Biological Oceanography

Research Trends

Contributors

L. Anderson
Feng Chen
F. Chever
D. Wayne Coats
F. Criado-Aldeanueva
J. Del Río Vera
John R. Dolan
Jessica Frost
F. Gaill
J. García-Lafuente
Beatriz Guijarro
Nianzhi Jiao
Jinjun Kan
G. S. Karabashev
Vassilis Kitidis
Rolf Koppelmann
N. Le Bris
Enric Massutí
Joan Moranta
Francesc Ordines
Antoni Quetglas
Rebecca F. Shipe
Günther Uher
María Valls
Chuanlun Zhang
Fan Zhang

LÉA P. MERTENS
Editor

NOVA

BIOLOGICAL OCEANOGRAPHY RESEARCH TRENDS

BIOLOGICAL OCEANOGRAPHY RESEARCH TRENDS

LÉA P. MERTENS
EDITOR

Nova Science Publishers, Inc.
New York

LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA

Biological oceanography research trends / Léa P. Mertens (editor).
p. cm.
Includes bibliographical references and index.
ISBN-13: 978-1-60692-698-7

1. Marine biology--Research. 2. Oceanography--Research. I. Mertens, Léa P.
QH91.5.B46 2006
578.77--dc22
2007034053

Published by Nova Science Publishers, Inc. ✣ New York

CONTENTS

PREFACE

Biological oceanography concerns the biology and ecology of oceanic, marine, coastal and estuarine organisms. These range from viruses and bacteria to microbes and phytoplankton, from zooplankton and benthic invertebrates to shellfish, fish and marine mammals. The organisms live in a dynamic fluid easily described as a chemical soup that covers ~71% of the earth's surface and is intimately coupled to the atmosphere, the seafloor and the land. Thus, to determine how organisms are influenced by their environment, biological oceanographers must function across many sub-disciplines such as biochemistry, genetics, physiology, behaviour, population dynamics and community ecology. They must be knowledgeable of ocean physics, chemistry, geology, and atmospheric and radiative processes.This new book presents the latest research in this field from around the world.

Expert Commentary - Long-term series of almost 15 years of altimetry data (1992-2005) have been used to study sea level trends over the Mediterranean Sea. Although sea level variations are mainly driven by the steric contribution, the mass-induced component plays some role in modulating its oscillation. A spatially averaged positive trend of 2 mm/year has been observed, 65% corresponding to the steric (mainly on thermal origin) contribution. A negative sea level trend from 2001 onwards is worth mentioning. Sea level rise is particularly important in the Levantine basin south of Crete with values up to 10 mm/year. Some other rising spots are localised throughout the Levantine basin and in the Adriatic and Alboran Seas, with more moderate values. Sea level drop areas are localised in the Balearic basin, between the Balearic Islands and the African coasts, and in the Ionian basin southeast of Italy and Sicily, where the highest negative trends (up to 10 mm/year) are reached mainly due to the mass-induced contribution. NAO might be ultimate responsible for some recent changes in sea level variations, being the easternmost and westernmost areas (Levantine and Balearic basins), the most sensitive to NAO control.

Chapter 1 - The Mediterranean (MED) is the largest of the peripheral seas to the main oceans, connecting with the Atlantic through the Strait of Gibraltar in its western basin. The functioning of this sea has been compared to an inverse estuarine because the outflow (evaporation) is higher than the inflows (raining and river discharges), giving rise to a high salinity (~38) and a continuous entrance of superficial Atlantic waters to compensate the losses from evaporation. The MED is also characterized by its poverty, which is not reflected in the diversity of species as it is in overall productivity. During its long history, the harvesting of living organisms was restricted to coastal areas until the first few decades of the 20^{th} century, when technology development allowed the exploitation of deep-sea resources.

Although demersal fisheries takes a great number of target species (more than 100 being of commercial value and abundant in the catches), the main landings in biomass correspond to pelagic fisheries, which take almost exclusively sardines and anchovies. Therefore, MED demersal fisheries can be classified as multi-specific, with many fleets based all along the 40000 km of coast. The diversity and economic importance of small-scale fisheries are essential features of the MED. In the western MED, this fishery is important in the number of boats and fishers, but the major catches and earnings comes from bottom trawl. By contrast, in the eastern MED the small-scale fishery is socio-economically more important than trawling and purse seining. Bottom trawlers work on soft bottoms of the continental shelf, targeting mainly fish and cephalopods, and the upper slope, where they take basically decapod crustaceans along a broad depth range down to almost 1000 m depth. Throughout this wide bathymetric range, different biological communities with their corresponding species are exploited. Although studies of the MED go far to ancient times, the systematic study of different aspects of this sea began at the end of the 19th century. The first reports of MED megafauna were basically qualitative studies, focussed on the compilation, catalogue and description of the different taxonomic groups. From the second half of the 20th century, studies on quantitative analysis of megafaunal communities, bathymetric distribution of their components and population dynamics of target species was largely developed. Nowadays, the research focus on the development of the ecosystem approach, the analysis of oceanographic and fishery time-series, the identification and characterisation of habitats of interest and the search for relationships among environmental factors and marine resource dynamics. In this respect, a multidisciplinary approximation is absolutely necessary in the new ecosystem view of marine research and management.

Chapter 2 - Cold temperatures, increased pressure, dim light and the absence of net primary production characterize the twilight zone between 200 and 1000 m of the open ocean. Below this depth is the dark zone consisting of stable abiotic conditions and perpetual darkness, with bioluminescence, the ability of organisms to produce their own light, being the only light source. These zones, also called the meso- and bathypelagic zones, are herein referred to as the deep-sea. The main food sources for organisms inhabiting these zones are vertically migrating animals, sinking carcasses and sinking particulate organic matter (POM) produced by autotrophic and heterotrophic organisms in the euphotic zone.

Approximately 90% of POM is remineralised as it sinks through the water column. Remineralization processes remain poorly characterized throughout the water column because they are difficult to observe and quantify. The character of the material transported to the deep-sea reflects the ecological structure of the upper ocean and influences the remineralization rate. Portions of sinking POM are transported from lower to higher trophic levels via the food chain. Longevity at these higher trophic levels probably exists in the deep-sea due to slow growth and maturity caused by low quantities of food reaching the deep.

A notable gap in understanding the linkage between trophic levels and remineralisation rates is the lack of information about gelatinous organisms. These organisms are widely distributed and occasionally occur in large numbers with biomass exceeding that of fish. This can greatly impact food web dynamics. A likely explanation for the paucity of knowledge of gelatinous organisms is the inherent difficulty of sampling them. Most gelatinous organisms are fragile and break into pieces when sampled with commonly used nets. Thus, information on their ecological role and physiological rates is very limited.

This paper will give an overview on deep-sea zooplankton ecology, its temporal distribution, and its role in organic matter cycling, while addressing gaps in knowledge. The following topics should be investigated in future research dealing with deep-sea zooplankton ecology: physiological rates and potentials of functional groups and key species, responses to different quantities and qualities of food (flux), responses to disturbances or changes in temperature, pH, O_2, pollutants etc. and, last but not least, effects of climate change on carbon transformation and storage in the twilight and dark zones.

Chapter 3 - Over the last three decades, aquatic photochemical reactions have been shown to be involved in the cycling of climatically active trace gases, metals and nutrients. More recently, these reactions have been shown to release a number of bioavailable nitrogen (N) species, including ammonium, nitrite and low molecular weight dissolved organic substances such as urea, amines and amino acids. In most of the surface ocean, the photic zone is either permanently or seasonally depleted of nutrients, and in particular N, which are limiting primary production. Under such conditions, the photochemical mineralisation of dissolved organic nitrogen (DON) may be a significant source of limiting N to primary producers. Extrapolations of local rates of photochemical N release in a diverse range of marine settings suggest that this process may account for up to 50 % of phytoplankton N requirements. However, assessments of the importance of photochemical N release on regional to global scales are hindered by insufficient information on its wavelength dependence and by the lack of robust concepts for the extrapolation of photochemical rates. In this chapter, the authors synthesise the presently available information on photochemical N release, its environmental controls and wavelength dependence, identify gaps in our understanding of the processes involved and discuss the ecological significance of photochemical DON mineralisation.

Chapter 4 - Highly productive colonies of *Alvinella pompejana* are found in one of the most extreme environments of the deep-sea. This annelid secretes a tube on the wall of hydrothermal smokers of the East Pacific Rise. Both the inner face of the tube and the dorsal part of the animal are colonized by an abundant microflora. The capacity of this symbiotic association to deal with strong thermal and chemical gradients, at the interface between the smoker wall and the surrounding seawater, remains poorly understood. Particularly, the mechanism by which chemoautotrophic microbes take advantage of this microenvironment have not yet been described. The tube is thought to be ventilated from a seawater-dominated medium overlying the colony. This provides protection against hot, acidic and strongly sulfidic fluids escaping from the mineral substrate. Here the authors have shown that the chemical properties of the tube wall are also likely to play a role in the supply of sulfide to sulfide-oxidizing primary producers in the tube. The permeability of the newly secreted tube wall to H_2S is remarkably high. This should favor sulfide diffusion through the tube wall from the highly sulfidic fluids venting from the smoker wall to the internal medium primarily composed of seawater. This process should have a determining impact on sulfide and electron acceptors availability in the tube, potentially maximizing the chemosynthetic energy budget for sulfide oxidizers. As the tube wall becomes progressively coated with minerals, its permeability to sulfide strongly decreases. This restricts H_2S diffusion to the anterior part of the tube and limits the diffusive flux to the internal microenvironment.

Chapter 5 - One important application of satellite ocean color observations is to advance the studies in biological-physical interaction (BPI) in the ocean. The satellite methods have to offer synopticity and space-time resolution of observations that are far beyond those of

conventional techniques thus affording the capability to cover the BPI processes at local, regional, and global scales. As yet, this offer relies on comparative study of remotely sensed distributions of sea surface temperature (SST) as a physical feature of the marine environmentandchlorophyll concentration C from ocean color as a measure of phytoplankton abundance. As a rule, the ocean color bears no direct indication of chlorophyll but strongly depends on products of phytoplankton degradation that, supposedly, closely co-vary with chlorophyll at least in open ocean waters. This concept underlies present-day algorithms of retrieval of C from ocean color. Available literature on properties of optically significant admixtures of sea water gives grounds to expect that determinations of chlorophyll, based on this concept, tend to diminish actual differences in variability of living and "dead" matter in the marine environment which contradicts the goals of BPI research. Another drawback of "BPI-from-space" approach is in the fact that ocean color originates from the upper part of the euphotic layer of the ocean. Therefore, such an approach yields incomplete information at sites where phytoplankton collects in the seasonal pycnocline which usually belongs to the lower part of the euphotic layer. Observations of variability in chlorophyll concentration, CDOM fluorescence, and water temperature, induced by a subsurface intrusion, illustrate these circumstances. To summarize briefly, the ocean color methods can be regarded as a valuable support and reconnaissance tool of BPI research but in situ instrumentation and techniques are the only means that provide data uniquely determined in physical nature which is of primary importance in the context of BPI.

Chapter 6 - Many planktonic microbes exhibit nearly global distributions. Apparent adaptability to wide ranges of environmental conditions in cosmopolitan species could involve physiological diversity among individuals. However, variability between individuals among microorganisms is rarely considered. We examined individual variability in the common marine ciliate *Euplotes vannus*. Digestion was followed in individual cells subjected to different levels of ambient food concentration. Average digestion times were not significantly different because, in the absence of food or in the presence of super-saturating food concentrations, there was a large variability between individuals. Some ciliates completed food vacuole processing under 1 h while others took as long as 17 h. The range of digestion times, which increased under extreme conditions, may correspond to range of growth rates or growth potentials among individuals.

Chapter 7 - Diatoms are important primary producers in global oceans and dominate in extensive areas of the western Atlantic ocean that are influenced by the low salinity, high dissolved silicon (dSi) waters of the Amazon River plume. Experiments were conducted to compare the Si uptake kinetics of diatoms in plume influenced waters and in nearby oceanic waters. At 5 oceanic stations, salinities ranged from 35.69 to 36.27, dSi concentrations ranged from 0.9 to 2.0 μM and biogenic silica (bSi) concentrations ranged from 0.02 to 0.07 μmol l^{-1}. At 6 plumewater stations, salinities ranged from 31.08 to 33.76, dSi concentrations ranged from 2.3 to 8.7 μM and biogenic silica (bSi) concentrations ranged from 2.3 to 8.7 μmol l-1. Si uptake kinetics were measured in multiple bottle incubations with dSi additions ranging from 1.25 to 40 μM. At oceanic stations, uptake kinetics generally fit the Michaelis-Menten function, with half saturation constants (Ks) between 13 and 25 μM and maximum biomass-specific uptake rates of 0.26 to 0.50 hr^{-1}. At two plumewater stations with high biogenic silica concentrations, uptake increased linearly with substrate concentration. At the remaining 4 plumewater stations, uptake kinetics fit the Michaelis-Menten function, and Ks values were

68.5 to 90.6 μM. In both environments, dSi concentrations were present at high concentrations relative to other macronutrients. We suggest that the observed inefficient uptake kinetics in both environments is an adaptation that allows diatom populations to be Si limited, which has been shown to be an adaptive advantage in phytoplankton competition.

Chapter 8 - This chapter will discuss recent progresses in marine microbial ecology. Foci will be placed on microbial processes and mechanisms related to carbon cycling in the ocean, including the newly recognized microbial light utilization in the surface ocean, archaeal carbon fixation and methane oxidation in the deep ocean and sediment, as well as lysis of host organisms by viroplanktons and its influence on carbon cycling in the water column. Key species or functioning groups of microorganisms include *Prochlorococcus* that possess unique photosynthesis pigments, the divinyl chlorophylls; aerobic anoxygenic phototrophic bacteria (AAPB) with bacterial chlorophyll *a*; rhodopsin containing proteobacteria (PR); nonthermophilic crenarchaeota that use ammonia as a major energy source for autotrophic growth; and ANME groups of archaea that oxidize methane for energy. The recent findings have raised challenges to the conventional concepts and theories. To face these challenges we propose novel models based on understandings of newly discovered microbial processes. For carbon cycling in the surface water of the ocean, a conceptual model is proposed based on bio-utilization of light, where bacteriochlorophyll *a*-induced anoxygenic phototrophy and proteiorhodopsin-based proton pump are considered as a novel mechanism for photosynthesis. For carbon sequestration below the surface water, a concept of non-sinking biological pumps is proposed, which is in contrast to the well known sinking flux-based conventional biological pump. Furthermore, a putative model of co-evolution of early life and the Earth is proposed based on the understanding of the ecological and molecular features of *Prochlorococcus* and AAPB in the present oceans. In addition to scientific frontiers, relevant state-of-the-art techniques in current microbial oceanography are also presented, including atomic force microscopy that bridges microorganisms to environments through analysis of cell surfaces; flow cytometry-based determination of bacterial membrane potential that provides continuous monitoring of cellular physiological response to environmental changes; time series-based infrared epifluorescence microscopy (TIREM) for accurate enumeration of bacteriochlorophyll containing microorganisms in the marine environment; meta-genomics and proteomics as a tool for insights into microbial community composition and functions; and microbial lipid biomarkers and isotope signatures to trace carbon flow at the molecular level.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 1-8
ISBN: 978-1-60021-935-1

Expert Commentary

STERIC AND MASS-INDUCED SEA LEVEL TRENDS OVER THE MEDITERRANEAN SEA FROM ALTIMETRY DATA

F. Criado-Aldeanueva[1], J. Del Río Vera[2] and J. García-Lafuente[1]
[1]Department of Applied Physics, University of Málaga, Spain
[2]Mission Planning and User Services Office, ESRIN/European Space Agency
Casella Postale64, 00044, Frascati (Rome) Italy

ABSTRACT

Long-term series of almost 15 years of altimetry data (1992-2005) have been used to study sea level trends over the Mediterranean Sea. Although sea level variations are mainly driven by the steric contribution, the mass-induced component plays some role in modulating its oscillation. A spatially averaged positive trend of 2 mm/year has been observed, 65% corresponding to the steric (mainly on thermal origin) contribution. A negative sea level trend from 2001 onwards is worth mentioning. Sea level rise is particularly important in the Levantine basin south of Crete with values up to 10 mm/year. Some other rising spots are localised throughout the Levantine basin and in the Adriatic and Alboran Seas, with more moderate values. Sea level drop areas are localised in the Balearic basin, between the Balearic Islands and the African coasts, and in the Ionian basin southeast of Italy and Sicily, where the highest negative trends (up to 10 mm/year) are reached mainly due to the mass-induced contribution. NAO might be ultimate responsible for some recent changes in sea level variations, being the easternmost and westernmost areas (Levantine and Balearic basins), the most sensitive to NAO control.

INTRODUCTION

The Mediterranean Sea, a semi-enclosed basin that extends over 3000 km in longitude and over 1500 km in latitude, communicates with the Atlantic Ocean through the Strait of Gibraltar and with the Black Sea through the Turkish Bosphorus and Dardanelles Straits. The Strait of Sicily separates the western and eastern Mediterranean basins.

The estimation of total sea level was primarily achieved by means of tide gauge stations located in coast worldwide but not able to measure in the open ocean. Trends considerably vary with location, although the accepted global range is 1-2 mm/year (Church et al., 2001) with lower values, about 0.5 mm/year for the Mediterranean Sea over the last decades (Cazenave et al., 2002). The launch in 1992 of ERS-1 and TOPEX/POSEIDON (T/P) satellites meant the start of altimetry missions and new efforts were devoted to this topic. Cazenave et al. (2002), merging 6 years (1993-1998) of T/P data with ERS-1 data (October 1992 to June 1996), have analysed sea level variations both in the Mediterranean and Black Seas. A mean sea level rise of approximately 7 mm/year is reported in the Mediterranean Sea, although its spatial distribution is far from uniform. While the Levantine basin is rising at a rate of 25-30 mm/year, the Ionian Sea is falling by 15-20 mm/year. In the western basin, sea level trends reported are significantly lower, some regions rising and others falling. Using larger data series -9 years of T/P, ERS-1 and ERS-2 (substitute of ERS-1 since June 1996) merged data-, Fenoglio-Marc (2002) reports more moderate trends: 2.2 mm/year for the entire Mediterranean, with lower values in the western basin (about 0.4 mm/year) and higher in the eastern (some 9.3 mm/year). The Ionian Sea is again found to be falling by 11.9 mm/year. Finally, with almost 15 years of altimetry data available, Vigo et al. (2005) have reported an abrupt and significant change in the sea level trend from mid-1999 in certain regions of the Mediterranean that claims for further research.

DATA AND METHODOLOGY

Total sea level has been determined from altimetry data from diverse satellite/missions (T/P, ERS-1/2, GFO, ENVISAT and JASON 1) collected through the merged AVISO products, freely available on www.aviso.oceanobs.com. Data are supplied in terms of sea level anomalies referred to a 7 years time average mean profile. Combining data from different missions significantly improves the estimation of mesoscale signals The AVISO regional solution for the Mediterranean Sea is used, with 1/8 x 1/8 degrees spatial resolution and weekly time resolution that covers the period 1992-2005.

In order to estimate the steric contribution ξ_S of the total sea level ξ_T, temperature and salinity profiles from the Jet Propulsion Laboratory ECCO model have been collected in a ten-day basis, with a spatial resolution of 1x1 degree and 46 depth levels with 10 meter resolution for the first 150 meters. Changes in the steric contribution (ξ_S) of total sea level (ξ_T) represent the effect of expansion and contraction of water column due to changes in density profiles (ρ), consequences of variations in temperature (T) and salinity (S) as modelled by (1).

$$\xi_S = -\frac{1}{\rho_0}\int_{-H}^{0}\frac{\partial\rho}{\partial T}\cdot T'(z)dz + \frac{1}{\rho_0}\int_{-H}^{0}\frac{\partial\rho}{\partial S}\cdot S'(z)dz \tag{1}$$

where $T'(z)$ and $S'(z)$ are temperature and salinity anomalies referred to their climatic mean value; ρ_0 represents a reference density and H is the bottom depth.

RESULTS

Figure 1 shows the spatially averaged steric contribution ξ_S during the analysed period computed from ECCO model. ξ_S describes a clear seasonal cycle with mean amplitude of 12.4 cm, maximum in the first half of September and minimum in the first half of March. The total sea level ξ_T computed from altimetry data also displays a seasonal oscillation (Figure 1), although its maxima and minima are not so well defined. Mean amplitude of some 9 cm is obtained, usually reaching maximum at mid-October and minimum at mid-April).

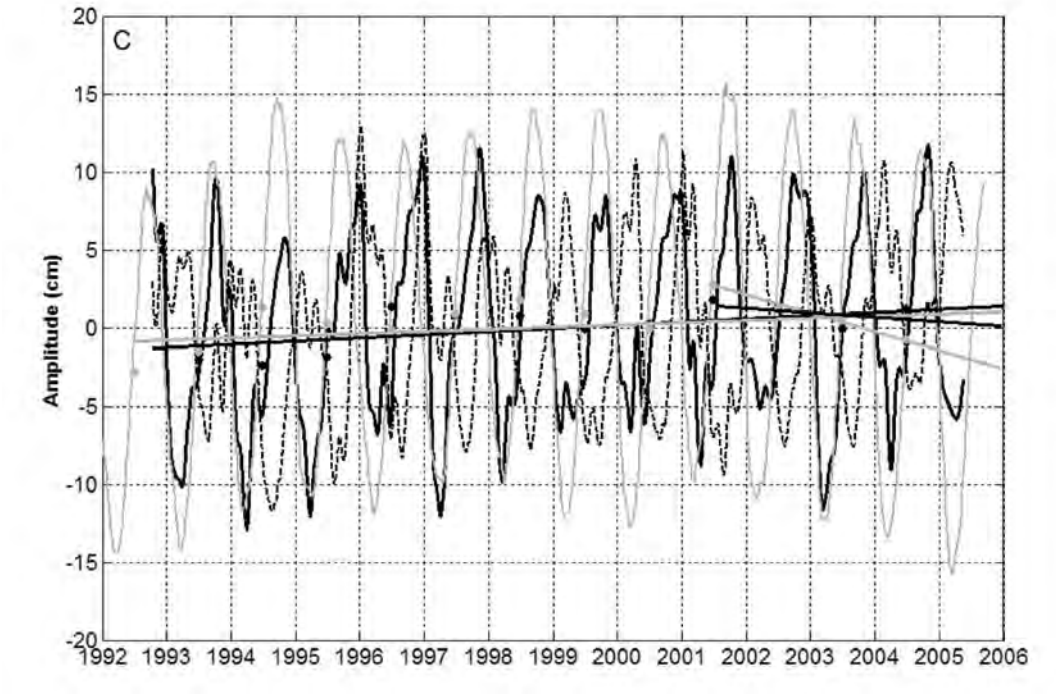


Figure 1. Spatially averaged total sea level (ξ_T, black thick line) from altimetry data due to the steric (ξ_S, grey thick line) and mass-induced (ξ_M, dashed line) contributions. Yearly mean values of ξ_T and ξ_S have been marked in their respective colour codes. Positive trends of 2 mm/yr and 1.3 mm/year have been found for ξ_T and ξ_S, respectively. Decreasing trends from 2001 onwards (more evident in the ξ_S signal) are also indicated.

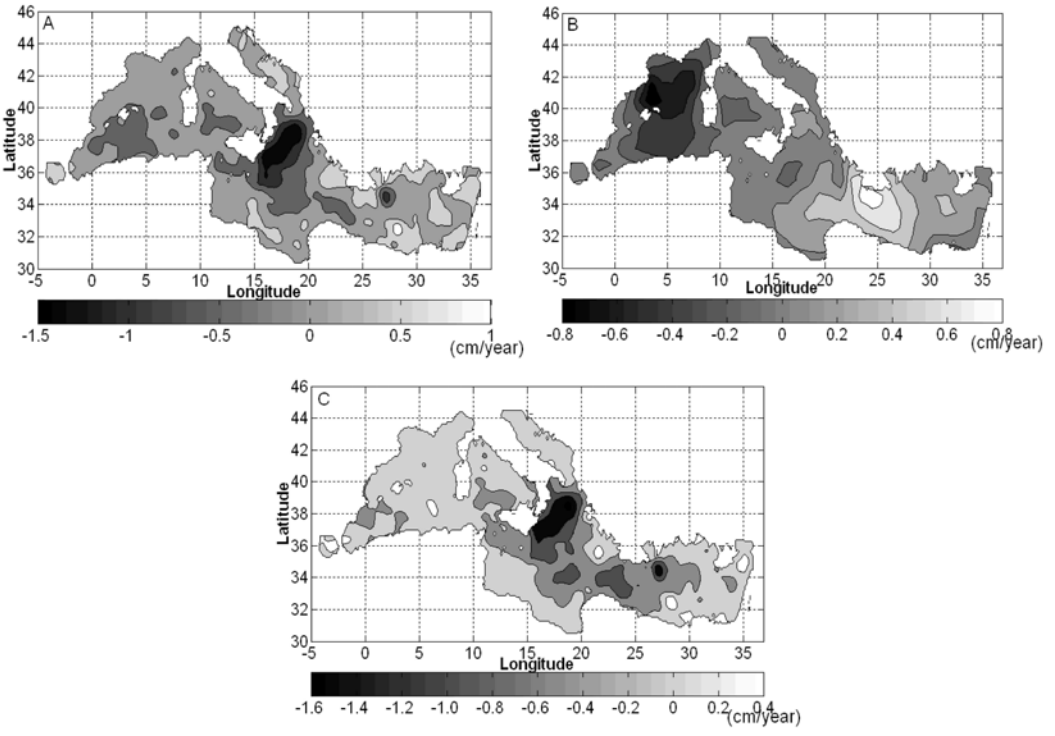


Figure 2. Trends spatial patterns for total sea level (ξ_T, panel A) due to the steric (ξ_S, panel B) and mass-induced (ξ_M, panel C) components. Sea level rise is particularly important in the Levantine basin (~10 mm/year), where both steric and mass-induced components cooperate, whereas sea level drop mainly concentrates in the Ionian basin (>10 mm/year), probably linked to a mass-induced origin.

Figure 1 shows similitude between ξ_T and ξ_S but it also evidences discrepancies. Not only ξ_S has greater amplitude but also its phase leads that of ξ_T by around 40-45 days. As the volume of water that determines the mean sea level in an oceanic basin (ξ_T) is the combination of two effects: steric, ξ_S and mass-induced, ξ_M, the latter is invoked to play some role in modulating ξ_T oscillation (Figure 1). ξ_M maxima and minima are reached in the first half of February and August, respectively, in good agreement with the previous results reported by García et al. (2006). As mentioned by these authors, when ξ_T is rising (falling), the Mediterranean is losing (gaining) mass and both steric and mass-induced contributions counteract each other to produce the net sea level variations.

A linear least squares fitting has been applied to obtain trends. For the period 1992-2005, positive trends of 2 mm/yr and 1.3 mm/year have been found for ξ_T and ξ_S, respectively. ξ_S trend only accounts for 65% of ξ_T trend, the remaining being accounted for ξ_M. The general sea drop starting from 1960 mentioned by Tsimplis and Baker (2000) is not observed in our data. To this regard, it is worth mentioning the change of sea level trend observed from 2001-2002 (Figure 1), when a negative trend, more evident in ξ_S, begins to appear. This change, also shown by Vigo et al. (2005), could be related to long period oscillations of Mediterranean Sea level with decadal frequencies such as the irregular SST oscillation with 60-70 year period with maximum around 2000 described by Moron (2003).

Some different regions are worth mentioning in the total sea level (ξ_T) spatial pattern (Figure 2a): sea level rise is particularly important in the Levantine basin south of Crete with

values up to 10 mm/year. Some other rising spots are localised throughout the Levantine basin as well as in the Adriatic and Alboran Seas with more moderate positive trends. Negative trends mainly concentrate in the Balearic basin, between the Balearic Islands and the African coasts (some 5 mm/year decreasing rate) and in the Ionian basin southeast of Italy and Sicily where the higher negative trends are reached (up to 10 mm/year). Spatial distribution of ξ_S trend for the period 1992-2005 (Figure 2b) shows two well-defined regions: the Levantine basin, where ξ_S is rising at a rate of 4-6 mm/year with peaks of 8 mm/year south of Greece and the Balearic basin, with a negative trend of 4-6 mm/year and peaks of 8 mm/year north of the Balearic Islands.

The good correspondence between ξ_T and ξ_S spatial patterns (Figures 2a and 2b) is broken in certain regions. The most noticeable is over the Ionian basin, where ξ_T does not appear to be linked to a thermosteric contraction of water column, but to the mass-induced component (ξ_M). ξ_M trends (Figure 2c) have been derived assuming a total trend composed by volume and mass trends. Some caution must be exerted for this consideration as noise may not be negligible at all. However, the high negative mass-induced trend over the Ionian basin (up to 12 mm/year) suggests that it really corresponds to a physical feature. ξ_M trend also exhibits a rising region north of Balearic Islands (some 4 mm/year) and some positive spots in the Levantine, Adriatic and Alboran basins.

DISCUSSION

The negative trend over the Ionian basin is likely related to the Eastern Mediterranean climate Transient (EMT). A shift in the formation site of deep and bottom waters from the Adriatic to the Aegean Sea (Roether et al., 1996; Malanotte-Rizzoli et al., 1999; Theocharis et al., 1999) have altered both the deep and upper conveyor belts of the Eastern Mediterranean. Different hypothesis such as internal redistribution of salt (Klein et al., 1999), changes in the local atmospheric forcing combined with long term salinity change (Theocharis et al., 1999) or changes in circulation patterns (Malanotte-Rizzoli et al., 1999) have been proposed concerning possible causes of this unique event, although it is still an open issue whether some of them are rather consequences than causes of the EMT. In any case, noticeable changes in circulation patterns over the Ionian basin have been reported in the last decades: at about 1986-87, a shift in the surface circulation in the Ionian basin, that changed from cyclonic to anticyclonic, began to appear (Pinardi et al., 1997; Malanotte-Rizzoli et al., 1997, 1999). This circulation pattern was well established until about March 1998, when it returned to cyclonic (Banca, 2000). These changes forced variations in the path of the Atlantic Ionian Stream (AIS) along the Ionian basin (Banca, 2000) and might result in a mass-induced negative trend in total sea level.

Tsimplis and Josey (2001) suggest that changes in total sea level could be linked with modifications in the large-scale meteorological forcing and, particularly, with the North Atlantic Oscillation (NAO). Positive NAO values mean positive pressure differences between Gibraltar and Iceland and are indicative of more and stronger winter storms crossing the Atlantic Ocean on a more northerly track, this resulting in warmer and wetter winters in Europe but colder and dryer winters over the Mediterranean (Hurrell, 1995), thus increasing the surface salinity. The higher state of NAO during recent decades (Hurrell, 1995) may also

be cooperating to the positive water column average salinity trend. Some other concomitant factors such as decrease in fresh water input as a result of human activities and decrease in precipitation since 1940 (Bethoux and Gentili, 1996, 1999) may be involved. Vignudelli et al. (1999) have demonstrated that NAO exerts an influence on the circulation of the northwestern Mediterranean Sea through air-sea interactions. Thus, ultimately NAO might be responsible for the recent changes detected in the Mediterranean thermohaline circulation as well as for the sea level variations. To explore this possibility, correlation between NAO index and total sea level ξ_T trends for the period 1992-2005 have been computed. Based on this correlation, the Mediterranean Sea is divided into three regions: the Central basin, which is not reactive to NAO and the easternmost and westernmost areas (Levantine and Balearic basins), in which the highest values (>0.3) are reached and NAO influence on ξ_T might be more effective.

CONCLUSION

The main conclusions of this work can be summarised as follows: from long-term series of almost 15 years (1992-2005) of altimetry data, positive trends of 2 mm/yr and 1.3 mm/year have been found over the whole Mediterranean for the total sea level and the steric contribution (mainly of thermal origin), respectively. Trend of the steric contribution only accounts for 65% of total sea level trend, the remaining being accounted for the mass-induced component. As previously showed by Vigo et al. (2005), a negative trend in total sea level, more evident in the thermosteric component, from 2001 onwards is also detected. Sea level rise is particularly important in the Levantine basin south of Crete with values up to 10 mm/year. Some other rising spots are localised throughout the Levantine basin and in the Adriatic and Alboran Seas, with more moderate positive trends. Negative trends mainly concentrate in the Balearic basin, between the Balearic Islands and the African coasts (some 5 mm/year decreasing rate) and in the Ionian basin southeast of Italy and Sicily where the higher negative trends are reached (up to 10 mm/year), the mass-induced contribution being widely dominant here. The negative trend over the Ionian basin was probably one consequence of the Eastern Mediterranean climate Transient, which has brought noticeable changes in the circulation patterns over this basin in the last decades (Pinardi et al., 1997; Malanotte-Rizzoli et al., 1997, 1999; Banca, 2000).

REFERENCES

Banca, B.M., 2000. Recent changes in dynamics of the Eastern Mediterranean affecting the water characteristics of adjacent basins. *CIESM Workshop Series* 2000, 27-31.

Bethoux, J.P. and Gentili, B., 1999. Functioning of the Mediterranean Sea: past and present changes related to freshwater input and climate changes. *Journal of Marine Systems* 20, 33-47.

Cazenave, A., Bonnefond, P., Mercier, F., Dominh, K. and Toumazou, V., 2002. Sea level variations in the Mediterranean Sea and Black Sea from satellite altimetry and tide gauges. *Global and Planetary Change* 34, 59-86.

Church, J. A., Gregory, J. M., Huybrechts, P., Kuhn, M., Lambeck, K., Nhuan, M. T., Qin, D. and Woodworth, P. L., 2001. *Changes in Sea Level. In Climate Change* (2001). The Scientific Basis. Contribution of Working Group 1 to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P., Dai, X., Maskell, K. and Johnson, C. I., eds. Cambridge University Press, 639-694.

Fenoglio-Marc, L., 2002. Long-term sea level change in the Mediterranean Sea from multi-satellite altimetry and tide gauges. *Physics and Chemistry of the Earth* 27, 1419-1431.

García, D., Chao, B.F., Del Río, J., Vigo, I. and García-Lafuente, 2006. On the steric and mass-induced contributions to the annual sea level variations in the Mediterranean Sea. Journal of Geophysical Research 111, C09030, doi:10.1029/2005JC002956.

Hurrell, J. W., 1995. Decadal trends in the North Atlantic Oscillation : regional temperatures and precipitation. *Science* 269, 676-679.

Klein, B., Roether, W., Manca, B., Bregant, D., Beitzel, V., Kovacevic, V. and Luchetta, A., 1999. The large deep water transient in the Eastern Mediterranean. *Deep-Sea Research I* 46, 371-414.

Malanotte-Rizzoli, P., Manca, B.B., D'Alcala, M.R., Theocharis, A., Bergamasco, A., Bregant, D., Budillon, G., Civitarese, G., Georgopoulos, D., Michelato, A., Sansone, E., Scarazzato, P. and Souvermezoglou, E., 1997. A synthesis of the Ionian hydrography, circulation and water mass pathways during POEM-phase I. *Progress in Oceanography* 39, 153-204.

Malanotte-Rizzoli, P., Manca, B.B., D'Alcala, M.R., Theocharis, A., Brenner, S., Budillon, G. and Ozsoy, E., 1999. The Eastern Mediterranean in the 80s and in the 90s: the big transition in the intermediate and deep circulations. *Dynamics of Atmospheres and Oceans* 29 (2-4), 365-395.

Moron, V., 2003. L'évolution séculaire des températures de surface de la mer Méditerranée (1856-2000). *Comptes Rendus Geoscience* 335, 721-727 (in French with abridged English version).

Pinardi, N., Korres, G., Lascaratos, A., Stanev, E. and Roussenov, V., 1997. Evidence for interannual variability of the Mediterranean Sea upper ocean circulation. *Geophysical Research Letters* 24 (4), 245-247.

Roether, W., Manca, B.B., Klein, B., Bregant, D., Georgopoulos, D., Beitzel, V., Kovacevic, V. and Luchetta, A., 1996. Recent changes in eastern Mediterranean Deep Waters. *Science* 271, 333-335.

Theocharis, A., Nittis, K., Kontoyiannis, H., Papageorgiou, E. and Balopoulos, E., 1999. Climatic changes in the Aegean Sea influence the Eastern Mediterranean thermohaline circulation (1986-1997). *Geophysical Research Letters* 26 (11), 1617-1620.

Tsimplis, M. N. and Baker, T.F., 2000. Sea level drop in the Mediterranea Sea: an indicator of deep water salinity and temperature changes? *Geophysical Research Letters* 27 (12), 1735-1738.

Tsimplis, M. N. and Josey, S.A., 2001. Forcing of the Mediterranean Sea by atmospheric oscillations over the North Atlantic. *Geophysical Research Letters* 28, 803-806.

Vignudelli, S., Gasparini, G. P., Astraldi, M., Schiano, M. E., 1999. A possible influence of the North Atlantic Oscillation on the circulation of western Mediterranean Sea. *Geophysical Research Letters* 26, 623-626.

Vigo, I., García, D. and Chao, B.F., 2005. Change of sea level trend in the Mediterranean and Black seas. *Journal of Marine Research* 63, 1085-1100.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 9-65
ISBN: 978-1-60021-935-1

Chapter 1

RESEARCH TRENDS ON DEMERSAL FISHERIES OCEANOGRAPHY IN THE MEDITERRANEAN

Joan Moranta, Antoni Quetglas, Enric Massutí, Beatriz Guijarro, Francesc Ordines and María Valls
IEO – Centre Oceanogràfic de les Illes Balears. Moll de Ponent s/n, 07015 Palma, Spain

ABSTRACT

The Mediterranean (MED) is the largest of the peripheral seas to the main oceans, connecting with the Atlantic through the Strait of Gibraltar in its western basin. The functioning of this sea has been compared to an inverse estuarine because the outflow (evaporation) is higher than the inflows (raining and river discharges), giving rise to a high salinity (~38) and a continuous entrance of superficial Atlantic waters to compensate the losses from evaporation. The MED is also characterized by its poverty, which is not reflected in the diversity of species as it is in overall productivity. During its long history, the harvesting of living organisms was restricted to coastal areas until the first few decades of the 20th century, when technology development allowed the exploitation of deep-sea resources. Although demersal fisheries takes a great number of target species (more than 100 being of commercial value and abundant in the catches), the main landings in biomass correspond to pelagic fisheries, which take almost exclusively sardines and anchovies. Therefore, MED demersal fisheries can be classified as multi-specific, with many fleets based all along the 40000 km of coast. The diversity and economic importance of small-scale fisheries are essential features of the MED. In the western MED, this fishery is important in the number of boats and fishers, but the major catches and earnings comes from bottom trawl. By contrast, in the eastern MED the small-scale fishery is socio-economically more important than trawling and purse seining. Bottom trawlers work on soft bottoms of the continental shelf, targeting mainly fish and cephalopods, and the upper slope, where they take basically decapod crustaceans along a broad depth range down to almost 1000 m depth. Throughout this wide bathymetric range, different biological communities with their corresponding species are exploited. Although studies of the MED go far to ancient times, the systematic study of

different aspects of this sea began at the end of the 19th century. The first reports of WMED megafauna were basically qualitative studies, focussed on the compilation, catalogue and description of the different taxonomic groups. From the second half of the 20th century, studies on quantitative analysis of megafaunal communities, bathymetric distribution of their components and population dynamics of target species were largely developed. Nowadays, the research focuses on the development of the ecosystem approach, the analysis of oceanographic and fishery time-series, the identification and characterisation of habitats of interest and the search for relationships among environmental factors and marine resource dynamics. In this respect, a multidisciplinary approximation is absolutely necessary in the new ecosystem view of marine research and management.

ENVIRONMENTAL CHARACTERISTICS

Geomorphology

The Mediterranean (MED) is the largest of the seas peripheral to the main oceans, with an area of about 2.5 million km^2 and a maximum east-west and north-south distance of approximately 3800 km and 900 km, respectively. The MED is limited by the Strait of Gibraltar, a shallow and narrow channel 320 m deep and 14 km wide, which contitute the only connection with the Atlantic Ocean. The north eastern limit connects the MED to the Black Sea through the Dardanelles Channel, where the maximum depth is 70 m. The MED and the Red Sea have been connected since 1869 by the Suez Canal, which is a large artificial canal 163 km long and 300 m wide at its narrowest point, with a depth of around 13 m.

Straits and channels are important geomorphologic characteristics, which play an essential role for the exchange of water masses and related properties internally between all the sub-basins and externally between the MED and the world ocean (Astraldi et al., 1999). From west to east, the most importants are: i) the Strait of Gibraltar, which constitutes the western limit of the MED; ii) the Balearic Channels, which control the meridional fluxes of the western MED (WMED); iii) the Sardinia Channel and the Strait of Sicily, which clearly separate the MED into two basins, the WMED and eastern MED (EMED); iv) the Otranto Strait, through which are conveyed the exchanges between the Adriatic sub-basin with the rest of the MED; v) and the Cretan Arc Strait, which is the interface between the Aegean Sea and the Levantine sub-basin (Figure 1).

The 200 m isobath is commonly used in the MED to separate the continental shelf, which represents less than 25% of the total area, from the slope, which extends to 60% of the whole basin. The continental slope is a relatively narrow zone, in which the change from 200 m to 2500 m occurs within a few tens of kilometres. The abyssal plains cover about 15% of the bottoms and are defined by several isobaths in the western and eastern basin, where they reach a maximum depth of 2855 and 5121 m, respectively.

The WMED is divided into five sub-basins: the Alboran, Algerian, Balearic, Liguro-Provençal and Tyrrhenian sub-basins (Figure 1). The Alboran Sea is the first MED sub-basin encountered by the inflowing Atlantic water and it is characterized by a very complex bottom topography with a maximum depth of 2000 m, together with the presence of the Alboran Island and the associated shallow banks that extend south-westward towards the African

coast. The Algerian sub-basin is situated off the southern Levantine coast of the Iberian Peninsula, between the Balearic Islands, the west of Sardinia and the coast of Algeria. This sub-basin has the maximum depth of the MED and is connected to the Balearic sub-basin by a series of sills in the arch of the Balearic Islands with the following depths: 800 m between Ibiza and the mainland, 600 m between Ibiza and Mallorca, and less than 100 m between Mallorca and Menorca. The Balearic sub-basin is bounded by the Iberian coast to the north and west, the Balearic Islands to the south, and the Liguro-Provençal sub-basin and the Gulf of Lions to the north and east, respectively. To the northeast, this sub-basin is open down to depths of greater than 2500 m. A distinctive feature of the Balearic sub-basin is the presence of big submarine canyons (Palanques et al., 2005), not present in the Algerian sub-basin (Acosta et al., 2002), which strongly influence the environmental conditions of deep-sea ecosystems (Puig et al., 2000; Canals et al., 2006). These geomorphological structures cause a highly dynamic water circulation close to the bottom and play an important role in the transport and concentration of sediment to greater depths. They have been shown to be areas of high productivity (Monaco et al., 1990; Bosley et al., 2004; Granata et al., 2004) that can act as recruitment grounds (Stefanescu et al., 1994). Finally, the Tyrrhenian sub-basin lies between the peninsula of Italy and the islands of Corsica and Sardinia.

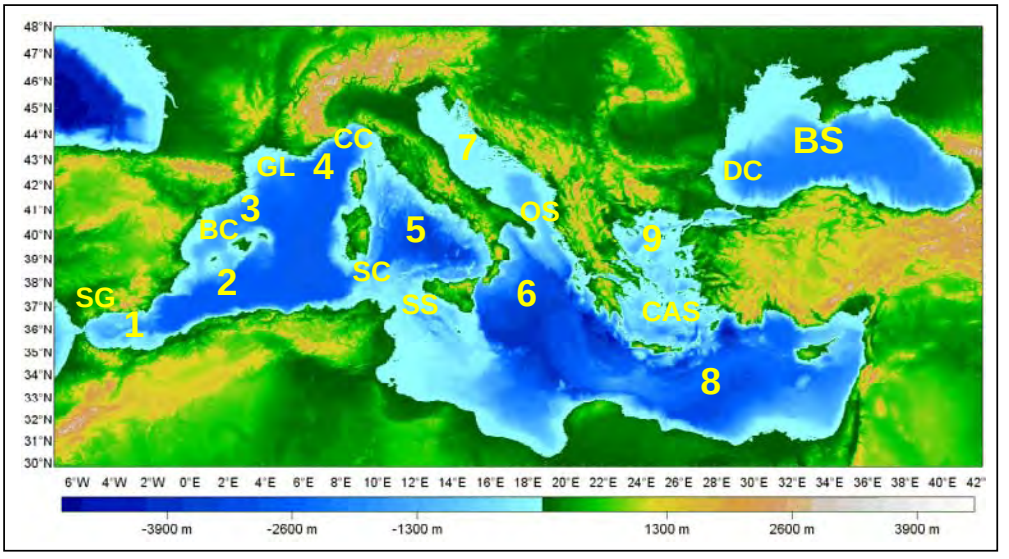


Figure 1. Map showing the different sub-basins in both the western and eastern Mediterranean basins (WMED and EMED, respectively) along with the main marine channels and straits. WMED includes five subbasins: 1) Alboran Sea; 2) Algerian; 3) Balearic; 4) Liguro-Provençal; and 5) Tyrrhenian. EMED contains four subbasins: 6) Ionian Sea; 7) Adriatic Sea; 8) Levantine; and 9) Aegean Sea. SG: Strait of Gibraltar, BC: Balearic Channels, CC: Corsica Channel, SC: Sardinia Channel, SS: Strait of Sicily, OS: Otranto Strait, CAS: Cretan Arc Straits, DC: Dardanelles Channel, GL: Gulf of Lions; BS: Black Sea. (Source: International Ocean Commisuion, International Hydrographic Organization and Britihs Ocanographic Data Cenre –IOC/IHO/BODC- ,2003: Centenary Edition of the GEBCO Digital Atlas, published on CD-ROM. Liverpool, UK).

The EMED is subdivided into four sub-basins: the Adriatic, Ionian, Levantine and Aegean sub-basins (Figure 1). The Adriatic Sea is a semi-enclosed, almost rectangular sub-basin (800 km long and 200 km wide) connected to the EMED Sea through the Strait of Otranto (75 km wide and 800 m deep at the sill). This sub-basin is divided into three compartments: i) the northern part, from the Gulf of Venice to the Ancona–Zadar transect, is shallow and gently sloping to an average depth of 35 m; ii) the Middle Adriatic has an average depth of 140 m and two SW–NE aligned depressions across the basin reaching 250 m depth; and iii) the Southern Adriatic part, extending down to the rise of the Otranto Strait with a depression of 1200 m depth in the middle. The Ionian sub-basin lies between the south of Italy and Greece and the Libian coast. This sub-basin shows the deepest record in the MED at the Hellenic Trough west of the submarine ridge between western Crete and Libya, which separate the Ionian sub-basin from the Levantine sub-basin. The Aegean sub-basin has a complex topographical structure, with irregular bathymetry, a multifarious coastline, a narrow continental shelf and hundreds of small and large islands (Poulos et al., 1997). The bottom topography consists of three main depressions: (i) the North Aegean Trough, which accommodates the Sporades Basin (1500 m maximum depth) and the Mount Athos Basin (1000 m maximum depth); (ii) the Chios Basin within the central part, with depths of up to 1100 m; and (iii) the Cretan Basin, which has the largest and deepest depression within the region, with water depths exceeding 2000 m in the northeast of Crete. At its northeastern extremity, the Aegean Sea exchanges flows with the Sea of Marmara, which acts as a conduit between the Aegean and Black Seas. To the south, the Aegean Sea communicates with the EMED Sea through the Cretan Arch Straits.

Hydrodynamics

The overall water mass dynamics of the MED are determined by physical processes typical of the open ocean, such as deep water formation and the overturning thermohaline circulation. At the same time, the dynamics of its regional sub-basins (mainly the Adriatic and Aegean) and the transport through the relatively shallow and narrow straits (Gibraltar, Sicily, Otranto, Kassos) are of primary importance for the circulation of the deep part of the MED (Demirov and Pinardi, 2002).

From the surface to the bottom, there are the following layers: i) Modified Atlantic Waters (MAW), warm and low salinity; ii) Western Mediterranean Intermediate Waters (WIW), with minimum temperature; iii) Levantine Intermediate Waters (LIW), with maximum temperature and salinity; iv) and Western (Eastern) MED Deep Waters (WMDW, EMDW) (Table 1). The upper layer normally extends down to a depth of around 150-200 m and shows high seasonal variations of temperature, being uniform during winter (13-15 ºC), but reaching temperatures as high as 20-25 ºC in summer which extend down to about 50 m, and shows a strong gradient between depths of 50 and 100 m in late summer (Pinot et al., 2002). Although the formation of deep-water masses in the MED results from winter cooling, the temperature in the deepest part of the western and eastern basins is only ~13 ºC, with a salinity of 38.5 to 38.6 (Carpine, 1970; Hopkins, 1985). Particularly, the WMED is characterised by a high environmental stability for both temperature and salinity below a depth of 200 m (Hopkins, 1985), although distinct oceanographic conditions with biological implications have been described in the area (Millot, 1987; Millot, 1999).

Table 1. Characteristic values of potential temperature (θ) and salinity (S) of the different water types and local values from the Balearic Islands, according to Lopez-Jurado et al. (2007))

Water mass	Values at origin	Local values
MAW	$15.0 < \theta < 18.0$ $36.15 < S < 36.50$	$15.0 < \theta < 28.0$ $36.50 < S < 37.50$
WIW	$12.5 < \theta < 13.0$ $37.90 < S < 38.30$	$12.5 < \theta < 13.0$ $37.90 < S < 38.30$
LIW	$14.0 < \theta < 15.0$ $38.70 < S < 38.80$	$13.0 < \theta < 13.4$ $38.45 < S < 38.60$
WMDW	$12.7 < \theta < 12.9$ $38.40 < S < 38.48$	$12.7 < \theta < 12.9$ $38.40 < S < 38.48$

MAW: Modified Atlantic Waters; WIW: Western Mediterranean Intermediate Waters; LIW: Levantine Intermediate Waters and WMDW: Western Mediterranean Deep Waters.

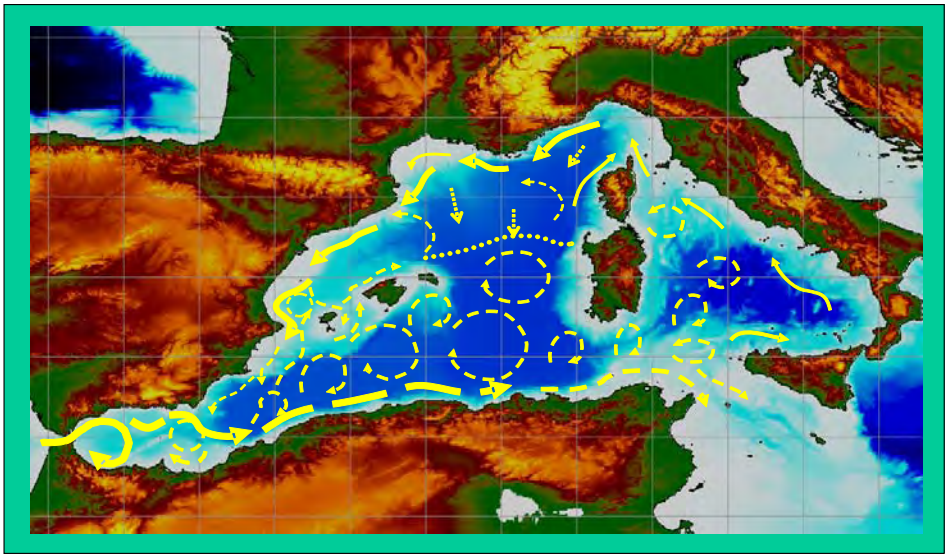


Figure 2. General circulation of the surface Modified Atlantic Water (MAW) in the western Mediterranean [Adapted from: López-Jurado et al. (2006). Wraping up of the IDEA Project: International Workshop on Environment, Demersal Resources and Fisheries. fttp://www.ba.ieo.es/idea/workshop/expos/Lopez-Jurado.pdf; modified from Millot (1999)].

The inflow of surface MAW in the MED, which are colder, less saline and richer in nutrients, through the Strait of Gibraltar drives the so-called Atlantic Strem System (ASS), and they circulate from the Alboran Sea, where the ASS becomes the Algerian current (AC), along the coast of Africa until the easternmost part of the EMED influencing the properties of the surface waters in the whole basin (Demirov and Pinardi, 2002). This surface water is modified during its journey eastward and it becomes saltier and denser due to the fact that evaporation in the MED is not compensated by river discharges and precipitation. In the area of Sicily, the AC splits into two branches (Figure 2). The first one goes north along the Tyrrhenian and Liguro-Provençal sub-basins to the Gulf of Lions and the Iberian coast before

returning to the Alboran Sea. The second one enters the EMED basin, where several cyclonic and anticyclonic gyres exist.

The Atlantic water in the Alboran Sea forms a quasi permanent anticyclonic gyre in the west and a more variable circuit in the east. In some cases, this eastern circuit is cyclonic with filaments extending eastwards from Cabo de Gata, seemingly in relation to strong westerly winds, although most of the time the circuit might be anticyclonic and is the origin of the AC. Due to these particular oceanographic conditions, the Alboran Sea can be considered an area of high productivity (similar to adjacent Atlantic waters) within the general oligotrophic context of the MED (Cartes et al., 2002). In the Balearic sub-basin, the circulation of the water masses is similar to a large cyclonic gyre, and is controlled by two permanent front systems following slope bathymetric contours: WIW flowing from the Gulf of Lions along the continental shelf-break and MAW entering into the Balearic sub-basin from the Algerian sub-basin, which acts as a reservoir for water of Atlantic origin (Millot, 1985) following the Balearic slope (Millot, 1987; Font, 1987; Pinot et al., 1995; Millot, 1999). The Balearic Channels play an essential role in this general scheme circulation as they are important passages for the meridional exchange between both water masses (Pinot et al., 2002). These frontal boundary regions, reinforced by the formation of a winter water mass with a minimum water column temperature, which can be compared to the mode waters of the North Atlantic (Pinot el al., 2002), are particularly relevant since they increase the biomass (Lhorenz et al., 1988) and enhance exceptional biologically active locations in the WMED (Estrada, 1996).

The circulation of the MAW in the EMED is still debated but a new hypothesis about the surface circulation in this basin has been proposed (Hamad et al., 2005). After “branching” in a more or less complex way at the entrance of the Channel of Sicily, MAW circulates mainly along the Ionian sub-basin. This sub-basin is a transition zone across which different water masses (e.g., MAW, LIW, EMDW) undergo transformations along their pathways between the EMED and WMED. Part of the MAW continues along the Tunisian coast while the rest, probably the larger part, follows the Tunisian continental slope, from where a northern interannual branch has been observed. Off Libya and Egypt, the circulation of MAW is markedly unstable, generating mesoscale anticyclonic eddies that extend down to the bottom. These eddies then accumulate in the Herodotus bathymetric trough (depths>3000 m in the Levantine sub-basin), forming the “Mersa Matruh Gyre”. Owing to much shallower depths, instability of the MAW circulation generates eddies that have smaller spatio-temporal scales and accumulate in the “Shikmona Gyre” area. In the northern part of the basin the circulation of MAW is clearly related to the zones of dense water formation, such as the southern Adriatic, the northern Levantine and the Aegean sub-basins.

Formation of both intermediate and deep waters takes place during the winter along the northern borders in both basins of the MED. Wintertime intermediate convection in the Levantine sub-basin produces LIW, which are transported westward in the layer between 300 and 500 m towards the Strait of Sicily and then towards the Gulf of Lions and Gibraltar (Demirov and Pinardi, 2002). Two meridional thermohaline cells form in both the WMED and EMED, driven by the process of deep water formation. The deep WMDW form in the Gulf of Lions by deep convection processes during cold winters due to the presence of a cyclonic gyre, which is an important preconditioning factor for this winter deep convection process (Leaman and Schott, 1991). The EMDW form in the Adriatic (Roether and Schlitzer, 1991) or the Aegean sub-basins (Roether et al., 1996) and then, through the relatively narrow and shallow straits, sink into deeper parts of the EMED.

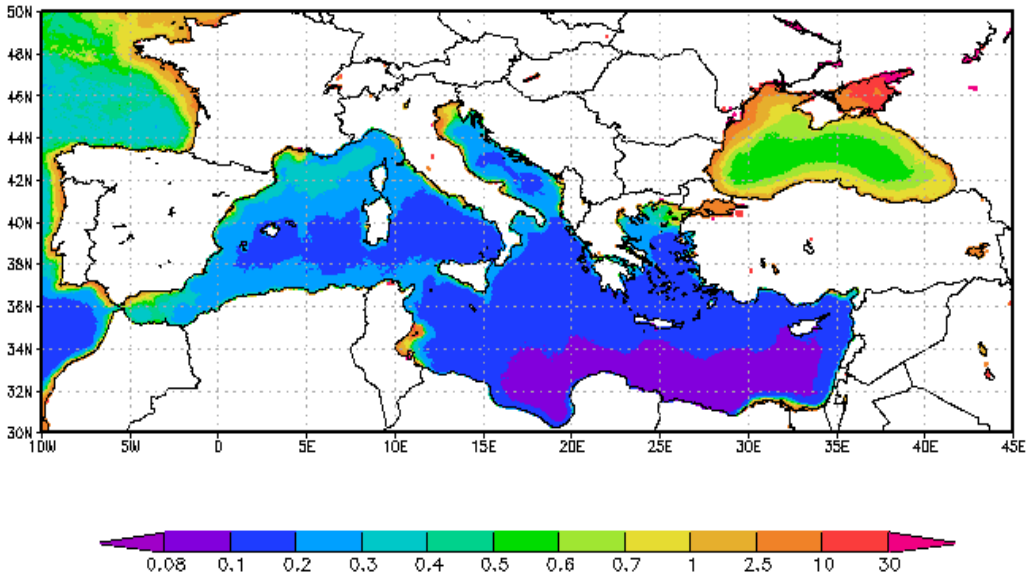


Figure 3. Satellite image showing the average monthly distribution of surface chlorophyll-a (mg/m^3) in the Mediterranean from January 2003 to December 2006 (Source: http://reason.gsfc.nasa.gov/Giovanni).

Productivity

The low biological productivity of MED waters compared to other areas elsewhere is well known (e.g. Margalef, 1985). However, this oligotrophic characteristic has been under discussion for a number of years. In fact primary production rates reported for the WMED are comparable to values reported for the rest of the world (Estrada, 1996), which could be due to the presence of a series of mechanisms enhancing fertility at certain times of the year in connection with hydrographic structures (Estrada, 1996; Bosc et al., 2004). During the winter, deep nutrient-rich waters reach the surface and allow spring algal blooms to occur. MED production is then characterized by two annual extremes in the nutritive limits. The first one corresponds to winter mixing and determines the initial stock of nutrients, since the quantity of nutrients brought into the photic zone will mainly depend on the depth reached by cooling waters. The second limit corresponds to the maximum summer stratification, characterised by the lowest availability of new nutrients.

Surface biomass and productivity for the WMED has been estimated from satellite imagery (Morel and André, 1991; Arnone, 1994). Surface productivity over deep-water varies seasonally, with maximum production of around 400 mgCm^{-2}d^{-1} in May and minimal production of <120 mgCm^{-2}d^{-1} in November (Morel and André, 1991). Satellite images show how the WMED area and, more so, the north-western part are surrounded by water with higher levels of chlorophyll pigmentation than elsewhere in the eastern part or along the southern coastline (Figure 3). Although, a decrease in integrated primary production, particulate carbon export and nutrient availability towards the EMED has been observed (Moutin and Raimbault, 2002), the estimated annual primary production of the Rhodes area (eastern Levantine sub-basin) has comparable values to those reported for the north-western

MED (Napolitano et al., 2000; Mouitin and Raimbault, 2002; Bosc et al., 2004). If it is assumed that 1% of surface production reaches a depth of 2000 m, then the maximum vertical flux would be 1.5-4 $mgCm^{-2}day^{-1}$ (Tyler, 2003).

DEMERSAL FISHERIES

Fishing Exploitation

Total annual landings of MED fisheries have steadily increased since 1950, remaining at around 1.5 million metric tonnes. Although MED fisheries only represent a small proportion of the world production (Figure 4), the mean prices of landings (which are mainly sold fresh) are well above the average prices of world markets. In the European Union, MED fisheries represent about 20% of the total catch but 35% of the total value of landings (Lleonart and Maynou, 2003). From the socio-economic point of view, fishing activities in the MED employ several hundred thousand persons. Although demersal fisheries takes a great number of target species, the main landings in biomass correspond to pelagic fisheries, that focus basically on two fish species, sardines and anchovies. These small pelagic species are caught with purse seines and pelagic trawl (Figure 5) and their stocks are in general linked to major rivers run-off (e.g. Ebro and Rhone) and upwelling areas.

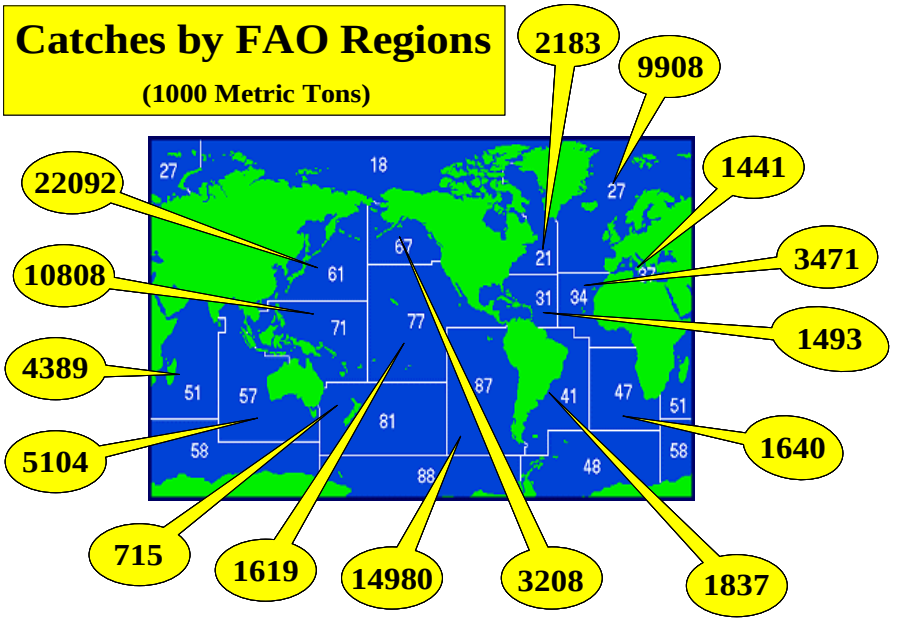


Figure 4. World marine fisheries production by FAO fishery regions in 2005 (Source: CIHEAM, 2003. Development and agri-food policies in the Mediterranean region. Annual Report. International Centre for Advanced Mediterranean Agronomic Studies; and FAO, 2007. Fishstat Plus 2.32. Fisheries and Aquaculture Information and Statistics Service; Food and Agriculture Department).

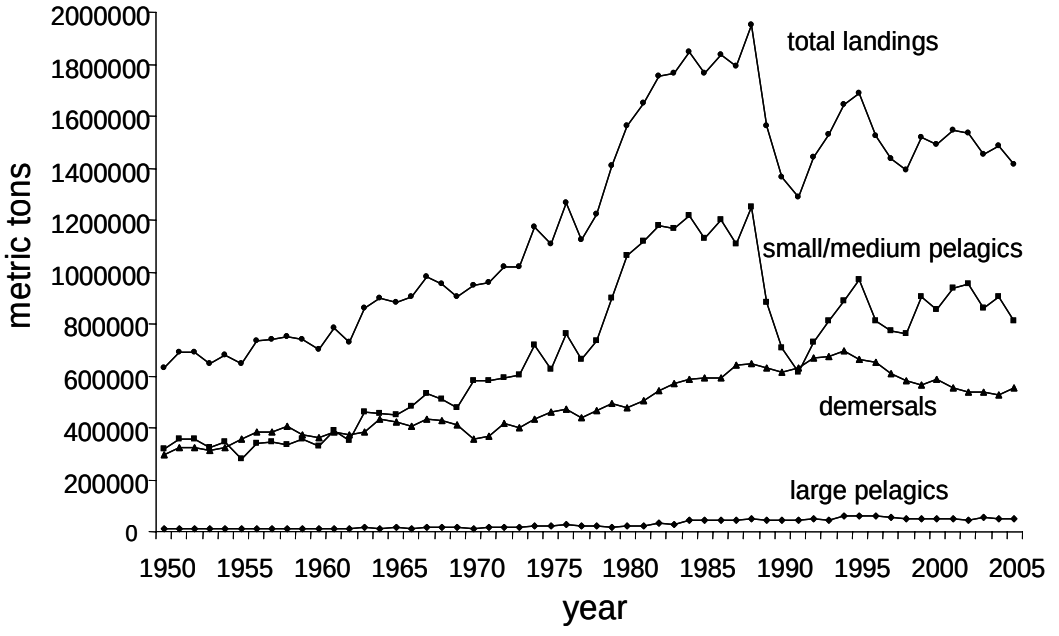


Figure 5. Demersal, pelagic (both large and small/medium species) and total landings of the Mediterranean fisheries, including the Black Sea, during the last half century (Source: FAO 2007. Fishstat Plus 2.32. Fisheries and Aquaculture Information and Statistics Service (FIES); Food and Agriculture Department. Modified from Lleonart and Maynou, 2003).

The presence of a high diversity of species and the absence of large monospecifc stocks comparable to those inhabiting some wide areas of the open oceans, is characterisitics of the MED demersal fisheries (Farrugio et al., 1993). There are many target demersal species, more than 100 being of commercial value and abundant in the landings (Lleonart and Maynou, 2003). Therefore, the MED demersal fisheries can be classified as multispecific (Caddy, 1993), with many fleets (the total number of fishing boats has been estimated at around 100000) based all along the 40000 km of coast (CIHEAM, 2003). MED fishing activities exhibit great variations in production methods from one area to another, which reflect the adaptation of human communities to the physical and biological environmental conditions (Farrugio et al., 1993). However, other aspects such as the social, economic and historical context of the neighbouring countries are important elements to be taken into account.

The diversity and economic importance of small-scale fisheries are also essential features of the MED. The small-scale fleets are generally composed by small and low tonnage boats (less than 10 m length), operating mostly at depths down to 100 m and less than 3 miles from the coast (Farrugio and Le Coree, 1993; Stergiou et al., 1996). This fleet performs daily trips and employ a highly diversified kinds of fishing gears (mainly nets, longlines and seines) varying seasonally and geographically. Although in the WMED the small-scale fishery is very important in number of boats and fishers, the major catches and earnings come from bottom trawls (Figure 6, Table 2). By contrast, in other parts of the MED, such as Greek waters, the small-scale fishery is socio-economically more important than trawling and purse seining, since it represents 87.5% of the total fleet, 57.5% of total fishing power, 67.3% of fishers and produces near half of the total wholesale value of catch (Stergiou et al., 1996).

The main target species, along with the gear used in taking them in the most important small-scale fisheries in both basins of the MED, are shown in Table 3. The bottom trawl fishery is developed on the continental shelf and upper slope down to approximately 800 m. Some of the units (small vessels) operate almost exclusively on the continental shelf (targeting red mullets *Mullus surmuletus* and *M. barbatus*, vulgaris common octopus *Octopus* spp., European hake *Merluccius merluccius* and sea breams Sparidae, Figure 7), some others (large vessels) work almost exclusively on the continental slope (targeting decapod crustaceans, especially Norway lobster *Nephrops norvegicus* and red shrimp *Aristeus antennatus*, Figure 8) and the rest can operate either on the continental shelf or on the slope fishing grounds, depending on the season, the weather conditions and also economic factors (e.g. market prices). Although landings from the slope are mainly composed of a few number of decapod crustacean species (Guijarro and Massutí, 2006) with high economic value, the landings from the continental shelf are the most important in terms of biomass, being composed of a high number of fish species and some cephalopods (Ordines et al., 2006).

