranzo, A.E. (1995) Response of *Pasteurella piscicida* and *Flexibacter maritimus* to skin mucus of marine fish. *Diseases of Aquatic Organisms* **21**, 103–108.

Magariños, B., Romalde, J.L., Noya, M., Barja, J.L. and Toranzo, A.E. (1996a) Adherence and invasive capacities of the fish pathogen *Pasteurella piscicida*. *FEMS Microbiology Letters* **138**, 29–34.

Magariños, B., Bonet, R., Romalde, J.L., Martínez, M.L., Congregado, F. and Toranzo, A.E. (1996b) Influence of the capsular layer on the virulence of *Pasteurella piscicida* for fish. *Microbial Pathogenesis* **21**, 289–297.

Magariños, B., Romalde, J.L., Cid, A. and Toranzo, A.E. (1997a) Viability of starved *Pasteurella piscicida* in seawater monitored by flow cytometry and the effects of antibiotics on its resuscitation. *Letters in Applied Microbiology* **24**, 122–126.

Magariños, B., Osorio, C.R., Toranzo, A.E. and Romalde, J.L. (1997b) Applicability of ribotyping for intraspecific classification and epidemiological studies of *Photobacterium damsela* subsp. *piscicida*. *Systematic and Applied Microbiology* **20**, 634–639.

Magariños, B., Couso, N., Noya, M., Merino, P., Toranzo, A.E. and Lamas, J. (2001) Effect of temperature on the development of pasteurellosis in carrier gilthead seabream (*Sparus aurata*). *Aquaculture* **195**, 17–21.

Magnadóttir, B., Bambir, S.H., Gudmundsdóttir, B.K., Pilstrom, L. and Helgason, S. (2002) Atypical *Aeromonas salmonicida* infection in naturally and experimentally infected cod, *Gadus morhua* L. *Journal of Fish Diseases* **25**, 583–597.

Magnússon, H.B., Fridjónsson, Ó.H., Andrésson, Ó.S., Benediktsdóttir, E., Gudmundsdóttir, S. and Andrésdottír, V. (1994) *Renibacterium salmoninarum*, the causal agent of bacterial kidney disease in salmonid fish, detected by nested reverse transcription-PCR of 16S rRNA sequences. *Applied and Environmental Microbiology* **60**, 4580–4583.

Maher, M., Palmer, R., Gannon, F. and Smith, T. (1995) Relationship of a novel bacterial fish pathogen to *Streptobacillus moniliformis* and the fusobacteria group, based on 16S ribosomal RNA analysis. *Systematic and Applied Microbiology* **18**, 79–84.

Maheshkumar, S., Goyal, S.M. and Economon, P.P. (1990) Concentration and detection of *Aeromonas salmonicida* from hatchery water. *Journal of Fish Diseases* **13**, 513–518.

Mahnken, C.V.W. (1975) Status report on commercial salmon culture in Puget Sound. *Commercial Fish Farmer, Aquaculture News* **2**, 8–11.

Mahoney, J.B., Midlige, F.H. and Deuel, D.G. (1973) A fin rot disease of marine and euryhaline fishes in the New York Bight. *Transactions of the American Fisheries Society* **102**, 597–605.

Mainous, M.E. and Smith, S.A. (2005) Efficacy of common disinfectants against *Mycobacterium marinum*. *Journal of Aquatic Animal Health* **17**, 284–288.

Majumdar, T., Ghosh, S., Pal, J. and Mazumder, S. (2006) Possible role of a plasmid in the pathogenesis of a fish disease caused by *Aeromonas hydrophila*. *Aquaculture* **256**, 95–104.

Mano, N., Inui, T., Arai, D., Hirose, H. and Deguchi, Y. (1996) Immune response in the skin of eel against *Cytophaga columnaris*. *Fish Pathology* **31**, 65–70.

Marco-Noales, E., Milán, M., Fouz, B., SanJuán, E. and Amaro, C. (2001) Transmission to eels, portals of entry, and putative reservoirs of *Vibrio vulnificus* serovar E (Biotype 2). *Applied and Environmental Microbiology* **67**, 4717–4725.

Marco-Noales, E., Biosca, E.G., Rojo, C. and Amaro, C. (2004) Influence of aquatic microbiota on the survival in water of the human and eel pathogen *Vibrio vulnificus* serovar E. *Environmental Microbiology* **6**, 364–376.

Markovic, M., Radojicic, M., Cosic, S. and Levnaic, D. (1996) Massive death of silver carp (*Hypophthalmichthys molitrix* Val.) and big head (*Aristichthys nobilis* Rich.) caused by *Pseudomonas fluorescens* bacteria. *Veterinarski Glasnik* **50**, 761–765.

Markwardt, N.M., Gocha, Y.M. and Klontz, G.W. (1989) A new application for coomassie brilliant blue agar: Detection of *Aeromonas salmonicida* in clinical samples. *Diseases of Aquatic Organisms* **6**, 231–233.

Marsden, M.J., Vaughan, L.M., Foster, T.J. and Secombes, C.J. (1996a) A live (*ΔaroA*) *Aeromonas salmonicida* vaccine for furunculosis preferentially stimulates T-cell responses relative to B-cell responses in rainbow trout (*Oncorhynchus mykiss*). *Infection and Immunity* **64**, 3863–3869.

Marsden, M.J., Collins, E. and Secombes, C.J. (1996b) Factors influencing the clearance of a genetically attenuated (*ΔaroA*) strain of *Aeromonas salmonicida* from rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **27**, 89–94.

Marsden, M.J., Freeman, L.C., Cox, D. and Secombes, C.J. (1996c) Non-specific immune responses in families of Atlantic salmon, *Salmo salar*, exhibiting differential resistance to furunculosis. *Aquaculture* **146**, 1–16.

Marsh, M.C. (1902) *Bacterium truttae*, a new bacterium pathogenic to trout. *Science* **16**, 706.

Marshall, S., Heath, S., Henríquez, V. and Orrego, C. (1998) Minimally invasive detection of *Piscirickettsia salmonis* in cultivated salmonids via the PCR. *Applied and Environmental Microbiology* **64**, 3066–3069.

Martin-Carnahan, A. and Joseph, S.W. (2005) Genus I: *Aeromonas* Stanier 1943, 213^{AL}. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 557–578.

Martínez, J.L., Casado, A. and Enríquez, R. (2004) Experimental infection of *Flavobacterium psychrophilum* in fins of Atantic salmon *Salmo salar* revealed by scanning electron microscopy. *Diseases of Aquatic Organisms* **59**, 79–84.

Martinez-Murcia, A.J., Esteve, C., Garay, E. and Collins, M.D. (1992) *Aeromonas allosaccharophila* sp. nov., a new mesophilic member of the genus *Aeromonas*. *FEMS Microbiology Letters* **91**, 199–206.

Martínez-Picardo, J., Blanch, A.R. and Jofre, J. (1994) Rapid detection and identification of *Vibrio anguillarum* by using a specific oligonucleotide probe complementary to 16S rRNA. *Applied and Environmental Microbiology* **60**, 732–737.

Martinsen, B., Myhr, E., Reed, E. and Håstein, T. (1991) In vitro antimicrobial activity of sarafloxacin against clinical isolates of bacteria pathogenic to fish. *Journal of Aquatic Animal Health* **3**, 235–241.

Masada, C.L., LaPatra, S.E., Morton, A.W. and Strom, M.S. (2002) An *Aeromonas salmonicida* type IV pilin is required for virulence in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **51**, 13–25.

Massad, G., Arceneaux, J.E.L. and Byers, B.R. (1991) Acquisition of iron from host sources by mesophilic *Aeromonas* species. *Journal of General Microbiology* **137**, 237–241.

Massias, B., Dumetz, F., Urdaci, M.-C. and Le Hénaff, M. (2004) Identification of P18, a surface protein produced by the fish pathogen *Flavobacterium psychrophilum*. *Letters in Applied Microbiology* **97**, 574–580.

Masterman, A.T. and Arkwright, J.A. (1911) *Report, Board of Agriculture and Fisheries, U.K.*

Masumura, K. and Wakabayashi, H. (1977) An outbreak of gliding bacterial disease in hatchery-borne red sea bream (*Pagrus major*) and gilthead (*Acanthopagrus schlegeli*) fry in Hiroshima. *Fish Pathology* **12**, 171–177.

Masumura, K., Yasunobu, H., Okada, N. and Muroga, K. (1989) Isolation of a *Vibrio* sp., the causative bacterium of intestinal necrosis of Japanese flounder larvae. *Fish Pathology* **24**, 135–141.

Mata, A.I., Gibello, A., Casamayor, A, Blanco, M.M., Domínquez, L. and Fernández-Garayzábal, J.F. (2004) Multiplex PCR assay for the detection of bacterial pathogens associated with warm-water streptococcosis in fish. *Applied and Environmental Microbiology* **70**, 3183–3187.

Mata, M., Skarmeta, A. and Santos, Y. (2002) A proposed serotyping scheme for *Flavobacterium psychrophilum*. *Letters in Applied Microbiology* **35**, 166–170.

Mateos, D. and Paniagua, C. (1995) Surface characteristics of *Aeromonas hydrophila* recovered from trout tissues. *Journal of General and Applied Microbiology* **41**, 249–254.

Mathew, J.A., Tan, Y.P., Rao, P.S.S., Lim, T.M. and Leung, K.Y. (2001) *Edwardsiella tarda* mutants defective in siderophore production, motility, serum resistance and catalase activity. *Microbiology* **147**, 449–457.

Matsuo, K. and Miyazono, I. (1993) The influence of long-term administration of peptidoglycan on disease resistance and growth of juvenile rainbow trout. *Nippon Suisan Gakkaishi* **59**, 1377–1379.

Matsuoka, S. (2004) Discharge of *Edwardsiella tarda* cells from experimentally infected Japanese flounder. *Fish Pathology* **39**, 9–13.

Matsuoka, S. and Kamada, S. (1995) Discharge of *Pasteurella piscicida* cells from experimentally infected yellowtail. *Fish Pathology* **30**, 221–225.

Matsuoka, S. and Nakai, T. (2004) Seasonal appearance of *Edwardsiella tarda* and its bacteriophages in the culture farms of Japanese flounder. *Fish Pathology* **39**, 145–152.

Matsuyama, H., Mangindaan, R.E.P. and Yano, Y. (1992) Protective effect of schizophyllan and scleroglucan against *Streptococcus* sp. infection in yellowtail (*Seriola quinqueradiata*). *Aquaculture* **101**, 197–203.

Matsuyama, T., Kamaishi, T., Ooseko, N., Kurohara, K. and Iida, T. (2005) Pathogenicity of motile and non-motile *Edwardsiella tarda* to some marine fish. *Fish Pathology* **40**, 133–135.

Matsuyama, T., Kamaishi, T. and Oseko, N. (2006) Rapid discrimination of fish pathogenic *Vibrio* and *Photobacterium* species by oligonucleotide DNA array. *Fish Pathology* **41**, 105–112.

Mattheis, T. (1964) Das Vorkommen von *Vibrio anguillarum* in Ostseefischen. *Zentralblatt für Fischerei N.F.* **XII**, 259–263.

Mauel, M.J. and Fryer, J.L. (2001) Amplification of a *Piscirickettsia salmonis*-like 16S rDNA product from bacterioplankton DNA collected from the coastal waters of Oregon, USA. *Journal of Aquatic Animal Health* **13**, 280–284.

Mauel, M.J., Giovannoni, S.J. and Fryer, J.L. (1996) Development of polymerase chain reaction assays for detection, identification and differentiation of *Piscirickettsia salmonis*. *Diseases of Aquatic Organisms* **26**, 189–195.

Mauel, M.J., Miller, D.L., Frazier, K., Liggett, A.D., Styer, L., Montgomery-Brock, D. and Brock, J. (2003) Characterization of a piscirickettsiosis-like disease in Hawaiian tilapia. *Diseases of Aquatic Organisms* **53**, 249–255.

Maugeri, T.L., Crisafi, E., Genovese, L. and Scoglio, M.E.R. (1983) Identification of *Vibrio anguillarum* with the API-20E system. *Microbiologica* **1**, 73–79.

Maulén, N.P., Morales, P.J., Aruti, D., Figueroa, J.E., Concha, M.I., Krauskopf, M. and Maulén, G. (1996) Identification of *Renibacterium salmoninarum* DNA fragment associated with bacterial internalization into CHSE-cultured cells. *FEMS Microbiology Letters* **135**, 37–43.

Mawdesley-Thomas, L.E. (1969) Furunculosis in the goldfish *Carasius auratus* (L.). *Journal of Fish Biology* **1**, 19–23.

McArdle, J.F. and Dooley-Martin, C. (1985) Isolation of *Yersinia ruckeri* type 1 (Hagerman strain) from goldfish *Carassius auratus* (L.). *Bulletin of the European Association of Fish Pathologists* **5**, 10–11.

McCarthy, D.H. (1975a) Detection of *Aeromonas salmonicida* antigen in diseased fish tissue. *Journal of General Microbiology* **88**, 185–187.

McCarthy, D.H. (1975b) Fish furunculosis. *Journal of the Institute of Fisheries Management* **6**, 13–18.

McCarthy, D.H. (1975c) Columnaris disease. *Journal of the Institute of Fisheries Management* **6**, 44–47.

McCarthy, D.H. (1976) Laboratory techniques for the diagnosis of fish-furunculosis and whirling disease. *Ministry of Agriculture, Fisheries and Foods, Fisheries Research Technical Report No.* **23**, 5 pp.

McCarthy, D.H. (1977a) The identification and significance of atypical strains of *Aeromonas salmonicida*. *Bulletin de l'Office International des Epizooties* **87**, 459–463.

McCarthy, D.H. (1977b) Some ecological aspects of the bacterial fish pathogen, *Aeromonas salmonicida*. *Society of Applied Bacteriology Symposium Aquatic Microbiology* **6**, 299–324.

McCarthy, D.H. (1978) A study of the taxonomic status of some bacteria currently assigned to the genus Aeromonas. Ph.D. thesis, Council of National Academic Awards, UK.

McCarthy, D.H. (1980) Some ecological aspects of the bacterial fish pathogen—*Aeromonas salmonicida*. In: *Aquatic Microbiology. Symposium of the Society of Applied Bacteriology No. 6*, pp. 299–324.

McCarthy, D.H. and Rawle, C.T. (1975) Rapid serological diagnosis of fish furunculosis caused by smooth and rough strains of *Aeromonas salmonicida*. *Journal of General Microbiology* **86**, 185–187.

McCarthy, D.H. and Roberts, R.J. (1980) Furunculosis of fish: The present state of our knowledge. In: Droop, M.A. and Jannasch, H.W. (eds), *Advances in Aquatic Microbiology*. London, Academic Press, pp. 293–341.

McCarthy, D.H. and Whitehead, P. (1977) An immuno-India ink technique for rapid laboratory diagnosis of fish furunculosis. *Journal of Applied Bacteriology* **42**, 429–431.

McCarthy, D.H., Stevenson, J.P. and Salsbury, A.W. (1974) Therapeutic efficacy of a potentiated sulphonamide in experimental furunculosis. *Aquaculture* **4**, 407–410.

McCarthy, D.H., Amend, D.F., Johnson, K.A. and Bloom, J.V. (1983) *Aeromonas salmonicida*: Determination of an antigen associated with protective immunity and evaluation of an experimental bacterin. *Journal of Fish Diseases* **6**, 155–174.

McCarthy, D.H., Cory, T.R. and Amend, D.F. (1984) Imunization of rainbow trout, *Salmo gairdneri* Richardson, against bacterial kidney disease: Preliminary efficacy evaluation. *Journal of Fish Diseases* **7**, 65–71.

McCarthy, Ú., Steiropoulos, N.A., Thompson, K.D., Adams, A., Ellis, A.E. and Ferguson, H.W. (2005) Confirmation of *Piscirickettsia salmonis* in European sea bass *Dicentrarchus labrax* and phylogenetic comparison with salmonid strains. *Diseases of Organisms* **64**, 107–119.

McCashion, R.N. and Lynch, W.H. (1987) Effects of polymyxin B nonapeptide on *Aeromonas salmonicida*. *Antimicrobial Agents and Chemotherapy* **31**, 1414–1419.

McCormick, J.I. and McLoughlin, M.F. (1993) The characterisation and pathogenicity of the first isolate of *Yersinia ruckeri* from rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Northern Ireland. *Bulletin of the European Association of Fish Pathologists* **13**, 138–140.

McCormick, J.I., Hughes, M.S. and McLoughlin, M.F. (1995) Identification of *Mycobacterium chelonae* in a cichlid oscar *Astronotus ocellatus* Cuvier, by direct cycle sequencing of

polymerase chain reaction amplified 16S rRNA gene sequences. *Journal of Fish Diseases* **18**, 459–461.

McCoy, R.H. and Pilcher, K.S. (1974) Peptone beef extract glycogen agar, a selective and differential *Aeromonas* medium. *Journal of the Fisheries Research Board of Canada* **31**, 1553–1555.

McCracken, A. and Fidgeon, S. (1977) The effect of storage on drugs incorporated into pelleted fish food. *Journal of Applied Bacteriology* **42**, 289–290.

McCraw, B.M. (1952) Furunculosis of fish. *United States Fish and Wildlife Service, Special Scientific Report* **84**, 87 pp.

McDaniel, D.W. (1972) *Hatchery Biologist, Quarterly Report*, First Quarter 1972.

McIntosh, D. and Austin, B. (1988) Comparison of methods for the induction, propagation and recovery of L-phase variants of *Aeromonas* spp. *Journal of Diarrhoeal Disease Research* **6**, 131–136.

McIntosh, D. and Austin, B. (1990a). Recovery of cell wall deficient forms (L-forms) of the fish pathogens *Aeromonas salmonicida* and *Yersinia ruckeri*. *Systematic and Applied Microbiology* **13**, 378–381.

McIntosh, D. and Austin, B. (1990b) Recovery of an extremely proteolytic form of *Serratia liquefaciens* as a pathogen of Atlantic salmon, *Salmo salar*, in Scotland. *Journal of Fish Biology* **36**, 765–772.

McIntosh, D. and Austin, B. (1991a) Atypical characteristics of the salmonid pathogen *Aeromonas salmonicida*. *Journal of General Microbiology* **137**, 1341–1343.

McIntosh, D. and Austin, B. (1991b) The role of cell wall deficient bacteria (L-forms; sphaeroplasts) in fish diseases. *Journal of Applied Bacteriology* **70**, 1S–7S.

McIntosh, D. and Austin, B. (1993) Potential use of vaccines based on cell-wall-defective or deficient (L-form) *Aeromonas salmonicida* for the control of furunculosis. *Journal of Aquatic Animal Health* **5**, 254–258.

McIntosh, D., Meaden, P.G. and Austin, B. (1996) A simplified PCR-based method for the detection of *Renibacterium salmoninarum* utilizing preparations of rainbow trout (*Oncorhynchus mykiss*, Walbaum) lymphocytes. *Applied and Environmental Microbiology* **62**, 3929–3932.

McIntosh, D., Flaño, E., Grayson, T.H., Gilpin, M.L. and Austin, B. (1997) Production of putative virulence factors by *Renibacterium salmoninarum* grown in cell culture. *Microbiology* **143**, 3349–3356.

McKell, J. and Jones, D. (1976) A numerical taxonomic study of *Proteus–Providencia* bacteria. *Journal of Applied Bacteriology* **41**, 143–161.

McKibben, C.L. and Pascho, R.J. (1999) Shedding of *Renibacterium salmoninarum* by infected chinook salmon *Oncorhynchus tschawytscha*. *Diseases of Aquatic Organisms* **38**, 75–79.

McVicar, A.H., Bruno, D.W. and Fraser, C.O. (1988) Fish diseases in the North Sea in relation to sewage sludge dumping. *Marine Pollution Bulletin* **19**, 169–173.

Mekuchi, T., Kiyokawa, T., Honda, K., Nakai, T. and Muroga, K. (1995a) Infection experiments with *Edwardsiella tarda* in the Japanese flounder. *Fish Pathology* **30**, 247–250.

Mekuchi, T., Kiyokawa, T., Honda, K., Nakai, T. and Muroga, K. (1995b) Vaccination trials in the Japanese flounder against edwardsiellosis. *Fish Pathology* **30**, 251–256.

Mellergaard, S. (1983) Purification and characterization of a new proteolytic enzyme produced by *Aeromonas salmonicida*. *Journal of Applied Bacteriology* **54**, 289–294.

Mellergaard, S. and Larsen, J.L. (1981) Haemagglutination activity of *Aeromonas salmonicida* and *Vibrio anguillarum* strains isolated from diseased rainbow trout (*Salmo gairdneri*). *Bulletin of the European Association of Fish Pathologists* **1**, 26–28.

Merino, S., Aguilar, A., Rubires, X., Simon-Pujol, D., Congregado, F. and Tomás, J.M. (1996) The role of the capsular polysaccharide of *Aeromonas salmonicida* in the adherence and invasion of fish cell lines. *FEMS Microbiology Letters* **142**, 185–189.

Merino, S., Aguilar, A., Rubires, X., Abitiu, N., Regué, M. and Tomás, J.M. (1997a) The role of the capsular polysaccharide of *Aeromonas hydrophila* serogroup O:34 in the adherence to and invasion of fish cell lines. *Research in Microbiology* **148**, 625–631.

Merino, S., Aguilar, A., Tomás, J.M., Bonet, R., Martinez, M.J., Simón-Pujol, D. and Congregado, F. (1997b) Complement resistance of capsulated strains of *Aeromonas salmonicida*. *Microbial Pathogenesis* **22**, 315–320.

Merino-Contreras, M.L., Guzman-Murillo, M.A., Ruiz-Bustos, E., Romera, M.J., Cadena-Roa, M.A. and Ascencio, F. (2001) Mucosal immune response of spotted sand bass *Paralabrax maculatofasciatus* (Steindachner, 1868) orally immunised with an extracellular lectin of *Aeromonas veronii*. *Fish & Shellfish Immunology* **11**, 115–126.

Mesa, M.G., Maule, A.G., Poe, T.P. and Schreck, C.B. (1999) Influence of bacterial kidney disease on smoltification in salmonids: Is it a case of double jeopardy? *Aquaculture* **174**, 25–41.

Meshram, S.U., Joshi, S., Kamdi, R. and Peshwe, S. (1998) *In vitro* interaction of microbial biopesticides with fish pathogens prevailing in aquaculture food industry. *Journal of Food Science and Technology, Mysore* **35**, 177–178.

Meyer, F.P. (1964) Field treatments of *Aeromonas liquefaciens* infections in golden shiners. *Progressive Fish Culturist* **26**, 33–35.

Meyer, F.P. and Bullock, G.L. (1973) *Edwardsiella tarda*, a new pathogen of channel catfish (*Ictalurus punctatus*). *Applied Microbiology* **25**, 155–156.

Meyers, T.R., Short, S., Farrington, C., Lipson, K., Geiger, H.J. and Gates, R. (1993) Comparison of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) for measuring the prevalences and levels of *Renibacterium salmoninarum* in wild and hatchery stocks of salmonid fishes in Alaska, U.S.A. *Diseases of Aquatic Organisms* **16**, 181–189.

Michel, C. (1979) Furunculosis of salmonids: Vaccination attempts in rainbow trout (*Salmo gairdneri*) by formalin-killed germs. *Annales de Recherche Vétérinaire* **10**, 33–40.

Michel, C. (1980) A standardized model of experimental furunculosis in rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 746–750.

Michel, C. (1981) A bacterial disease of perch (*Perca fluviatilis* L.) in an Alpine lake: Isolation and preliminary study of the causative organism. *Journal of Wildlife Diseases* **17**, 505–510.

Michel, C. and Dubois-Darnaudpeys, A. (1980) Persistence of the virulence of *Aeromonas salmonicida* kept in river sediments. *Annales de Recherche Vétérinaire* **11**, 375–386.

Michel, C. and Faivre, B. (1987) *In vitro* and *in vivo* study of an antimicrobial activity displayed by the redmouth disease agent, *Yersinia ruckeri*. *Annales Recherches Vetérinaire* **18**, 43–46.

Michel, C., Gerard, J.-P., Fourbet, B., Collas, R. and Chevalier, R. (1980) Emploi de la flumequine contre la furunculose des salmonides; essais therapeutiques et perspectives pratiques. *Bulletin Français de Pisciculture* **52**, 154–162.

Michel, C., Faivre, B. and De Kinkelin, P. (1986a) A clinical case of enteric redmouth in minnows (*Pimephales promelas*) imported in Europe as bait-fish. *Bulletin of the European Association of Fish Pathologists* **6**, 97–99.

Michel, C., Faivre, B. and Kerouault, B. (1986b) Biochemical identification of *Lactobacillus piscicola* strains from France and Belgium. *Diseases of Aquatic Organisms* **2**, 27–30.

Michel, C., Gonzalez, R., Bonjour, E. and Avrameas, S. (1990) A concurrent increasing of natural antibodies and enhancement of resistance to furunculosis in rainbow trout. *Annales de Recherche Vétérinaire* **21**, 211–218.

Michel, C., Nougayrède, P., Eldar, A., Sochon, E. and de Kinkelin, P. (1997) *Vagococcus salmoninarum*, a bacterium of pathological significance in rainbow trout *Oncorhynchus mykiss* farming. *Diseases of Aquatic Organisms* **30**, 199–208.

Michel, C., Messiaen, S. and Bernardet, J.-F. (2002) Muscle infections in imported neon tetra, *Paracheirodon innesi* Myers: Limited occurrence of microsporidia and predominance of severe forms of columnaris disease caused by an Asian genomovar of *Flavobacterium columnare*. *Journal of Fish Diseases* **25**, 253–263.

Michel, C., Bernardet, J.-F., Daniel, P., Chilmonczyk, S., Urdaci, M. and De Kinkelin, P. (2002a) Clinical and aetiological aspects of a summer enteritic syndrome associated with the sporulating segmented filamentous bacterium, "*Candidatus* Arthromitus" in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **25**, 533–543.

Midtlyng, P.J. (1996) A field study on intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. *Fish & Shellfish Immunology* **6**, 553–565.

Midtlyng, P.J., Reitan, L.J., Lillehaug, A. and Ramstad, A. (1996) Protection, immune responses and side effects in Atlantic salmon (*Salmo salar* L.) vaccinated against furunculosis by different routes. *Fish & Shellfish Immunology* **6**, 599–600.

Millan, M. (1977) New diseases in salmon culture in Spain. *Bulletin de l'Office International des Epizooties* **87**, 515–516.

Miller, J.D. and Neely, M.N. (2005) Large-scale screen highlights the importance of capsule for virulence in the zoonotic pathogen *Streptococcus iniae*. *Infection and Immunity* **73**, 921–934.

Milton, D.L., O'Toole, R., Hörstedt, P. and Wolf-Watz, H. (1996) Flagellin A is essential for the virulence of *Vibrio anguillarum*. *Journal of Bacteriology* **178**, 1310–1319.

Minagawa, T., Nakai, T. and Muroga, K. (1983) *Edwardsiella tarda* in eel culture environment. *Fish Pathology* **17**, 243–250.

Minami, T. (1979) *Streptococcus* sp., pathogenic to cultured yellowtail, isolated from fishes for diets. *Fish Pathology* **14**, 15–19.

Minami, T., Nakamura, M., Ikeda, Y. and Ozaki, H. (1979) A beta-hemolytic *Streptococcus* isolated from cultured yellowtail. *Fish Pathology* **14**, 33–38.

Mitchell, A.J. and Goodwin, A.E. (2000) The isolation of *Edwardsiella ictaluri* with a limited tolerance for aerobic growth from channel catfish. *Journal of Aquatic Animal Health* **12**, 297–300.

Mitchum, D.L. (1981) Concurrent infections: ERM and furunculosis found in emerald shiners. *Fish Health Section/American Fisheries Society Newsletter* **9**, 2.

Mitchum, D.L. and Sherman, L.E. (1981) Transmission of bacterial kidney disease from wild to stocked hatchery trout. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 547–551.

Mitchum, D.L., Sherman, L.E. and Baxter, G.T. (1979) Bacterial kidney disease in feral populations of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **36**, 1370–1376.

Mitoma, Y., Aoki, T. and Crosa, J.H. (1984) Phylogenetic relationships among *Vibrio anguillarum* plasmids. *Plasmid* **12**, 143–148.

Mittal, K.R., Lalonde, G., Leblanc, D., Olivier, G. and Lallier, R. (1980) *Aeromonas hydrophila* in rainbow trout: Relation between virulence and surface characteristics. *Canadian Journal of Microbiology* **26**, 1501–1503.

Miwa, S. and Mano, N. (2000) Infection with *Edwardsiella tarda* causes hypertrophy of liver cells in the Japanese flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* **42**, 227–231.

Miwa, S. and Nakayasu, C. (2005) Pathogenesis of experimentally induced bacterial cold water disease in ayu *Plecoglossus altivelis*. *Diseases of Aquatic Organisms* **67**, 93–104.