Table 2. Percentage of vessels and mean engine power (± standard error) and mean length (± standard error) by gear in the different geographical areas of the western Mediterranean delimited by the General Fishery Commission of the Mediterranean (GFCM)

		GSA1	GSA5	GSA6N	GSA6S	Total
GEAR (%)	Trawl	16.23	12.17	27.67	33.04	24.4
	Atisanal	66.59	84.39	61.93	58.78	64.9
	Purseine	15.16	3.17	9.45	7.63	9.7
	Difting longline	2.03	0.26	0.95	0.55	1
ENGINE NOMINAL POWER (average±s.e.)	Trawl	182.4±8.5	219.6±13.7	297.0±12.5	272.6±8.1	262.8±6.1
	Atisanal	39.6±1.4	40.4±1.8	43.5±1.4	51.8±2.2	44.2±0.9
	Purseine	134.2±7.3	83.4±14.1	321.4±27.8	274.1±15.2	224.2±11.2
	Difting longline	129.2±7.5	95.0	104.1±15.5	146.4±26.5	123.1±7.4
SHIP LENGTH (meters) (average±s.e.)	Trawl	15.7±0.3	17.0±0.4	17.1±0.3	17.9±0.2	17.3±0.1
	Atisanal	6.4±0.1	7.0±0.1	7.0±0.1	7.5±0.1	6.9±0.1
	Purseine	11.2±0.3	9.5±0.7	16.7±0.6	16.3±0.5	14.1±0.3
	Difting longline	15.2±0.7	10.4	11.2±0.9	15.5±1.9	13.8±0.6

GSA1: between strait of Gibraltar and Cape of Gata, GSA5: Baleric Islands; GSA6S: between Cape of Gata and Cape San Antonio, and GSA6N: between Cape San Antonio and Cape of Creus

Table 3. Main target species, gears used to take them, and some bibliographical references in the most important small-scale fisheries in both the western and eastern Mediterranean sub-basins

	Target species	Gears	Sources
Western Mediterranean	*Mullus* spp. (Fi), *Solea solea* (Fi), *Palinurus elephas* (Cr), *Sepia officinalis* (Ce)	gill nets, trammel nets	Demestre et al., 1997; Goñi and Latrouite, 2005; García-Rodríguez et al., 2006
	Octopus vulgaris (Ce)	trammel nets, longlines, pots	Sánchez and Obarti, 1993
	Merluccius merluccius (Fi)	gill nets, longlines	Aldebert et al., 1993; Martín et al., 1999
	Aphia minuta (Fi)	boat seines	La Mesa et al., 2005
	Coyphaena hippurus (Fi)	surrounding nets	Massutí and Morales-Nin, 1999
	Sparidae species (Fi), Scorpaenidae species (Fi)	trammel nets, gill nets, longlines	Iglesias et al., 1994; García-Rodríduez et al., 2006
	Plesionika edwardsii (Cr)	traps	García-Rodríguez et al., 2000
	Bolinus brandaris (Bi), *Chamelea gallina* (Bi), *Donax trunculus* (Bi), *Callista chione* (Bi), *Ruditapes decussatus* (Bi)	clam dredges	Ramón and Richardson, 1992; Martín et al., 1995; Ramón et al., 1995; Ramón et al., 2005.
Eastern Mediterranean	*Mullus* spp. (Fi), Mugillidae species (Fi), *Pagellus erythrinus* (Fi)	trammel nets	Stergiou et al., 2006
	Merluccius merluccius (Fi)	gill nets, longlines	Tzanatos et al., 2006
	Octopus vulgaris (Ce)	pots	Tzanatos et al., 2006
	Seriola dumerilii (Fi)	longlines	Tzanatos et al., 2006
	Sparidae species (Fi), *Sepia officinalis* (Ce)	trammel nets	Tzanatos et al., 2006
	Spicara smaris (Fi), *Sepia officinalis* (Ce)	beach seines	Belcari et al., 2002; Tzanatos et al., 2005
	Sparidae species (Fi), Scorpaenidae species (Fi), *Anguilla anguilla* (Fi)	longlines, lagoon seines	Tzanatos et al., 2005
	Katsuwonus pelamis (Fi), *Sarda sarda* (Fi)	trolling lines	Tzanatos et al., 2005
	Mullus spp. (Fi)	gill nets	Stergiou et al., 1996
	Penaeus kerathurus (Cr)	trammel nets	Conides et al., 2006
	Callista chione (Bi), *Venus verrucosa* (Bi)	divers	Metaxatos, 2004

Taxonomic group of each species is shown in parentheses after the name: Fi (fish), Cr (Crustacean), Ce (Cephalopod), Bi (Bivalve).

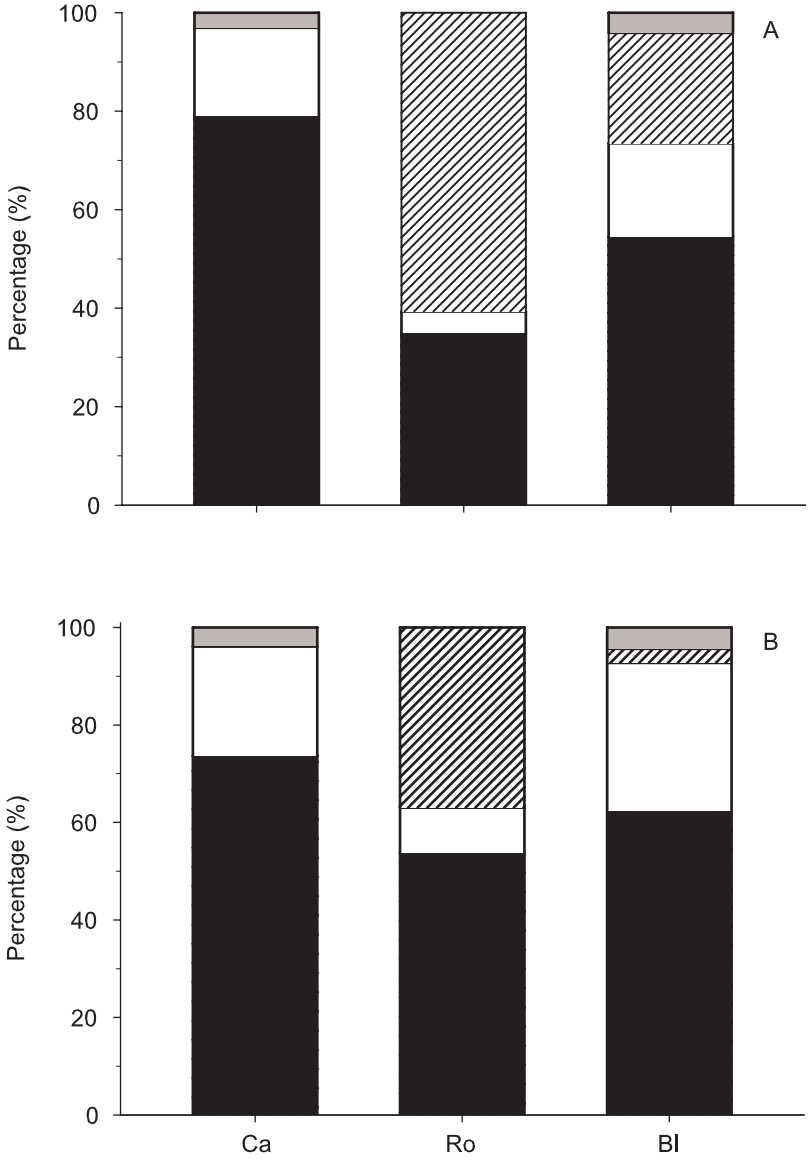


Figure 6. Percentage of landings (A, in weight) and earnings (B, in euros) in the four main fleets for the period 2000-2006, both pelagic (purse seine and drifting longline) and demersal (trawl and small-scale), of two commercially important ports of Iberian Coast (Ca:Sant Carles de la Ràpita, Ro: Roses) and the Balearic Islands (BI) in the western Mediterranean.

Figure 7. Example of the catch taken by the the bottom trawl fleet fishing on the continental shelf of the Balearic Islands (western Mediterranean) where it can be observed the great amount of red algae (top) and the multi-specific nature of the capture mainly composed by fishes (bottom).

Figure 8. Example of the catch taken by the the bottom trawl fleet fishing along different depths (400-800 m) on the upper slope of the Balearic Islands (western Mediterranean). While the decapod crustacean *Aristeus antennatus* predominates in the deepest depth (bottom), fishes are more abundant in shallower waters (top).

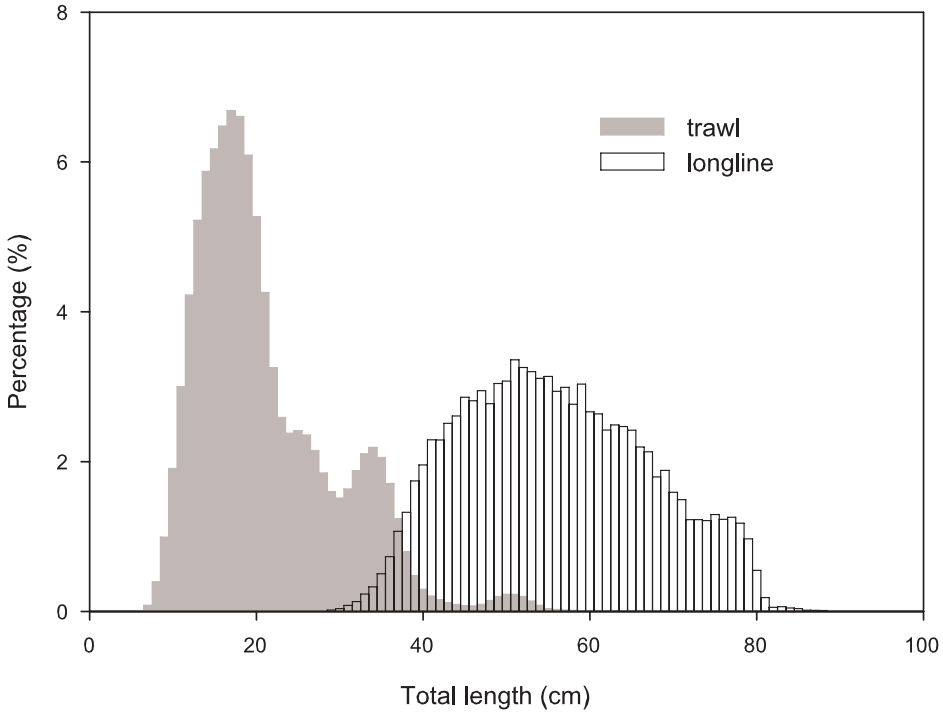


Figura 9. Length-frequency distributions of European hake *Merluccius merluccius* captured in the Gulf of Lions (north-western Mediterranean) with bottom trawl and long-line for the period 1998-2004.

Among fishes, red mullets and the European hake have a great economic importance in the WMED, being mainly exploited by trawl on the shallow shelf and the shelf-break, respectively (Oliver and Massutí, 1995). The bottom trawl fishery exploits mainly recruits and juveniles of these species (Figure 9), but the vulnerability to trawling appears to decline with fish size, because adults inhabit rocky bottoms (Abella et al., 1997; Martín et al., 1999). Therefore, adult fishing mortality in the MED has been typically low, and this ‘‘refuge effect’’ for adults has been put forward as the most plausible explanation for the apparent sustainability of red mullets and European hake fisheries, despite the high fishing pressure (Oliver, 1993; Abella et al., 1997). However, since 1980’s European hake is being regularly exploited with artisanal fixed gears (gill nets and bottom long-lines), formerly in the Gulf of Lions (Aldebert *et al.*, 1993) and more recently in some areas off the Iberian coast (García-Rodríguez *et al.*, 2006) and the northern Tyrrhenian Sea (Sbrana et al., 2007). These selective gears are used over fishing grounds not available to trawl (e.g. rocky bottoms and submarine canyons), and their catches are mainly based on adult hake (Figure 9). As example, length ranges of specimens captured in the Gulf of Lions with trawls, gill nets and long-lines are 10-30, 30-45 and 40-70 cm, respectively (GFCM, 2006). Moreover, to the growth over-fishing steady state argued for this species and other demersal resources in the MED (Lleonart and Maynou, 2003), a risk of recruitment over-exploitation in the Gulf of Lions has also been pointed out in recent years (Lleonart, 2004; GFCM, 2006) that could give rise to stock collapse. In fact, landings of European hake, which were mainly based on recruits, have declined markedly throughout the 1990s in some areas of the WMED (Lleonart and Lloret

2004; Lloret and Lleonart, 2002; Goñi et al., 2004), suggesting a growth overfishing (Oliver, 1993; Lleonart and Maynou, 2003) and more recently, due to the introduction of long-line that exploits large adult specimens, risk of recruitment overfishing (Lleonart, 2004; GFCM, 2006).

The decapod crustacean Norway lobster is fished all around the MED between 400 and 600 m depth. Sardà (1998) reviewed the biology and fishery of this species in the MED and showed a common life-cycle model throughout its distribution range and an exploitation level close to its carrying capacity. The red shrimp fishery was initiated in the 1940's (e.g. Oliver, 1953; Bas and Rubio, 1959; Bas, 1966) and it is now exploited on the upper continental slope, between 500 and 800 m depth (off the Catalan coast this can extend down to ~1000 m) along the whole Iberian coast and the Balearic Islands where red shrimp is by far the predominant species (Demestre and Lleonart, 1993; Sardà et al., 1994b; García-Rodríduez and Esteban, 1999; Carbonell et al., 1999; García-Rodríduez, 2003). In the central MED, both *A. antennatus* and *Aristeomorpha foliacea* are important catches of this fishery whose main fishing grounds are located along the Ionian Sea from coastal waters to 750 m (Sardà et al., 2004a; Ragonese et al., 2001; Sbrana et al., 2003; Belcari et al., 2003). By contrast, red shrimp populations in the EMED remain practically unexploited (D'Onghia et al., 2005; Papaconstantinou and Kapiris, 2003; Mytilineou et al., 2006) because the trawl fishing activities are mainly developed on the continental shelf and upper slope not exceeding generally 400-500 m in depth (Politou et al., 2004). In the last decades, a great number of studies have focused on the estimation of the exploitation level of red shrimp populations (Sardà et al., 2004a, and references cited therein). In spite of the high fishing pressure exerted on this species, results do not show overexploitation, but a tendency to go far from the optimum harvesting. This might be due to the wide depth range distribution in bathyal waters (down to 3300 m, Sardà et al., 2004a) and the fact that part of the population is not vulnerable to trawling (Sardà, 1993). Other important species captured as by-catch on the continental shelf and upper slope in MED trawl fisheries are the picarel *Spicara smaris,* the blue whiting *Micromesistius poutassou,* the great fork-beard *Phycis blennoides,* the blue-mouth *Helicolenus dactylopterus* and the rose shrimp *Parapenaeus longirostris* (Massutí et al., 1996b; Moranta et al., 2000; Guijarro and Massutí, 2006; Ordines et al., 2006).

Recreational fishery is also very important in the MED, being one of the most frequent leisure activities in coastal zones and involving a large number of persons and consequently high levels of fishing effort (Morales-Nin et al. 2005b). This refers to all types of fishing activities including amateur, tourism, and sport/competition, undertaken by any individual, with or without a boat, for leisure purposes and not involving the catch selling. A great variety of recreational fishing methods exits, but they can be classified in three main classes: fishing from a boat, fishing from shore and spearfishing. In spite of its importance, recreational fisheries have been poorly studied but a recent work developed in the Balearic Islands arise that 5% of the population participate of this leisure activity, amounting a minimum catch of 1200 metric tonnes, which represents 27.44% of the commercial landings (Morales-Nin et al., 2005b). This recreational fishery was highly seasonal, reflecting the seasonal variability in abundance of the incidental and key target species, which also varied with fishing method, depth, and bottom substrate. The main target species along with other fishing characteristics of the recreational fisheries from the Balearic Islands are shown in Table 4.

Table 4. Fishing classes along with their differential characteristics (bottom type and water column layer where the fishing is carried out) and most important target species in the main recreational fisheries developed in the Balearic Islands (western Mediterranean)

Fishing class	Bottom type	Water column	Target species
from shore	hard, rocky	bottom fishing	*Symphodus* spp., *Coris julis*, *Diplodus annularis*, *Serranus cabrilla*
		surface fishing	*Oblada melanura* and *Sarpa salpa*
	soft, sandy	bottom fishing	*Lithognathus mormyrus*, *Umbrina cirrosa*, *Sparus aurata*, *Diplodus* spp.
from boat	*Posidonia oceanica* beds	bottom fishing	*Serranus scriba*, *D. annularis*, *C. julis*
	soft, sandy	bottom fishing	*Bothus podas*, *Trachinus* spp., *Synodus saurus*, *Xyrichthys novacula*
	hard, rocky	bottom fishing	*Serranus cabrilla*, *Pagellus* spp., *Pagrus pagrus*, *Diplodus vulgaris*, *Spondyliosoma cantharus*.
	indistinct	surface	*Coryphaena hippurus*, *Seriola dumerilii*, *Trachurus* spp.
spearfishing	hard, rocky	bottom fishing	*Epinephelus marginatus*, *Sciaena umbra*, *Diplodus sargus*, *Octopus vulgaris*.

All the species shown are fishes, with the only exception of the cephalopod *Octopus vulgaris*.

Research, Assessment and Management

The MED has long tradition in fisheries oceanography investigations. Many travellers, humanists, historians, geographers, naturalists and biologists have described the marine living resources and the fishing exploitation in this “sea between the lands”. The Greek and Latin authors of the Antiquity, such as Aristotle (384-322 B.C.) and Pliny the Old (23 B.C.-79 A.C.), made the first observations and gave explanations on the ecology of some species. Afterwards, the books of the Mostassaf, who acted as inspector or consumer advocate in the Muslims markets, are inventories of the marine species fished, with data of their price and weight, along the Middle Age. During the Renaissance, the Occitan naturalist Rondelet made the first catalogue of the MED fauna in 1554, containing descriptions and fine illustrations of fishes and other marine creatures, obtained from markets and fishermen. At the second half of the 18th century and early 19th, other faunistic catalogues were prepared by other naturalists such as Linné in 1758, Delaroche in 1809 and Risso in 1810, among others. At this period, it was also initiated the abundant literature which describe the evolution of technological, economic and social aspects of the MED fisheries, pointing out the diversity of fishing activities, some of them still remain nowadays.

Since the 19th century, the invention and adoption of new fishing techniques, such as trawling which rapidly extended, motorisation of the boats and mechanization of fishing gears were the landmarks of a turning point in the history of MED fisheries (Farrugio et al., 1993). Since these authors, this 'technological revolution' coincided with a reinforced concern felt in the western world for physical oceanography and marine biology. On 1860, the recuperation of a submarine cable from 2000 m depth off Sardinia, which was covered by animals, allowed to reject the Azoic theory in the MED, which had been formulated some years ago by Edward Forbes in the Aegean sub-basin, suggesting that no life existed below 600 m depth. At the end of this century, the first scientific surveys were developed and numerous research marine stations were established along the MED coast. Those studies were fundamental to basic knowledge of the hydrographic, sedimentological and faunistic characteristics of the MED. Then, more or less connected to fishing activities, fisheries research evolved towards studies describing not only the fishing techniques, gears and boats, but also the biology of the main species. The highly multi-species nature of the MED catches favoured the studies of numerous species, not necessarily the most important commercial ones.

The second half of the 20th century, with the generalisation of the concepts of optimal exploitation and over-exploitation, represented the advent of modern fisheries research, and little by little systematic studies were developed with the aim of contributing to fisheries management (Farrugio et al., 1993). However, the passage from marine biology *sensu-stricto* to fisheries research is relatively new in the MED, for many countries being recent the research carried out specifically to support the management of exploited populations (Caddy, 1993). Initially, this research was oriented towards the evolution of yields and the demographic composition of catches. Then, the first attempts to apply population dynamic models to some exploited stocks were developed in the north-western MED countries (Oliver, 1993). In fact, no long data series are usually available, because such information has been obtained from on-going research projects, with no continuity in time, and only until recent decades monitoring plans have been implemented in some areas (Lleonart and Maynou, 2003). In this way, scientists have applied the methods developed for tropical fisheries, not only due to the scarcity of suitable data, but also because of the complexity of the multi-species artisanal fisheries (Lleonart and Maynou, 2003). According to these authors, who reviewed the fish stock assessments in the MED, the first approach was the application of production models under equilibrium conditions, a method not particularly useful because of the difficulties in effort estimation (Lleonart, 1993). The lack of long and reliable time series, joined to other shortcomings in data that prevent the use of more standard assessment methods (Lleonart and Maynou, 2003), led scientists from late 1980s to use pseudo-cohort and yield per recruit analyses in most cases. However, presently some few demersal stocks begin to be yearly assessed using standard virtual population analysis (e.g. GFCM, 2006). There also exist some few examples where other methodologies, such as non-equilibrium surplus production (Carbonell and Azevedo, 2003) and ad-hoc bio-economic models (Lleonart et al., 1996; Lleonart et al., 2003; Maynou et al., 2006) have been applied. Finally, it must be pointed out the very recent application of the Ecopath mass-balance model, which is reviewed in a posterior section, to characterise the functioning and structure of the exploited demersal ecosystems and to assess the impact of fishing exploitation (Coll et al., 2006).

The bottom trawl surveys, developed since 1994 by the European MED countries (Bertrand et al., 2002), and extended in recent years to Morocco and the eastern Adriatic

countries, also constitute a useful tool in the assessment of demersal fisheries. This fishery-independent data, promoted considering the difficulties of sampling commercial catches in the area, has provided indices of relative abundance and population structure, over space and time, of the main demersal species. This information has mainly been used to map the spatial distribution and to estimate time data of relative abundance of the main species (e.g. Abelló et al., 2002), as well as to characterise the benthic communities and demersal assemblages exploited (e.g. González and Sánchez, 2002; Bertrand et al., 2002; Biagi et al., 2002; Massutí and Reñones, 2005). Only more recently, trawl survey data has been coupled to population dynamic models to tune the indirect assessments (GFCM, 2006), being also used to estimate mortality rates and to assess the state of the fisheries, applying a variant of surplus production models (Abella et al., 1999) and the new stock assessment method direct survival analysis (Ferrandis and Hernández, 2007). Other important contribution of trawl surveys is the study of sensitive habitats and essential fish habitats (e.g. Colloca et al., 2004; Fiorentino *et al.*, 2003), of great interest nowadays within the context of the new ecosystem approach to fisheries (see below). Time series of relative abundance of recruits and adults, gathered during trawl surveys, have allowed studies of stock-recruitment relationships taking also into account environmental variability (e.g. Levi et al., 2003). These direct methods have been very useful to estimate the fishing impact on the biodiversity (e.g. Aldebert, 1997; Jukic-Peladic et al., 2001) and on the abundance of some vulnerable species such as the elasmobranches (e.g. Bertrand et al., 2000; Massutí and Moranta, 2003). The same data have also been used to estimate fishing power, recruitment indices and their relationships to environmental variables, by means of regression analysis, generalised linear models and time series analysis (e.g. Goñi et al., 1999; Lloret et al., 2000; Lloret et al., 2001; García-Rodríguez, 2003; Alemany and Alvarez, 2003; Goñi et al., 2004).

The management of the MED fisheries is based on effort control, no Total Allowable Catches (TACs) are implemented, except for the bluefin tuna and the swordfish. This kind of management can also be on the basis of the above mentioned general lack of data in the MED. Other technical measures, such as minimum landing sizes and minimum mesh sizes, are also implemented but not always enforced, and in all cases these measures are lower than in the Atlantic. Since Lleonart and Maynou (2003), most of the rules concerning the management of demersal fisheries have been developed for trawling, not only because it is the main gear contributing to demersal catches, but also because it presents poor selectivity in comparison with the most important artisanal gears (nets and lines). In the WMED, the effort control of the trawl fishery have been mainly based on the limitation of the number of boats and their engine power, the limitation on the horsepower of new vessels and the establishment of maximum time at sea (e.g. days per week and hours per day). However, the fishing mortality has increased continuously during recent decades, because technological progress has not been considered for management purposes (Sardà, 1998). In some areas, closed banned bottoms for trawling, usually the three first nautical miles from the coast or up to 50 m depth, are in force to protect *Posidonia oceanica* beds and recruitment grounds for some species. These measures have been reinforced with subsidised seasonal closures, but with no clear benefits for the exploited resources (Lleonart and Maynou, 2003). New recent measures have been considered for the management of the trawl fishery in the WMED, such as not allowing fishing below 1000 m depth (as precautionary, because trawl fleet have not yet explored these grounds) and over coraligenous bottoms and maërl beds, as well as the further introduction of square mesh cod-end, replacing the diamond mesh cod-end in force nowadays

(Council Regulation, EC Nº 1967/2006 of 21 December 2006). Moreover, it has been recomemdeed the establishment of three fisheries restricted areas in order to protect the deep-sea sensitive habitats (Recommendation GFCM/2006/3, FAO 2006): i) "Lophelia reef off Capo Santa Maria di Leuca", located in international waters of central MED in order to protect deep water coral reefs; ii) "The Nile Delta area cold hydrocarbon seeps", characterised by an exceptional concentration of cold hydrocarbon seeps which had favoured the development of unique living community; and iii) the "Erastothemus Sea Mount", located in the EMED basin to protect its deep sea sensitive habitat.

Acording to Lleonart and Maynou (2003), "the fisheries management is reactive, never adaptive and still less, precautionary". So, there is not pressure to assess the resources, acquisition of data suffering from the lack of interest of all sectors involved (administration, fishermen and scientists). Furthermore, due to the narrowness of the continental shelf, few stocks (excluding the large pelagics) are shared by two or more countries, which help to minimise the international concerns about the fishery resources. In recent years, periodical assessments are done within the framework of the Scientific Advisory Committee of the General Fisheries Commission for the MED (GFCM; www.faogfcm.org) in some areas of the WMED. This regional FAO body reviews the state of the MED resources, including their abundance and level of exploitation, as well as the state of the fisheries based thereon, and recommends appropriate measures related to the rational management of living marine resources, in particular by regulating fishing methods and gears. Up to 30 geographical sub-areas has been recognised, based on geographic, political and statistical considerations rather than biological or economic ones, as well as 42 species, considered as priority for assessment and management purposes. The interest criteria are based on the volume of landings and economic importance of the species in the GFCM region. Among the priority species, 20 of them are demersal fishes, decapod crustaceans and cephalopods. Although morphometric and genetic techniques have been applied for stock identification in the MED, studies on demersal species such as hake (Roldán *et al.*, 1998), Norway lobster (Castro *et al.*, 1998) and red shrimp (Sardà *et al.*, 1998) have not been conclusive. The first objective of the GFCM Sub-committee on Stock Assessment is to assess shared stocks of priority species in the MED.

In adition to the GFCM, the MED Science Commission CIESM (www.ciesm.org) is another international organism which plays an important role in the fishery oceanography research of the MED, supporting a network of several thousand marine researchers and running expert workshops, collaborative programs and regular congresses. Their scientific committees Living Resources and Marine Ecosystems, and Physics and Climate of the Ocean, are more directly involved in these kinds of studies and some workshops has been organized related with fisheries research in the last decade (e.g. CIESM, 1998, 1999, 2000)

Effects of Demersal Fisheries on Ecosystems

The effects of fishing exploitation on ecosystems are widely described woldwide and have been recently reviewed in the MED by Tudela (2000). This author analyse the fishing impact dealing with the main threats to marine biodiversity (including both vulnerable species and habitats) arising from selected fishing gears and practices in use in MED waters. According to this work, most of the major effects of fishing recorded around the world also occur in the MED.

Table 5. Percentage of discards of the total catch by depth stratum obtained in different ports of the western Mediterranean

Port	Year	Stratum A (<150 m)	Stratum B (151-350 m)	Stratum C (>350 m)
%Discards of total catch		%	%	%
Fuengirola[1]	1995-96	45	55	42
Santa Pola[1]	1995-96	23	56	24
Valencia[1]	1995-96	23	27	21
Palma[1]	1995-96	69	62	19
Alcúdia[1]	1995-96	55	44	14
Pisa[1]	1995-96	32	21	22
Vilanova[1]	1995-96	48	17	22
Mallorca[2]	1996-97	-	-	42
Vilanova[3]	1995-96	62.7	19.35	19.5

[1] Carbonell et al. (1998); [2] Moranta et al. (2000); [3] Sánchez et al. (2003).

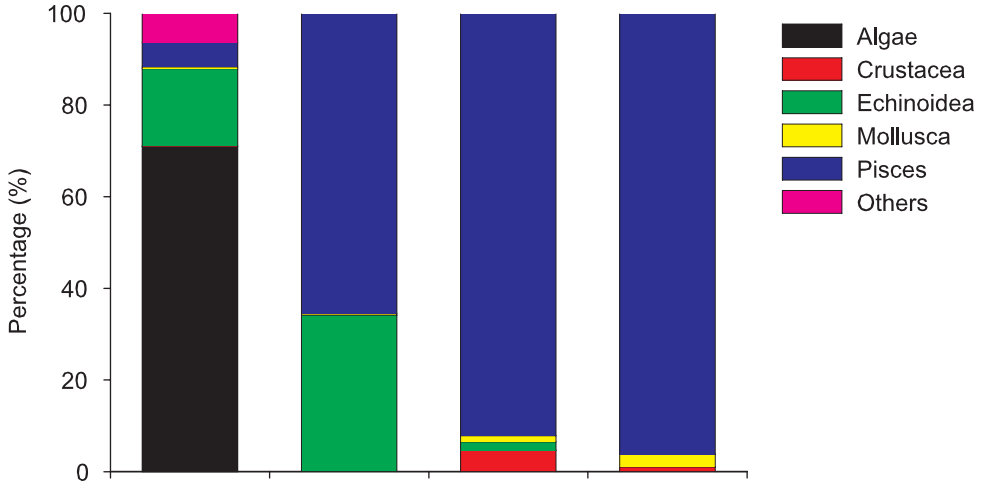


Figura 10. Faunistical composition of discards in the bottom trawl fishery of the Balearic Islands (western Mediterranean) in different bathymetric strata along the continental shelf and slope. The Others fraction includes Ascidiacea, Cnidaria, Porifera and Tentaculata. SS: shallow shelf (50-100 m); DS: deep shelf (101-200 m); US: upper slope (201-500 m); MS: middle slope (501-800 m).

By-catch and discards are one of the most undesirable consequences of fishing. Therefore, since the end of the 20th century there has been an increasing concern about the quantity of by-catch and discards resulting from commercial fisheries and the search for solutions inherent to this problem (FAO, 1996), overall taking into account that these fractions may contribute to biological overfishing and to alter the structure of marine ecosystems by favouring scavengers (Moranta et al., 2000; Demestre et al., 2000b). By-catch

is not exclusive to any particular gear type and region of the world, but an issue which affects all forms of fishing. Although all types of gear produce discards, bottom trawling has the greatest impact (Goñi, 1998; Tudela, 2000). The amount of discards produced by this fishery in the MED varies between depths and areas, but ranged between 10% and 75% of the total catch (Table 5, Figure 10) (Sartor et al. 1998; Moranta et al., 2000; Tudela, 2000; Machias et al., 2001; Carbonell et al., 2003; Sánchez et al., 2004; Guijarro and Massutí, 2006; Ordines et al., 2006).

One of the ways to reduce the impact of fishing on both target and non-target species is increasing the gear selectivity, that is, the capacity of fishing gears to capture a certain size rank of the population, determining the specific composition of the capture. Knowing the selectivity of the different gears is of great importance for a suitable fishing management since its improvement contributes to reduce the capture of juveniles, increases the yield of the target species, reduces the amount of discards and, consequently, the impact on the ecosystem. Selectivity studies are specially important in the MED owing to the multispecies and multigear characteristics of their demersal fisheries, in contrast with those fisheries targeting a single or few species. Nevertheless, the existing bibliography focussing on this aspect is still scarce and depends on the fishing gear considered. Probably because its low selectivity and gret impact, bottom trawl has received more attention than other gear types (e.g. Stergiou et al., 1997; Ragonese et al., 2001; Sardà et al. 2004c; Guijarro and Massutí, 2006; Ordines et al., 2006). These studies have pointed out that bottom trawl affects to more than 300 species among vertebrates and invertebrates, including the non-commercial species (e.g. Matarrese et al., 1996; Quetglas et al., 2000; Moranta et al., 2000; Abelló et al., 2002; Sánchez et al., 2004; Massutí and Reñones, 2005). In order to reduce this impact, many studies have been developed to improve the trawl net selectivity by different ways, such as increasing the mesh size (Sardà et al., 1993; Mytilineou et al., 1998; Ragonese et al., 2001; Ragonese et al., 2002; Carlucci et al., 2006), changing from diamond- to square-shaped mesh codends (Petrakis and Stergiou, 1997; Stergiou et al., 1997; Mallol et al., 2001; Bahamon et al., 2006; Guijarro and Massutí, 2006; Ordines et al., 2006; Sardà et al., 2006), using square mesh escape windows (Metin et al., 2005), changing the codend material or characteristics (Deval et al., 2006; Sala et al., 2007) or using sorting grids (Sardà et al., 2004d; Sardà et al., 2005; Sardà et al., 2006). Results showed that an increase in the mesh size or a change to square mesh improves selectivity, not only decreasing the proportion of discarded catch but also increasing the length at first capture (length at which 50% of specimens escape) of the main species. Moreover, square mesh was more efficient than using larger sizes of diamond mesh (Guijarro and Massutí, 2006; Ragonese and Bianchini, 2006). Selectivity studies on the gears used in the small-scale fishery (trammel nets, gill nets and longlines) are still scarce and focus basically on the analysis of the catch composition (Stergiou et al., 1996; Stergiou et al., 2002; Fabi et al., 2002; Stergiou et al., 2006). Taking into account the number of species affected, longlines have been found to be more selective than trammel nets and gill nets (Stergiou et al., 1996; Stergiou et al., 2002; Stergiou et al., 2006). Finally, Morales-Nin et al. (2005b) showed that recreational fishery presents a moderate selectivity compared to professional fishing gears (trawl and small-scale).

In many cases, the impact of bottom trawl on the ecosystems are analysed considering target species or major taxonomic groups. In this sense, Chondrichthyans represent an especially sensible group to fishing impacts and, although it typically appears in the by-catch of fisheries all around the world, remains almost totally unmanaged nowadays (Stevens et al.,

2000). Fishing impacts have in fact been evidenced throughout the last decade in highly exploited areas of the WMED such as the Gulf of Lions (Aldebert, 1997; Bertrand et al, 1998), by changes in their number of species and decreases in the abundance and biomass of some ones (e.g. *Raja clavata*). More recent works have analysed the demersal elasmobranch assemblages (Capapé et al., 2000; Relini et al., 2000; Bertrand et al., 2000; Massutí and Moranta, 2003), as well as the importance of this group in the by-catch and discards of trawling fisheries (Carbonell et al., 2003; Sánchez et al., 2004; Guijarro and Massutí, 2006).

Sea-floor organisms are also heavily impacted by bottom trawling, specially on the continental shelf, where seafloor communities are more developed and a large amount of benthic organisms is removed (Ordines et al., 2006). In some areas of the WMED, discards of flora and invertebrates on the continental shelf represent up to 67% of the bottom trawl catch, due fundamentally to rodophytic algae (Carbonell et al., 1998). Moreover, some of the communities affected on these fishing grounds have already been considered sensitive habitats, as is the case of maërl and *Leptometra phalangium* beds, or habitats of special interest because of their high productivity, such as the soft red algae bottoms on the shallow shelf which present high biomass indexes (Figure 11). All these habitats increase the structural complexity of the sediments, creating more shelter possibilities for recruits and it has also been suggested that highly productive areas, such as *L. phalangium* beds on the shelf break, are important spawning grounds (Colloca et al., 2004). Trawling on these communities cause physical damage, directly crushing the benthic organisms (De Biasi, 2004) or removing large amounts of them, such as red algae and echinoderms (Colloca et al., 2003; Ordines et al., 2006), which reduces the structural complexity of these bottoms. Trawling on maërl beds, mainly composed by slow-growth red algae species of Corallinacea, reduces the diversity and coverage of these algae, but also causes a loss of species richness, density and biomass of macrofauna species, and even a substitution of maërl by other algae species more resilient to trawling impacts like Peyssonneliacea (Bordehore et al., 2003). Similarly, since Smith et al. (2000), the impact of otter trawling on *L. phalangium* beds leads to a strong reduction of this crinoid and other echinoderms, along with a decrease in richness, abundance and biomass of other benthic species.

Although the effects of fishing activities on the seafloor communities are becoming clear, it is still to be determined how this degradation will affect the populations of the demersal resources related to these habitats. In fact, it was not until recent years that scientists began to explore the relationships between demersal resources and the ecosystems exploited (Demestre et al., 2000b; Colloca et al., 2003; Colloca et al., 2004; Massutí and Reñones, 2005; Ordines et al., 2007). Unravel the complexity of these relationships could be the key to the ecosystem approach to fisheries management, as it would imply that collateral impacts on the ecosystem due to fishing activities should not only be considered as a damage to the 'other' organisms present in the fisheries, but also an indirect impact on the species targeted that adds to the direct impact due to fishing mortality.

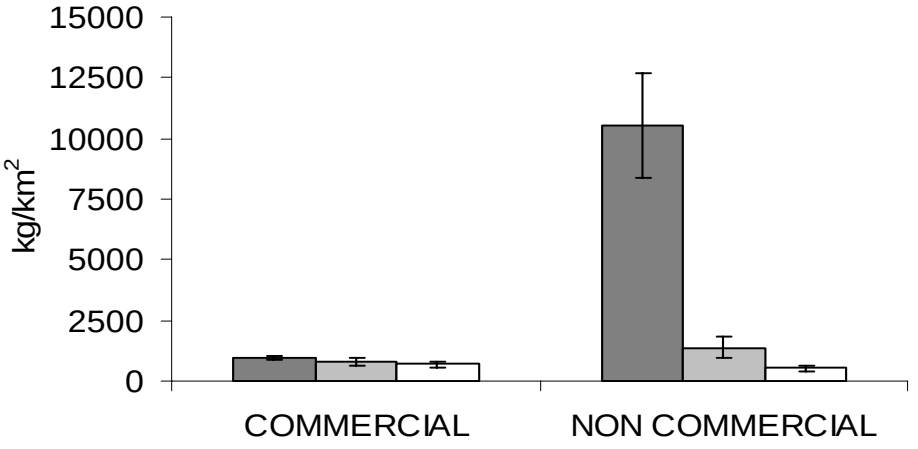


Figure 11. Biomass yields (kg/km^2) of both commercial and non-commercial species on soft red algae beds (dark grey), maërl beds (grey) and sandy-mud bottoms (white) from the fishing grounds off the Balearic Islands (western Mediterranean).

In addition to the direct effect of fishing gears on the ecosystems, it should also be considered other possible indirect effects on demersal fisheries, produced by the reduction of the population of some key species. The risk of stock collapse for a top predator species such as hake, could have important consequences on the whole ecosystem mediated by trophic cascade effects (Cury et al., 2003). In this sense, mean trophic level of fishery catches in the eastern Ionian sub-basin has been decreasing over the last 20 years (Stergiou and Koulouris, 2000), indicating that the effect of fishing down the food web (Pauly et al., 1998) is at play in the MED. Moreover, the decline of higher-order marine predators preying on epilpelagic species (e.g. common dolphin *Delphinus delphis*, swordfish *Xiphias gladius* and tuna *Thunnus thynnus*) is consistent with the hypothesis of prey depletion, likely resulting from intensive exploitation of local fish stocks, particularly anchovies and sardines (Berazi et al., 2006). These ecological changes, which might already have started in the MED (Caddy *et al.*, 1995), could be accelerated by the degradation of the coastal zone.

LIFE HISTORY TRAITS

From the second half of the nineteenth century, the recognition of the depletion of many fishing stocks and the need for manage them, made that many biological studies were mainly directed to exploited species. This implies that nowadays those aspects related to the general biology such as population structure, distribution, growth, diet and reproduction of the most important commercial species are relatively well-documented. However, the multispecific context of MED trawl fisheries prevents accurate studies on the plethora of the captured species and therefore they have been basically focused on the most important ones, either in biomass nor in economic value, such as hake, red mullets, red shrimp and Norway lobster.

Among them, the European hake has been one of the most intensively studied species during the last decade. Since trawlers base the exploitation on small, juvenile individuals,

many of these works deals with the biology of recruits, but larvae and pre-recruits have also received the attention of scientists. These works have focused on three principal issues, such as distribution, diet and growth. Spatial and temporal variability in abundance or distribution of different developmental stages, going from eggs to juveniles and adults, have been thoroughly treated (Relini et al., 2002; Olivar et al., 2003; Maynou et al., 2003; Goñi et al., 2004; Abella et al., 2005). Studies on diet have analyzed the prey composition and ontogenic changes (Bozzano et al., 1997) but also aspects related to feeding such as the influence of environmental variables (Cartes et al., 2004b), diel vertical migrations (Bozzano et al., 2005) and even the implications of retinal topography on feeding habits (Bozzano and Catalán, 2002). Age and growth have been determined using both otoliths and lenth-frequency methods but in spite of the efforts putted on this topic (Morales-Nin and Aldebert, 1997; García-Rodríguez and Esteban, 2002; Morales-Nin and Moranta, 2004; Palomera et al., 2005; Morales-Nin et al., 2005a; Morales-Nin et al., 2005c; Belcari et al., 2006), uncertainty remains on the growth rate and conclusive validation studies are still needed.

Owing to the high economic importance of the red shrimp there also exist abundant information on this crustacean. Although some of these works cover wide general aspects of the biology and fishery of the species, the majority of them deal with more specific aspects such as feeding (Cartes and Sardà, 1989; Carbonell et al., 1999), molting (Sardà et al., 1989), gonad development (Carbonell et al., 2006), seasonal and spatial large-scale mobility patterns (Sardà et al., 1997), population structure and dynamics (Sardà et al., 1994c; Sardà and Maynou, 1998a; D'Onghia et al., 2005), the internal structure of shoals (Sardà et al., 2003) or the presence of contaminants in deep sea populations of this crustacean (Rotllant et al., 2006). A bulk of works have also been published on morphometrics, many of them analyzing the rostrum as external key character (Sardà and Demestre, 1989; Sardà et al., 1995; Sardà et al., 1997; Bas and Sardà, 1998; Kapiris and Thessalou-Legaki, 2001).

The case of Norway lobster is similar to the red shrimp, since also its high economic value has resulted in the publication of many studies covering both the general biology and fishery (Merella et al., 1998; Sardà, 1998; Sardà et al., 1998a) and more specific aspects such as diet (Cristo and Cartes, 1998; Aguzzi et al., 2004), reproduction (Sardà, 1991; Relini et al., 1998; Bianchini et al., 1998; Rotllant et al., 2005), population structure and dynamics (Sardà et al., 1998b; Smith et al., 2003; Aguzzi et al., 2003; Aguzzi et al., 2004) and comparisons among different populations (Maltagliati et al., 1998; Castro et al., 1998; Stamatis et al., 2004; Stamatis et al., 2006).

Biological studies on red mullets are still scarce and the available ones center on the general life-history, spatial distribution and feeding habits. There exists a work dealing with various biological aspects of *M. surmuletus* such as the population structure, reproduction, age and growth (Reñones et al., 1995). Concerning *M. barbatus*, there are information on the population parameters obtained from length-frequency analysis (Ozbilgin et al., 2004), on the stock-recruitment relationship and how it is affected by sea surface temperature anomalies (Levi et al., 2003) and also on behavioral changes in this species induced by an invasive macroalga (Levi and Francour, 2004; Longepierre et al., 2005). Other works analyze the spatial segregation (Lombarte et al., 2000), the spatio-temporal distribution (Tserpes et al., 2002) and the resource partitioning (Vassilopoulou et al., 2001; Aguirre and Sánchez, 2005) of these two sympatric species.

Given that even a resumed and representative list of the available studies dealing with the biology of other species, neither with nor without commercial importance, would be out the

aims of the present work, the following are only some examples of papers published during the last years. There are studies on the general biology of fishes (Massutí et al., 1995; Morales-Nin et al., 1996b; D'Onghia et al., 1998a; D'Onghia et al., 2000; García-Rodríguez et al., 2005; D'Onghia et al., 2006), crustaceans (Campisi et al., 1998; D'Onghia et al., 1998a; Maiorano et al., 2002; Colloca, 2002; Maynou et al., 2004) and cephalopods (Quetglas et al., 1998a; Quetglas et al., 2005; Relini et al., 2006; Ceriola et al., 2006). There also exist studies treating more specific biological aspects such as diet (Quetglas et al., 1998b; Quetglas et al., 1999; Vassilopoulou, 2006), growth (Massutí et al., 2000; Quetglas and Morales-Nin, 2004), reproduction (Rotllant et al., 2002; Chatzispyrou and Megalofonou, 2005), distribution and population structure, and dynamics (Massutí et al., 2001; Katsanevakis and Verriopoulos, 2006).

DEMERSAL MEGAFAUNAL COMMUNITIES

The knowledge on megafaunal communities (fishes, crustaceans and cephalopods) in the MED are mainly limited to the bathymetric range in which the commercial fishing operates, usually up to 800 m depth (e.g. Maynou et al., 1996; Massutí et al., 1996b; Ungaro et al., 1999; Quetglas et al., 2000; Demestre et al., 2000a; Colloca et al., 2003; Kallianiotis et al., 2004; Labropoulou and Papaconstantinou, 2004). Below this depth, the major available information on quantitative aspects of biomass and abundance or bathymetric distribution and community structure of deep megafaunal groups is found in the north-western MED down to 2200 m depth (Abelló and Valladares, 1988; Villanueva, 1992; Stefanescu et al., 1992a; Stefanescu et al., 1992b; Cartes and Sardà, 1993; Stefanescu et al., 1993; Stefanescu et al., 1994; Villanueva, 1995; Moranta et al., 1998; Maynou and Cartes, 2000; Massutí et al., 2004; Cartes et al., 2004a) and just recently down to 4000 m depth in both the western and central basins (Sardà et al., 2004b). Nevertheless, the maximum depth, prospected using free-fall baited cameras and traps, has been investigated in the EMED where the bathymetric distribution of some megafaunal species has been extended beyond 4000 m depth (Jones et al., 2003).

As reported in other areas, depth gradient is the main factor affecting the composition and structure of megafaunal assemblages in the MED (Cartes and Sardà, 1993; Sardà et al., 1994a; Moranta et al., 1998; Maynou and Cartes, 2000; Kallianiotis et al., 2000; D'Onghia et al., 2004). However, other factors such as light intensity and bottom type can also influence the spatial distribution of demersal species. It has been hypothesised that demersal assemblages could be determined by the benthic communities where they inhabit (Demestre *et al.*, 2000; Colloca *et al.*, 2003; Massutí and Reñones, 2005). This could be of special interest on the continental shelf, where the benthos is more developed than on the slope. On shelf bottoms, light is considered the most important environmental factor affecting the distribution of benthic organisms (Martí *et al.*, 2005; Ballesteros, 2006), especially the macro-algae (Ordines et al., 2007).

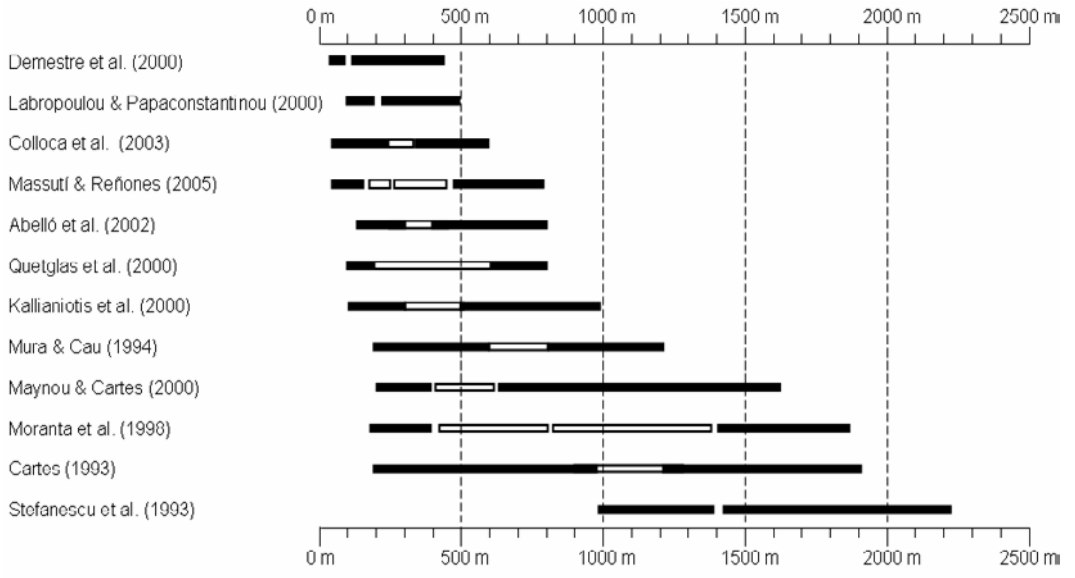


Figure 12. Summary of zonation schemes reported by some researchers in the Mediterranean for different taxonomic groups: fishes (Stefanescu et al., 1993; Moranta et al., 1998; Labropoulou and Papaconstantinou, 2000; Kallianiotis et al., 2000; Demestre et al., 2000a; Colloca et al., 2003; Massutí and Reñones, 2005), crustaceans (Cartes, 1993; Mura and Cau, 1994; Maynou and Cartes, 2000; Abelló et al., 2002), and cephalopods (Quetglas et al., 2000). Black and white sections indicate bathymetric ranges with relative homogeneous species composition and with high species turnover, respectively [adapted from Carney (2005)].

A comprehensive review about deep faunal zonation in the context of contemporary biogeography and oceanography studies, including the MED, has been provided by Carney (2005). According to this author, temperature, light, hydrostatic pressure and food availability are the main factors determining the zonation patterns, but others such as water mass, nature of substrate and biotic factors (predation, competition, dispersion, etc.) have also been frequently suggested. Depending on the taxonomic group and the geographical area, different depth faunal zones have been identified in the MED (Figure 12). For instance, four faunal zones has been identified in demersal fishes: two upper-slope zones at 200-400 m and 400-800 m; a middle slope zone at 800-1400 m; and lower slope, deeper than 1400 m (Moranta et al., 1998). The analysis of decapod crustaceans identified three main faunal zones in the south-west of the Balearic Islands: shelf-edge and upper-slope, 195-402 m; middle slope, 415-601 m; and lower middle slope, 626-1714 m (Maynou and Cartes, 2000). Cephalopods, which have motilities and feeding types similar to fish, showed a shelf fauna down to 100 m, a distinct upper slope fauna down to 800 and a wide band of transition in between (Quetglas et al., 2000). The different responses to depth gradient shown by each taxonomic group may be attributed to the distinct trophic levels occupied (Maynou and Cartes, 2000).

Fishes are the dominant taxonomic group on the continental shelf and slope in the WMED (Moranta et al., 2000; Ordines et al., 2006), but decapod crustaceans are also important, or even dominant in number, on the upper slope down to 800 m depth, being a sizeable fraction of the total megafaunal biomass (Cartes et al., 1994; Maynou et al., 1996; Maynou and Cartes, 2000). One of the most important features of fish communities is the

existence of a biomass peak around 1200 m depth (Stefanescu et al., 1993; Moranta et al., 1998; D'Onghia et al., 2004). Interestingly, Cartes et al. (2001) reported the existence of a suprabenthos biomass peak between 800 and 1300 m depth, the principal component of the Benthic Boundary Layer (Cartes, 1998a). As shown in several studies, the suprabenthos is a fundamental part of the diet of deep-water fishes (Macpherson, 1981; Carrassón and Matallanas, 1990; Carrassón and Cartes, 2002). Decapod crustaceans, which also prey on the suprabenthos (Cartes, 1994; Cartes, 1998b), peaks between 400-700 m (Maynou and Cartes, 2000), and Cartes et al. (2001) suggested that this may originate a phenomenon of aggressive exclusion due to the exploitation of similar resources. The decrease of fish species richness throughout the bathymetric range and the decrease of fish abundance on the upper slope, which remains constant below 500 m, is also characteristic of these megafaunal communities (Stefanescu et al., 1993; Moranta et al., 1998; D'Onghia et al., 2004).

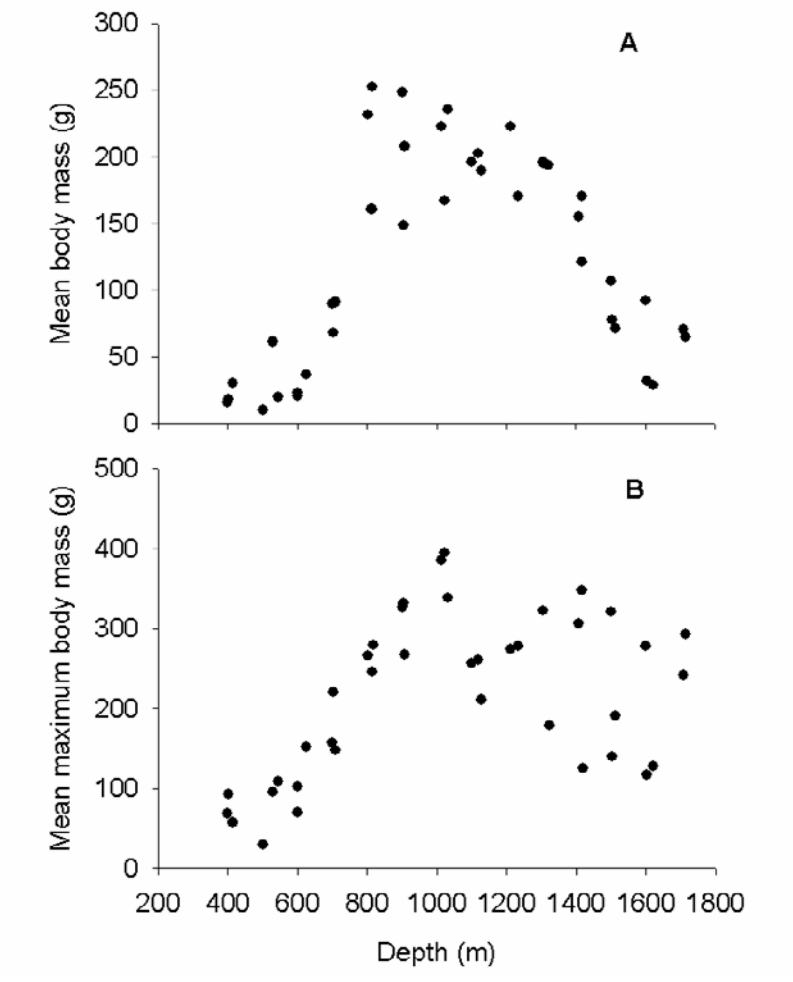


Figure 13. Trends in the mean body mass (A) and mean maximum body mass (B) of the demersal species from the continental slope community (400-1714 m depth) of the Balearic Islands (western Mediterranean). [Source: Moranta et al. (2004)].

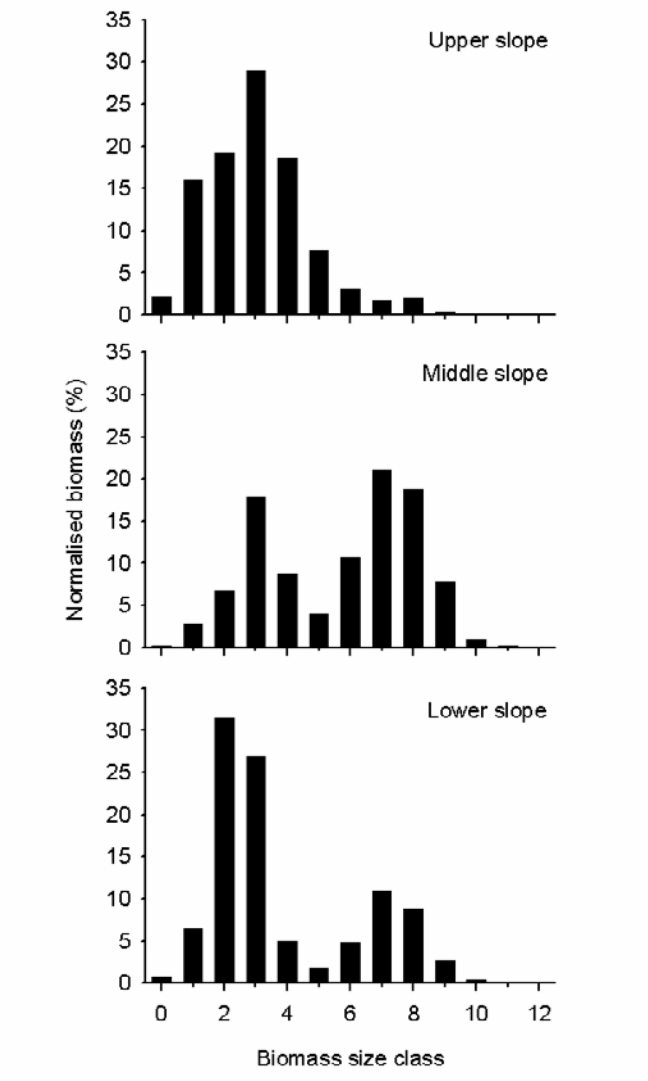


Figure 14. Normalised biomass spectra in the three demersal fish assemblages reported along the continental slope off the Balearic Islands (western Mediterranean): upper slope (400-800 m), middle slope (801-1400 m) and lower slope (1401-1714 m). The biomass size class corresponds to the $\log_2$ of weight [Source: Moranta et al. (2004)].

The peak of fish biomass on the middle slope is explained by species-specific bathymetric responses that leads to the predominance of larger individuals and larger species in this deep range (Figure 13). This is specially clear observing the normalised biomass spectra obtained in the upper, middle and lower slope (Figure 14). While the absence of large specimens on the upper slope could be explained by the high fishing exploitation rates (Moranta et al. 2004 and references cited therein), the low biomass levels found on the lower slope could be due to minor availability of trophic resources at these depths (Stefanescu et al., 1993; Carney, 2005). Such a reduced trophic resources on the lower slope could has imposed strong selection pressures for the diversification of fish trophic strategies, which may explain the coexistence of small and large-sized species (e.g. *Bathypterois mediterraneus*, *Lepidion*

lepidion, *Alepocephalus rostratus* and *Centroscymnus coelolepis* (Carrassón et al., 1992; Carrassón et al., 1997; Carrassón and Matallanas, 1998).

Geographical and temporal distribution studies in the MED have so far received scarce attention. Considering large spatial scales (macro-scales), megafaunal assemblage studies carried out in the WMED have detected geographical variations related to hydrodynamic conditions, surface production inputs and topography and bottom characteristics (Abelló et al., 2002; Massutí et al., 2004; Gaertner et al., 2005). However, it is not usual to detect geographical differences at meso-scales owing to the strong effect of depth, which masks the contribution of secondary environmental variables showing variations in closed areas such as hydrodynamics and geo-morphology. Most previous meso-scale studies did not consider this effect and thus spatial variations were mainly attributed to the depth gradient. Analysis of deep-sea demersal fish assemblages in the North Aegean Sea (Labropoulou and Papaconstantinou, 2000) and assemblages in the trawling fishing grounds off the Balearic Islands at 50-800 m depth (Massutí and Reñones, 2005) constitute notable exceptions. Labropoulou and Papaconstantinou (2000) found geographical differences in two areas 200 km apart, which were mainly attributed to bottom and oceanographic characteristics. Massutí and Reñones (2005) also found geographical differences between the western and eastern coast basins off Mallorca, which could be related to differences in habitat and macro-epibenthic communities.

Referring to temporal variations, there are fewer studies dealing with megafaunal communities (Cartes et al., 1994; Sardà et al., 1994a; Maynou et al., 1996; Ungaro et al., 1998; Cartes, 1998a; Maynou and Cartes, 2000; Kallianiotis et al., 2000) than on single species (e.g. (Demestre and Abelló, 1993; Sardà et al., 1994b; Morales-Nin et al., 1996a; Massutí et al., 1996a; Morales-Nin et al., 1996b; Labropoulou et al., 1997; Recasens et al., 1998; Matarrese et al., 1998; D'Onghia et al., 1998b; Maynou et al., 2003). Seasonal variations in demersal assemblages observed in the north-western MED have been related to seasonal changes in food resources (De Bovée et al. 1990, Cartes 1998a). This temporal variability is probably reinforced by the presence of submarine canyons, which are known to have strong seasonal variations in their physical conditions (e.g. bottom currents, sediment re-suspension and inputs of organic matter) related to discharges of continental run-off (Buscail et al. 1990, Monaco et al. 1990). Not surprisingly, seasonal cycles related to the spring peak of planktonic production (e.g. Estrada et al. 1985) have also been reported within the canyons for other taxonomic groups, such as hydromedusa (Gili et al. 2000). In general, the maximum accumulation of organic matter on the canyons' floors takes place in late spring (Buscail et al. 1990). The highest values of species richness and density were also observed in late spring in the submarine canyon, summer in the upper slope, and autumn in the middle slope, which implies a temporal delay in the transfer through the water column of the organic matter produced in spring in shallow waters (Moranta et al., in press). As discussed above, submarine canyons are characterised by a rapid sediment bypass to deeper zones, explaining the quick response of their fish assemblages to the peak of seasonal primary production in surface waters. The importance of canyons in determining temporal changes was also reflected in a comparison of the crustacean assemblages of two WMED areas (Maynou and Cartes, 2000). While seasonal variations were observed in the Balearic sub-basin, where there exist submarine canyons, no variations appeared in the Algerian sub-basin, an area without such geological structures and where marine snow has been identified as the single primary source of food (Polunin et al. 2001).

THE ECOSYSTEM APPROACH TO FISHERIES: PLACING RESOURCES IN ITS ENVIRONMENT

Environment and Population Dynamics Relationships

Faunistic assemblages have constituted the first step to go beyond individual approaches to the study of single species. A further step in the path to analyse the complexity present in the structure and dynamics of ecosystems is the study of organisms along with the biotic and abiotic characteristics of the habitats where they live. Relationships between environmental factors and fluctuations of fish stocks started to be recognized at the end of the 19th century and beginning of the 20th (Helland-Hansen and Nansen, 1909; Hjort, 1914). In spite of this early recognition, the study of the influence of environmental factors on living marine resources began to be developed only from the second half of the 20th century, in the Pacific Ocean (www.calcofi.org) and later in the North Atlantic Ocean (Stenseth et al., 2004). Nowadays, although fishing exploitation is considered to be one of the principal factors that determines the dynamics of demersal resources, it is also recognized that environmental variables, both abiotic (climate and hydrodynamics) and biotic (trophic resources and predators), can cause the observed oscillations at the intra- as well as the inter-annual level, not only in the population dynamics but also in the production of exploited species (e.g. Cushing, 1982; Laevastu and Favorite, 1988). Although environmental factors principally affect the mortality in the first phases of species development, which to a great extent determines their recruitment and abundance, they also influence other population parameters such as growth rates, distribution, migration, natural mortality and catchability. In the MED, a very important part of the demersal fisheries is based on the exploitation of young individuals (Lleonart and Maynou, 2003) and, therefore, they depend largely on the recruitment, which is one of the population parameters most vulnerable to environmental factors.

In the WMED, important fluctuations in the distribution, structure and population dynamics of the principal demersal species have been described at both the meso- and macro-scale, which affect the yield of the trawl fisheries (Caddy, 1993). These changes have been observed both in the species exploited on the shelf (e.g. Oliver, 1993; Quetglas *et al.*, 1998a; Lloret and Lleonart, 2002) and the slope (Carbonell et al., 1999; Abelló et al., 2002; Tobar and Sardà, 1992; Sardà et al., 1994, 1997; García-Rodríguez, 2003). The first authors reporting these fluctuations were Astudillo and Caddy (1988), who analysed the annual landings of hake and red mullet off the Balearic Islands from 1940 to 1975 and found a marked periodicity of 12-15 years. A similar situation was observed afterwards in the red shrimp in other areas of the western Mediterranean (Tobar and Sardà, 1987). Using spectral analysis, Quetglas et al. (1998) and Carbonell et al. (1999) obtained a period of 7.7 and 8.7 years in common octopus and red shrimp respectively (Figure 15), but they did not reported the causes of such fluctuations, only noting that they could be related to some regional climatic pattern rather than to a particular behaviour of a given species. More recently, the fluctuations observed in hake and red shrimp catches has been related with macro, meso-scale and local climatic indices, such as NAO (North-Atlantic Oscillation), MO (MED Oscillation) and IDEA index, respectively (Figure 16, Massutí et al., 2007). These authors showed how two oceanographic scenarios around the Balearic Islands could influence the population dynamics of hake and red shrimp, especially for recruitment, that seemed to be enhanced

during low NAO and IDEA indices periods. During these periods, colder-than-normal winter produces high generation of cold WIW in the Gulf of Lions, which flow southwards and reach the Balearic Islands channels in spring, increasing the productivity in the area. This oceanographic scenario could also be favourable to the distribution of hake on the fishing grounds where trawl fleet target this species, increasing its accessibility to the fishery. Both spawning stock and abundance of red shrimp seem to be also enhanced by high MO index periods, which could reflect the increased presence of the saline and warm LIW in the study area, extending over the fishing grounds of this species. Fluctuations of landings and environemental conditions in the WMED were also analysed by Lloret et al. (2001), which found that monthly catches and CPUE of some commercial species were significantly possitively correlated with run-off of local rivers and the wind mixing index during the spawning season, with time lags of less than a year. Their results showed that enhanced hydroclimatic conditions in the north-western MED were favourable for the productivity of the fish and invertebrate stocks, and suggested the presence of linkage between recruitment of MED species and local (river discharges, wind conditions) and global (NAO) environemental conditions.

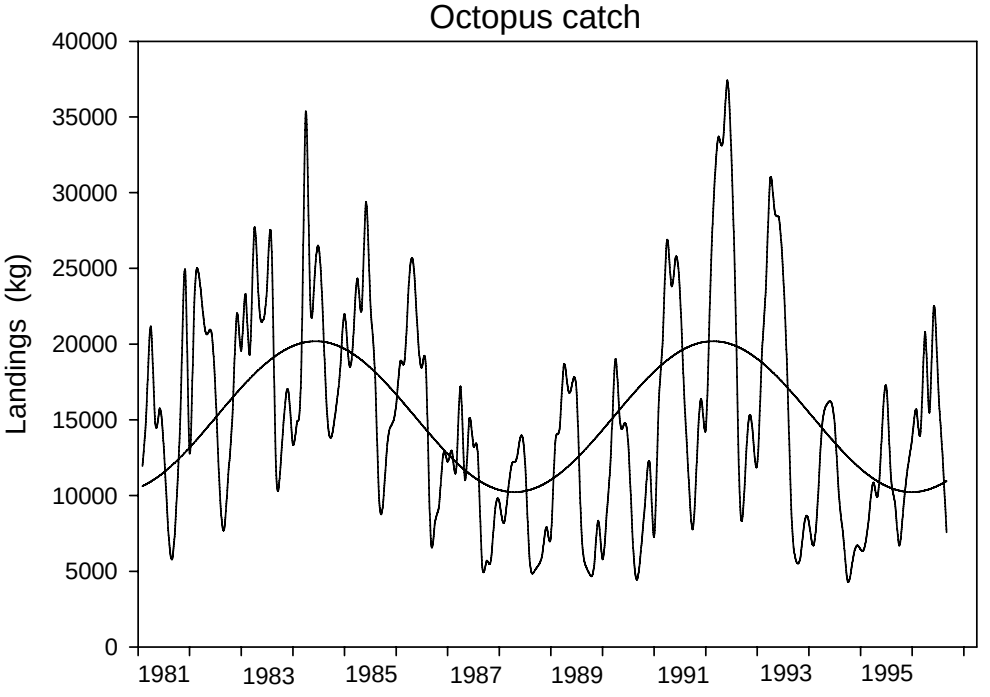


Figure 15. Monthly landings, from January 1981 to August 1996, and the sinusoidal function that best fits the data in common octopus *Octopus vulgaris* caught by trawlers on the continental shelf of the Balearic Islands (western Mediterranean). Spectrum analysis showed that this series had a significant periodicity of 92 months (7.7 years). [Source: Quetglas et al. (1998a)].