Miyata, M., Aoki, T., Inglis, V., Yoshida, T. and Endo, M. (1995) RAPD analysis of *Aeromonas salmonicida* and *Aeromonas hydrophila*. *Journal of Applied Bacteriology* **79**, 181–185.

Miyata, M., Inglis, V. and Aoki, T. (1996) Rapid identification of *Aeromonas salmonicida* subspecies *salmonicida* by the polymerase chain reaction. *Aquaculture* **141**, 13–24.

Miyazaki, T. and Egusa, S. (1976) Histopathological studies on *Edwardsiella tarda* infection of Japanese eel—1. Natural infection—suppurative interstitial nephritis. *Fish Pathology* **11**, 33–43.

Miyazaki, T. and Kaige, N. (1985) A histopathological study on motile aeromonad disease of Crucian carp. *Fish Pathology* **21**, 181–185.

Miyazaki, T., Jo, Y., Kubota, S.S. and Egusa, S. (1977) Histopathological studies on vibriosis of the Japanese eel *Anguilla japonica*. Part 1. Natural infection. *Fish Pathology* **12**, 163–170.

Miyoshi, Y. and Suzuki, S. (2003) PCR method to detect *Nocardia seriolae* in fish samples. *Fish Pathology* **38**, 93–97.

Mizuki, H., Whasio, S., Morita, T., Itoi, S. and Sugita, H. (2006) Distribution of the fish pathogen *Listonella anguillarum* in the Japnese flounder *Paralichthys olivaceus* hatchery. *Aquaculture* **261**, 26–32.

Mladineo, I., Miletic, I. and Bocina, I. (2006) *Photobacterium damselae* subsp. *piscicida* outbreak in cage-reared Atlantic bluefin tuna *Thunnus thynnus*. *Journal of Aquatic Anial Health* **18**, 51–54.

Moki, S.T., Nomura, T. and Yoshimizu, M. (1995) Effects of incubation temperature for isolation on auto-agglutination of *Aeromonas salmonicida*. *Fish Pathology* **30**, 67–68.

Moles, A. (1997) Effect of bacterial kidney disease on saltwater adaptation of coho salmon smolts. *Journal of Aquatic Animal Health* **9**, 230–233.

Møller, J.D., Larsen, J.L., Madsen, L. and Dalsgaard, I. (2003) Involvement of a sialic acid-binding lectin with hemagglutination and hydrophobicity of *Flavobacterium psychrophilum*. *Applied and Environmental Microbiology* **69**, 5275–5280.

Møller, J.D., Barnes, A.C., Dalsgaard, I. and Ellis, A.E. (2005) Characterisation of surface blebbing and membrane vesicles produced by *Flavobacterium psychrophilum*. *Diseases of Aquatic Organisms* **64**, 201–209.

Møller, V. (1955) Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. *Acta Pathologica et Microbiologica Scandinavia* **36**, 58–172.

Mooney, J., Powell, E., Clabby, C. and Powell, R. (1995) Detection of *Aeromonas salmonicida* in wild Atlantic salmon using a specific DNA probe test. *Diseases of Aquatic Organisms* **21**, 131–135.

Moore, W.E.C. and Holeman-Moore, L.V. (1986) Genus: *Eubacterium* Prévot 1938, 294^{AL}. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 2. Baltimore, MD, Williams & Wilkins, pp. 1353–1373.

Morgan, J.A.W., Cranwell, P.A. and Pickup, R.W. (1991) Survival of *Aeromonas salmonicida* in lake water. *Applied and Environmental Microbiology* **57**, 1777–1782.

Morgan, J.A.W., Rhodes, G. and Pickup, R.W. (1993) Survival of non-culturable *Aeromonas salmonicida* in lake water. *Applied and Environmental Microbiology* **59**, 874–880.

Morris, D.J., Turgut, E. and Thompson, K.D. (2002) The use of melanin bleaching for the immunohistochemical detection of *Renibacterium salmoninarum*. *Bulletin of the European Association of Fish Pathologists* **22**, 33–36.

Morrison, C., Cornick, J., Shum, G. and Zwicker, B. (1981) Microbiology and histopathology of "saddleback" disease of underyearling Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **4**, 243–258.

Morrison, E.E. and Plumb, J.A. (1994) Olfactory organ of channel catfish as a site of experimental *Edwardsiella ictaluri* infection. *Journal of Aquatic Animal Health* **6**, 101–109.

Morse, E.G., Greenwood, D.E., Meyers, E.P., Anderson, V.L. and Duncan, M.A. (1978) Experimental *Salmonella* infections in *Carassius auratus* goldfish. *Journal of Experimental Science and Health Part A. Environmental Science and Engineering* **13**, 325–335.

Moss, M.O. and Ryall, C. (1980) The genus *Chromobacterium*. In: Starr, M.P., Stolp, H., Trüper, H.G., Balows, A. and Schlegel, H.G. (eds), *The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria*, Vol. 2. Berlin, Springer-Verlag, pp. 1355–1364.

Mudarris, M. and Austin, B. (1989) Systemic disease in turbot *Scophthalmus maximus* caused by a previously unrecognised *Cytophaga*-like bacterium. *Diseases of Aquatic Organisms* **6**, 161–166.

Mudarris, M. and Austin, B. (1992) Histopathology of a gill and systemic disease of turbot (*Scophthalmus maximus*) caused by a *Cytophaga*-like bacterium (CLB). *Bulletin of the European Association of Fish Pathologists* **12**, 120–123.

Mudarris, M., Austin, B., Segers, P., Vancanneyt, M., Hoste, B. and Bernardet, J.-F. (1994) *Flavobacterium scophthalmum* sp. nov., a pathogen of turbot (*Scophthalmus maximus* L.). *International Journal of Systematic Bacteriology* **44**, 447–453.

Munn, C.B. (1978) Haemolysin production by *Vibrio anguillarum*. *FEMS Microbiology Letters* **3**, 265–268.

Munn, C.B. (1980) Production and properties of a haemolytic toxin by *Vibrio anguillarum*. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 69–74.

Munn, C.B. and Trust, T.J. (1984) Role of additional protein layer in virulence of *Aeromonas salmonicida*. In: Acuigrup (ed.), *Fish Diseases, Fourth COPRAQ Session*. Madrid, Editora ATP, pp. 69–73.

Munn, C.B., Ishiguro, E.E., Kay, W.W. and Trust, T.J. (1982) Role of surface components in serum resistance of virulent *Aeromonas salmonicida*. *Infection and Immunity* **36**, 1069–1075.

Munro, A.L.S. (1984) A furunculosis vaccine: Illusion or achievable objective. In: de Kinkelin, P.K. (ed.), *Symposium on Fish Vaccination*. Paris, Office International des Epizooties, pp. 97–120.

Munro, A.L.S., Hastings, T.S., Ellis, A.E. and Liversidge, J. (1980) Studies on an ichthyotoxic material produced extracellularly by the furunculosis bacterium *Aeromonas salmonicida*. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 98–106.

Muraoka, A., Ogawa, K., Hashimoto, S. and Kusuda, R. (1991) Protection of yellowtail against pseudotuberculosis by vaccination with a potassium thiocyanate extract of *Pasteurella piscicida* and co-operating protective effect of acid-treated, naked bacteria. *Nippon Suisan Gakkaishi* **57**, 249–253.

Muroga, K. (1975) Studies on *Vibrio anguillarum* and *V. anguillicida* infections. *Journal of the Faculty of Fisheries and Animal Husbandry, Hiroshima University* **14**, 101–205.

Muroga, K. (1990) Bacterial infections in cultured fishes in Japan. In: Hirano, R. and Hanyu, I. (eds), *The Second Asian Fisheries Forum*. Manila, Asian Fisheries Society, pp. 963–966.

Muroga, K. and De La Cruz, M. (1987) Fate and location of *Vibrio anguillarum* in tissues of artificially infected ayu (*Plecoglossus altivelis*). *Fish Pathology* **22**, 99–103.

Muroga, K. and Yasunobu, H. (1987) Uptake of bacteria by rotifer. *Nippon Suisan Gakkaishi* **53**, 2091.

Muroga, K., Jo, Y. and Sawada, T. (1975) Studies on red spot disease of pond cultured eels.—II. Pathogenicity of the causative bacterium *Pseudomonas anguilliseptica*. *Fish Pathology* **9**, 107–114.

Muroga, K., Jo, Y. and Nishibuchi, M. (1976a) Pathogenic *Vibrio* isolated from cultured eels. I. Characteristics and taxonomic status. *Fish Pathology* **12**, 141–145.

Muroga, K., Nishibuchi, M. and Jo, Y. (1976b) Pathogenic *Vibrio* isolated from cultured eels. II. Physiological characteristics and pathogenicity. *Fish Pathology* **12**, 147–151.

Muroga, K., Sugiyama, T. and Ueki, N. (1977a) Pasteurellosis in cultured black sea bream (*Mylio macrocephalus*). *Journal of the Faculty of Fisheries and Animal Husbandry, Hiroshima University* **16**, 17–21.

Muroga, K., Nakai, T. and Sawada, T. (1977b) Studies on red spot disease of pond cultured eels.—IV. Physiological characteristics of the causative bacterium *Pseudomonas anguilliseptica*. *Fish Pathology* **12**, 33–38.

Muroga, K., Takahashi, S. and Yamanoi, H. (1979) Non-cholera *Vibrio* isolated from diseased ayu. *Bulletin of the Japanese Society of Scientific Fisheries* **45**, 829–834.

Muroga, K., Lio-Po, G., Pitogo, C. and Imada, R. (1984a) *Vibrio* sp. isolated from milkfish (*Chanos chanos*) with opaque eyes. *Fish Pathology* **19**, 81–87.

Muroga, K., Yamanoi, H., Hironaka, Y., Yamamoto, S., Tatani, M., Jo, Y., Takahashi, S. and Hanada, H. (1984b) Detection of *Vibrio anguillarum* from wild fingerlings of ayu *Plecoglossus altivelis*. *Bulletin of the Japanese Society of Scientific Fisheries* **50**, 591–596.

Muroga, K., Iida, M., Matsumoto, H. and Nakai, T. (1986) Detection of *Vibrio anguillarum* from waters. *Bulletin of the Japanese Society of Scientific Fisheries* **52**, 641–647.

Muroga, K., Yasunobu, H., Okada, N. and Masumura, K. (1990) Bacterial enteritis of cultured flounder *Paralichthys olivaceus* larvae. *Diseases of Aquatic Organisms* **9**, 121–125.

Murray, C.B., Evelyn, T.P.T., Beacham, T.D., Barner, L.W., Ketcheson, J.E. and Prosperi-Porta, L. (1992) Experimental induction of bacterial kidney disease in chinook salmon by immersion and cohabitation challenges. *Diseases of Aquatic Organisms* **12**, 91–96.

Murray, R.G.E., Dooley, J.S.G., Whippey, P.W. and Trust, T.J. (1988) Structure of an S layer on a pathogenic strain of *Aeromonas hydrophila*. *Journal of Bacteriology* **170**, 2625–2630.

Mushiake, K., Muroga, K. and Nakai, T. (1984) Increased susceptibility of Japanese eel *Anguilla japonica* to *Edwardsiella tarda* and *Pseudomonas anguilliseptica* following exposure to copper. *Bulletin of the Japanese Society of Scientific Fisheries* **50**, 1797–1801.

Mushiake, K., Nakai, T. and Muroga, K. (1985) Lowered phagocytosis in the blood of eels exposed to copper. *Fish Pathlogy* **20**, 49–53.

Muthiara, L.W., Raymond, B.T., Dekievit, T.R. and Stevenson, R.H.W. (1993) Antibody specificities of polyclonal rabbit and rainbow trout antisera against *Vibrio ordalii* and serotype O:2 strains of *Vibrio anguillarum*. *Canadian Journal of Microbiology* **39**, 492–499.

Mutoloki, S., Brudeseth, B., Reite, O.B. and Evensen, Ø. (2006) The contribution of *Aeromonas salmonicida* extracellular products to the induction of inflammation in Atlantic salmon (*Salmo salar* L.) following vaccination with oil-based vaccines. *Fish & Shellfish Immunology* **20**, 1–11.

Myhr, E., Larsen, J.L., Lillehaug, A., Gudding, R., Heum, M. and Håstein, T. (1991) Characterization of *Vibrio anguillarum* and closely related species isolated from farmed fish in Norway. *Applied and Environmental Microbiology* **57**, 2756–2757.

Nagai, T. and Iida, Y. (2002) Occurrence of bacterial kidney disease in cultured ayu. *Fish Pathology* **37**, 77–81.

Nagai, T. and Sakamoto, T. (2006) Susceptibility and immune response to *Flavobacterium psychrophilum* between different stocks of ayu *Plecoglossus altivelis*. *Fish Pathology* **41**, 99–104.

Nagai, T., Iida, Y. and Yoneji, T. (2003) Field trials of a vaccine with water-soluble adjuvant for bacterial coldwater disease in ayu *Plecoglossus altivelis*. *Fish Pathology* **38**, 63–65.

Nagai, T., Tamura, T., Iida, Y. and Yoneji, T. (2004) Differences in susceptibility to *Flavobacterium psychrophilum* among three stocks of ayu *Plecoglossus altivelis*. *Fish Pathology* **39**, 159–164.

Nakai, T. and Muroga, K. (1979) Studies on red spot disease of pond cultured eels—V. Immune response of the Japanese eel to the causative bacterium *Pseudomonas anguilliseptica*. *Bulletin of the Japanese Society of Scientific Fisheries* **45**, 817–821.

Nakai, T. and Muroga, K. (1982) *Pseudomonas anguilliseptica* isolated from European eels (*Anguilla anguilla*) in Scotland. *Fish Pathology* **17**, 147–150.

Nakai, T., Muroga, K. and Wakabayashi, H. (1981) Serological properties of *Pseudomonas anguilliseptica* in agglutination. *Bulletin of the Japanese Society of Scientific Fisheries* **47**, 699–703.

Nakai, T., Muroga, K. and Wakabayashi, H. (1982a) An immuno-electrophoretic analysis of *Pseudomonas anguilliseptica*. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 363–367.

Nakai, K. Muroga, K., Ohnishi, K., Jo, Y. and Tanimoto, H (1982b) Studies on the red spot disease of pond cultured eels—IX. A field vaccination trial. *Aquaculture* **30**, 131–135.

Nakai, T., Kanno, T., Cruz, E.R. and Muroga, K. (1987) The effects of iron compounds on the virulence of *Vibrio anguillarum* in Japanese eels and ayu. *Fish Pathology* **22**, 185–189.

Nakai, T., Miyakawa, M., Muroga, K. and Kamito, K. (1989a) The tissue distribution of atypical *Aeromonas salmonicida* in artificially infected Japanese eels, *Anguilla japonica*. *Fish Pathology* **24**, 23–28.

Nakai, T., Muroga, K. and Masumura, K. (1989b) Immersion vaccination of juvenile rockfish *Sebastes schlegeli* by *Vibrio ordalii* and *Vibrio anguillarum* antigens. *Suisanzoshoku* **37**, 129–132.

Nakai, T., Sugimoto, R., Park, K.-H., Matsuoka, S., Mori, K., Nishioka, T. and Maruyama, K. (1999) Protective effects of bacteriophage on experimental *Lactococcus garvieae* infection in yellowtail. *Diseases of Aquatic Organisms* **37**, 33–41.

Nakajima, M. and Chikahata, H. (1979) Efficacy of oral and hyperosmotic vaccinations for vibriosis in ayu. *Fish Pathology* **14**, 9–13.

Nakajima, K., Muroga, K. and Hancock, R.E.W. (1983) Comparison of fatty acid, protein and serological properties distinguishing outer membrane of *Pseudomonas anguilliseptica* strains from those of fish pathogens and other pseudomonads. *International Journal of Systematic Bacteriology* **33**, 1–8.

Nakamura, Y. (1982) Doxycycline. *Fish Pathology* **17**, 67–76.

Nakamura, Y., Nakai, T. and Muroga, K. (1990) Induction of antibody producing cells in some fishes by immunization with *Vibrio anguillarum* O-antigen. *Fish Pathology* **25**, 225–230.

Nakatsugawa, T. (1983) *Edwardsiella tarda* isolated from cultured young flounder. *Fish Pathology* **18**, 99–101.

Nakatsugawa, T. (1994) Atypical *Aeromonas salmonicida* isolated from cultured spotted halibut. *Fish Pathology* **29**, 193–198.

Naudé, T.W. (1975) The occurrence and diagnosis of certain trout diseases. *Fish Farmer* **9**, 15–20.

Navarre, O. and Halver, J.E. (1989) Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. *Aquaculture* **79**, 207–221.

Nayak, D.K., Asha, A., Shankar, K.M. and Mohan, C.V. (2004) Evaluation of biofilm of *Aeromonas hydrophila* for oral vaccination of *Clarias batrachus*: A carnivore model. *Fish & Shellfish Immunology* **16**, 613–619.

Nedoluha, P.C. and Westhoff, D. (1995) Microbiological analysis of striped bass (*Morone saxatilis*) grown in flow-through tanks. *Journal of Food Protection* **58**, 1363–1368.

Nelson, D.R., Sadlowski, Y., Eguchi, M. and Kjelleberg, S. (1997) The starvation–stress response of *Vibrio (Listonella) anguillarum*. *Microbiology* **143**, 2305–2312.

Nelson, E.J. and Ghiorse, W.C. (1999) Isolation and identification of *Pseudoalteromonas piscicida* strain Cura-d associated with diseased damselfish (Pomacentridae) eggs. *Journal of Fish Diseases* **22**, 253–260.

Nelson, J.S., Kawahara, E., Kawai, K. and Kusuda, R. (1989) Macrophage infiltration in pseudotuberculosis of yellowtail, *Seriola quinqueradiata*. *Bulletin of Marine Sciences and Fisheries, Kochi University* **11**, 17–22.

Nematollahi, A., Decostere, A., Pasmans, F., Ducatelle, R. and Haesebrouck, F. (2003) Adhesion of high and low virulence *Flavobacterium psychrophilum* strains to isolated gill arches of rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **55**, 101–107.

Nematollahi, A., Pasmans, F., Haesebrouck, F. and Decostere, A. (2005) Early interactions of *Flavobacterium psychrophilum* with macrophages of rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **64**, 23–28.

Nematollahi, A., Pasmans, F., Van den Broeck, W., Ducatelle, R., Haesebrouck, F. and Decostere, A. (2005a) Association of *Flavobacterium psychrophilum* strains with intestinal explants of rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **67**, 67–72.

Nese, L. and Enger, Ø. (1993) Isolation of *Aeromonas salmonicida* from salmon lice *Lepeophtheirus salmonis* and marine plankton. *Diseases of Aquatic Organisms* **16**, 79–81.

Newman, S.G. and Majnarich, J.J. (1982) Direct immersion vaccination of juvenile rainbow trout, *Salmo gairdneri* Richardson, and juvenile coho salmon, *Oncorhynchus kisutch* (Walbaum), with a *Yersinia ruckeri* bacterin. *Journal of Fish Diseases* **5**, 339–341.

Newton, J.C., Wood, T.M. and Hartley, M.M. (1997) Isolation and partial characterization of extracellular proteases produced by isolates of *Flavobacterium columnare* derived from channel catfish. *Journal of Aquatic Animal Health* **9**, 75–85.

Nguyen, H.T. and Kanai, K. (1999) Selective agars for the isolation of *Streptococcus iniae* from Japanese flounder, *Paralichthys olivaceus*, and its cultural environment. *Journal of Applied Microbiology* **86**, 769–776.

Nielsen, B. and Dalsgaard, I. (1991) Plasmids in *Vibrio salmonicida* isolates from the Faroe Islands. *Bulletin of the European Association of Fish Pathologists* **11**, 206–207.

Nielsen, B., Olsen, J.E. and Larsen, J.L. (1993) Plasmid profiling as an epidemiological marker within *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* **15**, 129–135.

Nielsen, B., Olsen, J.E. and Larsen, J.L. (1994) Ribotyping of *Aeromonas salmonicida* subsp. *salmonicida*. *Diseases of Aquatic Organisms* **18**, 151–158.

Nielsen, M.E., Høi, L., Schmidt, A.S., Qian, D., Shimada, T., Shen, J.Y. and Larsen, J.L. (2001) Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that causes disease outbreaks in aquaculture production in the Zhajiang Province of China. *Diseases of Aquatic Organisms* **46**, 23–29.

Nieto, T.P. and Ellis, A.E. (1986) Characterization of extracellular metallo- and serine-proteases of *Aeromonas hydrophila* strain B_{51}. *Journal of General Microbiology* **132**, 1975–1979.

Nieto, T.P., Corcobado, M.J.R., Toranzo, A.E. and Barja, J.L. (1985) Relation of water temperature to infection of *Salmo gairdneri* with motile *Aeromonas*. *Fish Pathology* **20**, 99–105.

Nieto, T.P., López, L.R., Santos, Y., Núñez, S. and Toranzo, A.E. (1990) Isolation of *Serratia plymuthica* as an opportunistic pathogen in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **13**, 175–177.

Nieto, T.P., Santos, Y., Rodriguez, L.A. and Ellis, A.E. (1991) An extracellular acetylcholinesterase produced by *Aeromonas hydrophila* is a major lethal toxin for fish. *Microbial Pathogenesis* **11**, 101–110.

Nigrelli, R.F. (1943) Causes of disease and death of fishes in captivity. *Zoologica* **28**, 208–216.

Nigrelli, R.F. and Hutner, S.H. (1945) The presence of a myxobacterium, *Chondrococcus columnaris* (Davis) Ordal & Rucker (1944) on *Fundulus heteroclitus* (L.). *Zoologica* **30**, 101–103.

Nigrelli, R.F. and Vogel, H. (1963) Spontaneous tuberculosis in fishes and in other cold-blooded vertebrates with special reference to *Mycobacterium fortuitum* Cruz from fish and human lesions. *Zoologica (New York)* **48**, 130–143.

Nikl, L., Albright, L.J. and Evelyn, T.P.T. (1991) Influence of seven immunostimulants on the immune response of coho salmon to *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* **12**, 7–12.

Nikl, L., Evelyn, T.P.T. and Albright, L.J. (1993) Trials with an orally and immersion-administered β-1,3 glucan as an immunoprophylactic against *Aeromonas salmonicida* in juvenile chinook salon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms* **17**, 191–196.

Nilsson, W.B. and Strom, M.S. (2002) Detection and identification of bacterial pathogens of fish in kidney tissue using terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes. *Diseases of Aquatic Organisms* **48**, 175–185.

Nilsson, W.B., Gudkovs, N. and Strom, M.S. (2006) Atypical strains of *Aeromonas salmonicida* contain multiple copies of insertion element ISAsa4 useful as a genetic marker and a target for PCR assay. *Diseases of Aquatic Organisms* **70**, 209–217.

Ninomiya, K. and Yamamoto, M. (2001) Efficacy of oil-adjuvanted vaccines for bacterial hemorrhagic ascites in ayu *Plecoglossus altivelis*. *Fish Pathology* **36**, 183–185.

Ninomiya, M., Muraoka, A. and Kusuda, R. (1989) Effect of immersion vaccination of cultured yellowtail with a ribosomal vaccine prepared from *Pasteurella piscicida*. *Nippon Suissan Gakkaishi* **55**, 1773–1776.

Nishibuchi, M. and Muroga, K. (1977) Pathogenic *Vibrio* isolated from cultured eels. III. NaCl tolerance and flagellation. *Fish Pathology* **12**, 87–92.

Nishibuchi, M. and Muroga, K. (1980) Pathogenic *Vibrio* isolated from cultured eels. V. Serological studies. *Fish Pathology* **14**, 117–124.

Nishibuchi, M., Muroga, K., Seidler, R.J. and Fryer, J.L. (1979) Pathogenic *Vibrio* isolated from cultured eels. IV. Deoxyribonucleic acid studies. *Bulletin of the Japanese Society of Scientific Fisheries* **45**, 1469–1473.

Nishibuchi, M., Muroga, K. and Jo, Y. (1980) Pathogenic *Vibrio* isolated from cultured eels. VI. Diagnostic tests for the disease due to the present bacterium. *Fish Pathology* **14**, 125–131.

Nishimori, E., Kita-Tsukamoto, K. and Wakabayashi, H. (2000) *Pseudomonas plecoglossicida* sp. nov., the causative agent of bacterial haemorrhagic ascites of ayu, *Plecoglossus altivelis*. *International Journal of Systematic and Evolutionary Microbiology* **50**, 83–89.

Nitzan, S., Shwartsburd, B. and Heller, E.D. (2004) The effect of growth medium salinity of *Photobacterium damselae* subsp. *piscicida* on the immune response of hybrid bass (*Morone saxatilis* × *M. chrysops*). *Fish & Shellfish Immunology* **16**, 107–116.

Nomoto, R., Munasinghe, L.I., Shimahara, Y., Yasuda, H., Nakamura, A., Misawa, N., Itami, T. and Yoshida, T. (2004) Lancefield group C *Streptococcus dysgalactiae* infection responsible for fish mortalities in Japan. *Journal of Fish Diseases* **27**, 679–686.

Nomoto, R., Unose, N., Shimahara, Y., Nakamura, A., Hirae, T., Maebuchi, K., Harada, S., Misawa, N., Itami, T., Kagawa, H. and Yoshida, T. (2006) Characterization of Lancefield group C *Streptococcus dysgalactiae* isolated from farmed fish. *Journal of Fish Diseases* **29**, 673–682.

Nomura, S. and Saito, H. (1982) Production of the extracellular hemolytic toxin by an isolated strain of *Aeromonas salmonicida*. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 1589–1597.

Nomura, S., Fujino, M., Yamakawa, M. and Kawahara, E. (1988) Purification and characterization of salmolysin, an extracellular hemolytic toxin from *Aeromonas salmonicida*. *Journal of Bacteriology* **170**, 3694–3702.

Noonan, B. and Trust, T.J. (1995) The leucine zipper of *Aeromonas salmonicida* AbcA is required for the transcriptional activation of the P2 promoter of the surface-layer structural gene, *vapA*, in *Escherichia coli*. *Molecular Microbiology* **17**, 379–386.

Nordmo, R., Varma, K.J., Sutherland, I.H. and Brokken, E.S. (1994) Florfenicol in Atlantic salmon, *Salmo salar* L.: Field evaluation of efficacy against furunculosis in Norway. *Journal of Fish Diseases* **17**, 239–244.

Nordmo, R., Ramstad, A. and Holth Riseth, J.M. (1998) Induction of experimental furunculosis in heterogeneous test populations of Atlantic salmon (*Salmo salar* L.) by use of a cohabitation method. *Aquaculture* **162**, 11–21.

Norqvist, A., Hagström, Å. and Wolf-Watz, H. (1989) Protection of rainbow trout against vibriosis and furunculosis by the use of attenuated strains of *Vibrio salmonicida*. *Applied and Environmental Microbiology* **55**, 1400–1405.

Norqvist, A., Norman, B. and Wolf-Watz, H. (1990) Identification and characterization of a zinc metalloprotease associated with invasion by the fish pathogen *Vibrio anguillarum*. *Infection and Immunity* **58**, 3731–3736.

Norqvist, A., Bergman, A., Skogman, G. and Wolf-Watz, H. (1994) A field trial with the live attenuated fish vaccine strain *Vibrio anguillarum* VAN1000. *Bulletin of the European Association of Fish Pathologists* **14**, 156–158.

Novotny, A.J. (1978) Vibriosis and furunculosis in marine cultured salmon in Puget Sound. *Washington Marine Fisheries Review* **40**, 52–55.

Novotny, L., Dvorska, L., Lorencova, A., Beran, V., and Pavlik, I. (2004) Fish: A potential source of bacterial pathogens for human beings. *Veterinary Medicine* **49**, 343–358.

Noya, M., Magariños, B. and Lamas, J. (1995) Interactions between peritoneal exudate cells (PECs) of gilthead bream (*Sparus aurata*) and *Pasteurella piscicida*. A morphological study. *Aquaculture* **131**, 11–21.

Nusbaum, K.E. and Morrison, E.E. (2002) *Edwardsiella ictaluri* bacteraemia elicits shedding of *Aeromonas hydrophila* complex in latently infected channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* **25**, 343–350.

Nybelin, O. (1935) Untersuchungen über den bei Fischen Krankheitsegen den Spatpliz *Vibrio anguillarum*. *Meddelanden frau Statens Undersöknings—och Fasok—sanstalt für Sotvattenfisket Stockholm* **8**, 1–62.

Nzeako, B.C. (1991) Variation in *Aeromonas hydrophila* surface structures. *Bulletin of the European Association of Fish Pathologists* **11**, 176–179.

Oakey, H.J. and Owens, L. (2000) A new bacteriophage, VHML, isolated from a toxin-producing strain of *Vibrio harveyi* in tropical Australia. *Journal of Applied Microbiology* **89**, 702–709.