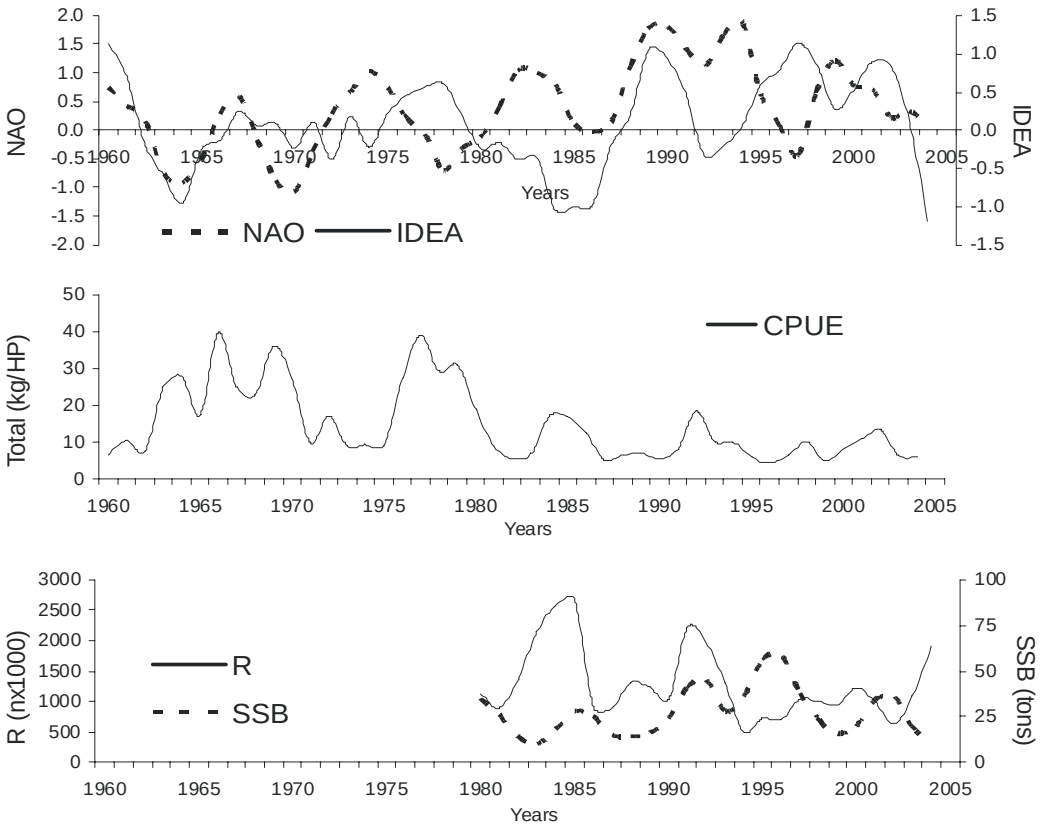


Figure 16. Available data series of environmental variables (three year running averaged NAO and IDEA indices) and population dynamics parameters for hake (CPUE: catch per unit effort; R: recruitment; SSB: spawning stock biomass) in the Balearic Islands [Source: Massutí et al. (2007)].

Other environmental factors, such as light and bottom characteristics have also been analysed. Seafloor topography and sediment characteristics mainly affect crustaceans, as they mark the distribution of red shrimp *A. antennatus* (Tudela *et al.*, 2003; Guijarro *et al.*, in press), or the abundance and some biological parameters of the Norway lobster (Aguzzi et al., 2003; Maynou and Sardà, 1997, 2001). The catchability of this benthic species is also affected by light intensity (Maynou and Sardà, 2001; Aguzzi et al., 2003). Sediment characteristics also play an important role in the nursery areas of European hake (Maynou et al., 2003). Finally, water masses characteristics have an influence not only on the abundance but also on the condition of demersal species, as more productive waters improve the condition both in fishes (Lloret et al., 2002, 2005; Hidalgo et al., 2007) and crustaceans (Guijarro et al., 2007), favouring also the recruitment (Bas and Calderón-Aguilera, 1989).

Ecosystem Tropho-dynamic Models

Tropho-dynamic models analyse the fluxes of matter and energy in the ecosystems based on the trophic relationships among their components. Nowadays, Ecopath with Ecosim (EwE; Christensen and Walters, 2004) is the most popular and widely used tropho-dynamic model

but, in spite of such popularity, it has scarcely been applied in the MED. Daskalov (2002) used this software to look for empirical evidence for ecosystem effects of fishing in the Black Sea, trying to explain the deteriorated environmental conditions observed during recent decades. He built a balanced model using 15 ecological groups and simulated the ecosystem dynamics over 30 years, assuming alternative scenarios of increasing fishing pressure and eutrophication. Based on the results obtained, the author invoked a trophic cascade produced by the removal of predators as a mechanism to explain the observed changes. Libralato et al. (2002) also used EwE to compare the energy flows of two habitats representative of two typical subsystems of the Venice Lagoon, the seagrass meadow, as a mature ecosystem, and the fishing grounds of a bivalve species, subjected to mechanical clam harvesting. Pinnegar and Polunin (2004) developed an aggregated biomass-based simulation model of an inflalittoral zone to carry out simulations where fishing intensity and catch selection were varied. The short recovery times reported by this work seems to indicate that the MED rocky littoral ecosystem is relatively resilient and mature compared to many other coastal and shelf systems. Finally, Coll et al. (2006) constructed a mass-balance model of the exploited continental shelf and upper slope of the north WMED in order to characterise its structure and functioning and to describe ecosystem impacts of fishing. The ecosystem was dominated by the pelagic domain, which accounted for the main biomass and catches and was where flows mainly occurred. The ecotrophic efficiencies and mortality rates suggested that the system is highly constrained by predators (natural predators and fishery) and was the intermediate-low development, in terms of Odum´s theory of ecosystem, was related to high fishing intensity. Following the authors, the low trophic level of the catch was in line with the long history of fishing exploitation in the area.

FUTURE TRENDS

Despite the long tradition in fisheries oceanography investigations and the progress done during the last decades, available information on this topic in the MED is still not comparable with that of the North Eastern Atlantic or the coasts of United States and Canada, even though problems related to reliability and accuracy of data have also been recognised in these areas. Except for some peculiarities related to the MED context (multispecies and multigear exploitation), research on fisheries oceanography in this sea will probably follow the same trends observed at the worldwide level. Presently ongoing studies and those carried out in the immediate future are surely going to be developed in the framework of the ecosystem approach to fisheries (EAF). Although studies on the biology of single species will continue to be done, mainly on certain aspects such as age, fecundity or distribution, the bulk of the marine research would be directed to those issues typically studied by ecology, such as relations among different species and among same species and their environment. To fulfil this new requirement, a strategy based upon innovative science that will address the complexity of marine ecosystems, linking ecological processes to ecosystem-level patterns, is needed (Cury, 2004). For instance, the construction of meaningful mass-balance models provides the basic constraints to understand processes within ecosystems (Cole, 2005). Moreover, development of ecosystem-based models appropriate to the MED is indispensable to provide useful tools to diagnose the health of the resource, to evaluate its potential, and

recommend management polices (Lleonart and Recasens, 1996). Thus, present and future research will also be characterized by the general application of analytical tools that have recently been introduced in ecological studies (geographical information systems, generalized additive models, artificial neural networks, genetic algorithms, and fuzzy logic), the introduction of new methodologies from other scientific fields and even the development of new ones. For this purpose, a multidisciplinary scientific expertise, through collaboration in interdisciplinary teams, is essential to obtain new knowledge and to address the impact of global changes on marine ecosystems (Cury, 2004; Stenseth et al., 2005). These interdisciplinary teams should be able to detect regime shifts that represent crucial ecological patterns for the EAF, as there are sudden changes in structure and functioning of marine ecosystems, but they should also be able to understand the nature of such ecosystem changes (Cury, 2004).

In the field of demersal fisheries assessment, some future lines can be discerned from ongoing studies in other geographical areas. In spite of the general present and future tendency to the EAF, monospecific assessment methods (production and analytical models) will probably continue to be applied, but they will certainly benefit from a series of analytical tools related to cope with uncertainty in the results such as bootstrap, Monte Carlo or Bayesian analysis (McAllister and Kirkwood, 1998). Standardisation of approaches to data collection, monitoring, statistics compilation and research are needed in light of the problems of over-fishing and environmental degradation facing the MED. The development of the EAF needs to integrate 'traditional' single-species objectives without leaving traditional approaches that have never been fully implemented. Thus, single-species models and operational single-species objectives must be embedded as an important component of the EAF (Mace, 2004). Approaches accounting for the multi-species characteristics of the MED fisheries need to be implemented, integrating biological and economic aspects (Lleonart and Maynou, 2003). Up to date, the lack of suitable data have prevented the use of approaches with high data requirements such as multispecies models (Hollowed et al., 2000), and this could also be challenged in the next future. The worldwide acceptance of the EAF, along with the general use of the tropho-dynamic model Ecopath with Ecosim (EwE) in the present marine research, will probably produce a bloom in the use of this software in future MED studies, an area where such methodology has still been poorly used, as was seen above. Along with this improvement, fishery studies should be extended to the African coast, where there is an important lack of information.

Other important aspects to be taken into account for stock assessment purposes are the spatio-temporal component of commercial catches with its inherent variability and the relationships between environment and marine resources, topics that have been poorly studied in the MED. However, both issues are very important in the area owing to two main features. Firstly, trawl catches are mainly composed by recruits of 0-1 years old (Lleonart and Maynou, 2003), and secondly many trawl landings in the WMED have shown cyclic fluctuations during recent decades, which do not seem to be totally dependent of fishing activities (e.g. Oliver, 1993; Lloret *et al.*, 2001). These characteristics make long term previsions, as those usually derived using the classical approaches, specially sensitive to recruitment fluctuations and to the spatio-temporal variation of the fishing pattern. It should be necessary to consider the ecosystem interactions (e.g. environmental conditions) that may produce changes in biomass, recruitment, growth and natural mortality rates. More research effort to improve the knowledge on stock-recruitment relationships and on population's compensatory density-

dependent controls is thus necessary. Therefore, assessment models addressing the spatial-temporal component should be developed to provide advice regarding the choice of an optimal effort partitioning by area, season and fishing gear, aimed at the maximisation of incomes in a precautionary context.

Finally, issues of capital importance in the present and next future are those associated to environmental conservation. These aspects will certainly be of paramount importance in the MED, an area supporting high population densities, especially in summer with tourism overcrowding, and threatened by many environmental problems such as construction, pollution, over-fishing and loss of biodiversity and natural habitats. Marine protected areas (MPAs), considered as no take zones, are an extreme case of the precautionary approach (Lauck et al. 1998), and provide a refuge in space rather than a refuge in numbers, the later being the aim of most tradicional fisheries management measures (CIESM, 2000). It is spected that MPAs may simultaneously satisfay various objectives of ecosystem-based approach to the management of marine resources: preserving ecosystem diversity and structure, rebuilding overexploited fish stocks or directly impacted species, maintaining within-species genetic variability and trohpic level balance (Gislason et al., 2000; Browman and Stergiou, 2004). As a whole, the MPAs in the MED are small in size, and there are no effective large-sized MPAs despite natural exceptions, such as deep waters areas (beyond 800 m depth) inaccessible to demersal fishing (CIESM, 2000). This is a fundamental aspect for the stablishment of new MPAs in the MED because if the choice of their location, size and number is well grouped in marine ecology and fishery oceanography, then MPAs stand to become an effective tool for conservation and management (Browman and Stergiou, 2004). Acoording to Browman and Stergiou (2004), in order for this to be realized, two closely related steps are required, the delimitation and definition of Large Marine Ecosystems (LME, e.g. Sherman and Duda, 1998) and a more ecological/oceanographyc orientation in fisheries science. LMEs are large regions of marine space ecompasing coastal areas from river basins and estuarines to the seaward boundaries of continental shelf and the outer margins of the major current systems characterised by: (i) bathymetry, (ii) hydrography, (iii) productivity, and (iv) trophically dependent populations. The future fisheries science approach require: (i) identifying and mapping the key faunistic component and the biodiversity hot pots, (ii) describing the life cycles of these key components, (iii) spatially mapping the life cycles of key species, and (iv) identifying the special oceanographyc features associated with the retention of and nursery areas of these key components.

REFERENCES

Abella, A., Belluscio, A., Bertrand, J., Carbonara, P.L., Giordano, D., Sbrana, M., Zamboni, A., 1999. Use of MEDITS trawl survey data and commercial fleet information for the assessment of some Mediterranean demersal resources. *Aquatic Living Resources* 12, 155-166.

Abella, A., Caddy, J.F., Serena, F., 1997. Do natural mortality and availability decline with age? An alternative yield paradigm for juvenile fisheries, illustrated by the hake *Merluccius merluccius* fishery in the Mediterranean. *Aquatic Living Resources* 10, 257-269.

Abella, A., Serena, F., Ria, M., 2005. Distributional response to variations in abundance over spatial and temporal scales for juveniles of European hake (*Merluccius merluccius*) in the Western Mediterranean Sea. *Fisheries Research* 71, 295-310.

Abelló, P., Carbonell, A., Torres, P., 2002. Biogeography of epibenthic crustaceans on the shelf and upper slope off the Iberian Peninsula Mediterranean coasts: implications for the establishment of natural management areas. *Scientia Marina* 66, 183-198.

Abelló, P., Valladares, F.J., 1988. Bathyal decapod crustaceans of the Catalan Sea (Northwestern Mediterranean). *Mésogée* 48, 97-102.

Acosta, A., Canals, M., López-Martínez, L., Muñóz, A., Herranz, P., Urgeles, R., Palomo, C., Casamor, J.L., 2002. The Balearic Promontory geomorphology (western Mediterranean): morphostructure and active processes. *Geomorphology* 49, 177-204.

Aguirre, H., Sánchez, P., 2005. Feeding resource partitioning between *Mullus barbatus* and *M. surmuletus* in the Catalan Sea (northwestern Mediterranean). *Ciencias Marinas* 31, 429-439.

Aguzzi, J., Sardà, F., Abelló, P., Company, J., Rotllant, G., 2003. Diel and seasonal patterns of *Nephrops norvegicus* (Decapoda: Nephropidae) catchability in the western Mediterranean. *Marine Ecology Progress Series* 258, 201-211.

Aguzzi, J., Sardà, F., Allue, R., 2004. Seasonal dynamics in *Nephrops norvegicus* (Decapoda: Nephropidae) catches off the Catalan coasts (Western Mediterranean). *Fisheries Research* 69, 293-300.

Aldebert, Y., Recasens, L., Lleonart, J., 1993. Analysis of gear interactions in a hake fishery: The case of the Gulf of Lions (NW Mediterranean). *Scientia Marina* 57, 207-217.

Aldebert, Y., 1997. Demersal resources of the Gulf of Lions (Mediterranean). Impact on fish diversity. *Vie et Milieu* 47, 275-284.

Aldebert, Y., Recasens, L., 1996. Comparison of methods for stock assessment of European hake *Merluccius merluccius* in the Gulf of Lions (Northwestern mediterranean). *Aquatic Living Resources* 9, 13-22.

Alemany, F., Alvarez, F., 2003. Determination of effective fishing effort on hake *Merluccius merluccius* in a Mediterranean trawl fishery. *Scientia Marina* 67, 491-499.

Arnone, R., 1994. The temporal and spatial variability of chlorophyll in the western Mediterranean. In: La violette, P.E. (Ed.), Seasonal and Interannual Variability of the Mediterranean Sea. *Coastal and Estuarine Studies. American Geophysical Union*, Washington DC, pp. 195-225.

Astraldi, M., Balopoulos, S., Candela, J., Font, J., Gacic, M., Gasparini, G.P., Manca, B., Theocharis, A., Tintoré, J., 1999. The role of straits and channels in understanding the characteristics of Mediterranean circulation. *Progress in Oceanography* 44, 65-108.

Astudillo, A., Caddy, J.F., 1988. Periodicidad de los desembarcos de merluza (*Merluccius merluccius*) y salmonete (*Mullus* spp.) en la Isla de Mallorca. *Int. Symp. Long Term Changes Mar. Fish Pop.*, Vigo, Spain, pp. 221-234 (in Spanish, with English Abstract).

Bahamon, N., Sardà, F., Suuronen, P., 2006. Improvement of trawl selectivity in the NW Mediterranean demersal fishery by using a 40 mm square mesh codend. *Fisheries Research* 81, 15-25.

Ballesteros, E., 2006. Mediterranean coralligenous assemblages: a syntesis of present knowledge. *Oceanography and Marine Biology*: *An Annual Review* 44, 123-195.

Bas, C., 1966. La gamba rosada (*Aristeus antennatus*). Publicaciones técnicas de la junta de estudios de pesca 1, 15pp.

Bas, C., Calderon-Aguilera, L.E., 1989. Effect of anthropogenic and environmental factors on the blue whiting *Micromesistius poutassou* off the Catalonian coast, 1950-1982. *Marine Ecology Progress Series* 54, 221-228

Bas, C., Rubio, M., 1959. Fishing grounds of the Spanish Catalonian coast. *Proceedings of the General Fishery Council for the Mediterranean* 5, 89.

Bas, C., Sardà, F., 1998. Long-term morphometric variations in a population of the deep-sea shrimp *Aristeus antennatus* (Risso, 1816) (Decapoda, Aristeidae). *Crustaceana* 71, 369-377.

Bearzi, G., Politi, E., Agazzi, S., Azzellino, A., 2006. Prey depletion caused by overfishing and the decline of marine megafauna in eastern Ionian Sea coastal waters (central Mediterranean). *Biological Conservation* 127, 373-382.

Belcari, P, Viva, C, Mori, M, De Ranieri, S., 2003. Fishery and biology of *Aristaeomorpha foliacea* (Risso, 1827) (Crustacea: Decapoda) in the Northern Tyrrhenian Sea (Western Mediterranean). *Journal of the Northwest Atlantic Fisheries Science* 31, 195-204.

Belcari, P., Ligas, A., Viva, C., 2006. Age determination and growth of juveniles of the European hake, *Merluccius merluccius* (L., 1758), in the northern Tyrrhenian Sea (NW Mediterranean). *Fisheries Research* 78, 211-217.

Belcari, P., Sartor P., Sánchez, P., Demestre, M., Tsangridis, A., Leondarakis, P., Lefkaditou, E., Papaconstantinou, C., 2002. Exploitation patterns of the cuttlefish, *Sepia officinalis* (Cephalopoda, Sepiidae), in the Mediterranean Sea. *Bulletin of Marine Science* 71, 187-196

Bertrand, J.A., Aldebert, Y., Souplet, A., 1998. Temporal variability of demersal species in the Gulf of Lions from trawl surveys (1983-1997). *IFREMER Actes de Colloques* 26, 153-164.

Bertrand, J.A., Gil de Sola, L., Papaconstantinou, C., Relini, G., Souplet, A., 2000. Contribution on the distribution of elasmobranchs in the Mediterranean (from the MEDITS surveys). *Biologia Marina Mediterranean* 7, 1-15.

Bertrand, J.A., Gil de Sola, L., Papaconstantinou, C., Relini, G., Souplet, A., 2002. The general specifications of the MEDITS surveys. *Scientia Marina* 66, 9-17.

Biagi, F., Sartor, P., Ardizzone, G.D., Belcari, P., Belluscio, A., Serena, F., 2002. Analysis of demersal assemblages off the Tuscany and Latium coasts (north-western Mediterranean). *Scientia Marina* 66, 233-242.

Bianchini, M.L., Di Stefano, L., Ragonese, S., 1998. Size and age at onset of sexual maturity of female Norway lobster *Nephrops norvegicus* L. (Crustacea: Nephropidae) in the Strait of Sicily (Central Mediterranean Sea). *Scientia Marina* 62, 151-159.

Bordehore C., Ramos-Esplá, A.A., Riosmena-Rodríguez, R., 2003. Comparative study of two maërl beds with different otter trawling history, southeast Iberian Peninsula. *Aquatic Conservation: Marine and Freshwater Ecosystems* 13, 43-54.

Bosc, E., Bricaud, A., Antoine, D., 2004. Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from four years of SeaWiFS observations. *Global Biogeochemical Cycles* 18, GB1005 1-17.

Bosley, K.L., Lavelle, J.W., Brodeur, R.D., Wakefield, W.W., Emmett, R.L., Baker, E.T., Rehmke, K.M., 2004. Biological and physical processes in and around Astoria submarine Canyon, Oregort, USA. *Journal of Marine Systems* 50, 21-37.

Bozzano, A., Catalán, I.A., 2002. Ontogenetic changes in the retinal topography of the European hake, *Merluccius merluccius*: implications for feeding and depth distribution. *Marine Biology* 141, 549-559.

Bozzano, A., Recasens, L., Sartor, P., 1997. Diet of the European hake *Merluccius merluccius* (Pisces: Merlucciidae) in the western Mediterranean (Gulf of Lions). *Scientia Marina* 61, 415-415.

Bozzano, A., Sardà, F., Rios, J., 2005. Vertical distribution and feeding patterns of the juvenile European hake, *Merluccius merluccius* in the NW Mediterranean. *Fisheries Research* 73, 29-36.

Broman H.I. Stergiou K.I., 2004. Marine protected areas as a central alement of ecosystem-based management: defining their location, size and number. In: Browman H.I., Stergiou K.I. (Eds.), Perspectives on ecosystem-based approaches to the management of marine resources. *Marine Ecology Progress Series* 274, 285-291.

Browman, H.I., Stergiou, K.I., 2004. Perspectives on ecosystem-based approaches to the management of marine resources. *Marine Ecology Progress Series* 274, 269-270.

Buscail, R., Pocklington, R., Daumas, R., Guidi, L., 1990. Fluxes and budget of organic-matter in the benthic boundary-layer over the Northwestern Mediterranean margin. *Continental Shelf Research* 10, 1089-1122.

Caddy, J.F., 1993. Some future perspectives for assessment and management of Mediterranean fisheries. *Scientia Marina* 57, 121-130.

Caddy, J.F., R. Refk and T. Do-Chi. 1995. Productivity estimates for the Mediterranean: evidence of accelerating ecological change. *Ocean and Coastal Management*, 26, 1-18.

Campisi, S., Mura, M., Cau, A., 1998. Biological aspects of *Plesionika antigai* (Zariquiey Alvarez, 1955) (Crustacea: Decapoda : Pandalidae) in central-western Mediterranean. *Journal of Natural History* 32, 1453-1462.

Canals, M., Puig, P., de Madron, X.D., Heussner, S., Palanques, A., Fabres, J., 2006. Flushing submarine canyons. *Nature* 444, 354-357.

Capapé, C., Tomasini, J.A., Quignard J.P., 2000. Les elasmobranches pleurotrêmes de la côte du Languedoc (France, Méridionale): observations biologiques et démographiques. *Vie et Milieu*, 50, 123-133.

Carbonell, A., Lloret, J., Demestre, M., 2007. Relationship between condition and recruitment success of red shrimp (*Aristeus antennatus*) in the Balearic Sea (Northwestern Mediterranean). *Journal of Marine Systems* (in press).

Carbonell, A., Alemany, F., Merella, P., Quetglas, A., Roman, E., 2003. The by-catch of sharks in the western Mediterranean (Balearic Islands) trawl fishery. *Fisheries Research* 61, 7-18.

Carbonell, A., Azevedo, M., 2003. Application of non-equilibrium production models to the red shrimp (*Aristeus antennatus*, Risso, 1816) fishery in the northwestern Mediterranean. *Fisheries Research* 65, 323-334.

Carbonell, A., Carbonell, M., Demestre, M., Grau, A., Monserrat, S., 1999. The red shrimp *Aristeus antennatus* (Risso, 1816) fishery and biology in the Balearic Islands, Western Mediterranean. *Fisheries Research* 44, 1-13.

Carbonell, A., Grau, A., Lauronce, V., Gomez, C., 2006. Ovary development of the red shrimp, *Aristeus antennatus* (Risso, 1816) from the northwestern Mediterranean Sea. *Crustaceana* 79, 727-743.

Carbonell, A., Martín, P., De Ranieri, S., Wedis, T., 1998. Discards of the western Mediterranean trawl fleets. *Rapport de la Commission Internationale pour l'Explration Scientifique de la Mer Méditerranée* 35, 392-393.

Carlucci, R., D'Onghia, G., Sion, L., Maiorano, P., Tursi, A., 2006. Selectivity parameters and size at first maturity in deep-water shrimps, *Aristaeomorpha foliacea* (Risso, 1827) and *Aristeus antennatus* (Risso, 1816), from the north-western Ionian sea (Mediterranean sea). *Hydrobiologia* 557, 145-154

Carney, R.S., 2005. Zonation of deep biota on continental margins. *Oceanography and Marine Biology: An Annual Review* 43, 211-278.

Carpine, C., 1970. Ecologie de l'étage bathyal dans la Mediterraneé occidentale. *Memoires de l'Institut Oceanographique (Monaco)* 2, 1-146.

Carrassón, M., Cartes, J.E., 2002. Trophic relationships in a Mediterranean deep-sea fish community: partition of food resources, dietary overlap and connections within the benthic boundary layer. *Marine Ecology Progress Series* 241, 41-55.

Carrassón, M., Matallanas, J., 1990. Preliminary data about the feeding habits of some deep-sea Mediterranean fishes. *Journal of Fish Biology* 36, 461-463.

Carrassón, M., Matallanas, J., 1998. Feeding habits of *Alepocephalus rostratus* (Pisces: Alepocephalidae) in the western Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* 78, 1295-1306.

Carrassón, M., Matallanas, J., Casadevall, M., 1997. Feeding strategies of deep-water morids on the western Mediterranean slope. *Deep-Sea Research I* 44, 1685-1699.

Carrassón, M., Stefanescu, C., Cartes, J.E., 1992. Diets and bathymetric distributions of two bathyal sharks of the Catalan deep sea. *Marine Ecology Progress Series* 82, 21-30.

Cartes, J.E., Madurell, T., Fanelli, E., López-Jurado, J.L., 2007. Dynamics of suprabenthos-zooplankton communities around the Balearic Islands (NW Mediterranean): influence of environmental variables and effects on the biological cycle of *Aristeus antennatus*. *Journal of Marine Systems* (in press).

Cartes, J.E., 1993. Deep-sea decapod fauna of the western Mediterranean: bathymetric distribution and biogeographic aspects. *Crustaceana* 65, 29-40.

Cartes, J.E., 1994. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea (western Mediterranean). *Marine Biology* 120, 639-648.

Cartes, J.E., 1998a. Dynamics of the bathyal Benthic Boundary Layer in the northwestern Mediterranean: depth and temporal variations in macrofaunal-megafaunal communities and their possible connections within deep-sea trophic webs. *Progress in Oceanography* 41, 111-139.

Cartes, J.E., 1998b. Feeding strategies and partition of food resources in deep-water decapod crustaceans (400-2300 m). *Journal of the Marine Biological Association of the United Kingdom* 78, 509-524.

Cartes, J.E., Company, J.B., Maynou, F., 1994. Deep-water decapod crustacean communities in the Northwestern Mediterranean: influence of submarine canyons and season. *Marine Biology* 120, 221-229.

Cartes, J.E., Gremare, A., Maynou, F., Villora-Moreno, S., Dinet, A., 2002. Bathymetric changes in the distributions of particulate organic matter and associated fauna along a deep-sea transect down the catalan sea slope (Northwestern Mediterranean). *Progress in Oceanography* 53, 29-56.

Cartes, J.E., Maynou, F., Morales-Nin, B., Massutí, E., Moranta, J., 2001. Trophic structure of a bathial benthopelagic boundary layer community south of the Balearic Islands (southwestern Mediterranean). *Marine Ecology Progress Series* 215, 23-35.

Cartes, J.E., Maynou, F., Moranta, J., Massutí, E., Lloris, D., Morales-Nin, B., 2004a. Patterns of bathymetric distribution among deep-sea fauna at local spatial scale: comparison of mainland vs. insular areas. *Progress in Oceanography* 60, 29-45.

Cartes, J.E., Rey, J., Lloris, D., de Sola, L.G., 2004b. Influence of environmental variables on the feeding and diet of European hake (*Merluccius merluccius*) on the Mediterranean Iberian coasts. *Journal of the Marine Biological Association of the United Kingdom* 84, 831-835.

Cartes, J.E., Sardà, F., 1989. Feeding ecology of the deep-water Aristeid Crustacean *Aristeus antennatus*. *Marine Ecology Progress Series* 54, 229-238.

Cartes, J.E., Sardà, F., 1993. Zonation of deep-sea decapod fauna in the Catalan Sea (Western Mediterranean). *Marine Ecology Progress Series* 94, 27-34.

Castro, M., Gancho, P., Henriques, P., 1998. Comparison of several populations of Norway lobster, *Nephrops norvegicus* (L.), from the Mediterranean and adjacent Atlantic. A biometrics study. *Scientia Marina* 62, 71-79.

Ceriola, L., Ungaro, N., Toteda, F., 2006. Some information on the biology of *Illex coindetii* Verany, 1839 (Cephalopoda, Ommastrephidae) in the South-Western Adriatic Sea (Central Mediterranean). *Fisheries Research* 82, 41-49.

Chatzispyrou, A., Megalofonou, P., 2005. Sexual maturity, fecundity and embryonic development of the spiny dogfish, *Squalus acanthias*, in the eastern Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* 85, 1155-1161.

Christensen, V., Walters, C.J., 2004. Ecopath with Ecosim: methods, capabilities and limitations. *Ecological Modelling* 172, 109-139.

CIESM, 1998. Gaps in Mediterranean Fishery Science. *CIESM Workshop Series* 5, 39 pp.

CIESM, 1999. Precautory Approach to local fisheries in the Mediterranean Sea. *CIESM Workshop Series* 7, 89 pp.

CIESM, 2000. Fishing down the Mediterranean food webs? *CIESM Workshop Series* 12, 99 pp.

CIHEAM, 2003. Development and agri-food policies in the Mediterranean region. Annual Report. *International Centre for Advanced Mediterranean Agronomic Studies*, Paris, 237 pp.

Cole, J.J., 2005. Communication between terrestrial and marine ecologist: loud, sometimes abrasive, but healthy and occasionally usefull. In: Stergiou K.I., Browman H.I., Bringing the gap between aquatic and terrestrial ecology. *Marine Ecology Progress Series* 304, 272-274

Coll, M., Palomera, I., Tudela, S., Sardà, F., 2006. Trophic flows, ecosystem structure and fishing impacts in the South Catalan Sea, Northwestern Mediterranean. *Journal of Marine Systems* 59, 63-96.

Colloca, F., 2002. Life cycle of the deep-water pandalid shrimp *Plesionika edwardsii* (Decapoda, Caridea) in the central Mediterranean Sea. *Journal of Crustacean Biology* 22, 775-783.

Colloca, F., Cardinale, M., Belluscio, A., Ardizzone, G.D., 2003. Pattern of distribution and diversity of demersal assemblages in the central Mediterranean Sea. *Estuarine, Coastal and Shelf Science* 56, 469-480.

Colloca, F., Carpentieri, P., Balestri, E., Ardizzone, G.D., 2004. A critical habitat for Mediterranean fish resources: shelf-break areas with *Leptometra phalangium* (Echinodermata : Crinoidea). *Marine Biology* 145, 1129-1142.

Conides A., Glamuzina B., Jug-Dujakovik J. Papaconstantinou C. and Kapiris K, 2006. Age, growth, and mortality of the karamote shrimp *Melicertus kerathurus* (Forskal, 1775), in the East Ionian Sea (western Greece). *Crustaceana* 79 (1), 33-52.

Cristo, M., Cartes, J.E., 1998. A comparative study of the feeding ecology of *Nephrops norvegicus* (L.), (Decapoda: Nephropidae) in the bathyal Mediterranean and the adjacent Atlantic. *Scientia Marina* 62, 81-90.

Cury, P.M., 2004. Tuning the ecoscope for the Ecosystem Approach to Fisheries. In: Browman H.I., Stergiou K.I. (Eds.), Perspectives on ecosystem-based approaches to the management of marine resources. *Marine Ecology Progress Series* 274, 272-275.

Cushing, D.H., 1982. *Climate and Fisheries.* Academic Press, London and New York.

Daskalov, G.M., 2002. Overfishing drives trophic cascade in the Black Sea. *Marine Ecology Progress Series* 225, 53-63.

De Biasi, A.M., 2004. Impact of experimental trawling on the benthic assemblage along the Tuscany coast (north Tyrrhenian Sea, Italy). *ICES Journal of Marine Science* 61, 1260-1266.

De Bovée, F., Guidi, L.D., Soyer, J., 1990. Quantitative distribution of deep-sea meiobenthos in the northwestern Mediterranean (Gulf of Lions). *Continental Shelf Research* 10, 1123-1145.

Demestre, M., Abelló, P., 1993. Growth and distribution of *Solenocera membranacea* (Risso, 1816) (Decapoda, Dendrobranchiata) in the northwestern Mediterranean Sea. *Scientia Marina* 57, 161-166.

Demestre, M., Lleonart, J., 1993. Population dynamics of *Aristeus antennatus* (Decapoda: Dendrobranchiata) in the northwestern Mediterranean. *Scientia Marina* 57, 183-189.

Demestre, M., Sánchez, P., Abelló, P., 2000a. Demersal fish assemblages and habitat characteristics on the continental shelf and upper slope of the north-western Mediterranean. *Journal of the Marine Biological Association of the United Kingdom* 80, 981-988.

Demestre, M., Sánchez, P., Kaiser, M.J., 2000b. The behavioural response of benthic scavengers to otter-trawling disturbance in the Mediterranean. In: Kaiser, M.J., Groot, S.J.d. (Eds.), *Effects of Fishing on Non-target Species and Habitats. Biological, Consevation and Socio-economic Issues.* Blackwell Science, Oxford, 121-129.

Demestre, M., Sbrana, M., Alvarez, F., Sánchez, P., 1997. Analysis of the interaction of fishing gear in *Mullus barbatus* fisheries of the western Mediterranean. *Journal of Applied Ichthyology* 13, 49-56.

Demirov, E., Pinardi, N., 2002. Simulation of the Mediterranean Sea circulation from 1979 to 1993: Part I. The interannual variability. *Journal of Marine Systems* 33, 23-50.

Deval, M.C., Bok, T., Ates, C., Ozbilgin, H., 2006. Selectivity of PE and PA material codends for rose shrimp (*Parapenaus longirostris*) in Turkish twin rigged beam trawl fishery. *Fisheries Research* 81, 72-79.

D'Onghia, G., Capezzuto, F., Mytilineou, C., Maiorano, P., Kapiris, K., Carlucci, R., Sion, L., Tursi, A., 2005. Comparison of the population structure and dynamics of *Aristeus antennatus* (Risso, 1816) between exploited and unexploited areas in the Mediterranean Sea. *Fisheries Research* 76, 22-38.

D'Onghia, G., Maiorano, P., Matarrese, A., Tursi, A., 1998a. Distribution, biology, and population dynamics of *Aristaeomorpha foliacea* (Risso, 1827) (Decapoda, Natantia, Aristeidae) in the north-western Ionian Sea (Mediterranean Sea). *Crustaceana* 71, 518-544.

D'Onghia, G., Mastrototaro, F., Maiorano, P., 2000. Biology of silver scabbard fish, *Lepidopus caudatus* (Trichiuridae), from the Ionian Sea (Eastern-Central Mediterranean). *Cybium* 24, 249-262.

D'Onghia, G., Politou, C.Y., Bozzano, A., Lloris, D., Rotllant, G., Sion, L., Mastrototaro, F., 2004. Deep-water fish assemblages in the Mediterranean Sea. *Scientia Marina* 68, 87-99.

D'Onghia, G., Sion, L., Maiorano, P., Mytilineou, C., Dalessandro, S., Carlucci, R., Desantis, S., 2006. Population biology and life strategies of *Chlorophthalmus agassizii* Bonaparte, 1840 (Pisces: Osteichthyes) in the Mediterranean Sea. *Marine Biology* 149, 435-446.

D'Onghia, G., Tursi, A., Marano, C.A., Basanisi, M., 1998b. Life history traits of *Hoplostethus mediterraneus* (Pisces: Beryciformes) from the North-western Ionian Sea (Mediterranean Sea). *Journal of the Marine Biological Association of the United Kingdom* 78, 321-339.

Estrada, M., 1996. Primary production in the northwestern Mediterranean. *Scientia Marina* 60, 55-64.

Estrada, M., Vives, F., and Alcaraz, M., 1985. Life and production in the open sea. In: Margalef, R. (Eds.), *The Western Mediterranean.* Pergamon Press Ltd, London, 150-200.

Fabi, G., Sbrana, M., Biagi, F., Grati, F., Leonori, I., Sartor, P., 2002. Trammel net and gill net selectivity for *Lithognathus mormyrus* (L., 1758), *Diplodus annularis* (L., 1758) and *Mullus barbatus* (L., 1758) in the Adriatic and Ligurian seas. *Fisheries Research* 54, 375-388.

FAO, 1996. *Report of the technical consultation on reduction of wastage in fisheries.* Tokyo, Japan, 2 October - 1 November 1996. 547,.27.

FAO, 2003. The ecosystem approach to fisheries. *FAO Technical Guidelines for Responsible Fisheries* 4, 112.

Farrugio, H., Le Coree, G., 1993. A sampling strategy and methodology for assessment and monitoring of Mediterranean small-scale fisheries. *Scientia Marina* 57, 131-137.

Farrugio, H., Oliver, P., Biagi, F., 1993. An overview of the history, knowledge, recent and future research trends in the Mediterranean fisheries. *Scientia Marina* 57, 105-119.

Ferrandis, E., Hernández, P., 2007. Direct Survival Analysis: a new stock assessment method. *Scientia Marina* 71, 175-185.

Florentino, F., Garofalo, G., De Santi, A., Bono, G., Giusto, G.B., Norrito, G., 2003. Spatio-temporal distribution of recruits (0 group) of *Merluccius merluccius* and *Phycis blennoides* (Pisces, Gadiformes) in the Strait of Sicily (Central Mediterranean). *Hydrobiologia* 503, 223-236.

Font, J., 1987. The path of the Levantine Intermediate Water to the Alboran Sea. *Deep-Sea Research* 34, 1745-1755.

Gaertner, J.C., Bertrand, J.A., Gil de Sola Simarro, L., Durbec, J.P., Ferrandis, E., Souplet, A., 2005. Large spatial scale variation of demersal fish assemblage structure on the continental shelf of the NW Mediterranean Sea. *Marine Ecology Progress Series* 297, 245-257.

García-Rodríguez, M., Esteban, A., Pérez-Gil J.L. Considerations on the biology of *Plesionika edwardsi* (Brandt, 1851) (Decapoda, Caridea, Pandalidae) from experimental

trap catches in the Spanish western Mediterranean Sea. 2000. *Scientia Marina* 64, 369-379.

García-Rodríguez, M., 2003. Characterisation and standardisation of a red shrimp, *Aristeus antennatus* (Risso, 1816), fishery off the Alicante gulf (SE Spain). *Scientia Marina* 67, 63-74.

García-Rodríguez, M., 2003. Characterisation and standardisation of a red shrimp, *Aristeus antennatus* (Risso, 1816), fishery off the Alicante gulf (SE Spain). *Scientia Marina* 67, 63-74.

García-Rodríguez, M., Esteban, A., 1999. On the biology and fishery of *Aristeus antennatus* (Risso, 1816), (Decapoda, Dendrobranchiata) in the Ibiza Channel (Balearic Islands, Spain). *Scientia Marina* 63, 27-37.

García-Rodríguez, M., Esteban, A., 2002. How fast does hake grow? A study on the Mediterranean hake (*Merluccius merluccius* L.) comparing whole otoliths readings and length frequency distributions data. *Scientia Marina* 66, 145-156.

García-Rodríguez, M., Fernandez, A.M., Esteban, A., 2006. Characterisation, analysis and catch rates of the small-scale fisheries of the Alicante Gulf (SE Spain) over a 10 years time series. *Fisheries Research* 77, 226-238.

García-Rodríguez, M., Pereda, P., Landa, J., Esteban, A., 2005. On the biology and growth of the anglerfish *Lophius budegassa* Spinola, 1807 in the Spanish Mediterranean: a preliminary approach. *Fisheries Research* 71, 197-208.

GFCM, 2006. Report of the ninth session of the Scientific Advisory Committee. Rome, 24-27 October 2006. *FAO Fisheries Report,* 814: 112 pp.

Gili, J.M., Pages, F., Bouillon, J., Palanques, A., Puig, P., Heussner, S., Calafat, A., Canals, M., Monaco, A., 2000. A multidisciplinary approach to the understanding of hydromedusan populations inhabiting Mediterranean submarine canyons. *Deep-Sea Research* 47, 1513-1533.

Gislason, H., Sinclair, M.M., 2000. Ecosystem effects of fishing. *ICES Journal of Marine Science* 57, 466-467.

González, M., Sánchez, P., 2002. Cephalopod assemblages caught by trawling along the Iberian Peninsula Mediterranean coast. *Scientia Marina* 66, 199-208.

Goñi, R., 1998. Ecosystem effects of marine fisheries: an overview. *Ocean & Coastal Management* 40, 37-64.

Goñi, R., Adlerstein, S., Álvarez, F., García, M., Sanchez, P., Sbrana, M., Maynou, F., Viva, C., 2004. Recruitment indices of European hake, *Merluccius merluccius* (Linnaeus 1758), in the Northwest Mediterranean based on landings from bottom-trawl multispecies fisheries. *ICES Journal of Marine Science* 61, 760-773.

Goñi, R., Álvarez, F., Adlerstein, S., 1999. Application of generalized linear modeling to catch rate analysis of Western Mediterranean fisheries: the Castellón trawl fleet as a case study. *Fisheries Research* 42, 291-302.

Goñi, R., Latrouite, D. 2005. Review of the biology, ecology and fisheries of *Palinurus spp*. species of European waters: *Palinurus elephas* (Fabricius, 1787) and *Palinurus mauritanicas* (Gruvel, 1911) *Cahiers de Biologie Marine* 46 (2), 127-142.

Granata, T.C., Estrada, M., Zika, U., Merry, C., 2004. Evidence for enhanced primary production resulting from relative vorticity induced upwelling in the Catalan Current. *Scientia Marina* 68, 113-119.

Guijarro, B., Massutí, E., 2006. Selectivity of diamond- and square-mesh codends in the deepwater crustacean trawl fishery off the Balearic Islands (western Mediterranean). *ICES Journal of Marine Science* 63, 52-67.

Guijarro, B., Massutí, E., Moranta, J., Díaz, P., 2007. Population dynamics of the red shrimp *Aristeus antennatus* in the Balearic Islands (Western Mediterranean): short espatio-temporal differences and influence of environmental factors. *Journal of Marine Systems.*

Hamad, N., Millot, C., Taupier-Letage, I., 2005. A new hypothesis about the surface circulation in the eastern basin of the Mediterranean Sea. *Progress in Oceanography* 66, 287-298.

Helland-Hansen, B. and Nansen, F. 1909. The Norwegian Sea: its physical oceanography based upon the norwegian researches 1900-1904. Report on Norwegian Fishery and Marine Investigations Vol 11 No.2 (http://web.gfi.uib.no/The%20Norwegian% 20Sea/TNS.htm).

Hidalgo, E. Massutí, J. Moranta, J. Cartes, J. Lloret, P. Oliver and B. Morales-Nin , 2007. Seasonal and short spatial patterns in European hake (*Merluccius merluccius*, L) recruitment process at the Balearic Islands (NW Mediterranean): the role of environment on distribution and condition. *Journal of Marine Systems* (in press).

Hjort, J. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. 1914. *Rapports et Proces-Verbaux des Reunions, Conseil International pour L'Exploration scientifique de la Mer Medeterranee.* Monaco 20:1-228.

Hollowed, A.B., Bax, N., Beamish, R., Collie, J., Fogarty, M., Livingston, P., Pope, J., Rice, J.C., 2000. Are multispecies models an improvement on single-species models for measuring fishing impacts on marine ecosystems? *ICES Journal of Marine Science* 57, 707-719.

Hopkins, T.S., 1985. Physiscs of the sea. In: Margalef, R. (Eds.), *Key Environments: Western Mediterranean.* Pergamon Press, New York, pp. 100-125.

Iglesias, M., Massutí, E., Reñones, O., Morales-Nin, B., 1994. Three small-scale fisheries based on the island of Majorca (NW Mediterranean). *Bolletí de la Societat d'Història Natural de les Illes Balears* 37, 35-58.

Jones, E.G., Tselepides, A., Babley P.M., Collins, M.A., Priede, I.G., 2003. Bathymetric distribution of some benthic and benthopelagic species attracted to baited cameras and traps in the deep eastern Mediterranean. *Marine Ecology Progress Series* 251, 75-86.

Jukic-Peladic, S., Vrgoc, N., Krstulovic-Sifner, S., Piccinetti, C., Piccinetti-Manfrin, G., Marano, G., Ungaro, N., 2001. Long-term changes in demersal resources of the Adriatic Sea: comparison between trawl surveys carried out in 1948 and 1998. *Fisheries Research* 53, 95-104.

Kallianiotis, A., Sophronidis, K., Vidoris, P., Tselepides, A., 2000. Demersal fish and megafaunal assemblages on the Cretan continental shelf and slope (NE Mediterranean): seasonal variation in species density, biomass and diversity. *Progress in Oceanography* 46, 429-455.

Kallianiotis, A., Vidoris, P., Sylaios, G., 2004. Fish species assemblages and geographical sub-areas in the North Aegean Sea, Greece. *Fisheries Research* 68, 171-187.

Kapiris, K., Thessalou-Legaki, M., 2001. Sex-related variability of rostrum morphometry of *Aristeus antennatus* (Decapoda : Aristeidae) from the Ionian Sea (Eastern mediterranean, Greece). *Hydrobiologia* 449, 123-130.

Katsanevakis, S., Verriopoulos, G., 2006. Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. *ICES Journal of Marine Science* 63, 151-160.

La Mesa, M., Arneri, E., Caputo, V., Iglesias, M., 2005. The transparent goby, *Aphia minuta*: Review of biology and fisheries of a paedomorphic European fish. *Reviews in Fish Biology and Fisheries* 15, 89-109.

Labropoulou M., Papaconstantinou, C., 2000. Community structure of deep-sea demersal fish in the North Aegean Sea (northeastern Mediterranean). *Hydrobiologia* 440, 281-296.

Labropoulou, M., Machias, A., Tsimenides, N., Eleftheriou, A., 1997. Feeding habits and ontogenetic diet shift of the striped red mullet, *Mullus surmuletus* Linnaeus, 1758. *Fisheries Research* 3, 257-267.

Labropoulou, M., Papaconstantinou, C., 2004. Community structure and diversity of demersal fish assemblages: the role of fishery. *Scientia Marina* 68, 215-226.

Laevastu, T., and F. Favorite. *1988. Fishing and stock fluctuations*. Fishing News Books, Ltd., Surrey, England. 239 pp.

Leaman, K.D., Schott, F.A., 1991. Hydrographic structure of the convective regime in the Gulf of Lions: winter 1987. *Journal Physical Oceanography* 21, 575– 598.

Levi, D., Andreoli, M.G., Bonanno, A., Fiorentino, F., Garofalo, G., Mazzola, S., Norrito, G., Patti, B., Pernice, G., Ragonese, S., Giusto, G.B., Rizzo, P., 2003. Embedding sea surface temperature anomalies into the stock recruitment relationship of red mullet (*Mullus barbatus* L. 1758) in the Strait of Sicily. *Scientia Marina* 67, 259-268.

Levi, F., Francour, P., 2004. Behavioural response of *Mullus surmuletus* to habitat modification by the invasive macroalga *Caulerpa taxifolia*. *Journal of Fish Biology* 64, 55-64.

Lhorenz, S.E., Wiesenburg D.A., De Palma I.P., Johnson K.S., 1988. Interrelationship among primary production, chlorophyll, and environmental conditions in frontal regions of the Western Mediterranean Sea. *Deep-Sea Research I* 35, 793-810.

Libralato, S., Pastres, R., Pranovi, F., Raicevich, S., Granzotto, A., Giovanardi, O., Torricelli, P., 2002. Comparison between the energy flow networks of two habitat in the Venice lagoon. P.S.Z.N. *Marine Ecology* 23, 228–236.

Lleonart, J., 1993. Methods to analyse the dynamics of exploited marine populations: Use and development of models. *Scientia Marina* 57, 261-267.

Lleonart, J., 2004. A review of Mediterranean shared stocks, assessment and management. In: Payne, A.I.L., O'Brien, C.M., Rogers, S.I. (Eds.), *Management of Shared Fish Stocks.* Blackwell, Oxford, pp. 113-130.

Lleonart, J., Franquesa, R., Salat, J., Oliver, P., 1996. "Heures" a bio-economic model for Mediterranean fisheries, towards an approach for the evaluation of management strategies. *Scientia Marina* 60, 427-430.

Lleonart, J., Maynou, F., 2003. Fish stock assessments in the Mediterranean: state of the art. *Scientia Marina* 67, 37-49.

Lleonart, J., Maynou, F., Recasens, L., Franquesa, R., 2003. A bioeconomic model for Mediterranean fisheries, the hake off Catalonia (western Mediterranean) as a case study. *Scientia Marina* 67, 337-351.

Lleonart J., Recasens L., 1996. Fisheries and the environment in the Mediterranean Sea. In: Caddy, J.F. (Ed.). Resource and environmental issues relevant to Mediterranean fisheries management. Studies and Reviews. *General Fisheries Council for the Mediterranean Rome FAO*, 66: 5-18.

Lloret, J., Galzin, R., Gil de Sola, L., Souplet A., and Demestre M. 2005. Habitat related differences in lipid reserves of some exploited fish species in the north-western Mediterranean continental shelf. *Journal of Fish Biology* 67, 51-65.

Lloret, J., Lleonart, J., 2002. Recruitment dynamics of eight fishery species in the north-western Mediterranean Sea. *Scientia Marina* 66, 77-82.

Lloret, J., Lleonart, J., Solé, I., 2000. Time series modelling of landings in Northwest Mediterranean Sea. *ICES Journal of Marine Science* 57, 171-184.

Lloret, J., Lleonart, J., Sole, I., Fromentin, J.M., 2001. Fluctuations of landings and environmental conditions in the north-western Mediterranean Sea. *Fisheries Oceanography* 10, 33-50.

Lloret, J., Palomera, I., Salat, J., Sole, I., 2004. Impact of freshwater input and wind on landings of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in shelf waters surrounding the Ebre (Ebro) River delta (north-western Mediterranean). *Fisheries Oceanography* 13, 102-110.

Lombarte, A., Recasens, L., Gonzalez, M., Gil de Sola, L., 2000. Spatial segregation of two species of Mullidae (*Mullus surmuletus* and *M. barbatus*) in relation to habitat. *Marine Ecology Progress Series* 206, 239-249.

Longepierre, S., Robert, A., Levi, F., Francour, P., 2005. How an invasive alga species (*Caulerpa taxifolia*) induces changes in foraging strategies of the benthivorous fish *Mullus surmuletus* in coastal Mediterranean ecosystems. *Biodiversity and Conservation* 14, 365-376.

López-Jurado, J.L., Marcos, M., Monserrat, S., 2007. Hydrographic conditions during the IDEA project. *Journal of Marine Systems* (in press).

Mace M.M., 2004. In defence of fisheries scientists, single species models and other scapagoats: confronting the real problems. In: Browman H.I., Stergiou K.I. (Eds.), Perspectives on ecosystem-based approaches to the management of marine resources. *Marine Ecology Progress Series* 274, 285-291.

Machias, A., Vassilopoulou, V., Vatsos, D., Bekas, P., Kallianiotis, A., Papaconstantinou, C., Tsimenides, N., 2001. Bottom trawl discards in the northeastern Mediterranean Sea. *Fisheries Research* 53, 181-195.

Macpherson, E., 1981. Resource partitioning in a mediterranean demersal fish community. *Marine Ecology Progress Series* 4, 183-193.

Maiorano, P., D'Onghia, G., Capezzuto, F., Sion, L., 2002. Life-history traits of *Plesionika martia* (Decapoda: Caridea) from the eastern-central Mediterranean sea. *Marine Biology* 141, 527-539.

Mallol, S., Casadevall, M., García, E., 2001. Comparison of discarded, escaped and landed fish using diamond and square mesh codends. *Rapport du Commission Internationale pour l'Exploration Scientifique de la mer Méditerranée* 36: 296.

Maltagliati, F., Camilli, L., Biagi, F., Abbiati, M., 1998. Genetic structure of Norway lobster, *Nephrops norvegicus* (L.) (Crustacea: Nephropidae), from the Mediterranean Sea. *Scientia Marina* 62, 91-99.

Margalef, R., 1985. Environmental control of the mesoscale distribution of primary producers and its bearing to primary production in the Western Mediterranean. In: Moraitou-Apostolopoulou, M., Kiortsis, V. (Eds.), *Mediterranean Marine Ecosystems.* Plenum Press, New York, pp. 213-229.

Martí, R., Uriz, M.J., Ballesteros, E., Turon, X., 2005. Seasonal variation in structure of three algal communities under different light conditions. *Estuarine Coastal and Shelf Science* 64, 613-622..

Martín, P., Sánchez, P., Ramón, M., 1995. Population structure and exploitation of *Bolinus brandaris* (Mollusca: Gastropoda) off the Catalan coast (northwestern Mediterranean). *Fisheries Research*, 23: 319-331.

Martín, P., Sartor, P., García-Rodríguez, M., 1999. Exploitation patterns of the European hake *Merluccius merluccius*, red mullet *Mullus barbatus* and striped red mullet *Mullus surmuletus* in the western Mediterranean. *Journal of Applied Ichthyology* 15, 24-28.

Massutí, E., Gordon, J.D.M., Moranta, J., Swan, S.C., Stefanescu, C., Merrett, N.R., 2004. Mediterranean and Atlantic deep-sea fish assemblages: differences in biomass composition and size-related structure. *Scientia Marina* 68, 101-115.

Massutí, E., Monserrat, S., Oliver, P., Moranta, J., López-Jurado, J.L., Marcos, M., Hidalgo, J.M., Guijarro, B. Carbonell, A., Pereda, P., 2007. *The influence of oceanographic scenarios on the population dynamics of demersal resources in the western Mediterranean: hypothesis for hake and red shrimp off Balearic Islands* (in press).

Massutí, E., Morales-Nin, B. (Eds.), 1999. Biology and Fishery of Dolphinfish and related species. *Scientia Marina* 63 (3-4), 181-472.

Massutí, E., Morales-Nin, B., Lloris, D., 1996a. Bathymetric distribution and recruitment patterns of *Phycis blennoides* (Pisces: Gadidae) from the slope of the northwestern Mediterranean. *Scientia Marina* 60, 481-488.

Massutí, E., Morales-Nin, B., Moranta, J., 2000. Age and growth of blue-mouth, *Helicolenus dactylopterus* (Osteichthyes: Scorpaenidae), in the western Mediterranean. *Fisheries Research* 46, 165-176.

Massutí, E., Morales-Nin, B., Stefanescu, C., 1995. Distribution and biology of five grenadier fish (Pisces, Macrouridae) from the upper and middle slope of the north-western Mediterranean. *Deep-Sea Research* I 42, 307-330.

Massutí, E., Moranta, J., 2003. Demersal assemblages and depth distribution of elasmobranchs from the continental shelf and slope off the Balearic Islands (western Mediterranean). *ICES Journal of Marine Science* 60, 753-766.

Massutí, E., Moranta, J., Gil de Sola, L., Morales-Nin, B., Prats, L., 2001. Distribution and population structure of the rockfish *Helicolenus dactylopterus* (Pisces : Scorpaenidae) in the western Mediterranean. *Journal of the Marine Biological Association of the United Kingdom* 81, 129-141.

Massutí, E., Reñones, O., 2005. Demersal resource assemblages in the trawl fishing grounds off the Balearic Islands (western Mediterranean). *Scientia Marina* 69, 167-181.

Massutí, E., Reñones, O., Carbonell, A., Oliver, P., 1996b. Demersal fish communities exploited on the continental shelf and slope off Majorca (Balearic Islands, NW Mediterranean). *Vie et Milieu* 46, 45-55.

Matarrese, A., D'Onghia, G., Basanisi, M., Mastrototaro, F., 1998. Spawning and recruitment of *Phycis blennoides* (Phycidae) from the north-western Ionian Sea (middle-eastern Mediterranean). *Italian Journal of Zoology* 65, 203-209.

Matarrese, A., D'Onghia, G., Tursi, A., Basanisi, M., 1996. New information on the ichthyofauna of the South-eastern Italian Coasts (Ionian Sea). *Cybium* 20, 197-211.

Maynou, F., Abelló, P., Sartor, P., 2004. A review of the fisheries biology of the mantis shrimp, *Squilla mantis* (L., 1758) (Stomatopoda, Squillidae) in the Mediterranean. *Crustaceana* 77, 1081-1099.

Maynou, F., Cartes, J.E., 2000. Community structure of bathyal decapod crustaceans off south-west Balearic Islands (western Mediterranean): seasonality and regional patterns in zonation. *Journal of the Marine Biological Association of the United Kingdom* 80, 789-798.

Maynou, F., Conan, G.Y., Cartes, J.E., 1996. Spatial structure and seasonality of decapod crustacean populations on the northwestern Mediterranean slope. *Limnology and Oceanography* 4, 113-125.

Maynou, F., Lleonart, J., Cartes, J.E., 2003. Seasonal and spatial variability of hake (*Merluccius merluccius* L.) recruitment in the NW Mediterranean. *Fisheries Research* 60, 65-78.

Maynou, F., Lleonart, J., Cartes, J.E., 2003. Seasonal and spatial variability of hake (*Merluccius merluccius* L.) recruitment in the NW Mediterranean. *Fisheries Research* 60, 65-78.

Maynou, F., Sardà, F., 2001. Influence of environmental factors on commercial trawl catches of *Nephrops norvegicus* (L.). *ICES Journal of Marine Science* 58, 1318-1325.

Maynou, F., Sardà, F., Tudela, S., Demestre, M., 2006. Management strategies for red shrimp (*Aristeus antennatus*) fisheries in the Catalan sea (NW Mediterranean) based on bioeconomic simulation analysis. *Aquatic Living Resources* 19, 161-171.

McAllister, M.K., Kirkwood, G.P., 1998. Bayesian stock assessment: a review and example application using the logistic model. *ICES Journal of Marine Science* 55, 1031-1060.

Merella, P., Alemany, F., Carbonell, A., Quetglas, A., 1998. Fishery and biology of Norway lobster *Nephrops norvegicus* (Decapoda: Nephropidae) in Mallorca (western Mediterranean). *Journal of Natural History* 32, 1631-1640.

Metaxatos, A., 2004. Population dynamics of the venerid bivalve *Callista chione* (L.) in a coastal area of the eastern Mediterranean. *Journal of Sea Research* 52, 293– 305

Metin, C., Ozbilgin, H., Tosunoglu, Z., Gokce, G., Aydin, C., Metin, G., Ulas, A., Kaykac, H., Lok, A., Duzbastilar, F.O., Tokac, A., 2005. Effect of square mesh escape window on codend selectivity for three fish species in the Aegean Sea. *Turkish Journal of Veterinary & Animal Sciences* 29, 461-468.

Millot, C., 1985. Some features of the Algerian current. *Journal of Geophysical Research Oceans* 90, 7169-7176.

Millot, C., 1987. Circulation in the Western Mediterranean Sea. *Oceanologica Acta* 10, 143-149.

Millot, C., 1999. Circulation in the Western Mediterranean Sea. *Journal of Marine Systems* 20, 423-442.

Monaco, A., Biscaye P., Soyer J., Pocklington R., Heussner S., 1990. Particle fluxes and ecosystem response on a continental margin: the 1985-1988 Mediterranean ECOMARGE experiment. *Continental Shelf Research* 10, 809-839.

Monserrat, S., López-Jurado, J.L. and Marcos, M., 2007. A mesoscale index to describe the regional ocean circulation around the Balearic Islands. *Journal of Marine Systems* (in press).

Morales-Nin, B., Aldebert, Y., 1997. Growth of juvenile *Merluccius merluccius* in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length-frequency analysis. *Fisheries Research* 30, 77-85.

Morales-Nin, B., Bjelland, R.M., Moksness, E., 2005a. Otolith microstructure of a hatchery reared European hake (*Merluccius merluccius*). *Fisheries Research* 74, 300-305.

Morales-Nin, B., Massutí, E., Stefanescu, C., 1996a. Bathymetric distribution and growth patterns of *Bathypterois mediterraneus* from the north-western Mediterranean Sea. *Journal of Fish Biology* 49, 276-288.

Morales-Nin, B., Massutí, E., Stefanescu, C., 1996b. Distribution and biology of *Alepocephalus rostratus* from the Mediterranean Sea. *Journal of Fish Biology* 48, 1097-1112.

Morales-Nin, B., Moranta, J., 2004. Recruitment and post-settlement growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. *Scientia Marina* 68, 399-409.

Morales-Nin, B., Moranta, J., García, C., Tugores, M.P., Grau, A.M., Riera, F., Cerdà, M., 2005b. The recreational fishery in Mallorca Island (Western Mediterranean): implications for coastal resources management. *ICES Journal of Marine Science* 62, 727-739.

Morales-Nin, B., Swan, S.C., Gordon, J.D.M., Palmer, M., Geffen, A.J., Shimmield, T., Sawyer, T., 2005c. Age-related trends in otolith chemistry of *Merluccius merluccius* from the north-eastern Atlantic Ocean and the western Mediterranean Sea. *Marine and Freshwater Research* 56, 599-607.

Moranta, J., Massutí, E., Morales-Nin, B., 2000. Fish catch composition of the deep-sea decapod crustacean fisheries in the Balearic Islands (western Mediterranean). *Fisheries Research* 45, 253-264.

Moranta, J., Palmer, M., Massutí, E., Stefanescu, C., Morales-Nin, B., 2004. Body fish size tendencies within and among species in the deep-sea of the western Mediterranean. *Scientia Marina* 68, 141-152.

Moranta, J., Stefanescu, C., Massutí, E., Morales-Nin, B., Lloris, D., 1998. Fish community structure and depth-related trends on the continental slope of the Balearic Islands (Algerian basin, western Mediterranean). *Marine Ecology Progress Series* 171, 247-259.

Morel, A., André, J.M., 1991. Pigment distribution and primary production in the western Mediterranean as derived and modelled from coastal zone colour scanner observations. *Journal of Geophysical Research* 96, 12695-12698.

Moutin, T., Raimbault, P., 2002. Primary production, carbon export and nutrients availability in western and eastern Mediterranean Sea in early summer 1996 (MINOS cruise). *Journal of Marine Systems* 33, 273-288.

Mura, M., Cau, A., 1994. Community structure of the decapod crustaceans in the middle bathyal zone of the Sardinian channel. *Crustaceana* 67, 259-266.

Mytilineou, C, Politou, CY, Fourtouni, A., 1998. Trawl selectivity studies on *Nephrops norvegicus* (L.) in the eastern Mediterranean Sea. *Scientia Marina* 62, 107-116.

Mytilineou, Ch., Kavadas, S., Politou, C.-Y., Kapiris, K., Tursi, A., Maiorano, P., 2006. Catch composition on red shrimps' (*Aristaeomorpha foliacea* and *Aristeus antennatus*) grounds in the Eastern Ionian Sea. *Hydrobiologia* 557, 155-160.

Napolitano, E., Oguz, T., Malanotte-Rizzoli, P., Yilmaz, A., Sansone, E., 2000. Simulations of biological production in the Rhodes and Ionian basins of the eastern Mediterranean. *Journal of Marine Systems* 24, 277-298.

Olivar, M.P., Quilez, G., Emelianov, M., 2003. Spatial and temporal distribution and abundance of European hake, *Merluccius merluccius*, eggs and larvae in the Catalan coast (NW Mediterranean). *Fisheries Research* 60, 321-331.

Oliver, M., 1953. Bionomía de los fondos de 300 a 600 m en el sur y suroeste de Mallorca. *Boletín del Instituto Español de Oceanografía* 63, 3-20.

Oliver, P., 1993. Analysis of fluctuations observed in the trawl fleet landings of the Balearic Islands. *Scientia Marina* 57, 219-227.

Oliver, P., Massutí, E., 1995. Biology and fisheries of western Mediterranean hake. In: Alheit, J., Pitcher, T.J. (Eds.), *Hake: Biology, Fisheries and Markets*. Chapman & Hall, London.

Ordines, F., Massutí, E., Guijarro, B., Mas, R., 2006. Diamond vs. square mesh codend in a multi-species trawl fishery of the western Mediterranean: effects on catch composition, yield, size selectivity and discards. *Aquatic Living Resources* 19, 329-338.

Ordines, F., Massutí, E., Quetglas, A., Moranta, J., 2007. Macro-epibenthic assemblages on the trawling grounds along the shallow continental shelf off the Balearic Islands (western Mediterranean). *Rapp. Comm. int. Mer Médit.* 38, 538.

Ozbilgin, H., Tosunoglu, Z., Bilecenoglu, M., Tokac, A., 2004. Population parameters of *Mullus barbatus* in Izmir Bay (Aegean Sea), using length frequency analysis. *Journal of Applied Ichthyology* 20, 231-233.

Palanques, A., García-Ladona, E., Gomis, D., Martín, J., Marcos, M., Pascual, A., Puig, P., Gili, J.M., Emelianov, M., Monserrat, S., Guillen, J., Tintore, J., Segura, M., Jordi, A., Ruiz, S., Basterretxea, G., Font, J., Blasco, D., Pages, F., 2005. General patterns of circulation, sediment fluxes and ecology of the Palamo's (La Fonera) submarine canyon, northwestern Mediterranean. *Progress in Oceanography* 66, 89-119.

Palomera, I., Olivar, M.P., Morales-Nin, B., 2005. Larval development and growth of the European hake *Merluccius merluccius* in the northwestern Mediterranean. *Scientia Marina* 69, 251-258.

Papaconstantinou, C, Kapiris, K, 2003. The biology of the giant red shrimp (*Aristaeomorpha foliacea*) at an unexploited fishing ground in the Greek Ionian Sea. *Fisheries Research* 62, 37-51.

Petrakis, G, Stergiou, K.I., 1997. Size selectivity of diamond and square mesh codends for four commercial Mediterranean fish species. *ICES Journal of Marine Science* 54, 13-23.

Pikitch, E.K., Santora, C., Babcock, E.A., Bakun, A., Bonfil, R., Conover, D.O., Dayton, P., Doukakis, P., Fluharty, D., Heneman, B., Houde, E.D., Link, J., Livingston, P.A., Mangel, M., McAllister, M.K., Pope, J., Sainsbury, K.J., 2004. Ecosystem-Based Fishery Management. *Science* 305, 346-347.

Pinnegar, J.K., Polunin, N.V.C., 2004. Predicting indirect effects of fishing in Mediterranean rocky littoral communities using a dynamic simulation model. *Ecological Modelling* 172, 249-267.

Pinot, J.M., Lopez-Jurado, J.L., Riera, M., 2002. The CANALES experiment (1996-1998). Interannual, seasonal, and mesoscale variability of the circulation in the Balearic Channels. *Progress in Oceanography* 55, 335-370.

Pinot, J.M., Tintoré, J., Gomis, D., 1995. Multivariate analysis of the surface circulation in the Balearic Sea. *Progress in Oceanography* 36, 343-376.

Politou, C.Y., Kapiris, K., Maiorano, P., Capezzuto, F., Dokos, J., 2004. Deep-sea Mediterranean biology: the case of *Aristaeomorpha foliacea* (Risso, 1827) (Crustacea : Decapoda : Aristeidae). *Scientia Marina* 68, 129-139.

Polunin, N.V.C., Morales-Nin B, Pawsey W.E., Cartes, J.E., Pinnegar, J.K., Moranta, J. 2001. Feeding relationships in Mediterranean bathyal assemblages elucidated by stable nitrogen and carbon isotope data. *Marine Ecology Progress Series* 220, 13-23.

Poulos, S.E., Drakopoulos, P.G., Collins, M.B., 1997. Seasonal variability in sea surface oceanographic conditions in the Aegean Sea (Eastern Mediterranean): an overview. *Journal of Marine Systems* 13, 225-244.

Puig, P., Palanques, A., Guillen, J., García-Ladona, E., 2000. Deep slope currents and suspended particle fluxes in and around the Foix submarine canyon (NW Mediterranean). *Deep-Sea Research Part* I 47, 343-366.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P., Sánchez, P., 1998a. Biology and fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, western Mediterranean). *Fisheries Research* 36, 237-249.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P., Sánchez, P., 1998b. Some aspects of the biology of *Todarodes sagittatus* (Cephalopoda : Ommastrephidae) from the Balearic sea (western Mediterranean). *Scientia Marina* 62, 73-82.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P., Sánchez, P., 1999. Diet of the European flying squid *Todarodes sagittatus* (Cephalopoda: Ommastrephidae) in the Balearic Sea (western Mediterranean). *Journal of the Marine Biological Association of the United Kingdom* 79, 479-486.

Quetglas, A., Carbonell, A., Sánchez, P., 2000. Demersal continental shelf and upper slope cephalopod assemblages from the Balearic Sea (North-Western Mediterranean). Biological aspects of some deep-sea species. *Estuarine, Coastal and Shelf Science* 50, 739-749.

Quetglas, A., González, M., Franco, I., 2005. Biology of the upper-slope cephalopod *Octopus salutii* from the western Mediterranean Sea. *Marine Biology* 146, 1131-1138.

Quetglas, A., Morales-Nin, B., 2004. Age and growth of the ommastrephid squid *Todarodes sagittatus* from the western Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* 84, 421-426.

Ragonese, S., Bianchini, M.L., Di Stefano, L., 2002. Trawl cod-end selectivity for deepwater red shrimp (*Aristaeomorpha foliacea*, Risso 1827) in the Strait of Sicily (Mediterranean Sea). *Fisheries Research* 57(2), 131-144.

Ragonese, S., Bianchini, ML., 2006. Trawl selectivity trials on the deep-water rose shrimp (*Parapenaeus longirostris*) in Sicilian waters. *Hydrobiologia* 557, 113-119.

Ragonese, S., Zagra, M., Di Stefano, L., Bianchini, M.L., 2001. Effect of codend mesh size on the performance of the deep-water bottom trawl used in the red shrimp fishery in the Strait of Sicily (Mediterranean Sea). *Hydrobiologia* 449, 279-291.

Ramón, M., Abelló P., Richardson, C.A., 1995. Population structure and growth of *Donax trunculus* (Bivalvia: Donacidae) in the western Mediterranean. *Marine Biology* 121, 665-671.

Ramón, M., Cano, J., Peña, J.B., Campos, M.J.,2005. Current status and perspectivas of mollusc (bivalves and gastropods) culture in the Spanish Mediterranean. *Boletín del Instituto Español de Oceanografía* 21(4), 343-355.

Ramón, M., Richardson, C.A., 1992. Age determination and shell growth of *Chamelea gallina* (Bivalvia:Veneridae) in the western Mediterranean. *Marine Ecology Progress Series* 89, 15-23

Recasens, L., Lombarte, A., Morales-Nin, B., Torres, G.J., 1998. Statiotemporal variation in the population structure of the European hake in the NW Mediterranean. *Journal of Fish Biology* 53, 387-401.

Relini, G., Biagi, F., Serena, F., Belluscio, A., Spedicato, M.T., Rinelli, P., Follesa, M.C., Piccinetti, C., Ungaro, N., Sion, L., Levi, D., 2000. I Selaci pescati con lo strascico nei mari italiani. *Biologia Marina Mediterranea* 7, 347-384.

Relini, L.O., Mannini, A., Fiorentino, F., Palandri, G., Relini, G., 2006. Biology and fishery of *Eledone cirrhosa* in the Ligurian Sea. *Fisheries Research* 78, 72-88.

Relini, L.O., Papaconstantinou, C., Jukic-Peladic, S., Souplet, A., Gil de Sola, L., Piccinetti, C., Kavadas, S., Rossi, M., 2002. Distribution of the Mediterranean hake populations (*Merluccius merluccius smiridus* Rafinesque, 1810) (Osteichthyes: Gadiformes) based on six years monitoring by trawl-surveys: some implications for management. *Scientia Marina* 66, 21-38.

Relini, L.O., Zamboni, A., Fiorentino, F., Massi, D., 1998. Reproductive patterns in Norway lobster *Nephrops norvegicus* (L.), (Crustacea Decapoda Nephropidae) of different Mediterranean areas. *Scientia Marina* 62, 25-41.

Reñones, O., Massutí, E., Morales-Nin, B., 1995. Life history of the red mullet *Mullus surmuletus* from the bottom-trawl fishery off the Island of Majorca (north-west Mediterranean). *Marine Biology* 123, 411-419.

Roether, W., Manca, B.B., Klein, B., Bregant, D., Georgopoulos, D., Beitzel, V., Kovacevic, V., Luchetta, A., 1996. Recent changes in the Eastern Mediterranean deep waters. *Science* 271, 333–335.

Roldán, M.I., García-Marin, J.L., Utter, F., Pla, C., 1998. Population genetic structure of European hake, *Merluccius merluccius*. *Heredity* 81, 327-334.

Rotllant, G., Abad, E., Sardà, F., Abalós, M., Company, J., Rivera, J., 2006. Dioxin compounds in the deep-sea rose shrimp *Aristeus antennatus* (Risso, 1816) throughout the Mediterranean Sea. *Deep-Sea Research Part I* 53, 1895-1906.

Rotllant, G., Moranta, J., Massutí, E., Sardà, F., Morales-Nin, B., 2002. Reproductive biology of three gadiform fish species through the Mediterranean deep-sea range (147-1850 m). *Scientia Marina* 66, 157-166.

Rotllant, G., Ribes, E., Company, J., Durfort, M., 2005. The ovarian maturation cycle of the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) (Crustacea, Decapoda) from the western Mediterranean Sea. *Invertebrate Reproduction & Development* 48, 161-169.

Sala, A., Lucchetti, A., Buglioni, G., 2007. The influence of twine thickness on the size selectivity of polyamide codends in a Mediterranean bottom trawl. *Fisheries Research* 83, 192-203.

Sánchez, P., Demestre, M., Martín, P., 2004. Characterisation of the discards generated by bottom trawling in the northwestern Mediterranean. *Fisheries Research* 67, 71-80.

Sánchez, P. and Obarti, R., 1993. The biology and fishery of Octopus vulgaris caught with clay pots on the Spanish Mediterranean coast. In: Okutani, T., R.K. O'Dor and T. Kubodera (Eds.) *Recent Advances in Fisheries Biology*. Tokai University Press, Tokyo, 477-487.

Sardà, F, Conan GY, Fuste X., 1993. Selectivity of Norway lobster *Nephrops norvegicus* (L.) in the northwestern Mediterranean. *Scientia Marina* 57, 167-174.

Sardà, F., 1991. Reproduction and molt synchronism in *Nephrops norvegicus* (L) (Decapoda, Nephropidae) in the Western Mediterranean: is spawning annual or biennial? *Crustaceana* 60, 186-199.

Sardà, F., 1993. Bio-ecological aspects of the decapod crustacean fisheries in the Western Mediterranean. *Aquatic Living Resources* 6, 229-305.

Sardà, F., 1998. *Nephrops norvegicus* (L): Comparative biology and fishery in the Mediterranean Sea. *Scientia Marina* 62, 1-143.

Sardà, F., Bacón, N., Sardà-Palomera, F., Moli, B., 2005. Commercial testing of a sorting grid to reduce catches of juvenile hake (*Merluccius merluccius*) in the western Mediterranean demersal trawl fishery. *Aquatic Living Resources* 18, 87-91.

Sardà, F., Bahamon, N., Moli, B., Sardà-Palomera, F., 2006. The use of square mesh codend and sorting grids to reduce catches of young fish and improve sustainability in a multispecies bottom trawl fishery in the Mediterranean. *Scientia Marina* 70, 347-353.

Sardà, F., Bas, C., Lleonart, J., 1995. Functional morphometry of *Aristeus antennatus* (Risso, 1816) (Decapoda, Aristeidae). *Crustaceana* 68, 461-471.

Sardà, F., Bas, C., Roldan, M.I., Pla, C., Lleonart, J., 1998a. Enzymatic and morphometric analyses in Mediterranean populations of the rose shrimp, *Aristeus antennatus* (Risso, 1816). *Journal of Experimental Marine Biology and Ecology* 221, 131-144.

Sardà, F., Calafat, A., Flexas, M.M., Tselepides, A., Canals, M., Espino, M., Tursi, A., 2004a. An introduction to Mediterranean deep-sea biology. *Scientia Marina* 68, 7-38.

Sardà, F., Cartes, J.E., Company, J.B., 1994a. Spatio-temporal variations in megabenthos abundance on three different habitats of the Catalan deep-sea (Western Mediterranean). *Marine Biology* 120, 211-219.

Sardà, F., Cartes, J.E., Norbis, W., 1994b. Spatio-temporal structure of the deep-water shrimp *Aristeus antennatus* (Decapoda: Aristeidae) population in the western Mediterranean. *Fishery Bulletin* 92, 599-607.

Sardà, F., Cartes, J.E., Norbis, W., 1994c. Spatiotemporal structure of the deep-water shrimp *Aristeus antennatus* (Decapoda, Aristeidae) population in the western Mediterranean. *Fishery Bulletin* 92, 599-607.

Sardà, F., Company, J., Castellon, A., 2003. Intraspecific aggregation structure of a shoal of a Western Mediterranean (Catalan coast) deep-sea shrimp, *Aristeus antennatus* (Risso, 1816), during the reproductive period. *Journal of Shellfish Research* 22, 569-579.

Sardà, F., Cros, M.L., Sese, B., 1989. Ca balance during molting in the prawn *Aristeus antennatus* (Risso, 1816) - the role of cuticle calcification in the life cycle of decapod crustaceans. *Journal of Experimental Marine Biology and Ecology* 129, 161-171.

Sardà, F., Demestre, M., 1989. Shortening of the rostrum and rostral variability in *Aristeus antennatus* (Risso, 1816) (Decapoda, Aristeidae). *Journal of Crustacean Biology* 9, 570-577.

Sardà, F., D'Onghia, G., Politou, C.Y., Tselepides, A. (Eds.), 2004b. Mediterranean deep-sea biology. *Scientia Marina* 68, 1-204.

Sardà, F., Lleonart, J., Cartes, J.E., 1998b. An analysis of the population dynamics of *Nephrops norvegicus* (L.) in the Mediterranean Sea. *Scientia Marina* 62, 135-143.

Sardà, F., Maynou, F., 1998a. Assessing perceptions: Do Catalan fishermen catch more shrimp on Fridays? *Fisheries Research* 36, 149-157.

Sardà, F., Maynou, F., Tallo, L., 1997. Seasonal and spatial mobility patterns of rose shrimp *Aristeus antennatus* in the western Mediterranean: results of a long-term study. *Marine Ecology Progress Series* 159, 133-141.

Sardà, F., Molí B. and Palomera I. 2004c. Selectivity of Norway lobster *Nephrops norvegicus* (L.) in the northwestern Mediterranean. *Scientia Marina* 57, 167-174.

Sardà, F., Moli, B., Palomera, I., 2004d. Preservation of juvenile hake (*Merluccius merluccius*, L.) in the western Mediterranean demersal trawl fishery by using sorting grids. *Scientia Marina* 68 (3), 435-444.

Sartor, P., Belcari, P., Carbonell, A., Gonzalez, M., Quetglas, A., Sánchez, P., 1998. The importance of cephalopods to trawl fisheries on the Western Mediterranean. *South African Journal of Marine Science* 20, 67-72.

Sbrana, M, Belcari, P., De Ranieri, S., Sartor, P., Viva, C., 2007. Comparison of the catches of European hake (*Merluccius merluccius*, L. 1758) taken with experimental gillnets of different mesh sizes in the northern Tyrrhenian Sea (western Mediterranean). *Scientia Marina* 71, 47-56.

Sbrana, M, Sartor P, Belcari P, 2003. Analysis of the factors affecting crustacean trawl fishery catch rates in the northern Tyrrhenian Sea (western Mediterranean). *Fisheries Research* 65, 271-284.

Sherman K, Duda AM (1999) An ecosystem approach to global assessment and management of coastal waters. *Marine Ecology Progress Series* 190, 271–287.

Smith, C.J., Marrs, S.J., Atkinson, R.J.A., Papadopoulou, K.N., Hills, J.M., 2003. Underwater television for fisheries-independent stock assessment of *Nephrops norvegicus* from the Aegean (eastern Mediterranean) Sea. *Marine Ecology Progress Series* 256, 161-170.

Smith, C.J., Papadopoulou, K.N., Diliberto, S., 2000. Impact of otter trawling on an eastern Mediterranean commercial trawl fishing ground. *ICES Journal of Marine Science* 57, 1340-1351.

Stamatis, C., Triantafyllidis, A., Moutou, K.A., Mamuris, Z., 2004. Mitochondrial DNA variation in northeast atlantic and mediterranean populations of norway lobster, *Nephrops norvegicus*. *Molecular Ecology* 13, 1377-1390.

Stamatis, C., Triantafyllidis, A., Moutou, K.A., Mamuris, Z., 2006. Allozymic variation in Northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. *ICES Journal of Marine Science* 63, 875-882.

Stefanescu, C., Lloris, D., Rucabado, J., 1992a. Deep-living demersal fishes in the Catalan Sea (western Mediterranean) below a depth of 1000 m. *Journal of Natural History* 26, 197-213.

Stefanescu, C., Lloris, D., Rucabado, J., 1993. Deep-sea fish assemblages in the Catalan Sea (western Mediterranean) below a depth of 1000 m. *Deep-Sea Research I* 40, 695-707.

Stefanescu, C., Morales-Nin, B., Massutí, E., 1994. Fish assemblages on the slope in the Catalan Sea (Western Mediterranean): influence of a submarine canyon. *Journal of the Marine Biological Association of the United Kingdom* 74, 499-512.

Stefanescu, C., Rucabado, J., Lloris, D., 1992b. Deep-size trends in western Mediterranean demersal deep-sea fishes. *Marine Ecology Progress Series* 81, 205-213.

Stenseth N.C., Mysterud A., Duran J.M., Hjermann D. Ø, Ottersen G., 2005. Uniting ecologist into a smooth, tasty and potentet blend. In: Stergiou K.I., Browman H.I., Bringing the gap between aquatic and terrestrial ecology. *Marine Ecology Progress Series* 304, 289-292

Stenseth, N.C., Ottersen, G., Hurrell, J.W. Belgrano, A., 2004. *Marine ecosystems and climate variation.* Oxford University Press Inc., New York, 252 pp.

Stergiou K.I., Koulouris M. 2000. Fishing down the marine food webs in the Hellenic Seas. Fishing down the Mediterranean food webs? *CIEM Workshop Series* 12, 73-78.

Stergiou, K.I., Moutopoulos, D.K., Erzini, K., 2002. Gill net and longlines fisheries in Cyclades waters (Aegean Sea): species composition and gear competition. *Fisheries Research* 57, 25-37.

Stergiou, K.I., Moutopoulos, D.K., Soriguer, M.C., Puente, E., Lino, P.G., Zabala, C., Monteiro, P., Errazkin, L.A., Erzini, K., 2006. Trammel net catch species composition, catch rates and metiers in southern European waters: A multivariate approach. *Fisheries Research* 79, 170-182.

Stergiou, K.I., Petrakis, G., Politou, C.Y. 1996. Small-scale fisheries in the South Euboikos Gulf (Greece): species composition and gear competition. *Fisheries Research* 26, 325-336.

Stergiou, K.I., Politou, C.Y., Christou, E.D., Petrakis, G., 1997. Selectivity experiments in the NE Mediterranean: the effect of trawl codend mesh size on species diversity and discards. *ICES Journal of Marine Science* 54, 774-786.

Stergiou, K.I., Petrakis, G., Politou, C.Y., 1997. Size selectivity of diamond and square mesh cod-ends for *Nephrops norvegicus* in the Aegean Sea. *Fisheries Research* 29, 203-209.

Stevens, J.D., Bonfil, R., Dulvy, N.K., Walker, P.A., 2000. The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science* 57, 476-494.

Tobar, R., Sardà, F., 1992. Annual and diel light cycle as a predictive factor in deep-water fisheries for the prawn *Aristeus antennatus* Risso, 1816. *Fisheries Research* 15, 169-179.

Tserpes, G., Fiorentino, F., Levi, D., Cau, A., Murenu, M., Zamboni, A., Papaconstantinou, C., 2002. Distribution of *Mullus barbatus* and *M. surmuletus* (Osteichthyes: Perciformes) in the Mediterranean continental shelf: implications for management. *Scientia Marina* 66, 39-54.

Tudela, S., 2000. *Ecosystem effects of fishing in the Mediterranean: an analysis of the major threats of fishing gear and practices to biodiversity and marine habitats.* FAO Fisheries Department (EP/INT/759/GEF), Rome, Italy, 45 pp.

Tudela, S., Sardà, F., Maynou, F., Demestre, M., 2003. Influence of submarine canyons on the distribution on the deep-water shrimp, *Aristeus antennatus* (Risso, 1816) in the NW Mediterranean. *Crustaceana* 76, 217-225.

Tyler, P.A., 2003. The peripherial deep seas. In: Tyler, P.A. (Eds.), Ecosystems of the deep oceans. *Ecosystems of the world* 28. Elsevier Science B.V., Amsterdam, 261-293 pp.

Tzanatos, E., Dimitriou, E., Katselis, G., Georgiadis, M., Koutsikipoulos, C., 2005. Composition, temporal dynamics and regional characteristics of small-scale fisheries in Greece. *Fisheries Research* 73, 147-158.

Tzanatos, E., Somarakis, S., Tserpes, G., Koutsikipoulos, C., 2006. Identifying and classifying small-scale fisheries metiers in the Mediterranean: A case study in the Patraikos Gulf, Greece. *Fisheries Research* 81, 158-168.

Ungaro, N., Marano, C.A., Marsan, R., Martino, M., Marzano, M.C., Strippoli, G., Vlora, A., 1999. Analysis of demersal species assemblages from trawl surveys in the South Adriatic sea. *Aquatic Living Resources* 12, 177-185.

Ungaro, N., Marano, G., Vlora, A., Martino, M., 1998. Space-time variations of demersal fish assemblages in the South-western Adriatic Sea. *Vie et Milieu* 48, 191-201.

Vassilopoulou, V., 2006. Dietary habits of the deep-sea flatfish *Lepidorhombus boscii* in north-eastern Mediterranean waters. *Journal of Fish Biology* 69, 1202-1220.

Vassilopoulou, V., Papaconstantinou, C., Christides, G., 2001. Food segregation of sympatric *Mullus barbatus* and *Mullus surmuletus* in the Aegean Sea. *Israel Journal of Zoology* 47, 201-211.

Villanueva, R., 1992. Deep-sea cephalopods of the north-western Mediterranean: indications of up-slope ontogenetic migration in two bathybenthic species. *Journal of Zoology*, London 227, 267-276.

Villanueva, R., 1995. Distribution and abundance of bathyal sepiolids (Mollusca: Cephalopoda) in the northwestern Mediterranean. *Bulletin du Musée Oceanographique de Monaco* 19-26.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 67-130
ISBN: 978-1-60021-935-1

Chapter 2

THE ECOLOGICAL ROLE OF ZOOPLANKTON IN THE TWILIGHT AND DARK ZONES OF THE OCEAN

Rolf Koppelmann [*] ***and Jessica Frost*** [†]
University of Hamburg, Institute for Hydrobiology and Fisheries Science
Grosse Elbstrasse 133, 22767 Hamburg, Germany

ABSTRACT

Cold temperatures, increased pressure, dim light and the absence of net primary production characterize the twilight zone between 200 and 1000 m of the open ocean. Below this depth is the dark zone consisting of stable abiotic conditions and perpetual darkness, with bioluminescence, the ability of organisms to produce their own light, being the only light source. These zones, also called the meso- and bathypelagic zones, are herein referred to as the deep-sea. The main food sources for organisms inhabiting these zones are vertically migrating animals, sinking carcasses and sinking particulate organic matter (POM) produced by autotrophic and heterotrophic organisms in the euphotic zone.

Approximately 90% of POM is remineralised as it sinks through the water column. Remineralization processes remain poorly characterized throughout the water column because they are difficult to observe and quantify. The character of the material transported to the deep-sea reflects the ecological structure of the upper ocean and influences the remineralization rate. Portions of sinking POM are transported from lower to higher trophic levels via the food chain. Longevity at these higher trophic levels probably exists in the deep-sea due to slow growth and maturity caused by low quantities of food reaching the deep.

A notable gap in understanding the linkage between trophic levels and remineralisation rates is the lack of information about gelatinous organisms. These organisms are widely distributed and occasionally occur in large numbers with biomass exceeding that of fish. This can greatly impact food web dynamics. A likely explanation

[*] Phone: +49 40 42838 6679; Fax: +49 40 42838 6618; E-mail: koppelmann@uni-hamburg.de
[†] jessica.frost@uni-hamburg.de

for the paucity of knowledge of gelatinous organisms is the inherent difficulty of sampling them. Most gelatinous organisms are fragile and break into pieces when sampled with commonly used nets. Thus, information on their ecological role and physiological rates is very limited.

This paper will give an overview on deep-sea zooplankton ecology, its temporal distribution, and its role in organic matter cycling, while addressing gaps in knowledge. The following topics should be investigated in future research dealing with deep-sea zooplankton ecology: physiological rates and potentials of functional groups and key species, responses to different quantities and qualities of food (flux), responses to disturbances or changes in temperature, pH, O_2, pollutants etc. and, last but not least, effects of climate change on carbon transformation and storage in the twilight and dark zones.

1. INTRODUCTION

The pelagic environment is the part of the open ocean comprising the water column. It is the largest ecosystem on earth and can be divided into different zones (Figure 1). The euphotic, also known as epipelagic, zone from the surface down to approximately 200 m is illuminated with enough light for photosynthesis. Plants and animals are largely concentrated in this zone. Most of the food for animals living in deeper parts of the ocean is produced in this zone. The environment below the euphotic zone is referred to as the deep-sea in this paper. The twilight, also known as mesopelagic, zone between 200 and 1000 m of the open ocean is the area where only dim light penetrates the water and a net primary production is no longer possible. In the dark, also known as bathypelagic, zone, below 1000 m, light is virtually absent. It is completely dark being only lit by bioluminescence, the light produced by the animals themselves. A specialized zone is the dynamic environment between the deep water and the sea-floor called the benthic boundary layer. This zone is of considerable interest because of the presence of very strong gradients of energy, dissolved and solid chemical components, suspended and particulate organic matter, and the number of organisms living there.

Seasonal changes of plankton abundance in the euphotic zone are primarily due to changing abiotic parameters (temperature, light, nutrients) and are well known for different areas of the ocean (e.g. Parsons and Lalli 1988, Newton *et al.* 1994). While the chemical and physical properties in the twilight zone are still influenced by abiotic and biotic activities at the surface and large areas of oxygen minimum occurring in some ocean regions, these properties hardly vary in the dark zone. In this zone, seasonal differences have been rarely observed. Temperature is generally low (< 6-7 °C) and salinity (~35 PSU) and oxygen concentrations are stable throughout the year (Svedrup *et al.* 1942, Menzies 1965, Mantyla and Reid 1983, Tyler 1995). In some semi-enclosed parts of the ocean, like the Mediterranean and the Red Seas, however, higher temperatures and salinities are observed in the deep-sea (Weikert and Koppelmann 1996). The distribution of animals inhabiting the benthic boundary layer is also influenced by tidal currents and benthic storms. Since no surface light penetrates the deep-sea, annual cycles cannot be triggered by changes in light intensity or length of daytime.

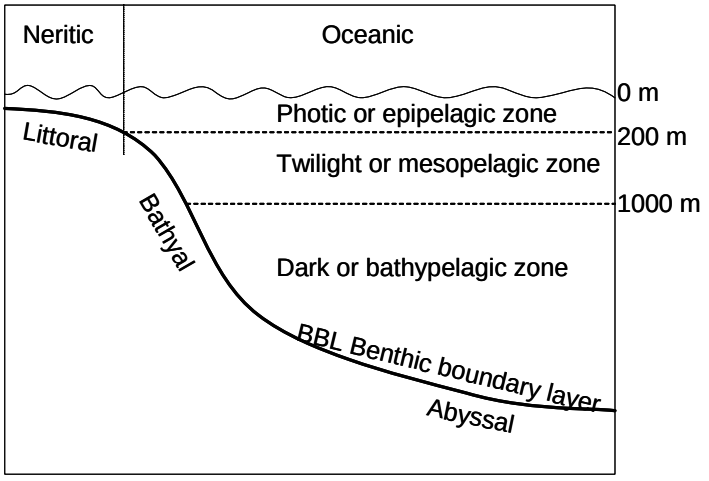


Figure 1. Habitat zones in the open ocean.

Despite these relatively stable conditions, living in the deep-sea is physiologically challenging due to limited resources. The deep-sea ecosystem depends mostly on organic material from the epipelagic zone. Food is transferred to greater depths by the vertical migration of plankton and micronekton (Vinogradov 1968, Vinogradov and Tseitlin 1983) in the form of gut contents or as a result of predation at depth (Angel 1989, Longhurst and Harrison 1989). Food is also transferred to a larger extent by the sinking of particulate organic material (Angel 1984, Fowler and Knauer 1986). Approximately 90% of the sinking POM is remineralized on its way through the water column, and the amount of available food decreases exponentially with increasing depth. This paper will give an overview on deep-sea zooplankton ecology, the temporal variability in distribution exemplified for three different oceanic regions, and its role in organic matter cycling, while addressing current gaps in knowledge.

2. TEMPORAL TRENDS IN DEEP-SEA ZOOPLANKTON DISTRIBUTION

The concept of the deep-sea as a constant environment, not subjected to temporal and spatial changes, has been revised extensively over the past 25 years. Episodic, mainly seasonal, variations in sinking particulate organic fluxes, extending from small particles to large phytodetritus and gelatinous agglomerates, are reported to arrive at the abyssal sea-floor (Billett *et al.* 1983, Smith *et al.* 2001). Spring bloom settlement, produces significant changes in the abundance and structure of the benthic community (Billett *et al.* 1988, Tyler *et al.* 1992, Loubere 1998), stimulating reproduction of some macrobenthic species and fishes (Bishop and Shalla 1994 and literature quoted therein) and sediment community oxygen consumption, particularly by microbial constituents (Lochte 1992). Wigham *et al.* (2003) reported on long-term changes in the megafaunal community at the 5000 m deep Porcupine Abyssal Plain in the NE Atlantic in relation to the inter-annual variability of food supply. However, it is difficult to differentiate between effects of long-term changes and stochastic events, like food falls (Billett *et al.* 2006), in deep-sea ecosystems.

In this chapter, we will present temporal variations in deep-sea zooplankton distribution by reviewing our studies from the temperate NE Atlantic (Christiansen *et al.* 1999, Koppelmann and Weikert 1999), the monsoon-driven Arabian Sea (Fabian *et al.* 2005, Koppelmann and Weikert 2005, Koppelmann *et al.* 2003a, 2005) and the semi-enclosed eastern Mediterranean Sea (Koppelmann and Weikert 2007, Weikert *et al.* 2001). In all regions, zooplankton sampling was performed by similar sampling strategies using the same methods. Mesozooplankton samples were taken by oblique stratified hauls (towing speed: 2 knots) with the use of a $1m^2$-MOCNESS (Multiple Opening and Closing Net and Environmental Sensing System; Wiebe *et al.* 1985) equipped with 9 or 18 nets of 333-µm mesh. Veering and heaving speed of the winch was 0.5 m s^{-1}, heaving speed was reduced to 0.2-0.3 m s^{-1} when the net passed the 450 m depth. Generally, sampling intervals were 50 m in the upper 450 m, 150 m between 450 and 1050 m, 200 m between 1050 and 2250 m, and 250 or 500 m below this depth. Upon recovery of the MOCNESS, nets were rinsed with seawater and plankton were preserved immediately in a 4% formaldehyde-seawater solution buffered with sodiumtetraborate (Steedman 1976). After placing the fractions in 70% ethanol for 30 seconds and drying them on cleansing paper, the material was wet-weighed on an analytical balance. Then, the samples were transferred into a sorting fluid comprised of 0.5% propylene-phenoxetol, 5.0% propylene glycol and 94.5% fresh water for taxonomical investigations (Steedman 1976).

2.1. Temperate NE Atlantic

Information on temporal changes of deep-sea plankton communities at lower latitudes is scarce. Data which may reflect responses to pulses of food supply were reported for bathypelagic mesozooplankton with respect to composition, total respiratory carbon demand and alterations of the carbon flux for the abyssal sediment community in the NE Atlantic (Koppelmann and Weikert 1999). Deevey and Brooks (1971) suggested seasonal variations in Sargasso Sea zooplankton at least as deep as 2000 m.

In the temperate NE Atlantic, the phytoplankton bloom varies in timing by as much as six weeks each year (Rice *et al.* 1986) but it usually begins in early May. Seasonally pulsed inputs have also been reported from other parts of the Atlantic and Pacific Oceans (Gooday 1996, Smith *et al*. 2001). These observations confirm the classical idea that "at some places of the ocean, a periodical variation of supply of food from the surface layer may give rise to a little annual excitement" at bathyal depths (Mosely 1880).

2.1.1. Hydrographical Setting

Koppelmann and Weikert (1999) reported seasonal changes of deep-sea zooplankton at the BIOTRANS site (47° N, 20° W) in the NE Atlantic in 1992 (Figure 2). The BIOTRANS site is located in the deep west European Basin (water depths > 4500 m), which reveals a variable bottom topography structured by a system of ridges and furrows (Heinrich 1986). The hydrography at this open ocean site was well explored during the NOAMP project (Northeast Atlantic Monitoring Program; Mittelstaedt *et al.* 1986). It exhibits a stable hydrographic environment, which is typical of the deep open sea. Different types of water masses were identified: surface water from 0 m to 150 m, North Atlantic Central Water between 150 m and 650 m, Mediterranean Water between 650 m and 1250 m, Labrador

Water from 1700 m to 2000 m, Middle North Atlantic Deepwater between 2300 m and 2800 m, and bottom water below 4000 m. The bottom water presents a stable hydrographic environment with temperatures between 2.54 and 2.63 °C, salinities between 34.90 to 34.91, and an oxygen content of 5.5 to 5.7 ml O_2 l^{-1} (Mittelstaedt *et al.* 1986).

2.1.2. Zooplankton Distribution

Figure 3 describes the bathymetric distributions of mesozooplankton abundance in terms of total biomass and individual numbers with respect to spring and summer. In 1992, the standing stock in the upper 750 m was higher by a factor of 4.2 in spring than in summer (Table 1). The respective biomass was higher by a factor of 2.0 in spring. Between 750 and 1050 m depth, seasonal differences were less obvious. In the dark zone, the summer concentrations of total zooplankton and biomass exceeded the spring values of the same year. This variance was caused by elevated concentrations in the layers between 1050 m and 2250 m (Figure 3). In this upper bathypelagic zone, the abundance was higher by a factor of 3.8 in summer, while the biomass was higher by a factor of 2.9. In detail, polychaetes were 9.3 times more abundant in summer than in spring, followed by the cyclopoid copepods (7.8) and siphonophore fragments (6.8). Malacostracs (4.5) and calanoid copepods (3.5) held an intermediate position (Table 1). The summer/spring ratios of ostracods (1.7) and chaetognaths (1.4) were least. The changes in total abundance of mesozooplankton in the 1050-2250 m layer were primarily caused by calanoid copepods, which comprised 81.0% of the total standing stock in spring and 76.4% in summer. Members of Metridinidae were abundant in the 1050-2250 m layer, comprising 14.4% of the calanoid standing stock during spring but 59.1% during summer. In absolute numbers, the standing stock of the Metridinidae, which were almost exclusively presented by the genus *Metridia*, increased by a factor of 13.5, from 360 Ind. m^{-2} in spring to 4870 ind. m^{-2} in summer. The ontogenetical composition of the genus was examined by one profile in spring and summer each (Figure 4). In spring, adult females contributed 5.1% to the generic standing stock, adult males 7.5%, CV copepodites 54.6% and younger stages 32.8%. In summer, juvenile *Metridia* CV copepodites dominated (97.7%) in relative and absolute numbers: adult females (0.03%) and males (0.1%) were nearly absent, while younger stages were minor (2.2%). Below 2250 m, the biomass concentrations were quite similar in spring and summer, whereas the abundance was slightly higher in summer (Table 1).

2.1.3. Ecological Relevance

Boreal regions of the open ocean are known for increased zooplankton abundances at depths greater than 1000 m during autumn and winter, where abundances are coupled with a faunistic change in general (e.g. Vinogradov 1968). In most cases, the phenomenon is due to diapausing stages of some prominent epipelagic, herbivorous/omnivorous calanoid copepod species like *Calanus finmarchicus* and *C. helgolandicus,* which descend at the end of the vegetation period (e.g. Vinogradov 1968, Hirche 1983, Kaartvedt 1996, Bonnet *et al.* 2005). In contrast, the increment in the standing stock of mesozooplankton in the dark zone of the BIOTRANS area in summer 1992 was not due to a shortage of food. Instead, a transient mass injection of phytoplankton spring bloom material was found at the sea-floor during the summer survey (Pfannkuche *et al.* 1993). The incidental analysis of the guts of bathypelagic copepods from our 1992 spring samples (Koppelmann 1994) revealed material that was similar in its composition to phytodetritus flocs collected at the bathyal deep-sea floor in 1986

in succession of a spring bloom (Thiel *et al.* 1988). Although information is scarce, loss of sinking surface-born material in the dark zone, in general, is evidenced by algal cells found in some deep-sea phaeodarians and particle-feeding copepods, which indicates a surface origin of the ingested particles (Gowing and Wishner 1992).

Table 1. Standing stocks (ind. m^{-2} of given depth-ranges) of zooplankton groups in the NE Atlantic in spring and summer 1992 (modified after Koppelmann and Weikert 1999)

Depth-zone m	Season	Biomass mg m^{-2}	Total Abundance Ind. m^{-2}	Ostracoda Ind. m^{-2}	Calanoida Ind. m^{-2}	Cyclopoida Ind. m^{-2}	Malacostraca Ind. m^{-2}	Chaetognatha Ind. m^{-2}	Polychaeta Ind. m^{-2}	Siphonophora Ind. m^{-2}
0-750	Spring	19475.4	27614.6	1982.1	19229.7	2498.1	406.1	1004.7	n/d	404.8
	Summer	9687.2	6565.7	662.6	4818.9	258.6	455.0	283.6	n/d	165.6
750-1050	Spring	2195.5	2018.4	111.4	1631.6	67.2	41.3	86.9	n/d	31.1
	Summer	2281.9	2032.4	204.5	1546.0	95.2	38.7	120.8	n/d	74.2
1050-2250	Spring	3520.9	3090.2	143.1	2501.8	245.1	51.9	115.9	12.1	31.1
	Summer	10468.3	10784.0	262.1	8237.6	1660.6	262.7	199.3	79.9	219.6
2250-4250	Spring	1507.5	687.6	65.9	526.1	45.1	14.1	25.0	3.2	5.4
	Summer	1393.7	1019.2	86.3	806.1	47.0	16.8	40.5	6.0	6.2

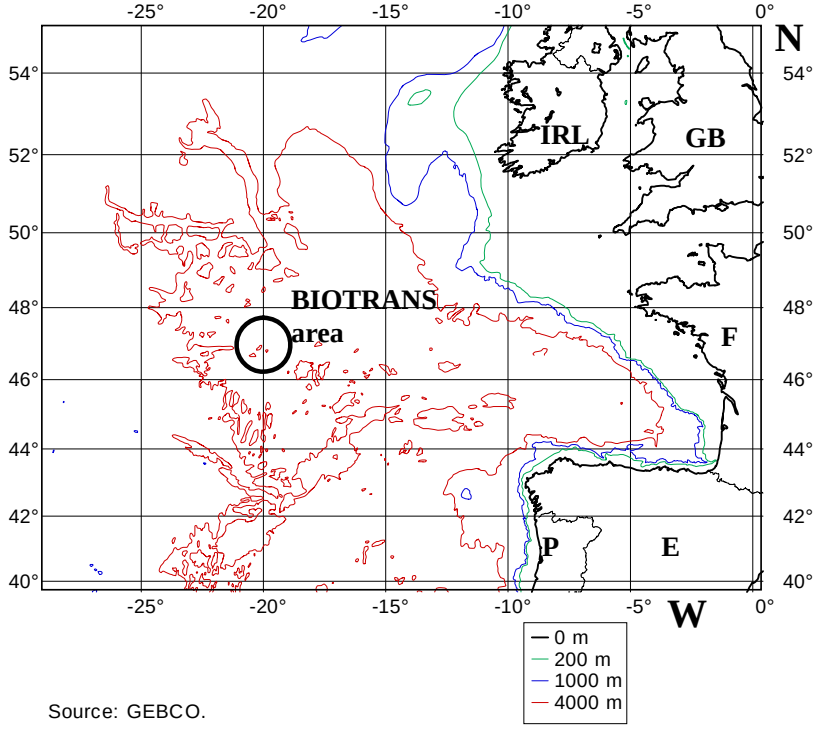


Figure 2. Area of investigation in the NE Atlantic.

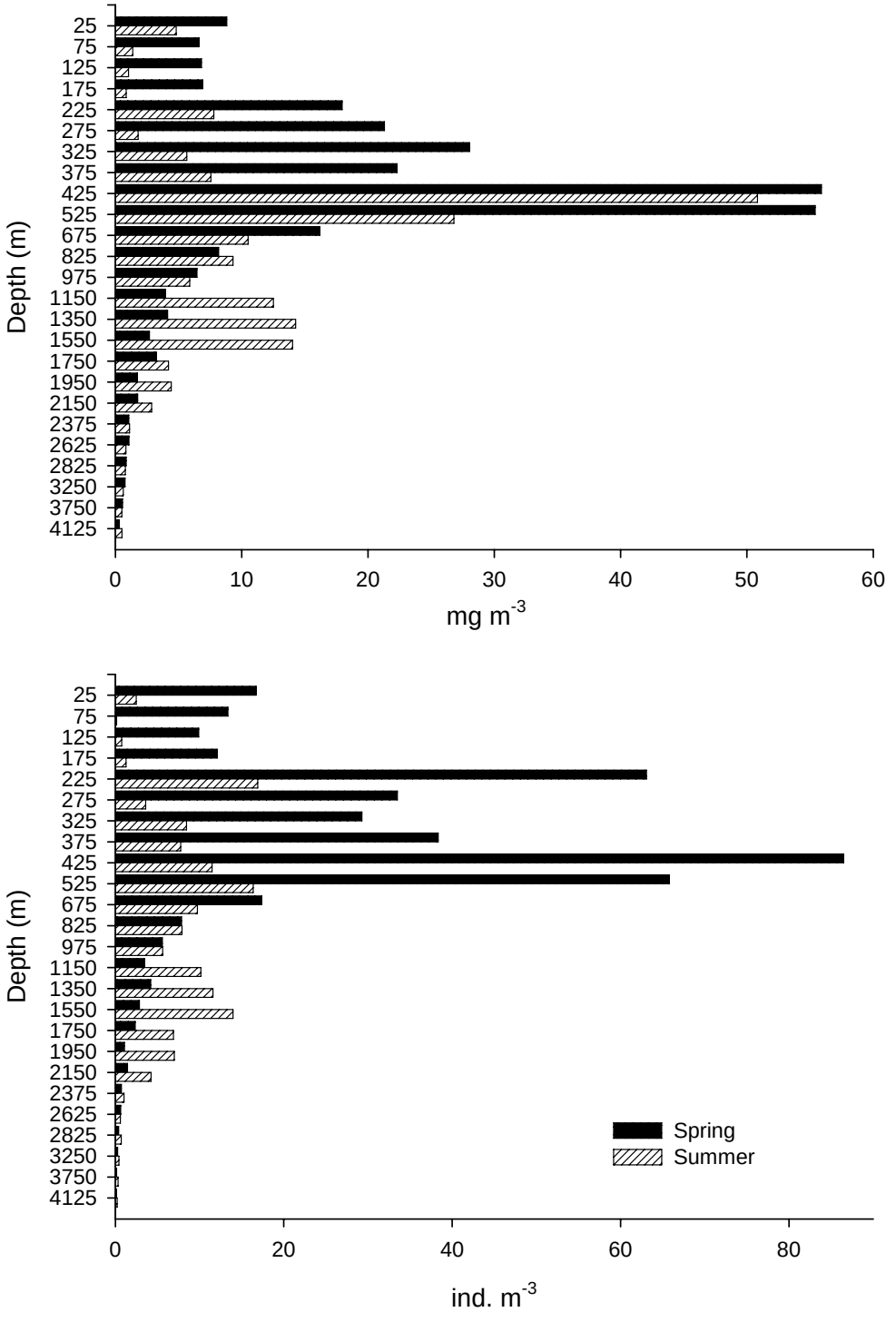


Figure 3. Biomass and abundance of zooplankton in the NE Atlantic in spring and summer 1992 at the mid-depths of different sampling zones. Above 1000 m only daytime values.

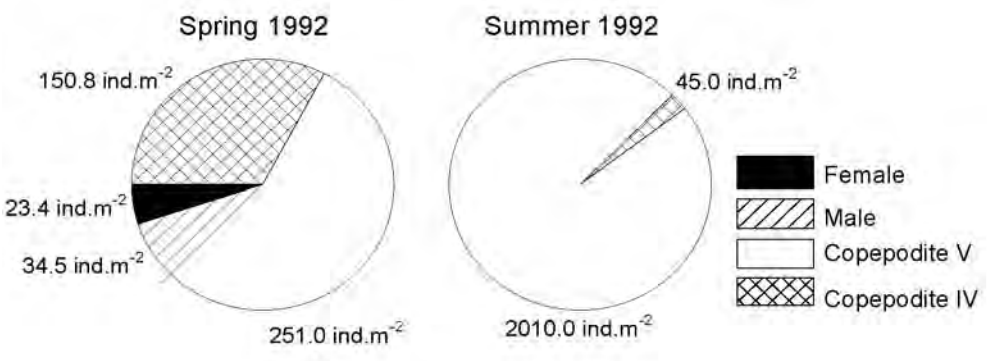


Figure 4. Standing stocks of *Metridia* spp. (sexes, CV, CIV) between 1050 and 2250 m in spring and summer 1992 in the NE Atlantic.

There was not a well-established single bloom event in the BIOTRANS area in spring 1992. Due to several storm events, the surface layer was mixed in intervals and a number of episodic and temporally-variable phytoplankton blooms occurred during the study period (Koeve *et al.* 1993). This suggests a series of smaller flux events of phytodetritus or any other kind of phytoplankton-derived material deposited into the deep-sea over an extended period of time. Despite the high average settling rate of 100 m d^{-1} (Billett *et al.* 1983, Alldredge and Silver 1988), the access time on potential food particles by bathypelagic organisms to initiate reproduction seems to be sufficient.

Unlike the situation at high latitudes, the summer increase of the bathypelagic community in the BIOTRANS area was accompanied by an increase of virtually all major zooplankton groups, with calanoid copepods (Table 1), predominantly *Metridia* species, being the main contributors. A seasonal and/or diel feeding migration by encompassing the upper 1000 m is unlikely. As exemplified for the principal zooplankton taxon, the calanoid Metridinidae, the enrichment in zooplankton abundance in the upper bathypelagic zone was most likely due to reproduction. The slight prevalence of males during both seasonal surveys contrasts the high proportion of females among sexes, which is typical of deep-sea zooplankton (Wishner 1980 and literature cited therein). In the course of reproduction, males are generally assumed to appear prior to females in calanoid copepods, indicating the close onset of mating coupled with low adult numbers (Kouwenberg 1993). This finding and the absolute and relative increase of CVs from spring to summer would point towards a reproduction cycle not exceeding 140 days for the deep-living *Metridia* population. The time span agrees with calculations on the generation times of boreal zooplankton (Diel 1988 and literature cited therein). The response of the genus appears to be typically r-selected.

The reasons for an absence of a seasonal increase of zooplankton in the lower bathypelagic zone are uncertain. Changes could have appeared later in the year due to a longer development time of deep-living bathypelagic zooplankton, and/or could not be observed with the method used (i.e. the mesh was too coarse to catch naupliar and early copepodid stages). Another explanation relies on the increasing evidence that, in general, the lower bathypelagic zone differs ecologically from the upper bathypelagic zone, which is probably reflected by a reduced decrease of zooplankton at depths below 2500 m (Koppelmann and Weikert 1992). This difference may be due to large-scale resuspension of particles from the sea-floor by abyssal storms, faunistic changes and/or decreased predation

pressure (Weikert and Koppelmann 1996). Assuming relatively high background values of suspended matter, the events of increased detrital input may not be sufficient to elicit a seasonal pulse in the mesozooplankton of the deep-sea at the BIOTRANS site, or the native fauna is not capable due to its feeding behavior to use fast sinking large material. Whatsoever, if there is no reaction, then this would indicate a marked shift towards a predominantly K-selected fauna in an environment of increased stability. However, at the bathyal deep-sea floor where detrital particles accumulate episodically, the resulting concentrations might again be high enough to trigger a seasonal reaction by the benthic and benthopelagic faunas.

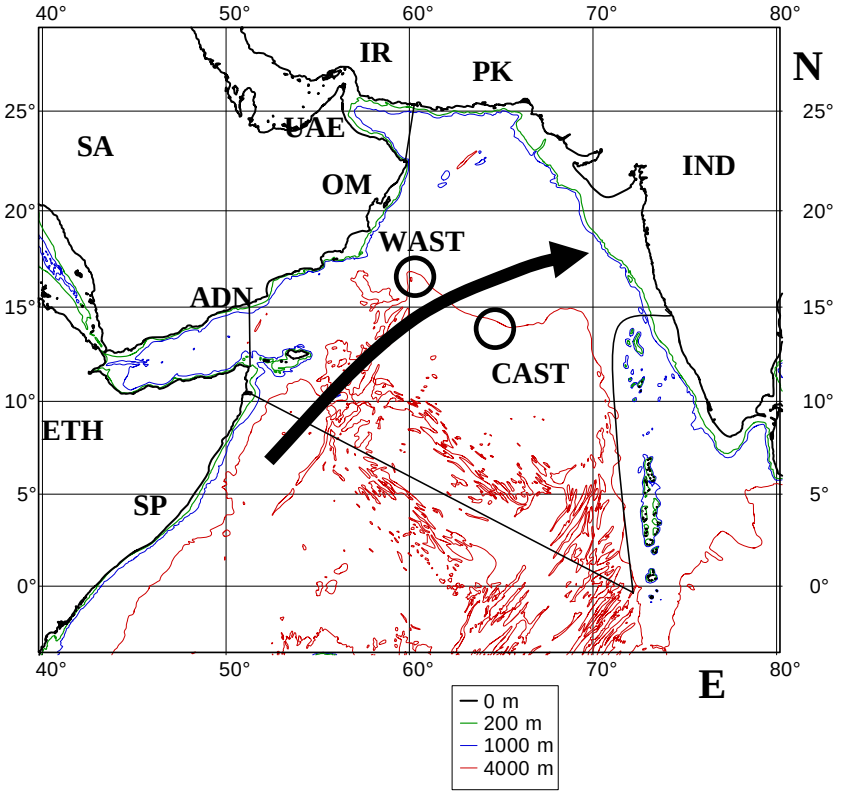
At the benthic boundary layer, there is some indication of increasing densities of nekton (e.g. Christiansen *et al.* 1990, Bagley *et al.* 1994, Priede 1994) and plankton (Angel 1990 and literature cited therein) towards the bottom. This increase may be attributed to an accumulation and resuspension of organic matter and to interactions between pelagos and benthos. A medium-scale variability, possibly generated by the bottom topography, was found for abyssal near-bottom mesozooplankton in the NE Atlantic (Christiansen *et al.* 1999). Hence, it can also be expected that at least the near-bottom zooplankton and nekton, like the benthos, is affected by temporal and spatial variations in the deposition of organic matter to the sea-floor. The detection of a seasonal deposition of phytodetritus (e.g. Billett *et al.* 1983, Lampitt 1985, Thiel *et al.* 1988) and its impact on the benthos (Gooday and Turley 1990, Pfannkuche 1993) led to the conclusion that life in the deep-sea is not as constant as previously thought.

2.2. Monsoon-driven Arabian Sea

In recent years, detailed studies of the Joint Global Ocean Flux Study (JGOFS; SCOR 1992) have improved our knowledge of zooplankton distribution and ecology in the epipelagic and mesopelagic zones of the Arabian Sea (Madhupratap and Haridas 1990, Kidwai and Amjad 2000, Madhupratap *et al.* 2001 and literature cited in these studies), but depths below 1000 m have been largely neglected. Quantitative information on zooplankton living in the dark zone is based on single vertical profiles of biomass from different stations, which are composed of coarse sampling intervals integrating some hundreds of meters (Vinogradov 1968). In this section, we will show that temporal differences exist in the deep Arabian Sea and that primary production and particle flux rates affect the abundance and distribution of mesozooplankton in the large habitat zones at two stations situated in different productivity regimes, where a good coverage of abiotic and biogeochemical data exists (Haake *et al.* 1993, Rixen *et al.* 1996).

2.2.1. Boundaries and Topography of the Arabian Sea

The Indian Ocean covers approximately 20% of the total ocean area of the world. It is the youngest of the world's three major oceans and its development began about 150 million years ago (mya) by the breakup of the large, ancient continent Gondwana. The ocean first opened approximately 125 mya and most of the Indian Ocean basin is less than 80 million years old. The present configuration was formed ca. 36 mya. The Arabian Sea is situated in the northwestern part of the Indian Ocean.



Source: GEBCO.

Figure 5. Areas of investigation in the Arabian Sea. WAST = Western Arabian Sea Station. CAST = Central Arabian Sea Station. The thin lines show the boarders of the Arabian Sea. The arrow indicates the direction of the Findlater Jet during the SW monsoon.

The Arabian Sea (Figure 5) is bound to the south by an imaginary line from Ras Hafun (Somalia) to the Addu Atoll (Maldives; 0°42' S, 73°09' E), thence up the western edge of the Maldives and the Laccadives to the Indian coast at 14°48' N, 74°07' E. To the east it is bound by India, to the north by Pakistan and Iran, and to the west by the Arabian Peninsula and the Horn of Africa (International Hydrographic Bureau 1953). The Gulf of Oman connects the Arabian Sea with the Persian Gulf via the Strait of Hormuz, while the Gulf of Aden connects it with the Red Sea via the Strait of Bab el-Mandeb. Most of the Arabian Sea has depths that exceed 3000 m (Figure 5), and the 1000 m contour line reaches close to the coast except off Pakistan and India. The Carlsberg Ridge, a submarine belt of elevated seafloor, on the southern border of the Arabian Sea divides the Arabian Basin from the Somali Basin.

2.2.2. Monsoonal Forcing, Currents and Water Masses

The Arabian Sea is a tropical ocean that is influenced by seasonal changing monsoon conditions which lead to a total reversal of the surface current system. During November to February, high atmospheric pressure over Asia and low pressure over an area from northern Australia to approximately 10° S brings the NE monsoon winds and a wet season for southern Indonesia and northern Australia. Between May to September the conditions change; low pressure over Asia and high pressure over Australia. This causes a very strong SW monsoon

with wind speeds up to 24 knots and a wet season in South Asia. The onset of the SW monsoon varies inter-annually by a 30-50 days oscillation (Krishnamurti *et al.* 1985 a,b). The intensity of the monsoon strength is related to the bi-annual oscillation in the tropical atmosphere (Cadet and Diehl 1984, Dube *et al.* 1990). On a longer time-scale, a link with the El Niño/Southern Oscillation pattern remains under discussion (Barnett 1985, Webster and Yang 1992). Calm inter-monsoon periods exist between the NE and the SW monsoons.

During the weak NE monsoon, the drift current flows south along the Indian coast and turns west at about 10° N. One branch flows into the Gulf of Aden and the other south along the Somali coast. This pattern changes drastically with the introduction of the strong SW monsoon winds, upon which surface currents react almost immediately. A branch of the South Equatorial Current now turns north between 5° S and the equator, and then flows as the Somali Current with speeds up to 7 knots along the coast into the Arabian Sea. Beyond Socotra, the Somali Current becomes part of an anticyclonic circulation to the northeast along the coast of Arabia and then south along the coast of India (Wyrtki 1973, Tchernia 1980). Pronounced upwelling occurs along the African and Arabian coast during this period (Sastry and D'Souza 1972, Smith and Bottero 1977, Tsai *et al.* 1992). A very narrow atmospheric jet at a low altitude (Figure 5), known as the Findlater Jet (Findlater 1969, 1974), drives an open ocean upwelling by Ekman pumping to the NW of the axis and a downwelling SE of the axis during the SW monsoon season (Smith and Bottero 1977, Luther *et al.* 1990, Brock *et al.* 1991, Brock and McClain 1992). Moreover, filaments of nutrient-rich water from coastal upwelling can reach the open sea (Manghnani *et al.* 1998, Lee *et al.* 2000, Toon *et al.* 2000). Little is known about the circulation of deep and bottom waters in the Arabian Sea. Warren (1994) suggests that bottom water upwells in the Arabian Sea at a rate of 5-10 * 10^{-5} cm s^{-1} into the bottom of the North Indian deep water.The circulation in the layer north of 10° N consists of a poleward western boundary current and a weaker equatorwards flow to the east.

Shetye *et al.* (1994) gave a review on circulation and water masses of the Indian Ocean. The annual precipitation is less than 10 cm in the northern and western part of the Arabian Sea and up to 200 cm in the area of the Bay of Bengal and the equatorial Indian Ocean, whereby the annual evaporation over this area is almost uniform, ca.150 cm. This leads to an increase in salinity in the northern Arabian Sea and the resulting water mass is called the Arabian Sea High Salinity Water (ASHSW). The higher precipitation in the southern and eastern part of the northern Indian Ocean forms the Equatorial Surface Water (Darbyshire 1967). This water moves into the Arabian Sea during the NE monsoon either with the surface drift or with the current along the coast of India. The denser ASHSW sinks below the Equatorial Surface Water and its core is marked by a sub-surface salinity maximum (Rochford 1964).

Sea surface temperature (SST) ranges between 24-25 °C in January and February and >28 °C in June (Robinson 1960). According to Colborn (1976), four phases of the annual cycle of SST can be described: a warming from February to May, cooling from May to August, warming from September to mid-November, and cooling from Mid-November to January. These changes in temperature are associated with variations in wind speed due to monsoonal conditions and upwelling phenomena (Rixen *et al.* 1996).

From sources of the Persian Gulf and the Red Sea, a horizontal salinity gradient can be found at subsurface depths. Warm, saline Persian Gulf Water (PGW) flows through the Strait of Hormuz into the Gulf of Oman where it sinks to depths between 200 and 250 m. Spread of this water leads to a salinity maximum, which can be found between 200 and 400 m in the

northern Arabian Sea. The high salinity Red Sea Water (RSW) enters the Gulf of Aden at 36 PSU and 15 °C. This water mass can be found between 500 m in the North (18° N) and 800 m near the equator. At 2500 m, the core of the Indian Ocean Deep Sea Water can be found with a salinity of 34.77 to 34.78 PSU and a temperature between 1.91 to 2.11 °C (Wyrtki 1971). The bottom water of the Arabian Sea is derived from south of the equator, with salinity varying around 34.73 and temperature between 1.09-1.33 °C.

2.2.3. Primary Production and Particle Flux

Gilson (1937) reviewed the knowledge of the nitrogen cycle of that time and discussed the conditions for the production of plankton at different areas and times in the Arabian Sea. During the International Indian Ocean Expedition (IIOE), the primary production in the Arabian Sea was extensively measured by various authors, which resulted in a summary of collected data (Babenerd and Krey 1974) and a Phytoplankton Production Atlas (Krey and Babenerd 1976). Later, the distribution of phytoplankton in the Arabian Sea was described on the basis of 850 samples collected during two cruises of Russian research vessel in 1966 and 1972 (Kuz'menko 1977). Most of these data were reviewed by Banse (1987) in his description of the seasonality of phytoplankton chlorophyll in the central and northern Arabian Sea. In the entire Arabian Sea north of approximately 10° N, a bloom period was detected during the SW monsoon, including the regions of coastal upwelling. A winter bloom was present in the northernmost region of the Arabian Sea, possibly due to new nutrient supply by convective overturn (Madhupratap *et al.* 1996, Prasana Kumar and Prasad 1996). The inter-monsoon periods exhibited overall low plankton concentrations.

Several papers (Banse and McClain 1986, Brock *et al.* 1991, Brock and McClain 1992) described phytoplankton blooms in the Arabian Sea from CZCS (Coastal Zone Color Scanner) data. Banse and English (2000) reviewed the available literature and modified the data with a more conservative cloud screen than used for the NASA Global Data Set in order to describe the geographical differences in seasonality of CZCS-derived phytoplankton pigments in the Arabian Sea from 1978 to 1986. The authors confirmed the pattern of elevated phytoplankton chlorophyll concentrations during the SW monsoon almost everywhere in the Arabian Sea. New data from the SeaWiFS (Sea-viewing Wide Field-of-view Sensor) instrument also show this pattern.

The pattern of seasonally changing primary production rates is also reflected by particle flux rates measured in the deep-sea by sediment traps deployed at 2800-3000 m depth (Haake *et al.* 1993). Haake *et al.* (1993) stated that enhanced fluxes exist during the two monsoons, while lower fluxes exist during the two inter-monsoon periods in the western and central Arabian Sea. In the eastern part of the Arabian Sea, peak fluxes exist during the late SW monsoon until the end of October, whereas particle fluxes are variable during other times. Honjo *et al.* (1999) measured particle flux rates as a part of the US-JGOFS Arabian Sea Process Study at meso- and bathypelagic depths in the western Arabian Sea along a transect quasi-perpendicular to the Oman coast between October 1994 and December 1995. These results also fit the described pattern of larger fluxes during the SW monsoon rather than during the NE monsoon. Lee *et al.* (1998) linked these flux rates with concomitantly measured primary production data and sediment accumulation rates. The authors stated that the organic carbon fluxes generally decreased with increasing distance from the shore, with the largest gradient between the surface and the sediment at the offshore station.

2.2.4. Zooplankton Distribution

An on-/offshore gradient with lowest values of zooplankton abundance in oceanic waters was observed during the IIOE (Cushing 1973). Recently, this feature was confirmed for the upper 1000 m of the water column along a line from 18° N, 58° E-14° N, 65° E and further south to 10° N, 65° E by Smith *et al.* (1998) and Wishner *et al.* (1998) and at the Somali coast by Couwelaar (1997) for several seasons. In contrast, Madhupratap *et al.* (1996) and Padmavati *et al.* (1998) did not find significant differences between coastal and offshore regions in the central and eastern Arabian Sea during the winter monsoon in 1995.

In the Arabian Sea, sampling was regionally limited and occurred mainly in onshore waters. Also, the NE monsoon and inter-monsoon periods are poorly sampled in the open waters. Sufficient data for a winter-summer comparison were only available for the Somali Current area (Baars and Osterhuis 1998). Using JGOFS data sets from the Arabian Sea, Koppelmann *et al.* (2003a) investigated the effects of monsoon-induced changes in the water column on the abundance and distribution of deep-living zooplankton. During three cruises with the *RV's Meteor* and *Sonne*, deep-sea zooplankton were systematically sampled in the Arabian Sea. The cruises covered the autumn inter-monsoon in October 1995 (*RV Meteor* cruise 33/1; Lochte *et al.* 1996), the spring inter-monsoon in April 1997 (*RV Sonne* cruise 118; Pfannkuche and Utecht 1998), and the NE monsoon in February 1998 (*RV Sonne* cruise 129; Pfannkuche and Utecht 1999). The data was fitted into a single hypothetical year. Although inter-annual variability exists in atmospheric forcing (Rixen *et al.* 1996), the biannual change in wind direction and monsoonal circulation is a regular event in the Arabian Sea (Fieux and Stommel 1977, Schott and McCreary 2001), which provides a strong trigger signal for biological reactions.

During the investigations, higher zooplankton abundance and biomass values were found at station WAST (Western Arabian Sea Station) than at station CAST (Central Arabian Sea Station). Both sites are influenced by the low-level Findlater Jet (Findlater 1969, 1974) blowing from SW during the summer monsoon (Figure 5). The jet drives nutrient-rich waters into the open-ocean (Morrisson *et al.* 1999), causing high primary productivity and high particle flux rates in the area of station WAST (Haake *et al.* 1993). CAST is located on the SE side of the Findlater Jet, where the mixed layer was deeper and primary production and abundance of zooplankton were diminished compared to values at WAST. The difference was greatest in October, intermediate in April, and minor in February. Koppelmann *et al.* (2003a) compiled primary production data, other zooplankton data, and flux data from different sources and added these data into their scheme of a single hypothetical year.

The epipelagic zooplankton data indicated temporal changes in zooplankton biomass and abundance at the two sites. Highest values occurred in October at WAST (Figure 6), following the high productive summer SW monsoon, whereas at CAST (Figure 6), high values were found in October and February (NE monsoon). At both stations, lowest values were found during the spring inter-monsoon, indicating an in-phase coupling of primary production and zooplankton abundance in the upper 150 m (Koppelmann *et al.* 2003a). Although data suggest that seasonal differences in mesozooplankton biomass and individual counts were weak in the Arabian Sea (~3 fold) compared to changes up to 1-2 orders of magnitude in temperate oceans (Cushing 1975), values were in the same order as described for other tropical areas (2-3 fold; Cushing 1975). Seasonal variability in biomass of epipelagic zooplankton in the Arabian Sea has already been reported by Smith *et al.* (1998), Wishner *et al.* (1998) and Stelfox *et al.* (1999), showing highest values during the SW monsoon. Their

data also show that seasonal peaks in biomass of zooplankton varied depending on the sampling location.

Several authors have reported a low seasonal response of zooplankton biomass in the surface and near-surface waters of the Arabian Sea (Madhupratap *et al.* 1996, Baars and Osterhuis 1998, Wishner *et al.* 1998, Baars 1999) and the northern Somali Basin (e.g. Couwelaar 1997) despite strong seasonality in physical forcing (Rixen *et al.* 1996, Weller *et al.* 1998), primary production (Cushing 1973, Brock *et al.* 1993), and export flux (Haake *et al.* 1993, Lee *et al.* 1998, Honjo *et al.* 1999). This phenomenon was termed the 'Arabian Sea Paradox' (Baars and Brummer 1995). The results presented by Koppelmann *et al.* (2003a) show low zooplankton abundance during the spring inter-monsoon in the western and central Arabian Sea, hence the concept of steady zooplankton biomass is not supported. Furthermore, Luo *et al.* (2000) stated that zooplankton biomass during the SW monsoon could be even as much as 5 fold greater than during the spring inter-monsoon at some sites in the upper 200 m.

At mesopelagic depths, temporal differences were not obvious at CAST (Figure 6). At WAST, however, lowest biomass and individual standing stocks were found during the inter-monsoon in April between peaks of POC flux (Koppelmann *et al.* 2003a). According to Wishner *et al.* (1998), low values occurred also during the late SW and early NE monsoons. The authors stated that overall seasonal trends were weak in the mesopelagic zone (200-1000 m) with high biomass in the >2 mm size fraction during the spring intermonsoon and a biomass peak in August for the smaller size classes. Tseitlin and Rudyakov (1999), by using the very large database archived by various Russian expeditions, also found some seasonal fluctuation in the mesopelagic zone (100-1000 m) in the northern region (0°-15° N) of the Indian Ocean. During the SW monsoon, zooplankton biomass was higher and decreased more abruptly with depth as compared to the NE monsoon period.

In the bathypelagic zone, Koppelmann *et al.* (2003a) found statistically significant differences in abundance between seasons at both stations, indicating a change in production of the overlying surface layer. At WAST, however, differences in the decrease of abundance with depth were detected between February and April and February and October, which would indicate a change in processes (e.g. Roe 1988). Perhaps this decrease was caused by an observed change in the relative contribution of mesozooplankton size classes in the epipelagic zone at station WAST (Koppelmann et al. 2003a), which possibly altered the particle flux. These data suggest that the bathypelagic community responds to increased or modified fluxes of POM.

2.2.5. Ecological Relevance

At twilight and dark depths, spatial differences in zooplankton abundance were detectable in the Arabian Sea, reflecting the annual productivity at the surface. In contrast to results from the NE Atlantic (Koppelmann and Weikert 1999), a direct coupling of mesozooplankton abundance with the seasonal variability in POC flux was not found in the Arabian Sea though significant temporal differences existed in the dark zone. Zooplankton profiles from the Arabian Sea and other oceans show a reduced decline of abundance and biomass with increasing depth starting at about 2250 to 2500 m, where a faunistic change seems to occur (Weikert and Koppelmann 1996). The question remains whether different remineralization modes of the deep-sea biota are active in the dark zone. Also Antia *et al.* (2001) suggested that the midwater fauna between the bottom of the euphotic zone and ~2000 m efficiently utilizes sinking particles until a threshold value of particles is reached that ultimately settle to

the abyssal sea-floor. Yamaguchi *et al.* (2002) reported from the North Pacific Ocean an increase of bacterial biomass with depth in relation to the biomass of protozoans and mesozooplankton, contributing up to 80% to the total biomass between 3000 and 4000 m. These findings are supported by Koppelmann *et al.* (2005). The distribution of free-living bacteria and mesozooplankton was investigated at WAST and CAST in February 1998 during the late NE monsoon (Figure 7). In the epipelagic zone (0-150 m), bacterial abundance was 0.6-2.2 * 10^{11} cells m^{-3} and mesozooplankton abundance was 200-700 ind. m^{-3}. At the core of the oxygen minimum zone (150-400 m), mesozooplankton abundance decreased sharply, whereas bacterial biomass increased at WAST and CAST. At greater depths (>400 m), mesozooplankton abundance decreased more distinctly with increasing depth, whereas bacteria remained relatively stable below 700 m. The result is a relatively higher share and greater importance of bacteria in the bathypelagic zone (>1000 m). In order to get better insights into the functioning of the biological pump and to understand the underlying mechanisms and processes, it is necessary to investigate the abundance and physiological activity from bacteria to fishes in the deep-sea.

2.2.6. The Vertical Distribution of Specific Taxa in the Arabian Sea under Special Consideration of the Oxygen Minimum Zone (OMZ)

Oxygen is an intrinsic element for most of the metazoan fauna and hence, it controls its distribution. Longhurst (1967) observed that most zooplankton were excluded from waters containing less than 0.2 ml O_2 l^{-1}. Values below this concentration were defined by Fiadero and Strickland (1968) as zones of hypoxia. The absence of measurable quantities of oxygen from open ocean waters was first mentioned by Schmidt (1925). Schmidt (1925) found that no O_2 exist in a layer between 400 and 500 m over a total depth of 3140 m in the Gulf of Panama. In fact, during a cruise aboard U.S.S. *Albatross*, Agassiz (1891) already found an azoic zone between 366 and 732 m in the Gulf of Panama. In the years after 1925, considerable attention was given to the minima and maxima of O_2 content occurring over large parts of the oceans, which were explained by biological activity as well as by circulation (Sverdrup 1938, Wüst 1935). The distribution of oxygen and hypoxia in the world oceans was reviewed by several authors (Wyrtki 1962, Kamykowski and Zentara 1990, Olson *et al.* 1993).

A very extensive and pronounced OMZ exists over a vast area in the Arabian Sea, extending from just below the mixed layer down to over 1000 m depth. This OMZ was first described by Sewell and Fage (1948) from a data set obtained by the John Murray Expedition between 1933 and 1934. Some years later, Vinogradov and Voronina (1962) also reported on the OMZ in the Arabian Sea, which may cause an occasional massive fish kill in the area.