Oakey, H.J., Ellis, J.T. and Gibson, L.F. (1998) The development of random DNA probes specific for *Aeromonas salmonicida*. *Journal of Applied Microbiology* **84**, 37–46.

O'Brien, D., Mooney, J., Ryan, D., Powell, E., Hiney, M., Smith, P.R. and Powell, R. (1994) Detection of *Aeromonas salmonicida*, causal agent of furunculosis in salmonid fish, from the tank effluent of hatchery-reared Atlantic salmon smolts. *Applied and Environmental Microbiology* **60**, 3874–3877.

O'Farrell, C.L. and Strom, M.S. (1999) Differential expression of the virulence-associated protein p57 and characterization of its duplicated gene *msa* in virulent and attenuated strains of *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* **38**, 115–123.

Ogara, W.O., Mbuthia, P.G., Kaburia, H.F.A., Sørum, H., Kagunya, D.K., Nduthu, D.I. and Colquhoun, D. (1998) Motile aeromonads associated with rainbow trout (*Oncorhynchus mykiss*) mortality in Kenya. *Bulletin of the European Association of Fish Pathologists* **18**, 7–9.

O'Hici, B., Olivier, G. and Powell, R. (2000) Genetic diversity of the fish pathogen *Aeromonas salmonicida* demonstrated by random amplified polymorphic DNA and pulsed-field gel electrophoresis analyses. *Diseases of Aquatic Organisms* **39**, 109–119.

Ohnishi, K. and Muroga, K. (1976) *Vibrio* sp. as a cause of disease in rainbow trout cultured in Japan. 1. Biochemical characteristics. *Fish Pathology* **11**, 159–165.

Ohnishi, K., Watanabe, K. and Jo, Y. (1982) *Pasteurella* infection in young black seabream. *Fish Pathology* **16**, 207–210.

Ohtsuka, H., Nakai, T., Muroga, K. and Jo, Y. (1984) Atypical *Aeromonas salmonicida* isolated from diseased eels. *Fish Pathology* **19**, 101–107.

Oladosu, G.A., Ayinla, O.A. and Ajiboye, M.O. (1994) Isolation and pathogenicity of a *Bacillus* sp. associated with a septicaemic condition in some tropical freshwater fish species. *Journal of Applied Ichthyology* **10**, 69–72.

Olafsen, J.A., Christie, M. and Raa, J. (1981) Biochemical ecology and psychrotrophic strains of *Vibrio anguillarum* isolated from outbreaks of vibriosis at low temperature. *Zentralblatt für Bakteriologie and Hygiene, Erste Abteilung Originale C* **2**, 339–348.

Olea, I., Bruno, D.W. and Hastings, T.S. (1993) Detection of *Renibacterium salmoninarum* in naturally infected Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum) using an enzyme-linked immunosorbent assay. *Aquaculture* **116**, 99–110.

O'Leary, W.M., Panos, C. and Helz, G.E. (1956) Studies on the nutrition of *Bacterium salmonicida*. *Journal of Bacteriology* **72**, 673–676.

O'Leary, P.J. (1977) Enteric redmouth of salmonids: A biochemical and serological comparison of selected isolates. M.S. thesis, Oregon State University.

Olesen, J.E. and Larsen, J.L. (1993) Ribotypes and plasmid content of *Vibrio anguillarum* strains in relation to serovar. *Applied and Environmental Microbiology* **59**, 3863–3870.

Oliver, J.D., Warner, R.A. and Cleland, D.R. (1983) Distribution of *Vibrio vulnificus* and other lactose-fermenting vibrios in the marine environment. *Applied and Environmental Microbiology* **45**, 985–998.

Olivier, G. (1990) Virulence of *Aeromonas salmonicida*: Lack of relationship with phenotypic characteristics. *Journal of Aquatic Animal Health* **2**, 119–127.

Olivier, G., Evelyn, T.P.T. and Lallier, R. (1985a) Immunogenicity of vaccines from a virulent and an avirulent strain of *Aeromonas salmonicida*. *Journal of Fish Diseases* **8**, 43–55.

Olivier, G., Evelyn, T.P.T. and Lallier, R. (1985b) Immunity to *Aeromonas salmonicida* in coho salmon (*Oncorhynchus kisutch*) induced by modified Freunds complete adjuvant: Its non-specific nature and the probable role of macrophages in the phenomenon. *Developmental and Comparative Immunology* **9**, 419–432.

Olivier, G., Eaton, C.A. and Campbell, N. (1986) Interaction between *Aeromonas salmonicida* and peritoneal macrophages of brook trout (*Salvelinus fontinalis*). *Veterinary Immunology and Immunopathology* **12**, 223–234.

Olivier, G., Griffiths, S.G., Fildes, J. and Lynch, W.H. (1992) The use of Western blot and electroimmunotransfer blot assays to monitor bacterial kidney disease in experimentally challenged Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **15**, 229–241.

Olsen, A.B., Melby, H.P., Speilberg, L., Evensen, Ø. and Håstein, T. (1997) *Piscirickettsia salmonis* infection in Atlantic salmon *Salmo salar* in Norway: Epidemiological, pathological and microbiological findings. *Diseases of Aquatic Organisms* **31**, 35–48.

Olsen A.B., Mikalsen, J., Rode, M., Alfjorden, A., Hoel, E., Straum-Lie, K., Haldorsen, R. and Colquhoun, D.J. (2006) A novel systemic granulomatous inflammatory disease in farmed Atlantic cod, *Gadus morhua* L., associated with a bacterium belonging to the genus *Francisella*. *Journal of Fish Diseases* **29**, 307–311.

Olsen, A.B., Birkbeck, T.H., Nilsen, H.K., MacPherson, H.L., Wangel, C., Myklebust, C., Laidler, L.A., Aarflot, L., Thoen, E., Nygård, S., Thayumanavan, T. and Colquhoun, D.J. (2006a) Vaccine-associated systemic *Rhodococcus erythropolis* infection in farmed Atlantic salmon *Salmo salar*. *Diseases in Aquatic Organisms* **72**, 9–17.

Olsen, J.E. and Larsen, J.L. (1990) Restriction fragment length polymorphism of the *Vibrio anguillarum* serovar 01 virulence plasmid. *Applied and Environmental Microbiology* **56**, 3130–3132.

Olson, B.H. (1978) Enhanced accuracy of coliform testing in seawater by a modification of the Most Probable Number method. *Applied and Environmental Microbiology* **36**, 438–444.

Olsson, J.C., Jöborn, A., Westerdahl, A., Blomberg, L., Kjelleberg, S. and Conway, P.L. (1996) Is the turbot, *Scophthalmus maximus* (L.), intestine a portal of entry for the fish pathogen *Vibrio anguillarum*. *Journal of Fish Diseases* **19**, 225–234.

Olsson, J.C., Jöborn, A., Westerdahl, A., Blomberg, L., Kjelleberg, S. and Conway, P.L. (1998) Survival, persistence and proliferation of *Vibrio anguillarum* in juvenile turbot, *Scophthalmus maximus* (L.), intestine and faeces. *Journal of Fish Diseases* **21**, 1–9.

Onarheim, A.M. (1992) The glucan way to fish health. *Fish Farming International* **19**, 32–33.

Onishi, and Shiro (1978) Streptococcal infections in cultured sweetfish and herring. Abstract presented at the Spring Meeting of the Japanese Marine Products Association, p. 48.

Ooyama, T., Kera, A., Okada, T., Inglis, V. and Yoshida, T. (1999) The protective immune response of yellowtail *Seriola quinqueradiata* to the bacteria fish pathogen *Lactococcus garvieae*. *Diseases of Aquatic Organisms* **37**, 121–126.

Ooyama, T., Hirokawa, Y., Minami, T., Yasuda, H., Nakai, T., Endo, M., Ruangpan, L. and Yoshida, T. (2002) Cell-surface properties of *Lactococcus garvieae* strains and their immunogenicity in the yellowtail *Seriola quinqueradiata*. *Diseases of Aquatic Organisms* **51**, 169–177.

Oppenheimer, C.H. (1962) Marine fish diseases. In: *Fish as Food*, Vol. 2. New York, Academic Press, pp. 541–572.

Ordal, E.J. and Earp, B.J. (1956) Cultivation and transmission of etiological agent of bacterial kidney disease in salmonid fishes. *Proceedings of the Society of Experimental Biology and Medicine* **92**, 85–88.

Ordal, E.J. and Rucker, R.R. (1944) Pathogenic myxobacteria. *Proceedings of the Society of Experimental Biology and Medicine* **56**, 15–18.

Ortega, C., Ruiz, I., De Blas, I., Muzquiz, J.L., Fernandez, A. and Alonso, J.L. (1996) Furunculosis control using a paraimmunization stimulant (Baypamun) in rainbow trout. *Veterinary Research (Paris)* **27**, 561–568.

Osorio, C.R., Collins, M.D., Toranzo, A.E., Barja, J.L. and Romalde, J.L. (1999) 16S rRNA gene sequence analysis of *Photobacterium damselae* and nested PCR method for rapid detection of the causative agent of fish pasteurellosis. *Applied and Environmental Microbiolgy* **65**, 2942–2946.

Osorio, C.R., Toranzo, A.E., Romalde, J.L. and Barja, J.L. (2000) Multiplex PCR assay for *ureC* and 16S rRNA genes clearly discriminates between both subspecies of *Photobacterium damselae*. *Diseases of Aquatic Organisms* **40**, 177–183.

Ostland, V.E., LaTrace, C., Morrison, D. and Ferguson, H.W. (1999) *Flexibacter maritimus* associated with a bacterial stomatitis in Atlantic salmon smolts reared in net-pens in British Columbia. *Journal of Aquatic Animal Health* **11**, 35–44.

Ostland, V.E., Byrne, P.J., Hoover, G. and Ferguson, H.W. (2000) necrotic myositis of rainbow trout, *Oncorhynchus mykiss* (Walbaum): Proteolytic characteristics of a crude extracellular preparation from *Flavobacterium psychrophilum*. *Journal of Fish Diseases* **23**, 329–336.

Ostland, V.E., Stannard, J.A., Creek, J.J., Hedrick, R.P., Ferguson, H.W., Carlberg, J.M. and Westerman, M.E. (2006) Aquatic *Francisella*-like bacterium associated with mortalities of intensively cultured hybrid striped bass *Morone chrysops* × *M. Saxatilis*. *Diseases of Aquatic Organisms* **72**, 135–145.

Otis, V.S. and Behler, J.L. (1973) The occurrence of salmonellae and *Edwardsiella* in the turtles of New York zoological park. *Journal of Wildlife Diseases* **9**, 4–6.

O'Toole, R., Milton, D.L. and Wolf-Watz, H. (1996) Chemotactic motility is required for invasion of the host by the fish pathogen *Vibrio anguillarum*. Molecular Microbiology 19, 625–637.

O'Toole, R., Milton, D.L., Hörstedt, P. and Wolf–Watz, H. (1997) RpoN of the fish pathogen *Vibrio (Listonella) anguillarum* is essential for flagellum production and virulence by the water-borne but not intraperitoneal route of inoculation. *Microbiology* **143**, 3849–3859.

O'Toole, R., Lundberg, S., Fredriksson, S.Å., Jansson, A., Nilsson, B. and Wolf-Watz, H. (1999) The chemotactic response of *Vibrio anguillarum* to fish intestinal mucus is mediated by a combination of multiple mucus components. *Journal of Bacteriology* **181**, 4308–4317.

O'Toole, R., von Hofsten, J., Rosqvist, R., Olsson, P.-R. and Wolf-Watz, H. (2004) Visualisation of zebrafish infection by GFP-labelled *Vibrio anguillarum*. *Microbial Pathogenesis* **37**, 41–46.

Otte, E. (1963) Die heutigen Ansichten über die Atiologie der Infektiösen Bachwassersucht der Karpfen. *Wiener Tierärztliche Monatsschrift* **50**, 995–1005.

Owens, L., Austin, D.A. and Austin, B. (1996) Effect of strain origin on siderophore production in *Vibrio harveyi* isolates. *Diseases of Aquatic Organisms* **28**, 93–106.

Pacha, R.E. (1961) Columnaris disease in fishes in the Columbia River Basin. Ph.D. thesis, University of Washington, Seattle.

Pacha, R.E. (1968) Characteristics of *Cytophaga psychrophila* (Borg) isolated during outbreaks of bacterial cold-water disease. *Applied Microbiology* **16**, 97–101.

Pacha, R.E. and Kiehn, E.D. (1969) Characterization and relatedness of marine vibrios pathogenic to fish: Physiology, serology and epidemiology. *Journal of Bacteriology* **100**, 1242–1247.

Pacha, R.E. and Ordal, E.J. (1963) Epidemiology of columnaris disease in salmon. *Bacteriological Proceedings*, p. 3.

Pacha, R.E. and Ordal, E.J. (1967) Histopathology of experimental columnaris disease in young salmon. *Journal of Comparative Pathology* **77**, 419–423.

Pacha, R.E. and Ordal, E.J. (1970) Myxobacterial diseases of salmonids. In: Snieszko, S.F. (ed.), *A Symposium on Diseases of Fishes and Shellfishes*. Washington, D.C., American Fisheries Society, Special Publication No. 5, pp. 243–257.

Pacha, R.E. and Porter, S. (1968) Characteristics of myxobacteria isolated from the surface of freshwater fish. *Applied Microbiology* **16**, 1901–1906.

Paclibare, J.O., Evelyn, T.P.T., Albright, L.J. and Prosperi-Porta, L. (1994) Clearing of the kidney disease bacterium *Renibacterium salmoninarum* from seawater by the blue mussel *Mytilus edulis*, and the status of the mussel as a reservoir of the bacterium. *Diseases of Aquatic Organisms* **18**, 129–133.

Padilla, D., Real, F., Gómez, V., Sierra, E., Acosta, B., Déniz, S. and Acosta, F. (2005) Virulence factors and pathogenicity of *Hafnia alvei* for gilthead seabrea, *Sparus aurata* L. *Journal of Fish Diseases* **28**, 411–417.

Padrós, F., Zarza, C., Dopazo, L., Cuadrado, M. and Crespo, S. (2006) Pathology of *Edwardsiella tarda* infection in turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases* **29**, 87–94.

Pakingking, R., Takano, R., Nishizawa, T., Mori, K.-I., Iida, Y., Arimoto, M. and Muroga, K. (2003) Experimental coinfection with aquabirnavirus and viral hemorrhagic septicemia virus (VHSV), *Edwardsiella tarda* or *Streptococcus iniae* in Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* **38**, 15–21.

Palleroni, N.J. (1984) Genus I: *Pseudomonas* Migula 1894, 237^{AL} (Nom. Cons. Opin. 5, Jud. Comm. 1952, 237). In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 141–199.

Palleroni, N.J. (2005) Genus I: *Pseudomonas* Migula 1894, 237^{AL} (Nom. Cons., Opin 5 of the Jud. Comm. 1952, 121). In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 323–379.

Palmer, R. and Smith, P.R. (1980) Studies on vaccination of Atlantic salmon against furunculosis. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 107–112.

Palmer, R., Drinan, E. and Murphy, T. (1994) A previously unknown disease of farmed Atlantic salmon: Pathology and establishment of bacterial aetiology. *Diseases of Aquatic Organisms* **19**, 7–14.

Palmer, R., Ruttledge, M., Callanan, K. and Drinan, E. (1997) A piscirickettsiosis-like disease in farmed Atlantic salmon in Ireland: Isolation of the agent. *Bulletin of the European Association of Fish Pathologists* **17**, 68–72.

Panangala, V.S., Shelby, R.A., Shoemaker, C.A., Klesius, P.H., Mitra, A. and Morrison, E.E. (2006) Immunofluorescent test for simultaneous detection of *Edwardsiella ictaluri* and *Flavobacterium columnare*. *Diseases of Aquatic Organisms* **68**, 197–207.

Pang, L., Zhang, X.-H., Zhong, Y., Chen, J., Li, Y. and Austin, B. (2006) Identification of *Vibrio harveyi* using PCR amplification of the *toxR* gene. *Letters in Applied Microbiology* **43**, 249–255.

Paniagua, C., Rivero, O., Anguita, J. and Naharro, G. (1990) Pathogenicity factors and virulence for rainbow trout (*Salmo gairdneri*) of motile *Aeromonas* spp. isolated from a river. *Journal of Clinical Microbiology* **28**, 350–355.

Parisot, T.J. and Wood, E.M. (1960) A comparative study of the causative agent of a mycobacterial disease of salmonid fishes. II. A description of the histopathology of the disease in chinook salmon (*Oncorhynchus tshawytscha*) and a comparison of the strain characteristics of the fish disease with leprosy and human tuberculosis. *Annual Review of Respiratory Diseases* **82**, 212–222.

Park, K.H., Kato, H., Nakai, T. and Muroga, K. (1998) Phage typing of *Lactococcus garvieae* (formerly *Enterococcus seriolicida*) a pathogen of cultured yellowtail. *Fisheries Science (Tokyo)* **64**, 62–64.

Park, S.C. and Nakai, T. (2003) Bacteriophage control of Pseudomonas plecoglossicida infection in ayu Plecoglossus altivelis. *Diseases of Aquatic Organisms* **53, 33–39.**

Park, S.C., Shimamura, I., Fukunaga, M., Mori, K.-I. and Nakai, T. (2000) Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, for disease control. *Applied and Environmental Microbiology* **66**, 1416–1422.

Park, S.C., Shimamura, I., Hagihira, M. and Nakai, T. (2000a) A brown pigment-producing strain of *Pseudomonas plecoglossicida* isolated from ayu with hemorrhagic ascites. *Fish Pathology* **35**, 91–92.

Park, S.C., Nakai, T. and Yuasa, A. (2002) Relationship between in vitro motility of *Pseudomonas plecoglossicida* and clinical conditions in affected ayu. *Fish Pathology* **37**, 77–81.

Park, S.I. (1986) Distribution of *Edwardsiella tarda* in eel culture pond and its pathogenicity to eel. Doctoral Thesis, Faculty of Agriculture, University of Tokyo.

Park, S.-I., Wakabayashi, H. and Watanabe, Y. (1983) Serotype and virulence of *Edwardsiella tarda* isolated from eels and their environment. *Fish Pathology* **18**, 85–89.

Parker, N.D. and Munn, C.B. (1985) Cell surface properties of virulent and attenuated strains of *Aeromonas salmonicida*. In: Ellis, A.E. (ed.), *Fish and Shellfish Pathology*. London, Academic Press, pp. 97–105.

Pascho, R.J., Elliott, D.G., Mallett, R.W. and Mulcahy, D. (1987) Comparison of five techniques for the detection of *Renibacterium salmoninarum* in adult coho salmon. *Transactions of the American Fisheries Society* **116**, 882–890.

Pascho, R.J., Landolt, M.L. and Ongerth, J.E. (1995) Inactivation of *Renibacterium salmoninarum* by free chlorine. *Aquaculture* **131**, 165–175.

Pascho, R.J., Chase, D. and McKibben, C.L. (1998) Comparison of the membrane-filtration fluorescent antibody test, the enzyme-linked immunosorbent assay, and the polymerase chain reaction to detect *Renibacterium salmoninarum* in salmonid ovarian fluid. *Journal of Veterinary Diagnostic Investigation* **10**, 60–66.

Pasnik, D.J., Evans, J.J. and Klesius, P.H. (2005) Duration of protective antibodies and correlation with survival in Nile tilapia *Oreochromis niloticus* following *Streptococcus algalactiae* vaccination. *Diseases in Aquatic Organisms* **66**, 129–143.

Pasnik, D.J. and Smith, S.A. (2005) Immunogenic and protective effects of a DNA vaccine for *Mycobacterium marinum* in fish. *Veterinary Immunology and Immunopathology* **103**, 195–206.

Pasnik, D.J. and Smith, S.A. (2006) Immune and histopathologic responses of DNA-vaccinated hybrid striped bass *Morone saxatilis* × *M. chrysops* after acute *Mycobacterium marinum* infection. *Diseases of Aquatic Organisms* **73**, 33–41.

Pasnik, D.J., Evans, J.J., Panangala, V.S., Klesius, P.H., Shelby, R.A. and Shoemaker, C.A. (2005a) Antigenicity of *Streptococcus agalactiae* extracellular products and vaccine efficacy. *Journal of Fish Diseases* **28**, 205–212.

Pate, M., Jencic, V., Zolnir-Dovc, M. and Ocepek, M. (2005) Detection of mycobacteria in aquarium fish in Slovenia by culture and molecular methods. *Diseases of Aquatic Organisms* **64**, 29–35.

Paterson, W.D. (1974) Biochemical and serological differentiation of several pigment-producing aeromonads. *Journal of the Fisheries Research Board of Canada* **31**, 1259–1261.

Paterson, W.D. (1982) Furunculosis and other associated diseases caused by *Aeromonas salmonicida*. In: Anderson, D.P., Dorson, M. and Dubouget, P. (eds), *Antigens of Fish Pathogens: Development and Production for Vaccines and Serodiagnostics*. Lyon, France, Collection Foundation Marcel Merieux, pp. 119–137.

Paterson, W.D. and Fryer, J.L. (1974a) Immune response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* endotoxin. *Journal of the Fisheries Research Board of Canada* **31**, 1743–1749.

Paterson, W.D. and Fryer, J.L. (1974b) Immune response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* cells administered intraperitoneally in Freund's complete adjuvant. *Journal of the Fisheries Research Board of Canada* **31**, 1751–1755.

Paterson, W.D., Gallant, C., Desautels, D. and Marshall, L. (1979) Detection of bacterial kidney disease in wild salmonids in the Margaree River system and adjacent waters using

an indirect fluorescent antibody technique. *Journal of the Fisheries Research Board of Canada* **36**, 1464–1468.

Paterson, W.D., Douey, D. and Desautels, D. (1980) Relationships between selected strains of typical and atypical *Aeromonas salmonicida*, *Aeromonas hydrophila*, and *Haemophilus piscium*. *Canadian Journal of Microbiology* **26**, 588–598.

Paterson, W.D., Lall, S.P. and Desautels, D. (1981) Studies on bacterial kidney disease in Atlantic salmon (*Salmo salar*) in Canada. *Fish Pathology* **15**, 283–292.

Pathiratne, A., Widanapathirana, G.S. and Chandrarkanthi, W.H.S. (1994) Association of *Aeromonas hydrophila* with epizootic ulcerative syndrome (EUS) in freshwater fish in Sri Lanka. *Journal of Applied Ichthyology* **10**, 204–208.

Paula, S.J., Duffey, P.S., Abbott, S.L., Kokka, R.P., Oshio, L.S., Janda, J.M., Shimada, T. and Sakazaki, R. (1988) Surface properties of autoagglutinating mesophilic aeromonads. *Infection and Immunity* **56**, 2658–2665.

Pavan, M.E., Abbott, S.L., Zorzópulus, J. and J.M. Janda (2000) *Aeromonas salmonicida* subsp. *pectinolyticus* subsp. nov., a new pectinase-positive subspecies isolated from a heavily polluted river. *International Journal of Systematic and Evolutionary Microbiology* **50**, 1119–1124.

Pazos, F., Santos, Y., Magariños, B., Bandín, I., Núñez, S. and Toranzo, A.E. (1993) Phenotypic characteristics and virulence of *Vibrio anguillarum*-related organisms. *Applied and Environmental Microbiology* **59**, 2969–2976.

Pedersen, K. and Larsen, J.L. (1995) Evidence for the existence of distinct populations of *Vibrio anguillarum* serogroup O1 based on plasmid contents and ribotypes. *Applied and Environmental Microbiology* **61**, 2292–2296.

Pedersen, K., Kofod, H., Dalsgaard, I. and Larsen, J.L. (1994) Isolation of oxidase-negative *Aeromonas salmonicida* from disease turbot *Scophthaslmus maximus*. *Diseases of Aquatic Organisms* **18**, 149–154.

Pedersen, K., Dalsgaard, I. and Larsen, J.L. (1996a) Characterization of atypical *Aeromonas salmonicida* isolates by ribotyping and plasmid profiling. *Journal of Applied Bacteriology* **80**, 37–44.

Pedersen, K., Koblavi, S., Tiainen, T. and Larsen, J.L. (1996b) Restriction fragment length polymorphism of the pMJ101-like plasmid and ribotyping in the fish pathogen *Vibrio ordalii*. *Epidemiology and Infection* **117**, 386–391.

Pedersen, K., Tiainen, T. and Larsen, J.L. (1996c) Plasmid profiles, restriction length polymorphisms and O-serotypes among *Vibrio anguillarum* isolates. *Epidemiology and Infection* **117**, 471–478.

Pedersen, K., Dalsgaard, I. and Larsen, J.L. (1997a) *Vibrio damsela* associated with diseased fish in Denmark. *Applied and Environmental Microbiology* **63**, 3711–3715.

Pedersen, K., Gram, L., Austin, D.A. and Austin, B. (1997b) Pathogenicity of *Vibrio anguillarum* serogroup O1 compared to plasmids, outer membrane protein profile, and siderophore production. *Journal of Applied Microbiology* **82**, 365–371.

Pedersen, K., Verdonck, L., Austin, B., Austin, D.A., Blanch, A.R., Grimont, P.A.D., Jofre, J., Koblavi, S., Larsen, J.L., Tiainen, T., Vigneulle, M. and Swings, J. (1998) Taxonomic evidence that *Vibrio carchariae* Grimes *et al.* 1985 is a junior synonym of *Vibrio harveyi* (Johnson & Shunk 1936) Baumann *et al.* 1981. *International Journal of Systematic Bacteriology* **48**, 749–758.

Penner, J.L. (1984) Genus XII: *Providencia* Ewing 1962, 96^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 494–496.

Penner, J.L. (2005) Genus XX: *Providencia* Ewing 1962, 96^{AL}. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 753–759.

Perera, R.P., Johnson, S.K., Collins, M.D. and Lewis, D.H. (1994) *Streptococcus iniae* associated with mortality in *Tilapia nilotica* × *T. aurea* hybrids. *Journal of Aquatic Animal Health* **6**, 335–340.

Peréz, M.J., Rodríguez, L.A. and Nieto, T.P. (1998) The acetylcholinesterase ichthyotoxin is a common component of the extracellular products of Vibrionaceae strains. *Journal of Applied Microbiology* **84**, 47–52.

Petrie, J., Bruno, D.W. and Hastings, T.S. (1996) Isolation of *Yersinia ruckeri* from wild Atlantic salmon, *Salmo salar* L., in Scotland. *Bulletin of the European Association of Fish Pathologists* **16**, 83–84.

Phipps, B.M., Trust, T.J., Ishiguro, E.E. and Kay, W.W. (1983) Purification and characterization of the cell surface virulent A protein from *Aeromonas salmonicida*. *Biochemistry* **22**, 2934–2939.

Pickup, R.W., Rhodes, G., Cobban, R.J. and Clarke, K.J. (1996) The postponement of nonculturability in *Aeromonas salmonicida*. *Journal of Fish Diseases* **19**, 65–74.

Pier, G.B. and Madin, S.H. (1976) *Streptococcus iniae* sp. nov., a beta hemolytic streptococcus from an Amazon freshwater dolphon, *Inia geoffrensis*. *International Journal of Systematic Bacteriology* **26**, 545–553.

Piganelli, J.D., Wiens, G.D. and Kaattari, S.L. (1999) Elevated temperature treatment as a novel method for decreasing p57 on the cell surface of *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* **36**, 29–35.

Pippy, J.H.C. (1969) Kidney disease in juvenile Atlantic salmon (*Salmo salar*) in the Margaree River. *Journal of the Fisheries Research Board of Canada* **26**, 2535–2537.

Pippy, J.H.C. and Hare, G.M. (1969) Relationship of river pollution to bacterial infection in salmon (*Salmo salar*) and suckers (*Catostomus commersoni*). *Transactions of the American Fisheries Society* **98**, 685–690.

Pirhonen, J., Schreck, C.B. and Gannam, A. (2000) Appetite of chinook salmon (*Oncorhynchus tshawytscha*) naturally infected with bacterial kidney disease. *Aquaculture* **189**, 1–10.

Planas, M., Pérez-Lorenzo, M., Vázquez, J.A. and Pintado, J. (2005) A model for experimental infections with *Vibrio (Listonella) anguillarum* in first feeding turbot (*Scophthalmus maximus* L.) larvae under hatchery conditions. *Aquaculture* **250**, 232–243.

Plehn, M. (1909) Die Furunculose-epidemie der Salmoniden in Suddeutschland. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Originale, Abteilung 1* **L11**, 468.

Plehn, M. (1911) Die Furunculose der Salmoniden. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Originale, Abteilung 1* **60**, 609–624.

Plumb, J.A. (1984) Immunization of five species of warm water fish against *Edwardsiella ictaluri*. *Journal of Fish Diseases* **6**, 261–266.