The influence of the OMZs on the distribution of zooplankton is discussed in several papers for different parts of the world oceans (e.g. Childress 1968, Judkins 1980, Weikert 1980, Sameoto 1986, Wishner *et al.* 1995, Saltzman and Wishner 1997 a,b) and the Arabian Sea (Vinogradov and Voronina 1962, Madhupratap and Haridas 1990, Kinzer *et al.* 1993, Böttger-Schnack 1994, 1996, Koppelmann and Weikert 1997, Herring 1998, Padmavati *et al.* 1998, Wishner 1998, Morrison *et al.* 1999, Fabian *et al.* 2005). At the upper and lower OMZ interfaces, organic matter and nitrifying bacteria accumulate, causing zones of enhanced biological activity (Karl and Knauer 1984, Ward *et al.* 1989, Lipschultz *et al.* 1990, Wishner *et al.* 1995).

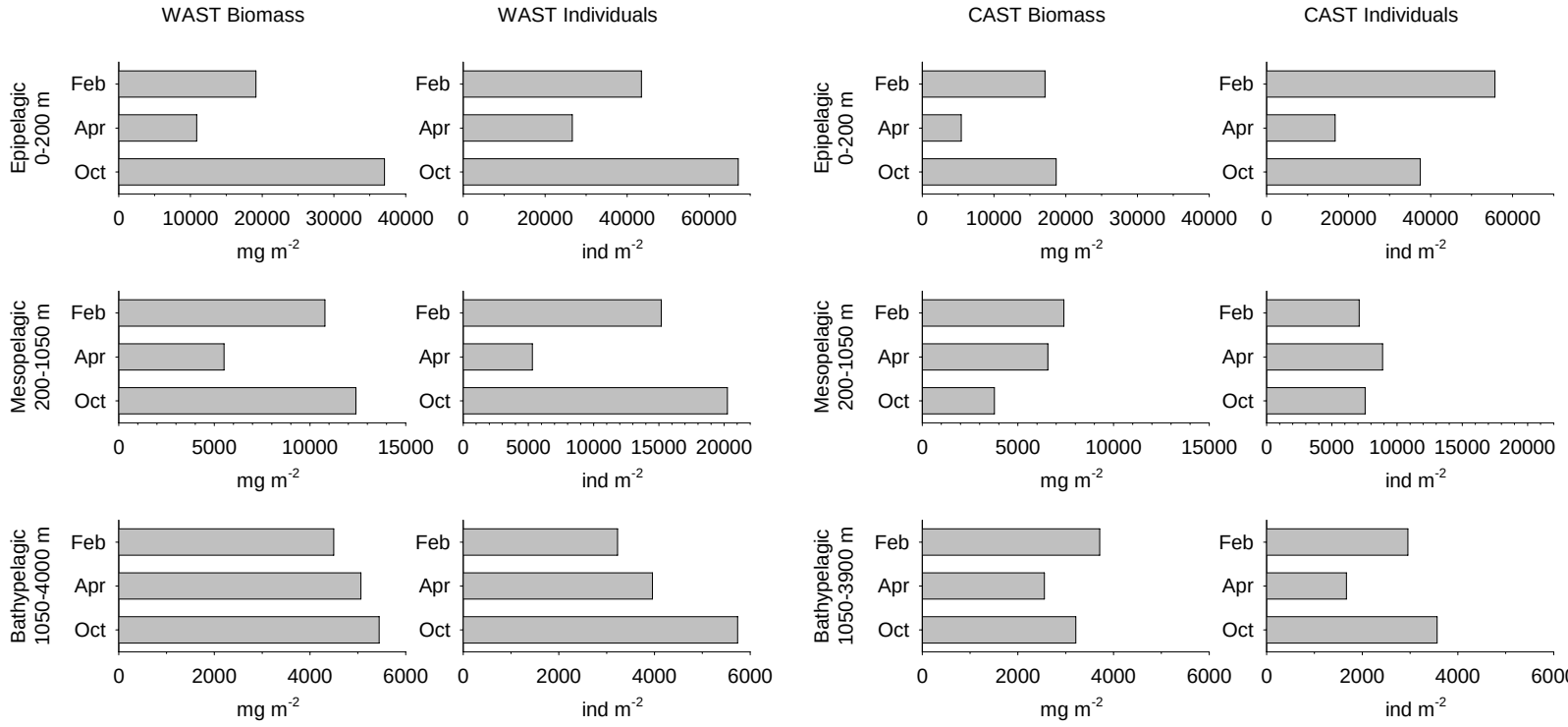


Figure 6. Standing stocks (mg m^{-2} and ind. m^{-2}) of zooplankton in different habitat zones in the Arabian Sea during an idealized year (February 1998, April 1997, October 1995).

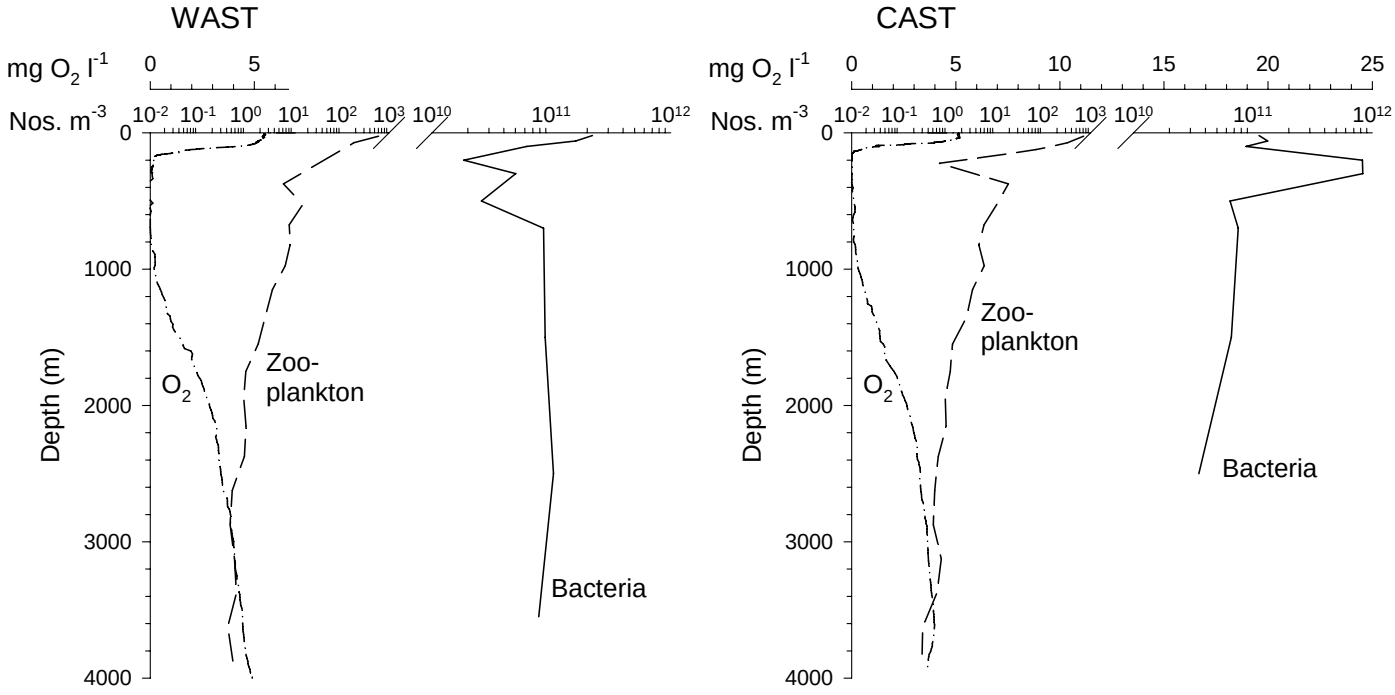


Figure 7. Vertical distributions of zooplankton and bacteria numbers over the full depth at stations WAST (Western Arabian Sea Station) and CAST (Central Arabian Sea Station) in February 1998 in relation to the oxygen concentration.

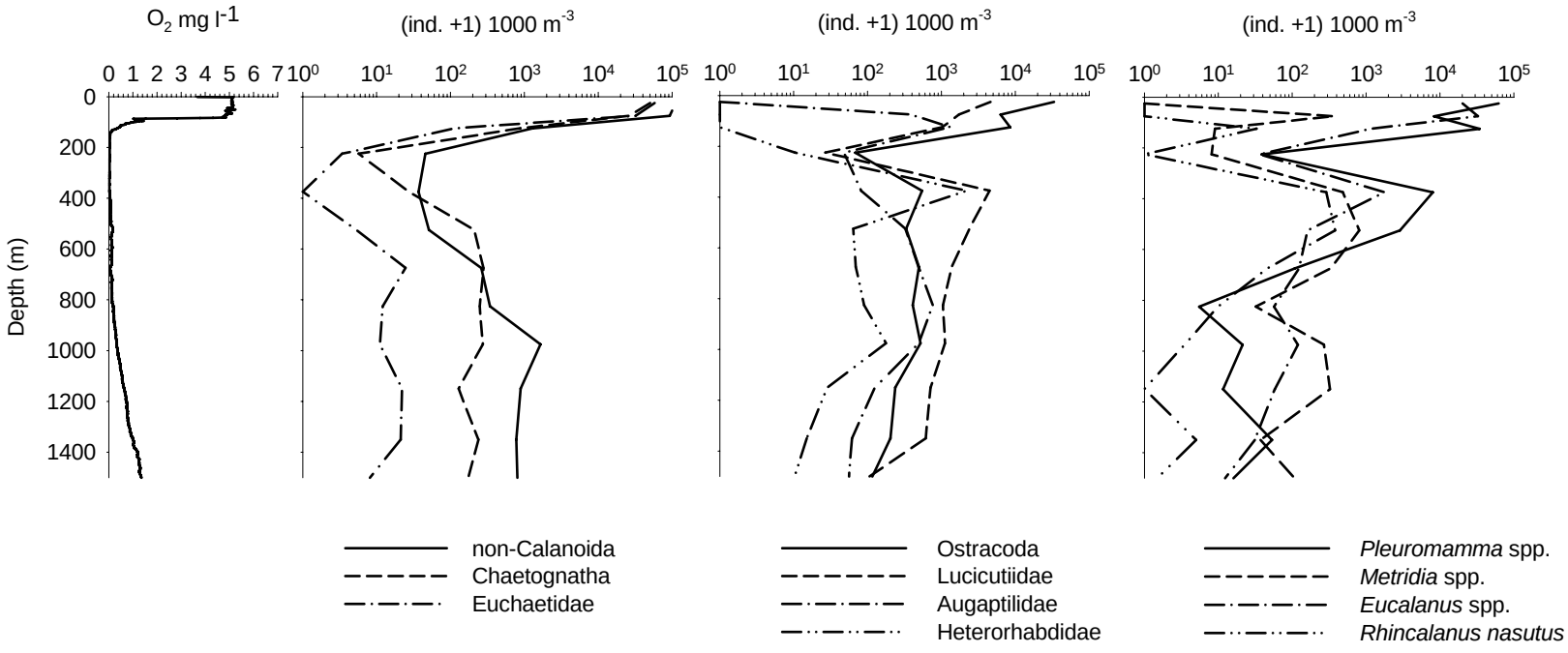


Figure 8. Vertical distribution of specific zooplankton taxa in the oxygen minimum zone (OMZ) of the Arabian Sea (modified after Fabian *et al*. 2005).

The concentrations of all major groups of zooplankton were reduced in the OMZ (Fabian *et al.* 2005). Among them, non-calanoid copepods, chaetognaths and the calanoid family Euchaetidae showed the widest vertical extension of decreased abundance in the OMZ (Figure 8). Chaetognaths, Euchaetidae and many species of non-calanoid copepods are supposed to be carnivorous; possibly the oxygen demands of these predators cannot be fulfilled in the OMZ. In turn, this might explain why non-calanoid copepod and chaetognath abundance often markedly increased just below the OMZ (Figure 8). The existence of enhanced individual concentrations at the lower boundary of the OMZ was first suggested by Vinogradov and Voronina (1962) for the Arabian Sea. Also, Morrison *et al.* (1999) observed such an increase for some zooplankton groups. The authors opine that there is some evidence that the increase is correlated with increased oxygen values close to the base of the OMZ, allowing the mentioned groups to satisfy their oxygen requirements.

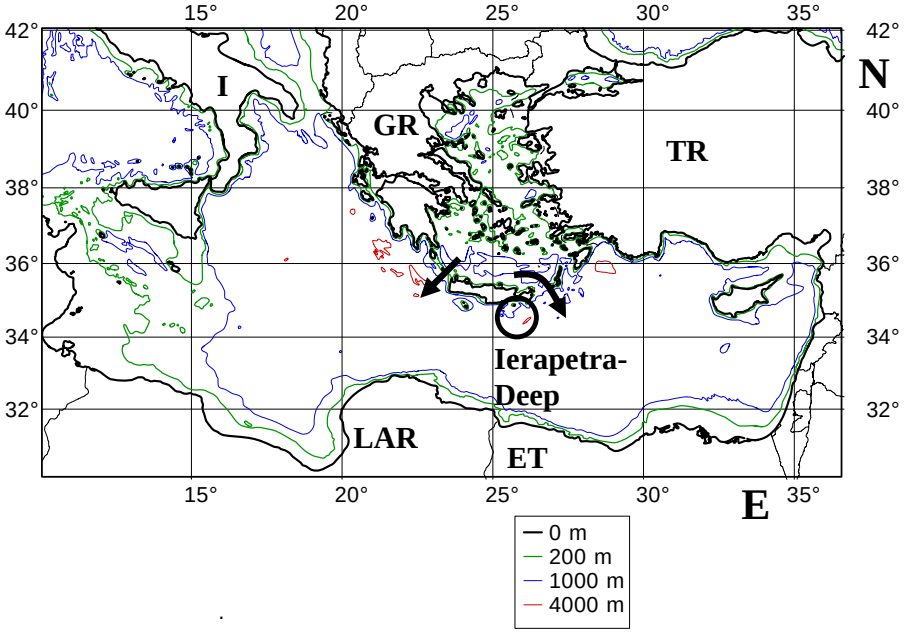
Some species have evolved mechanisms to survive low oxygen concentrations. Calanoid copepods reached a relative abundance of up to 95% in the OMZ. An increase of calanoid copepods in OMZs was also reported from the SE Arabian Sea (Madhupratap and Haridas 1990) and the tropical eastern Pacific (Sameoto 1986, Saltzman and Wishner 1997a,b). For the Arabian Sea, Vinogradov and Voronina (1962) remarked that *Rhincalanus nasutus* was common at oxygen concentrations lower than 0.2 mg O_2 l^{-1}. Fabian *et al.* (2005) found that Ostracoda, *Pleuromamma* spp., *Metridia* spp., *Eucalanus* spp., *Rhincalanus nasutus*, *Lucicutia* spp., Augaptilidae, and Heterorhabdidae decreased in abundance at the upper boundary of the OMZ but are again common within the OMZ (Figure 8). Weikert (1980) stated that *R. nasutus* represented 20% of the copepods in the core of the OMZ (1.3-1.9 mg O_2 l^{-1}) in the Red Sea. Longhurst (1976) and Judkins (1980) found *Calanus* and *Eucalanus* to be abundant in the OMZ (0.3-0.7 mg O_2 l^{-1}) off Baja California in the Pacific. Also mesopelagic fishes can appear in high numbers in OMZs (Cornejo and Koppelmann 2006 and literature cited therein). Wishner *et al.* (2000) reported *Lucicutia grandis* as a typical inhabitant of the OMZ of the Arabian Sea living in the depth range of 300-1000 m and representing 12.8% of all calanoid copepods as an average.

The copepod *Lucicutia grandis* has been studied extensively as an indicator species of oceanic OMZs (Wishner *et al.* 2000). This large (prosome length 4-5 mm) omnivorous calanoid lives mainly near the lower interface of the OMZ where it eats a variety of zooplankton and particulate material (Gowing and Wishner 1998). Feeding and reproduction of *L. grandis* occurs during all seasons but is triggered, in part, by the greater input of sinking POM during monsoonal periods (Wishner *et al.* 2000). Juveniles live higher up in the water column and in waters with lower oxygen levels than do the adults (Wishner *et al.* 2000).

Koppelmann and Weikert (2005) described the vertical distribution of *L. grandis*, a steady resident in the oceanic OMZ, and *Calanoides carinatus*, a transient resident in the oceanic OMZ, during three different monsoon periods in the central Arabian Sea over the full-depth of 3950 m and discussed their ecological implications. *C. carinatus* is smaller in size than *L. grandis* (prosome length 2 mm) and shows ontogenetic migration in coastal upwelling regions (Verheye *et al.* 1991), with dormancy in deep waters during periods of low phytoplankton abundance. Development from egg to adult is between one and three weeks depending on the temperature (Peterson and Painting 1990). This rapidly growing herbivore, with increases in dry body weight of 14 to 23% per day (Smith 1984), impacts the phytoplankton and zooplankton communities and, hence, the abundance of commercially important fishes (Smith 1995). In the Indian Ocean, *C. carinatus* colonizes surface waters

along the coasts of the southern Arabian Peninsula and the Horn of Africa during the summer upwelling season (SW monsoon), reaching standing stocks as large as 9000 ind. m^{-2} in the upper 200 m off Somalia (Stephens *et al.* 1992, Smith 2001). From December to April, most of the population diapauses as copepodite V (CV) stages at depths below 400 m (Smith 2001). Based on model results, ca. 36% of the diapausing population off Somalia is transported into the interior of the Arabian Sea (Idrisi *et al.* 2004). In fact, Padmavati *et al.* (1998) and Koppelmann and Weikert (2005) found this species in the central Arabian Sea at twilight depths.

The relative contribution of *L. grandis* (<6%) and *C. carinatus* (<2%) to the total zooplankton biomass in the twilight zone (150-1000 m) is small; however, these species are important bioindicators of the modes of flux in the open Arabian Sea. *L. grandis* modify organic material in the mesopelagic zone on its route to deeper waters and the sea-floor, but rates and pathways of biological transfer processes in this zone are sparsely known. By their bodies, *C. carinatus* transports organic material from the shelf waters into the center of the Arabian Sea. Thus, this species acts as an indicator of lateral transport mechanisms. The contribution of *C. carinatus* to the nutrition of the deep-sea is difficult to assess due to the limited information about transport rates. The role of *L. grandis* and *C. carinatus* in the Arabian Sea ecosystem should be further investigated to gain better insights into midwater flow budgets and to validate model results of on-/offshore transport mechanisms (Idrisi *et al.* 2004).



Source: GEBCO.

Figure 9. Sampling site in the eastern Mediterranean. The arrows indicate the deep-water outflow during the EMT (Eastern Mediterranean Transient) in the early 90's.

2.3. Semi-enclosed Eastern Mediterranean

The Mediterranean Sea is a semi-enclosed basin in an arid region. Due to its antiestuarine circulation and low external nutrient supply, the production decreases from the Strait of Gibraltar in the west to the coast of Asia Minor in the east (Turley 1999). Phytoplankton and bacterial production in the western part of the basin are up to three times higher than in the eastern Mediterranean Sea (Turley *et al.* 2000), which is one of the most oligotrophic ocean regions in the world (Dugdale and Wilkerson 1988).

The eastern Mediterranean consists of two major basins (Figure 9): The Ionian Basin east of the Strait of Sicily and between the coasts of Italy, Greece and Lybia and the Levantine Basin south of Asia Minor. Both basins are separated by the Cretan Passage, a submarine ridge between Crete and Barqah (Lybia). The Aegean Sea is located north of the island of Crete between the coasts of Greece and Turkey. It is connected to the main basins by the Straits of the Cretan Arc. The Adriatic Sea is located in the northwest between Italy, Croatia and Albania. It is connected to the main basins by the Strait of Otranto. The deep-water body below approximately 750 m of the Ionian and Levantine Basins in the eastern Mediterranean has higher values of salinity (38.65 PSU) and temperature (~14 °C) in comparison with the Atlantic Ocean. In this environment, the planktonic fauna is impoverished compared to the open ocean in terms of abundance and species richness (Scotto di Carlo *et al.* 1991). Life in the deep basins was extinguished ca. 8000 years ago during the last glaciation, and the shallow sill at the Strait of Gibraltar hampers re-colonization from the Atlantic Ocean. Hence, the deep-water zooplankton consists mainly of mesopelagic species and a small number of true deep-sea species like *Lucicutia longiserrata* (Weikert and Trinkaus 1990, Weikert and Koppelmann 1993).

2.3.1. Hydrographical Setting and the Eastern Mediterranean Transient (EMT)

The Adriatic Sea has been historically considered as the main source of deep and bottom water in the Ionian and Levantine Basins. A single coherent convection cell connected both basins, and the turnover time below 1200 m was 125 years (Roether and Schlitzer 1991). Three main water masses can be characterized in the basins: Surface water of Atlantic origin (AW) down to the thermocline, Levantine Intermediate Water (LIW) between the thermocline and approximately 1200 m depth and Eastern Mediterranean Deep Water (EMDW) below 1200 m. Apart from hydrological discontinuities induced by the formation of cyclonic and anticyclonic mesoscale eddies (Robinson *et al.* 2001, Hamad *et al.* 2005), the stability of the deep-water body was significantly interrupted in 1988/89 by the EMT. An extended dry period between 1988 and 1993 and exceptional cold winters in 1987 and 1992-1993 led to the formation of high-density water in the Aegean Sea (Lascaratos *et al.* 1999, Theocharis *et al.* 1999). This dense water flowed out through the Cretan Arc Straits (Figure 9) as Cretan Intermediate Water (CIW) and Cretan Deep Water (CDW) into the Ionian and Levantine Basins (Roether *et al.* 1996, Lascaratos *et al.* 1999), which replaced the formerly Adriatic Water in the deep interior of the basins and probably lifted the nutricline partly into the euphotic zone (Klein *et al.* 1999). In 1995, the entire bottom layer of the Levantine Basin was engulfed with CDW. In the Ionian, the CDW had reached the Straits of Sicily and Otranto (Theocharis *et al.* 2002). The total outflow of Aegean water into the eastern Mediterranean basins has been estimated to be around $2.3*10^{14}$ m^3 between 1988 and 1995 (Roether *et al.* 1996), corresponding to a mean rate of approximately 1 Sverdrup (10^6 m^3 s^{-1}). This

phenomenon was named the Eastern Mediterranean Transient (EMT). The highest flow rate with over 3 Sverdrup (Robinson *et al.* 2001, Roether *et al.* 2007) was detected in 1993 during notice of the mass occurrence of zooplankton species in the deep Levantine Sea (Weikert 1996, Weikert *et al.* 2001). Increased water densities were already observed in the southern Aegean Sea in 1959-65 and 1970-73 under extreme meteorological conditions, however, without affecting the entire eastern Mediterranean (Robinson *et al.* 2001). Since 1995, the outflowing CDW was no longer dense enough to reach the bottom of the Ionian and Levantine Basins, instead ventilated layers between 1500 and 2500 m (Theocharis *et al.* 2002).

2.3.2. Temporal Distribution of Specific Taxa

There are three main species of calanoid copepods in the eastern Mediterranean. Their population cores are concentrated at different depth layers (Weikert and Trinkaus 1990). *Haloptilus longicornis* is most abundant in the epipelagic zone, with the highest abundance at 100 m (Figure 10). *Eucalanus monachus* occurred in highest numbers around 600 m, while *Lucicutia longiserrata* only lives below 1000 m.

In June 1993, during the EMT, the distribution pattern changed dramatically due to the mass occurrence of two inter-zonal calanoid copepods (Figure 10). *Calanus helgolandicus*, which was found only sporadically as single individuals before the EMT (1987), appeared in high numbers (~6000 Ind. 1000 m^{-3}) especially in the dark zone, below 1000 m. *Eucalanus monachus* still showed a peak of abundance in the twilight zone, but in the dark zone, the abundance increased to approximately 6000 ind. 1000 m^{-3}. Both species caused a spectacular increase in mesozooplankton biomass and abundance and the outstanding mode of the mesozooplankton profiles below 1000 m.

Calanus helgolandicus was found for the first time in abundant numbers and at greatest depths in the Levantine Sea in June 1993. Weikert *et al.* (2001) hypothesized that this species, when expatriated by currents from the northern temperate parts of the eastern Mediterranean and transported into the Levantine basin, found favorable conditions after the hydrographical change associated with the EMT. Results from the survey in January 1998 underlay a potential transport from the Aegean Sea into the Ierapetra Deep through the Kasos Strait (Bonnet *et al.* 2005).

Eucalanus monachus, on the other hand, has been well-documented by historical and recent cruises to be indigenous in the Levantine and Ionian Seas and numerically conspicuous in intermediate and deep waters (Delalo 1966, Weikert and Trinkaus 1990, Scotto di Carlo *et al.* 1991, Pancucci-Papadopoulou *et al.* 1992). Both calanoids, *Eucalanus monachus* and *Calanus helgolandicus*, are ontogenetic migrators, which are well recognized to diapause in winter as CVs in dormancy at depth (Hirche 1983, Smith 1995). Weikert *et al.* (2001) hypothesized that the unusual distribution pattern of the individuals in 1993 was caused by water masses cascading during the EMT from the Aegean Sea into the adjacent Ierapetra Deep and/or strong convective mixing in the area of the anticyclonic Ierapetra gyre, where the species were found in high numbers. Both calanoid copepods may have contributed to the supply of food in the deep-sea environment, and indeed, some carnivorous species like Aetidaeidae and *Heterorhabdus* spp. were found in increased numbers during the main phase of the EMT in 1993 (Koppelmann and Weikert 2007).

During the post-EMT phase, the distribution of mesozooplankton was again similar to the situation before the EMT (Figure 10). *Calanus helgolandicus* was only found in sparse

numbers, *Haloptilus longicornis* peaked at 100 m, and *Eucalanus monachus* was dominant in the twilight zone. *Lucicutia longiserrata*, however, occurred in higher abundances in the dark zone as before the EMT (Figure 10). The species was predominant, with abundances between 200 to 300 Ind. 1000 m^{-3}. Adults had their maximum abundances in the upper dark zone between 1400 and 2400 m, while juveniles lived somewhat deeper in the water column with abundances around 200 Ind. 1000 m^{-3} (Figure 11). As a final result, the standing stock of *Lucicutia longiserrata* below 1050 m was ca. 6-fold higher after the EMT than before the EMT, whereas it was least during the EMT (Koppelmann and Weikert 2007).

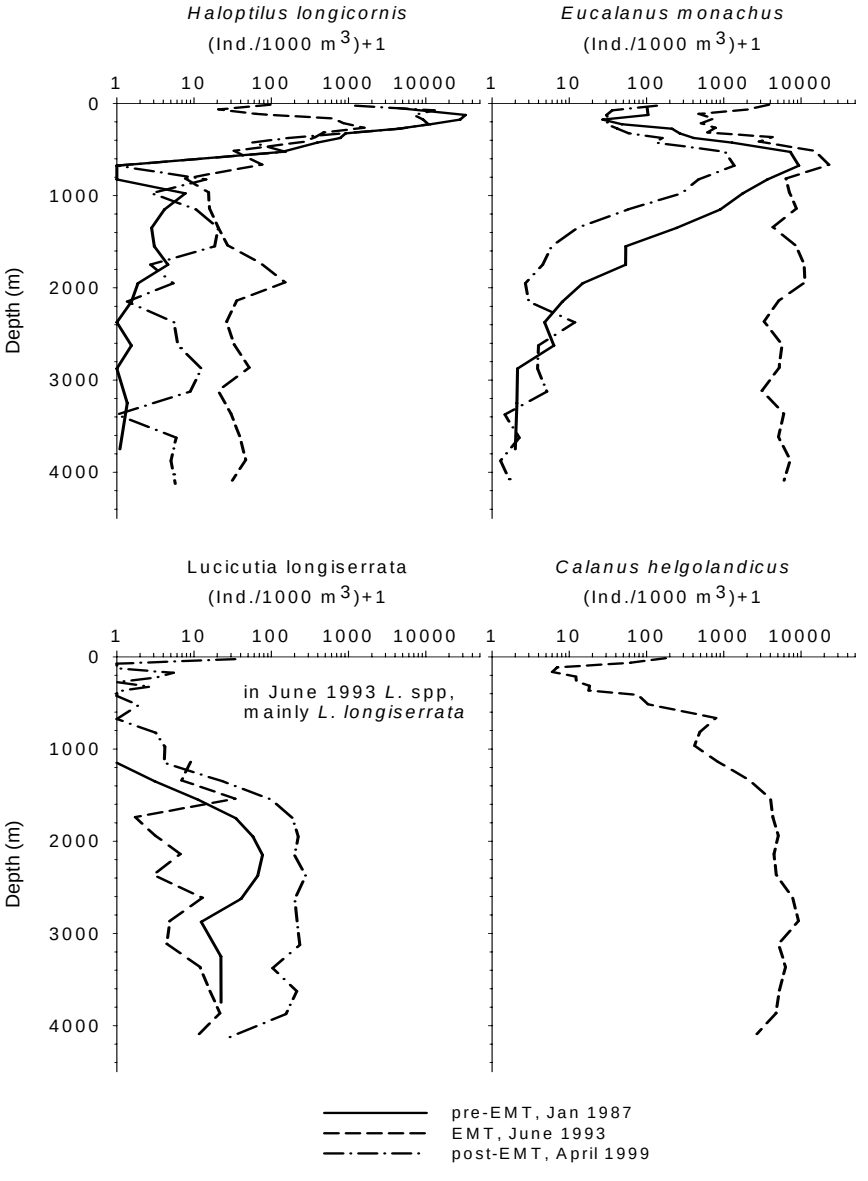


Figure 10. Vertical distribution of zooplankton main taxa in the eastern Mediterranean during the pre-EMT (Eastern Mediterranean Transient), EMT, and post-EMT phases (modified after Koppelmann and Weikert 2007).

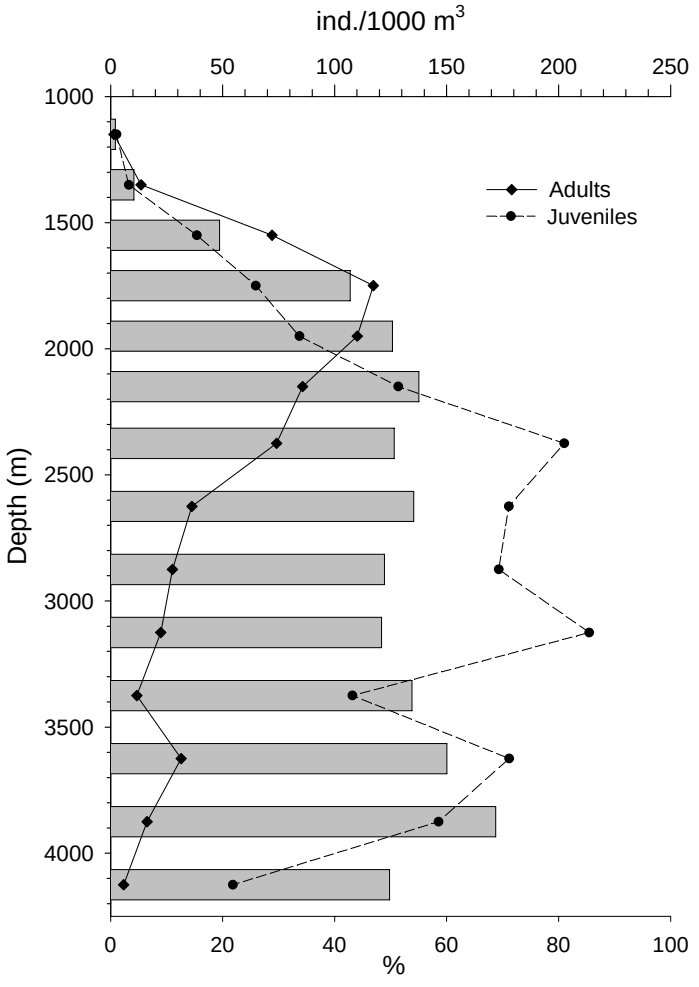


Figure 11. Vertical distribution of *Lucicutia longiserrata* adults and juveniles in the dark zone (>1000 m) of the eastern Mediterranean during the post-EMT (Eastern Mediterranean Transient) phase in April 1999 and the relative contribution (bars) of this species to the total zooplankton (after Koppelmann *et al*. 2004).

2.3.3. Ecological Relevance

There is sufficient evidence that the higher abundances of the bathypelagic species *Lucicutia longiserrata* was influenced by the EMT on a larger temporal scale due to increased nutrition in the deep-sea. Klein *et al*. (2003) found a decrease in oxygen concentration in the eastern Mediterranean at depths below 2200 m between 1995 and 1999 when the output of dense waters from the Cretan Sea had subsided. The authors hypothesized that the decrease in oxygen was caused by the consumption of POM and dissolved organic carbon (DOC), which was transported by the EMT outflow into deeper water layers and by an increased metabolism of deep-sea zooplankton.

Thought is emerging that deep-sea ecosystems are fragile and that the deep-sea community is extremely sensitive to a wide range of disturbances (Gage and Tyler 1991). Long-term investigations of deep-sea biology in the eastern Mediterranean provide the opportunity to study the response of a deep-sea ecosystem to climate variability on a decadal

scale (Danovaro *et al.* 2001). Although triggers may be different, the effects of increased food supply are most evident in food-limited areas like oligotrophic deep-seas (Danovaro *et al.* 1999). Small changes, however, in the physical characteristics of deep waters can also alter the steady state of important biogeochemical and functional variables (Danovaro *et al.* 2001).

Although the data set presented by Koppelmann and Weikert (2007) is small, providing enticing but not incontrovertible support for the ideas about zooplankton responses, there are evidences that the deep-sea ecosystem is able to respond quickly to changes in the environment and that memory of these changes persists. The question of whether or not the biological phenomena observed in the course of the EMT are episodic events or are due to a shift in climate is open for discussion. There is, however, strong evidence that local meteorological conditions may affect the hydrography and biology of the entire eastern Mediterranean. The results suggest that biological changes in the pelagic realm can be easily monitored in the deep eastern Mediterranean due to the hydrographically extreme yet "simply structured" ecosystem (Weikert and Koppelmann 1996).

3. ROLE OF DEEP-SEA ZOOPLANKTON IN THE PELAGIC ORGANIC MATTER CYCLE

Since the beginning of industrialization, atmospheric carbon dioxide (CO_2) concentration has been increased by fossil fuel burning and deforestation. In 2006, the CO_2 concentration has reached 380 ppm, which is 100 ppm higher than in pre-industrial times and the actual increase is faster than hitherto assumed (Raupach *et al*. 2007). This is the highest CO_2 concentration of the last 600 000 years. The global carbon cycle is one of the main topics of "IGBP-Global Change" programs (IGBP 1990). There is growing acceptance that global warming is caused by the "greenhouse effect" of this gas and that climate change is human-driven. An estimate for the years 2000-2005 shows that 7.2 Gt (billion tons) carbon per year are released into the atmosphere by fossil fuel burning and cement emissions, which was 28% higher in 2005 than in 1990 during the Kyoto protocol base year (UNESCO-SCOPE 2006). The world ocean absorbs approximately 2.2 Gt C a^{-1} of which 1.6 Gt C a^{-1} remains in deep and intermediate waters.

The deep-sea harbors the largest ecosystem on earth. Although the abundance and the metabolic rates of pelagic organisms living in this ecosystem are low, they affect the oceanic carbon cycle due to the vast extension of the environment (Rowe 1981). Apart from its intrinsic scientific interest, detailed information on their metabolic needs and generation times is necessary to understand and to model the role of the organisms within the oceanic matter cycle (Sasaki *et al.* 1988, Lampitt 1992).

CO_2 is transformed into organic carbon via photosynthetic activity by phytoplankton in the euphotic zone of the oceans (Figure 12). Phytoplankton is consumed by herbivorous organisms that are fed upon by omnivorous and carnivorous animals. Ungrazed cells, viral lysis, transparent exopolymer particles (TEP), carcasses and fecal material produce POM. POM is consumed by detritivorous zooplankton, and then remineralized by bacterial activity, or sinks to greater depths. POM is partly transformed into dissolved organic matter (DOM) by metabolic activity, which fuels the microbial loop (Williams 1990, Ducklow 2000). POM cycling in the ocean's interior is controlled by the interaction of physical, chemical and

biological forces. Various groups of organisms like bacteria, protozoa and metazoa use carbohydrates as energetic resources and change the shape and composition of sinking particulate material. Other pathways are the vertical migrations of living animals on different time scales.

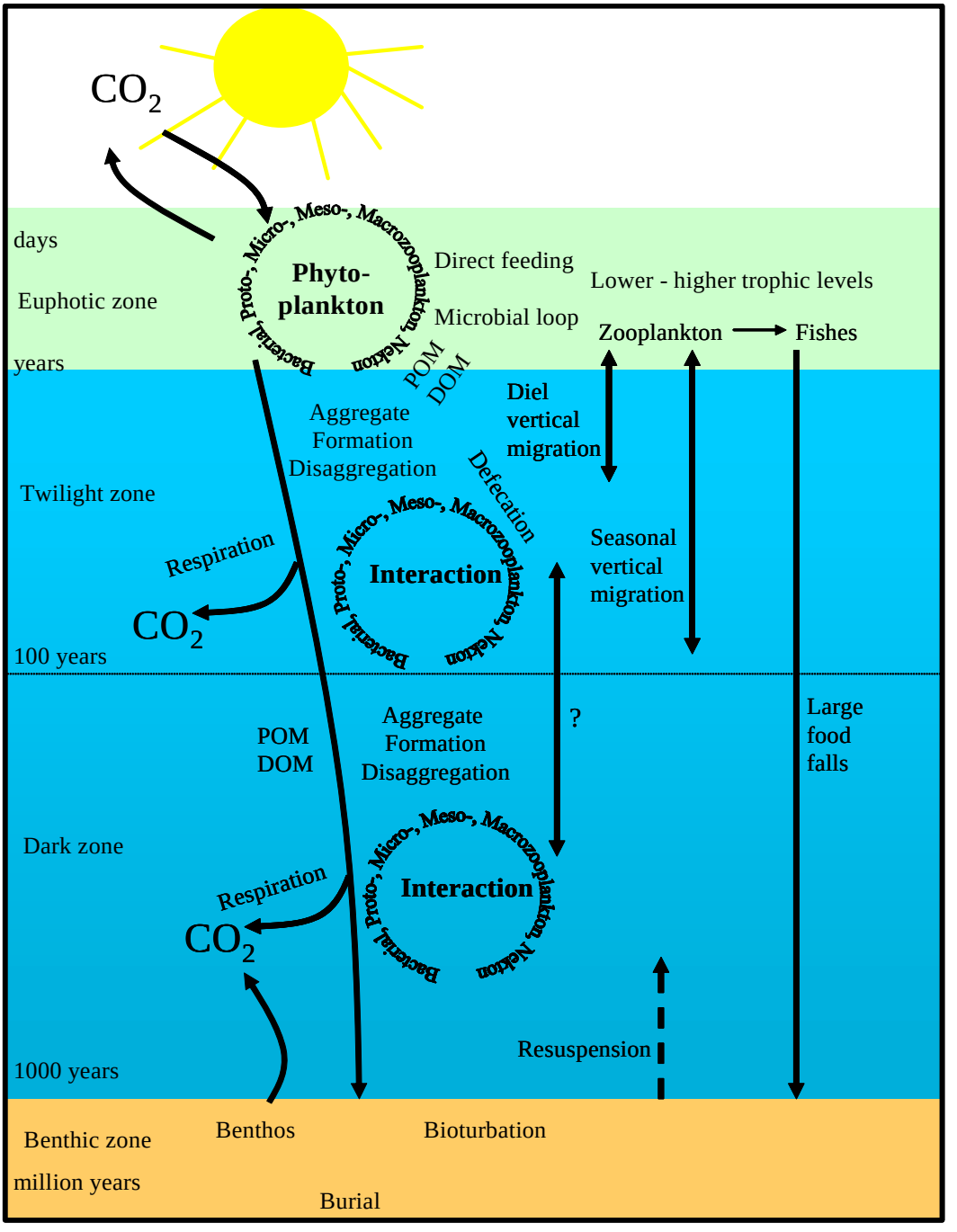


Figure 12. Hypothetical pathways of carbon in the open ocean and storage time of CO_2 in different compartments of the water column and the benthic zone.

On the way through the water column, these particles are remineralized, modified or built up to new living biomass by the activity of microorganisms, zooplankton and nekton. The modification and transfer of biomass is influenced by trophic relationships, whereby parts of the organic carbon are released as CO_2 by the metabolism of the heterotrophic organisms. This amount of carbon is "lost" from the sinking flux and fuels the dissolved inorganic carbon pool of the ocean's interior. These processes have been studied for some sites in the open ocean (e.g. Lampitt 1992, Koppelmann and Weikert 1999, Koppelmann *et al.* 2000, Yamaguchi *et al.* 2002, Koppelmann *et al.* 2004), but knowledge about the interaction between POM and organisms is still scarce in the deep-sea.

The dark zone below 1000 m is the most poorly known ecosystem on earth. The vast volume of approximately 988 million km^3 (i.e. 73% of the total ocean volume) and hence the enormous capacity of storing carbon, today approx. 38 000 Gt C are stored in the deep-water (93% of the carbon stored in the oceans; SCOR 1992), makes this zone very important for balancing the carbon flow in the ocean. In the epi- and mesopelagic zones, carbon is bound for months or several years, while in the sediments it is bound for millions of years (Figure 12). The storage time of carbon in the dark zone is several hundreds of years (Lampitt and Antia 1997).

3.1. Metabolic Consumption

Steinberg *et al.* (1997) showed that zooplankton consume 6-43% of discarded larvacean houses in the upper mesopelagic zone in the Monterey Bay off California. Similar values of 38% and 32% of POC consumption by copepods in the twilight zone were found by Sasaki *et al.* (1988) and Yamaguchi *et al.* (2002), respectively, in the western Pacific Ocean using carbon consumption rates calculated from copepod dry-weight and water temperature (Ikeda 1974). Carbon remineralization rates based on direct mesozooplankton respiration measurements (Smith 1982, Smith *et al.* 1986, Wishner and Gowing 1987) or ETS (Electron Transport System Activity) values resulted in lower contributions in the dark zone of the NE Atlantic (Lampitt 1992, Christiansen *et al.* 2001) and the Arabian Sea (Koppelmann *et al.* 2000). One exception was found in the upper bathypelagic zone (1000-2250 m) of the BIOTRANS site in the NE Atlantic (Koppelmann and Weikert 1999), when high remineralization rates were found in summer 1992, which were associated with an elevated standing stock of mesozooplankton after the sinking of spring phytodetritus (Figure 3).

Bishop (1989) compared equations on the relationship of carbon flux and depth published by various authors and concluded that the one by Martin *et al.* (1987) best fitted the available data. This equation was used to compare the carbon requirements in the bathypelagic zone of the Arabian Sea with carbon losses in this depth zone (Koppelmann *et al.* 2000). This formula was also used for particle flux comparisons by Jickells *et al.* (1996) and Lampitt and Antia (1997). The flux rates at 1050 m and 3900 m (CAST) and 4000 m (WAST) were extrapolated from the organic carbon flux in the short-term sediment traps 500 m above the bottom. Subtracting the deeper flux rates from the 1050 m flux rates gives the total carbon losses in the dark zone. The equation results in carbon losses of 13.6 mg C m^{-2} d^{-1} in April 1997 and 35.1 mg C m^{-2} d^{-1} in October 1995. The respective values for April 1997 and October 1995 at CAST were 3.5 and 10.7 mg C m^{-2} d^{-1}. The metabolic requirements of the mesozooplankton integrated over the whole bathypelagic water column were 4.0% and 5.4% of the measured

carbon losses at WAST in April 1997 and October 1995, respectively (Figure 13). At CAST, 8.4% of the carbon was respired by the mesozooplankton in April 1997 and 10.3% in October 1995. Respective values from the temperate NE Atlantic (BIOTRANS) indicate relatively higher demands of 23.3% in spring and 52.4% in summer during the mass development of *Metridia* species (Koppelmann and Weikert 1999). This pattern reinforces the statement by Koppelmann and Weikert (1997) that the relative portion of bathypelagic zooplankton on the total water column stock is higher in the NE Atlantic than in the Arabian Sea at WAST and CAST. Lampitt (1992) found a value very similar to those of the Arabian Sea: 9% of the carbon was remineralized in a 900 m thick stratum 100 m above the sea-bed at the less productive 5440 m deep Madeira Abyssal Plain. However, the author stated that higher mineralization rates possibly occur further up in the water column. Overall, the spatial and temporal changes of carbon flux in the Arabian Sea correspond well with the requirements measured for mesozooplankton.

Standardized carbon consumption rates of zooplankton in the eastern Mediterranean (Figure 13) were higher than rates measured in the deep open ocean (Koppelmann *et al.* 2004), which is most likely due to the elevated temperature of ~14 °C in the bathypelagic zone of the eastern Mediterranean. The absolute rates, however, were very low, reflecting the oligotrophic character of the basin. Integrated over the bathypelagic zone, the mesozooplankton contributes to 23.1% of the carbon losses from the sinking flux, with highest values of 54.5% between 3000 and 3250 m. At depths below 2500 m, *L. longiserrata* juveniles gained importance in absolute and relative numbers (Figure 10) and may have contributed to the peak observed in community carbon demand at great depths (Halsband-Lenk *et al.* 2003). The prevailing late developmental stages (copepodites IV-V) may indicate increased consumption during gametogenesis (Mayzaud *et al.* 2002, Rey-Rassat *et al.* 2002).

The remaining carbon losses in the dark zone are most likely due to requirements by other size classes, by incorporation of new body substances, excretion, defecation, and the transfer to the dissolved organic carbon pool. However, as discussed by Lampitt (1992), defecation can be disregarded in this context as particles produced within the stratum presumably behave in the same way as particles entering the stratum. Growth and excretion may also be disregarded on the assumption that the faunal community is in steady state and that excretory products are utilized within the stratum by other faunal groups. Nevertheless, additional studies of *in-situ* and ETS respiration rates of different zooplankton and nekton size classes at different stations and times are necessary to modify the respiration-ETS and the mesozooplankton-microplankton ratios.

Based on the results from the eastern Mediterranean, Koppelmann *et al.* (2004) hypothesized that the deep pelagic biota efficiently use the sinking flux in the oligotrophic Levantine Sea and thus, act as a filter for the nutrition of the benthos, which amplifies the oligotrophic character of the eastern Mediterranean. Indeed, the benthos community is extremely impoverished in the eastern Mediterranean (Thiel 1983, Tselepides and Eleftheriou 1992), possibly due to a highly reduced food input caused by efficient organic matter recycling by deep-water organisms. The increased recycling efficiency of pelagic organisms in the dark zone may facilitate starvation of the benthos in the nutrient-poor Levantine Basin.

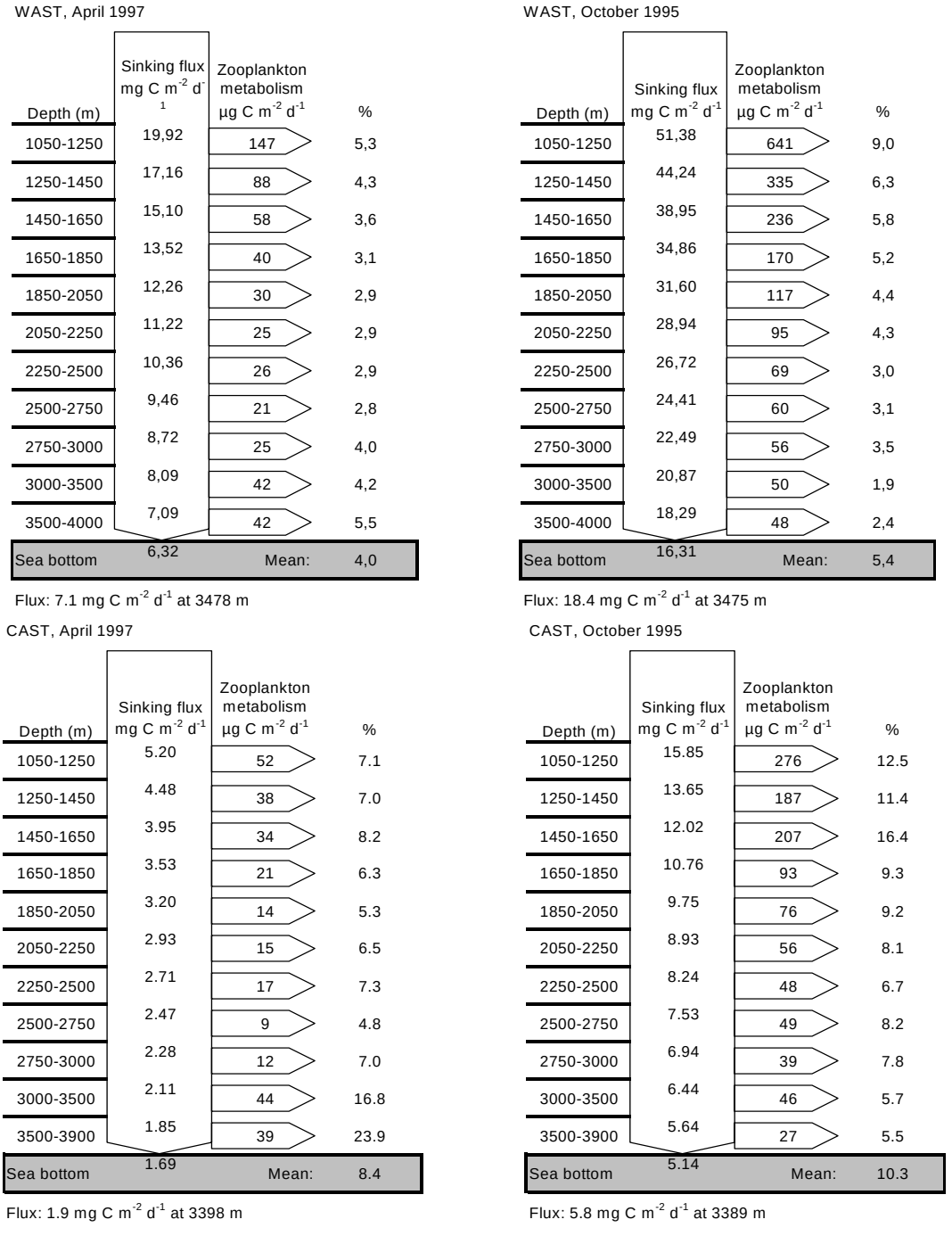
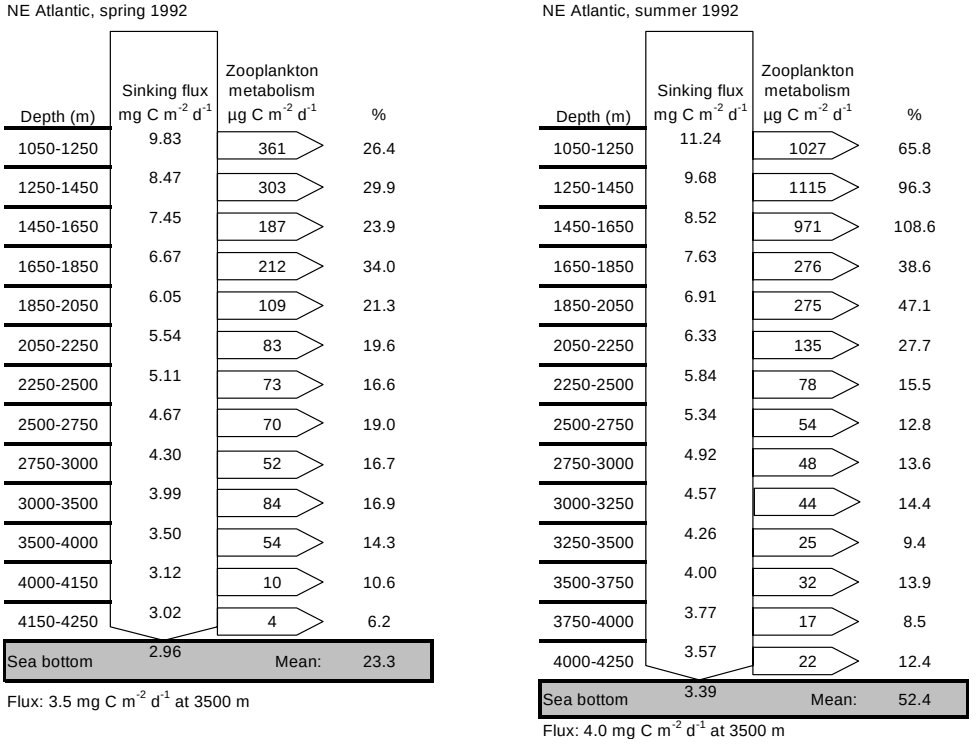


Figure 13. Carbon requirements of zooplankton in different depth-zones of the Arabian Sea, the NE Atlantic, and the eastern Mediterranean and the relative contribution of zooplankton to carbon losses from the sinking particulate organic carbon (POC) flux.



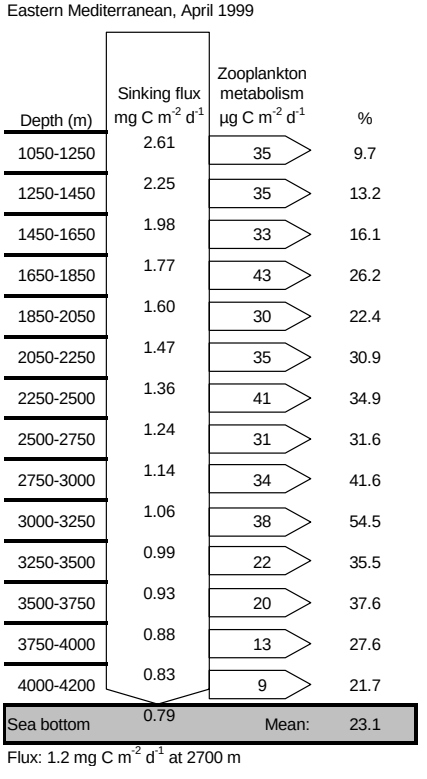


Figure 13. (Continued)

Respired carbon can be stored for periods on the order of 1000 years in the open seas, which is the estimated time scale of mixing for the ocean (Broecker and Peng 1982, Southam and Peterson 1985). Thus, the bathypelagic zooplankton is expected to be an important element of a large-scale acting mechanism by which the carbon can be redistributed to the sea surface and atmosphere. Even that part of the settling carbon, which is formed into new dissolved and particulate organic substances by the deep-living fauna, will not necessarily be removed from the ocean-atmosphere system by export to the sea-floor and subsequent incorporation to the sediment (Bender and Heggie 1984). The losses by metabolism (respiration and excretion) would counteract the rapid transfer of POM to depth by the "ladder of migration" (Vinogradov 1962). However, the ecological relevance of this migration in bathyal and abyssal depths is disputed (Wakefield and Smith 1990) and has to be substantiated by detailed data sets.

From the magnitude of the observed changes in bathypelagic zooplankton abundance and respiration in the investigated seas, the knowledge about seasonal reactions of deep living zooplankton is important for modelling the ecology of the deep-sea system, as the fauna may exert control on the magnitude and timing of the sediment community activity. Investigations on the flux of particles, well-coordinated in space and time with fine-stratified zooplankton sampling during different seasons and in different regions of the ocean, are necessary to elucidate the suggested coupling of episodes of increased particle flux with the increment of bathypelagic organisms. The improved faunal assessment of deep-living zooplankton and its structures together with measurements of reproduction and physiological activities (respiration, excretion) are of principle relevance for a better understanding of the function of the deep-sea.

3.2. Somatic Organic Matter Transfer

The use of the natural abundance of stable isotopes in ecological research has expanded rapidly in the past 20 years. For example, stable isotopes are used in studies on global element cycles, past climatic conditions, hydrothermal vent systems, and to study plant and animal ecology (Fry 2006, Lajtha and Michener 2006). The relative amount of the heavier $\delta^{13}C$ and $\delta^{15}N$ isotopes is enriched within the food web by the organism relative to the diet (Michener and Schell 1994). Due to these reasons, stable isotope tracing is an important tool in studies of aquatic food webs in limnic as well as marine ecosystems (e.g. Yoshii *et al.* 1999, Tamelander *et al.* 2006). Since the $\delta^{15}N$ values of animals reflect their diets, the isotopic signature of an organism provides integrated information about its feeding habits over longer time periods. Stable isotope based food web studies on zooplankton and their size class focus mainly on the upper layers of the water column (Sholto-Douglas *et al.* 1991, Fry and Quiñones 1994). In the deep-sea, stable isotopes were used as tracers of sewage-derived organic material in a benthic deep-sea food web (van Dover *et al.* 1992). However, there is only limited information on the enrichment of stable isotopes in deep-sea zooplankton throughout the water column (Koppelmann *et al.* 2000, Koppelmann *et al.* 2003b).

The deep-sea ecosystem depends mostly on organic material from the epipelagic zone. The food is transferred to greater depths by the vertical migration of plankton and micronekton (Vinogradov 1968, Vinogradov and Tseitlin 1983) either in the form of gut

contents or as a result of predation (Angel 1989, Longhurst and Harrison 1989), but to a larger extent by sinking of POM (Angel 1984, Fowler and Knauer 1986). The investigations of $\delta^{15}N$ values are helpful to understand the particle dynamics in the ocean (Altabet 1988, Altabt *et al.* 1991, Schäfer and Ittekkot 1995, Schäfer *et al.* 1998, Voss *et al.* 1996).

The stable isotopic composition of POM in sediment traps was investigated concomitantly with zooplankton sampling in the Arabian Sea (Koppelmann *et al.* 2000). Delta ^{15}N values were 6.9 ‰ and 7.4 ‰ in April 1997 and February 1998, respectively, in approximately 3400 m depth (500 meters above bottom). Assuming that POM is the main source of food (i.e. the primary diet) reaching the deep-sea (Angel 1990), the trophic level of the different zooplankton size classes can be calculated by the following formula:

$$HTL = (\delta^{15}N_{ZOO} - \delta^{15}N_{POM}) / 3.6$$

where HTL is the heterotrophic level, $\delta^{15}N_{ZOO}$ and $\delta^{15}N_{POM}$ are the stable isotope values of zooplankton and POM, respectively, and 3.6 is the mean value of trophic enrichment in per mill supported by the findings of Minagawa and Wada (1984) and Hobson and Welch (1992). Based on the HTL of the zooplankton size classes, and assuming a transfer efficiency factor (TE) of 0.1-0.2 between trophic levels (e.g. Blackburn 1981, Pauly and Christensen 1995), the amount of material entering the trophic web can be estimated.

$$\text{Required organic material} = \text{Zooplankton biomass} * (1/TE)^{HTL}$$

The results of the calculations show the high amount of material needed for the higher trophic levels in the deep bathypelagic zone (Table 2). The results from the eastern Mediterranean also show high demands for the deep-living zooplankton of the size range 0.5-1 mm, which mainly consists of *Lucicutia longiserrata.* This area, however, was influenced by the EMT, which transports organic material into the deep-sea environment. Note that the diet of the animal consists of a variety of sources. In other words, their diet is provided via the food chain by lower or even the same trophic levels. Cannibalism and feeding on carcasses are other dietary pathways that exist.

There are two major uncertainties in the above calculated results: (1.) It is not known whether the trophic level increase of 3.4‰ and 3.8‰ published by Minagawa and Wada (1984) and Hobson and Welch (1992), respectively, can also be applied to the deep bathypelagic zooplankton community. (2.) The general assumption of 10-20% transfer efficiency between trophic levels is uncertain for the food-limited deep-sea environment (Blackburn 1981, Pauly and Christensen 1995). Transfer efficiency values might be higher in food-limited environments like the deep-sea, where the organisms may have developed energy saving mechanisms (Childress and Thuesen 1992).

Table 2. Estimates of organic matter required to maintain the standing stock of zooplankton at a given trophic level

Area	Month, Year	Depth range (m)	Zooplankton size range (mm)	Zooplankton standing stock (mg C m^{-2})[a]	$\delta^{15}N$ (weighted by biomass)	D15N POM	HTL	Organic matter required (mg C m^{-2})
Arabian Sea, WAST	February 1998	2500-4000	0.5-5	37.95	15.06[b]	7.4[b]	2.13	1170
Arabian Sea, WAST	April 1997	2500-4000	0.5-5	32.96	14.96	7.4	2.10	968
Arabian Sea, CAST	February 1998	2500-3900	0.5-5	31.02	14.54	7.4	1.98	751
Arabian Sea, CAST	April 1997	2500-3900	0.5-5	23.68	14.82	6.9	2.20	817
Eastern Mediterranean	April 1999	2500-4250	0.5-1[c]	9.28	11.42	1.7	2.69	704

a) converted from wet weight measurements using conversion factors measured concomitantly with the samples.

b) data from April 1997.

c) biomass >1 mm too low to measure stable isotopes.

WAST = Western Arabian Sea Station. CAST = Central Arabian Sea Station. HTL = Heterotrophic level. POM = Particulate organic matter

Nevertheless, assuming that the results are correct in their order of magnitude, this suggests that it takes a longer time to provide the food for the organisms of the larger zooplankton size-classes (i.e. the higher trophic levels) than for the smaller ones, provided that the animals do not undergo vertical migration to reach other feeding areas nor have access to other food sources. More data on the biomass and stable isotopic composition of certain groups of zooplankton on the species level is needed together with information on the feeding behavior, set in context with flux rates of organic matter, to gain insight of the life cycles, transfer efficiencies and trophic enrichment factors of deep-sea zooplankton.

3.3. Longevity at Depths

Due to the low amount of organic carbon available for higher trophic levels, it seems quite reasonable to suggest that these organisms have long life spans. Longevity of deep-sea organisms was described for some fish species (38–130 years, Koslow 1996, Allain and Lorance 2000) Recently, the life span of a marine benthic vestimentiferan tube worm (*Lamellibrachia* sp.) two meters in length living around hydrocarbon seeps on the Louisiana continental slope at a depth around 550 m, was estimated to range between 170 and 250 years (Bergquist *et al.* 2000). Observations of benthic macro- and megafauna also indicate that K-strategies like slow growth and extended longevity have evolved in Polar Regions and in the deep-sea (Thiel *et al.* 1996). Our coarse estimates of required organic material to maintain the standing stock of zooplankton (Table 2) and the estimated input into the system by sinking

organic particles show that it would take 10-35 months to provide these amounts of organic matter (Figure 14).

Although high age is not the general rule in the deep-sea (Gage and Tyler 1991), there are some indications that extended life spans seem to exist also in mesozooplankton living at depths below 2500 m. Longevity and, hence, long regeneration times make the deep-sea fauna vulnerable to large-scale disturbances of the environment by man, such as contamination by deep-sea waste dumping and deep-sea mining (Thiel *et al.* 2001). Moreover, in the case of industrial exploitation of deep-sea resources, the information on life spans of deep-sea animals is essential to evaluate potential human impacts and maintain sustainability of the local community.

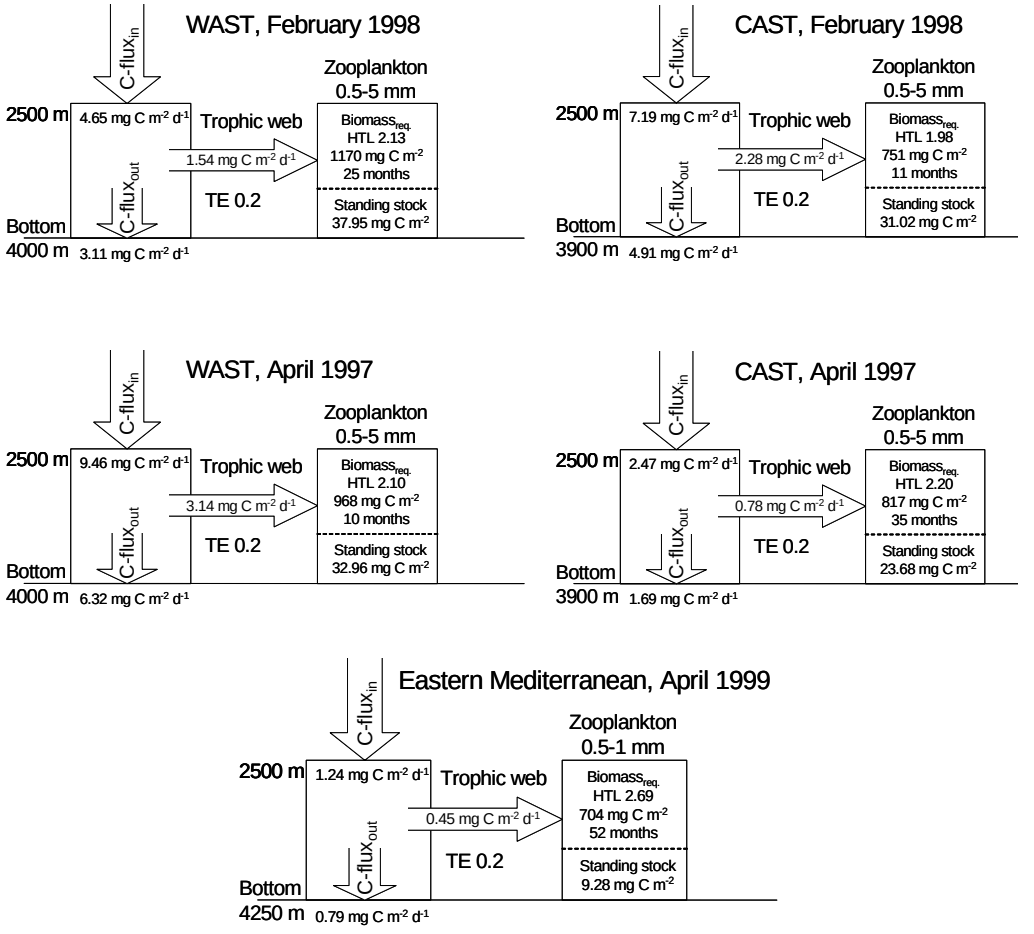


Figure 14. Estimates of carbon requirements of zooplankton to maintain the standing stock at a given trophic level and time needed to provide these amounts of carbon from the sinking POC flux.

4. GELATINOUS ORGANISMS - UNDERESTIMATED CONTRIBUTORS TO THE PELAGIC ORGANIC MATTER CYCLE?

We have learned through the previous chapters that temporal differences exist among deep-sea zooplankton and that these differences affect carbon utilization rates. This information, however, is primarily based on hard-bodied zooplankton. Literature related to soft-bodied gelatinous zooplankton, with special emphasis on abundance, distribution, carbon utilization rates and their ability to consume dissolved organic carbon, is even more scarce as compared to the available literature on hard-bodied zooplankton. A likely explanation for the paucity of information about gelatinous zooplankton is the inherent difficulty in sampling them due to their fragility and transparency. In this chapter, we present a general introduction to gelatinous organisms, including current knowledge and technological advances in sampling equipment. For the purpose of this paper, greater emphasis was placed on deep-sea gelatinous zooplankton and their potential role in the cycling of pelagic organic matter.

4.1. Introduction to Gelatinous Zooplankton

Gelatinous zooplankton consist of an assemblage of animals that are transparent, radially symmetrical invertebrates, owning soft bodies lacking hard skeletal components, and delicate with tissues composed of 95% or more of water, comparable to the consistency of gelatin. Unlike fish and hard-bodied zooplankton, which retain their form outside of water, gelatinous zooplankton require the support of their aqueous environment to avoid collapsing into an unidentifiable ‘‘blob’’. Gelatinous zooplankton are fairly large animals, ranging from centimeters to meters in diameter. These animals do not swim rapidly but remain buoyant because the mixture of salts in their gelatinous tissue is lighter than the weight of the salt in the seawater within which they swim. However, they are not able to swim against currents and therefore belong to the plankton. Gelatinous zooplankton can enhance their ability to remain buoyant by excluding certain ions (primarily sulfate) or accumulating other ions, like ammonium, in their tissues (Wrobel and Mills 1998; http://www.msc.ucla.edu/oceanglobe /investigations.htm).

The term gelatinous zooplankton encompasses the two phyla Cnidaria and Ctenophora and members of Tunicata. The phylum Cnidaria includes Anthozoans, Cubomedusae, Hydromedusae and Scyphomedusae. The phylum Ctenophora is distantly related to cnidarians and consists of ctenophores. Tunicata are a subphylum within the phylum Chordata. The class Thaliacea consists of salps and doliolids. Salps may occur as single individuals or chains of individuals. A unique characteristic of doliolids is their ability to feed on planktonic particles using currents created by cilia rather than using their muscle bands to pulse the body like most members of gelatinous zooplankton. The class Larvacea/ Appendicularia consists of appendicularians, which are unique among the tunicates because they take the form of a "tadpole" with a tail containing a notochord.

Gelatinous zooplankton are ubiquitous in marine environments with few occurring in freshwater. Habitats range from coastal nearshore to the open ocean and down into the deep pelagic zone. Species found within such habitats typically have a wide tolerance for environmental variation (Wrobel and Mills 1998). Gelatinous zooplankton deserve their

reputation as the most enigmatic of plankton because they are difficult to capture, preserve and quantify, yet they often constitute a predatory biomass large enough to exert strong top-down influences on productivity and survival of zooplankton and ichthyoplankton. Furthermore, gelatinous zooplankton can exert bottom-up influences on energy transfer to fish and higher predators by altering fluxes of particles, carbon and nutrients. All of these influences remain poorly quantified due to a lack of reliable data (ICES 2003, Frost 2006).

4.2. Advantages of Deep-sea Gelatinous Zooplankton

Within the last 25 years, research into gelatinous zooplankton has revealed a list of adaptations that are believed to allow these organisms to successfully inhabit the vast, cold, dark, high-pressured, food-limited environment of the deep-sea. As compared to shallow-living organisms, depth-related physiological adaptations of deep-sea gelatinous zooplankton include size, watery tissues and flimsy bodies, decreased metabolic demands, bioluminescence as a basis for communication, and chemoreception.

According to Osborn and Barber (2004), one key to the proliferation of larger-sized gelatinous organisms in the twilight zone is the reduction of shear forces at depths below the mixed layer. Large size improves feeding success by increasing the capture rate of particles and prey. Size enhancement is achieved by producing tissue of dilute organic biomass, which offers little palatability for potential predators like fish and crustaceans. The advantage of this adaptive approach is that enhanced size is achieved without a comparable increase in metabolic demand. Thus the physics of the ocean's deep-sea encourages body forms and feeding structures that would be impractical and uncommon at shallower depths (Robison 2004). For instance, the giant siphonophores *Praya* and *Apolemia* have been witnessed to reach lengths of 30 m and 10 m, respectively. Robison (2004) describes *Deepstaria*, a semaeostome medusa, as having extremely pliant bell tissues that can stretch to four or five times its contracted diameter of a meter or more, presumably enabling it to engulf a wide range of prey types. Furthermore, *Stygiomedusa*, another large semaeostome, has bell diameters up to 1.5 m and overall lengths of 4 m or more. The girths of these medusae are large enough to provide ecological substrate for other species. In the case of the siphonophores, juvenile fishes and small amphipods (*Cystisoma*) have been known to shelter themselves along the lengths of the colonies (Robison 2004).

Owning flimsy bodies mostly comprised of water requires low levels of energy allowing gelatinous zooplankton to fast for long periods of time. Investigations into the role of lower temperatures, decreased light levels, increased pressure, and reduced food levels with greater depth ultimately suggest that the decrease in metabolic rate with increasing depth is largely due to a reduced need for locomotory skills. Childress (1995) and Seibel *et al.* (1997, 2000) have demonstrated that below 1000 m, when ambient light is absent, the decline in metabolism stops. Another energy saving tool for gelatinous zooplankton is their reliance on transparency rather than locomotion to avoid detection by their sighted predators and prey. Unlike their surface-dwelling counterparts whose predatory/prey responses are often based on locomotion triggered by vision, deep-sea gelatinous zooplankton lack image-forming eyes. Instead, they rely on other means of communication, like bioluminescence, and have evolved a sensory system to differentiate between bright and dark light intensities (Robison 2004, Nouvian 2006 and contributors therein).

Bioluminescence, a chemical process by which organisms of the deep-sea generate their own light, serves many life-sustaining functions. These functions include locating food, attracting mates and being a defense mechanism. Recently, Haddock *et al.* (2005) discovered a deep-sea siphonophore, *Erenna sp.*, using red-emitting bioluminescent appendages that act like lures for fish prey. A camouflage trick called counterillumination is used as a defense mechanism by many organisms living in the twilight depths of 200 and 1000 m. Through the use of ventral photophores, counterillumination can allow an organism to mask the opacity of its silhouette by adjusting the intensity of its light emission to that of the level of ambient light from above, protecting itself so as to go unseen by potential predators. One of the best examples of bioluminescence as a defense mechanism is the rotary disk display by the common deep-sea scyphozoan, *Atolla wyvillei*, when it is threatened. During this display, thousands of blue lights flash and can attract the attention of larger animals over a distance of 100 m in order to eat predators of *A. wyvillei*, like the mesopelagic shrimp *Notostomus robustus* (Larson *et al.* 1991, Moore *et al.* 1993, Axelsen 2005). At least 90% of deep-sea inhabitants are capable of producing their own light in one fashion or another, making bioluminescence the most widespread form of communication in the deep-sea (Robison 2004, Haddock *et al.* 2005, Nouvian 2006 and contributors therein).

Like bioluminescence, chemoreception, is another form of sensory communication that likely evolved in the deep-sea in the absence of sunlight as a visual cue. Tamburri *et al.* (2000) provides the only direct experimental evidence of chemoreception in deep-sea fauna through a study on specimens of the hydromedusa *Mitrocoma cellularia*. Hydromedusae were collected by a remote operated vehicle (ROV), transported to a laboratory, and when subjected to substrate- and solution-borne prey extract, they responded just as they would have to the smell and taste of actual prey. Naïve experimental *M. cellularia* showed no response to controls that contained no prey extract. To validate the study's findings, similar experiments were conducted in the ocean, on specimens of free-swimming *M. cellularia* that were neither collected nor restrained, yielding the same results as the laboratory findings (Tamburri *et al.* 2000; Robison 2004).

Sensory communication, like chemoreception and bioluminescence, is probably in wide use for intraspecific interactions concerning aggregation, feeding, mating, and simple recognition. Like shallow-water organisms, deep-sea soft-bodied gelatinous organisms may exploit conspicuous chemical warning signals as well as toxins as defense mechanisms against predators. Future deep-sea studies, as suggested by Robison (2004), would include molecular techniques that may allow thorough investigations into species identification, the relatedness of known taxa, and population dynamics to be made *in situ*, which would further our understanding of the adaptations and success of deep-sea gelatinous organisms. The physiological adaptations discussed above illustrate how dynamic and vigorous gelatinous zooplankton can be to a once long perceived lifeless world or, as we know it, the deep-sea.

4.3. Trophic Position

Gelatinous zooplankton consume at each level of the trophic structure within marine ecosystems. Tunicates, with special emphasis on salps, are herbivores, while members of the phyla Cnidaria and Ctenophora are almost entirely carnivores. Members of the class

Hydromedusae demonstrate patterns of omnivory, whereas members of the class Larvacea are primarily detrivores (Sommer and Stibor 2002, Robison 2004, Arai 2005).

In contrast to linearly theoretical food chains, food webs practically represent those species that do not conveniently fit into the conventional trophic levels as set by food chains. In other words, the shortcoming of food chains is that they do not recognize the complexity of interactions that comprise reality, e.g. organisms are consumed by more than one predator and consume more than one type of prey. Food webs are described as energy systems with multiple and shifting interactions between organisms because more than one predator species may consume them. As some organisms grow, they change diets and thus trophic levels as a consequence. Another example of the dynamic nature of food webs is the functional response of an organism when the relative quantity of their food item changes. Competition for food between different predators can develop when their shared food becomes limited. The biomass of top-level species may also be altered due to competition among organisms. For example gelatinous predators, including ctenophores, chaetognaths, medusae and siphonophores, and other carnivorous invertebrate zooplankton compete for copepods, euphausiids and other prey items shared by larval fish (Robison 2004). The best-known evidence of the consequence of such competitive relationships among food webs in the open ocean is the collapse of the Black Sea fishing industry by the invasion of a ctenophore, *Mnemiopsis leidyi* (Vinogradov *et al.* 1989). Tragedies like what happened to the Black Sea fishing industry have driven the scientific community to improve not only their efforts into the study of gelatinous zooplankton but also the technology with which to sample them.

The discovery of a large and complex gelatinous fauna in the deep-sea of Monterey Bay, California, USA, is one of the key ecological advances enabled by progressive technology. Robison (2004) coins the term “jelly web” and provides data from long-term studies conducted in Monterey Bay that show nutrient energy entering the jelly web through two principal pathways: crustacean and gelatinous grazers. Both groups of grazers are consumed by a variety of gelatinous predators, which are themselves consumed by other gelatinous predators. As much as a quarter of total pelagic biomass may be incorporated into the bodies of gelatinous organisms and they can seasonally dominate the second and third trophic levels of mesopelagic communities. However, the residence time and fate beyond the third and fourth trophic levels of the web are still largely unknown. Furthermore, even with a 10-year, ROV-based quantitative time series of video observations and transects in Monterey Bay, the nature and extent of the gelatinous portion of the web has been seriously underestimated and our understanding is still incomplete. Unfortunately to date, there exist few other long-term, extensive studies into deep-sea gelatinous community trophic structures as performed by the Monterey Bay Aquarium Research Institute.

4.4. Organic Matter Utilization

The occurrences of outbreaks (a.k.a. blooms) of gelatinous zooplankton, namely medusae (a.k.a. jellyfish), have the potential to yield a significant flux of organic matter to the deep-sea floor. Gelatinous zooplankton are now being recognized as playing an important role in the transfer of organic matter to the sea-floor by way of fecal aggregates and mucous sheets (Wiebe *et al.* 1979, Robison *et al.* 2005). Fecal aggregates and mucous sheets sink rapidly to the deep-sea floor, provide a labile food source for benthic organisms and make a major

contribution to oceanic biogeochemical fluxes (McCave 1975, Wiebe *et al.* 1976, Honjo 1978, 1980, Dunbar and Berger 1981, Iseki 1981, Pfannkuche and Lochte 1993, Billett *et al.* 2006). Most investigations related to fecal aggregates have been on those of crustacean origin (e.g. Fowler and Small 1972, Turner 1977, Honjo and Roman 1978, Small *et al.* 1979, Turner and Ferrante 1979, Komer *et al.* 1981, Tanoue and Hara 1986). Considerable attention, however, now targets the contribution from gelatinous zooplankton (Alldredge 1976, Wiebe *et al.* 1979, Pomeroy and Deibel 1980, Bruland and Silver 1981, Iseki 1981, Silver and Bruland 1981, Madin 1982, Matsueda *et al.* 1986, Madin *et al.* 2006). Many gelatinous zooplankton are mucus-net feeders and, during blooms, their prolific egesta (feeding nets and external houses) and degesta (feces) will constitute important components of the local particulate organic matter (POM; Morris *et al.* 1988). Still, scarcely documented is the role carcasses of jellyfish play in the downward transfer of carbon. Even less is known about rates in production and use of dissolved organic carbon (DOC) by gelatinous organisms.

NE Atlantic Ocean

Cacchione *et al.* (1978) recorded the occurrence of salp carcasses rolling along the seabed in the Hudson submarine canyon, New York, USA, at a depth of 3400 m. In the same general area, Wiebe *et al.* (1979) estimated a flux of 3.5 mg C m^{-2} d^{-1} in the carcasses of the salps in addition to 12 mg C m^{-2} d^{-1} of rapidly sinking fecal aggregates from a bloom during a mid-summer period. These findings suggest that salp carcasses may provide more than half of the daily energy requirements of the benthic microfauna. Since the longevity of the salp bloom was unknown, Billett *et al.* (2006) calculated the flux of salp feces/carcasses potentially provided a carbon input of about 1 g C m^{-2} by taking data of Wiebe *et al.* (1979) and estimating a two-month salp bloom. Their calculation is equivalent to approximately half the mean annual downward flux of organic carbon in the area as measured by near-bottom sediment traps (Biscaye *et al.* 1988). While observations made during the *Challenger* expedition indicated that salp carcasses could be transported rapidly to abyssal depths and might act as a good food source for benthic fauna (Moseley 1880), gathering supportive evidence that carcasses provide a significant transport pathway for carbon in the oceans is slow-footed. Whale carcasses have been a notable exception (Smith and Baco 2003). With regards to global significance to carbon cycling, however, fauna that occur in great abundance, like jellyfish, have a greater potential to significantly affect carbon cycling and recycling. Unfortunately, direct quantitative observations are rare.

Arabian Sea

Very few data exists on the effects of blooms of jellyfish on carbon flux and on the deep-sea fauna. Of current literature, Billett *et al.* (2006) describe large numbers of the scyphomedusan, *Crambionella orsini*, found dead on the sea floor over a wide area of the Arabian Sea off the coast of Oman at depths between 300 and 3300 m. In this study, photographic transects were used to estimate the standing stock of carbon in jelly detritus deposited on the sea-floor. These estimates ranged from 1.5 to 78 g C m^{-2}. The total annual downward flux of organic carbon as measured by sediment traps at 3100 m off the southeastern coast of Oman during the mid-1990s ranged from 4.3 to 4.8 g C m^{-2} a^{-1} (Honjo *et al.* 1999). By comparing to the highest estimates from the two studies, there is more than an order of magnitude in excess. Thus, jelly detritus in the Arabian Sea may provide a major transport pathway for organic carbon into the deep-sea (Billett *et al.* 2006).

It is uncertain what relevance the observation in the Gulf of Oman has in comparison to other oceanic areas. The authors suggest that it is possible that the jelly detritus occurred only as a result of an intense OMZ. If OMZs are key factors in delaying the rate of degradation of jellyfish as they pass through the water column, then it might be expected that the deposition of jelly detritus is more significant in OMZ regions. Jellyfish blooms, however, are widespread in the world oceans (Mills 2001). With this in mind, jellyfish carcasses, and bodies of other forms of gelatinous zooplankton, could potentially be important vectors of carbon transport to the deep-sea in all areas of the world oceans (Billett *et al.* 2006).

Mediterranean Sea

Morris *et al.* (1988) collected sediment-trap samples, which contained large amounts of organic aggregates and fecal pellets, during and after the occurrence of a salp (*Salpa fusiformis*) bloom in the Bay of Villefranche, Mediterranean Sea in April/May 1985. Salps are active herbivores, occurring in the offshore waters of the Ligurian Sea between April and June (Braconnot 1963, Choe 1985). At its maximum abundance, the salp population is distributed from the coast to at least 18 miles offshore (Nival *et al.* 1985). Sediment-trap aggregates were rich in carbohydrates and mineral grains and had similar rates of sedimentation (900-2100 m d^{-1}) to those of the fecal pellets (1000-2000 m d^{-1}). The study's results from mineralogical and organic chemical analyses indicate the potential effect of these mucus-rich aggregates on local biogeochemical fluxes.

Total organic matter (TOM; expressed as relative % dry matter) from two trap samples taken during the salp bloom measured 18% and 24%, respectively. Insoluble carbohydrate was 11% and 14%, while protein was 6% and 8%, respectively. When comparing these values to those of a trap sample taken six days post-bloom, both TOM and insoluble carbohydrate percentages were lower than those of bloom samples. The post-bloom protein percentage was 7% (Morris *et al.* 1988).

Several investigators have studied the production and sinking rates of POM created by salp blooms (Cacchione *et al.* 1978, Wiebe *et al.* 1979, Pomeroy and Deibel 1980). Salps, at low densities, are known for being ubiquitous throughout the world's oceans. Episodic blooms, however, are also known to occur at continental shelf and slope areas (Thompson 1942, Foxton 1966, Berner 1967, Binet 1976, Wiebe *et al.* 1979, Billett *et al.* 2006). Although infrequent, the pulsed nature of these blooms and the magnitude of the organic material produced can dramatically affect the local flux of surface primary production to deep-sea benthic communities and underlying sediments (Wiebe *et al.* 1979, Madin 1982, Morris *et al.* 1988).

A study by La Ferla and Azzaro (2004) highlights the metabolic carbon dioxide production of the Mediterranean Sea. Data from their case study illustrates the "peculiarity" of the deep Mediterranean waters with regards to other oceanic deep waters. Data within the case study suggests accelerated remineralization rates as having considerable importance to the Mediterranean deep layers with regards to similar oceanic depths (also in La Ferla *et al.* 2003). Of the world's oceans, the Mediterranean Sea has the highest remineralization ratio of 47% (expressed as percentages of depth-integrated deep to depth-integrated shallow), while the Indian Ocean (24.8), Pacific Ocean (21.1) and the Alantic Ocean (14.9) follow (Packard *et al.* 1988, Naqvi *et al.* 1996, Azzaro 1997, La Ferla and Azzaro 2004). Since the Mediterranean surface water is known for its oligotrophy, this acceleration seems mainly caused by the important advective processes occurring in the Mediterranean rather than by

export production from the upper layers. Such evidence is explained partially by the availability of organic carbon suitable for remineralization and the high temperatures of the Mediterranean deep-sea (~13 °C), which aids in accelerating microbial metabolism (Seritti *et al.* 2003, Koppelmann *et al.* 2004).

Pacific Ocean

Robison *et al.* (2005), highlights the importance of the mucous feeding nets of larvaceans in transporting carbon rapidly to the deep-sea floor. The authors calculated an average carbon flux to be 7.6 g C m^{-2} a^{-1} via discarded giant larvacean "houses," which is roughly equivalent to the seafloor carbon flux at mid-slope depths in Monterey Bay, California, USA (7.2 g C m^{-2} a^{-1}; Pilskaln *et al.* 1996).

Nordic Fjords

Hansson and Norrman (1995) performed a series of incubation experiments to measure the rate of release of DOC by the scyphozoan *Aurelia aurita* that were collected in 1991-1992 in Gullmarsfjorden, Sweden. Data from those experiments showed adult medusae (9.5 to 18 cm in diameter from the Skagerrak) release DOC at a rate ranging from 0.70 to 1.6 mg C ind^{-1} d^{-1}. Not only is this range similar to the amount of carbon allocated to reproduction, but also is equivalent to 2.5 and 7.1% of the carbon assimilated in one season (June to September).

In addition to determining DOC release rates by *A. aurita*, the authors found that bacterial growth was stimulated by the presence of the scyphozoan. Minerals bound in the prey items of *A. aurita* may be remineralized to the pelagic microbial community as dissolved organic matter (DOM), which can then be consumed by planktonic microbes as described by Azam *et al.* (1983). As a consequence, *A. aurita* has the potential to influence the microbial food web via the release of DOM to the microbial community (Hansson and Norrman 1995).

Mucus generated by *A. aurita*, which more easily allows prey items to adhere to the medusa, is likely the main source of the DOC observed (Schneider 1989a). Leakage of mucoid particulates associated with feeding may prove to be a key source of DOC, but it is often neglected in measurements of nutrient excretion by medusae. Some medusae excrete considerable amounts of ammonia and phosphate (Schneider 1989b), but their quantitative release and uptake of organic carbon is not well documented.

To clarify carbon use and remineralization processes in the open ocean, recent efforts point toward the understanding of the trophic role of medusae in the pelagic food web (e.g. Frost 2006, Madin *et al.* 2006). It remains unclear to what extent the carbon consumed by *A. aurita* is transferred to other planktonic organisms. One way to address the cost of losing carbon to the water-column is to relate the amount of carbon released by *A. aurita* to the total energy budget of the scyphozoan (Hansson and Norrman 1995). Consequently, more data is necessary to produce reliable correlations between carbon use and loss to the pelagic environment.

Remarks

Although a thorough search for the literature above was performed, readers should not consider it a completely exhaustive one. The examples highlighted above illustrate recent and future turns in the study of gelatinous zooplankton and their importance to the pelagic ecosystem. There is a wealth of knowledge that remains unclear, unsolved and undiscovered.

4.5. Technological Advances and What Lies Ahead

Increasing awareness and appreciation of how gelatinous zooplankton affect the health of the oceans has largely been due to *in situ* observational techniques and to rigorous investigations of trophic interactions (Hamner *et al.* 1975, CIESM 2001, Haddock 2004). In the mid-1970s, observations on the biology and distribution of gelatinous zooplankton by SCUBA divers stimulated speculation about the structure of tropical, oceanic ecosystems. It became evident that conventional sampling with plankton nets did not quantify gelatinous zooplankton accurately due to their patchy distributions, delicate construction and escape responses. Furthermore, as gelatinous zooplankton feed, they create mucous aggregates containing particulate matter that are also irregularly distributed, delicate and significantly undersampled by plankton nets (Hamner *et al.* 1975). Although blue-water SCUBA diving was one of the first methods for observing gelatinous zooplankton *in situ*, it provides a limited view of their diversity because many species occur below safe diving depths or are overlooked because they are small and transparent (Båmstedt *et al.* 2003, Haddock 2004).

Solutions to the challenges of sampling gelatinous zooplankton and the resulting lack of rigorous data include altering conventional plankton nets and developing new techniques (Figure 15). Various kinds and sizes of closed cod ends on plankton nets help minimize damage to gelatinous fauna. In addition, video, real-time image analysis, holographic sampling systems and multi-frequency acoustics offer significant promise as replacements or complements to nets (Smith and Rumohr 2005). These new techniques allow for continuous or semi-continuous sampling across a range of spatial scales. Such sampling will help decipher the infamous patchy distributions of gelatinous zooplankton and provide insights into the relationships between gelatinous zooplankton, hydrographic structures and other mesozooplankton (ICES 2003).

The question still remains, why do we need to learn more about gelatinous zooplankton? Perhaps the best, irrefutable reason for understanding their effects on the health of the ocean comes from the devastating impact that high densities can have on human activities. Local fisheries, like the tragedy of the Black Sea, are not the only victims of blooms of gelatinous zooplankton; coastal economies dependent on tourism can suffer as well. Summer is a prime season for both tourists and gelatinous zooplankton blooms. The idea of being entangled in a bloom of stinging medusae is enough to deter any beachcomber.

Research into what drives these episodic blooms can help create reliable numerical models to better predict when and where they might occur. Other ‘‘hot topics’’ with respect to gelatinous zooplankton include cataloging deep-sea faunal diversity and behavior and resolving the role of gelatinous zooplankton in carbon flux as part of addressing global warming. Deep-sea faunal research has revealed a number of never-before-seen or described species of gelatinous zooplankton. For example, scientists at the Monterey Bay Aquarium Research Institute (MBARI) have identified two new species of deep-sea gelatinous zooplankton, *Tiburonia granrojo* and *Stellamedusa ventana*, since 2003 (Matsumoto *et al.* 2003, Raskoff and Matsumoto 2004). These discoveries would not have been possible without such tools and techniques as described in Figure 15.

Sampling and observational techniques for gelatinous zooplankton
(modified from G. Gorsky, CIESM 2001; Frost 2006)

- **Net Sampling**
  - i. *Standard WPII nets* with mesh size larger than 200µm are used for meso-zooplankton sampling. Net sampling often destroys fragile gelatinous structures but adapted cod ends could reduce the damage.

  - ii. *Large collector nets* (20-60 l) are used for sampling large gelatinous organisms. Their use is limited due to handling difficulties. When sampling dense populations of medusae, trawl nets are more appropriate.
  - iii. *Multiple nets (***MOCNESS**, BIONESS, Hydrobios) are poorly adapted for sampling gelatinous plankton, especially for taxonomic purposes, due to their destructive nature.
- **Optical Devices**
  - i. *Aircraft observations* can determine the extent and spatiotemporal evolution of large outbreaks of gelatinous animals near the water's surface.
  - ii. *Autonomous underwater vehicles (AUVs)* are considered the technology of the future in oceanography (Rife and Rock 2001). Eventually, these vehicles will be able to detect patches of organisms and provide images for taxonomic identification.
  - iii. *Bioluminescence,* especially the patterns of bioluminescent organelles, can be used as a taxonomic descriptor and optical methods to acquire and treat these signals are in development (Mixed Light Imaging System-MLIS, Widder 1992; see also http://www.hboi.edu/marinesci/biolum.html).

  - iv. *Submersibles,* whether manned or remotely operated vehicles (**ROV**s), are well suited for qualitative *in situ* studies of large gelatinous animals. They provide high-quality images and undamaged animals. As compared to manned submersibles, ROVs have narrower fields of vision, slower reaction times and more laborious manipulation.
  - v. *Video profiling* promises to be an area of intensive development in the future. The Video Plankton Recorder (**VPR**) can visualize and quantify small zooplankton. The Underwater Video Profiler (UVP) can visualize macrozooplankton from the surface to a depth of 1000 m. With the rapid progress of imaging technologies, the quality of data collected by both instruments is constantly improving. The instruments support rapid assessment of dominant populations but do not reliably identify rare or new species.

- **Acoustics**

  Acoustics are not well suited for the study of gelatinous animals because most of them are permeable to sound and do not produce a well-defined backscatter. Some patterns of echoes may be specific to a faunal group but variability is often high.

Figure 15. Sampling and observational techniques for gelatinous zooplankton (modified from G. Gorsky, CIESM 2001; Frost 2006). Image credits: Universität Hamburg - Institut für Hydrobiologie und Fischereiwissenschaft (IHF).

Within the events described above, we can find several ecological questions that seldom receive rigorous answers. How are patches of gelatinous zooplankton distributed across various scales in three-dimensional space? Furthermore, how does this distribution change through time and do the patches have a significant and changing substructure? What hydrographic, biological and behavioral influences create and maintain discrete patches and any associated substructure? What are the magnitudes of the top-down and bottom-up effects from such patches? What quantities of carbon and nutrients become ‘‘bound’’ in the gelatinous zooplankton and where do they go? At a minimum, reliable answers to such questions require combining large-scale sampling of gelatinous zooplankton, small-scale sampling and enumeration of specific taxa, and laboratory experiments and measurements on healthy animals to elucidate physiological and trophic states. Ultimately, we need sophisticated sampling techniques that support well-designed natural and manipulative experiments targeting gelatinous zooplankton if we truly want to assess their ecological role in oceanic systems.

With recent and future advances in technology, now is an opportune time for significant advances in our understanding of these enigmatic, delicate and beautiful animals (Haddock 2004).

5. CONCLUSION

Temporal and stochastic changes like increasing reproduction and abundance can be observed through the biota living in the deep-sea despite relatively stable abiotic conditions. The magnitude of these changes, however, decreases with increasing depth. It is not surprising that the animals in the well-lit surface layer of the temperate oceans are showing temporal trends, since the environmental conditions there are subject to seasonal changes like the length of daytime, temperature and the availability and mobilization of nutrients, etc. These effects also influence the animals in the twilight zone because they are closely associated with the euphotic zone via daily and seasonal migrating zooplankton and fishes within these zones. Migration into the dark zone, below 1000 m, was often postulated (Vinogradov 1968), but no direct evidence exists.

Organisms inhabiting the dark zone primarily depend on organic matter and dead corpses sinking from the surface into deeper waters. This sinking occurs in temporally changing quantities and qualities. A temporal reaction on such fluxes was observed in the zooplankton assemblage of the open ocean in the upper part of the dark zone between 1000 and 2500 m, which is also called the upper bathypelagic zone. Below these depths, temporal variations are rarely visible. Finally, when the sinking organic material reaches the bottom, where it accumulates, temporal changes again occur (Wigham *et al.* 2003, Billett *et al.* 2006). Such a "bottom effect" may impact the water-column some hundred meters above the sea bottom.

Based on the knowledge gained so far, we will open the following hypotheses for discussion:

1. There are strong indications that some faunal elements of the zooplankton community in the upper bathypelagic zone of the open ocean respond quickly to increasing availability of food by increasing reproduction. These organisms produce

high amounts of offspring so that some may survive. The reproduction strategy is *r-selected.*

2. The organisms in the lower bathypelagic realm do not show the same response. The abundance is spatially and temporally more or less stable throughout the water column (Weikert and Koppelmann 1996, Koppelmann and Weikert 1999, Koppelmann *et al.* 2003a). Only low numbers of offspring are produced. The production strategy is *K-selected.* This is biologically a "quiet" zone, meaning that predation pressure is reduced and most of the animals are scavengers.
3. Predation by gelatinous organisms and the production of mucous alters the mode of carbon flux in the deep-sea enormously. However, due to methodological difficulties their contribution is hitherto underestimated.
4. In semi-enclosed basins with a high deep-water temperature, like the eastern Mediterranean, the "true" deep-sea fauna is impoverished and most of the animals are immigrants from shallower layers. In this environment, significant changes in abiotic parameters and an increased availability of food, as caused by the EMT (Roether *et al.* 1996, Weikert *et al.* 2001), results in long-term changes in the abundance of selected species. These changes will be memorized over an extended time-span of several years.

Knowledge of trophic changes in deep-water environments and the investigation of key taxa, including gelatinous organisms, will help us to better understand the future of deep-sea biota in a changing environment caused by CO_2 emissions and global warming. Based on increases in pH, changes in organic matter inputs both in quality and quantity, and changes in hydrography, it will become necessary to learn more about the vulnerability of deep-water ecosystems. In order to accomplish this, we have to understand the effects of climate change on the distribution and physiological capacity of the deep-sea fauna.

Investigations of deep-water animals are technologically challenging tasks (see also Figure 15) because it is hard to catch these animals alive and healthy for experiments. Pressure-retaining sampling chambers (e.g. Youngbluth 1984) and pressure aquaria, as well as *in-situ* laboratories, are necessary to gain sophisticated knowledge on the biology, physiology, and ecology of these organisms. Deep-sea laboratories can be deployed and maintained using remotely operating vehicles (ROV's). In addition, future experiments can be conducted by the use of autonomous underwater vehicles (AUV's).

ACKNOWLEDGEMENTS

We wish to thank all colleagues, technicians, students and the staff of the various research vessels for making the research successful, for their contribution in taking and sorting the samples, and for discussions of the results. Our thank goes as well to the editor for giving the opportunity to write this contribution. Section 4 is part of a thesis by J.F.

REFERENCES

Agassiz, A. (1891). Three letters from the Albatross. *Bulletin of the Museum of comparative Zoology, 4*, 185-200.

Alldredge, A.L. (1976). Discarded appendicularian houses as sources of food, surface habitats and particulate organic matter in planktonic environments. *Limnology and Oceanography, 21*, 14-23.

Alldredge, A.L. & Silver, M.W. (1988). Characteristics, dynamics and significance of marine snow. *Progress in Oceanography, 20*, 41-82.

Allain, V. & Lorance, P. (2000). Age estimation and growth of some deep-sea fish from the Northeast Atlantic Ocean, In: M. Gayet, J.Y. Sire (Eds.). *First meeting on Ichthyology in France, RIF 2000, Vol. 24* (pp 7-16). Paris: Societe francaise d'Ichtyologie.

Altabet, M.A. (1988). Variations in nitrogen isotopic composition between sinking and suspended particles: implications for nitrogen cycling and particle transformation in the open ocean. *Deep-Sea Research I, 35*, 535-554.

Altabet, M.A., Deuser, W.G., Honjo, S. & Stienen, C. (1991). Seasonal and depth-related changes in the source of sinking particles in the North Atlantic. *Nature, 354*, 136-139.

Angel, M.V. (1984). Detrital organic fluxes through pelagic ecosystems. In: M.J.R. Fasham (Ed.). *Flows of energy and materials in marine ecosystems* (pp 475-516). Plenum Press.

Angel, M.V. (1989). Does mesopelagic biology affect the vertical flux? In: W.H. Berger, V. Smetacek, G. Wefer (Eds.), *Productivity of the ocean: present and past* (pp 155-173). John Wiley & Sons.

Angel, M.V. (1990). Life in the benthic boundary layer: connections to the mid-water and sea floor. *Philosophical Transactions of the Royal Society London A, 331*, 15-28.

Antia, A.N., Koeve, W., Fischer, G., Blanz, T., Schulz-Bull, D., Scholten, J., Neuer, S., Kremling, K., Kuss, J., Peinert, R., Hebbeln, D., Bathmann, U., Conte, M., Fehner, U. & Zeitzschel, B. (2001). Basin-wide particulate carbon flux in the Atlantic Ocean: Regional export patterns and potential for atmospheric CO_2 sequestration. *Global Biogeochemical Cycles, 15*, 845-862.

Arai, M.N. (2005). Predation on pelagic coelenterates: a review. *Journal of the marine biological Association of U.K., 85*, 523-536.

Axelsen, H. (2005). Identification of the medusas *Periphylla periphylla* and *Atolla sp.* in the diet of the mesopelagic shrimp *Notostomus robustus*, from the mid-Atlantic ridge, using DNA techniques. University of Bergen, Master student study, unpublished.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. & Thingstad, F. (1983). The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series, 10*, 257-263.

Azzaro, M. (1997). Attività respiratoria (ETSa) e biomassa microbica (ATP) nel Mediterraneo Orientale. *Thesis*, Natural Science Univ., Messina, Italy, unpublished, 68 pp.

Baars, M. (1999). On the paradox of high mesozooplankton biomass, throughout the year in the western Arabian Sea: Re-analysis of IIOE data and comparison with newer data. *Indian Journal of Marine Sciences, 28*, 125-137.

Baars, M.A. & Brummer, G.-J.A. (1995). Workshop review results from Netherlands Indian Ocean Programme. *U.S. JGOFS News, 6*, 10-11.

Baars, M. & Osterhuis, S.S. (1998). Zooplankton biomass in the 200 m in and outside the seasonal upwelling areas of the western Arabian Sea. In: A.C. Pierrot-Bults, S. v. d. Spoel (Eds.), *Pelagic Biogeography ICoPB II* (pp 36-49). Paris: UNESCO.

Babenerd, B. & Krey, J. (1974). *Indian Ocean: collected data on primary production, phytoplankton pigments, and some related factors.* Kiel: Institut für Meereskunde an der Universität Kiel, Germany. 521 pp.

Bagley, P.M., Smith, A. & Priede, I.G. (1994). Tracking movements of deep demersal fishes in the Porcupine Seabight, North-East Atlantic Ocean. *Journal of the marine biological Association of U.K.*, *74*, 473-480.

Båmstedt, U. Kaartvedt, S. & Youngbluth, M. (2003). An evaluation of acoustic and video methods to estimate the abundance and vertical distribution of jellyfish. *Journal of Plankton Research*, *25*, 1307-1318.

Banse, K. (1987). Seasonality of phytoplankton chlorophyll in the central and northern Arabian Sea. *Deep-Sea Research I*, *34*, 713-723.

Banse, K. & English, D.C. (2000). Geographical differences in seasonality of CZCS-derived phytoplankton pigment in the Arabian Sea for 1978-1986. *Deep-Sea Research II*, *47*, 1623-1677.

Banse, K. & McClain, C.R. (1986). Satellite-observed winter blooms of phytoplankton in the Arabian Sea. *Marine Ecology Progress Series, 34,* 201-211.

Barnes, R.D. (1980). *Invertebrate Zoology*, 4th Edition. Saunders College, PA.

Barnett, T. P. (1985). Variations in near-global sea level pressure. *Journal of Atmospheric Sciences, 42,* 478-500.

Bender, M.L. & Heggie, D.T. (1984). Fate of organic carbon reaching the deep sea floor: a status report. *Geochimica et Cosmochimica Acta*, *48*, 977-986.

Bergquist, D.C., Williams, F.M. & Fisher, C.R. (2000). Longevity record for deep-sea invertebrate. *Nature*, *403*, 499-500.

Berner, L. (1967). Distributional atlas of Thaliacea in the California current region. *California coop. ocean. Fisheries Investigation Atlas, 8*, 1-322.

Billett, D.S.M., Lampitt, R.S., Rice, A.L. & Mantoura, R.F.C. (1983). Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature*, *302*, 520-522.

Billett, D.S.M., Llewellyn, C., Watson, J. (1988). Are deep-sea holothurians selective deposit feeders? In: R.D. Burke, P.V. Mladenov, P. Lampert, R.L. Ž Parsley (Eds.), *Echinoderm Biology* (pp 421-429). Rotterdam: Balkema.

Billett, D.S.M., Bett, B.J., Jacobs, C.L., Rouse, I.P. & Wigham, B.D. (2006). Mass deposition of jellyfish in the deep Arabian Sea. *Limnology and Oceanography*, *51*, 2077-2083

Binet, D. (1976). Contribution a l'ecologie de quelques taxons du zooplankton de Cote d'Ivoire. II: Dolioles-Salpes-Appendiculaires. *Docums scient. Centre Rech. Océanogr., Abidjan*, *7*, 45-61.

Biscaye, P.E., Anderson, R.F. & Deck, B.L. (1988). Fluxes of particles and constituents to the eastern United States continental slope and rise: SEEP-1. *Continental Shelf Research*, *8*, 855-904.

Bishop, J.K.B. (1989). Regional extremes in particulate matter composition and flux: effects on the chemistry of the ocean interior. In: W.H. Berger, V.S. Smetacek, G. Wefer (Eds.), *Productivity of the Oceans: Present and Past* (pp 117-137). John Wiley & Sons.

Bishop, J.D.D. & Shalla, S.H. (1994). Discrete seasonal reproduction in an abyssal peracarid crustacean. *Deep-Sea Research I*, *41*, 1789-1800.

Blackburn, M. (1981). Low latitude gyral regions. In: A.R. Longhurst (Ed.), *Analysis of marine systems. 1. Marine ecology* (pp 3-29). London: Acdemic Press.

Bonnet, D., Richardson, A., Harris, R., Hirst, A., Beaugrand, G., Edwards, M., Ceballos, S., Diekmann, R., López-Urrutia, A., Valdes, L., Carlotti, F., Molinero, J.C., Weikert, H., Greve, W., Lucic, D., Albaina, A., Yahia, N.D., Umani, S.F., Miranda, A., Santos, A.d., Cook, K., Robinson, S. & Fernández de Puelles, M.L. (2005). An overview of *Calanus helgolandicus* ecology in European waters. *Progress in Oceanography, 65,* 1-53.

Böttger-Schnack, R. (1994). The microcopepod fauna in the eastern Mediterranean and Arabian Seas: a comparision with the Red Sea fauna. *Hydrobiologia, 292/293*, 271-282.

Böttger-Schnack, R. (1996). Vertical structure of small metazoan plankton, especially non-calanoid copepods. I. Deep-Arabian Sea. *Journal of Plankton Research, 18,* 1073-1101.

Braconnot, J.C. (1963). Eticle du cycle annuel des salpes et des dolioles en rade de Villefranche-sur-Mer. *J . Cons. perm. int. Explor. Mer., 28,* 21-36.

Brock, J. C. & McClain, C.R. (1992). Interannual variability of the southwest monsoon phytoplankton bloom in the northwest Arabian Sea. *Journal of Geophysical Research, 97,* 733-750.

Brock, J. C., McClain, C.R., Luther, M.E. & Hay, W.W. (1991). The phytoplankton bloom in the northwest Arabian Sea during the southwest monsoon of 1979. *Journal of Geophysical Research, 96,* 20623-20642.

Brock, J., Sathyendranath, S. & Platt, T. (1993). Modelling the seasonality of subsurface light and primary production in the Arabian Sea. *Marine Ecology Progress Series, 101*, 209-221.

Broecker, W.S. & Peng, T.H. (1982). *Tracers in the Sea.* Palisades, N.Y.: Lamont-Doherly Geological Observatory.

Bruland, K.W. & Silver, M.W. (1981). Sinking rates of fecal pellets from gelatinous zooplankton (salps, pteropods, doliolids). *Marine Biology, 63,* 295-300.

Cacchione, D.A., Rowe, G.T. & Malahoff, A. (1978). Submersible investigation of the outer Hudson submarine canyon. In: D.J. Stanley & G. Kelling (Eds.), *Sedimentation in submarine canyons, fans and trenches* (pp 42-50). Dowden: Hutchinson & Ross.

Cadet, D. L. & Diehl, B.C. (1984). Interannual variability of surface fields over the Indian Ocean during recent decades. *Monthly Weather Revue, 112,* 1921-1935.

Childress, J.J. (1968). Oxygen Minimum Layer: Vertical distribution and respiration of the Mysid *Gnathophausia ingens*. *Science, 160,* 1242-1243.

Childress, J.J. (1995). Are there physiological and biochemical adaptations of metabolism in deep-sea animals? *Trends in Ecology and Evolution, 10,* 30-36.

Childress, J.J. & Thuesen, E.V. (1992). Metabolic potential of deep-sea animals: regional and global scales. In: G.T. Rowe & V. Pariente (Eds.), *Deep-sea food chains and the global carbon cycle* (pp 217-236). Dordrecht: Kluwer Academic Publisher..

Choe, S.M. (1985). Contribution a l'etude biologique et ecologique des tuniciers pelagiques salpides (Thalices), en mer Ligure (Mediterranee). *Thèse de l'Universite Paris VI.*

Christiansen, B., Pfannkuche, O. & Thiel, H. (1990). Vertical distribution and population structure of the necrophagous amphipod *Eurythenes gryllus* in the West European Basin. *Marine Ecology Progress Series, 66,* 35-45.

Christiansen, B., Drüke, B., Koppelmann, R. & Weikert, H. (1999). The bear-bottom zooplankton at the abyssal BIOTRANS site, northeast Atlantic: composition, abundance and variability. *Journal of Plankton Research, 21,* 1847-1863.

Christiansen, B., Beckmann, W. & Weikert, H. (2001). The structure and carbon demand of the bathyal benthic boundary layer community: a comparison of two oceanic locations in the NE-Atlantic. *Deep-Sea Research II*, *48*, 2409-2424.

Chun, C. (1880). *Die Ctenophoren des Golfes von Neapel. Vol. 1*. Leipzig: Wilhelm Engelmann.

CIESM, 2001. Gelatinous zooplankton outbreaks: theory and practice. *CIESM Workshop Series*, Naples, Italy. 29 August-1 September 2001.

Colborn, J. G. (1976). *The Thermal Structure of the Indian Ocean.* Honolulu: The University Press of Hawaii. 173 pp.

Cornejo, R. & Koppelmann R. (2006). Horizontal and vertical distribution of mesopelagic fishes with special reference to *Vinciguerria lucetia* Garman 1899 (Phosichthyidae: Pisces) in the Humboldt Current Region off Peru. *Marine Biology, 149,* 1519-1537.

Couwelaar, M.v. (1997). Zooplankton and micronekton biomass off Somalia and in the southern Red Sea during the SW monsoon of 1992 and the NE monsoon of 1993. *Deep-Sea Research II*, *44*, 1213-1234.

Cushing, D.H. (1973). Production in the Indian Ocean and transfer from the primary to the secondary level. In: B. Zeitzschel (Ed.), *The biology of the Indian Ocean* (pp 475-486). Berlin: Springer.

Cushing, D.H. (1975). *Marine ecology and fisheries*. Cambridge: Cambridge University Press.

Danovaro, R., Dinet, A., Duineveld, G. & Tselepides, A. (1999). Benthic response to particulate fluxes in different trophic environments: a comparison between the Gulf of Lions-Catalan Sea (western-Mediterranean) and the Cretan Sea (eastern-Mediterranean). *Progress in Oceanography*, *44*, 287-312.

Danovaro, R., Dell'Anno, A., Fabiano, M., Pusceddu, A. & Tselepides, A. (2001). Deep-sea ecosystem response to climate changes: the eastern Mediterranean case study. *Trends in Ecology and Evolution*, *16*, 505-510.

Darbyshire, M. (1967). The surface waters off the coast of Kerala, south-west India, *Deep-Sea Research, 14*, 295-320.

Deevey, G.B. & Brooks, A.L. (1971). The annual cycle in quantity and composition of the zooplankton of the Sargasso Sea off Bermuda. II. The surface to 2,000 m. *Limnology and Oceanography*, *16*, 927-943.

Delalo, E.P. (1966). The zooplankton of the Eastern Mediterranean (Levantine Sea and Gulf of Sirte) (in Russian). *Issledovaniya Planktona Yuzhnikh Morei Okeanograficheskaya Komissiya Akademiya Nauk SSSR, 7,* 62–81.

Diel, S. (1991). Zur Lebensgeschichte dominanter Copepodenarten (*Calanus finmarchicus*, *C. glacialis*, *C. hyperboreus*, *Metridia longa*) in der Framstraße. *Berichte zur Polarforschung*, *88*, 1-113.

Dover, C.L.v., Grassle, J.F., Fry, B., Garritt, R.H. & Starczak, V.R. (1992). Stable isotope evidence for entry of sewage-derived organic marterial into a deep-sea food web. *Nature, 360*, 153-156.

Dube, S.K., Luther, M.E. & O'Brien, J.J. (1990). Relationships between interannual variability in the Arabian Sea and Indian summer monsoon rainfall. *Journal of Meteorology and Atmospheric Physics*, *44*, 153-165.

Ducklow, H. (2000). Bacterial production and biomass in the oceans. In: D.L. Kirchman (Ed.), *Microbial Ecology of the Oceans* (pp 85-120). New York: Wiley.

Dugdale, R.C. & Wilkerson, F.P. (1988). Nutrient sources and primary production in the Eastern Mediterranean. *Oceanologica Acta, Sp. Vol.*, 179-184.

Dunbar, R.B., Berger, W.H. (1981). Faecal pellet flux to modern bottom sediments of Santa Barbara Basin (California) based on sediment trapping. *Bulletin of the Geological Society of America, 92*, 212-218.

Fabian, H., Koppelmann, R. & Weikert, H. (2005). Full-depths zooplankotn composition at two deep sites in the western and central Arabian Sea. *Indian Journal of Marine Sciences, 34*, 174-187.

Fiadero, M. & Strickland, J.D.H. (1968). Nitrate reduction and the occurrence of a deep nitrite maximum in the ocean off the west coast of South America. *Deep-Sea Research, 26*, 187–201.

Fieux, M. & Stommel, H. (1977). Onset of the Southwest Monsoon over the Arabian Sea from marine reports of surface winds: structure and variability. *Monthly Weather Revue, 105*, 231-236

Findlater, J. (1969). A major low-level air current near the Indian Ocean during the northern summer. *Quarterly Journal of the Royal Meteorological Society, 95*, 362-380

Findlater, J. (1974). The low-level cross-equatorial air current of the western Indian Ocean during the northern summer. *Weather, 29*, 411-416.

Fowler, S.W. & Knauer, G.A. (1986). Role of large particles in the transport of elements and organic compounds through the oceanic water column. *Progress in Oceanography, 16*, 147-194.

Fowler, S.W. & Small, L.F. (1972). Sinking rates of euphausiid faecal pellets. *Limnology and Oceanography, 17*, 293-296.

Foxton, P. (1966). The distribution and life history of *Salpa thompsoni* Foxton, with observations on a related species, *Salpa gerlachei* Foxton. *Discovery Report, 34*, 1- 116

Frost, J.R.. (2006). Gelatinous Zooplankton: Evolution of Sampling Techniques. *Eur-Oceans Newsletter, 3*, 9-11

Fry, B. (2006). *Stable isotope ecology. Environmental Science.* New York: Springer.

Fry, B. & Quiñones, R.B. (1994). Biomass spectra and stable isotope indicators of trophic level in zooplankton of the northwest Atlantic. *Marine Ecology Progress Series, 112*, 201-204.

Gage J.D. & Tyler P.A. (1991). *Deep-sea Biology: A Natural History of Organisms at the Deep-sea Floor.* Cambridge: Cambridge University Press, 504 pp.

Gilson, H.C. (1937). The nitrogen cycle. *Scientific Reports of the John Murray Expedition. 1932-1934, 2*, 21-81.

Gooday, A.J. (1996). Epifaunal and shallow infaunal foraminiferal communities at three abyssal NE Atlantic sites subject to different phytodetritus input regimes. *Deep-Sea Research I, 43*, 1395-1421.

Gooday, A.J. & Turley, C.M. (1990). Responses by benthic organisms to inputs of organic material to the ocean floor. a review. *Philosophical Transactions of the Royal Society London A, 331*, 119-138.

Gowing, M.M. & Wishner, K.F. (1992). Feeding ecology of benthopelagic zooplankton on an eastern tropical Pacific seamount. *Marine Biology, 112*, 451-467.

Gowing, M.M. & Wishner, K.F. (1998). Feeding ecology of the copepod *Lucicutia aff. L. grandis* near the lower interface of the Arabian Sea oxygen minimum zone. *Deep-Sea Research II, 45*, 2433-2459.

Haake, B., Ittekkot, V., Rixen, T., Ramaswamy, Nair & Curry (1993). Seasonality and interannual variability of particle fluxes to the deep Arabian Sea. *Deep-Sea Research I, 40*, 1323-1344.

Haddock, S.H.D. (2004). A golden age of gelata: past and future research on planktonic ctenophores and cnidarians. *Hydrobiologia, 530/531*, 549-556.

Haddock, S.H.D., Dunn, C.W., Pugh, P.R. & Schnitzler, C.E. (2005). Bioluminescent and red-fluorescent lures in a deep-sea siphonophore. *Science, 309*, 263.

Halsband-Lenk, C., Koppelmann, R. & Weikert, H. (2003). Carbon requirements of deep sea zooplankton estimated from ETS measurements in relation to size fraction and taxonomic composition. In: A. Yilmaz (Ed.), *Oceanography of Eastern Mediterranean and Black Sea* (pp 805-813). Ankara: TUBITAK.

Hamad N., Millot C., Taupier-Letage I. (2005) A new hypothesis about the surface circulation in the eastern basin of the Mediterranean Sea. *Progress in Oceanography, 66*, 287-298.

Hamner, W.M., Madin, L.P., Alldredge, A.L., Gilmer, R.W. & Hamner, P.P. (1975). Underwater observations of gelatinous zooplankton: sampling problems, feeding biology, and behavior. *Limnology and Oceanography, 20*, 907-917.

Hansson, L.J. & Norrman, B. (1995). Release of dissolved organic carbon (DOC) by the scyphozoan jellyfish *Aurelia aurita* and its potential influence on the production of planktic bacteria. *Marine Biology, 121*, 527-532.

Heinrich, H. (1986). Bathymetrie und Geomorphologie des NOAMP-Gebietes, Westeuropäisches Becken (17° W bis 22° W, 46° N bis 49° N). *Deutsche hydrographische Zeitung, 39*, 183-196.

Herring, P.J., Fasham, M.J.R., Weeks, A.R., Hemmings, J.C.P., Roe, H.S.J., Pugh, P.R., Holley, S., Crisp, N.A. & Angel, M.V. (1998). Across-slope relations between the biological populations, the euphotic zone and the oxygen minimum layer off the coast of Oman during the southwest monsoon (August, 1994). *Progress in Oceanography, 41*, 69-109.

Hirche, H.-J. (1983). Overwintering of *Calanus finmarchicus* and *Calanus helgolandicus*. *Marine Ecology Progress Series, 11*, 281-290.

Hobson, K.A. & Welch, H.E. (1992). Determination of trophic relationships within a high Artic marine food web using delta13C and delta15N analysis. *Marine Ecology Progress Series, 84*, 9-18.

Honjo, S. (1978). Sedimentation of materials in the Sargasso Sea at a 5367 m deep station. *Journal of Marine Research, 36*, 469-492.

Honjo, S. & Roman, M.R. (1978). Marine copepod faecal pellets: production, preservation and sedimentation. *Journal of Marine Research, 36*, 45-57.

Honjo, S. (1980). Material fluxes and modes of sedimentation in the mesopelagic and bathypelagic zones. *Journal of Marine Research, 38*, 53-97.

Honjo, S., Dymond, J., Prell, W. & Ittekkot, V. (1999). Monsoon-controlled export fluxes to the interior of the Arabian Sea. *Deep-Sea Research II, 46*, 1859-1902.

ICES (2003). *Report of the Working Group on Zooplankton Ecology*. Gijón, Spain. 24-26 February 2003.

Idrisi, N., Olascoaga, J., Garraffo, Z., Olson, D.B. & Smith, S.L. (2004). Mechanisms for emergence from diapause of *Calanoides carinatus* in the Somali current. *Limnology and Oceanography, 49*, 1262-1268.

IGBP (1990). The International Geosphere-Biosphere Programme: A study of global change. The initial core projects. *IGBP Report, 12*.

Ikeda, T. (1974). Nutritional ecology of marine zooplankton. *Memoirs of the Faculty of Fisheries, Hokkaido University, 22*, 1-97.

Iseki, K (1981). Particulate organic matter transport to the deep sea by salp faecal pellets. *Marine Ecology Progress Series, 5*, 55-60.

Jickells, T.D., Newton, P.P., King, P., Lampitt, R.S. & Boutle, C. (1996). A comparison of sediment trap records of particle fluxes from 19 to 48° N in the northeast Atlantic and their relation to surface water productivity. *Deep-Sea Research I, 43*, 971-986.

Judkins, D.C. (1980). Vertical distribution of zooplankton in relation to the oxygen minimum off Peru. *Deep-Sea Research I, 27A*, 457-487.

Kaartvedt, S. (1996). Habitat preference during overwintering and timing of seasonal vertical migration of Calanus finmarchicus. *Ophelia, 44*, 145-156.

Kamykowski, D. & Zentara, S.-J. (1990). Hypoxia in the world ocean as recorded in the historical data set. *Deep-Sea Research I, 37*, 1861-1874.

Karl, D.M. & Knauer, G.A. (1984). Vertical distribution, transport, and exchange of carbon in the northeast Pacific Ocean: evidence for multiple zones of biological activity, *Deep-Sea Research I, 31*, 221-243.

Kidwai, S. & Amjad, S. (2000). Zooplankton: pre-southwest and northeast monsoons of 1993 to 1994, from the North Arabian Sea. *Marine Biology, 136*, 561-571.

Kinzer, J., Böttger-Schnack, R. & Schulz, K. (1993). Aspects of horizontal distribution and diet of myctophid fish in the Arabian Sea with reference to the deep water oxygen deficiency. *Deep-Sea Research II, 40*, 783-800.

Klein, B., Roether, W., Manca, B.B., Bregant, D., Beitzel, V., Kovacevic, V. & Luchetta, A. (1999). The large deep-water transient in the Eastern Mediterranean. *Deep-Sea Research I, 46*, 371-414.

Klein, B., Roether, W., Kress, N., Manca, B.B., Ribera d'Alcala, M., Souvermezoglou, A., Theocharis, A., Civitarese, G. & Luchetta, A. (2003). Accelerated oxygen consumption in eastern Mediterrean deep waters following the recent changes in thermohaline circulation. *Journal of Geophysical Research, 108*, doi10.1029/2002JC001454.

Koeve, W., Podewski, S., Pollehne, B., Zeitzschel, B., Jochem, F., Kähler, P., Dettmar, A., Deckers, M., Haupt, O., Reitmeier, S., Fritsche, P., Werner, R. & Sellmer, C. (1993). Planktological investigations during the Winter-Spring-Summer transition at 47° N, 20° W (JGOFS). *Meteor Berichte, 93-4*, 100-115.

Komer, P.D., Morse, A.P., Small, L.F. & Fowler, S.W. (1981). An analysis of sinking rates of natural copepod and euphausiid faecal pellets. *Limnology and Oceanography, 26*, 173-181.

Koppelmann, R., 1994. Saisonale Veränderungen in bathypelagischen Zooplanktonbeständen des Nordostatlantiks. Hamburg: *Dissertation, Fachbereich Biologie, Universität Hamburg*, 130 pp.

Koppelmann, R. & Weikert, H. (1992). Full-depth zooplankton profiles over the deep bathyal of the NE Atlantic. *Marine Ecology Progress Series, 86*, 263-272.

Koppelmann, R. & Weikert, H. (1997). Deep Arabian Sea mesozooplankton distribution. Intermonsoon, October 1995. *Marine Biology, 129*, 549-560.

Koppelmann, R. & Weikert, H. (1999). Temporal changes of deep-sea mesozooplankton abundance in the temperate NE Atlantic and estimates of the carbon budget. *Marine Ecology Progress Series, 179*, 27-40.

Koppelmann, R. & Weikert, H. (2000). Transfer of organic matter in the deep Arabian Sea zooplankton community: insights from $\delta^{15}N$ analysis. *Deep-Sea Research II, 47*, 2653-2672.

Koppelmann, R. & Weikert, H. (2005). Temporal and vertical distribution of two ecologically different calanoid copepods (*Calanoides carinatus* KRØYER 1849 and *Lucicutia grandis* GIESBRECHT 1895) in the deep waters of the central Arabian Sea. *Marine Biology, 147*, 1173-1178.

Koppelmann, R. & Weikert, H. (2007). Spatial and temporal distribution patterns of deep-sea mesozooplankton in the eastern Mediterranean - indications of a climatically induced shift? *Marine Ecology, 28*, 259-275.

Koppelmann, R., Schäfer, P. & Schiebel, R. (2000). Organic carbon losses measured by heterotrophic activity of mesozooplankton and $CaCO_3$ flux in the bathypelgic zone of the Arabian Sea. *Deep-Sea Research II, 47*, 169-187.

Koppelmann, R., Fabian, H. & Weikert, H. (2003a). Temporal variability of deep-sea zooplankton in the Arabian Sea. *Marine Biology, 142*, 959-970.

Koppelmann, R., Weikert, H. & Lahajnar, N. (2003b). Vertical distribution of mesozooplakton and its $\delta^{15}N$ signature at a deep-sea site in the Levantine Sea (eastern Mediterranean) in April 1999. *Journal of Geophysical Research, 108*, doi:10.1029/2002JC001351.

Koppelmann, R., Weikert, H. & Halsband-Lenk, C. (2004). Mesozooplankton community respiration and its relation to particle flux in the oligotrophic eastern Mediterranean. *Global Biogeochemical Cycles, 18*, GB1039, doi:10.1029/2003GB002121.

Koppelmann, R., Zimmermann-Timm, H. & Weikert, H. (2005). Bacterial and zooplankton distribution in deep waters of the Arabian Sea. *Deep-Sea Research I, 52*, 2184-2192.

Koslow, J.A. (1996). Energetic and life-history patterns of deep-sea benthic, benthopelagic and seamount-associated fish. *Journal of Fish Biology, 49 (Suppl. A)*, 54-74.

Kouwenberg, J. (1993). Sex ratio of calanoid copepods in relation to population composition in the northwestern Mediterranean. *Crustaceana, 64*, 281-299.

Krey, J., & Babenerd, B. (1976). *Phytoplankton production atlas of the International Indian Ocean Expedition*. Paris: Intergovernmental Oceanographic Commission. 70 pp.

Krishnamurti, T.N., Jayakumar, P.K., Sheng, J., Surgi, N. & Kumar, A. (1985a). Divergent circulations on the 30 to 50 day time scale. *Journal of Atmospheric Science, 42*, 364-375.

Krishnamurti, T.N., Oosterhof, D.K. & Mehta, A.V. (1985b). Air-sea interaction on the time scale of 30-50 days. *Journal of Atmospheric Science, 45*, 1304-1322.

Kuz'menko, L.O. (1974). Primary productivity of the northern Arabian Sea. *Oceanology, 20*, 164-167.

La Ferla, R. & Azzaro, M. (2001). Microbial respiration in the Levantine Sea: evolution of the oxidative processes in relation to the main Mediterranean water masses. *Deep-Sea Research I, 48*, 2147-2159.

La Ferla, R., Azzaro, M., Civitarese, G. & Ribera D'Alcalà, M. (2003) Distribution patterns of carbon oxidation in the Eastern Mediterranean Sea: evidence of changes in remineralization processes. *Journal of Geophysical Research, 108*, doi: 10.1029/2002JC001602.

La Ferla, R. & Azzaro, M. (2004). Metabolic CO_2 production in the Mediterranean Sea: A case study for estimating carbon budget in the sea. *Scientia Marina*, *68*, 57-64.

Lajtha, K. & Michener, R.H. (2006). *Stable isotopes in ecology and environmental science*. Oxford: Blackwell.

Lampitt, R.S. (1985). Evidence for the seasonal deposition of detritus to the deep-sea floor and its subsequent resuspension. *Deep-Sea Research I*, *32*, 885-897.

Lampitt, R.S. (1992). The contribution of deep-sea macroplankton to organic remineralization: results from sediment trap and zooplankton studies over the Madeira Abyssal Plain. *Deep-Sea Research I*, *39*, 221-233.

Lampitt, R.S. & Antia, A.N. (1997). Particle flux in deep seas: regional characteristics and temporal variability. *Deep-Sea Research I*, *44*, 1377-1403.

Larson, R.J., Mills, C.E. & Harbison, G.R. (1991). Western Atlantic midwater hydrozoan and scyphozoan medusae: *in situ* studies using manned submersibles. *Hydrobiologia*, *216/217*, 311-317

Lascaratos, A., Roether, W., Nittis, K. & Klein, B. (1999). Recent changes in deep water formation and spreading in the eastern Mediterranean Sea: a review. *Progress in Oceanography*, *44*, 5-36.

Lee, C., Murray, D.W., Barber, R.T., Buesseler, K.O., Dymond, J., Hedges, J.I., Honjo, S., Manganini, S.J., Marra, J., Moser, C., Peterson, M.L., Prell, W.L. & Wakeham, S.G. (1998). Particulate organic carbon fluxes: compilation of results from the 1995 US JGOFS Arabian Sea process study. *Deep-Sea Research II*, *45*, 2489-2501.

Lee, C.M., Jones, B.H., Brink, K.H. & Fischer, A.S. (2000). The upper-ocean response to monsoonal forcing in the Arabian Sea: seasonal and spatial variabilty. *Deep-Sea Research II*, *47*, 1177-1226.

Lipschultz, F., Wofsy, S.C., Ward, B.B., Codispoti, L.A., Fredrich, G. & Elkins, J.W. (1990). Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the Eastern Tropical South Pacific Ocean. *Deep-Sea Research I*, *37*, 1513-1541.

Lochte K. (1992) Bacterial standing stock and consumption of organic carbon in the benthic boundary layer of the abyssal North Atlantic. In: G.T. Rowe, V. Pariente (Eds.). *Deep-sea food chains and the global carbon cycle* (pp 1-10). Dordrecht: Kluwer Academic Publ.

Lochte, K., Halbach, P. & Flemming, B. (1996). *Biogeochemical fluxes in the deep-sea and investigations of geological structures in the Indian Ocean. Cruise No. 33, 22 September - 30 December 1995*. Hamburg: Meteor-Berichte, 160 pp.

Longhurst, A.R. (1967). Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum. *Deep-Sea Research*, *14*, 51-63.

Longhurst, A R. (1976). Interactions between zooplankton and phytoplankton profiles in the eastern tropical Pacific Ocean, *Deep-Sea Research*, *23*, 729-754.

Longhurst, A.R. & Harrison, W.G. (1989). The biological pump: Profiles of plankton production and consumption in the upper ocean. *Progress in Oceanography*, *22*, 47-123.

Loubere, P. (1998) The impact of seasonality on the benthos as reflected in the assemblages of deep-sea foraminifera. *Deep-Sea Research I*, *45*, 409-432.

Luo, J., Ortner, P.B., Forcucci, D. & Cummings, S.R. (2000). Diel vertical migration of zooplankton and mesopelagic fish in the Arabian Sea. *Deep-Sea Research II*, *47*, 1451-1473.

Luther, M.E., O'Brien, J.J. & Prell, W.L. (1990). Variability in upwelling fields in the northwestern Indian Ocean over the past 18,000 years; 1, Model experiments. *Paleoceanography, 5*, 433-445.

Madhupratap, M. & Haridas, P. (1990). Zooplankton, especially calanoid copepods in the upper 1000 m of the south-east Arabian Sea. *Journal of Plankton Research, 12*, 305-321.

Madhupratap, M., Prasanna Kumar, S., Bhattathiri, P.M.A., Dileep Kumar, M., Raghukumar, S., Nair, K.K.C. & Ramaiah, N. (1996). Mechanism of the biological response to winter cooling in the northeastern Arabian Sea. *Nature, 384*, 549-552.

Madhupratap, M., Gopalakrishnan, T.C., Haridas, P. & Nair, K.K.C. (2001). Mesozooplankton biomass, composition and distribution in the Arabian Sea during the Fall Intermonsoon: implications of oxygen gradients. *Deep-Sea Research II, 48*, 1345-1368.

Madin, L.P. (1982). Production, composition and sedimentation of salp fecal pellets in oceanic waters. *Marine Biology, 67*, 39-45.

Madin, L.P., Kremer, P., Wiebe, P.H., Purcell, J.E., Horgan, E.H. & Nemazie D.A. (2006). Periodic swarms of the salp *Salpa aspera* in the Slope Water off the NE United States: Biovolume, vertical migration, grazing, and vertical flux. *Deep-Sea Research I, 53*, 804-819.

Manghnani, V., Morrison, J.M., Hopkins, T.S. & Böhm, E. (1998). Advection of upwelled waters in the form of plumes off Oman during the Southwest Monsoon. *Deep-Sea Research II, 45*, 2027-2052.

Mantyla, A.W. & Reid, J.R. (1983). Abyssal characteristics of the world ocean waters. *Deep-Sea Research I, 30*, 805-833.

Martin, J.H., Knauer, G.A., Karl, D.M. & Broenkow, W.W. (1987). VERTEX: carbon cycling in the northeast Pacific. *Deep-Sea Research I, 34*, 267-285.

Matsueda, H., Handa, N., Inoue, I. & Takano, H. (1986). Ecological significance of salp fecal pellets collected by sediment traps in the eastern North Pacific. *Marine Biology, 91*, 421-431.

Matsumoto, G.I., Raskoff, K. & Lindsay, D. (2003). *Tiburonia granrojo* n. sp., a mesopelagic scyphomedusa from the Pacific Ocean representing the type of a new subfamily (class Scyphozoa: order Semaeostomeae: family Ulmaridae: subfamily Tiburoniinae subfam. nov.). *Marine Biology, 143*, 73-77.

Mayzaud, P., Razouls, S., Errhif, A., Tirelli, V. & Labat, J.P. (2002). Feeding, respiration and egg production rates of copepods during austral spring in the Indian sector of the Antarctic Ocean: role of the zooplankton community in carbon transformation. *Deep-Sea Research I, 49*, 1027-1048.

McCave, I.N. (1975). Vertical flux of particles in the ocean. *Deep-Sea Research, 22*, 491-502.

Menzies, R.J. (1965). Conditions for the existence of life on the abyssal sea floor. *Oceanography and Marine Biology Annual Revue, 3*, 195-210.

Michener, R.H. & Schell, D.M., 1994. Stable isotopes ratios as tracers in marine aquatic food webs. In: K. Lajtha, R.H. Michener, R.H. (Eds.). *Stable isotopes in ecology and environmental research* (pp 138-157). Oxford: Blackwell Scientific Publications, Oxford.

Mills, C.E. (2001). Jellyfish blooms: Are populations increasing globally in response to changing ocean conditions? *Hydrobiologia, 451*, 55-68.

Minagawa, M. & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, *48*, 1135-1140.

Mittelstaedt, E., Bock, M., Bork, I., Klein, H., Nies, H. & Schauer, U. (1986). *Ausbreitungsbedingungen für Stoffe in großen Ozeantiefen. Nordostatlantisches Monitoring Programm*. Hamburg: DHI, 58 pp.

Moore, P.G., Rainbow, P.S. & Larson, R.J. (1993). The mesopelagic shrimp *Notostomus robustus* Smith (Decapoda:Oplophoridae) observed *in situ* feeding on the medusan *Atolla wyvillei* Haeckel in the Northwest Atlantic, with notes on gut contents and mouthpart morphology. *Journal of Crustacean Biology*, *13*, 690-696.

Morris, R.J., Bone, Q. Head, R., Braconnot, J.C. & Nival, P. (1998). Role of salps in the flux of organic matter to the bottom of the Ligurian Sea. *Marine Biology*, *97*, 237-241.

Morrison, J.M., Codispoti, L.A., Smith, S.L., Wishner, K., Flagg, C., Gardner, W.D., Gaurin, S., Naqvi, S.W.A., Manghnani, V., Prosperie, L. & Gundersen, J.S. (1999). The oxygen minimum zone in the Arabian Sea during 1995. *Deep-Sea Research II*, *46*, 1903-1931.

Moseley, H.N. (1880). Deep-sea dredging and life in the deep sea. *Nature*, *21*, 543-547//569-572//591-593.