Plumb, J.A. and Sanchez, D.J. (1983) Susceptibility of five species of fish to *Edwardsiella ictaluri*. *Journal of Fish Diseases* **6**, 261–266.

Plumb, J.A. and Vinitnantharat, S. (1993) Vaccination of channel catfish, *Ictalurus punctatus* (Rafinesque), by immersion and oral booster against *Edwardsiella ictaluri*. *Journal of Fish Diseases* **16**, 65–71.

Plumb, J.A., Schachte, J.H., Gaines, J.L., Peltier, W. and Carrol, B. (1974) *Streptococcus* sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. *Transactions of the American Fisheries Society* **103**, 358–361.

Poort, M.J., Whipps, C.M., Watral, V.G., Font, W.F. and Kent, M.L. (2006) Molecular characterization of *Mycobacterium* species in non-native poeciliids in Hawaii using DNA sequences. *Journal of Fish Diseases* **29**, 181–185.

Popoff, M. (1969) Étude sur les *Aeromonas salmonicida*. I. Caractères biochimiques et antigéniques. *Annales de Recherches Vétérinaire* **3**, 49–57.

Popoff, M. (1970) Bacterioses, généralitiés—*Aeromonas* et Aeromonose. Mimeograph, p. 38. Institut National de la Recherche Agronomique.

Popoff, M. (1971a) Étude sur les *Aeromonas salmonicida*. II. Caractérisation des bactériophages actifs sur les "*Aeromonas salmonicida*" et lysoypie. *Annales de Recherches Vétérinaire* **2**, 33–45.

Popoff, M. (1971b) Interest diagnostique d'un bactériophage specifique des *Aeromonas salmonicida*. *Annales de Recherches Vétérinaire* **2**, 137–139.

Popoff, M. (1984) Genus III: *Aeromonas* Kluyver & van Niel 1936, 398[AL]. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 545–548.

Popoff, M.Y. and Le Minor, L.E. (2005) Genus XXXIII: *Salmonella* Lignières 1900, 389[AL]. In: Brenner, D.J., Krieg, N.R. and Staley, J.T. (eds), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 2, *The Proteobacteria*, Part B, *The Gammaproteobacteria*. New York, Springer-Verlag, pp. 764–799.

Poppe, T.T., Håstein, T. and Salte, R. (1985) Hitra disease (haemorrhagic syndrome) in Norwegian salmon farming: Present status. In: Ellis, A.E. (ed.), *Fish and Shellfish Pathology*. New York, Academic Press, pp. 223–229.

Pot, B., Devriese, L.A., Ursi, D., Vandamme, P., Haesebrouck, F. and Kersters, K. (1996) Phenotypic identification and differentiation of *Lactococcus* strains isolated from animals. *Systematic and Applied Microbiology* **19**, 213–222.

Powell, J.L. and Loutit, M.W. (1994a) Development of a DNA probe using differential hybridization to detect the fish pathogen *Vibrio anguillarum*. *Microbial Ecology* **28**, 365–373.

Powell, J.L. and Loutit, M.W. (1994b) The detection the fish pathogen *Vibrio anguillarum* in water and fish using a species specific DNA probe combined with membrane filtration. *Microbial Ecology* **28**, 375–383.

Powell, M., Carson, J. and van Gelderen, R. (2004) Experimental induction of gill disease in Atlantic salmon *Salmo salar* smolts with *Tenacibaculum maritimum*. *Diseases of Aquatic Organisms* **61**, 179–185.

Powell, M., Overturf, K., Hogge, C. and Johnson, K. (2005) Detection of *Renibacterium salmoninarum* in Chinook salmon, *Oncorynchus tshawytscha* (Walbaum), using quantitative PCR. *Journal of Fish Diseases* **28**, 615–622.

Powell, M.D., Harris, J.O., Carson, J. and Hill, J.V. (2005a) Effects of gill abrasion and experimental infection with *Tenacibaculum maritimum* on the respiratory physiology of Atlantic salmon *Salmo salar* affected with amoebic gill disease. *Diseases of Aquatic Organisms* **63**, 169–174.

Prabakaran, M., Binuramesh, C., Steinhagen, D. and Michael, R.D. (2006) Immune response and disease resistance of *Oreochromis mossambicus* to *Aeromonas hydrophila* after exposure to hexavalent chromium. *Diseases of Aquatic Organisms* **68**, 189–196.

Prasad, S., Morris, P.C., Hansen, R., Meaden, P.G. and Austin, B. (2005) A novel bacteriocin-like substance from a pathogenic strain of *Vibrio harveyi*. *Microbiology* **151**, 3051–3058.

Pratschner, G.A. (1978) The relative resistance of six transferrin phenotypes of coho salmon (*Oncorhynchus kisutch*) to cytophagosis, furunculosis, and vibriosis. M.Sc. thesis, University of Washington, Seattle.

Pujalte, M.J., Sitjá-Bobadilla, A., Macián, M.C., Belloch, C., Álvarez-Pellitero, P., Pérez-Sánchez, J., Uruburu, F. and Garay, E. (2003) Virulence and molecular typing of *Vibrio harveyi* strains isolated from cultured dentex, gilthead sea bream and European sea bass. *Systematic and Applied Microbiology* **26**, 284–292.

Puttinaowarat, S., Thompson, K.D., Kolk, A. and Adams, A. (2002) Identification of *Mycobacterium* spp. isolated from snakehead, *Channa striata* (Fowler), and Siamese fighting fish, *Betta splendens* (Regan), using polymerase chain reaction–reverse cross blot hybridization (PCR–RCBH). *Journal of Fish Diseases* **25**, 235–243.

Pybus, V., Loutit, M.W. and Tagg, J.R. (1994) Siderophore production by New Zealand strains of *Vibrio anguillarum*. *New Zealand Journal of Marine and Freshwater Research* **28**, 309–315.

Pychynski, T., Malanowska, T. and Kozlowski, M. (1981) Bacterial flora in branchionecrosis of carp (particularly *Bacillus cereus* and *Bacillus subtilis*). *Medycyna Weterynaryjna* **37**, 742–743.

Pyle, S.W. and Shotts, E.B. (1980) A new approach for differentiating flexibacteria isolated from cold-water and warm-water fish. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 1040–1042.

Pyle, S.W. and Shotts, E.B. (1981) DNA homology studies of selected flexibacteria associated with fish disease. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 146–151.

Pylkkö, P., Pohjanvirta, T., Madetoja, J. and Pelkonen, S. (2005) Characterisation of atypical *Aeromonas salmonicida* infection in Arctic charr *Salvelinus alpinus* and European grayling *Thymallus thymallus*. *Diseases of Aquatic Organisms* **66**, 121–128.

Raa, J., Rørstad, G., Engstad, R. and Robertsen, B. (1990) The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. *Disease in Asian Aquaculture*. Bali, Indonesia.

Rabb, L., Cornick, J.W. and MacDermott, L.A. (1964) A macroscopic slide agglutination test for the presumptive diagnosis of furunculosis in fish. *Progressive Fish Culturist* **26**, *118–120*.

Rahman, M.H. and Kawai, K. (2000) Outer membrane proteins of *Aeromonas hydrophila* induce protective immunity in goldfish. *Fish & Shellfish Immunology* **10**, 379–382.

Rahman, M.H., Kawai, K. and Kusuda, R. (1997) Virulence of starved *Aeromonas hydrophila* to cyprinid fish. *Fish Pathology* **32**, 163–168.

Rahman, M.H., Suzuki, S. and Kawai, K. (2001). The effect of temperature on *Aeromonas hydrophila* infection in goldfish, *Carassius auratus*. *Journal of Applied Ichthyology* **17**, 282–285.

Rahman, M.H., Kuroda, A., Dijkstra, J.M., Kiryu, I., Nakanishi, T. and Ototake, M. (2002) The outer membrane fraction of *Flavobacterium psychrophilum* induces protective immunity in rainbow trout and ayu. *Fish & Shellfish Immunology* **12**, 169–179.

Rahman, M.H., Colque-Navarro, P., Kühn, I., Huys, G., Swings, J. and Möllby, R. (2002a) Identification and characterization of pathogenic *Aeromonas veronii* biovar sobria associated with epizootic ulcerative syndrome in fish in Bangladesh. *Applied and Environmental Microbiology* **68**, 650–655.

Rajan, P.R., Lin, Y.H.-Y., Ho, M.-S. and Yang, H.-L. (2003) Simple and rapid detection of *Photobacterium damselae* subsp. *piscicida* by a PCR technique and plating method. *Letters in Applied Microbiology* **95**, 1375–1380.

Rangdale, R.E. and Way, K. (1995) Rapid identification of *C. psychrophila* from infected spleen tissue using an enzyme-linked immunosorbent assay. *Bulletin of the European Association of Fish Pathologists* **15**, 213–216.

Rangdale, R.E., Richards, R.H. and Alderman, D.J. (1996) Isolation of *Cytophaga psychrophila*, causal agent of rainbow trout fry syndrome (RTFS) from reproductive fluids and egg

surfaces of rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the European Association of Fish Pathologists* **16**, 63–67.

Rangdale, R.E., Richards, R.H. and Alderman, D.J. (1997a) Minimum inhibitory concentrations of selected antimicrobial compounds against *Flavobacterium psychrophilum* the causal agent of rainbow trout fry syndrome (RTFS). *Aquaculture* **158**, 193–201.

Rangdale, R.E., Richards, R.H. and Alderman, D.J. (1997b) Colonisation of eyed rainbow trout ova with *Flavobacterium psychrophilum* leads to rainbow trout fry syndrome in fry. *Bulletin of the European Association of Fish Pathologists* **17**, 108–111.

Ranger, B.S., Mahrous, E.A., Mosi, L., Adusumilli, S., Lee, R.E., Colorni, A., Rhodes, M. and Small, P.L.C. (2006) Globally distributed mycobacterial fish pathogens produce a novel plasmid-encoded toxic macrolide, mycolactone F. *Infection and Immunity* **74**, 6037–6045.

Ransom, D.P. (1978) Bacteriologic, immunologic and pathologic studies of *Vibrio* sp. pathogenic to salmonids. Ph.D. thesis, Oregon State University, Corvallis.

Ransom, D.P., Lannan, C.N., Rohovec, J.S. and Fryer, J.L. (1984) Comparison of histopathology caused by *Vibrio anguillarum* and *Vibrio ordalii* and three species of Pacific salmon. *Journal of Fish Diseases* **7**, 107–115.

Rao, P.S.S., Lim, T.M. and Leung, K.Y. (2001) Opsonized virulent *Edwardsiella tarda* strains are able to adhere to and survive and replicate within fish phagocytes but fail to stimulate reactive oxygen intermediates. *Infection and Immunity* **69**, 5689–5697.

Rao, P.S.S., Yamada, Y., Tan, Y.P. and Leung, K.Y. (2004) Use of proteomics to identify novel virulence determinants that are required for *Edwardsiella tarda* pathogenesis. *Molecular Microbiology* **53**, 573–586.

Rasch, M., Buch, C., Austin, B., Slierendrecht, W., Ekmann, K.S., Larsen, J.L., Johansen, C., Riedel, K., Eberl, L., Givskov, M. *et al.* (2004) An inhibitor of bacterial quorum sensing reduces mortalities caused by vibriosis in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Systematic and Applied Microbiology* **27**, 350–359.

Rashid, M.M., Honda, K., Nakai, T. and Muroga, K. (1994a) An ecological study on *Edwardsiella tarda* in flounder farms. *Fish Pathology* **29**, 221–227.

Rashid, M.M., Mekuchi, T., Nakai, T. and Muroga, K. (1994b) A serological study on *Edwardsiella tarda* strains isolated from diseased Japanese flounder (*Paralichthys olivaceus*). *Fish Pathology* **29**, 277.

Rasmussen, H.B. and Larsen, J.L. (1987) Further antigenic analyses of the fish pathogenic bacterium *Vibrio anguillarum*. *Current Microbiology* **16**, 145–148.

Ravelo, C., Magariños, B., López-Romalde, Toranzo, A.E. and Romalde, J.L. (2003) Molecular fingerprinting of fish-pathogenic *Lactococus garvieae* strains by random amplified polymorphic DNA analysis. *Journal of Clinical Microbiology* **41**, 751–756.

Real, F., Acosta, B., Déniz, S., Oros, J. and Rodriguez, E. (1994) *Aeromonas salmonicida* infection in *Sparus aurata* in the Canaries. *Bulletin of the European Association of Fish Pathologists* **14**, 153–155.

Reali, D., Pretti, C., Tavanti, L. and Cognetti-Varriale, A.M. (1997) *Pasteurella piscicida* (Janssen & Surgalla, 1964): A simple method of isolation and identification from rearing-ponds. *Bulletin of the European Association of Fish Pathologists* **17**, 51–53.

Reddacliff, G.L., Hornitsky, M., Carson, J., Petersen, R. and Zelski, R. (1993) Mortalities of goldfish, *Carassius auratus* (L.), associated with *Vibrio cholerae* (non-O1). *Journal of Fish Diseases* **16**, 517–520.

Rehnstam, A.-S., Norquist, A., Wolf-Watz, H. and Hagström, Å. (1989) Identification of *Vibrio anguillarum* in fish by using partial 16S rRNA sequences and a specific 16S rRNA oligonucleotide probe. *Applied and Environmental Microbiology* **55**, *1907–1910.*

Reichenbach, H. (1989) Genus I: *Cytophaga* Winogradsky 1929, 577^{AL}, emend. In: Staley, J.T., Bryant, M.P., Pfennig, N. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 3. Baltimore, MD, Williams & Wilkins, pp. 2015–2020.

Reid, H.I., Griffen, A.A. and Birkbeck, T.H. (2004) Isolates of *Piscirickettsia salmonis* from Scotland and Ireland show evidence of clonal diversity. *Applied and Environmental Microbiology* **70**, 4393–4397.

Report (1969) *Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine (Swann Report)*. London, H.M.S.O.

Rhodes, L.D., Coady, A.M. and Strom, M.S. (2002) Expression of duplicate *msa* genes in the salmonid pathogen *Renibacterium salmoninarum*. *Applied and Environmental Microbiology* **68**, 5480–5487.

Rhodes, L.D., Coady, A.M. and Deinhard, R.K. (2004) Identification of a third *msa* gene in *Renibacterium salmoninarum* and the associated virulence phenotype. *Applied and Environmmental Microbiology* **70**, 6488–6494.

Rhodes, L.D., Rathbone, C.K., Corbett, S.C., Harrell, L.W. and Strom, M.S. (2004a) Efficacy of cellular vaccines and genetic adjuvants against bacterial kidney disease in chinook salmon (*Oncorhynchus tshawytscha*). *Fish & Shellfish Immunology* **16**, 461–474.

Rhodes, M.W., Kator, H., Kotob, S., van Berkum, P., Kaattari, I., Vogelbein, W., Quinn, F., Floyd, M.M., Butler, W.R. and Ottinger, C.A. (2003) *Mycobacterium shottsii* sp. nov., a slowly growing species isolated from Chesapeake Bay striped bass (*Morone saxatilis*). *International Journal of Systematic and Evolutionary Microbiology* **53**, 421–424.

Rhodes, M.W., Kator, H., Kaattari, I., Gauthier, D., Vogelbein, W. and Ottinger, C.A. (2004b) Isolation and characterization of mycobacteria from striped bass *Morone saxatilis* from the Chesapeake Bay. *Diseases of Aquatic Organisms* **61**, 41–51.

Rhodes, M.W., Kator, H., McNabb, A., Deshayes, C., Reyrat, J.-M., Brown-Elliott, A., Wallace Jr, R., Trott, K.A., Parker, J.M., Lifland, B. *et al.* (2005) *Mycobacterium pseudoshottsii* sp. nov., a slowly growing chromogenic species isolated from Chesapeake Bay striped bass (*Morone saxatilis*). *International Journal of Systematic and Evolutionary Microbiology* **55**, 1139–1147.

Richards, C. (1984) Genus VI: *Enterobacter* Hormaeche & Edwards 1960, 7215^{AL}; Nom. Cons. Opin. 28, Jud. Comm. 1963, 38. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. I. Baltimore, MD, Williams & Wilkins, pp. 465–469.

Richards, R.H. and Roberts, R.J. (1978) Bacteriology of teleosts. In: Roberts, R.J. (ed.), *Fish Pathology*. London, Bailliere Tindall, pp. 183–204.

Rintamäki-Kinnunen, P., Bernardet, J.-F. and Bloigu, A. (1997) Yellow pigmented filamentous bacteria connected with farmed salmonid fish mortality. *Aquaculture* **149**, 1–14.

Ritter, D.B. and Gerloff, R.K. (1966) Deoxyribonucleic acid hybridization among some species of the genus *Pasteurella*. *Journal of Bacteriology* **92**, 1838–1939.

Roald, S.O. (1977) Effects of sublethal concentrations of lignosulphonates on growth, intestinal microflora and some digestive enzymes of rainbow trout (*Salmo gairdneri*). *Aquaculture* **12**, 327–335.

Roald, S.O. and Håstein, T. (1980) Infection with an acinetobacter-like bacterium in Atlantic salmon (*Salmo salar*) broodfish. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 154–156.

Roberts, M.S. (1983) A report of an epizootic in hatchery rainbow trout, *Salmo gairdneri* Richardson, at an English trout farm, caused by *Yersinia ruckeri*. *Journal of Fish Diseases* **6**, 551–552.

Roberts, R.J. (1976) Bacterial diseases of farmed fishes. In: Skinner, F.A. and Carr, J.G. (eds), *Microbiology in Agriculture, Fisheries and Food*. London, Academic Press, pp. 55–61.

Roberts, R.J. (1978) *Fish Pathology*. London, Bailliere Tindall.

Roberts, R.J. and Horne, M.T. (1978) Bacterial meningitis in farmed rainbow trout, *Salmo gairdneri* Richardson, affected with chronic pancreatic necrosis. *Journal of Fish Diseases* **1**, 157–164.

Roberts, S.D. (1980) A method of reducing the carrier state of *Aeromonas salmonicida* in juvenile Pacific salmon. M.S. thesis, University of Idaho.

Robertsen, B., Rørstad, G., Engstad, E. and Raa, J. (1990) Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. *Journal of Fish Diseases* **13**, 391–400.

Robertson, M.H., Clarke, I.R., Coghlan, J.D. and Gill, O.N. (1991) Leptospirosis in trout farmers. *Lancet* **ii**, 626–627.

Robertson, P.A.W., Austin, D.A. and Austin, B. (2005) Prevention of ulcer disease in goldfish by means of vaccination. *Journal of Aquatic Animal Health* **17**, 203–209.

Robinson, J.A. and Meyer, F.P. (1966) Streptococcal fish pathogen. *Journal of Bacteriology* **92**, 512.

Robohm, R.A., Brown, C. and Murchelano, R.A. (1979) Comparison of antibodies in marine fish from clean and polluted rivers of the New York Bight—relative levels against 36 bacteria. *Applied and Environmental Microbiology* **38**, 248–257.

Rockey, D.D., Fryer, J.L. and Rohovec, J.S. (1988) Separation and in vivo analysis of two extracellular proteases and the T-hemolysin from *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* **5**, 197–204.

Rockey, D.D., Shook, L.A., Fryer, J.L. and Rohovec, J.S. (1989) Salmonid serum inhibits hemolytic activity of the secreted hemolysin of *Aeromonas salmonicida*. *Journal of Aquatic Animal Health* **1**, 263–268.

Rockey, D.D., Dungan, C.F., Lundar, T. and Rohovec, J.S. (1991) Monoclonal antibodies against *Aeromonas salmonicida* lipopolysacharide identify differences among strains. *Diseases of Aquatic Organisms* **10**, 115–120.

Rodger, H.D. and Drinan, E.M. (1993) Observation of a rickettsia-like organism in Atlantic salmon, *Salmo salar* L., in Ireland. *Journal of Fish Diseases* **16**, 361–369.

Rodgers, C.J. (1990) Immersion vaccination for control of fish furunculosis. *Diseases of Aquatic Organisms* **8**, 69–72.

Rodgers, C.J. (1992) Development of a selective-differential medium for the isolation of *Yersinia ruckeri* and its application in epidemiological studies. *Journal of Fish Diseases* **15**, 243–254.

Rodgers, C.J. and Austin, B. (1982) Oxolinic acid for control of enteric redmouth disease in rainbow trout. *Veterinary Record* **112**, 83.

Rodgers, C.J. and Austin, B. (1985) Oral immunisation against furunculosis: An evaluation of two field trials. In: Manning, M.J. and Tatner, M.F. (eds), *Fish Immunology*. London, Academic Press, pp. 185–194.

Rodgers, C.J., Pringle, J.H., McCarthy, D.H. and Austin, B. (1981) Quantitative and qualitative studies of *Aeromonas salmonicida* bacteriophage. *Journal of General Microbiology* **125**, 335–345.

Rodkhum, C., Hirono, I., Stork, M., DiLorenzo, M., Crosa, J.H. and Aoki, T. (2006) Putative virulence-related genes in *Vibrio anguillarum* identified by random genome sequencing. *Journal of Fish Diseases* **29**, 157–166.

Rodrigues, P.N.S. and Pereira, F.A. (2004) Effect of dietary iron overload on *Photobacterium damselae* subsp. *piscicida* pathogenicity in sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* **27**, 673–676.

Rodriguez, L.A., Fernandez, A.I.G., Santos, Y. and Nieto, T.P. (1990) Surface properties of *Serratia plymuthica* strains isolated from rainbow trout. In: Lésel, R. (ed.), *Microbiology in Poecilotherms*. Amsterdam, Elsevier, pp. 265–268.

Rodriguez, L.A., Ellis, A.E. and Nieto, T.P. (1993a) Effects of the acetylcholinesterase toxin of *Aeromonas hydrophila* on the central nervous system of fish. *Microbial Pathogenesis* **14**, 411–415.

Rodriguez, L.A., Fernandez, A.I.G. and Nieto, T.P. (1993b) Production of the lethal acetylcholinesterase toxin by different *Aeromonas hydrophila* strains. *Journal of Fish Diseases* **16**, 73–78.

Rødsaether, M.C., Olafsen, J., Raa, J., Myhre, K. and Steen, J.B. (1977) Copper as an initiating factor of vibriosis (*Vibrio anguillarum*) in eel (*Anguilla anguilla*). *Journal of Fish Biology* **10**, 17–21.

Rogers, W.A. (1981) Serological detection of two species of *Edwardsiella* infecting catfish. *Developments in Biological Standardization* **49**, 169–172.

Rogosa, M., Franklin, J.G. and Perry, K.D. (1961) Correlation of the vitamin requirements with cultural and biochemical characters of *Lactobacillus* spp. *Journal of General Microbiology* **25**, 473–482.

Romalde, J.L. and Toranzo, A.E. (1993) Pathological activities of *Yersinia ruckeri*, the enteric redmouth (ERM) bacterium. *FEMS Microbiology Letters* **112**, 291–300.

Romalde, J.L., Conchas, R.F. and Toranzo, A.E. (1991) Evidence that *Yersinia ruckeri* possesses a high affinity iron uptake system. *FEMS Microbiology Letters* **80**, 121–126.

Romalde, J.L., Barja, J.L., Magariños, B. and Toranzo, A.E. (1994) Starvation-survival processes of the bacterial fish pathogen *Yersinia ruckeri*. *Systematic and Applied Microbiology* **17**, 161–168.

Romalde, J.L., Magariños, B., Fouz, B., Bandín, I., Núñez, S. and Toranzo, A.E. (1995) Evaluation of BIONOR Mono-kits for rapid detection of bacterial fish pathogens. *Diseases of Aquatic Organisms* **21**, 25–34.

Romalde, J.L., Magariños, B., Villar, C., Barja, J.L. and Toranzo, A.E. (1999) Genetic analysis of turbot pathogenic *Streptococcus parauberis* strains by ribotyping and random amplified polymorphic DNA. *FEMS Microbiology Letters* **459**, 297–304.

Romalde, J.L., Magariños, B., Lores, F., Osorio, C.R. and Toranzo, A.E. (1999a) Assessment of a magnetic bead–EIA based kit for rapid diagnosis of fish pasteurellosis. *Journal of Microbiological Methods* **38**, 147–154.

Romalde, J.L., Lores, F., Magariños, B., Barja, J.L. and Toranzo, A.E. (2000) Study of cell surface associated virulence factors of *Streptococcus parauberis* strains pathogenic for turbot. *Bulletin of the European Association of Fish Pathologists* **20**, 244–251.

Roode, M.C. (1977) *Streptococcus* sp. infection in rainbow trout. *Fish Farmer* **18**, 6–8.

Rørvik, K.-A., Dehli, A., Thomasen, M., Ruyter, B., Steien, S.H. and Salte, R. (2003) Synergistic effects of dietary iron and omega-3 fatty acid levels on survival of farmed Atlantic salmon, *Salmo salar* L., during natural outbreaks of furunculosis and cold water vibriosis. *Journal of Fish Diseases* **26**, 477–485.

Rose, A.S., Ellis, A.E. and Munro, A.L.S. (1989) The infectivity of different routes of exposure and shedding rates of *Aeromonas salmonicida* subsp. *salmonicida* in Atlantic salmon, *Salmo salar* L., held in sea water. *Journal of Fish Diseases* **12**, 573–578.

Rose, A.S., Ellis, A.E. and Munro, A.L.S. (1990a) Evidence against dormancy in the bacterial fish pathogen *Aeromonas salmonicida* subsp. *salmonicida*. *FEMS Microbiology Letters* **68**, 105–108.

Rose, A.S., Ellis, A.E. and Munro, A.L.S. (1990b) The survival of *Aeromonas salmonicida* subsp. *salmonicida* in seawater. *Journal of Fish Diseases* **13**, 205–214.

Ross, A.D. (1962) Isolation of a pigment-producing strain of *Aeromonas liquefaciens* from silver salmon (*Oncorhynchus kisutch*). *Journal of Bacteriology* **84**, 590–591.

Ross, A.D. (1966) Endotoxin studies. In: *Progress in Sport Fishery Research*. Resource Publication **39**, 77 pp.

Ross, A.J. and Brancato, F.P. (1959) *Mycobacterium fortuitum* Cruz from the tropical fish *Hyphessobrycon innesi*. *Journal of Bacteriology* **78**, 392–395.

Ross, A.J. and Johnson, H.E. (1962) Studies of transmission of mycobacterial infections of chinook salmon. *Progressive Fish Culturist* **24**, 147–149.

Ross, A.J. and Klontz, G.W. (1965) Oral immunization of rainbow trout (*Salmo gairdneri*) against an etiological agent of "redmouth disease". *Journal of the Fisheries Research Board of Canada* **22**, 713–719.

Ross, A.J. and Smith, C.A. (1972) Effect of two iodophors on bacterial and fungal fish pathogens. *Journal of the Fisheries Research Board of Canada* **29**, 1359–1361.

Ross, A.J. and Toth, R.J. (1974) *Lactobacillus*: A new fish pathogen? *Progressive Fish Culturist* **36**, 191.

Ross, A.J., Rucker, R.R. and Ewing, W.H. (1966) Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Microbiology* **12**, 763–770.

Rouhbakhsh-Khaleghdoust, A. (1975) The incidence of *Clostridium botulinum* type E in fish and bottom deposits in the Caspian Sea coastal water. *Pahlavi Medical Journal* **6**, 550–556.

Ruangpan, L., Kitao, T. and Yoshida, T. (1986) Protective efficacy of *Aeromonas hydrophila* vaccines in Nile tilapia. *Veterinary Immunology and Immunopathology* **12**, 345–350.

Rucker, R.R. (1949) A streptomycete pathogenic to fish. *Journal of Bacteriology* **58**, 659–664.

Rucker, R.R. (1959) Vibrio infections among marine and fresh-water fish. *Progressive Fish Culturist* **21**, 22–25.

Rucker, R.R. (1963) Status of fish diseases and relation to production. *Report of the Second Governor's Conference on Pacific Salmon*, Seattle, January 1963, pp. 98–101.

Rucker, R.R. (1966) Redmouth disease of rainbow trout (*Salmo gairdneri*). *Bulletin de l'Office International des Epizooties* **65**, 825–830.

Rucker, R.R., Bernier, A.F., Whipple, W.J. and Burrows, R.E. (1951) Sulfadiazine for kidney disease. *Progressive Fish Culturist* **13**, 135–137.

Rucker, R.R., Earp, B.J. and Ordal, E.J. (1953) Infectious diseases of Pacific salmon. *Transactions of the American Fisheries Society* **83**, 297–312.

Runyon, E.H., Wayne, L.G. and Kubica, G.P. (1974) Genus I: *Mycobacterium* Lehmann & Neumann 1896. In: Buchanan, R.E. and Gibbons, N.E. (eds), *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore, MD, Williams & Wilkins, pp. 682–701.

Ryan, J.M. and Bryant, D.R. (1997) Fish tank granuloma: A frequently misdiagnosed infection of the upper limb. *Journal of Accident and Emergency Medicine* **14**, 398–399.

Saeed, M. (1983) Chemical characterization of the lipopolysaccharides of *Edwardsiella ictaluri* and the immune response of channel catfish to this function and to whole cell antigen with histopathological comparisons. Ph.D. dissertation, Auburn University.

Saeed, M.O. (1995) Association of *Vibrio harveyi* with mortalities in cultured marine fish in Kuwait. *Aquaculture* **136**, 21–29.

Saeed, M.O. and Plumb, J.A. (1987) Serological detection of *Edwardsiella ictaluri* Hawke lipopolysaccharide antibody in serum of channel catfish *Ictalurus punctatus* Rafinesque. *Journal of Fish Diseases* **10**, 205–209.

Saeed, M.O., Alamoudi, M.M. and Al-Harbi, A.H. (1987) A *Pseudomonas* associated with disease in cultured rabbitfish *Siganus rivulatus* in the Red Sea. *Diseases in Aquatic Organisms* **3**, 177–180.

Sae-Oui, D., Muroga, K. and Nakai, T. (1984) A case of *Edwardsiella tarda* infection in cultured colored carp *Cyprinus carpio*. *Fish Pathology* **19**, 197–199.

Sahoo, P.K. (2003) Immunostimulating effect of triiodothyronine: Dietary administration of triiodothyronine to rohu (*Labeo rohita*) enhances immunity and resistance to *Aeromonas hydrophila* infection. *Journal of Applied Ichthyology* **19**, 118–122.

Sahoo, P.K. and Mukherjee, S.C. (2002) The effect of dietary immunomodulation upon *Edwardsiella tarda* vaccination in healthy and immunocompromised Indian major carp (*Labeo rohita*). *Fish & Shellfish Immunology* **12**, 1–16.

Sakai, D.K. (1977) Causative factors of *Aeromonas salmonicida* in salmonid furunculosis: Extracellular protease. *Scientific Reports of the Hokkaido Fish Hatchery* **32**, 61–89.

Sakai, D.K. (1978) Colliquative activity of purified protease for muscular tissue in *Aeromonas salmonicida* subsp. *salmonicida*. *Scientific Reports of the Hokkaido Fish Hatchery* **33**, 55–73.

Sakai, D.K. (1984) The non-specific activation of rainbow trout, *Salmo gairdneri* Richardson complement by *Aeromonas salmonicida* extracellular products and the correlation of complement activity with the inactivation of lethal toxicity products. *Journal of Fish Diseases* **7**, 329–338.

Sakai, D.K. (1985) Significance of extracellular protease for growth of a heterotrophic bacterium, *Aeromonas salmonicida*. *Applied and Environmental Microbiology* **50**, 1031–1037.

Sakai, D.K. (1986a) Kinetics of adhesion associated with net electrical charges in agglutinating *Aeromonas salmonicida* cells and their spheroplasts. *Bulletin of the Japanese Society of Scientific Fisheries* **52**, 31–36.

Sakai, D.K. (1986b) Electrostatic mechanism of survival of virulent *Aeromonas salmonicida* strains in river water. *Applied and Environmental Microbiology* **51**, 1343–1349.

Sakai, D.K. (1987) Adhesion of *Aeromonas salmonicida* strains associated with net electrostatic charges to host tissue cells. *Infection and Immunity* **55**, 704–710.

Sakai, M. (1999) Current research status on fish immunostimulants. *Aquaculture* **172**, 63–92.

Sakai, M. and Kobayashi, M. (1992) Detection of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in salmonid fish, from pen-cultured coho salmon. *Applied and Environmental Microbiology* **58**, 1061–1063.

Sakai, M., Atsuta, S. and Kobayashi, M. (1986) A streptococcal disease of cultured Jacopever, *Sebastes schlegeli*. *Suisanzoshuku (Aquiculture)* **34**, 171–177.

Sakai, M., Kubota, R., Atsuta, S. and Kobayashi, M. (1987) Vaccination of rainbow trout *Salmo gairdneri* against β-haemolytic streptococcal disease. *Nippon Suisan Gakkaishi* **53**, 1373–1376.

Sakai, M., Atsuta, S. and Kobayashi, M. (1989a). *Pseudomonas fluorescens* isolated from the diseased rainbow trout, *Oncorhynchus mykiss*. *Kitasato Archives of Experimental Medicine* **62**, 157–162.

Sakai, M., Atsuta, S. and Kobayashi, M. (1989b) Comparison of methods used to detect *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease. *Journal of Aquatic Animal Health* **1**, 21–24.

Sakai, M., Atsuta, S. and Kobayashi, M. (1989c) Bacterial kidney disease in Masu salmon, *Oncorhynchus masou*. *Physiology and Ecology, Japan, Special Volume* **1**, 577–586.

Sakai, M., Atsuta, S. and Kobayashi, M. (1989d) Protective immune response in rainbow trout *Oncorhynchus mykiss*, vaccinated with β-haemolytic streptococcal bacterin. *Fish Pathology* **24**, 169–173.

Sakai, M., Atsuta, S. and Kobayashi, M. (1989e) Attempted vaccination of rainbow trout *Oncorhynchus mykiss* against bacterial kidney disease. *Nippon Suisan Gakkaishi* **55**, 2105–2109.

Sakai, M., Sugawara, M., Atsuta, S. and Kobayashi, M. (1990) The confirmatory diagnosis of bacterial kidney disease (BKD) using dot and western blotting assay. *Bulletin of the European Association of Fish Pathologists* **10**, 77–80.

Sakai, M., Otubo, T., Atsuta, S. and Kobayashi, M. (1993a) Enhancement of resistance to bacterial infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum), by oral administration of bovine lactoferrin. *Journal of Fish Diseases* **16**, 239–247.

Sakai, M., Atsuta, S. and Kobayashi, M. (1993b) The immune response of rainbow trout (*Oncorhynchus mykiss*) injected with five *Renibacterium salmoninarum* bacterins. *Aquaculture* **113**, 11–18.

Sakai, M., Atsuta, S. and Kobayashi, M. (1994) Survival of fish pathogen *Edwardsiella tarda* in sea water and fresh water. *Bulletin of the European Association of Fish Pathologists* **14**, 188–190.

Sakai, M., Yoshida, T., Atsuta, S. and Kobayashi, M. (1995) Enhancement of resistance to vibriosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum) by oral administration of *Clostridium butyricum* bacterin. *Journal of Fish Diseases* **18**, 187–190.

Sakai, M., Konno, T., Tassakka, A.C.M.A.R., Ponpornpisit, A., Areechon, N., Katagiri, T., Yoshida, T. and Endo, M. (2005) Characterization of a *Mycobacterium* sp. isolated from guppy *Poecilia reticulata*, using 16S ribosomal RNA and its internal transcribed spacer sequences. *Bulletin of the European Association of Fish Pathologists* **25**, 64–69.

Sakai, T., Kanai, K., Osatomi, K. and Yoshikoshi, K. (2004) Identification and characterization of a fimbrial gene cluster of *Edwardsiella tarda* expressing mannose-resistant haemagglutination. *Fish Pathology* **39**, 87–93.

Sakai, T., Oseko, N. and Iida, T. (2006) Rapid and simple method for the detection of *Yersinia ruckeri*, the causal agent of enteric redmouth disease. *Fish Pathology* **41**, 127–130.

Sakai, T., Yamada, H., Shimizu, H., Yuasa, K., Kamaishi, T., Oseko, N. and Iida, T. (2006a) Characteristics and pathogenicity of brown pigment-producing *Vibrio anguillarum* isolated from Japanese flounder. *Fish Pathology* **41**, 77–79.

Sakata, T., Nakaji, M. and Kakimoto, D. (1978) Microflora in the digestive tract of marine fish—I. General characterization of the isolates from yellowtail. *Memoirs of the Faculty of Fisheries of Kagoshima University* **27**, 65–71.

Sakata, T., Sugita, H., Mitsuoka, T., Kakimoto, D. and Kadota, H. (1980) Isolation of obligate anaerobes from the intestinal tracts of freshwater fish. *Bulletin of the Japanese Society of Scientific Fisheries* **46**, 511.

Sakata, T., Matsuura, M. and Shimokawa, Y. (1989) Characteristics of *Vibrio damsela* isolated from diseased yellowtail *Seriola quinqueradiata*. *Nippon Suisan Gakkaishi* **55**, 135–141.

Sakazaki, R. (1984) Genus IV: *Citrobacter* Werkman & Gillen 1932, 17315^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. I. Baltimore, MD, Williams & Wilkins, pp. 458–461.

Sakazaki, R. and Tamura, K. (1975) Priority of the specific epithet *anguillimortiferum* over the specific epithet *tarda* in the name of the organism presently known as *Edwardsiella tarda*. *International Journal of Systematic Bacteriology* **25**, 219–220.

Salati, F., Ikeda, Y. and Kusuda, R. (1987a) Effect of *Edwardsiella tarda* lipopolysaccharide immunization on phagocytosis in the eel. *Nippon Suisan Gakkaishi* **53**, 201–204.

Salati, F., Hamaguchi, M. and Kusuda, R. (1987b) Immune response of red sea bream to *Edwardsiella tarda* antigens. *Fish Pathology* **22**, 93–98.

Salati, F., Watanabe, K., Kawai, K. and Kusuda, R. (1989a) Immune response of ayu against *Vibrio anguillarum* lipopolysaccharide. *Nippon Suisan Gakkaishi* **55**, 45–49.

Salati, F., Kawai, S. and Kusuda, R. (1989b) Characteristics of the lipopolysaccharide from *Pasteurella piscicida*. *Fish Pathology* **24**, 143–147.

Salinas, P.C. and Crosa, J.H. (1995) Regulation of *angR*, a gene with regulatory and biosynthetic functions in the pJM1 plasmid-mediated iron uptake system of *Vibrio anguillarum*. *Gene* **160**, 17–23.

Salonius, K., Siderakis, C., MacKinnon, A.M. and Griffiths, S.G. (2005) Use of *Arthrobacter davidanieli* as a live vaccine against *Renibacterium salmoninarum* and *Piscirickettsia salmonis* in salmonids. In: Midlyng, P.J. (ed.), *Progress in Fish Vaccinology*. Basel, Switzerland, Karger, pp. 189–197.

Salte, R., Rørvik, K.-A., Reed, E. and Norberg, K. (1994) Winter ulcers of the skin in Atlantic salmon, *Salmo salar* L.: Pathogenesis and possible aetiology. *Journal of Fish Diseases* **17**, 661–665.

Samuelsen, O.B., Hjeltnes, B. and Glette, J. (1998) Efficacy of orally administered florfenicol in the treatment of furunculosis in Atlantic salmon. *Journal of Aquatic Animal Health* **10**, 56–61.

Sanarelli, G. (1891) Über einen neuen Mikroorganisms des Wassers, welcher für Thiere mit veraenderlicher und konstanter Temperatur pathogen ist. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten and Hygiene* **9**, 193–228.

Sanders, J.E. and Fryer, J.L. (1978) *Corynebacterium salmoninus* sp. nov. the causative agent of bacterial kidney disease: Selected biochemical properties and pathogenesis in salmonid fishes. *Proceedings of the Joint 3rd Biennial Fish Health Section/American Fisheries Society and 9th Annual Midwest Fish Disease Workshops, Kansas City*, pp. 28–33.

Sanders, J.E. and Fryer, J.L. (1980) *Renibacterium salmoninarum* gen. nov., sp. nov., the causative agent of bacterial kidney disease in salmonid fishes. *International Journal of Systematic Bacteriology* **30**, 496–502.

Sanders, J.E., Pilcher, K.S. and Fryer, J.L. (1978) Relation of water temperature to bacterial kidney disease in coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*O. nerka*) and steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **35**, 8–11.

Sandvick, O. and Hagan, O. (1968) Serological studies on proteinases produced by *Aeromonas salmonicida* and other aeromonads. *Acta Veterinaria Scandinavica* **9**, 1–9.

Sano, M., Nakano, H., Kimura, T. and Kusuda, R. (1994) Therapeutic effect of fosfomycin on experimentally induced pseudotuberculosis in yellowtail. *Fish Pathology* **29**, 187–192.

Sato, N., Yamane, N. and Kawamura, T. (1982) Systemic *Citrobacter freundii* infection among sunfish *Mola mola* in Matsushima aquarium. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 1551–1557.

Santos, Y., Toranzo, A.E., Dopazo, C.P., Nieto, T.P. and Barja, J.L. (1987) Relationship among virulence for fish, enterotoxigenicity, and phenotypic characteristics of motile *Aeromonas*. *Aquaculture* **67**, 29–39.

Santos, Y., Toranzo, A.E., Barja, J.L., Nieto, T.P. and Villa, T.G. (1988) Virulence properties and enterotoxin production of *Aeromonas* strains isolated from fish. *Infection and Immunity* **56**, 3285–3293.

Santos, Y., Bandín, I., Núñez, S., Nieto, T.P. and Toranzo, A.E. (1991) Serotyping of motile *Aeromonas* species in relation to virulence phenotype. *Bulletin of the European Association of Fish Pathologists* **11**, 153–155.

Santos, Y., Romalde, J.L., Bandín, I., Magariños, B. Núñez, S., Barja, J.L. and Toranzo, A.E. (1993) Usefulness of the API-20E system for the identification of bacterial fish pathogens. *Aquaculture* **116**, 111–120.

Santos, Y., García-Marquez, S., Pereira, P.G., Pazos, F., Riaza, A., Silva, R., El Morabit, A. and Ubeira, F.M. (2005) Efficacy of furunculosis vaccines in turbot, *Scophthalmus max-*

imus (L.): Evaluation of immersion, oral and injection delivery. *Journal of Fish Diseases* **28**, 165–172.

Sanz, F. (1991) Rainbow trout mortalities associated with a mixed infection with *Citrobacter freundii* and IPN virus. *Bulletin of the European Association of Fish Pathologists* **11**, 222.

Sarti, M., Giorgetti, G. and Manfrin, A. (1992) Method for the rapid diagnosis of visceral myxobacteriosis in reared trout in Italy. *Bulletin of the European Association of Fish Pathologists* **12**, 53.

Sasaki, T. and Aita, A. (1975) A study on fish diseases. Part 1. *Edwardsiella* isolated from the sea urchin. *Japanese Journal of Microbiology* **30**, 368.

Sato, N., Yamane, N. and Kawamura, T. (1982) Systemic *Citrobacter freundii* infection among sunfish *Mola mola* in Matsushima aquarium. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 1551–1557.

Savan, R., Igarashi, A., Matsuoka, S. and Sakai, M. (2004) Sensitive and rapid detection of edwardsiellosis in fish by a loop-mediated isothermal amplification method. *Applied and Environmental Microbiology* **70**, 621–624.

Sawyer, E.S. and Strout, R.G. (1977) Survival and growth in vaccinated, medicated and untreated coho salmon (*Oncorhynchus kisutch*) exposed to *Vibrio anguillarum*. *Aquaculture* **10**, 311–315.

Sawyer, E.S., Strout, R.G. and Coutermarsh, B.A. (1979) Comparative susceptibility of Atlantic salmon (*Salmo salar*) and coho (*Oncorhynchus kisutch*) salmon to three strains of *Vibrio anguillarum* from the Maine–New Hampshire coast. *Journal of the Fisheries Research Board of Canada* **36**, 280–282.

Scallan, A. and Smith, P.R. (1985) Control of asymptomatic carriers of *Aeromonas salmonicida* in Atlantic salmon smolts with flumequine. In: Ellis, A.E. (ed.), *Fish and Shellfish Pathology*. New York, Academic Press, pp. 119–127.

Schachte, J.H. (1978) Immunization of channel catfish, *Ictalurus punctatus*, against two bacterial diseases. *Marine Fisheries Review* **40**, 18–19.

Schachte, J.H. and Mora, E.C. (1973) Production of agglutinating antibodies in the channel catfish (*Ictalurus punctatus*) against *Chondrococcus columnaris*. *Journal of the Fisheries Research Board of Canada* **30**, 116–118.

Schäfer, J.-W., Alvarado, V., Enriquez, R. and Monrás, M. (1990) The "coho salmon syndrome" (CSS): A new disease in Chilean salmon reared in sea water. *Bulletin of the European Association of Fish Pathologists* **10**, 130.

Schäperclaus, W. (1930) *Pseudomonas punctata* als Krankheitserreger bei Fischen. *Zeitung für Fischerei* **28**, 289–370.

Schäperclaus, W. (1959) Grossversuche mit Streptomycin zur Bekämpfung der infektiösen Bauchwassersucht des Karpfens. *Deutsches Fischeren Zeitung* **6**, 176–179.

Schäperclaus, W. (1979) *Fischkrankheiten*. Berlin, Akademie-Verlag.

Schäperclaus, W. and Brauer, M. (1964) Bedeutung der Fluoreszenten für die Entstehung and Bekämpfung der Infektiösen Bauchwassersucht der Karpfen. *Zeitschrift für Fischerei* **12**, 75–76.

Schiewe, M.H. (1981) Taxonomic status of marine vibrios pathogenic for salmonid fish. *Developments in Biological Standardization* **49**, 149–158.

Schiewe, M.H. and Crosa, J.H. (1981) Molecular characteristic of *Vibrio anguillarum* biotype 2. *Canadian Journal of Microbiology* **27**, 1011–1018.

Schiewe, M.H., Crosa, J.H. and Ordal, E.J. (1977) Deoxyribonucleic acid relationships among marine vibrios pathogenic to fish. *Canadian Journal of Microbiology* **23**, 954–958.

Schiewe, M.H., Trust, T.J. and Crosa, J.H. (1981) *Vibrio ordalii* sp. nov.: A causative agent of vibriosis in fish. *Current Microbiology* **6**, 343–348.

Schmidtke, L.M. and Carson, J. (1994) Characteristics of *Vagococcus salmoninarum* isolated from diseased salmonid fish. *Journal of Applied Bacteriology* **77**, 229–236.

Schmidtke, L.M. and Carson, J. (1995) Characteristics of *Flexibacter psychrophilus* isolated from Atlantic salmon in Australia. *Diseases of Aquatic Organisms* **21**, 157–161.

Schneck, J.L. and Caslake, L.F. (2006) Genetic diversity of *Flavobacterium columnare* isolated from fish collected from warm and cold water. *Journal of Fish Diseases* **29**, 245–248.

Schneider, R. and Nicholson, B.L. (1980) Bacteria associated with fin rot disease in hatchery reared Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 1505–1513.

Schubert, R.H.W. (1961) Über die biochemischen Merkmale von *Aeromonas salmonicida*. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene 1, Originale* **183**, 485–494.

Schubert, R.H.W. (1967a) The taxonomy and nomenclature of the genus *Aeromonas* Kluyver & van Niel 1936. Part 1. Suggestions on the taxonomy and nomenclature of the aerogenic *Aeromonas* species. *International Journal of Systematic Bacteriology* **17**, 23–37.

Schubert, R.H.W. (1967b) The taxonomy and nomenclature of the genus *Aeromonas* Kluyver & van Niel 1936. Part II. Suggestions on the taxonomy and nomenclature of the anaerogenic *Aeromonas* species. *International Journal of Systematic Bacteriology* **17**, 237–279.

Schubert, R.H.W. (1974) Genus II: *Aeromonas* Kluyver & van Niel 1936, 398. In: Buchanan, R.E. and Gibbons, N.E. (eds), *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore, MD, Williams & Wilkins, pp. 345–348.

Scott, M. (1968) The pathogenicity of *Aeromonas salmonicida* (Griffin) in sea and brackish water. *Journal of General Microbiology* **50**, 864–868.

Sealey, W.M., Lim, C. and Klesius, P.H. (1997) Influence of the dietary level of iron from iron methionine and iron sulfate on immune response and resistance of channel catfish to *Edwardsiella ictaluri*. *Journal of the World Aquaculture Society* **28**, 142–149.

Sealey, W.M. and Gatlin III, D.M. (2002) Dietary supplementation of vitamin C and/or vitamin E before or after experimental infection with *Streptococcus iniae* has limited effects on survival of hybrid striped bass. *Journal of Aquatic Animal Health* **14**, 165–175.

Secades, P. and Guijarro, J.A. (1999) Purification and characterization of an extracellular protease from the fish pathogen *Yersinia ruckeri* and effect of culture conditions on production. *Applied and Environmental Microbiology* **65**, 3936–3975.

Secades, P., Alvarez, B. and Guijarro, J.A. (2001) Purification and characterization of a psychrophilic, calcium-induced, growth-phase-dependent metalloprotease from the fish pathogen *Flavobacterium psychrophilum*. *Applied and Environmental Microbiology* **67**, 2436–2444.

Secades, P., Alvarez, B. and Guijarro, J.A. (2003) Properties of a new psychrophilic metalloprotease (Fpp2) in the fish pathogen *Flavobacterium psychrophilum*. *FEMS Microbiology Letters* **226**, 273–279.

Seljestokken, B., Bergh, Ø., Melingen, G.O., Rudra, H., Olsen, R.H. and Samuelsen, O.B. (2006) Treating experimentally induced vibriosis (*Listonella anguillarum*) in cod, *Gadus morhua* L., with florfenicol. *Journal of Fish Diseases* **29**, 737–742.

Selvaraj, V., Sampath, K. and Sekar, V. (2005) Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. *Fish & Shellfish Immunology* **19**, 293–306.

Sendra, R.M., Esteve, C. and Alcaide, E. (1997) Enzyme-linked immunosorbent assay for detection of *Aeromonas hydrophila* serogroup O:19. *FEMS Microbiology Letters* **157**, 123–129.

Senson, P.R. and Stevenson, R.M.W. (1999) Production of the 57 kDa major surface antigen by a non-agglutinating strain of the fish pathogen *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* **38**, 23–31.

Shah, K.L. and Tyagi, B.C. (1986) An eye disease in silver carp, *Hypophthalmichthys molitrix*, held in tropical ponds, associated with the bacterium *Staphylococcus aureus*. *Aquaculture* **55**, 1–4.

Sharma, V.K., Kaura, Y.K. and Singh, I.P. (1974) Frogs as carriers of *Salmonella* and *Edwardsiella*. *Antonie van Leeuwenhoek* **40**, 171–175.

Shao, M., Ma, Y., Liu, Q. and Zhang, Y. (2005) Secretory expression of recombinant proteins in an attenuated *Vibrio anguillarum* strain for potential use in vaccines. *Journal of Fish Diseases* **28**, 723–728.

Shaw, D.H. and Hodder, H.J. (1978) Lipopolysaccharides of the motile aeromonads: Core oligosaccharide analysis as an aid to taxonomic classification. *Canadian Journal of Microbiology* **24**, 864–868.

Shayegani, M., Stone, W.B., DeForge, I., Root, T., Parsons, L.M. and Maupin, P. (1986) *Yersinia enterocolitica* and related species isolated from wildlife in New York state. *Applied and Environmental Microbiology* **52**, 420–424.

Sheeran, B. and Smith, P.R. (1981) A second extracellular proteolytic activity associated with the fish pathogen *Aeromonas salmonicida*. *FEMS Microbiology Letters* **11**, 73–76.

Sheeran, B., Drinan, E. and Smith, P.R. (1984) Preliminary studies on the role of extracellular proteolytic enzymes in the pathogenesis of furunculosis. In: Acuigrup (ed.), *Fish Diseases, Fourth COPRAQ Session*. Madrid, Editora ATP, pp. 89–100.

Shelby, R.A., Klesius, P.H., Shoemaker, C.A. and Evans, J.J. (2002) Passive immunization of tilapia, *Oreochromis niloticus* (L.), with anti-*Streptococcus iniae* whole sera. *Journal of Fish Diseases* **25**, 1–6.

Shewan, J.M. (1961) The microbiology of sea-water fish. In: Bergström, G. (ed.), *Fish as Food*, Vol. 1. London, Academic Press, pp. 487–560.

Shewan, J.M., Hobbs, G. and Hodgkiss, W. (1960) The *Pseudomonas* and *Achromobacter* groups of bacteria in the spoilage of marine white fish. *Journal of Applied Bacteriology* **23**, 463–468.

Shieh, H.S. (1985) Vaccination of Atlantic salmon, *Salmo salar* L., against furunculosis with protease from an avirulent strain of *Aeromonas salmonicida*. *Journal of Fish Biology* **27**, 97–101.

Shieh, H.S. and MacLean, J.R. (1975) Purification and properties of an extra-cellular protease of *Aeromonas salmonicida*, the causative agent of furunculosis. *International Journal of Biochemistry* **6**, 653–656.

Shima, T., Kodama, H., Iwasaki, T., Watarai, S. and Asagi, M. (2006) Adherence of *Lactococcus garvieae* to the intestinal and brain gangliosodes of the yellowtail, *Seriola quinqueradiata* Temminck & Schlegel. *Journal of Fish Diseases* **29**, 249–253.

Shimahara, Y., Yasuda, H., Nakamura, A., Itami, T. and Yoshida, T. (2005) Detection of antibody responses against *Nocardia seriolae* by enzyme-linked immunosorbent assay (ELISA) and a preliminary vaccine trial in yellowtail *Seriola quinqueradiata*. *Bulletin of the European Association of Fish Pathologists* **25**, 270–275.

Shinobu, R. (1965) Taxonomy of the whorl-forming Streptomycetaceae. *Memoirs of Osaka University Liberal Arts, Education and Natural Sciences* **14**, 72–201.

Shiomitsu, K., Kusuda, R., Osuga, H. and Munekiyo, M. (1980) Studies on chemotherapy of fish disease with erythromycin—II. Its clinical studies against streptococcal infection in cultured yellowtails. *Fish Pathology* **15**, 17–23.

Shiraki, K., Miyamoto, F., Sato, T., Sonezaki, I. and Sano, K. (1970) Studies on a new chemotherapeutic agent nifurprazine (HB-115) against infectious diseases. Part 1. *Fish Pathology* **4**, 130–137.

Shoemaker, C.A., Arias, C.R., Klesius, P.H. and Welker, T.L. (2005) technique for identifying *Flavobacterium columnare* using whole-cell fatty acid profiles. *Journal of Aquatic Animal Health* **17**, 267–274.

Shoemaker, C.A., Vandenberg, G.W., Désormeaux, A., Klesius, P.H. and Evans, J.J. (2006) Efficacy of *Streptococcus iniae* modified bacterin delivered using Oralject™ technology in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **255**, 151–156.

Shotts, E.B. and Rimler, R. (1973) Medium for the isolation of *Aeromonas hydrophila*. *Applied Microbiology* **26**, 550–553.

Shotts, E.B. and Waltman, W.D. (1990) A medium for the selective isolation of *Edwardsiella ictaluri*. *Journal of Wildlife Diseases* **26**, 214–218.

Shotts, E.B., Hsu, T.C. and Waltman, W.D. (1984) Extracellular proteolytic activity of *Aeromonas hydrophila* complex. *Fish Pathology* **20**, 37–44.

Shotts, E.B., Hsu, T.C. and Waltman, W.D. (1985) Extracellular proteolytic activity of *Aeromonas hydrophila* complex. *Fish Pathology* **20**, 37–44.

Shotts, E.B., Blazer, V.S. and Waltman, W.D. (1986) Pathogenesis of experimental *Edwardsiella ictaluri* infections in channel catfish (*Ictalurus punctatus*). *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 36–42.

Shotts, E.B., Talkington, F.D., Elliott, D.G. and McCarthy, D.H. (1980) Aetiology of an ulcerative disease in goldfish, *Carassius auratus* (L.): Characterization of the causative agent. *Journal of Fish Diseases* **3**, 181–186.

Siddall, R., Pike, A.W. and McVicar, A.H. (1994) Parasites of flatfish in relation to sewage dumping. *Journal of Fish Biology* **45**, 193–209.

Siegel, D.C. and Congleton, J.L. (1997) Bactericidal activity of juvenile chinook salmon macrophages against *Aeromonas salmonicida* after exposure to live or heat killed *Renibacterium salmoninarum* or to soluble proteins produced by *Renibacterium salmoninarum*. *Journal of Aquatic Animal Health* **9**, 180–189.

Simidu, U. and Egusa, S. (1972) A re-examination of the fish-pathogenic bacterium that has been reported as a *Pasteurella* species. *Bulletin of the Japanese Society of Scientific Fisheries* **38**, 803–812.

Simón, M., Mathes, A., Blanch, A. and Engelhardt, H. (1996) Characterization of a porin from the outer membrane of *Vibrio anguillarum*. *Journal of Bacteriology* **178**, 4182–4188.

Simón, M., Jofre, J. and Blanch, A.R. (1998) Evaluation of the immunospecificity of the porin Oml of *Vibrio anguillarum* serotype O1. *Journal of Applied Microbiology* **84**, 709–714.

Singer, J.T., Schmidt, K.A. and Reno, P.W. (1991) Polypeptides p40, pOM2, and pAngR are required for iron uptake and for virulence of the marine fish pathogen *Vibrio anguillarum* 775. *Journal of Bacteriology* **173**, 1347–1352.