Naqvi, S.W.A., Shailaja, M.S., Dileep Kumar, M. & Sen Gupta, R. (1996). Respiration rates in subsurface waters of the northern Indian Ocean: evidence for low decomposition rates of organic matter with the water column in the Bay in Bengal. *Deep-Sea Research II, 43*, 73-81.

Newton, P.P., Lampitt, R.S., Jickells, T.D., King, P. & Boutle, C. (1994). Temporal and spatial variability of biogenic particle fluxes during JGOFS northeast Atlantic process studies at 47° N, 20° W. *Deep-Sea Research I, 41*, 1617-1642.

Nival, P., Braconnot, J.C., Anderson, V., Oberdorff, T., Choe, S.M. & Laval, P. (1985). Estimation de l'impact des salpes sur le phytoplankton en mer Ligure. *Rapports et Proces-Verbaux des Reunions, Conseil International pour L'Exploration scientifique de la Mer Mediterranee*, *29*, 283-286.

Nouvian C. (2006). *The deep. Leben in der Tiefsee.* Knesebeck-Verlag.

Osborn, T. & Barber, R.T. (2003). Why are large, delicate, gelatinous organisms so successful in the ocean's interior? In: L. Seuront, P. Sutton (Eds.), *Handbook of Scaling Methods in Aquatic Ecology* (pp 329-332). Boca Raton, FL: CRC Press.

Olson, D.B., Hitchcock, G.L., Fine, R.A. & Warren, B.A. (1993). Maintenance of the low-oxygen layer in the central Arabian Sea. *Deep-Sea Research II, 40*, 673-685.

Packard, T.T., Denis, M. & Garfield, P. (1988). Deep-ocean metabolic CO_2 production: calculations from ETS activity. *Deep-Sea Research I, 35*, 371-382.

Padmavati, G., Haridas, P., Nair, K.K.C., Gopalakrishnan, T.C., Shiney, P. & Madhupratap, M. (1998). Vertical distribution of mesozooplankton in the central and eastern Arabian Sea during the winter monsoon. *Journal of Plankton Research*, *20*, 343-354.

Pancucci-Papadopoulou, M.A., Siokou-Frangou, I., Theocharis, A. & Georgopoulos, D. (1992). Zooplankton vertical distribution in relation to the hydrology in the NW Levantine and SE Aegean seas (spring 1986). *Oceanologica Acta*, *15*, 365-381.

Parsons, T.R. & Lalli, C.M. (1988). Comparative oceanic ecology of the plankton communities of the subartic Atlantic and Pacific Oceans. *Oceanography and Marine Biology Annual Revue*, *26*, 317-359.

Pauly, D. & Christensen, V. (1995). Primary production required to sustain global fisheries. *Nature, 374*, 255-257.

Peterson, W.T. & Painting, S.J. (1990). Developmental rates of the copepods *Calanus australis* and *Calanoides carinatus* in the laboratory, with discussion of methods used for development of development time. *Journal of Plankton Research, 12*, 283-293.

Pfannkuche, O. (1993). Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47° N, 20° W. *Deep-Sea Research I, 40*, 135-149.

Pfannkuche, O. & Lochte, K. (1993). Open ocean pelago-benthic coupling: cyanobacteria as tracers of sedimenting salp faeces. *Deep-Sea Research, 40*, 727-737.

Pfannkuche, O. & Utecht, C. (1998). *FS Sonne - Fahrtbericht/Cruise Report SO 118 BIGSET (Biogeochemical transport of matter and energy in the deep sea). Muscat (Oman) - Muscat (Oman), 31.03.-11.05.1997*. Kiel: GEOMAR Report 71.

Pfannkuche, O. & Utecht, C. (1999). *FS Sonne - Fahrtbericht/Cruise Report SO 129 BIGSET (Biogeochemical transport of matter and energy in the deep sea). Muscat (Oman) - Dubai (VAE), 30.01.-09.03.1998*. Kiel: GEOMAR Report 80.

Pfannkuche, O., Rheinheimer, G. & Thiel, H. (1993). BIO-C-FLUX. Biologischer Kohlenstoffffluß in der bodennahen Wasserschicht des küstenfernen Ozeans. Abschlußbericht für den Förderzeitraum 1.1.1990 - 31.12. 1992. *Berichte aus dem Institut für Meereskunde der Universität Kiel, 242*, 1-130.

Pilskaln, C.H., Paduan, J.B., Chavez, F.P., Anderson, R.Y. & Berelson, W.M. (1996). Carbon export and regeneration in the coastal upwelling system of Monterey Bay, central California. *Journal of Marine Research, 54*, 1149-1178.

Pomeroy, L.R. & Deibel, D. (1980). Aggregation of organic matter by pelagic tunicates. *Limnology and Oceanography, 35*, 643-652.

Prasanna Kumar, S. & Prasad, T.G. (1996). Winter cooling in the northern Arabian Sea. *Current Science, 71*, 834-841.

Priede, I.G. (1994). Tracking of scavenging fishes in the abyss. *Endeavour, New Series, 18***,** 74-79.

Raskoff, K.A. & Matsumoto, G. I. (2004). *Stellamedusa ventana*, a new mesopelagic scyphomedusae from the eastern Pacific representing a new subfamily, the Stellamedusinae. *Journal of the Marine Biological Association of U.K., 84*, 1-6.

Raupach, M.R., Marland, G., Ciais, P., Le Quéré, C, Canadell, J.G., Klepper, G. & Field, C.B. (2007). Global and regional drivers of accelerating CO_2 emissions. *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.0700609104

Rey-Rassat, C., Irigoien, X., Harris, R. & Carlotti, F. (2002). Energetic cost of gonad development in *Calanus finmarchicus* and *C. helgolandicus*. *Marine Ecology Progress Series, 238*, 301-306.

Rife, J. & Rock, S. (2001). *Visual tracking of Jellyfish in Situ*. Proceedings of the International Conference of Image Processing.

Rixen, T., Haake, B., Ittekkot, V., Guptha, M.V.S., Nair, R.R. & Schlüssel, P. (1996). Coupling between SW monsoon-related surface and deep ocean processes as discerned from continuous particle flux measurements and correlated satellite data. *Journal of Geophysical Research, 101*, 28569-28582.

Robinson, M.K. (1960). Indian Ocean vertical temperature sections. *Deep-Sea Research I, 6*, 249-258.

Robinson, A.R., Leslie, W.G., Theocharis, A. & Lascaratos, A. (2001). Mediterranean Sea Circulation. In: *Encyclopedia of Ocean Sciences* (pp 1689-1706). Academic Press.

Robison, B.H. (2004). Deep pelagic biology. *Journal of Experimental Marine Biology and Ecology, 300,* 253-272.

Robison, B.H., Reisenbichler, K.R. & Sherlock, R.E. (2005). Giant larvacean houses: Rapid carbon transport to the deep sea floor. *Science, 308,* 1609-1611.

Rochford, D.J. (1964). Salinity maxima in the upper 1000 m of the north Indian Ocean. *Australian Journal of Marine and Freshwater Research, 15,* 1–24.

Roe, H.S.J. (1988). Midwater biomass profiles over the Madeira Abyssal Plain and the contribution of copepods. *Hydrobiologia, 167/68,* 169-181.

Roether, W. & Schlitzer, R. (1991). Eastern Mediterranean deep water renewal on the basis of chlorofluoromethane and tritium data. *Dynamics of Atmospheres and Oceans, 15*, 333-354.

Roether, W., Klein, B., Beitzel, V., Manca, B.B., Kovacevic, V., Bregant, D., Luchetta, A. & Georgopoulos, D. (1996). Recent changes in Eastern Mediterranean deep waters. *Science, 271*, 333-335.

Roether W., Klein B., Manca B.B., Theocharis A., Kioroglou S. (in press) Transient Eastern Mediterranean deep water response to the massive dense-water output of the Aegean in the 1990s. *Progress in Oceanography*.

Rowe, G.T. (1981). The deep-sea ecosystem. In: A.R. Longhurst (Ed.), *Analysis of marine ecosystems. 1. Marine ecology* (pp 235-267). London: Academic Press.

Saltzman, J. & Wishner, K.F. (1997a). Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 1. General trends. *Deep-Sea Research I, 44,* 907-930.

Saltzman, J. & Wishner, K.F. (1997b). Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 2. Vertical distribution of copepods. *Deep-Sea Research I, 44*, 931-954.

Sameoto, D.D. (1986). Influence of the biological and physical environment on the vertical distribution of mesozooplankton and micronekton in the eastern tropical Pacific. *Marine Biology, 93*, 263-279.

Sasaki, H., Hattori, H. & Nishizawa, S. (1988). Downward flux of particulate organic matter and vertical distribution of calanoid copepods in the Oyashio Water in summer. *Deep-Sea Research I, 35*, 505-515.

Sastry, J.S. & D'Souza, R.S. (1972) Oceanography of the Arabian Sea during the southwest monsoon – III: Salinity. *Indian Journal of Meteorology and Geophysics, 23,* 479–490.

Schäfer, P. & Ittekkot, V. (1995). Isotopic biogeochemistry of nitrogen in the northern Indian Ocean. *Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg, 78,* 67-93.

Schäfer, P., Ittekkot, V., Gravenhorst, G., Langel, R. & Reineking, A. (1998). Variations of d15N-values and hydrolyzable amino acids in settling particles in the ocean. *Isotopes in Environmental and Health Studies, 34,* 191-199.

Schmidt, J. (1925). On the contents of oxygen in the ocean on both sides of Panama. *Science, 61,* 592-593.

Schneider, G. (1989a). Estimation of food demands of *Aurelia aurita* medusae populations in the Kiel Bight/western Baltic. *Ophelia, 31,* 17-27.

Schneider, G. (1989b). The common jellyfish *Aurelia aurita*: standing stock excretion and nutrient regeneration in the Kiel Bight, Western Baltic. *Marine Biology*, *100*, 507-514.

Schott, F.A. & McCreary, J.P.J. (2001). The monsoon circulation of the Indian Ocean. *Progress in Oceanography, 51*, 1-123.

SCOR (1992) Joint Global Ocean Flux Study. Implementation Plan. Scientific Commitee on Oceanic Research. *JGOFS Report, 9*.

Scotto di Carlo, B., Ianora, A., Mazzochi, M.G. & Scardi, M. (1991). Atlantis II Cruise: uniformity of deep copepod assemblages in the Mediterranean Sea. *Journal of Plankton Research, 13*, 263 - 277.

Seibel, B.A., Thuesen, E.V., Childress, J.J. & Gorodezky, L.A. (1997). Decline in pelagic cephalopod metabolism with habitat depth reflects differences in locomotory efficiency. *Biological Bulletin, 192*, 262-278.

Seibel, B.A., Thuesen, E.V. & Childress, J.J. (2000). Light-limitation on predator-prey interactions: consequences for metabolism and locomotion of deep-sea cephalopods. *Biological Bulletin, 198*, 284-298.

Seritti, A., Manca, B.B., Santinelli, C., Murru, E., Boldrin, A. & Nannicini, L. (2003). Relationships between dissolved organic carbon (DOC) and water mass structures in the Ionian Sea (winter 1999). *Journal of Geophysical Research, 108*, doi:10.1029/2002JC001345.

Sewell, R.B.S. & Fage, L. (1948). Minimum oxygen layer in the ocean. *Nature, 162*, 949-951.

Shetye, S.R., Gouveia, A.D. & Shenoi, S.S.C. (1994). Circulation and water masses of the Arabian Sea. *Proceedings of the Indian Academy of Science (Earth and Planetary Sciences), 103*, 107-123.

Sholto-Douglas, A.D., Field, J.G., James, A.G. & Merwe, N.J.v.d. (1991). 13C/12C and 15N/14N isotope ratios in the Southern Benguela Ecosystem: indicators of food web relationships among different size-classes of plankton and pelagic fish//differences between fish muscle and bone collagen tissues. *Marine Ecology Progress Series, 78*, 23-31.

Silver, M.W. & Bruland, K.W. (1981). Differential feeding and fecal pellet composition of salps and pteropods, and the possible origin of the deep-water flora and olive-green "cells". *Marine Biology, 62*, 263-273.

Small, L.F., Fowler, S.W. & Unlü, M.Y. (1979). Sinking rates of natural copepod fecal pellets. *Marine Biology, 51*, 233-241.

Smith, C.J. & Rumohr, H. (2005). Imaging Techniques. In: A. Eleftheriou, A. MacIntyre (Eds.), *Methods for the Study of Marine Benthos* (pp 87-112). Blackwell.

Smith, C.R. & Baco, A.R. (2003). *Oceanogr*. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology: An Annual Revue, 41*, 311-354.

Smith, K.L. (1982). Zooplankton of a bathyal benthic boundary layer: In situ rates of oxygen consumption and ammonium excretion. *Limnology and Oceanography, 27*, 461-471.

Smith, K.L., Carlucci, A.F., Williams, P.M., Henrichs, S.M., Baldwin, R.J. & Graven, D.B. (1986). Zooplankton and bacterioplankton of an abyssal benthic boundary layer: in situ rates of metabolism. *Oceanologica Acta, 9*, 47-55.

Smith, K.L., Kaufmann, R.S., Baldwin, R.S. & Carlucci, A.F. (2001). Pelagic-benthic coupling in the abyssal eastern North Pacific: An 8-year time-series study of food supply and demand. *Limnology and Oceanography, 46*, 543-556.

Smith, R.L. & Bottero, J.S. (1977). On upwelling in the Arabian Sea. In: M. Angel (Ed.), *A Voyage of Discovery* (pp. 291-304). New York: Pergamon.

Smith, S.L. (1984). Biological indication of active upwelling in the northwestern Indian Ocean in 1964 and 1979, and a comparison with Peru and northwest Africa. *Deep-Sea Research I, 31,* 951-967.

Smith, S.L. (1995). The Arabian Sea: mesozooplankton response to seasonal climate in a tropical ocean. *ICES Journal of marine Science, 52,* 427-438.

Smith, S.L. (2001). Understanding the Arabian Sea: Reflections on the 1994-1996 Arabian Sea Expedition. *Deep-Sea Research II, 48,* 1385-1402.

Smith, S.L., Codispoti, L.A., Morrison, J.M. & Barber, R.T. (1998). The 1994-1996 Arabian Sea Expedition: An integrated, interdisciplinary investigation of the response of the northwestern Indian Ocean to monsoonal forcing. *Deep-Sea Research II, 45,* 1905-1915.

Sommer, U. & Stibor, H. (2002). Copepoda -- Cladocera -- Tunicata: The role of three major mesozooplankton groups in pelagic food webs. *Ecological Research, 17,* 161-174.

Southam, J.R. & Peterson, W.H. (1985). Transient response of the marine carbon cycle. *Geophysical Monographs, 32,* 89-98.

Steedman, H.F. (1976). *Zooplankton fixation and preservation.* Paris: Unesco Press.

Steinberg, D.K., Silver, M.W. & Pilskaln, C.H. (1997). Role of mesopelagic zooplankton in the community metabolism of giant larvacean house detritus in Monterey Bay, California, USA. *Marine Ecology Progress Series, 147,* 167-179.

Stelfox, C.E., Burkill, P.H., Edwards, E.S., Harris, R.P. & Sleigh, M.A. (1999). The structure of zooplankton communities, in the 2 to 2000 µm size range, in the Arabian Sea during and after the SW monsoon, 1994. *Deep-Sea Research II, 46,* 815-842.

Stephen, R., Saraladevi, K., Meenakshikunjamma, P.P., Gopalakrishnan, T.C. & Saraswathy, M. (1992). In: B.N. Desai (Ed.), *Oceanography of the Indian Ocean* (pp 143-156). New Delhi: Oxford & IBH Publ. Co.

Sverdrup, H.U. (1938). On the explanation of the oxygen minima and maxima in the oceans. *J. Cons. perm. Int. Explor. Mer, 13,* 163-172.

Svedrup, H.U., Johnson, M.W. & Fleming, R.H. (1942). *The oceans.* Engelwood Cliffs, NJ: Prentice-Hall.

Tamburri, M.N., Halt, M.N. & Robison, B.H. (2000). Chemically regulated feeding by a midwater medusa. *Limnology and Oceanography, 45,* 1661-1666.

Tamelander, T., Renaud, P.E., Hop, H., Carroll, M.L., Ambrose, W.G. & Hobson, K.A. (2006). Trophic relationships and pelagic-benthic coupling during summer in the Barents Sea Marginal Ice Zone, revealed by stable carbon and nitrogen isotope measurements. *Marine Ecology Progress Series, 310,* 33-46

Tanoue, E. & Hara, S. (1986). Ecological implications of fecal pellets produced by the Antarctic krill *Euphausia superba* in the Antarctic Ocean. *Marine Biology, 91,* 359-369.

Tchernia, P. (1980). Descriptive regional oceanography. Oxford: Pergamon, University Press.

Theocharis, A., Balopoulos, E., Kioroglou, S., Kontoyiannis, H. & Iona, A. (1999). A synthesis of the circulation and hydrography of the South Aegean Sea and the Straits of the Cretan Arc (March 1994-January 1995). *Progress in Oceanography, 44,* 469-509.

Theocharis, A., Klein, B., Nittis, K. & Roether, W. (2002). Evolution and status of the Eastern Mediterranean Transient (1997-1999). *Journal of Marine Systems, 33-34,* 91-116.

Thiel, H. (1983). Meiobenthos and nanobenthos of the deep sea. In: G.T. Rowe (Ed.), *Deep-Sea Biology* (pp 167-228). New York: John Wiley.

Thiel, H., Pfannkuche, O., Schriever, G., Lochte, K., Gooday, A., Hemleben, C., Mantoura, R.F.G., Turley, C.M., Patching, J.W. & Riemann, F. (1988). Phytodetritus on the deep-sea floor in a central oceanic region of the Northeast Atlantic. *Biological Oceanography*, *6*, 203-239.

Thiel, H., Pörtner, H.O. & Arntz, W.E. (1996). Marine life at low temperatures - a comparison of polar and deep-sea characteristics. In: F. Uiblein, J. Ott, M. Stachowitsch, M. (Eds.), *Deep-sea and extreme shallow-water habitats: affinities and adaptations* (pp 183-219). Biosystematics and Ecology Series, *11*.

Thiel, H. & Forschungsgruppe Tiefsee-Umweltschutz (2001). Evaluation of the environmental consequences opf polymetallic nodule mining based on the results of the TUSCH Research Association. *Deep-Sea Research II*, *48*, 3433-3452.

Thompson, H. (1942). Pelagic tunicates in the plankton of south eastern Australian waters, and their place in oceanographical studies. *Bull. Counc. scient. ind. Res. Melb.*, *153*, 1-56.

Toon, R.K., Lohrenz, S.E., Rathbun, C.E., Wood, A.M., Arnone, R.A., Jones, B.H., Kindle, J.C. & Weidemann, A.D. (2000). Photosynthesis - irradiance parameters and community structure associated with coastal filaments and adjacent waters in the northern Arabian Sea. *Deep-Sea Research II*, *47*, 1249-1277.

Tsai, P.T.H., O'Brien, J.J. & Luther, M.E. (1992). The 26-day oscillation observed in the satellite sea surface temperature measurements in the equatorial western Indian Ocean. *Journal of Geophysical Research, 91.Q*, 9605-9618.

Tseitlin, V.B. & Rudyakov, Y.A. (1999). Seasonal variations of vertical distribution of zooplankton in the mesopelagic zone of the Indian Ocean. *Oceanology*, *39*, 521-527.

Tselepides, A. & Eleftheriou, A. (1992). South Aegean (Eastern Mediterranean) continental slope benthos: Macroinfaunal - Environmental relationships. In: G.T. Rowe, V. Pariente (Eds.), *Deep-sea food chains and the global carbon cycle* (pp 139-156). Dordrecht: Kluwer Academic Publisher.

Turley, C.M. (1999). The changing Mediterranean Sea - a sensitive ecosystem? *Progress in Oceanography*, *44*, 387-400.

Turley, C.M., Bianchi, M., Christaki, U., Conan, P., Harris, J.R.W., Psarra, S., Ruddy, G., Stutt, E.D., Tselepides, A. & Wambeke, F.v. (2000). Relationship between primary producers and bacteria in an oligotrophic sea-the Mediterranean and biogeochemical implications. *Marine Ecology Progress Series*, *193*, 11-18.

Turner, J.T. (1977). Sinking rates of fecal pellets from the marine copepod *Pontella meadii*. *Marine Biology*, *40*, 249-259.

Tyler, P.A. (1995). Conditions for the existence of life at the deep-sea floor: an update. *Oceanography and Marine Biology Annual Revue*, *33*, 221-244.

Tyler, P.A., Gage, J.D. & Billett, D.S.M. (1992). Reproduction and recruitment in deep-sea invertebrate populations in the NE Atlantic: a review of options. In: G. Colombo, I. Ferrari, V.U. Ceccherelli, R. Rossi, R. (Eds.), *Marine Eutrophication and Population Dynamics* (pp 257-262). Copenhagen:. Olsen & Olsen.

UNESCO-SCOPE (2006). *The Global Carbon Cycle*. UNESCO-SCOPE Policy Briefs October 2006 – No. 2. Paris: UNESCO-SCOPE.

Verheye, H.M., Hutchings, L. & Peterson, W.T. (1991). Life history and population maintenance strategies of *Calanoides carinatus* (Copepoda: Calanoida) in the southern Benguela ecosystem. *South African Journal of Marine Science, 11*, 179-191.

Vinogradov, M.E. (1962). Feeding of the deep-sea zooplankton. *Rapports P.-V. Conseil permanent International Exploration de la Mer, 153*, 114-120.

Vinogradov, M.E. (1968). *Vertical distribution of the Oceanic zooplankton.* Academy of Science of the U.S.S.R., Institut of Oceanography. In Russian, translated by Israel Program for Scientific Translation Ltd. Jerusalem: Keter Press.

Vinogradov, M.E. & Tseitlin, V.B. (1983). Deep-sea pelagic domain (aspects of bioenergetics). In: G.T. Rowe (Ed.), *Deep-Sea Biology* (pp 123-165). New York: John Wiley.

Vinogradov, M.E. & Voronina, N.M. (1962). Influence of the oxygen deficit on the distribution of plankton in the Arabian Sea. *Deep-Sea Research I, 9*, 523-530.

Vinogradov, M.Y., Shushkina, E.A., Musaeva, E.I. & Sorokin, P.Y. (1989). A newly acclimated species in the Black Sea: the ctenophore *Mnemiopsis leidyi* (Ctenophora: Lobata). *Oceanology, 29*, 200-224.

Voss, M., Altabet, M.A. & Bodungen, B.v. (1996). Delta15N in sedimenting particles as indicator of euphotic-zone processes. *Deep-Sea Research I, 43*, 33-47.

Wakefield, W.W. & Smith, K.L. (1990). Ontogenetic vertical migration in *Sebastolobus altivelis* as a mechanism for transport of particulate organic matter at continental slope depths. *Limnology and Oceanography, 35*, 1314-1328.

Ward, B.B., Glover, H.E. & Lipschultz, F. (1989). Chemoautotrophic activity and nitrification in the oxygen minimum zone off Peru. *Deep-Sea Research I, 36*, 1031-1051.

Warren, B.A. (1994). Driving the meridional overturning in the Indian Ocean. *Deep-Sea Research, 41*, 1349–1360.

Webster, P.J. & Yang, S. (1992). Monsoon and ENSO: Selectively interactive systems. *Quarterly Journal of the Royal. Meteorological Society, 118*, 877-926.

Weikert, H. (1980). The oxygen minimum layer in the Red Sea: Ecological implications of the zooplankton occurence in the area of the Atlantis II Deep. *Meeresforschung, 28*, 1-9.

Weikert, H. (1996). Changes in Levantine deep-sea zooplankton. In: P. Malanotte-Rizzoli, A.R. Robinson, M. Denis, M. (Eds.), *International POEM-BC/MTP Symposium. Biological processes in the eastern Mediterranean interaction with hydrological structures* (pp 99-101). Molitg les Bains: UNESCO press.

Weikert, H. & Koppelmann, R. (1993). Vertical structural patterns of deep-living zooplankton in the NE Atlantic, the Levantine Sea and the Red Sea: a comparision. *Oceanologica Acta, 16*, 163-177.

Weikert, H. & Koppelmann, R. (1996). Mid-water zooplankton profiles from the temperate ocean and partially landlocked seas. A re-evaluation of interoceanic differences. *Oceanologica Acta, 19*, 657-664.

Weikert, H. & Trinkaus, S. (1990). Vertical mesozooplankton abundace and distribution in the deep Eastern Mediterranean Sea SE of Crete. *Journal of Plankton Research, 12*, 601-628.

Weikert, H., Koppelmann, R. & Wiegratz, S. (2001). Evidence of episodic changes in deep-sea zooplankton abundance and composition in the Levantine Sea (Eastern Mediterranean). *Journal of Marine Systems, 30*, 221-239.

Weller, R.A., Baumgartner, M.F., Josey, S.A., Fischer, A.S. & Kindle, J.C. (1998). Atmospheric forcing in the Arabian Sea during 1994-1995: observations and comparisons with climatology and models. *Deep-Sea Research II*, *45*, 1961-1999.

Widder, E.A. (1992). Mixed light imaging system for recording bioluminescence behaviors. *Journal of the Marine Biological Association of U.K.*, *72*, 131-138.

Wiebe, P.H., Boyd, S.H. & Winget, C. (1976). Particulate matter sinking to the deep-sea floor at 2000 m in the Tongue of the Ocean, Bahamas, with a description of a new sedimentation trap. *Journal of Marine Research*, *34*, 341-354.

Wiebe, P.H., Madin, L.P., Haury, L.R., Harbison, G.R. & Philbin, L.M. (1979). Diel vertical migration by *Salpa aspersa* and its potential for large-scale particulate organic matter transport to the deep sea. *Marine Biology*, *53*, 249-255

Wiebe, P.H., Morton, A.W., Bradley, A.M., Backus, R.H., Craddock, J.E., Barber, V., Cowles, T.J. & Flierl, G.R. (1985). New developments in the MOCNESS, an apparatus for sampling zooplankton and micronekton. *Marine Biology*, *87*, 313-323.

Wigham, B.D., Tyler P.A. & Billett D.S.M. (2003). Reproductive biology of the abyssal holothurian *Amperima rosea*: an opportunistic response to variable flux of surface derived organic matter? *Journal of the Marine Biological Association UK*, *83*, 175–188.

Williams, P.J.l.B. (1990). The importance of losses during microbial growth: commentary on the physiology, measurements and ecology of the release of dissolved organic material. *Marine Microbial Food Webs*, *4*, 175-206.

Wishner, K.F. (1980). Aspects of the community of deep-sea, benthopelagic plankton with special attention to gymnopleid copepods. *Marine Biology*, *60*, 179-187.

Wishner, K.F. & Gowing, M.M. (1987). In situ filtering and ingestion rates of deep-sea benthic boundary-layer zooplankton in Santa Catalina Basin. *Marine Biology*, *94*, 357-366.

Wishner, K.F., Ashjian, C.J., Celfman, C., Gowing, M.M., Kann, K., Levin, L.A., Mullineaux, L.S. & Saltzman, J. (1995). Pelagic and benthic ecology of the lower interface of the Eastern Tropical Pacific oxygen minimum zone. *Deep-Sea Research I*, *42*, 93-115.

Wishner, K.F., Gowing, M.M. & Gelfman, C. (1998). Mesozooplankton biomass in the upper 1000 m in the Arabian Sea: overall seasonal and geographic patterns, and relationship to oxygen gradients. *Deep-Sea Research II*, *45*, 2405-2432.

Wishner, K.F., Gowing, M.M. & Gelfman, C. (2000). Living in suboxia: Ecology of an Arabian Sea oxygen minimum zone copepod. *Limnology and Oceanography*, *45*, 1576-1593.

Wrobel, D. & Mills, C. (1998*). Pacific Coast pelagic invertebrates. A guide to the common gelatinous animals*. Monterey: Monterey Bay Aquarium.

Wüst, G. (1935). Schichtung und Zirkulation des Atlantischen Ozeans. Das Bodenwasser und die Stratosphäre. *Wissenschaftliche Ergebnisse der Deutschen Atlantik Expedition "METEOR" 1925-1927*, *6*, 1-288.

Wyrtki, K. (1962). The oxygen minima in relation to ocean circulation. *Deep-Sea Research I*, *9*, 11-23.

Wyrtki, K. (1971). *Oceanographic Atlas of the International Indian Ocean Expedition*. Washington: National Science Foundation.

Wyrtki, K. (1973). Physical Oceanography of the Indian Ocean. In: B. Zeitzschel (Ed.), *The biology of the Indian Ocean* (pp 18-36). New York: Springer-Verlag.

Yamaguchi, A., Watanabe, Y., Ishida, H., Harimoto, T., Furusawa, H., Suzuki, S., Ishizaka, J., Ikeda, T. & Takahashi, M.M. (2002). Structure and size distribution of plankton communities down to the greater depths in the western North Pacific Ocean. *Deep-Sea Research II*, *49*, 5513-5529.

Yoshii, K., Melnik, N.G., Timoshkin, O.A., Bondarenko, N.A., Anoshko, P.N., Yoshioka, T. & Wada, E. (1999). Stable isotope analyses of the pelagic food web in Lake Baikal. *Limnology and Oceanography*, *44*, 502-511.

Youngbluth, M.J. (1984). Manned submersibles and sophisticated instrumentation: Tools for oceanographic reearch. *Proceedings of SUBTECH 1983 Symposium, Society of underwater technology, London*, 335-344.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 131-156
ISBN: 978-1-60021-935-1

Chapter 3

PHOTOCHEMICAL MINERALISATION OF DISSOLVED ORGANIC NITROGEN

***Vassilis Kitidis*[1,*] *and Günther Uher*[2]**
[1]Plymouth Marine Laboratory, Prospect Place, Plymouth, PL1 3DH, United Kingdom
[2]School of Marine Science and Technology, Ridley Building, Claremont Road, Newcastle University, NE1 7RU, United Kingdom

ABSTRACT

Over the last three decades, aquatic photochemical reactions have been shown to be involved in the cycling of climatically active trace gases, metals and nutrients. More recently, these reactions have been shown to release a number of bioavailable nitrogen (N) species, including ammonium, nitrite and low molecular weight dissolved organic substances such as urea, amines and amino acids. In most of the surface ocean, the photic zone is either permanently or seasonally depleted of nutrients, and in particular N, which are limiting primary production. Under such conditions, the photochemical mineralisation of dissolved organic nitrogen (DON) may be a significant source of limiting N to primary producers. Extrapolations of local rates of photochemical N release in a diverse range of marine settings suggest that this process may account for up to 50 % of phytoplankton N requirements. However, assessments of the importance of photochemical N release on regional to global scales are hindered by insufficient information on its wavelength dependence and by the lack of robust concepts for the extrapolation of photochemical rates. In this chapter, we synthesise the presently available information on photochemical N release, its environmental controls and wavelength dependence, identify gaps in our understanding of the processes involved and discuss the ecological significance of photochemical DON mineralisation.

* Corresponding author. Tel: +44 1752 633100; Fax: +44 1752 633101; E-mail: vak@pml.ac.uk

INTRODUCTION

Dissolved organic matter (DOM) constitutes a large reservoir of organic carbon and nitrogen in terrestrial and marine waters. For example, the respective oceanic carbon and nitrogen reservoirs in DOM are in the order of 685 Pg C (Hansell & Carlson 2001) and 60 to 530 Pg N (Capone 2000) [1 Pg is 10^{15} g]. DOM is subject to active biogeochemical cycling resulting in significant, global fluxes of carbon and nitrogen (Benner 2004; Berman & Bronk 2003; McCarthy et al. 1998). For example, the annual oceanic production of 'new' DOC is estimated at 1.2 Pg C $year^{-1}$ (Hansell & Carlson 2001). DOM consists of a multitude of identifiable biomolecules such as amino acids and carbohydrates. However, bulk DOM largely comprises of more complex compounds of higher molecular weight, which remain difficult to characterise at the molecular level (Benner 2002; Bronk 2002; Thurman 1985). Given the difficulties in its chemical characterisation, DOM is often operationally described in terms of its carbon (C) and nitrogen (N) pools, termed DOC and DON, respectively. Arguably, DOC and DON concentration values alone convey little information on chemical and biological reactivity of the DOM pool. Nevertheless, the fact that the marine DON reservoir exceeds the nitrogen content of marine biota by three orders of magnitude (Soederlund & Svensson 1976) has stimulated interest into biogeochemical DON cycling and especially photochemical transformations that release bioavailable nitrogen, potentially from otherwise refractory DOM.

A fraction of the DOM pool is chromophoric (CDOM), i.e. colour-bearing. The characteristic yellow-brown colour of DOM lead to the alternative terms '*gelbstoff*', '*gilvin*' and '*yellow substance*' (Kalle 1966; Kirk 1976). The optical absorbance properties of CDOM also define its key role in aquatic photochemistry. CDOM is the main UV-absorbing constituent in a range of natural waters, where it undergoes photochemical breakdown, is involved in the formation of short-lived, reactive species and acts as a photosensitiser, transferring energy to other redox processes (Blough & Vecchio 2002; Nelson & Siegel 2002). Thereby, CDOM is involved in DOM photodegradation and the formation of a number of low molecular weight photoproducts, which include climatically active trace gases as well as biologically labile compounds and plant nutrients that might stimulate the growth of microbial and planktonic communities (Kieber et al. 1989; Mopper & Kieber 2002; Moran & Zepp 1997).

Aquatic photochemical reactions mediate the transformation of DON into a number of inorganic and organic N compounds including ammonium (NH_4^+), nitrite (NO_2^-), urea ($CO(NH_2)_2$), amines ($R\text{-}NH_2$) and amino acids ($R\text{-}(COOH)NH_2$) (Bushaw-Newton & Moran 1999; Bushaw et al. 1996; Kieber et al. 1999; Spokes & Liss 1996; Tarr et al. 2001). The effects of these photochemical transformations on the Earth system are two-fold. Firstly, the photoproducts mentioned above contribute to phytoplankton and microbial nutrition and therefore ecosystem productivity. Secondly, aqueous ammonia ($NH_{3(g)}$) may be emitted to the atmosphere where it contributes to non-sea-salt aerosol formation and pH regulation, particularly in the remote marine environment (Coffman & Hegg 1995; Junge & Ryan 1958; Scott & Hobbs 1967). $NH_{3(g)}$ forms mainly fine mode aerosols [NH_4NO_3 and $(NH_4)_2SO_4$]. These aerosols may act as cloud condensation nuclei in the marine boundary layer and are therefore intimately linked with regulating Earth's reflectivity (albedo) through cloud formation.

In many marine and limnetic environments, the water column is permanently or seasonally stratified due to local climatic factors (insolation and water column mixing). The surface mixed layer is thereby separated from deeper water by a density gradient (pycnocline). Under such conditions, phytoplankton and microbial uptake of nutrients may lead to nutrient depletion in the surface mixed layer (nutrient depletion may extend below the surface mixed layer if the photic zone extends deeper). The regional balance between biological nutrient demand and biogeochemical nutrient supply determines which nutrients are 'limiting' primary production. Hence, the limiting nutrient becomes depleted while other nutrients remain unutilised. A detailed review of nutrient limitation is beyond the scope of this chapter, but the majority of the world ocean primary production is thought to be limited by the availability of N, though iron (Fe) and phosphorous (P) are limiting or co-limiting in certain regions or over geological timescales (Krom et al. 1991; Martin 1990; Martin et al. 1991; Tyrrell 1999; Tyrrell & Law 1997). N limitation of ecosystem productivity is also found in limnetic pelagic environments, although P limitation or N and P co-limitation are more common (Elser et al. 1990; Elser et al. 1995; Guildford & Hecky 2000). In the marine environment, dissolved inorganic N (DIN: NH_4^++NO_2^-+NO_3^-), for example in the north-east Atlantic Ocean and North Sea in summer is typically depleted with concentrations below a few tens of nmol L^{-1} while phosphate remains unutilised (Woodward & Owens 1990; Woodward & Rees 2001). However, DON concentrations in the oceanic surface mixed layer are usually around 6 μmol L^{-1} (Bronk 2002), i.e 2-3 orders of magnitude higher than those of DIN. The pronounced concentration difference between DIN and DON during periods of nutrient depletion suggests that even modest transformations of DON to DIN may lead to a significant supply of nutrients for primary production. This notion is consistent with bulk DON turnover times in the order of months in coastal waters (Bronk 2002) and with a modelling study of diverse, contrasting ecosystems by Blackford et al. (2004), which suggests that approximately 5 % of DON may be abiotically remineralised on a daily basis (Blackford et al. 2004). Although this process is not explicitly identified by Blackford et al. (2004), it may be partly attributed to DON photomineralisation, particularly in optically clear, stratified waters that are typically encountered at temperate latitudes during summer. Importantly, photochemical DON transformations are thought to convert non-labile DON to bio-labile inorganic and organic forms, which may have a significant impact on planktonic and microbial production, e.g. (Bushaw et al. 1996).

Recent reviews suggest that 12-72 % of DON may be bioavailable (Antia et al. 1991; Bronk 2002; Bronk et al. 2006). It is therefore critical to assess whether the supply of bioavailable nitrogen from photochemical DON transformations enhances planktonic production or whether plankton are capable of utilising ambient DON in the absence of photochemical transformations. A number of studies have used microbial assays to address this question (Bertilsson et al. 1999; Bushaw-Newton & Moran 1999; Bushaw et al. 1996). In such biological assays, growth media simulate nitrogen limitation and microbial growth on previously irradiated DON is compared to growth on non-irradiated DON. Differences in population growth are then usually ascribed to bioavailable N release during prior irradiation (see below: Microbial assays). This approach allows a qualitative assessment of the overall impact of DOM photomineralisation. However, interpretations of such assays may be complicated by simultaneous changes in bioavailability of nitrogen, carbon and phosphorus and the possible formation of potent oxidants and organic toxins during prior irradiation. A more mechanistic approach used in chemical studies involves the experimental determination

of the rates of photochemical N release (e.g. NH_4^+) and assessment of their significance with respect to other sources of bioavailable N in a given ecosystem. In this chapter, we refer to the microbial assays as 'biological studies' and those studies identifying specific photoproducts as 'chemical studies'. Notably, both these approaches have demonstrated increased as well as reduced N bioavailability following irradiation. Ultimately, a quantitative understanding of DON photomineralisation is required for the successful parameterisation of photochemical N release in biogeochemical ecosystem models and their use in assessing its possible impacts on ecosystem dynamics and primary production. Modelling efforts in this respect require at least a 'quasi-mechanistic' understanding of the processes involved, i.e. an understanding of major environmental controls that facilitates the development of robust concepts for the parameterisation of photochemical N release.

CHEMICAL STUDIES - GLOBAL DISTRIBUTION AND RATES

Figure 1 shows the global distribution of previous 'chemical studies' reporting photochemical DON mineralisation to NH_4^+ (photoammonification), NO_2^- and low molecular weight DON (LMW DON). The geographic coverage of these studies shows a number of interesting features: a) a bias towards inland or coastal waters, b) a northern hemisphere bias, c) a lack of studies with samples from the Indo-Pacific and d) a lack of studies with samples from high latitude areas (Figure 1). Of these, the most significant is arguably the paucity of marine data. Though a number of studies have investigated coastal photoammonification (Gardner et al. 1998; Morell & Corredor 2001; Vähätalo & Zepp 2005), water samples of salinities more typical of marine waters have been investigated in only two studies thus far, i.e. one study of a coastal lagoon [S > 33; (Buffam & McGlathery 2003)] and a further study from open ocean waters in the Eastern Mediterranean (Kitidis et al. 2006).

Tables 1 and 2 provide an overview of photochemical N release rates reported in the literature. Among the processes of photochemical N release from DON, photoammonification is the most widely studied both in terms of geographic coverage and ecosystem type. In general, photoammonification rates exceed photochemical production rates of other known products of photochemical N release. For example, studies using fulvic acid solutions from a temperate estuary showed that NH_4^+ photoproduction was four times higher than concurrent NO_2^- photoproduction (Bushaw et al. 1996; Kieber et al. 1999). Similarly, Gao and Zepp (1998) reported that NH_4^+ photoproduction rates from the Satilla River (USA, Georgia) exceeded those of NO_2^- and NO_3^- by at least one order of magnitude and Tarr et al. (2001) found that NH_4^+ photoproduction rates were up to 3 orders of magnitude higher than amino acid photoproduction rates from riverine fulvic acids. However, other experiments suggest that NH_4^+ photoproduction rates do not always exceed the production rates of NO_2^- or LMW DON (see below: Nitrite). Furthermore, previous studies show that the balance between NH_4^+ and NO_2^- photoproduction may vary both seasonally and geographically, i.e. with ecosystem type.

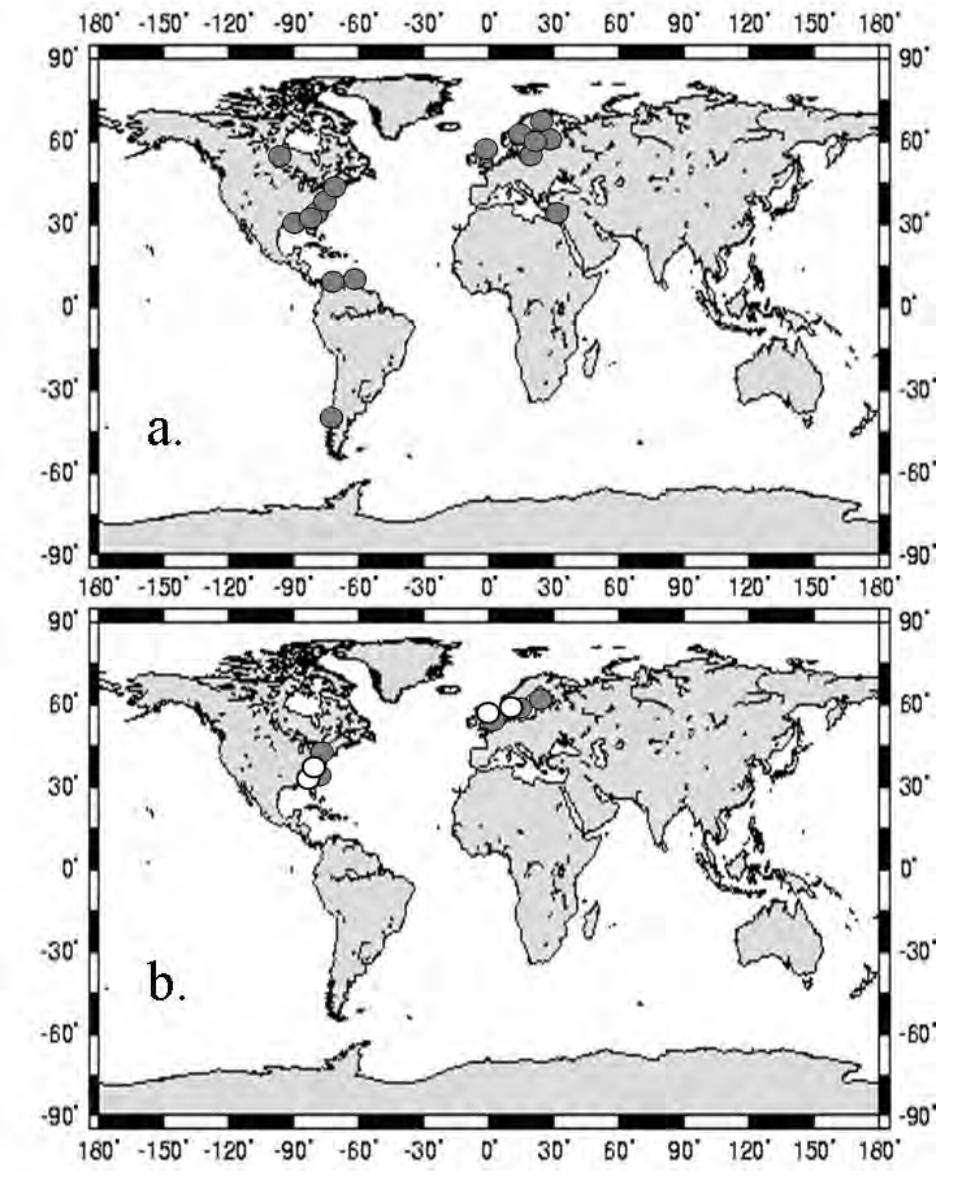


Figure 1. The geographic origin of samples reporting (a.) photoammonification (filled circles) and (b.) nitrite photoproduction (filled circles) and low molecular weight DON (empty circles). Further sites in the 'Eastern Tropical Pacific and South Atlantic Bight' [Bronk et. al. unpublished, in (Bronk 2002)] are omitted from this figure due to missing location details.

NH_4^+ photoproduction rates may vary from one to several hundred nmol L^{-1} h^{-1} (Table 1). However, one freshwater study reported exceptionally high photoproduction rates of up to 4700 nmol L^{-1} h^{-1} (Wang et al. 2000). These rates refer to irradiations of samples diluted with analytical-grade water, where observed rates were corrected for sample dilution. Such a correction assumes that photoammonification rates are proportional to substrate concentration alone. However, sample dilution with analytical grade water may have changed other sample characteristics that affect photochemical NH_4^+ production, such as trace metal content, pH,

buffering capacity and ionic strength (Gao & Zepp 1998; Wang et al. 2000), and therefore the exceptionally high rates in Wang et al. (2000) should be viewed with care. Since photochemical rates depend on spectral irradiance characteristics and light dose and most previous studies have used a variety of light sources (natural versus artificial, different spectral characteristics), caution must also be exercised when comparing the rates listed in Table 1. Nevertheless, some rough conclusions can be drawn from Table 1. Firstly, the highest rates of NH_4^+ photoproduction (where observed) are found in inland waters even if the exceptionally high rates by Wang et al. (2000) are not considered. Secondly, NH_4^+ photoproduction rates in marine waters (salinity >30) are generally one order of magnitude lower than in inland freshwaters (up to 3 orders of magnitude lower in some cases). Thirdly, results from estuarine studies are highly variable and include net photochemical NH_4^+ consumption. Although these observations suggest a general trend of decreasing photoammonification rate from inland waters to the marine environment, NH_4^+ photoproduction rates are not inversely correlated with salinity.

Table 1. Published NH_4^+ production rates (nmol L^{-1} h^{-1}) in freshwater, estuarine (salinity <30) and marine aquatic environments listing sample origin and publication

NH_4^+ photoproduction rates (nmol L^{-1} h^{-1})	Reference
Freshwater Samples (River and Lake)	
50-370	(Bushaw et al. 1996)
90	(Gao & Zepp 1998)
0	(Jørgensen et al. 1998)
0	(Bertilsson et al. 1999)
110-4700	(Wang et al. 2000)
0	(Wiegner & Seitzinger 2001)
>0, no rate data given	(Zagarese et al. 2001)
0-70	(Grzybowski 2002)
-370-0 † [hypolimnion]	(Vähätalo et al. 2003)
59-480 † [epilimnion]	(Vähätalo et al. 2003)
Estuarine (salinity <30)	
-70-220	(Gardner et al. 1998)
7-60	(Bushaw-Newton & Moran 1999)
12-115	(Morell & Corredor 2001)
-294-34.8	(Koopmans & Bronk 2002)
0	(Grzybowski 2002)
7-37 ‡	(Vähätalo & Zepp 2005)
10-35	(Stedmon et al. 2007)
Marine (salinity >30)	
1-46	(Buffam & McGlathery 2003)
0.7-2.9	(Kitidis et al. 2006)

† Rate normalised to the same irradiance as Bushaw et al. (1996).

‡ Estimated hourly rate based on NH_4^+ concentration increase of 500-2500 nmol L^{-1} during average 2.8 day incubations. This Table excludes unpublished data referred to by Bronk (2002) as the reference is lacking location details.

Table 2. Published NO_2^- and low molecular weight DON (LMW DON)production rates (nmol L^{-1} h^{-1})

NO_2^- photoproduction rates (nmol L^{-1} h^{-1})	**Reference**
<12 [SPE HS †]	(Spokes & Liss 1996)
1.4-6.7 [filtered and SPE HS]	(Kieber et al. 1999)
16-21 ₣ [filtered freshwater]	(Wiegner & Seitzinger 2001)
0-55 ‡ ₣ [filtered freshwater]	(Vähätalo et al. 2003)
0 ₣ [filtered lagoon water, salinity < 33]	(Buffam & McGlathery 2003)
LMW DON photoproduction rates (nmol L^{-1} h^{-1})	
29 [urea]	(Jørgensen et al. 1998)
-6-3 [urea]	(Kitidis 2002)
0-41 [dissolved primary amines]	(Bushaw-Newton & Moran 1999)
0.0-9.5 [amino acids]	(Tarr et al. 2001)
0.8-1.1 [amino acids]	(Buffam & McGlathery 2003)

† Solid phase extracted humic substances (SPE HS).

‡ Rate normalised to the same irradiance as Bushaw et al. (1996).

The initial sample treatment is indicated for the listed NO_2^- photoproduction rates in brackets. The nitrogen species measured are identified in brackets under LMW DON photoproduction rates. (₣): Reported as NO_3^-+NO_2^-.

The limited data available on NO_2^- and LMW DON products of bulk DON photomineralisation (Table 2) so far do not allow us to discern differences in photochemical N release between ecosystems. A number of studies have reported photochemical production or consumption of total nitrates (NO_3^-+NO_2^-). Given that DIN photochemical reactions result only in NO_3^- formation from NO_2^- or vice versa without affecting the sum of (NO_3^-+NO_2^-), any concentration changes in total nitrates may be attributed to DON photomineralisation. Since available studies only provide evidence for photochemical nitrite release from DON photodegradation, we assume that photochemical increases in the concentration of total nitrates may be attributed to NO_2^- photoproduction from DON.

In this chapter we focus mainly on a review of the current quasi-mechanistic understanding of DON phototransformations, but also refer to microbial assay work where appropriate. We also deal with known N photoproducts separately in order to give a detailed and specific account of our current knowledge on ammonium, nitrite and LMW DON photoproduction.

AMMONIUM

Environmental Controls or Reaction Mechanism?

The observed variability in photoammonification rates raises questions about the reaction mechanisms involved and their environmental controls. While the actual reaction mechanisms of ammonium photoproduction in natural waters are as yet unknown, possible pathways have been suggested in previous work. Bushaw et al. (1996) suggested a multi-step reaction mechanism involving the photo-chemical reaction of carbonyl groups with organic amines to

form an imine, or Schiff-base (azomethine), which subsequently hydrolyses to release NH_4^+. This reaction scheme is outlined in the following example with an aldehyde (R_1-CHO) and primary amine (R_2-NH_2) as substrates,

$$R_1\text{-CHO} + R_2\text{-NH}_2 \overset{+h\nu}{\leftrightarrow} R_1\text{-CH(OH)-NH-}R_2 \leftrightarrow R_1\text{-CH=N-}R_2 + H_2O \quad (1)$$

$$R_1\text{-CH=N-}R_2 + 2\ H_2O \rightarrow R_1\text{-CHO} + R_2\text{-OH} + NH_3 \quad (2)$$

where R_1 and R_2 are aliphatic or aromatic moieties. Given photochemical production rates of free low molecular weight carbonyls in the range of 1-100 nmol L^{-1} h^{-1} (Kieber et al. 1990), and concentrations of primary amines of up to 470 nmol L^{-1} in estuarine waters (Bronk 2002), ammonium formation from organic carbonyls and amines seems plausible. Analysis of solid phase extracted marine DOM has shown that carbonyl groups constitute 3-5 % of humic carbon, while amine groups are also associated with humic substances (Benner 2002). The functional groups involved in reactions (1) and (2), may therefore originate directly from high molecular weight marine DOM. However, it is less clear, if densities of carbonyl and amine functional groups in higher molecular weight DOM are high enough to support ammonium photoproduction. Furthermore, nucleophilic additions of amines to carbonyl compounds as in equation (1) are generally acid catalysed, reversible reactions (March 1985) that may only be forced towards imine formation in the presence of excess concentrations of the reactants or by product removal (Le Chatelier principle). The environmental relevance of this reaction pathway therefore remains uncertain.

Indeed, the reverse of the reaction scheme in equations (1) and (2), i.e. nucleophic addition of ammonia to dissolved organic carbonyl functions, was also proposed as a mechanism for the incorporation of nitrogen into marine humic substances (Kieber et al. 1997). Moreover, there is evidence of multiple formation pathways (Tarr et al. 2001). While the detailed reaction mechanisms of photochemical ammonium production are as yet unclear, previous work reported evidence for photosensitised formation pathways, the involvement of hydroxyl radicals and iron, and the effects of pH.

The photosensitised oxidation of methionine to 3-methylthiopropionaldehyde (methional) in aqueous solution with riboflavin as the photosensitiser provided the first evidence for photochemical ammonium formation from amino acids (Enns & Burgess 1965). Methionine photooxidation by riboflavin is thought to involve interaction between substrate and sensitiser triplet states rather than singlet oxygen and proceeds under both aerobic and anaerobic conditions (Sysak et al. 1977). Similarly, riverine humic and fulvic acid extracts were shown to photosensitise the formation of NH_4^+ from 100 µmol L^{-1} solutions of dihydroxy-phenyl-alanine, methionine and tyrosine (Tarr et al. 2001). Based on the inhibition of ammonium photoproduction from amino acids in the presence of propanol (an $^{\bullet}OH$ scavenger), Tarr et al. (2001) suggested that this NH_4^+ producing pathway proceeds via $^{\bullet}OH$ radicals derived from DOM photodegradation. However, not all amino acids yielded ammonium. For example tryptophan showed similar NH_4^+ production rates in the presence or absence of organics, while glycine, phenylalanine and histidine did not yield NH_4^+ (Tarr et al. 2001). The behaviour of various amino acids with respect to ammonium photoproduction is summarised in Table 3. It appears that neither aromaticity nor the nature of the amino acid nitrogen are directly linked to NH_4^+ photoproduction. For example, of the two N-heterocyclic aromatic

amino acids investigated, tryptophan produces ammonium while histidine does not. In summary, DOM may either act as a photosensitiser or as a source of reactive oxidants such as $^{\bullet}OH$, that further react with amino acids, although the nature of DOM photosensitisation in natural waters remains unresolved. In addition, further research is required to identify functional groups and reaction mechanisms involved in ammonium photoproduction.

Some information about the reactive intermediates involved in photoammonification was obtained from experiments with known radical scavengers. Irradiations using propan-1-ol (n-propanol) as a hydroxyl radical ($^{\bullet}OH$) scavenger showed reduced photoammonification rates, but did not suppress ammonium formation altogether (Tarr et al. 2001). This observation suggests the presence of at least two mechanisms of NH_4^+ photoproduction, one of which is $^{\bullet}OH$ mediated. The $^{\bullet}OH$ mediated pathway may also be responsible for continued NH_4^+ production in the dark after irradiation, as reported by Wang et al. (2000). Wang et al. (2000) suggested that dark NH_4^+ production may be accounted for by $^{\bullet}OH$ radicals produced from the transition metal catalysed decomposition of hydrogen peroxide (H_2O_2) [photo-Fenton reaction, (Zepp et al. 1992)]. Photochemically produced H_2O_2 in aquatic systems has a half-life in the order of several hours (Häkkinen et al. 2004; Sikorski & Zika 1993) and could therefore potentially sustain the production of $^{\bullet}OH$ in the dark, following irradiation. In contrast to Wang et al. (2000), a recent study in the oligotrophic Eastern Mediterranean did not observe post-irradiation (dark) NH_4^+ production (Kitidis et al. 2006). Since the decomposition of hydrogen peroxide is mediated by inorganic Fe, this difference may be due to low concentrations of ambient Fe in seawater as compared to river water. Alternatively, low ambient concentration of H_2O_2 in the Eastern Mediterranean may explain the absence of post-irradiation photoammonification.

Transition metals such as iron (Fe) have also been implicated in ammonium photoproduction in freshwaters. Gao and Zepp (1998) removed dissolved inorganic Fe from Satilla River samples (USA, Georgia) via complexation with fluoride and observed a 30% decrease in photoammonification rate at pH 7. Removal of Fe also reduced the pH dependence of NH_4^+ photoproduction by approximately 50% over the pH range from 7 to 4 (Gao & Zepp 1998). This finding suggests that the pH dependence of NH_4^+ photoproduction may in part be linked to the pH dependence of the Fe^{2+} - Fe^{3+} redox equilibrium. At low pH, the equilibrium shifts towards Fe^{2+} and would therefore favour the photo-Fenton reaction scheme proposed by Tarr et al. (2001), which involves $^{\bullet}OH$ radical formation from the reaction of hydrogen peroxide with Fe^{2+}.

Previous work on photoammonification has reported variable patterns of ammonium concentration increase with time that may be indicative of the presence of multiple formation pathways or competing, abiotic ammonium removal (Figure 2). Only a few studies reported an approximately linear increase in ammonium concentration over the first few hours of light exposure (Kitidis et al. 2006; Vähätalo et al. 2003; Wang et al. 2000). In contrast, ammonium concentrations in irradiated samples from a boreal pond showed a sigmoidal pattern with irradiation time, which was characterised by an initial lag-phase with little NH_4^+ concentration increase (<4 h), followed by a period of substantial photoproduction that appeared to reach an asymptotic limit (Bushaw et al. 1996), possibly due to substrate exhaustion. A similar asymptotic behaviour was observed by Vähätalo and Zepp (2005) in irradiations of Baltic Sea water, although the time resolution of their sampling intervals does not allow conclusions about the possible presence of an initial lag phase. Interestingly, irradiations of riverine, estuarine and near-coastal samples in Kitidis (2002) also exhibited lag

and production phases similar to those in Bushaw et al. (1996), but showed decreasing ammonium concentrations after the production phase, indicative of photochemical consumption. In other studies, dark and post-irradiation uptake of NH_4^+ was shown and attributed to the incorporation of NH_4^+ into the organic matter matrix (Kieber et al. 1997; Thorn & Mikita 1992; Wang et al. 2000). Interestingly, Koopmans and Bronk (2002) showed net photochemical NH_4^+ consumption in some groundwater and estuarine samples and production in others (Koopmans & Bronk 2002), and found a significant negative correlation between the initial NH_4^+ concentration and photoammonification rates in their dataset. Wang et al. (2000) suggested that NH_4^+ production and consumption processes occur simultaneously, and that the balance between these determines the net production or consumption of NH_4^+. Vähätalo et al. (2003) also highlighted high initial NH_4^+ concentration in irradiated hypolimnion waters as a possible cause for increased photoconsumption resulting in net photochemical uptake of NH_4^+ in these samples. Contrasting patterns of NH_4^+ production and consumption would certainly contribute to the variable patterns of net NH_4^+ change depicted in Figure 2. For example, both NH_4^+ production and consumption rates might vary with reactant concentrations such as NH_4^+ (Koopmans & Bronk 2002). However, the underlying reasons for the variability in photochemical NH_4^+ production and consumption patterns remain unclear and require further study.

Table 3. Amino acids used in irradiation experiments investigating NH_4^+ photoproduction (Tarr et al. 2001)

Amino Acid	NH_4^+ photoproduction		Hydropathy Index
	in pure water	in presence of organics	
Dihydroxy-Phenylalanine	-	-	2.8
Methionine	-	+	1.9
Glycine	-	-	-0.4
Tryptophan	+	no change	-0.9
Tyrosine	+	+	-1.3
Histidine	-	-	-3.2

Some amino acids were found to produce NH_4^+ in pure water (+) which was further enhanced in the presence of organics (+), while other compounds did not produce NH_4^+ in pure water or in the presence of organics (-). The amino acids are listed in order of decreasing hydrophobic nature as indicated by their hydropathy index (Kyte & Doolittle 1982).

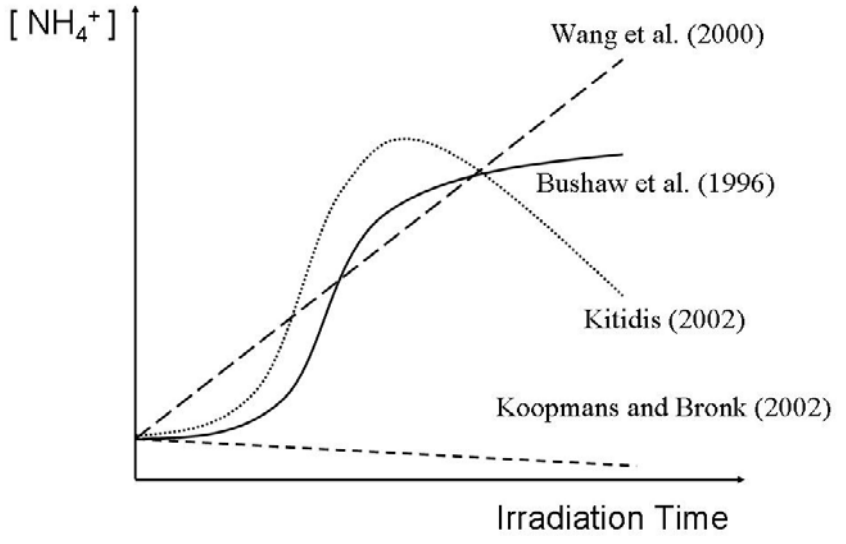


Figure 2. Kinetic variability of photoammonification reported in the literature. Sigmoidal production (solid line) reported by Bushaw et al. (1996); linear production (long-dash line) reported by Wang et al. (2000); bell-shaped production (dotted line) reported by Kitidis (2002); consumption (short-dash line) reported by Koopmans and Bronk (2002).

Bushaw et al. (1996) proposed that the carbonyl moieties required in step 1 of their proposed scheme were derived from the photochemical oxidation of DOM (Kieber et al. 1990). Given that photoproduction rates of low molecular weight carbonyl compounds are closely correlated with CDOM optical properties and photobleaching at mid-UV wavelengths, it seems plausible to expect similar relationships between NH_4^+ photoproduction and CDOM characteristics. Following this line of argument and the notion that spectral CDOM photodegradation and NH_4^+ photoproduction are dominated by mid-UV wavelengths, various studies reported photoammonification rates normalised to the initial CDOM absorption coefficient at 350 nm (a_{350}) (Bushaw-Newton & Moran 1999; Bushaw et al. 1996; Wang et al. 2000). However, available evidence on CDOM-photoammonification relationships appears to be variable if not contradictory at times. While dilution series of solid phase extracted DOM indicated that photoammonification rates increase proportionally with DOM levels (Bushaw et al. 1996), analysis of samples from different study sites suggests significant regional variations in the relationship between photoammonification rates and a_{350}. In agreement with this finding, an analysis of previous work concluded that the variability in NH_4^+ photoproduction rates cannot be ascribed to CDOM variability alone (Grzybowski 2003). Scant evidence from recent marine studies, however, suggests that combinations of CDOM optical properties with characteristics of bulk DOM composition or photochemical reactivity might be useful predictors of photoammonification rates on regional scales. Buffam and McGlathery (2003) found that photoammonification rates increased proportionally with increasing rates of CDOM absorbance loss at 280 nm. Furthermore, Kitidis et al. (2006) reported that photoammonification rates were correlated with the ratio of initial CDOM absorption coefficient at 300 nm divided by initial DOC concentration (CDOM:DOC), i.e. photoammonification rates increased with increasing DOC normalised CDOM absorbance (specific CDOM absorbance). Given that preferential loss of CDOM absorbance during DOM

photodegradation leads to decreasing specific CDOM absorbance and photoreactivity with continuing light exposure (Moran et al. 2000), the results of Buffam and Mc Glathery (2004) and Kitidis et al. (2006) suggest that NH_4^+ photoproduction rates are implicitly linked with the radiation exposure history of DOM. In agreement with this notion, the absence of detectable photoammonification in estuarine samples collected in August (Bushaw-Newton & Moran 1999) may have been due to decreased photoreactivity resulting from progressive DOM photodegradation during summer. These interpretations, however, contrast with results by Koopmans and Bronk (2002), who only observed NH_4^+ photoproduction in 2 out of 13 irradiated groundwater samples with no prior irradiance exposure. In summary, these findings suggest that CDOM optical characteristics and prior irradiation exposure don't fully reflect compositional differences relevant to NH_4^+ photoproduction, although regional relationships might exist.

Other controlling variables are also implicated in photoammonification, including pH dependent formation (Gao & Zepp 1998; Wang et al. 2000) and the identification of reactive intermediates such as iron (Fe) (Gao & Zepp 1998) and hydroxyl radical ($^{\bullet}OH$) (Tarr et al. 2001). Gao and Zepp (1998) and Wang et al. (2000) investigated the pH dependence of photoammonification rates during irradiations of acidified river water samples (pH range 2-7). Decreasing the pH from 7 to 4 increased photoammonification rates 1.6 fold and and 10 fold in irradiations of samples from the Pearl (USA, Louisiana) and Satilla Rivers (USA, Georgia), respectively (Gao & Zepp 1998; Wang et al. 2000). This pH dependence may be an important control in some polluted, acidified freshwaters that may show pH values <3. However, assuming that the pH dependence of photoammonification in seawater is similar to that in freshwaters, the comparatively narrow range of seawater pH [mostly pH 8.1 - 8.3, (Royal Society 2005)] suggests small variations in photoammonification rate in the order of ± 2-30 %.

Despite this progress in establishing the major environmental controls of NH_4^+ photoproduction, our understanding of photoammonification in seawater is compounded by the fact that the majority of the above studies were carried out using freshwater or estuarine samples. The contributions of different photoammonification pathways in seawater with contrasting CDOM characteristics (Green & Blough 1994) and significantly lower concentrations of amino acids (<1 μmol L^{-1}) (Bronk 2002) and Fe (<10 nmol L^{-1}) (Sunda 2001) remain unknown.

Wavelength Dependence

Knowledge of the wavelength dependence of NH_4^+ photoproduction is essential for modelling purposes at regional to global scales. The wavelength dependence of natural water photo-processes is usually expressed in the form of apparent quantum yield spectra. In the case of NH_4^+ photoproduction, the apparent quantum yield (AQY) at a given wavelength is defined as moles of NH_4^+ produced per mole of photons absorbed in solution. Unfortunately, AQY spectra of NH_4^+ photoproduction have so far not been determined experimentally. However, a number of studies have carried out irradiations using longpass optical filters that provide information on the broadband wavelength dependence of NH_4^+ photoproduction. Bushaw et al. (1996) used longpass filters with wavelength cutoffs at 320 nm and 360 nm in parallel with full spectrum irradiations of freshwater samples. These authors showed that 26-

35 % and 47-83 % of the observed full spectrum NH_4^+ photoproduction were due to wavelengths below 320 nm and below 360 nm respectively. Similarly, Kitidis et al. (2006) found that 76 % of the full spectrum NH_4^+ photoproduction in marine samples from the Eastern Mediterranean were due to wavelengths below 360 nm. These results indicate that NH_4^+ photoproduction is dominated by mid-UV radiation, consistent with wavelength dependencies of other aquatic photochemical processes.

In the absence of experimentally determined AQYs, recent work estimated AQY spectra from full spectrum irradiations, spectral solar irradiance and the assumption that the wavelength dependence of AQYs of NH_4^+ photoproduction ($\varphi_{NH4+,\,\lambda}$) is described by an exponential function,

$$\phi_{NH4+,\ \lambda} = c \times e^{-d \times \lambda} \tag{3}$$

where $\varphi_{NH4+,\,\lambda}$ is the AQY at wavelength λ (nm), c is a dimensionless constant and d (nm^{-1}) is a spectral slope parameter that describes the decrease of AQYs with increasing wavelength (nm^{-1}) (Vähätalo & Zepp 2005). Assuming that the wavelength integrated ammonium photoproduction, calculated from measured spectral irradiance, CDOM absorbance and apparent quantum yields is equivalent to the full spectrum NH_4^+ photoproduction measured in irradiation experiments, parameters c and d in equation (3) can then be obtained by a regression procedure. For ten irradiation experiments with samples from the north-eastern Baltic Sea (salinity < 6.3), the mean $\varphi_{NH4+,\,\lambda}$ at 350 nm ($\varphi_{NH4+,\,350}$) obtained by this procedure was 1.4×10^{-5}, and the fit parameters c and d where 0.93 ± 0.06 and 0.0322 ± 0.001 nm^{-1}, respectively. This parameterisation of photoammonification AQY may be widely applicable in coastal waters, given that $\varphi_{NH4+,\,350}$ for the Baltic Sea was in reasonable agreement with the value of $\varphi_{NH4+,\,350}$ (0.9×10^{-5}) for Satilla River water, calculated from an independent data set published by Gao and Zepp (1998). Further data from irradiation experiments using Baltic Sea water (salinity < 7.7) and the technique employed by Vähätalo and Zepp (2005) for estimating $\varphi_{NH4+,\,\lambda}$ suggest that the parameters c and d do not show significant inter annual or basin-scale variability (Stedmon et al. 2007). The approach used by Vähätalo and Zepp (2005) for estimating $\varphi_{NH4+,\,\lambda}$ in their Baltic Sea samples has the advantage of circumventing the time consuming and analytically complex experimental determination of photoammonification AQYs. Nevertheless, experimentally determined apparent quantum yield spectra of ammonium photoproduction remain highly desirable, particularly for coastal and open ocean waters that experience periods of significant nutrient depletion.