Singleton, F.L., Attwell, R.W., Jangi, M.S. and Colwell, R.R. (1982a) Influence of salinity and organic nutrient concentration on survival and growth of *Vibrio cholerae* in aquatic microcosms. *Applied and Environmental Microbiology* **43**, 1080–1085.

Singleton, F.L., Attwell, R., Jangi, S. and Colwell, R.R. (1982b) Effects of temperature and salinity on *Vibrio cholerae* growth. *Applied and Environmental Microbiology* **44**, 1047–1058.

Siwicki, A.K., Klein, P., Morand, M., Kiczka, W. and Studnicka, M. (1998) Immunostimulatory effects of dimerized lysozyme (KLP-602) on the nonspecific defense mechanisms and protection against furunculosis in salmonids. *Veterinary Immunology and Immunopathology* **61**, 369–378.

Skarmeta, A.M., Bandín, I., Santos, Y. and Toranzo, A.E. (1995) *In vitro* killing of *Pasteurella piscicida* by fish macrophages. *Diseases of Aquatic Organisms* **23**, 51–57.

Skarmeta, A.M., Henriquez, V., Zahr, M., Orrego, C. and Marshall, S.H. (2000) Isolation of a virulent *Piscirickettsia salmonis* from the brain of naturally infected coho salmon. *Bulletin of the European Association of Fish Pathologists* **20**, 261–264.

Skerman, V.B.D., McGowan, V. and Sneath, P.H.A. (1980) Approved lists of bacterial names. *International Journal of Systematic Bacteriology* **30**, 225–420.

Skirpstunas, R.T. and Baldwin, T.J. (2002) *Edwardsiella ictaluri* invasion of IEC-6, Henle 407, fathead minnow and channel catfish enteric epiuthelial cells. *Diseases of Aquatic Organisms* **51**, 161–167.

Skirpstunas, R.T. and Baldwin, T.J. (2003) Antibodies against affinity-purified, surface-exposed outer membrane proteins of *Edwardsiella ictaluri* block invasion into fathead minnow epithelial cells. *Journal of Aquatic Animal Health* **15**, 92–97.

Skov, M.N., Pedersen, K. and Larsen, J.L. (1995) Comparison of pulsed-field gel electrophoresis, ribotyping, and plasmid profiling for typing of *Vibrio anguillarum* serovar O1. *Applied and Environmental Microbiology* **61**, 1540–1545.

Smibert, R.M. and Krieg, W.R. (1981) 20. General characterization. In: Gerhardt, P. (ed.), *Manual of Methods for General Bacteriology*. Washington, D.C., American Society of Microbiology, pp. 409–443.

Smith (1939) Cited by McFaddin, T.W. (1969).

Smith, H.L. and Goodner, I.K. (1958) Detection of bacterial gelatinases by gelatin-agar plate method. *Journal of Bacteriology* **76**, 662–665.

Smith, I.W. (1961) A disease of finnock due to *Vibrio anguillarum*. *Journal of General Microbiology* **74**, 247–252.

Smith, I.W. (1962) Furunculosis in kelts. *Department of Agriculture and Fisheries for Scotland, Freshwater and Salmon Fisheries Research* **27**, 1–5.

Smith, I.W. (1963) The classification of *Bacterium salmonicida*. *Journal of General Microbiology* **33**, 263–274.

Smith, I.W. (1964) The occurrence and pathology of Dee disease. *Department of Agriculture and Fisheries for Scotland, Freshwater Salmon Fisheries Research* **34**, 1–12.

Smith, P. and Davey, S. (1993) Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress-inducible furunculosis by a fluorescent pseudomonad. *Journal of Fish Diseases* **16**, 521–524.

Smith, P.A., Lannan, C.N., Garces, L.H., Jarpa, M., Larenas, J., Caswell-Reno, P., Whipple, M. and Fryer, J.L. (1995) Piscirickettsiosis: A bacterin field trial in coho salmon (*Oncorhynchus kisutch*). *Bulletin of the European Association of Fish Pathologists* **14**, 137–141.

Smith, P.A., Pizarro, P., Ojeda, P., Contreras, J., Oyanedel, S. and Larenas, J. (1999) Routes of entry of *Piscirickettsia salmonis* in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **37**, 165–172.

Smith, P.A., Rojas, M.E., Guajardo, A., Contreras, J., Morales, M.A. and Larenas, J. (2004) Experimental infection of coho salmon *Oncorhynchus kisutch* by exposure of skin, gills and itestine with *Piscirickettsia salmonis*. *Diseases of Aquatic Organisms* **61**, 53–57.

Smith, P.D., McCarthy, D.H. and Paterson, W.D. (1980) Further studies on furunculosis vaccination. In: Ahne, W. (ed.), *Fish Diseases, Third COPRAQ Session*. Berlin, Springer-Verlag, pp. 113–119.

Smith, P.R. (1991) Stress test picks up furunculosis carriers. *Fish Farmer* **14**, 42.

Smith, P.R., Brazil, G.M., Drinan, E.M., O'Kelly, J., Palmer, R. and Scallan, A. (1982) Lateral transmission of furunculosis in sea water. *Bulletin of the European Association of Fish Pathologists* **3**, 41–42.

Smith, S.K., Sutton, D.C., Fuerst, J.A. and Reichelt, J.L. (1991) Evaluation of the genus *Listonella* and reassignment of *Listonella damsela* (Love *et al.*) MacDonell & Colwell to the genus *Photobacterium* as *Photobacterium damsela* comb. nov. with an emended description. *International Journal of Systematic Bacteriology* **41**, 529–534.

Smith, W.W. (1942) Action of alkaline acriflavine solution on *Bacterium salmonicida* and trout eggs. *Proceedings of the Society of Experimental Biology and Medicine* **51**, 324–326.

Sneath, P.H.A. (1984) Genus: *Chromobacterium* De Ley, Segers & Gillis 1978, 164^{AL}. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 376–377.

Snieszko, S.F. (1954) Therapy of bacterial fish diseases. *Transactions of the American Fisheries Society* **83**, 313–330.

Snieszko, S.F. (1957) Genus IV: *Aeromonas* Kluyver & van Niel 1936. In: Breed, R.S., Murray, E.G.D. and Smith, N.R. (eds), *Bergey's Manual of Determinative Bacteriology*, 7th edn. Baltimore, MD, Williams & Wilkins, pp. 189–193.

Snieszko, S.F. (1958a) Fish furunculosis. *United States Department of the Interior, Fish and Wildlife Service, Fisheries Leaflet* **467**, 4.

Snieszko, S.F. (1958b) Columnaris disease of fishes. *United States Department of the Interior, Fish and Wildlife Service, Fisheries Leaflet* **46**, 1–3.

Snieszko, S.F. (1964) Remarks on some facts of epizootiology of bacterial fish diseases. *Developments in Industrial Microbiology* **5**, 97–100.

Snieszko, S.F. (1978) Control of fish diseases. *Marine Fisheries Review* **40**, 65–68.

Snieszko, S.F. and Bullock, G.L. (1957) Treatment of sulfonamide-resistant furunculosis in trout and determination of drug sensitivity. *United States Department of the Interior, Fish and Wildlife Service, Fisheries Leaflet* **125**, 555–564.

Snieszko, S.F. and Friddle, S.B. (1949) Prophylaxis of furunculosis in brook trout (*Salvelinus fontinalis*) by oral immunization and sulphamerazine. *Progressive Fish Culturist* **1**, 161–168.

Snieszko, S.F. and Griffin, P.J. (1951) Successful treatment of ulcer disease in brook trout with terramycin. *Science* **112**, 717–718.

Snieszko, S.F. and Griffin, P.J. (1955) Kidney disease in brook trout and its treatment. *Progressive Fish Culturist* **17**, 3–13.

Snieszko, S.F. and Hoffman, G.L. (1963) Control of fish diseases. *Laboratory Animal Care* **13**, 197–206.

Snieszko, S.F., Griffin, P. and Friddle, S.B. (1950) A new bacterium (*Haemophilus piscium* n. sp.) from ulcer disease of trout. *Journal of Bacteriology* **59**, 699–710.

Snieszko, S.F., Dunbar, C.E. and Bullock, G.L. (1959) Resistance to ulcer disease and furunculosis in eastern brook trout, *Salvelinus fontinalis*. *Progressive Fish Culturist* **21**, 111–116.

Snieszko, S.F., Bullock, G.L., Hollis, E. and Boone, J.G. (1964a) *Pasteurella* sp. from an epizootic of white perch (*Roccus americanus*) in Chesapeake Bay tidewater areas. *Journal of Bacteriology* **88**, 1814–1815.

Snieszko, S.F., Bullock, G.L., Dunbar, C.E. and Pettijohn, L.L. (1964b) Nocardial infection in hatchery-reared fingerling rainbow trout (*Salmo gairdneri*). *Journal of Bacteriology* **88**, 1809–1810.

Sobhana, K.S., Mohan, C.V. and Shankar, K.M. (2002) Effect of dietary vitamin C on the disease susceptibility and inflammatory response of mrigal, *Cirrhinus mrigala* (Hamiton) to experimental infection of *Aeromonas hydrophila*. *Aquaculture* **207**, 225–238.

Soffientino, B., Gwaltney, T., Nelson, D.R., Specker, J.L., Maule, M. and Gómez-Chiarri, M. (1999) Infectious necrotizing enteritis and mortality caused by *Vibrio carchariae* in summer

flounder *Paralichthys dentatus* during intensive culture. *Diseases of Aquatic Organisms* **38**, 201–210.

Soltani, M., Munday, B. and Carson, J. (1994) Susceptibility of some freshwater species of fish to infection by *Cytophaga johnsonae*. *Bulletin of the European Association of Fish Pathologists* **14**, 133–135.

Soltani, M., Shanker, S. and Munday, B.L. (1995) Chemotherapy of *Cytophaga/Flexibacter*-like bacteria (CFLB) infections in fish: Studies validating clinical efficacies of selected antimicrobials. *Journal of Fish Diseases* **18**, 555–565.

Song, Y.L. and Kou, G.H. (1981) Immune response of eels (*Anguilla japonica*) against *Aeromonas hydrophila* and *Edwardsiella anguillimortiferum* (*E. tarda*) infection. *Proceedings of the Republic of China/U.S. Cooperative Science Seminar on Fish Diseases, National Science Council Series* **3**, 107–115.

Song, Y.L., Kou, G.H. and Chen, K.Y. (1982) Vaccination conditions for the eel (*Anguilla japonica*) with *Edwardsiella anguillimortiferum* bacterin. *CAPD (Taiwan) Fisheries Series No. 8, Fish Disease Research* **4**, 18–25.

Song, Y.L., Fryer, J.L. and Rohovec, J.S. (1988) Comparison of gliding bacteria isolated from fish in North America and other areas of the Pacific rim. *Fish Pathology* **23**, 197–202.

Sørensen, U.B.S. and Larsen, J.L. (1986) Serotyping of *Vibrio anguillarum*. *Applied and Environmental Microbiology* **51**, 593–597.

Sorimachi, M., Maeno, Y., Nakajima, K., Inouye, K. and Inui, Y. (1993) Causative agent of jaundice of yellowtail, *Seriola quinqueradiata*. *Fish Pathology* **28**, 119–124.

Sørum, H., Hvaal, A.B., Heum, M., Daae, F.L. and Wiik, R. (1990) Plasmid profiling of *Vibrio salmonicida* for epidemiological studies of cold-water vibriosis in Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). *Applied and Environmental Microbiology* **56**, 1033–1037.

Sørum, H., Myhr, E., Zwicker, B.M. and Lillehaug, A. (1993a) Comparison by plasmid profiling of *Vibrio salmonicida* strains isolated from diseased fish from different North European and Canadian coastal areas of the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 247–250.

Sørum, H., Kvello, J.H. and Håstein, T. (1993b) Occurrence and stability of plasmids in *Aeromonas salmonicida* subsp. *salmonicida* isolated from salmonids with furunculosis. *Diseases of Aquatic Organisms* **16**, 199–206.

Sørum, H., Holstad, G., Lunder, T. and Håstein, T. (2000) Grouping by plasmid profiles of atypical *Aeromonas salmonicida* isolated from fish, with special reference to salmonid fish. *Diseases of Aquatic Organisms* **41**, 159–171.

Sousa, J.A., Magariños, B., Eiras, J.C., Toranzo, A.E. and Romalde, J.L. (2001) Molecular characterization of Portuguese strains of *Yersinia ruckeri* isolated from fish culture systems. *Journal of Fish Diseases* **24**, 151–159.

Sövényi, J.F., Yamamoto, H., Fujimoto, S. and Kusuda, R. (1990) Lymphomyeloid cells, susceptibility to erythrodermatitis of carp and bacterial antigens. *Developmental and Comparative Immunology* **14**, 185–200.

Spanggaard, B., Huber, I., Nielsen, J., Nielsen, T. and Gram, L. (2000) Proliferation and location of *Vibrio anguillarum* during infection of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **23**, 423–427.

Speare, D.J. (1997) Differences in patterns of meningoencephalitis due to bacterial kidney disease in farmed Atlantic andchinook salmon. *Research in Veterinary Science* **62**, 79–80.

Speare, D.J., Brocklebank, J., Macnair, N. and Bernard, K.A. (1995) Experimental transmission of a salmonid *Rhodococcus* sp. isolate to juvenile Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **18**, 587–597.

Spence, K.D., Fryer, J.L. and Pilcher, K.S. (1965) Active and passive immunization of certain salmonid fishes against *Aeromonas salmonicida*. *Canadian Journal of Microbiology* **11**, 397–405.

Speyerer, P.D. and Boyle, J.A. (1987) The plasmid profile of *Edwardsiella ictaluri*. *Journal of Fish Diseases* **10**, 461–469.

Stackebrandt, E., Wehmeyer, U., Nader, H. and Fiedler, F. (1988) Phylogenetic relationship of the fish pathogenic *Renibacterium salmoninarum* to *Arthrobacter*, *Micrococcus* and related taxa. *FEMS Microbiology Letters* **50**, 117–120.

Stamm, J.M., Hanson, C.W., Chu, D.T.W., Bailer, R., Vojtko, C. and Fernandes, P.B. (1986) *In vitro* evaluation of A-56619 (Difloxacin) and A-56620: New aryl-fluoroquinolones. *Antimicrobial Agents and Chemotherapy* **29**, 193–200.

Stanier, R.Y. (1942) The *Cytophaga* group: A contribution to the biology of myxobacteria. *Bacteriological Reviews* **6**, 143–196.

Stanier, R.Y., Palleroni, M.J. and Doudoroff, M. (1966) The aerobic pseudomonads: A taxonomic study. *Journal of General Microbiology* **43**, 159–271.

Stanley, C., Hiney, M., Gilroy, D., Powell, R., Padley, D. and Smith, P. (2002) Furunculosis-inducing ability of microcosms seeded with *Aeromonas salmonicida* correlates with colony-forming ability but not with PCR and ELISA data. *Aquaculture* **210**, 35–48.

Stanley, L.A., Hudson, J.S., Schwedler, T.E. and Hayasaki, S.S. (1994) Extracellular products associated with virulent and avirulent strains of *Edwardsiella ictaluri* from channel catfish. *Journal of Aquatic Animal Health* **6**, 36–43.

Starliper, C.E. (1996) Genetic diversity of North American isolates of *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* **27**, 207–213.

Starliper, C.E. (2001) Isolation of *Serratia liquefaciens* as a pathogen of Arctic char, *Salvelinus alpinus* (L.). *Journal of Fish Diseases* **24**, 53–56.

Starliper, C.E. (2001a) The effect of depuration on transmission of *Aeromonas salmonicida* between the freshwater bivalve *Amblema plicata* and Arctic charr. *Journal of Aquatic Animal Health* **13**, 56–62.

Starliper, C.E., Schill, W.B., Shotts, E.B. and Waltman, W.D. (1988) Isozyme analysis of *Edwardsiella ictaluri*. *Microbios Letters* **37**, 81–87.

Starliper, C.E., Shotts, E.B. and Brown, J. (1992) Isolation of *Carnobacterium piscicola* and an unidentified Gram-positive bacillus from sexually mature and post-spawning rainbow trout (*Oncorhynchus mykiss*). *Diseases of Aquatic Organisms* **13**, 181–187.

Staroscik. A.M., Denkin, S.M. and Nelson, D.R. (2005) regulation of the *Vibrio anguillarum* metalloprotease EmpA by posttranslational modification. *Journal of Bacteriology* **187**, 2257–2260.

Starr, M.P. and Chatterjee, A.V. (1972) The genus *Erwinia*: Enterobacteria pathogenic to plants and animals. *Annual Review of Microbiology* **26**, 289–426.

Steigerwalt, A.G., Fanning, G.R., Fife-Asbury, M.A. and Brenner, D.J. (1976) DNA relatedness among species of *Enterobacter* and *Serratia*. *Canadian Journal of Microbiology* **22**, 121–137.

Steine, N.O., Melingen, G.O. and Wergeland, H.I. (2001) Antibodies against *Vibrio salmonicida* lipopolysaccharide (LPS) and whole bacteria in sera from Atlantic salmon (*Salmo salar* L.) vaccinated during the smolting and early post-smolt period. *Fish & Shellfish Immunology* **11**, 39–52.

Stensvåg, K., Arnesen, S.M., Bøgwald, J. and Jørgensen, T.Ø. (1999) Localization of purified surface molecules (lipopolysaccharide and A-protein) from *Aeromonas salmonicida* in the kidney and spleen from Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **22**, 337–349.

Stevenson, L.H. (1978) A case for bacterial dormancy in aquatic systems. *Microbial Ecology* **4**, 127–133.

Stevenson, R.M.W. and Allan, B.J. (1981) Extracellular virulence factors in *Aeromonas hydrophila* disease processes in salmonids. *Developments in Biological Standardization* **49**, 173–180.

Stevenson, R.M.W. and Airdrie, D.E. (1984a) Serological variation among *Yersinia ruckeri* strains. *Journal of Fish Diseases* **7**, 247–254.

Stevenson, R.M.W. and Airdrie, D.E. (1984b) Isolation of *Yersinia ruckeri* bacteriophages. *Applied and Environmental Microbiology* **47**, 1201–1205.

Stevenson, R.M.W. and Daly, J.G. (1982) Biochemical and serological characteristics of Ontario isolates of *Yersinia ruckeri*. *Canadian Journal of Fisheries and Aquatic Science* **39**, 870–876.

Stewart, D.J., Waldemariam, K., Dear, G. and Mochaba, F.M. (1983) An outbreak of "Sekiten-byo" among cultured European eels, *Anguilla anguilla* L., in Scotland. *Journal of Fish Diseases* **6**, 75–76.

Stine, C.B., Baya, A.M., Salierno, J.D., Kollner, M. and Kane, A.S. (2005) Mycobacterial infection in laboratory-maintained Atlantic menhaden. *Journal of Aquatic Animal Health* **17**, 380–385.

Stoffregen, D.A., Chako, A.J., Backman, S. and Babish, J.G. (1993) Successful therapy of furunculosis in Atlantic salmon, *Salmo salar* L., using the fluoroquinolone antimicrobial agent enrofloxacin. *Journal of Fish Diseases* **16**, 219–228.

Stoffregen, D.A., Backman, S.C., Perham, R.E., Bowser, P.R. and Babish, J.G. (1996) Initial disease report of *Streptococcus iniae* infection in hybrid striped (sunshine) bass and successful therapeutic intervention with the fluoroquinolone antibacterial enrofloxacin. *Journal of the World Aquaculture Society* **27**, 420–434.

Stringer-Roth, K.M., Yunghans, W. and Caslake, L.F. (2002) Differences in chondroitin AC lyase activity of *Flavobacterium columnare* isolates. *Journal of Fish Diseases* **25**, 687–691.

Strohl, W.R. and Tait, L.R. (1978) *Cytophaga aquatilis* sp. nov., a facultative anaerobe isolated from the gills of freshwater fish. *International Journal of Systematic Bacteriology* **28**, 293–303.

Stuart, C.A., van Stratum, E. and Rustigian, R. (1945) Further studies on urease production by *Proteus* and related organisms. *Journal of Bacteriology* **49**, 437.

Stuber, K., Burr, S.E., Braun, M., Wahli, T. and Frey, J. (2003) Type III secretion genes in *Aeromonas salmonicida* subsp. *salmonicida* are located on a large thermolabile virulence plasmid. *Journal of Clinical Microbiology* **41**, 3854–3856.

Sugita, A. (1996) A case of streptococcicosis in dusky spinefoot. *Fish Pathology* **31**, 47–48.

Sugita, H., Matsuo, N., Hirose, Y., Iwato, M. and Deguchi, Y. (1997) *Vibrio* sp. strain NM10, isolated from the intestine of a Japanese coastal fish, has an inhibitory effect against *Pasteurella piscicida*. *Applied and Environmental Microbiology* **63**, 4986–4989.

Sugiyama, A. and Kusuda, R. (1981a) Studies on the characters of *Staphylococcus epidermidis* isolated from diseased fishes—II. Serological properties of the isolates. *Fish Pathology* **16**, 25–33.

Sugiyama, A. and Kusuda, R. (1981b) Studies on the characters of *Staphylococcus epidermidis* isolated from diseased fishes—II. A comparative study on the serological properties between the isolates and the strains of human origin. *Fish Pathology* **16**, 35–41.

Sukenda and Wakabayashi, H. (2000) Tissue distribution of *Pseudomonas plecoglossicida* in experimentally infected ayu *Plecoglossus altivelis* studied by real-time quantitative PCR. *Fish Pathology* **35**, 223–228.

Sukenda and Wakabayashi, H. (2001) Adherence and infectivity of green fluorescent protein-labeled *Pseudomonas plecoglossicida* to ayu *Plecoglossus altivelis*. *Fish Pathology* **36**, 161–167.

Suomalainen, L.-R., Tiirola, M. and Valtonen, E.T. (2005) Treatment of columnaris disease of rainbow trout: Low pH and salt as possible tools. *Diseases of Aquatic Organisms* **65**, 115–120.

Suomalainen, L.-R., Tiirola, M. and Valtonen, E.T. (2005a) Influence of rearing conditions on *Flavobacterium columnare* infection of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* **28**, 271–278.

Suomalainen, L.-R., Reunanen, H., Ritva Ijäs, E., Valtonen, T. and Tiirola, M. (2006) Freezing induces biased results in the molecular detection of *Flavobacterium columnare*. *Applied and Environmental Microbiology* **72**, 1702–1704.

Suomalainen, L.-R., Kunttu, H., Valtonen, E.T., Hirvelä-Koski, V. and Tiirola, M. (2006a) Molecular diversity and growth features of *Flavobacterium columnare* strains isolated in Finland. *Diseases in Aquatic Organisms* **70**, 55–61.

Suomalainen, L.-R., Tiirola, M. and Valtonen, E.T. (2006b) Chondroitin AC lyase activity is related to virulence of fish pathogenic *Flavobacterium columnare*. *Journal of Fish Diseases* **29**, 757–763.

Suprapto, H., Hara, T., Nakai, T. and Muroga, K. (1996) Purification of a lethal toxin of *Edwardsiella tarda*. *Fish Pathology* **31**, 203–207.

Sussman, A.S. and Halvorson, H.O. (1966) *Spores, Their Dormancy and Germination*. New York, Harper & Row, 356 pp.

Suzuki, M., Nakagawa, Y., Harayama, S. and Yamamoto, S. (2001) Phylogentic analysis and taxonomy of marine *Cytophaga*-like bacteria: Proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **51**, 1639–1652.

Suzumoto, B.K., Schreck, C.B. and McIntyre, J.D. (1977) Relative resistance of three transferrin genotypes of coho salmon (*Oncorhynchus kisutch*) and their hematological responses to bacterial kidney disease. *Journal of the Fisheries Research Board of Canada* **34**, 1–8.

Svendsen, Y.S., Dalmo, R.A. and Bøgwald, J. (1999) Tissue localization of *Aeromonas salmonicida* in Atlantic salmon, *Salmo salar* L., following experimental challenge. *Journal of Fish Diseases* **22**, 125–131.

Swaim, L.E., Connolly, L.E., Volkman, H.E., Humbert, O., Born, D.E. and Ramakrishnan, L. (2006) *Mycobacterium marinum* infection of adult zebra fish causes caseating granulomatous tuberculosis and is moderated by adaptive immunity. *Infection and Immunity* **74**, 6108–6117.

Swain, P. and Nayak, S.K. (2003) Comparative sensitivity of different serological tests for seromonitoring and surveillance of *Edwardsiella tarda* infection of Indian major carps. *Fish & Shellfish Immunology* **15**, 333–340.

Sykes, J.B. (1976) *The Concise Oxford Dictionary of Current English*, 6th edn. Oxford, U.K., Oxford University Press.

Tajima, K., Yoshimizu, M., Ezura, Y. and Kimura, T. (1981) Studies on the causative organisms of vibriosis among the pen cultured coho salmon (*Oncorhynchus kisutch*) in Japan. *Bulletin of the Japanese Society of Scientific Fisheries* **47**, 35–42.

Tajima, K., Takahashi, T., Ezura, Y. and Kimura, T. (1984) Enzymatic properties of the purified extracellular protease of *Aeromonas salmonicida*, Ar-4 (EFDL). *Bulletin of the Japanese Society of Scientific Fisheries* **50**, 145–150.

Tajima, K., Ezura, Y. and Kimura, T. (1987a) The possible use of a thermolabile antigen in detection of *Vibrio anguillarum*. *Fish Pathology* **22**, 237–242

Tajima, K., Ezura, Y. and Kimura, T. (1987b) Pathogenicity of a non-protease secreting strain of *Aeromonas salmonicida*. *Bulletin of the Faculty of Fisheries, Hokkaido University* **38**, 139–150

Takemaru, I. and Kusuda, R. (1988) Chemotherapeutic effect of josamycin against natural streptococcal infection in cultured yellowtail. *Bulletin of the Japanese Society of Scientific Fisheries* **54**.

Talaat, A.M., Trucksis, M., Kane, A.S. and Reimschuessel, R. (1999) Pathogenicity of *Mycobacterium fortuitum* and *Mycobacterium smegmatis* to goldfish *Carassius auratus*. *Veterinary Microbiology* **66**, 151–164.

Tan, E., Low, K.W., Wong, W.S.F. and Leung, K.Y. (1998) Internalization of *Aeromonas hydrophila* by fish epidermal cells can be inhibited with a tyrosine kinase inhibitor. *Microbiology* **144**, 299–307.

Tan, Y.P., Zheng, J., Tung, S.L., Rosenshine, I. and Leung, K.Y. (2005) Role of type III secretion in *Edwardsiella tarda* virulence. *Microbiology* **151**, 2301–2313.

Taniguchi, M. (1982a) Experiment on peroral inoculation via food to induce yellowtail streptococcicosis. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 1717–1720.

Taniguchi, M. (1982b) Influence of food condition on artificial peroral infection of yellowtail streptococcicosis. *Bulletin of the Japanese Society of Scientific Fisheries* **48**, 1721–1723.

Taniguchi, M. (1983) Feeding experiment of yellowtail using frozen diet. *Bulletin of the Japanese Society of Scientific Fisheries* **49**, 611–615.

Tatani, M., Muroga, K., Sugiyama, T. and Hiramoto, Y. (1985) Detection of *Vibrio anguillarum* from reared fry and fingerlings of ayu. *Aquaculture* **33**, 59–66.

Tatner, M.F. (1987) The quantitative relationship between vaccine dilution, length of immersion time and antigen uptake, using a radiolabelled *Aeromonas salmonicida* bath in direct immersion experiments with rainbow trout, *Salmo gairdneri*. *Aquaculture* **62**, 173–185.

Tatner, M.F. (1989) The antibody response of intact and short term thymectomised rainbow trout (*Salmo gairdneri*) to *Aeromonas salmonicida*. *Developmental and Comparative Immunology* **13**, 387.

Tatner, M.F., Johnson, C.M. and Horne, M.T. (1984) The tissue localization of *Aeromonas salmonicida* in rainbow trout, *Salmo gairdneri* Richardson, following three methods of administration. *Journal of Fish Biology* **25**, 95–108.

Taylor, P.W. (2004) Detection of *Flavobacterium psychrophilum* in eggs and sexual fluids of Pacific salmonids by a polymerase chain reaction assay: Implications for vertical transmission of bacterial coldwater disease. *Journal of Aquatic Animal Health* **16**, 104–108.

Taylor, P.W. and Winton, J.R. (2002) Optimization of nested polymerase chain reaction assay for identification of *Aeromonas salmonicida*, *Yersinia ruckeri*, and *Flavobacterium psychrophilum*. *Journal of Aquatic Animal Health* **14**, 216–224.

Taylor. S.L., Jaso-Friedmann, L., Allison, A.B., Eldar, A. and Evans, D.L. (2001) *Streptococcus iniae* inhibition of apoptosis of non-specific cytotoxic cells: A mechanism of activation of innate immunity in teleosts. *Diseases of Aquatic Organisms* **46**, 15–21.

Tebbit, G.L., Erickson, J.D. and vande Water, R.B. (1981) Development and use of *Yersinia ruckeri* bacterins to control enteric redmouth disease. *Developments in Biological Standardization* **49**, 395–401.

Temprano, A., Yugueros, J., Hernanz, C., Sánchez, M., Berzal, B., Luengo, J.M. and Naharro, G. (2001) Rapid identification of *Yersinia ruckeri* by PCR amplification of *yruI-yruR* quorum sensing. *Journal of Fish Diseases* **24**, 253–261.

Temprano, A., Riaño, J., Yugueros, J., González, P., de Castro, L., Villena, A., Luengo, J.M. and Naharro, G. (2005) Potential use of a *Yersinia ruckeri* O1 auxotrophic *aroA* mutant as a live attenuated vaccine. *Journal of Fish Diseases* **28**, 419–428.

Teshima, C., Kudo, S., Ohtani, Y. and Saito, A. (1992) Kidney pathology from the bacterium *Hafnia alvei*: Experimental evidence. *Transactions of the American Fisheries Society* **121**, 599–607.

Teska, J.H., Shotts, E.B and Hsu, T.C. (1989) Automated biochemical identification of bacterial fish pathogens using the Abbott Quantum II. *Journal of Wildlife Diseases* **25**, 103–107.

Teska, J.D., Twerdok, L.E., Beaman, J., Curry, M. and Finch, R.A. (1997) Isolation of *Mycobacterium abscessus* from Japanese meduka. *Journal of Aquatic Animal Health* **9**, 234–238.

Teskeredzic, E., Grahek, D., Malnar, L., Teskeredzic, Z. and Hacmanjek, M. (1993) Bakterijska bolest americkog somica (*Amiurus nebulosus* L.). *Ribarstvo* **48**, 5–11.

Thomas-Jinu, S. and Goodwin, A.E. (2004) Acute columnaris infection in channel catfish, *Ictalurus punctatus* (Rafinesque): Efficacy of practical treatment for warmwater aquaculture ponds. *Journal of Fish Diseases* **27**, 23–28.

Thompson, F.L., Hoste, B., Vandemeulebroecke, K., Engelbeen, K., Denys, R. and Swings, J. (2002) *Vibrio trachuri* Iwamoto *et al.* 1995 is a junior synonym of *Vibrio harveyi* (Johnson & Shunk 1936) Baumann *et al.* 1981. *International Journal of Systematic and Evolutionary Microbiology* **52**, 973–976.

Thomson, R., Macpherson, H.L., Riaza, A. and Birkbeck, T.H. (2005) *Vibrio splendidus* type 1 as a cause of mortalities in hatchery-reared larval turbot, *Scophthalmus maximus* (L.). *Journal of Applied Microbiology* **99**, 243–250.

Thornton, J.C., Garduño, R.A., Newman, S.G. and Kay, W.W. (1991) Surface-disorganized, attenuated mutants of *Aeromonas salmonicida* as furunculosis live vaccines. *Microbial Pathogenesis* **11**, 85–99.

Thornton, J.C., Garduño, R.A., Carlos, S.J. and Kay, W.W. (1993) Novel antigens expressed by *Aeromonas salmonicida* grown *in vivo*. *Infection and Immunity* **61**, 4582–4589.

Thornton, J.C., Garduño, R.A. and Kay, W.W. (1994) The development of live vaccines for furunculosis lacking the A-layer and O-antigen of *Aeromonas salmonicida*. *Journal of Fish Diseases* **17**, 195–204.

Thornton, J.M., Austin, D.A., Austin, B. and Powel, R. (1999) Small subunit rRNA gene sequences of *Aeromonas salmonicida* subsp. *smithia* and *Haemophilus piscium* reveal pronounced similarities with *A. salmonicida* subsp. *salmonicida*. *Diseases of Aquatic Organisms* **35**, 155–158.

Thorsen, B.K., Enger, Ø., Norland, S. and Hoff, K.J. (1992) Long-term starvation survival of *Yersinia ruckeri* at different salinities studied by microscopical and flow cytometric methods. *Applied and Environmental Microbiology* **58**, 1624–1628.

Thune, R.L., Graham, T.E., Riddle, L.M. and Amborski, R.L. (1982a) Extracellular products and endotoxin from *Aeromonas hydrophila*: Effect on age-0 channel catfish. *Transactions of the American Fisheries Society* **111**, 404–408.

Thune, R.L., Graham, T.E., Riddle, L.M. and Amborski, R.L. (1982b) Extracellular proteases from *Aeromonas hydrophila*: Partial purification and effects on age-0 channel catfish. *Transactions of the American Fisheries Society* **111**, 749–754.

Thune, R.L., Johnson, M.C., Graham, T.E. and Amborski, R.L. (1986) *Aeromonas hydrophila* β-haemolysin: Purification and examination of its role in virulence in O-group channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* **9**, 55–61.

Thune, R.L., Collins, R.A. and Peña, M.P. (1997) A comparison of immersion, immersion/oral combination and injection methods for the vaccination of channel catfish *Ictalurus punctatus* against *Edwardsiella ictaluri*. *Journal of the World Aquaculture Society* **28**, 193–201.

Thune, R.L., Fernandez, D.H. and Battista, J.R. (1999) An *aroA* mutant of *Edwardsiella ictaluri* is safe and efficacious as a live, attenuated vaccine. *Journal of Aquatic Animal Health* **11**, 358–372.

Thune, R.L., Fernandez, D.H., Hawke, J.P. and Miller, R. (2003) Construction of a safe, stable, efficacious vaccine against *Photobacterium damselae* subsp. *piscicida*. *Diseases of Aquatic Organisms* **57**, 51–58.

Thyssen, A., Van Eygen, S., Hauben, L., Goris, J., Swings, J. and Ollevier, F. (2000) The application of AFLP for taxonomic and epidemiological studies of *Photobacterium damselae* subsp. *piscicida*. *International Journal of Systematic and Evolutionary Microbiology* **50**, 1013–1019.

Tiainen, T., Pedersen, K. and Larsen, J.L. (1995) Ribotyping and plasmid profiling of *Vibrio anguillarum* serovar O2 and *Vibrio ordalii*. *Journal of Applied Bacteriology* **73**, 384–392.

Tiainen, T., Pedersen, K. and Larsen, J.L. (1997) Immunological reactivity of *Vibrio anguillarum* sero-subgroups O2a and O2b, and comparison of their lipopolysaccharide profiles. *Current Microbiology* **34**, 38–42.

Teixeira, L.M., Merquior, V.L.C., Vianni, M. da C.E., Carvalho, M. da G.S., Fracalanzza, S.E.L., Steigerwalt, A.G., Brenner, D.J. and Facklam, R.R. (1996) Phenotypic and genotypic characterization of atypical *Lactococcus garvieae* strains isolated from water buffalos with subclinical mastitis and confirmation of *L. garvieae* as the senior subjective synonym of *Enterococcus seriolicida*. *International Journal of Systematic Bacteriology* **46**, 664–668.

Tiirola, M., Tellervo Valtonen, E., Rintamäki-Kinnunen and Kulomaa, M.S. (2002) Diagnosis of flavobacteriosis by direct amplification of rRNA genes. *Diseases of Aquatic Organisms* **51**, 93–100.

Tison, D.L., Nishibuchi, M., Greenwood, J.D. and Seidler, R.J. (1982) *Vibrio vulnificus* biogroup 2: A new biogroup pathogenic for eels. *Applied and Environmental Microbiology* **44**, 640–646.

Titball, R.W. and Munn, C.B. (1981) Evidence for two haemolytic activities from *Aeromonas salmonicida*. *FEMS Microbiology Letters* **12**, 27–30.

Titball, R.W. and Munn, C.B. (1983) Partial purification and properties of a haemolytic activity (T-lysin) from *Aeromonas salmonicida*. *FEMS Microbiology Letters* **20**, 207–210.

Titball, R.W. and Munn, C.B. (1985a) The purification and some properties of H-lysin from *Aeromonas salmonicida*. *Journal of General Microbiology* **131**, 1603–1609.

Titball, R.W. and Munn, C.B. (1985b) Interactions of extracellular products from *Aeromonas salmonicida*. In: Ellis, A.E. (ed.), *Fish and Shellfish Pathology*. London, Academic Press, pp. 61–68.

Todd, C. (1933) The presence of a bacteriophage for *B. salmonicida* in river waters. *Nature (London)* **131**, 360.

Tolmasky, M.E. and Crosa, J.H. (1984) Molecular cloning and expression of genetic determinants for the iron uptake system mediated by the *Vibrio anguillarum* plasmid pJM1. *Journal of Bacteriology* **160**, 860–866.

Tolmasky, M.E. and Crosa, J.H. (1995) Iron transport genes of the pJM1-mediated iron uptake system of *Vibrio anguillarum* are included in a transposonlike structure. *Plasmid* **37**, 180–190.

Toranzo, A.E., Barja, J.L. and Hetrick, F.H. (1982) Survival of *Vibrio anguillarum* and *Pasteurella piscicida* in estuarine and freshwaters. *Bulletin of the European Association of Fish Pathologists* **3**, 43–45.

Toranzo, A.E., Barja, J.L., Colwell, R.R. and Hetrick, F.M. (1983a) Characterization of plasmids in bacterial fish pathogens. *Infection and Immunity* **39**, 184–192.

Toranzo, A.E., Barja, J.L., Potter, S.A., Colwell, R.R., Hetrick, F.M. and Crosa, J.H. (1983b) Molecular factors associated with virulence of marine vibrios isolated from striped bass in Chesapeake Bay. *Infection and Immunity* **39**, 1220–1227.

Toranzo, A.E., Santos, Y., Nieto, T.P. and Barja, J.L. (1986) Evaluation of different assay systems for identification of environmental *Aeromonas* strains. *Applied and Environmental Microbiology* **51**, 652–656.

Toranzo, A.E., Baya, A.M., Roberson, B.S., Barja, J.L., Grimes, D.J. and Hetrick, F.M. (1987) Specificity of slide agglutination test for detecting bacterial fish pathogens. *Aquaculture* **61**, 81–97.

Toranzo, A.E., Baya, A.M., Romalde, J.J. and Hetrick, F.M. (1989) Association of *Aeromonas sobria* with mortalities of adult gizzard shad, *Dorosoma cepedianum* Lesueur. *Journal of Fish Diseases* **12**, 439–448.

Toranzo, A.E., Romalde, J.L., Núñez, S., Figueras, A. and Barja, J.L. (1993) An epizootic in farmed, market-size rainbow trout in Spain caused by a strain of *Carnobacterium piscicola* of unusual virulence. *Diseases of Aquatic Organisms* **17**, 87–99.

Toranzo, A.E., Devesa, S., Romalde, J.L., Lamas, J., Riaza, A., Leiro, J. and Barja, J.L. (1995) Efficacy of intraperitoneal vaccination and immersion vaccination against *Enterococcus* sp. infections in turbot. *Aquaculture* **134**, 17–27.

Touraki, M., Mourelatos, S., Karamanlidou, G., Kalaitzopoulou, S. and Kastritsis, C. (1996) Bioencapsulation of chemotherapeutics in *Artemia* as a means of prevention and treatment of infectious disease in marine fish fry. *Aquacultural Engineering* **15**, 133–147.

Toyama, T., Kita-Tsukamoto, K. and Wakabayashi, H. (1994) Identification of *Cytophaga psychrophila* by PCR targeted 16S ribosomal RNA. *Fish Pathology* **29**, 271–275.

Toyama, T., Tsukamoto, K.K. and Wakabayashi, H. (1996) Identification of *Flexibacter maritimus*, *Flavobacterium branchiophilum* and *Cytophaga columnaris* by PCR targeted 16 ribosomal DNA. *Fish Pathology* **31**, 25–33.

Traxler, G.S. and Li, M.F. (1972) *Vibrio anguillarum* isolated from a nasal abscess of the cod fish (*Gadus morhua*). *Journal of Wildlife Diseases* **8**, 207–214.

Treasurer, J. and Cox, D. (1991) The occurrence of *Aeromonas salmonicida* in wrasse (Labridae) and implications for Atlantic salmon farming. *Bulletin of the European Association of Fish Pathologists* **11**, 208–210.

Trüper, H.G. and De'Clari, L. (1997) Taxonomic note: Necessary correction of specific epiphets formed as substantives (nouns) "in apposition". *International Journal of Systematic Bacteriology* **47**, 908–909.

Trust, T.J. (1975) Bacteria associated with the gills of salmonid fishes in freshwater. *Journal of Applied Bacteriology* **38**, 225–233.

Trust, T.J. and Sparrow, R.A.H. (1974) The bacterial flora in the alimentary tract of freshwater salmonid fishes. *Canadian Journal of Microbiology* **20**, 1219–1228.

Trust, T.J., Bull, L.M., Currie, B.R. and Buckley, J.T. (1979) Obligate anaerobic bacteria in the gastro-intestinal microflora of the grass carp, *Ctenopharyngodon idella*, goldfish *Carassius auratus* and rainbow trout *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* **36**, 1174–1179.

Trust, T.J., Ishiguro, E.E. and Atkinson, H.M. (1980a) Relationship between *Haemophilus piscium* and *Aeromonas salmonicida* revealed by *Aeromonas hydrophila* bacteriophage. *FEMS Microbiology Letters* **9**, 199–201.

Trust, T.J., Khouri, A.G., Austen, R.A. and Ashburner, L.D. (1980b) First isolation in Australia of atypical *Aeromonas salmonicida*. *FEMS Microbiology Letters* **9**, 39–42.

Trust, T.J., Howard, P.S., Chamberlain, J.B., Ishiguro, E.E. and Buckley, J.T. (1980c) Additional surface protein in autoaggregating strains of atypical *Aeromonas salmonicida*. *FEMS Microbiology Letters* **9**, 35–38.

Trust, T.J., Courtice, I.D., Khouri, A.G., Crosa, J.H. and Schiewe, M.H. (1981) Serum resistance and haemagglutination ability of marine vibrios pathogenic for fish. *Infection and Immunity* **34**, 702–707.

Trust, T.J., Kay, W.W. and Ishiguro, E.E. (1983) Cell surface hydrophobicity and macrophage association of *Aeromonas salmonicida*. *Current Microbiology* **9**, 315–318.

Tsoumas, A., Alderman, D.J. and Rodgers, C.J. (1989) *Aeromonas salmonicida*: Development of resistance to 4-quinolone antimicrobials. *Journal of Fish Diseases* **12**, 493–507.

Uddin, N., Chowdhury, B.R. and Wakabayashi, H. (1997) Optimum temperatures for the growth and protease production of *Aeromonas hydrophila*. *Fish Pathology* **32**, 117–120.

Udey, L.R. (1977) Pathogenic, antigenic and immunogenic properties of *Aeromonas salmonicida* studied in juvenile coho salmon (*Oncorhynchus kisutch*). Ph.D. thesis, Oregon State University, Corvallis.

Udey, L.R. (1982) A differential medium for distinguishing A/r^+ from A/r^- phenotypes in *Aeromonas salmonicida*. In: *Proceedings of the 13th Annual Conference and Workshop and 7th Eastern Fish Health Workshop*. Baltimore, International Association for Aquatic Animal Medicine, p. 41.

Udey, L.R. and Fryer, J.L. (1978) Immunization of fish with bacterins of *Aeromonas salmonicida*. *Marine Fisheries Review* **40**, 12–17.

Udey, L.R., Young, E. and Sallman, B. (1976) *Eubacterium* sp. ATCC 29255: An anaerobic bacterial pathogen of marine fish. *Fish Health News* **5**, 3–4.

Udey, L.R., Young, E. and Sallman, B. (1977) Isolation and characterization of an anaerobic bacterium, *Eubacterium tarantellus* sp. nov., associated with striped mullet (*Mugil cephalus*) mortality in Biscayne Bay, Florida. *Journal of the Fisheries Research Board of Canada* **34**, 402–409.

Ugajin, M. (1979) Studies on the taxonomy of major microflora on the intestinal contents of salmonids. *Bulletin of the Japanese Society of Scientific Fisheries* **45**, 721–731.

Ugajin, M. (1981) Studies on *Streptococcus* sp. as a causal agent of an epizootic among the cultured ayu (*Plecoglossus altivelis*) in Tochigi Prefecture, Japan, 1980. *Fish Pathology* **16**, 119–127.

Ullah, M.A. and Arai, T. (1983a) Pathological activities of the naturally occurring strains of *Edwardsiella tarda*. *Fish Pathology* **18**, 65–70.

Ullah, M.A. and Arai, T. (1983b) Exotic substances produced by *Edwardsiella tarda*.. *Fish Pathology* **18**, 71–75.

Umbreit, W.W. and Ordal, E.J. (1972) Infection of goldfish with *Vibrio anguillarum*. *American Society of Microbiology News* **32**, 93–96.

Umbreit, T.H. and Tripp, M.R. (1975) Characterization of the factors responsible for death of fish infected with *Vibrio anguillarum*. *Canadian Journal of Microbiology* **21**, 1272–1274.

Umelo, E. and Trust, T.J. (1997) Identification and molecular characterization of two randomly located flagellin genes from *Aeromonas salmonicida* A449. *Journal of Bacteriology* **179**, 5292–5299.

Umelo, E. and Trust, T.J. (1998) Physical map of the chromosome of *Aeromonas salmonicida* and genomic comparisons between *Aeromonas* strains. *Microbiology* **144**, 2141–2149.

Urakawa, H., Kita-Tsukamoto, K., Stevens, S.E., Ohwada, K. and Colwell, R.R. (1998) A proposal to transfer *Vibrio marinus* (Russell 1891) to a new genus *Moritella* gen. nov. as *Moritella marina* comb. nov. *FEMS Microbiology Letters* **165**, 373–378.

Uribe, J.C., Vial, M.V., Carvajal, J., Onate, R. and Teuber, C. (1995) Increasing prevalence of BKD: An inadequate management during salmon transfer to seawater. *Medio Ambiente* **12**, 60–66.

Valdez, I.E. and Conroy, D.A. (1963) The study of a tuberculosis-like condition in neon tetras (*Hyphessobrycon innesi*) II. Characteristics of the bacterium isolated. *Microbiología Española* **16**, 249–253.

Valheim, M., Håstein, T., Myhr, E., Speilberg, L. and Ferguson, H.W. (2000) *Varracalbmi*: A new bacterial panophthalmitis in farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **23**, 61–70.

Valla, S., Frydenlund, K., Coucheron, D.H., Haugan, K., Johansen, B., Jørgensen, T., Knudsen, G. and Strøm, A. (1992) Development of a gene transfer system for curing plasmids in the marine fish pathogen *Vibrio salmonicida*. *Applied and Environmental Microbiology* **58**, 1980–1985.

Van Alstine, J.M., Trust, T.J. and Brooks, D.E. (1986) Differential partition of virulent *Aeromonas salmonicida* and attenuated derivatives possessing specific cell surface alterations in polymer aqueous-phase systems. *Applied and Environmental Microbiology* **51**, 1309–1313.

Vandamme, P., Bernardet, J.-F., Segers, P., Kersters, K. and Holmes, B. (1994) New perspectives in the classification of the flavobacteria: Description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *International Journal of Systematic Bacteriology* **44**, 827–831.

Vandamme, P., Devriese, L.A., Kersters, B.P.K. and Melin, P. (1997) *Streptococcus difficile* is a nonhemolytic group B, type Ib streptococcus. *International Journal of Systematic Bacteriology* **47**, 81–85.

Vandepitte, J., VanDamme, L., Fofana, Y. and Desmyter, J. (1980) *Edwardsiella tarda* et *Plesiomonas shigelloides*. Leur rôle comme agents de diarrhées et leur épidemiologie. *Bulletin de Societé Pathologie Exotique* **73**, 139–149.

Vandepitte, J., Lemmens, P. and DeSwert, L. (1983) Human edwardsiellosis traced to ornamental fish. *Journal of Clinical Microbiology* **17**, 165–167.

Van Duijn, C. (1981) Tuberculosis in fishes. *Journal of Small Animal Practice* **22**, 391–411.

Vatsos, I.N., Thompson, K.D. and Adams, A. (2001) Adhesion of the fish pathogen *Flavobacterium psychrophilum* to unfertilized eggs of rainbow trout (*Oncorhynchus mykiss*) and n-hexadecane. *Letters in Applied Microbiology* **33**, 178–182.

Vatsos, I.N., Thompson, K.D. and Adams, A. (2003) Starvation of *Flavobacterium psychrophilum* in broth, stream water and distilled water. *Diseases of Aquatic Organisms* **56**, 115–126.

Vatsos, I.N., Thompson, K.D. and Adams, A. (2006) Colonization of rainbow trout, *Oncorhynchus mykiss* (Walbaum), eggs by *Flavobacterium psychrophilum*, the causative agent of rainbow trout fry syndrome. *Journal of Fish Diseases* **29**, 441–444.

Vaughan, L.M., Smith, P.R. and Foster, T.J. (1993) An aromatic-dependent mutant of the fish pathogen *Aeromonas salmonicida* is attenuated in fish and is effective as a live vaccine against the salmonid disease furunculosis. *Infection and Immunity* **61**, 2172–2181.

Vedros, N.A. (1984) Genus I: *Neisseria* Trevisan 1885, 105[AL]. In: Krieg, N.R. and Holt, J.G. (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Baltimore, MD, Williams & Wilkins, pp. 290–296.

Veenstra, J., Rietra, P.J.G.M., Stoutenbeek, C.P., Coster, J.M., de Gier, H.H.W. and Dirks-Go, S. (1992) Infection by an indole-negative variant of *Vibrio vulnificus* transmitted by eels. *Journal of Infectious Diseases* **166**, 209–210.

Velji, M.I., Albright, L.J. and Evelyn, T.P.T. (1990) Protective immunity in juvenile coho salmon *Oncorhynchus kisutch* following immunization with *Vibrio ordalii* lipolysaccharide or from exposure to live *V. ordalii* cells *Diseases of Aquatic Organisms* **9**, 25–29

Velji, M.I., Albright, L.J. and Evelyn, T.P.T. (1991) Nature of the immune response in coho salmon *Oncorhynchus kisutch* following vaccination with *Vibrio ordalii* lipolysaccharides by two different routes. *Diseases of Aquatic Organisms* **11**, 79–84.

Velji, M.I., Albright, L.J. and Evelyn, T.P.T. (1992) Immunogenicity of various *Vibrio ordalii* fractions in coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* **12**, 97–101.

Venegas, C.A., Contreras, J.R., Larenas, J.J. and Smith, P.A. (2004) DNA hybridization assays for the detection of *Piscirickettsia salmonis* in salmonid fish. *Journal of Fish Diseases* **27**, 431–435.

Vervarcke, S., Lescroart, O., Ollevier, F., Kinget, R. and Michoel, A. (2004) Vaccination of African catfish with *Vibrio anguillarum* O2: 1. ELISA development and response to IP and immersion vaccination. *Journal of Applied Ichthyology* **20**, 128–133.

Vervarcke, S., Ollevier, F., Kinget, R. and Michoel, A. (2005) Mucosal response in African catfish after administration of *Vibrio anguillarum* O2 antigens via different routes. *Fish & Shellfish Immunology* **18**, 125–133.

Vethaak, A.D. (1992) Diseases of flounder (*Platichthys flesus* L.) in the Dutch Wadden Sea, and their relation to stress factors. *Netherlands Journal of Sea Research* **29**, 257–272.

Vethaak, A.D. and ap Rheinallt, T. (1992) Fish disease as a monitor for marine pollution: The case of the North Sea. *Reviews in Fish Biology and Fisheries* **2**, 1–32.

Vethaak, A.D. and Jol, J.G. (1996) Diseases of flounder *Platichthys flesus* in Dutch coastal and estuarine waters, with particular reference to environmental stress factors. 1. Epizootiology of gross lesions. *Diseases of Aquatic Organisms* **26**, 81–97.

Vethaak, A.D., Bucke, D., Lang, T., Wester, P., Johl, J. and Carr, M. (1992) Fish disease monitoring along a pollution transect: A case study using dab *Limanda limanda* in the German Bight, North Sea. *Marine Ecology Progress Series* **91**, 173–192.

Vethaak, A.D., Jol, J.G., Meijboom, A., Eggens, M.L., ap Rheinallt, T., Wester, P.W., Van De Zande, T., Bergman, A., Dankers, N., Ariese, F. *et al.* (1996) Skin and liver diseases induced in flounder (*Platichthys flesus*) after long-term exposure to contaminated sediments in large-scale mesocosms. *Environmental Health Perspectives* **104**, 1218–1229.

Vigneulle, M. (1990) Yersinose des salmonides: Etude comparée de différents modes de vaccination. *Ichtyophysiologica Acta* **13**, 43–58.

Vigneulle, M. and Baudin-Laurençin, F. (1995) *Serratia liquefaciens*: A case report in turbot (*Scophthalmus maximus*) cultured in floating cages in France. *Aquaculture* **132**, 121–124.

Vigneulle, M. and Gérard, J.P. (1986) Incidence d'un apport polyvitaminique sur la yersiniose expérimentale de la truite arc-en-ciel (*Salmo gairdneri*). *Bulletin de l'Academie Vétérinaire de France* **59**, 77–86.

Vik-Mo, F.T,, Bergh, Ø. and Samuelsen, O.B. (2005) Efficacy of orally administered flumequine in the treatment of vibriosis caused by *Listonella anguillarum* in Atlantic cod *Gadus morhua*. *Diseases of Aquatic Organisms* **67**, 87–92.

Villamil, L., Figueras, A., Toranzo, A.E., Planas, M. and Novoa, B. (2003) Isolation of a highly pathogenic *Vibrio pelagius* strain associated with mass mortalities of turbot, *Scophthalmus maximus* (L.) larvae. *Journal of Fish Diseases* **26**, 293–303.

Villamil, L., Figueras, A., Aranguren, R. and Novoa, B. (2003a) Non-specific immune response of turbot, *Scophthalmus maximus* (L.), experimentally infected with a pathogenic *Vibrio pelagius*. *Journal of Fish Diseases* **26**, 321–329.

Vinitnantharat, S. and Plumb, J.A. (1993) Protection of channel catfish *Ictalurus punctatus* following natural exposure to *Edwardsiella ictaluri* and effects of feeding antigen on antibody titer. *Diseases of Aquatic Organisms* **15**, 31–34.

Vipond, R., Bricknell, I.R., Durant, E., Bowden, T.J., Ellis, A.E., Smith, M. and MacIntyre, S. (1998) Defined deletion mutants demonstrate that the major secreted toxins are not essential for the virulence of *Aeromonas salmonicida*. *Infection and Immunity* **66**, 1990–1998.

Vivas, J., Carracedo, C., Riaño, J., Razquin, B.E., López-Fierro, P., Acosta, F., Naharro, G. and Villena, A.J. (2004) Behavior of an *Aeromonas hydrophila aroA* live vaccine in water microcosms. *Applied and Environmental Microbiology* **70**, 2702–2708.

Vivas, J., Riaño, J., Carracedo, B., Razquin, B.E., López-Fierro, P., Naharro, G. and Villena, A.J. (2004a) The auxotrophic *aroA* mutant of *Aeromonas hydrophila* as a live attenuated vaccine against *A. salmonicida* infections in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology* **16**, 193–206.

Vivas, J., Razquin, B., López-Fierro, P. and Villena, A.J. (2005) Modulation of the immune response to an *Aeromonas hydrophila aroA* live vaccine in rainbow trout: Effect of culture media on the humoral immune response and complement consumption. *Fish & Shellfish Immunology* **18**, 223–233.

Vladik, P., Vitovac, J. and Carvinka, S. (1974) The taxonomy of gram-positive immobile diplobacilli isolated from necrotizing nephroses in American char and rainbow trout. *Veterinami Medicina (Praha)* **19**, 233–238.

Voigt, H.-R. (1994) Fish surveys in the Vaike Vain Strait between the islands of Saaremaa and Muhu, western Estonia. *Proceedings of the Estonian Academy of Science and Ecology* **4**, 128–135.

Von Betegh, L. (1910) Weitere Beiträge zur experimentellen Tuberkulose der Meeresfischen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung I* **53**, 54.

Von Graevenitz, A. (1990) Revised nomenclature of *Campylobacter laridis*, *Enterobacter intermedium*, and "*Flavobacterium branchiophila*". *International Journal of Systematic Bacteriology* **40**, 211.

Wahli, T., Burr, S.E., Pugovkin, D., Mueller, O. and Frey, J. (2005) *Aeromonas sobria*, a causative agent of disease in farmed perch, *Perca fluviatilis* L. *Journal of Fish Diseases* **28**, 141–150.

Wakabayashi, H. and Egusa, S. (1966) Characteristics of a myxobacterium, *Chondrococcus columnaris*, isolated from diseased loaches. *Bulletin of the Japanese Society of Scientific Fisheries* **32**, 1015–1022.

Wakabayashi, H. and Egusa, S. (1972) Characteristics of a *Pseudomonas* sp. from an epizootic of pond-cultured eels (*Anguilla japonica*). *Bulletin of the Japanese Society of Scientific Fisheries* **38**, 577–587.

Wakabayashi, H. and Egusa, S. (1973) *Edwardsiella tarda* (*Paracolobactrum anguillimortiferum*) associated with pond-cultured eel diseases. *Bulletin of the Japanese Society of Scientific Fisheries* **39**, 931–936.

Wakabayashi, H., Egusa, S. and Fryer, J.L. (1980) Characteristics of filamentous bacteria isolated from the gills of salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 1499–1504.

Wakabayashi, H., Hikida, M. and Masumura, K. (1984) *Flexibacter* infections in cultured marine fish in Japan. *Helgoländer Meeresuntersuchungen* **37**, 587–593.

Wakabayashi, H., Hikida, M. and Masumura, K. (1986) *Flexibacter maritimus* sp. nov., a pathogen of marine fishes. *International Journal of Systematic Bacteriology* **36**, 396–398.

Wakabayashi, H., Huh, G.J. and Kimura, N. (1989) *Flavobacterium branchiophila* sp. nov., a causative agent of bacterial gill disease of freshwater fishes. *International Journal of Systematic Bacteriology* **39**, 213–216.

Wakabayashi, H., Toyama, T. and Iida, T. (1994) A study on serotyping of *Cytophaga psychrophila* isolated from fishes in Japan. *Fish Pathology* **29**, 101–104.

Wakabayashi, H., Sawada, K., Ninomiya, K. and Nishimori, E. (1996) Bacterial hemorrhagic ascites of ayu caused by *Pseudomonas* sp. *Fish Pathology* **31**, 239–240.

Wallbanks, S., Martinez-Murcia, A.J., Fryer, J.L., Phillips, B.A. and Collins, M.D. (1990) 16S rRNA sequence determination for members of the genus *Carnobacterium* and related lactic acid bacteria and description of *Vagococcus salmoninarum* sp. nov. *International Journal of Systematic Bacteriology* **40**, 224–230.

Walters, G.R. and Plumb, J.A. (1978) Modified oxidation/fermentation medium for use in the identification of bacterial fish pathogens. *Journal of the Fisheries Research Board of Canada* **35**, 1629–1630.

Waltman, W.D. and Shotts, E.B. (1984) A medium for the isolation and differentiation of *Yersinia ruckeri*. *Canadian Journal of Fisheries and Aquatic Sciences* **41**, 804–806.

Waltman, W.D. and Shotts, E.B. (1986a) Antimicrobial susceptibility of *Edwardsiella ictaluri*. *Journal of Wildlife Diseases* **22**, 173–177.

Waltman, W.D. and Shotts, E.B. (1986b) Antimicrobial susceptibility of *Edwardsiella tarda* from the United States and Taiwan. *Veterinary Microbiology* **12**, 277–282.

Waltman, W.D., Shotts, E.B. and Blazer, V.S. (1985) Recovery of *Edwardsiella ictaluri* from danio (*Danio devario*). *Aquaculture* **46**, 63–66.

Waltman, W.D., Shotts, E.B. and Hsu, T.C. (1986) Biochemical characteristics of *Edwardsiella ictaluri*. *Applied and Environmental Microbiology* **51**, 101–104.

Waltman, W.D., Shotts, E.B. and Wooley, R.E. (1989) Development and transfer of plasmid-mediated antimicrobial resistance in *Edwardsiella ictaluri*. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1114–1117.

Wang, G.-L., Yuan, S.-P. and Jin, S. (2005) Nocardiosis in large yellow croaker, *Larimichthys crocea* (Richardson). *Journal of Fish Diseases* **28**, 339–345.

Wang, S.-Y., Lauritz, J., Jass, J. and Milton, D.L. (2003) Role of the major outer-membrane protein from *Vibrio anguillarum* in bile resistance and biofilm formation. *Microbiology* **149**, 1061–1071.

Wang, W.-S., Chang, Y.-C., Shieh, M.-T. and Lin, C.-C. (1996) *Staphylococcus epidermidis* and cestode infection of cultured grass carp (*Ctenopharyngodon idella*) in Taiwan. *Reports on Fish Disease Research* **17**, 57–63.

Ward, P.D., Waters, C.A. and Sweeney, K.J. (1985) Autoagglutination of virulent *Aeromonas salmonicida* strains lacking additional surface layer. In: Ellis, A.E. (ed.), *Fish and Shellfish Pathology*. London, Academic Press, pp. 107–117.

Warren, J.W. (1963) Kidney disease of salmonid fishes and the analysis of hatchery waters. *Progressive Fish Culturist* **25**, 121–131.

Waterstrat, P., Ainsworth, J. and Capley, G. (1989) Use of an indirect enzyme-linked immunosorbent assay (ELISA) in the detection of channel catfish, *Ictalurus punctatus* (Rafinesque), antibodies to *Edwardsiella ictaluri*. *Journal of Fish Diseases* **12**, 87–94.

Weber, J.M. and Zwicker, B.M. (1979) *Aeromonas salmonicida* in Atlantic salmon (*Salmo salar*): Occurrence of specific agglutinins to three bacterial pathogens. *Journal of the Fisheries Research Board of Canada* **36**, 1102–1107.

Wedemeyer, G.A. and Ross, A.J. (1973) Nutritional factors in the biochemical pathology of corynebacterial kidney disease in the coho salmon (*Oncorhynchus kisutch*). *Journal of the Fisheries Research Board of Canada* **30**, 296–298.

Weeks-Perkins, B.A. and Ellis, A.E. (1995) Chemotactic responses of Atlantic salmon (*Salmo salar*) macrophages to virulent and attenuated strains of *Aeromonas salmonicida*. *Fish & Shellfish Immunology* **5**, 313–323.

Weiland, L.K., Mesa, M.G. and Maule, A.G. (1999) Influence of infection with *Renibacterium salmoninarum* on susceptibility of juvenile spring chinook salmon to gas bubble trauma. *Journal of Aquatic Animal Health* **11**, 123–129.

Weinstein, M.R., Litt, M., Kertesz, D.A., Wyper, P., Rose, D., Coulter, M., McGeer, A., Facklam, R., Ostach, C., Willey, B.M. *et al.* (1997) Invasive infections due to a fish pathogen, *Streptococcus iniae*. *New England Journal of Medicine* **337**, 589–594.

Welch, T.J. and Wiens, G.D. (2005) Construction of a virulent, green fluorescent protein-tagged *Yersinia ruckeri* and detection in trout tissues after intraperitoneal and immersion challenge. *Diseases of Aquatic Organisms* **67**, 267–272.

Welker, T.L., Shoemaker, C.A., Arias, C.R. and Klesius, P.H. (2005) Transmission and detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. *Diseases of Aquatic Organisms* **63**, 129–138.

Wells, N.A. and ZoBell, C.E. (1934) *Achromobacter ichthyodermis*, n. sp., the etiological agent of an infectious dermatitis of certain marine fishes. *Proceedings of the National Academy of Science, U.S.A.* **20**, 123–126.

Wenzel, S., Bach, R. and Müller-Prasuhn, G. (1971) Farmed trout as carriers of *Clostridium botulinum* and the cause of botulism—IV. Sources of contamination and contamination paths in fish farming and processing stations; ways to improve hygiene. *Archives für Lebensmittelhygiene* **22**, 131.

West, P.A. and Lee, J.V. (1982) Ecology of *Vibrio* species, including *Vibrio cholerae*, in natural waters of Kent, England. *Journal of Applied Bacteriology* **52**, 435–448.

West, P.A., Lee, J.V. and Bryant, T.N. (1983) A numerical taxonomic study of species of *Vibrio* isolated from the aquatic environment and birds in Kent, England. *Journal of Applied Bacteriology* **55**, 263–282.

Westerdahl, A., Olsson, J.C., Kjelleberg, S. (1991) Isolation and characterization of turbot (*Scophthalmus maximus*) associated bacteria with inhibitory effects against *Vibrio anguillarum*. *Applied and Environmental Microbiology* **57**, 2232–2228.

Westfall, B.A. (1945) Coagulation film anoxia in fishes. *Ecology* **26**, 283–287.

Whipps, C.M., Watral, V.G. and Kent, M.L. (2003) Characterization of a *Mycobacterium* sp. in rockfish, *Sebastes alutus* (Gilbert) and *Sebastes reedi* (Westrheim & Tsuyuki), using rDNA sequences. *Journal of Fish Diseases* **26**, 241–245.

Wiens, G.D. and Kaattari, S.L. (1991) Monoclonal antibody characterization of a leukoagglutinin produced by *Renibacterium salmoninarum*. *Infection and Immunity* **59**, 631–637.

Wiens, W.D., Chien, M.-S., Winton, J.R. and Kaattari, S.L. (1999) Antigenic and functional characterization of p57 produced by *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* **37**, 43–52.

Wiens, G.D., Pascho, R. and Winton, J.R. (2002). A single ala^{139}-to-glu substitution in the *Renibacterium salmoninarum* virulence-associated protein p57 results in antigenic variation and is associated with enhanced p57 binding to Chinook salmon leukocytes. *Applied and Environmental Microbiology* **68**, 3969–3977.

Wiik, R. and Egidius, E. (1986) Genetic relationships of *Vibrio salmonicida* sp. nov. to other fish-pathogenic vibrios. *International Journal of Systematic Bacteriology* **36**, 521–523.

Wiik, R., Andersen, K., Daae, F.L. and Hoff, K.A. (1989) Virulence studies based on plasmid profiles of the fish pathogen *Vibrio salmonicida*. *Applied and Environmental Microbiology* **55**, 819–825.

Wiklund, T. (1995a) Survival of "atypical" *Aeromonas salmonicida* in water and sediment microcosms of different salinities and temperatures. *Diseases of Aquatic Organisms* **21**, 137–143.

Wiklund, T. (1995b) Virulence of "atypical" *Aeromonas salmonicida* isolated from ulcerated flounder *Platichthys flesus*. *Diseases of Aquatic Organisms* **21**, 145–150.

Wiklund, T. and Bylund, G. (1993) Skin ulcer disease of flounder *Platichthys flesus* in the northern Baltic Sea. *Diseases of Aquatic Organisms* **17**, 165–174.

Wiklund, T. and Dalsgaard, I. (1995) Atypical *Aeromonas salmonicida* associated with ulcerated flatfish species in the Baltic sea and the North Sea. *Journal of Aquatic Animal Health* **7**, 218–224.

Wiklund, T. and Dalsgaard, I. (1998) Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish hosts. *Diseases of Aquatic Organisms* **32**, 49–69.

Wiklund, T. and Dalsgaard, I. (2003) Association of *Flavobacterium psychrophilum* with rainbow trout (*Oncorhynchus mykiss*) kidney phagocytes *in vitro*. *Fish & Shellfish Immunology* **15**, 387–395.

Wiklund, T. and Lönnström, L. (1994) Occurrence of *Pseudomonas anguilliseptica* in Finnish fish farms during 1986–1991. *Aquaculture* **126**, 211–217.

Wiklund, T., Sazonov, A., L'Iniova, G.P., Pugaewwa, V.P., Zoobaha, S.V. and Bylund, G. (1992) Characteristics of *Aeromonas salmonicida* subsp. *salmonicida* isolated from wild Pacific salmonids in Kamchatka, Russia. *Bulletin of the European Association of Fish Pathologists* **12**, 76–79.

Wiklund, T., Lönnström, L. and Niiranen, H. (1993) *Aeromonas salmonicida* subsp. *salmonicida* lacking pigment production, isolated from farmed salmonids in Finland. *Diseases of Aquatic Organisms* **15**, 219–223.

Wiklund, T., Dalsgaard, I., Eerola, E. and Olivier, G. (1994) Characteristics of "atypical" cytochrome–oxidase negative *Aeromonas salmonicida* isolated from ulcerated flounder (*Platichthys flesus* L.). *Journal of Applied Bacteriology* **76**, 511–520.

Wiklund, T., Madsen, L., Bruun, M.S. and Dalsgaard, I. (2000) Detection of *Flavobacterium psychrophilum* from fish tissue and water samples by PCR amplification. *Journal of Applied Microbiology* **88**, 299–307.

Williams, A.M., Fryer, J.L. and Collins, M.D. (1990) *Lactococcus piscium* sp. nov., a new *Lactococcus* species from salmonid fish. *FEMS Microbiology Letters* **68**, 109–114.

Williams, M.A. and Briggs, G.M. (1963) A new mineral mixture for experimental rat diets and evaluation of other mineral mixtures. *Federal Proceedings* **22**, 601–608.

Williams, M.J., Azadi, P. and Lawrence, M.L. (2003) Comparison of cellular and extracellular products expressed by virulent and attenuated strains of *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* **15**, 264–273.

Williams, P.J., Courtenay, S.C. and Vardy, C. (1997) Use of enrofloxacin to control atypical *Aeromonas salmonicida* in Atlantic tom cod. *Journal of Aquatic Animal Health* **9**, 216–222.

Williams, S.T., Locci, R., Vickers, J., Schofield, G.M., Sneath, P.H.A. and Mortimer, A.M. (1985) Probabilistic identification of *Streptoverticillium* species. *Journal of General Microbiology* **131**, 1681–1689.

Williamson, I.J.F. (1928) Furunculosis of the salmonidae. *Fisheries Board of Scotland, Salmon Fisheries* **5**, 1–17.

Williamson, I.J.F. (1929) A study of bacterial infection in fish and certain lower invertebrates. *Fisheries Board of Scotland, Salmon Fisheries* **11**, 3.

Wilson, A. and Horne, M.T. (1986) Detection of A-protein in *Aeromonas salmonicida* and some effects of temperature on A-layer assembly. *Aquaculture* **56**, 23–27.

Winter, G.W., Schreck, C.B. and McIntyre, J.D. (1979) Resistance of different stocks and transferrin genotypes of coho salmon, *Oncorhynchus kisutch*, and steelhead trout, *Salmo gairdneri*, to bacterial kidney disease and vibriosis. *Fishery Bulletin* **77**, 795–802.

Winton, J.R., Rohovec, J.S. and Fryer, J.L. (1983) Bacterial and viral diseases of cultured salmonids in the Pacific Northwest. In: Crosa, J.H. (ed.), *Bacterial and Viral Diseases of Fish: Molecular Studies*. Seattle, Washington Sea Grant Communications, pp. 1–20.

Wise, D.J., Tomasso, J.R., Schwedler, T.E., Blazer, V.S. and Gatlin III, D.M. (1993) Effect of vitamin E on the immune response of channel catfish to *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* **5**, 183–188.

Wise, D.J., Schwedler, T.E. and Terhune, J.S. (1997) Uptake and clearance of *Edwardsiella ictaluri* in the peripheral blood of channel catfish *Ictalurus punctatus* fingerlings during immersion challenge. *Journal of the World Aquaculture Society* **28**, 45–51.

Withler, R.E. and Evelyn, T.P.T. (1990) Genetic variation in resistance to bacterial kidney disease within and between two strains of coho salmon from British Columbia. *Transactions of the American Fisheries Society* **119**, 1003–1009.

Witt, D. and Stackebrandt, E. (1990) Unification of the genera *Streptoverticillium* and *Streptomyces*, and amendation of *Streptomyces* Waksman & Henrici 1943, 339^{AL}. *Systematic and Applied Microbiology* **13**, 361–371.

Wobeser, G. and Atton, F.M. (1973) An outbreak of columnaris disease in white suckers (*Catostomus commersoni*) in Saskatchewan. *Journal of the Fisheries Research Board of Canada* **30**, 681–683.

Wold, A. (1950) A promising drug: Chloromycetin. *Aquarium*, September 1950.

Wolf, K. (1966) Bacterial kidney disease of salmonid fishes. *United States Department of the Interior, Fish and Wildlife Service, Fisheries Leaflet* **8.**

Wolf, K. (1981) Chlamydia and rickettsia of fish. *Fish Health News* **10**, 1–5.

Wolf, K. and Dunbar, C.E. (1959) Test of 34 therapeutic agents for control of kidney disease in trout. *Transactions of the American Fisheries Society* **88**, 117–124.

Wolf, K. and Snieszko, S.F. (1963) Uses of antibiotics and other antimicrobials in therapy of diseases of fishes. *Antimicrobial Agents and Chemotherapy*, **161**, 597–603.

Wolf, L.E. (1954) Development of disease resistant strains of fish. *Transactions of the American Fisheries Society* **83**, 342–349.

Wolf, M.K. and Crosa, J.H. (1986) Evidence for the role of a siderophore in promoting *Vibrio anguillarum* infections. *Journal of General Microbiology* **132**, 2949–2952.

Wolke, R.E. (1975) Pathology of bacterial and fungal diseases affecting fish. In: Ribelin, W.E. and Migaki, G. (eds), *The Pathology of Fishes*. Wisconsin, University of Wisconsin Press, pp. 76–78.

Wong, K.R., Green, M.J. and Buckley, J.T. (1989) Extracellular secretion of cloned aerolysin and phospholipase by *Aeromonas salmonicida*. *Journal of Bacteriology* **171**, 2523–2527.

Woo, N.Y.S., Ling, J.L.M. and Lo, K.M. (1995) Pathogenic *Vibrio* spp. in the sea bream, *Sparus sarba*. *Journal of Sun Yetsen University Supplement* **3**, 192–193.

Wood, E.M. and Yasutake, W.T. (1956) Histopathology of kidney disease in fish. *American Journal of Pathology* **32**, 845–857.

Wood, J.W. (1968) *Diseases of Pacific Salmon: Their Prevention and Treatment*. Washington, D.C., State of Washington Department of Fisheries, Hatchery Division.

Wood, J.W. (1974) *Diseases of Pacific Salmon, Their Prevention and Treatment*. Washington, D.C., Department of Fisheries.

Wood, J.W. and Ordal, E.J. (1958) Tuberculosis in Pacific salmon and steelhead trout. Fish Commission of Oregon Control 25, 1–38.

Wood, J.W. and Wallis, J. (1955) Kidney disease in adult chinook salmon and its transmission by feeding to young chinook salmon. *Fisheries Commission of Oregon, Research Briefs* **6**, 32–40.

Wood, P.A. and Kaattari, S.L. (1996) Enhanced immunogenicity of *Renibacterium salmoninarum* in chinook salmon after removal of the bacterial cell surface associated 57 kDa protein. *Diseases of Aquatic Organisms* **25**, 71–97.

Wood, P.A., Wiens, G.D., Rohovec, J.S. and Rockey, D.D. (1995) Identification of an immunologically cross reactive 60-kilodalton *Renibacterium salmoninarum* protein distinct from p57: Implications for immunodiagnostics. *Journal of Aquatic Animal Health* **7**, 95–103.

Wood, S.C., McCashion, R.N. and Lynch, W.H. (1986) Multiple low-level antibiotic resistance in *Aeromonas salmonicida*. *Antimicrobial Agents and Chemotherapy* **29**, 992–996.

Woodall, A.N. and Laroche, G. (1964) Nutrition of salmonid fishes. XI. Iodide requirements of chinook salmon. *Journal of Nutrition* **824**, 475–482.

Wooster, G.A and Bowser, P.R. (1996) The aerobiological pathway of a fish pathogen: Survival and dissemination of *Aeromonas salmonicida* in aerosols and its implications in fish health management. *Journal of the World Aquaculture Society* **27**, 7–14.

Wu, H., Ma, Y., Zhang, Y. and Zhang, H. (2004) Complete sequence of virulence plasmid pEIB1 from the marine fish pathogen *Vibrio anguillarum* strain MVM425 and location of its replication region. *Microbiology* **97**, 1021–1028.

Wyatt, L.E., Nickelson, R. and Van Derzant, C. (1979) *Edwardsiella tarda* in freshwater catfish and their environment. *Applied and Environmental Microbiology* **38**, 710–714.

Xu (1975) Studies on the gill diseases of the grass carp (*Ctenopharynogodon idellus*). 1. Isolation of a myxobacterial pathogen. *Acta Hydrobiologica Sinica* **5**, 315–329.

Xu, H.-S., Roberts, N., Singleton, F.L., Attwell, R.W., Grimes, D.J. and Colwell, R.R. (1982) Survival and viability of non-culturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microbial Ecology* **8**, 313–323.

Yamada, Y., Kaku, Y. and Wakabayashi, H. (2000 Phylogenetic intrarelationships of atypical *Aeromonas salmonicida* isolated in Japan as determined by 16S rDNA sequencing. *Fish Pathology* 35, 35–40.

Yamanoi, H., Muroga, K. and Takahashi, S. (1980) Physiological characteristics and pathogenicity of NAG vibrio isolated from diseased ayu. *Fish Pathology* **15**, 69–73.

Yano, T., Matsuyama, H. and Mangindaan, R. (1991) Polysaccharide induced protection of carp, *Cyprinus carpio* L, against bacterial infection. *Journal of Fish Diseases* **14**, 577–582.

Yasumoto, S., Yoshimura, T. and Miyazaki, T. (2006) Oral immunization of common carp with a liposome vaccine containing *Aeromonas hydrophila* antigens. *Fish Pathology* **41**, 45–49.

Yasunaga, N., Hatai, K. and Tsukahara, J. (1983) *Pasteurella piscicida* from an epizootic of cultured red seabream. *Fish Pathology* **18**, 107–110.

Yasunobu, H., Muroga, K. and Maruyama, K. (1988) A bacteriological investigation on the mass mortalities of red seabream *Pagrus major* larvae with intestinal swelling. *Suisanzoshoku* **1**, 11–20.

Yasunobu, H., Arikawa, Y., Furutsuka-Uozumil, K., Dombo, M., Iida, T., Mahmoud, M.M., Okuda, J. and Nakai, T. (2006) Induction of hemagglutinating activity of *Edwardsiella tarda* by sodium chloride. *Fish Pathology* **41**, 29–34.

Ye, J., Foo, R.W.T., Lo, K.M., Zeng, J.S., Ling, J.M.L., Woo, N.Y.S. and Xu, H.S. (1997) Studies on the pathogens of vibriosis in cultured sea bream (*Sparus sarba*) in Hong Kong. *Journal of Marine Science*.

Yeh, H.-Y., Shoemaker, C.A. and Klesius, P.H. (2006) Sensitive and rapid detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus* by a loop-mediated isothermal amplification method. *Journal of Applied Microbiology* **100**, 919–925.

Yii, K.-C., Yang, T.I. and Lee, K.-K. (1997) Isolation and characterization of *Vibrio carchariae*, a causative agent of gastroenteritis in the groupers, *Epinephelus coioides*. *Current Microbiology* **35**, 109–115.

Yoshida, T., Kruger, R. and Inglis, V. (1995) Augmentation of non-specific protection in African catfish, *Clarias gariepinus* (Burchell), by the long-term oral administration of immunostimulants. *Journal of Fish Diseases* **18**, 195–198.

Yoshida, T., Endo, M., Sakai, M. and Inglis, V. (1997) A cell capsule with possible involvement in resistance to opsonophagocytosis in *Enterococcus seriolicida* isolated from yellowtail *Seriola quinqueradiata*. *Diseases of Aquatic Organisms* **29**, 233–235.

Yoshimizu, M., Ji, R., Nomura, T. and Kimura, T. (1987) A false positive reaction in the indirect fluorescent antibody test for *Renibacterium salmoninarum* ATCC 33209 caused by a *Pseudomonas* sp. *Scientific Report of the Hokkaido Salmon Hatchery* **41**, 121–127.

Young, C.L. and Chapman, G.B. (1978) Ultrastructural aspects of the causative agent and of bacterial kidney disease in brook trout (*Salvelinus fontinalis*). *Journal of the Fisheries Research Board of Canada* **35**, 1234–1248.

Yuasa, K., Kitancharoen, N., Kataoka, Y. and Al-Murbaty, F.A. (1999) *Streptococcus iniae*, the causative agent of mass mortality in rabbotfish *Siganus canaliculatus* in Bahrain. *Journal of Aquatic Animal Health* **11**, 87–93.

Yuasa, K., Kholidin, E.B., Panigoro, N. and Hatai, K. (2003) First isolation of *Edwardsiella ictaluri* from cultured striped catfish *Pangasius hypophthalmus* in Indonesia. *Fish Pathology* **38**, 181–183.

Yugueros, J., Temprano, A., Luengo, J.M. and Naharro, G. (2001) Molecular cloning of *Yersinia ruckeri aroA* gene: A useful taxonomic tool. *Journal of Fish Diseases* **24**, 383–390.

Yuksel, S.A., Thompson, K.D., Ellis, A.E. and Adams, A. (2001) Purification of *Piscirickettsia salmonis* and associated phage particles. *Diseases of Aquatic Organisms* **44**, 231–235.

Zamora, J. and Enriquez, R. (1987) *Yersinia enterocolitica*, *Yersinia fredericksenii* and *Yersinia intermedia* in *Cyprinus carpio* (L.). *Journal of Veterinary Medicine B* **34**, 155–157.

Zappulli, V., Patarnello, T., Patarnello, P., Frassineti, F., Franch, R., Manírin, A., Castagnaro, M. and Bargelloni, L. (2005) Direct identification of *Photobacterium damselae* subsp. *piscicida* by PCR-RFLP analysis. *Diseases of Aquatic Organisms* **65**, 53–61.

Zhao, J. and Aoki, T. (1989) A specific DNA hybridization probe for detection of *Pasteurella piscicida*. *Diseases of Aquatic Organisms* **7**, 203–210.

Zhang, X.-H. and Austin, B. (2000) Pathogenicity of *Vibrio harveyi* to salmonids. *Journal of Fish Diseases* **23**, 93–102.

Zhang, X.-H., Meaden, P.G. and Austin, B. (2001) Duplication of hemolysin genes in a virulent isolate of *Vibrio harveyi*. *Applied and Environmental Microbiology* **67**, 3161–3167.

Zhang, Y.L., Ong, C.T. and Leung, K.Y. (2000) Molecular analysis of genetic differences between virulent and avirulent strains of *Aeromonas hydrophila* isolated from diseased fish. *Microbiology* **46**, 999–1009.

Zhang, Y.L., Lau, Y.L., Arakawa, E. and Leung, K.Y. (2003) Detection and genetic analysis of group II capsules in *Aeromonas hydrophila*. *Microbiology* **149**, 1051–1060.

Zhu, K., Chi, Z., Li, J., Zhang, F., Li, M., Yasoda, H.N. and Wu, L. (2006) The surface display of haemolysin from *Vibrio harveyi* on yeast cells and their potential applications as live vaccines in marine fish. *Vaccine* **24**, 6046–6052.

Zimmermann, O.E.R. (1890) Die Bakterien unserer Trink- und Nutzwässer insbesondere der Wassers der Chemnitzer Wasseleitung. 1. Reihe. *Elfter Bericht der Naturwissenschaftlichen Gesellschaft zu Chemnitz* **1**, 38–39.

Zlotkin, A., Eldar, A., Ghittino, C. and Bercovier, H. (1998) Identification of *Lactococcus garvieae* by PCR. *Journal of Clinical Microbiology* **36**, 983–985.

Zlotkin, A., Chilmonczyk, S., Eyngor, M., Hurvitz, A., Ghittino, C. and Eldar, A. (2003) Trojan horse effect: Phagocyte-mediated *Streptococcus iniae* infection of fish. *Infection and Immunity* **71**, 2318–2325.

Zhong, Y., Zhang, X.-H., Chen, J., Chi, Z., Sun, B., Li, Y. and Austin, B. (2006) Over-expression, characterization, and pathogenicity of *Vibrio harveyi* hemolysin VHH. *Infection and Immunity* **74**, 6001–6005.

Zhou, Y.-C., Wang, J., Zhang, B. and Su, Y.-Q. (2002) Ultrasonic immunization of sea bream, *Pagrus major* (Temminck & Schlegel), with a mixed vaccine against *Vibrio alginolyticus* and *V. anguillarum*. *Journal of Fish Diseases* **25**, 325–331.

Zorrilla, A., Balebona, M.C., Moriñigo, M.A., Sarasquette, C. and Borrego, J.J. (1999) Isolation and characterization of the causative agent of pasteurellosis, *Photobacterium damsela* subsp. *piscicida*, from sole, *Solea senegalensis* (Kaup). *Journal of Fish Diseases* **22**, 167–172.

Zorrilla, A., Arijo, S., Chabrillon, M., Diaz, P., Martinez-Manzanares, E., Balebona, M.C. and Moriñigo, M.A. (2003) *Vibrio* species isolated from diseased farmed sole, *Solea senegalensis* (Kaup), and evaluation of the potential virulence role of their extracellular products. *Journal of Fish Diseases* **26**, 103–108.

Index