Biogeochemical and Ecological Significance of Ammonium Photoproduction

NH_4^+ photoproduction affects the biogeochemical cycling of aquatic DON and may provide a significant source of bioavailable nitrogen to phytoplankton and microbial communities, with possible consequences for ecosystem productivity. The role of photochemistry in DON biogeochemical cycling may be assessed on the basis of available photoproduction rate data and DON concentrations. Vähätalo et al. (2003) reported a daily photochemical conversion of 3 % of DON to NH_4^+ in epilimnion waters of Lake Valkea-Kotinen in southern Finland, indicating a DON turnover time of approximately one month.

For the eastern Baltic Sea, it was estimated that 10-18 % of the DON was photolabile with respect to NH_4^+ photoproduction and that 15-27 % of that was photomineralized to NH_4^+ in the 10 m deep surface mixed layer during summer (Vähätalo & Zepp 2005). A realistic turnover time for the entire photolable DON was estimated to be more than 10 years, given that the breakdown of stratification in autumn led to mixing of photodegraded DON in surface waters with non-photodegraded DON in deep waters until the photolytic surface layer developed again in the following spring (Vähätalo & Zepp 2005). Data from the euphotic layer of the eastern Mediterranean (140 m) suggest bulk DON turnover times of approximately eight years assuming that all the ambient DON could be photomineralized to NH_4^+ (Kitidis et al. 2006).

These limited data indicate turnover times that are several orders of magnitude longer than microbial turnover times of rapidly cycled DON fractions such as free dissolved amino acids [~1 h, (Bronk 2002)]. However, estimates of photochemical turnover compare favourably to previous estimates of bulk DON turnover in the surface ocean [~1-18 years, (Bronk 2002)] and are therefore consistent with the notion that photodegradation may have a significant role in the biogeochemical cycling of DON. Another consequence of slow photochemical DON turnover is that river-borne, terrestrial DON is likely to undergo transport into offshore regions, where nitrogen limitation is more prevalent and photochemical transformations are favoured by deeper light penetration. In addition, DON photomineralisation is likely to sustain NH_4^+ production beyond the spring phytoplankton bloom typically found in temperate marine systems and into the summer when nitrogen limitation occurs. The notion of offshore photomineralisation of riverborne DON was first put forward by Bushaw et al. (1996), who estimated that NH_4^+ photoproduction from fluvial DON exported onto the continental shelf of the south eastern United States accounted for an additional 20 % of total riverine inorganic nitrogen supply to the region. Likewise, Morell and Corredor (2001) estimated that photomineralisation of DON exported from the Orinoco river into the eastern Caribbean Sea may supply up to 50 % of the annual nitrogen requirement of phytoplankton growth within the Orinoco plume (Morell & Corredor 2001). While the assessments of Bushaw et al. (1996) and Morell and Corredor (2001) are based on extrapolations of riverine and estuarine rate data to coastal regions, recent work reported ammonium production rate measurements from the Baltic Sea (Stedmon et al. 2007; Vähätalo & Zepp 2005). Vähätalo and Zepp (2005) estimated that photoammonification supplied 1-2 % of daily phytoplankton nitrogen requirements in the eastern Baltic Sea in summer, but was of the same order of magnitude as atmospheric nitrogen deposition (Stedmon et al. 2007; Vähätalo & Zepp 2005). Significantly the latter is dominated by wet deposition during overcast days, while photoammonification is most pronounced on clear days with comparatively little atmospheric N deposition (Vähätalo & Zepp 2005). Under favourable conditions ammonium photoproduction might therefore be the dominant source of new nitrogen to open areas of the Baltic Sea that don't receive significant inputs of bioavailable riverine DON (Vähätalo and Zepp, 2005). Similarly, photoammonification in the ultra-oligotrophic eastern Mediterranean was of the same order of magnitude as atmospheric nitrogen deposition and was estimated to supply approximately 12±5 % of the annual nitrogen requirements of new production (Kitidis et al. 2006). Given the four fold difference between winter and summer irradiance levels over the eastern Mediterranean, ammonium photoproduction might be particularly important during the summer season, when nutrient depletion is strongest. Assessments of the overall ecological significance of photochemical N

release in the eastern Mediterranean is challenging in the light of prevailing phosphorus limitation (Krom et al. 2004; Krom et al. 1991; Thingstad & Mantoura 2005). However, given suggestions that phytoplankton are seasonally N and P co-limited in this region (Thingstad et al. 2005; Zohary et al. 2005), a significant ecosystem role for ammonium photoproduction remains plausible. In contrast to coastal and shelf areas, ammonium photoproduction appears to be less important in near-coastal waters that are subject to significant N inputs. For example, results from Hog Island Bay, a coastal lagoon (USA, Virginia) suggest that NH_4^+ photoproduction was only of minor importance compared to other inorganic nitrogen sources to this ecosystem (Buffam and McGlathery, 2003). In addition, high turbidity and water column light attenuation in near-coastal waters may reduce the depth of the photolytic surface layer and thereby the areal photoammonification rate, i.e. depth integrated over the photolytic zone. Surface rates are typically scaled for water column light attenuation in order to provide areal rate estimates. Thus, the highest surface rate (382 μmol L^{-1} d^{-1}) reported by Stedmon et al. (2007) was found in the more turbid eastern Baltic Sea, while the highest depth integrated, areal rate (237 μmol m^{-2} d^{-1}), was found in the western Baltic Sea where water column light attenuation was lower. At even lower light attenuation in the optically clear Mediterranean, Kitidis et al. (2006) also reported a depth integrated photoammonification rate of 237 μmol m^{-2} d^{-1}, although the surface rate (15 μmol L^{-1} d^{-1}) was one order of magnitude lower than the corresponding surface rate for the Baltic Sea (321 μmol L^{-1} d^{-1}) given by Stedmon et al. (2007).

The studies outlined above illustrate the potential importance of ammonium photoproduction as a source new nitrogen, but also highlight considerable variability in current assessments of the potential importance of ammonium photoproduction to regional nitrogen budgets. Some of this variability might arise from the approaches used to extrapolate the limited, available rate data to wider scales. Bushaw et al. (1996) and Morell and Corredor (2001) for example based their assessments of coastal photoammonification on photoproduction rate data from freshwater or estuarine samples, and extrapolated these to coastal marine waters by assuming that ammonium production rates can be predicted from CDOM absorbance or from fluorescence characteristics in combination with salinity as a tracer of terrestrial freshwater inputs. These assumption, however, may not always be justified, as indicated by our earlier discussion of ammonium production data in Table 1. Furthermore, these studies imply that photoammonification is dominated by the photomineralisation of terrestrial DON while the respective contribution of marine DON is negligible. Given that photochemical NH_4^+ release from autochthonous marine DON was shown to be a significant source of nitrogen in the eastern Mediterranean (Kitidis et al. 2006), excluding ammonium photoproduction from marine-derived DOM would therefore result in underestimation.

Unfortunately, photoammonification data for nitrogen limited open ocean systems are currently lacking, and still require experimental confirmation despite evidence of a significant photochemical NH_4^+ source. For example, high temporal resolution data of NH_3 concentrations in the marine boundary layer above open ocean areas of the temperate south Atlantic Ocean show a clear diurnal pattern with up to 5-fold higher concentration during daytime compared to night time (Norman & Leck 2005). This concentration difference was attributed to a daytime increase in the oceanic source of NH_3 to the marine boundary layer (Norman & Leck 2005). Photoammonification along with light-inhibition of biological NH_4^+ uptake may lead to NH_4^+ accumulation in surface waters and thereby contribute significantly

to diurnal variability of sea surface ammonium. In the light of global change, open ocean ammonium photoproduction might also be affected by ocean acidification caused by uptake of anthropogenic CO_2 (Royal Society 2005). Assuming that the pH dependence of ammonium photoproduction in seawater is similar to that in river water (Gao and Zepp, 1998; Wang et al.; 2000), projected pH decreases of up to 0.5 units over the next century (Caldeira & Wickett 2003) would result in a 26 to 160 % increase in NH_4^+ photoproduction rates. Under future global warming scenarios, enhanced nutrient depletion in the surface mixed layer as a consequence of enhanced thermal stratification may potentially increase the ecological significance of NH_4^+ photoproduction, particularly under increased UV radiation (Zepp et al. 1995). However, predictive understanding of future changes requires further studies of open ocean photoammonification and their environmental controls.

NITRITE

Nitrite (NO_2^-) is photochemically produced in aquatic environments either directly from DON (equation 4) or through photolysis of nitrate (equation 5) (Kieber et al. 1999; Mack & Bolton 1999; Spokes & Liss 1996; Wiegner & Seitzinger 2001; Zafiriou & True 1979a). These production pathways occur simultaneously with photochemical NO_2^- oxidation to NO_3^- (equation 6) (Zafiriou & True 1979b).

$$\mathrm{DON} + h\nu \rightarrow \mathrm{NO_2^-} \quad (4)$$

$$\mathrm{NO_3^-} + h\nu \rightarrow \mathrm{NO_2^-} \quad (5)$$

$$\mathrm{NO_2^-} + h\nu \rightarrow \mathrm{NO_3^-} \quad (6)$$

Both net production and consumption of NO_2^- and nitrates, i.e. NO_2^-+NO_3^-, have been observed in irradiation experiments (Jørgensen et al. 1998; Kieber et al. 1999; Spokes & Liss 1996; Vähätalo et al. 2003; Wiegner & Seitzinger 2001). Net photochemical nitrite consumption was attributed to increasing nitrite photo-oxidation rates with increasing substrate concentrations, i.e. photochemical nitrite oxidation at high nitrite levels may exceed production from nitrate photolysis and DON. This idea is strongly supported by the finding that photochemical nitrite production rates are negatively correlated with initial NO_2^- concentrations (Kieber et al. 1999). Net photochemical production of nitrite therefore depends on the initial levels of NO_2^- and NO_3^-, but also on the nature and abundance of DON, which may act both as substrate and photosensitiser (Kieber et al. 1999). At low nitrate levels, The NO_2^- concentration threshold, above which net consumption of NO_2^- is observed depends on the photoreactivity of DON with regard to NO_2^- photoproduction.

NO_3^- and NO_2^- have weak absorption maxima at 310 nm and 360 nm respectively. The molar extinction coefficient of NO_2^- at 360 nm is only one third of that of NO_3^- at 310 nm (Mack & Bolton 1999). While the photochemical reactions between NO_3^- and NO_2^- have been studied extensively [see review by Mack and Bolton (1999)], the environmental controls of photochemical production of NO_2^- from DON (Table 2) remain largely unexplored. Spokes and Liss (1996) added solid phase extracted humic acids to deionised water and seawater

solutions of nitrate and found that the rate of NO_2^- photoproduction increased proportionally to the amount of organics added, with solutions in deionised water showing higher rates compared to solutions in seawater. The latter was attributed to the removal of photochemically produced reactive species such as H_2O_2 and the superoxide radical (O_2^-) by transition metals (Mn and Fe) in the seawater matrix (Spokes & Liss 1996). Similarly, free radical quenching in seawater is thought to contribute to lower NO_2^- photolysis rates in seawater matrices (Zafiriou & True 1979b). While previous studies demonstrate the involvement of DOM in nitrite photoproduction (Spokes & Liss 1996), they don't allow to distinguish between DOM-substrate and DOM-photosensitiser effects. Kieber *et al*. (1999) speculated that nitrite may be formed from the cleavage of humic nitro groups by photochemically generated singlet oxygen. Given that singlet oxygen is derived from interactions between dissolved oxygen and triplet states within dissolved humics (Zepp et al. 1985), such a formation pathway would suggest that DOM acts simultaneously as substrate and photosensitiser. However, irradiations of oxygen saturated and anoxic Gulf Stream water augmented with DOM showed similar nitrite photoproduction rates, indicating that nitrite production did not proceed via singlet oxygen and other oxygen derived transients in these samples (Kieber et al. 1999).

Variable results from lake studies suggest that regional CDOM characteristics may be important in determining net NO_2^- photoproduction. For example, Vähätalo *et al*. [2003] showed seasonally varying photoproduction of $NO_3^- + NO_2^-$ in hypolimnion water from a humic rich lake in southern Finland, with highest rates occurring in July and August. In contrast, Jørgensen *et al*. (1998) found net photochemical consumption of $NO_3^- + NO_2^-$ in hypolimnion waters from a lake in southern Sweden. It is conceivable that such seasonal and regional differences in NO_2^- photoproduction are due to changes in the chemical composition of DON.

Parallel sunlight irradiations of forest runoff with a range of longpass UV filters (412, 317, 280 nm) showed no discernable difference in $NO_3^- + NO_2^-$ production (Wiegner & Seitzinger 2001). These results therefore suggest that, in contrast to photoammonification, nitrite photoproduction is dominated by visible wavelengths. Maximum photoproduction in the visible would be inconsistent with nitrite formation from nitrate and humic substances photolysis, because these chromophores absorb predominantly in the UV. While the involvement of as yet unidentified chromophores in the visible domain cannot be excluded, dominance of visible over UV wavelengths seems unlikely for most freshwater and marine systems. Further studies of the wavelength dependence of nitrite photoproduction are required to resolved this discrepancy.

LOW MOLECULAR WEIGHT ORGANIC N

In addition to ammonium and nitrite, photochemical DON transformations result in the production of a number of low molecular weight dissolved organic nitrogen (LMW-DON) compounds, including urea, primary amines and amino acids (Table 2). Published information relating to these photoproducts is scant compared to ammonium and nitrite, but deserves our attention, particularly in view of the bioavailability of these compounds.

Urea photoproduction of 29 nmol L^{-1} h^{-1} was first shown during natural sunlight incubations of filtered freshwater samples from a lake in southern Sweden (Jørgensen et al. 1998). However, irradiations of water samples from the Tyne estuary (NE England) and adjacent coastal North Sea waters showed urea photoproduction of approximately 3 nmol L^{-1} h^{-1} in only one out of three samples, while the other two showed no concentration change or net loss of urea (Kitidis 2002). Therefore, the exceptionally sparse information to date on urea photoproduction remains inconclusive with regard to the potential ecological importance of this process.

The photoproduction of primary amines and amino acids has received more attention than that of urea. Bushaw-Newton and Moran (1999) observed photoproduction of primary amines in 2 out of 4 irradiation experiments with solutions of solid phase extracted, estuarine DON reconstituted in a seawater matrix. The reported photoproduction rates of up to 41 nmol L^{-1} h^{-1}, were comparable to simultaneously determined photoammonification rates of up to 60 nmol L^{-1} h^{-1}. Koopmans and Bronk (2002) tested for the photochemical production of dissolved primary amines in 9 groundwater and 5 estuarine samples, but only observed their photochemical production in one of the groundwater samples. Amino acid photoproduction was reported by Tarr et al. (2001) for river and estuarine samples as well as for solid phase extracted Suwanee River fulvic acid. In total, seven amino acids were released photochemically, including glutamic acid, asparagine, serine, histidine, citrulline, alanine and norvaline. However, associated photoproduction rates were approximately three orders of magnitude lower than rates of concurrent photoammonification. It has been suggested that some primary amines may be derived from amino acids or proteins which in turn may be photochemically released from humic substances (Bushaw-Newton & Moran 1999). Similarly, Tarr et al. (2001) suggested that amino acids were photochemically released through the cleavage of peptide or amide bonds resulting in the formation of carboxylic and amine groups. The proposed reaction mechanisms for the photoproduction of primary amines (Bushaw-Newton & Moran 1999) and amino acids (Tarr et al. 2001) are outlined in Figure 3. Both assume photochemically mediated hydrolysis of peptide or amide bonds, although the nature of the assumed photochemical mediation remains unclear.

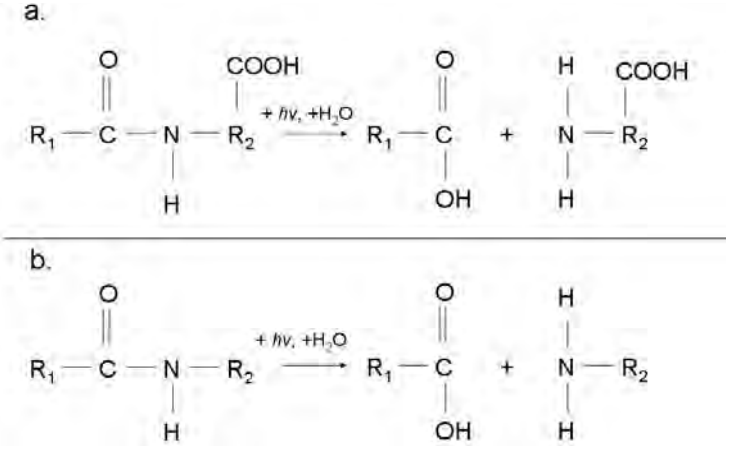


Figure 3. The reaction scheme proposed for a. the photoproduction of amino acids (Tarr et al. 2001) and b. the photoproduction of primary amines (Bushaw-Newton & Moran 1999). Both reactions proceed via hydrolysis of peptide or amide bonds.

Even though photoproduction of LMW DON has been shown for freshwaters, estuarine waters and groundwaters, the associated photochemical rates are usually lower than the respective rates of NH_4^+ photoproduction (see above: Chemical studies - global distribution and rates), so that they are thought to have lesser ecological significance.

MICROBIAL ASSAYS

Microbial assays might be useful tools for assessing the overall effects of DOM photodegradation and photochemical N release on planktonic communities. Bacterial growth rates and abundance were monitored during such studies over incubation periods of 4-8 days (Bertilsson et al. 1999; Bushaw-Newton & Moran 1999; Bushaw et al. 1996). Bushaw-Newton and Moran (1999) irradiated solutions of solid-phase extracted (SPE), estuarine DOM reconstituted in seawater and subsequently inoculated these with bacteria from the original sample under four different treatments; An unamended control treatment, a nitrogen limited (PO_4^{3-} added), a phosphorous limited (NH_4NO_3 added) and a final treatment to which both NH_4NO_3 and PO_4^{3-} were added (Bushaw-Newton & Moran 1999). In addition, four treatments as above were set up with non-irradiated samples. Bushaw-Newton and Moran (1999) found that the effect of prior irradiation was most pronounced in the nitrogen limited treatments (with added PO_4^{3-}). In these samples, bacterial abundance increased by 41 % in the irradiated treatment compared to the non-irradiated treatment of samples collected in winter. The bacterial community in these samples was therefore N and P co-limited. When PO_4^{3-} was added to the irradiated treatment, bacteria were able to utilise photochemically produced nitrogen, including NH_4^+ and primary amines, which were shown to be produced during irradiation (Bushaw-Newton & Moran 1999). Bushaw-Newton and Moran (1999) estimated that ammonium and dissolved primary amines accounted for approximately one third of photochemically produced bioavailable nitrogen in these experiments. However, bacterial assays carried out with samples collected during summer (August) showed that irradiation had no effect on the bioavailability of DON (Bushaw-Newton & Moran 1999).

Bushaw et al. (1996) carried out similar microbial incubations of irradiated freshwater SPE DOM with two additional treatments; A carbon rich treatment (glucose added) and a treatment to which glucose, NH_4NO_3 and PO_4^{3-} were added. Bushaw et al. (1996) also observed significantly enhanced bacterial growth in irradiated sample treatments where PO_4^{3-} was added (up to 4-folf higher compared to non-irradiated treatments with added PO_4^{3-}), and thus concluded that photochemical mineralisation of DON resulted in sufficient bioavailable N to fuel microbial growth. Addition of both NO_3^- and PO_4^{3-} to the irradiated sample also resulted in 3-fold increased bacterial growth compared to the non irradiated sample, suggesting that bacteria were utilising photochemically produced carbon. This was confirmed in the final treatment where glucose, NO_3^- and PO_4^{3-} addition was found to have no effect on bacterial growth between the irradiated and non-irradiated samples (Bushaw et al. 1996).

Bertilsson et al. (1999) set up parallel nitrogen limited (PO_4^{3-} and glucose added) and carbon limited microbial incubations (PO_4^{3-} and NO_3^- added). In contrast to these studies, Bertilsson et al. (1999) found that irradiation had no effect on bacterial growth under nitrogen limited conditions, but did result in enhanced growth under carbon limitation. This finding

was further supported by the absence of photoammonification during concurrent photochemical studies (Bertilsson et al. 1999).

By comparing microbial growth on irradiated and non-irradiated samples, one can asses the effect of prior irradiation on the bioavailability of DOM. Furthermore, comparison of the different treatments in each study allows assessments of photochemically induced changes in elemental bioavailability, carbon, nitrogen or phosphorous. However, prior irradiation of natural waters may produce harmful oxidants that may inhibit microbial growth [e.g. $^{\bullet}OH$, H_2O_2] and therefore lead to erroneous conclusions with regard to changes in DOM bioavailability. Though most of these reactive oxidants are short lived (fractions of a second), it has been shown that photochemically produced H_2O_2 can have a half-life of up to 2.5 days in lake waters (Häkkinen et al. 2004). This observation clearly demonstrates that photochemically produced oxidants may interfere with biological growth during incubations over a few days. Some caution is therefore advised in the interpretation of such experiments, particularly where irradiation is not found to enhance microbial growth.

Vähätalo and Zepp (2005) followed a different approach with Baltic Sea samples by separating the recalcitrant from bioavailable DON through long term (up to 5 month) microbial incubations prior to irradiation experiments during which NH_4^+ photoproduction was observed. This 'pre-treatment' removed approximately 5-13 % of the initial DON, thus indicating that 87-95 % of Baltic Sea DON was recalcitrant towards biological uptake. The potentially 'photoammonificable' fraction of the remaining material was estimated at 10-18 % of the initial ambient DON, and thus exceeded the labile DON fraction that could be removed during the initial microbial incubation (Vähätalo & Zepp 2005). While it remains unclear to what extent the biolabile and photolabile fractions of bulk DON overlap, these results clearly support the notion that DON photodegradation is a potentially important source of bioavailable N.

Taken together, the microbial assays described above suggest a potentially significant ecological role for photochemical DON mineralisation. This finding is in agreement with, and confirms, assessments based on experimental determinations of photochemical N release rates (see above: Biogeochemical and Ecological significance of ammonium photoproduction).

CONCLUSION

Photochemical mineralisation of DON has the potential to enhance productivity in aquatic ecosystems, particularly in settings where microbial or planktonic production is nitrogen limited. DON photochemical mineralisation and photoammonification in particular is likely to be impacted by the predicted change in oceanic pH due to the uptake of present and future increased CO_2 in the atmosphere and the change in UV radiation as well as ever increasing anthropogenic organic matter inputs to aquatic ecosystems. At present, information relating to the pathways of photochemical DON mineralisation is limited mostly to freshwaters and estuarine environments and limited largely to photochemical NH_4^+ production. The apparent lack of information on NO_2^- and LMW DON photoproduction, is further confounded by evidence suggesting that NO_2^- may at times be the dominant product of DON photomineralisation. In the case of photoammonification, evidence suggests that ambient pH, Fe, NH_4^+, and DOM composition play important roles in regulating NH_4^+

production rates. Although, such information is lacking from the marine environment, attention has recently turned towards quantifying photoammonification in seawater (Kitidis et al. 2006; Stedmon et al. 2007; Vähätalo & Zepp 2005). Notably, this effort has led to the development of formulations relating to the wavelength dependence of photoammonification (Vähätalo & Zepp 2005) and also demonstrated photochemical NH_4^+ production from autochthonous, marine DON (Kitidis et al. 2006). It is expected that wider application of modern, sophisticated analytical techniques for DOM characterisation such as Electro Spray Ionization Mass Spectrometry will provide some insight into molecular DOM composition and its role in aquatic biogeochemistry, e.g. (Seitzinger et al. 2005). Cheaper and more rapid techniques such as fluorescence spectroscopy of DOM with parallel factor analysis (Stedmon et al. 2003) also hold much promise for DOM characterisation with regard to its photochemical mineralisation (Stedmon et al. 2007).

So far, the limited, available information in areas relating to the environmental controls and wavelength dependence of DON photomineralisation have largely precluded the inclusion of this process in marine, biogeochemical ecosystem models. However, further progress towards the inclusion of photochemical N release terms into ecosystem models is required in these areas, in order to facilitate a predictive understanding of current and future ecosystem responses to photochemical N release.

REFERENCES

Antia, N. J., Harrison, P. J. & Oliveira, L. 1991 The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. *Phycologia* 30, 1-89.

Benner, R. 2002 Chemical Composition and Reactivity. In *Biogeochemistry of Marine Dissolved Organic Matter* (ed. D. A. Hansell & C. A. Carlson), pp. 774. London: Academic Press.

Benner, R. 2004 What happens to terrestrial organic matter in the ocean? *Marine Chemistry* 92, 307-310.

Berman, T. & Bronk, D. A. 2003 Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquatic Microbial Ecology* 31, 279-305.

Bertilsson, S., Stepanauskas, R., Cuadros-Hansson, R., Graneli, W., Wikner, J. & Tranvik, L. 1999 Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. *Aquatic Microbial Ecology* 19, 47-56.

Blackford, J. C., Allen, J. I. & Gilbert, F. J. 2004 Ecosystem dynamics at six contrasting sites: a generic modelling study. *Journal of Marine Systems* 52, 191-215.

Blough, N. V. & Vecchio, R. D. 2002 Chromophoric DOM in the Coastal Ocean. In *Biogeochemistry of Marine Dissolved Organic Matter* (ed. D. A. Hansell & C. A. Carlson), pp. 509-546. London: Academic Press.

Bronk, D. A. 2002 Dynamics of DON. In *Biogeochemistry of Marine Dissolved Organic Matter* (ed. D. A. Hansell & C. A. Carlson), pp. 774. London: Academic Press.

Bronk, D. A., See, J. H., Bradley, P. & Killberg, L. 2006 DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences Discussions* 3, 1247-1277.

Buffam, I. & McGlathery, K. J. 2003 Effect of ultraviolet light on dissolved nitrogen transformations in coastal lagoon water. *Limnology and Oceanography* 48, 723-734.

Bushaw-Newton, K. L. & Moran, M. A. 1999 Photochemical formation of biologically available nitrogen from dissolved humic substances in coastal marine systems. *Aquatic Microbial Ecology* 18, 285-292.

Bushaw, K. L., Zepp, R. G., Tarr, M. A., Shulz-Jander, D., Bourbonniere, R. A., Hodson, R. E., Miller, W. L., Bronk, D. A. & Moran, M. A. 1996 Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. *Nature* 381, 404-407.

Caldeira, K. & Wickett, M. E. 2003 Anthropogenic carbon and ocean pH. *Nature* 425, 365-365.

Capone, D. G. 2000 The Marine Microbial Nitrogen Cycle. In *Microbial Ecology of the Oceans* (ed. D. L. Kirchman), pp. 455-493. New York: Wiley-Liss Inc.

Coffman, D. J. & Hegg, D. A. 1995 A Preliminary-Study of the Effect of Ammonia on Particle Nucleation in the Marine Boundary-Layer. *Journal of Geophysical Research-Atmospheres* 100, 7147-7160.

Elser, J. J., Goldman, C. R. & Marzolf, E. R. 1990 Phosphorus and nitrogen limitation of phytoplankton growth in fresh waters of North America- A review and critique of experiment enrichments. *Canadian Journal of Fisheries Aquatic Science* 47, 1468-1477.

Elser, J. J., Stabler, L. B. & Hassett, R. P. 1995 Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: a comparative study. *Aquatic Microbial Ecology* 9, 105-110.

Enns, K. & Burgess, W. H. 1965 The Photochemical Oxidation of Ethylenediaminetetraacetic Acid and Methionine by Ryboflavin. *Journal of the American Chemical Society* 87, 5766-5770.

Gao, H. & Zepp, R. G. 1998 Factors Influencing Photoreactions of Dissolved Organic Matter in a Coastal River of the Southeastern United States. *Environmental Science and Technology* 32, 2940-2946.

Gardner, W. S., Cavaletto, J. F., Bootsma, H. A., Lavrentyev, P. J. & Troncone, F. 1998 Nitrogen cycling rates and light effects in tropical Lake Maracaibo, Venezuela. *Limnology and Oceanography* 43, 1814-1825.

Green, S. A. & Blough, N. V. 1994 Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. *Limnology and Oceanography* 39, 1903-1916.

Grzybowski, W. 2002 The significance of dissolved organic matter photodegradation as a source of ammonium in natural waters. *Oceanologia* 44, 355-365.

Grzybowski, W. 2003 Are data on light-induced ammonium release from dissolved organic matter consistent? *Chemosphere* 52, 933-936.

Guildford, S. J. & Hecky, R. E. 2000 Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnology and Oceanography* 45, 1213-1223.

Häkkinen, P. J., Anesio, A. M. & Graneli, W. 2004 Hydrogen peroxide distribution, production, and decay in boreal lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 61, 1520-1527.

Hansell, D. A. & Carlson, C. A. 2001 Marine Dissolved Organic Matter and the Carbon Cycle. *Oceanography* 14, 41-50.

Jørgensen, N. O. G., Tranvik, L., Edling, H., Graneli, W. & Lindell, M. 1998 Effects of sunlight on occurence and bacterial turnover of specific carbon and nitrogen compounds in lake water. *FEMS Microbiology Ecology* 25, 217-227.

Junge, C. & Ryan, T. G. 1958 Study of the SO_2 oxidation in solution and its role in atmospheric chemistry. *Quarterly Journal of the Royal Meteorological Society* 84, 46-55.

Kalle, K. 1966 The problem of gelbstoff in the sea. *Oceanography and Marine Biology Annual Review* 4, 91-104.

Kieber, D. J., McDaniel, J. & Mopper, K. 1989 Photochemical source of biological substrates in sea water : implications for carbon cycling. *Nature* 341, 637-639.

Kieber, R. J., Hydro, L. H. & Seaton, P. J. 1997 Photooxidation of triglycerides and fatty acids in seawater: Implications toward the formation of marine humic substances. *Limnology and Oceanography* 42, 1454-1462.

Kieber, R. J., Li, A. & Seaton, P. J. 1999 Production of Nitrite from the Photodegradation of Dissolved Organic Matter in Natural Waters. *Environmental Science and Technology* 33, 993-998.

Kieber, R. J., Zhou, X. L. & Mopper, K. 1990 Formation of Carbonyl-Compounds from UV-Induced Photodegradation of Humic Substances in Natural-Waters - Fate of Riverine Carbon in the Sea. *Limnology and Oceanography* 35, 1503-1515.

Kirk, J. T. O. 1976 Yellow substance (gelbstoff) and its contribution to the attenuation of photosynthetically active radiation in some inland and coastal south-eastern Australian waters. *Australian Journal of Marine and Freshwater Research* 27, 61-71.

Kitidis, V. 2002 CDOM dynamics and photoammonification in the marine environment. In *Marine Science and Technology*, pp. 180. Newcastle upon Tyne, UK: University of Newcastle.

Kitidis, V., Uher, G., Upstill-Goddard, R. C., Mantoura, R. F. C., Spyres, G. & Woodward, E. M. S. 2006 Photochemical production of ammonium in the oligotrophic Cyprus Gyre (Eastern Mediterranean). *Biogeosciences* 3, 439-449.

Koopmans, D. J. & Bronk, D. A. 2002 Photochemical production of dissolved inorganic nitrogen and primary amines from dissolved organic nitrogen in waters of two estuaries and adjacent surficial groundwaters. *Aquatic Microbial Ecology* 26, 295-304.

Krom, M. D., Herut, B. & Mantoura, R. F. C. 2004 Nutrient budget for the Eastern Mediterranean: Implications for phosphorus limitation. *Limnology and Oceanography* 49, 1582-1592.

Krom, M. D., Kress, N., Brenner, S. & Gordon, L. I. 1991 Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnology and Oceanography* 36, 424-432.

Kyte, J. & Doolittle, R. F. 1982 A simple method for displaying the hydropathic character of a protein. *Journal of Molecular Biology* 157, 105-132.

Mack, J. & Bolton, J. R. 1999 Photochemistry of nitrite and nitrate in aqueous solution: a review. *Journal of Photochemistry and Photobiology A: Chemistry* 128, 1-13.

March, J. 1985 *Advanced organic chemistry: Reactions, Mechanisms and Structure*. New York: John Wiley and Sons.

Martin, J. H. 1990 Glacial to interglacial CO_2 change: the iron hypothesis. *Paleoceanography* 5, 1-13.

Martin, J. H., Gordon, R. M. & Fitzwater, S. E. 1991 The Case for Iron. *Limnology and Oceanography* 36, 1793-1802.

McCarthy, M. D., Hedges, J. I. & Benner, R. 1998 Major bacterial contribution to marine dissolved organic nitrogen. *Science* 281, 231-234.

Mopper, K. & Kieber, D. J. 2002 Photochemistry and the Cycling of Carbon, Sulfur, Nitrogen and Phosphorus. In *Biogeochemistry of Marine Dissolved Organic Matter* (ed. D. A. Hansell & C. A. Carlson), pp. 455-507. London: Academic Press.

Moran, M. A., Sheldon, W. M. & Zepp, R. G. 2000 Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. *Limnology and Oceanography* 45, 1254-1264.

Moran, M. A. & Zepp, R. G. 1997 Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography* 42, 1307-1316.

Morell, J. M. & Corredor, J. E. 2001 Photomineralization of fluorescent organic matter in the Orinoco River plume: Estimation of ammonium release. *Journal of Geophysical Research* 106, 16807-16813.

Nelson, N. B. & Siegel, D. A. 2002 Chromophoric DOM in the Open Ocean. In *Biogeochemistry of Marine Dissolved Organic Matter* (ed. D. A. Hansell & C. A. Carlson), pp. 774. London: Academic Press.

Norman, M. & Leck, C. 2005 Distribution of marine boundary layer ammonia over the Atlantic and Indian Oceans during the Aerosols99 cruise. *Journal of Geophysical Research* 110, D16302.

Royal Society. 2005 Ocean acidification due to increasing atmospheric carbon dioxide, pp. 68. London: Royal Society (www.royalsoc.ac.uk).

Scott, W. D. & Hobbs, P. V. 1967 The Formation of Sulphate in Water Droplets. *Journal of the Atmospheric Sciences* 24, 54-58.

Seitzinger, S. P., Hartnett, H., Lauck, R., Mazurek, M., Minegishi, T., Spyres, G. & Styles, R. 2005 Molecular-level chemical characterization and bioavailability of dissolved organic matter in stream water using electrospray-ionization mass spectrometry. *Limnology and Oceanography* 50, 1-12.

Sikorski, R. J. & Zika, R. G. 1993 Modeling Mixed-Layer Photochemistry of H_2O_2 - Optical and Chemical Modeling of Production. *Journal of Geophysical Research-Oceans* 98, 2315-2328.

Soederlund, R. & Svensson, B. H. 1976 The global nitrogen cycle. *Ecological Bulletin* 22, 23-73.

Spokes, L. J. & Liss, P. S. 1996 Photochemically induced redox reactions in seawater, II. Nitrogen and iodine. *Marine Chemistry* 54, 1-10.

Stedmon, C. A., Markager, S. & Bro, R. 2003 Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry* 82, 239-254.

Stedmon, C. A., Markager, S., Tranvik, L., Kronberg, L., Slätis, T. & Martinsen, W. 2007 Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea. *Marine Chemistry* 104, 227-240.

Sunda, W. G. 2001 Bioavailability and Bioaccumulation of Iron in the Sea. In *The Biogeochemistry of Iron in Seawater* (ed. D. R. Turner & K. A. Hunter), pp. 41-84. Chichester: John Wiley & Sons Ltd.

Sysak, P. K., Foote, C. S. & Ching, T. Y. 1977 Chemistry of Singlet Oxygen .25. Photooxygenation of Methionine. *Photochemistry and Photobiology* 26, 19-27.

Tarr, M., Wang, W., Bianchi, T. S. & Engelhaupt, E. 2001 Mechanisms of ammonia and amino acid photoproduction from aquatic humic and colloidal matter. *Water Research* 35, 3688-3696.

Thingstad, T. F., Krom, M. D., Mantoura, R. F. C., Flaten, G. A. F., Groom, S., Herut, B., Kress, N., Law, C. S., Pasternak, A., Pitta, P., Psarra, S., Rassoulzadegan, F., Tanaka, T., Tselepides, A., Wassmann, P., Woodward, E. M. S., Riser, C. W., Zodiatis, G. & Zohary, T. 2005 Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean. *Science* 309, 1068-1071.

Thingstad, T. F. & Mantoura, R. F. C. 2005 Titrating excess nitrogen content of phosphorous-deficient eastern Mediterranean surface water using alkaline phosphatase activity as a bio-indicator. *Limnology and Oceanography: Methods* 3, 94-100.

Thorn, K. A. & Mikita, M. A. 1992 Ammonia fixation by humic substances: a nitrogen-15 and carbon-13 NMR study. *The science of the total environment* 113, 67-87.

Thurman, E. M. 1985 *Organic geochemistry of natural waters*. Dordrecht,: Martinus Nijhoff.

Tyrrell, T. 1999 The relative influences of nitrogen and phosphorous on oceanic primary production. *Nature* 400, 525-531.

Tyrrell, T. & Law, C. S. 1997 Low nitrate : phosphate ratios in the global ocean. *Nature* 387, 783-796.

Vähätalo, A. V., Salonen, K., Muenster, U., Jarvinen, M. & Wetzel, R. G. 2003 Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. *Archiv für Hydrobiologie* 156, 287-314.

Vähätalo, A. V. & Zepp, R. G. 2005 Photochemical Mineralization of Dissolved Organic Nitrogen to Ammonium in the Baltic Sea. *Environmental Science and Technology* 39, 6985-6992.

Wang, W., Tarr, M. A., Bianchi, T. S. & Engelhaupt, E. 2000 Ammonium Photoproduction from Aquatic Humic and Colloidal Matter. *Aquatic Geochemistry* 6, 275-292.

Wiegner, T. N. & Seitzinger, S. P. 2001 Photochemical and microbial degradation of external dissolved organic matter inputs to rivers. *Aquatic Microbial Ecology* 24, 27-40.

Woodward, E. & Owens, N. 1990 Nutrient depletion studies in offshore North Sea areas. . *Netherlands Journal of Sea Research* 25, 57-63.

Woodward, E. M. S. & Rees, A. P. 2001 Nutrient distributions in an anticyclonic eddy in the northeast Atlantic Ocean, with reference to nanomolar ammonium concentrations. *Deep Sea Research II* 48, 775-793.

Zafiriou, O. C. & True, M. B. 1979a Nitrate Photolysis in Seawater by Sunlight. *Marine Chemistry* 8, 33-42.

Zafiriou, O. C. & True, M. B. 1979b Nitrite Photolysis in Seawater by Sunlight. *Marine Chemistry* 8, 9-32.

Zagarese, H. E., Diaz, M., Pedroso, F., Ferraro, M., Cravero, W. & Tartarotti, B. 2001 Photodegradation of natural organic matter exposed to fluctuating levels of solar radiation. *Journal of Photochemistry and Photobiology B: Biology* 61, 35-45.

Zepp, R. G., Callaghan, T. V. & Erickson, D. J. 1995 Effects of increased solar ultraviolet radiation on biogeochemical cycles. *AMBIO* 24 181-187.

Zepp, R. G., Faust, B. C. & Hoigne, J. 1992 Hydroxyl Radical Formation in Aqueous Reactions (pH3-8) of Iron(II) with Hydrogen Peroxide: The Photo-Fenton Reaction. *Environmental Science and Technology* 26, 313-319.

Zepp, R. G., Schlotzhauer, P. F. & Sink, R. M. 1985 Photosensitized Transformations Involving Electronic-Energy Transfer in Natural-Waters - Role of Humic Substances. *Environmental Science & Technology* 19, 74-81.

Zohary, T., Herut, B., Krom, M. D., Mantoura, R. F. C., Pitta, P., Psarra, S., Rassoulzadegan, F., Stambler, N., Tanaka, T., Thingstad, T. F. & Woodward, E. M. S. 2005 Is the Eastern Mediterranean N & P co-limited in summer? The response of P supplemented water to added ammonia in an on-board microcosm experiment. *Deep Sea Research II* 52, 3024-3040.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 157-175
ISBN: 978-1-60021-935-1

Chapter 4

SULFIDE DIFFUSION AND CHEMOAUTOTROPHY REQUIREMENTS IN AN EXTREMOPHILIC WORM TUBE

N. Le Bris[1], L. Anderson[2], F. Chever[1] and F. Gaill[2]
[1]IFREMER, Département Etude des Ecosystèmes Profonds, Technopôle Brest-Iroise, BP70, 29280 Plouzané, France
[2]UMR 7138, Adaptation aux Milieux Extrêmes, Université Pierre et Marie Curie, 7 quai Saint-Bernard, 75005 Paris, France

ABSTRACT

Highly productive colonies of *Alvinella pompejana* are found in one of the most extreme environments of the deep-sea. This annelid secretes a tube on the wall of hydrothermal smokers of the East Pacific Rise. Both the inner face of the tube and the dorsal part of the animal are colonized by an abundant microflora. The capacity of this symbiotic association to deal with strong thermal and chemical gradients, at the interface between the smoker wall and the surrounding seawater, remains poorly understood. Particularly, the mechanism by which chemoautotrophic microbes take advantage of this microenvironment have not yet been described. The tube is thought to be ventilated from a seawater-dominated medium overlying the colony. This provides protection against hot, acidic and strongly sulfidic fluids escaping from the mineral substrate. Here we have shown that the chemical properties of the tube wall are also likely to play a role in the supply of sulfide to sulfide-oxidizing primary producers in the tube. The permeability of the newly secreted tube wall to H_2S is remarkably high. This should favor sulfide diffusion through the tube wall from the highly sulfidic fluids venting from the smoker wall to the internal medium primarily composed of seawater. This process should have a determining impact on sulfide and electron acceptors availability in the tube, potentially maximizing the chemosynthetic energy budget for sulfide oxidizers. As the tube wall becomes progressively coated with minerals, its permeability to sulfide strongly decreases. This restricts H_2S diffusion to the anterior part of the tube and limits the diffusive flux to the internal microenvironment.

Keywords: Alvinella, tube, East Pacific Rise, sulfide oxidizing bacteria, mineralization, H_2S permeability

1. INTRODUCTION

The habitat of *Alvinella pompejana* is one of the most chemically and thermally extreme encountered in the deep-sea. This annelid is found exclusively on active hydrothermal 'smokers' from the East Pacific Rise. *Alvinella* colonies occupy intense venting areas, where they are exposed to particularly acidic (pH down to 4), sulfidic (up to millimolar levels), and hot (temperatures often >100 °C) fluids (review in Le Bris and Gaill, 2007). This animal inhabits the tube it secretes at the surface of the mineral substrate forming the hydrothermal edifice (Figure 1). The tube wall is composed of fibrous protein layers (Gaill and Hunt, 1991). This structure displays an outstanding chemical and thermal resistance, and is thought to play a major role in protecting the animal from the harsh surrounding conditions (Gaill and Hunt, 1986). Within months, minerals are formed on the internal and external surfaces of the tube wall (Zbinden et al. 2001; 2003). This progressive mineralization of the tube is the first step towards its 'fossilization', initiated by the degradation of its organic structure and the formation of minerals between tube layers, before its complete encrustation by chimney accretion (Maginn et al. 2002).

Abundant microbial communities have been shown to colonize this microenvironment. It was hypothesized that *Alvinella pompejana* which spends most of the time inside its tube (Chevaldonné and Jolivet, 1993) may farm these associated microbial communities (Desbruyères et al. 1998). The potential importance of autotrophic carbon fixation in the worm microhabitat is, therefore, a key issue in understanding the capacity of this symbiotic association to cope with extreme surrounding conditions (Le Bris and Gaill, 2007). Gaill et al. (1988) have described dense microbial mats, both on the inner face of the tube and attached to the back of the animal. The dominant members of the *A. pompejana* associated microflora were shown to belong to the epsilon subdivision of Proteobacteria (Haddad et al. 1995; Cary et al. 1997). Attempts to culture these phylotypes remain unsuccessful (Jeanthon, 2000), but molecular data of cultured free-living and non-cultured epibiotic members of the epsilon-proteobacteria have provided new clues regarding microbial activity in the tube. Particularly, the results of Campbell et al. (2003) have confirmed the assumption that chemoautotrophic activity can be sustained in the tube by both the dominant epibionts and free-living bacteria. These authors also showed that genes coding for the key metabolic enzymes of the rTCA cycle, an alternative carbon fixation pathway to the Calvin cycle, were expressed. In a recent study, Hügler et al. (2005) revealed that this metabolic pathway can sustain high rates of carbon fixation by two chemolithoautotrophic sulfur oxidizers belonging to the epsilon-proteobacteria. Such as produced by this two strains, abundant filamentous sulfur has been described on both the inner surface of the tube and the animal's back (Gaill and Hunt, 1991). If not yet demonstrated, it can reasonably be hypothesized that sulfide oxidizing metabolisms contributes to fuels chemoautotrophic primary production in the *Alvinella pompajana* tube.

Sulfide oxidation is one of the most energetic chemical processes that can be used to fix CO_2 chemolithoautotrophically (Jannash, 1995). Information on the availability of sulfide and appropriate elecron acceptors in the *Alvinella pompejana* microhabitat is however required to

quantitatively appreciate the importance of this process. From the analysis of geochemical tracers of seawater in fluids collected inside the tubes, Di Meo-Savoie et al. (2004) revealed that the internal medium was composed up to 72 to 91 % seawater but still contained sulfide at concentrations ranging from 60 to 360µM. Despite this high seawater contribution, no oxygen was detected in the tubes by these authors, suggesting its total consumption by abiotic and biological processes. Additionally, the tube was shown to partition the environment into chemically contrasting niches (Le Bris et al. 2005). The near-neutral pH measured inside the tube that was consistent with a seawater-dominated medium, strongly departed from the acidic values (pH < 5) measured outside the tube indicating a predominant hydrothermal influence on this outer medium. Diffusion of H_2S across the tube wall could potentially result from the difference of about 1mM of sulfide between both sides of the tube wall which was predicted from these observations. The aim of this study was to assess the importance of this process with respect to the energy requirement for autotrophic carbon fixation using sulfide as an electron donor in the *Alvinella pompejana* tube. To address this question, we determined the permeability of the tube wall to H_2S, in relation to its degree of mineralization. In an attempt to appreciate the role of sulfide diffusion through the tube wall on energy budgets, the evolution of chemical conditions was modeled over time for an intermittent renewal regime of this medium.

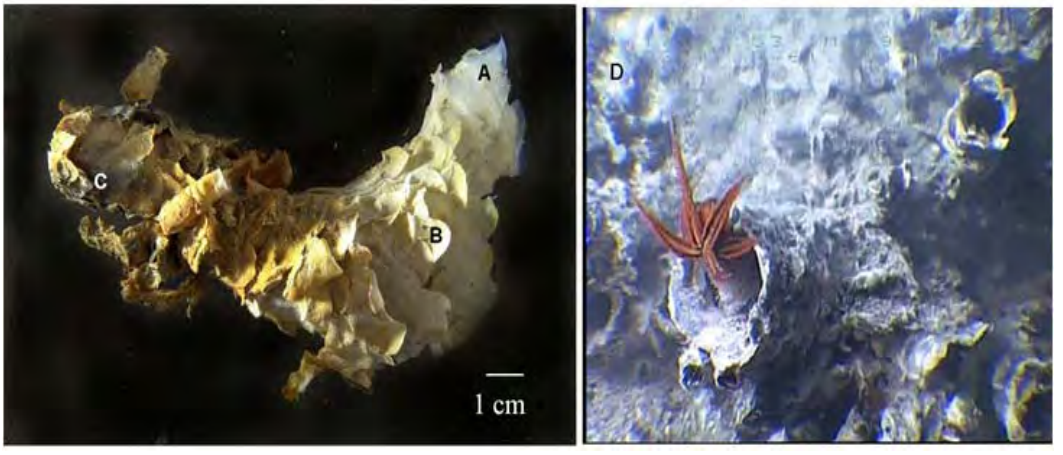


Figure 1. Picture of the *Alvinella pompejana* tube sample used forn this study. Permeability experiments were done on scaly sub-samples for the most recent part (A - translucent scales), the intermediate region (B - white scales) and the posterior region (C - black scales). Image capture showing a brief appearance of a worm at the opening of its tube on the sampled chimney (D).

2. MATERIAL AND METHODS

2.1. Tube Sampling

Alvinella pompejana tubes were sampled during the Biospeedo cruise in 2004, at the Hubbs site found on the 17 °S segment of the East Pacific Rise. Samples were collected from the surface of a large ‘snow-ball’-type smoker (~ 7 meters high) which was almost entirely

covered with tubes. Animals and their tubes were grabbed with the manipulator arm of the submersible Nautile, and placed in a closed box for recovery. Once on-board, tubes were selected and placed in individual containers filled with 10 % formalin buffered with seawater for preservation.

This study considered a 15 cm tube portion characteristic of *Alvinella pompejana* tubes. The *Alvinella pompejana* tube is made of layered 'scales' (Desbruyères et al. 1998). 'Scales' were sub-sampled from the tube at three levels relating to the distance from the hydrothermal chimney wall: 1) furthest from chimney wall at the tube opening (transparent portion); 2) at middle distance (white portion); and 3) closest to the chimney wall (black portion) (Figure 1). As previously described (Gaill and Hunt, 1991; Zbinden et al. 2003), these levels are characteristic of tube secretion stages which correlate to the protein layer deposition and mineral formation.

2.2. Permeability Measurements

Individual 'scales' from different parts of one tube were considered for this study. Each 'scale' represents the most homogeneous tube sub-sample possible. These individual 'scales' also display a good mechanical stability, enabling them to be manipulated easily with minimal risk of cracking. Permeation experiments were done on several of these sub-samples from the three portions of the tube.

'Scale' sub-samples of about 4 mm diameter were cut and clamped within the two parts of an insert. The thickness of the tube sample was measured using calipers prior to placement into the insert. The insert was carefully positioned into the diffusion chamber in order to expose the outer surface of the tube sample to the sulfide solution (Easymount, Harvard Apparatus). The thermostatted holder allowed the temperature in the chamber to be regulated to 32 °C. This value was considered as an intermediate representative from the temperature range measured inside *Alvinella pompejana* tubes (Cary et al. 1998, Le Bris and Gaill 2007).

A tube surface of 2 mm in diameter was exposed to the solutions filling the two reservoirs of the diffusion chamber. The volume of solution in each reservoir was 4 ml. The outer tube surface was exposed to a sulfide solution (S_1), while the inner surface was exposed to a solution initially devoid of sulfide (S_0). The increase of the sulfide concentration in this solution was monitored using an H_2S amperometric microelectrode (Unisense, Dk). Each experiment lasted 20 to 30 minutes after starting the tube's exposure to sulfide. Prior to the experiment, the S_0 solution was deoxygenated by nitrogen gas bubbling. Contamination with atmospheric oxygen was later minimized by closing the chambers with rubber stoppers, but could not be fully prevented. As empirically determined for a standard sulfide solution, this resulted in a 2 % min^{-1} decrease of the sulfide concentration which was later corrected for.

Solutions were prepared from ultrapure water, combined with NaCl (35 g l^{-1}) and $NaHCO_3$ (2. 10^{-3} M) to approach the ionic strength and carbonate buffer strength of seawater. The pH of the two solutions was set to an appropriate value using HCl (1M). Their characteristics reflected a compromise between expected natural exposure conditions, and the optimal conditions enabling sulfide diffusion to be monitored with sufficient precision. Fluids escaping from *Alvinella pompejana* colonies or circulating outside tubes were shown to display sulfide concentrations ranging from several hundreds of µM to 1.5 mM and acidic pH (4 to 5) (Le Bris and Gaill, 2007). This sulfide concentration range was however inadequate

for this experiment, since the diffusion rate of hydrogen sulfide was similar in this case to its oxidation rate by oxygen in the solution S_0. To reduce the relative impact of oxidation on the measured sulfide flux through the tube wall, and reasonably limit the experiment time, the concentration of sulfide in S_1 was set to 10 mM. This solution was prepared from a Na_2S stock solution of about 100 mM standardized using the iodometric method. This initially highly alkaline solution was neutralized to a pH of 7. The formation of a white precipitate, presumed to be elemental sulfur resulting from rapid sulfide oxidation, prevented us to acidify solution S_1 beyond this value. At this pH, H_2S contributes only half of the total sulfide concentration (c.a. 5 mM), i.e. 3 to 5 times the maximum H_2S levels observed in the *Alvinella* environment. Conversely, the pH of solution S_0 had to be maintained to a value between 4 and 5 in order to maintain free sulfide predominantly under the H_2S form and to circumvent the effect of slight pH changes on amperometric measurements. This was considered as the best option to ensure high precision in the determination of permeability values, despite a higher pH (~ 6.5) would have better matched the *in situ* conditions. In this study, we assumed that such differences in pH with respect to the real conditions did not substantially modify the permeability of the tube wall to sulfide.

2.3. Permeability and Diffusion Coefficient Calculation

According to Fick's First Law, the H_2S permeability of tube sub-samples, p ($cm.s^{-1}$), was defined as:

$$p = J/ (S. \Delta C) \quad (1)$$

J ($mol.s^{-1}$) is the sulfide flux through the tube wall. It is determined as the product of the [H_2S] increase rate ($mol.L^{-1}.s^{-1}$) and the volume of the 'internal' solution S_0. S is the surface of tube in contact with the solution (cm^2) and ΔC the H_2S concentration gradient between both sides of the tube wall. The difference in the concentration of the H_2S form alone was accounted to calculate permeability, since this species is the only sulfide form that is expected to diffuse through the hydrophobic tube wall. This assumption was checked by preliminary tests using alkaline sulfide solutions. The concentration of sulfide in S_0 is negligible in this first stage of exposure, therefore ΔC is considered equal to the concentration of H_2S in the solution S_1 ($mol.L^{-1}$). The diffusion coefficient D ($cm^2.s^{-1}$) is defined as the product of the permeability and the thickness of the tube sample (e in cm):

$$D = p. e \quad (2)$$

2.4. Microscopy and Microanalysis

2.4.1. Sample Preparation

Samples fixed for morphological observation were dehydrated in an ethanol and propylene oxide series with osmium, and then embedded in resin (Epoxy 812 AGAR, medium hardness). Semi-thin (800 nm thick) and ultra-thin sections (50-80 nm thick) were obtained from Reichert-Jung Ultramicrotome (Ultracut E) diamond knives Diatome HISTO

and Ultra 35° respectively. Semi-thin sections were stained with toluidine blue for basic observation by light microscopy.

2.4.2. SEM and X-ray Microanalysis

After cutting, the surface of the resin blocks was sufficiently polished for X-ray microanalyses (XRA) without the need for further grinding or polishing. The black tube sub-sample could not be cut with the diamond knife, owing to the large amount of mineralization. Instead this block was cut into 1 mm transverse slices using a diamond rotary saw (PRESI Mecatome P100), and polished with rotating abrasive disks, and further polished using aluminium oxide and a velour disk (on a PRESI Mecapol P230, Grenoble, France). The polished sections were mounted on round metal stubs with TEMP-FIX (EMS, "EUROMEDEX"), and three of their four sides were painted with silver "Quick Drying" paint. In order to protect the sample and prevent surface flaring during electron bombardment in the scanning electron microscope (JEOL JSM-840A), sections were carbon coated by rotative shadowing in a BALZERS BAF-400 unit prior to observation. X-ray microanalyses and elemental mapping (later referred as XRA) were performed using an attached LINK Pentafet detector (with the added possibility for Berium (Be) window removal or replacement by a thin Be window to detect low energy x-rays), and (EDAX) LINK eXl-10 energy dispersive X-ray analyzer. The functionality of the microscope allows the probe current to be optimized to seek particular element signatures, therefore the electron energy was mounted to 39keV for heavier elements such as zinc and iron, and lowered to 19keV/10keV (+ thin Be window) to seek lighter elements such as phosphorous.

2.5. Chemical Modeling

Chemical modeling was performed using the PHREEQC code (Parkhurst and Appelo, 1999). This calculation code was used to simulate the temporal changes induced by the simultaneous diffusion of sulfide from the outside and the consumption of oxygen and sulfide through abiotic sulfide oxidation. This code does not include corrections to thermodynamic data for the effect of pressure. At moderate temperatures, equilibrium calculations in solution were shown to be very similar to those obtained using the SOLMINEQ code at 260 MPa (Le Bris et al., 2003). More generally, we assumed that high pressure would have only a minor effect on the results.

The composition of the initial solutions used in the calculation was defined using ranges determined from measurements taken in the immediate environment of several alvinellid colonies (Le Bris et al. 2005; Le Bris and Gaill, 2007). The external medium was assumed to be 1 mM in sulfide, while the internal concentration was set to 10 % of this value, 100 µM, considering that this medium is composed of about 90 % seawater (Di Meo-Savoie et al. 2004). Accordingly, the oxygen concentration was set to 100 µM, based on the regional deep-sea seawater oxygen content of 110 µM (Sarradin et al. 1998). The seawater major ion composition at salinity of 35 was taken from (Millero, 1996).

The increase of H_2S concentration inside the tube with time can be expressed after Ficks's law as:

$$d[H_2S]_{int}{}^{t} = ([H_2S]_{ext} - [H_2S]_{int}{}^{t}) \,.\, p.S/V \, dt \qquad (3)$$

Including this equation to the PHREEQC input file enables the calculation of sulfide concentration variation within the tube. The permeability coefficient was either set equal to the value empirically determined for the anterior (white portion) or posterior part of the tube (black portion). S and V are, respectively, the surface of tube wall exposed to the outer medium and the corresponding volume inside the tube. The ratio S/V is independent of the length of tube considered and is equal to 4/d, where d is the tube diameter, here defined as 1.5 cm. This value corresponds to the diameter of the central part of the tube studied and is a reasonable estimation for the average tube diameter in a mature *A. pompejana* colony. The model was run for the two permeability coefficients corresponding to the white and black portions of the tube. The transparent portion was not considered, since it is not expected to be significantly exposed to the sulfide-rich fluids circulating in the main body of the colony (Le Bris et al. 2005).

Abiotic sulfide oxidation of sulfide by oxygen was also accounted for, using the rate given by Zhang and Millero (1994). According to these authors, Fe^{II} is the most active in catalyzing this process among various metals. In the *Alvinella* habitat, dissolved ferrous iron concentration can easily reach 100 µM or more (Le Bris and Gaill, 2007). In our simulations, we limited its value to 10 µM which is the upper limit of the range on which the catalytic rate was quantified. Extrapolating this rate to higher contents may largely overestimate the effect of reduced iron on the oxidation of sulfide (authors unpub. results). The calculation was performed setting the temperature to 30 °C, consistently with the temperature for which the permeability coefficient was determined. Our simulation assumed that thermal equilibrium is achieved between the inside and outside of the tube.

We assumed that, in this oxygen-limited environment, partial oxidation of hydrogen sulfide to elemental sulfur (S^0) according to reaction (4) was likely to be the dominant step for sulfide oxidation. Additionally, H_2S oxidation with nitrate was also accounted (5).

$$H_2S + ½\ O_2 \leftrightarrows S^0 + H_2O \quad (4)$$

$$½\ H^+ + H_2S + ¼\ NO_3^- \leftrightarrows S^0 + ¼\ NH_4^+ + ¾\ H_2O \quad (5)$$

The maximum energy that can be released from these reactions in the considered medium, ΔE_{O2} and ΔE_{NO3-}, can be quantified from the amount of H_2S that can be oxidized and the Gibbs free energy of the reaction (4) and (5) per mole of H_2S: ΔG_{O2} and ΔG_{NO3-}:

$$\Delta E_{O2} = \varepsilon_{O2} . \Delta G_{O2} \quad (6)$$

$$\Delta E_{NO3-} = \varepsilon_{NO3-} . \Delta G_{NO3-} \quad (7)$$

The factor ε_{O2} represents the maximum concentration of sulfide that can be oxidized by oxygen per volume unit. Since the oxidation of 1 mole H_2S only requires 0.5 mole O_2, ε_{O2} is equal to $[H_2S]$ if this concentration is lower than $2[O_2]$, and equal to $2[O_2]$ in the other case. When not completely oxidized after total O_2 consumption, the remaining sulfide, $[H_2S]$-$2[O_2]$, can be oxidized using nitrate. The amount of sulfide that can be oxidized by nitrate, ε_{NO3-}, is defined as the lowest of the remaining sulfide concentration, $[H_2S]$-$2[O_2]$, and $4[NO_3^-]$. This reflects the fact that only ¼ mole of nitrate is needed to oxidize one mole of sulfide.

Finally, chemical thermodynamics defines Gibbs free energies for reaction (4) and (5) as:

$$\Delta G_{O2}(T) = \Delta G°_{O2}(T) - RT \ln [a(H_2S).\, a(O_2)^{1/2}] \quad (8)$$

$$\Delta G_{NO3-}(T) = \Delta G°_{NO3-}(T) - RT \ln [a(H_2S).\, a(NO_3^-)^{1/4}.a(H^+)^{1/2} / a(NH_4^+)^{1/4}] \quad (9)$$

$\Delta G°$ (T) is the standard free energy of the reaction, a(x) the activity of the species x in the medium, R the Planck constant and T the temperature in Kelvin. The activity of elemental sulfur is set to one. Activities of nitrate and ammonium ions were assumed to be constant and equal to their concentrations. 30µM and 5 µM respectively were estimated for these compounds in the inner-medium according to published data (Di Meo-Savoie et al., 2004). The standard free energy can be calculated at any given temperature from its value at 25 °C, $\Delta G°$(298 K), and the standard enthalpy of the reaction $\Delta H°$(298K), according to the Vant'Hoff law. The thermodynamic constants used in these calculations ($\Delta G°$(298K) and $\Delta H°$(298K)) were all obtained from the database of the calculation code PHREEQC (Parkhust and Appelo, 1999). At 30 °C the calculated standard Gibbs free energies for the reactions (4) and (5) are, respectively, 184. 5 and 157.9 kJ.mol^{-1}.

3. RESULTS

3.1. Permeability of the Tube Wall to Sulfide

H_2S concentration in the solution S_0 exhibited a very characteristic change in the first 40-50 minutes after the introduction of the sulfide solution at the opposite side of the tube wall. Consistent with Fick's first law (see 2.3), the H_2S concentration displayed a linear increase, but this occurred after a certain time lapse where H_2S remained undetectable (Figure 2). This delay widely varied among experiments, ranging from < 5 min to up to 30 minutes. When the chamber containing the sulfide solution was rinsed with a diluted HCl solution between experiments, however, a quite reproducible time lapse was observed for successive experiments on a same tube sample. When the sulfide solution was kept in place, and only the solution S_0 alone was renewed, this delay tended to decrease significantly from one experiment to the other. Following this first step is a second stage of linear increase of H_2S concentration. A slight curvature of the sulfide curve was observed about 20 min after the beginning of the concentration rise.

The calculated permeability widely varies among sub-samples originating from the different parts of the tube (Table 1). When plotted against the corresponding wall thickness, a marked decrease was observed from the thinner translucent scales of the tube opening, to the thicker black scales of its posterior part (Figure 3). Remarkably, the permeability of the black mineralized scales was equal or close to zero. In this case, hydrogen sulfide remained undetectable or stayed below 0.6 µM, even after 50 minutes exposure of the external face to the 10 mM sulfide solution.

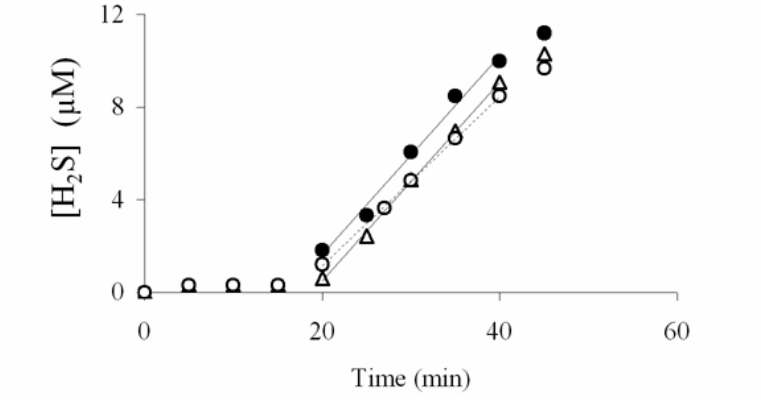


Figure 2. Increase of H_2S with time in the solution S_0 by diffusion through the the tube sub-sample.

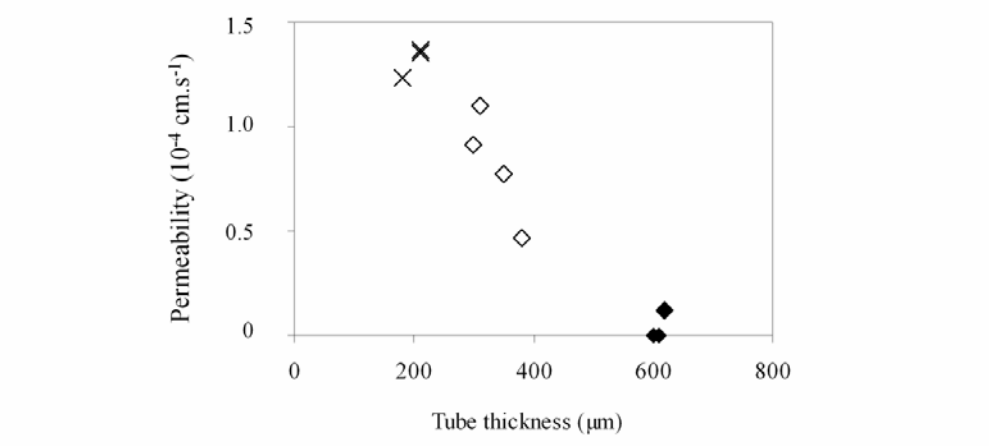


Figure 3. H_2S permeability as a function of tube thickness for the three types of sub-samples: translucent scales (crosses), white scales (open diamonds) and black scales (black diamonds).

Table 1. Mean permeability (p) and H_2S diffusion coefficient (D) for different types of tube sample (the uncertainty is defined as $2\sigma/\sqrt{n}$, where σ is the standard deviation for a series of n experiments)

Type	e (µm)	p (10^{-5} cm s^{-1})	D (10^{-6} $cm^2.s^{-1}$)	n
transparent	199 ± 20	14.1 ± 1.9	2.5 ± 0.8	8
white	329 ± 20	8.6 ± 1.7	2.8 ± 0.4	7
black	610 ± 20	0.4 ± 0.5	0. 25 ± 0.3	6

The diffusion coefficient is independent of variation in tube thickness and provides a more representative idea of the tube material properties with respect to H_2S diffusion. As shown in Table 1, the diffusion coefficient is ten times lower for the mineralized scales than for the two other types of samples. Contrastingly, the white and translucent tube samples have

very similar diffusion coefficients (not statistically different according to the uncertainties on these values).

3.2. Distribution of Elements within Tube Wall

3.2.1. General Features of Tube Samples

Optical microscopy and SEM imagery reveals a strongly banded internal tube structure for the white and black sections (Figures 4B and 4C). This structure is barely visible in the transparent sub-sample (Figure 4A). Standard microscopy clearly shows the structural differences between the different tube sub-samples. Of particular note is the presence of intense mineralization in half of the black tube sub-sample (Figure 4C).

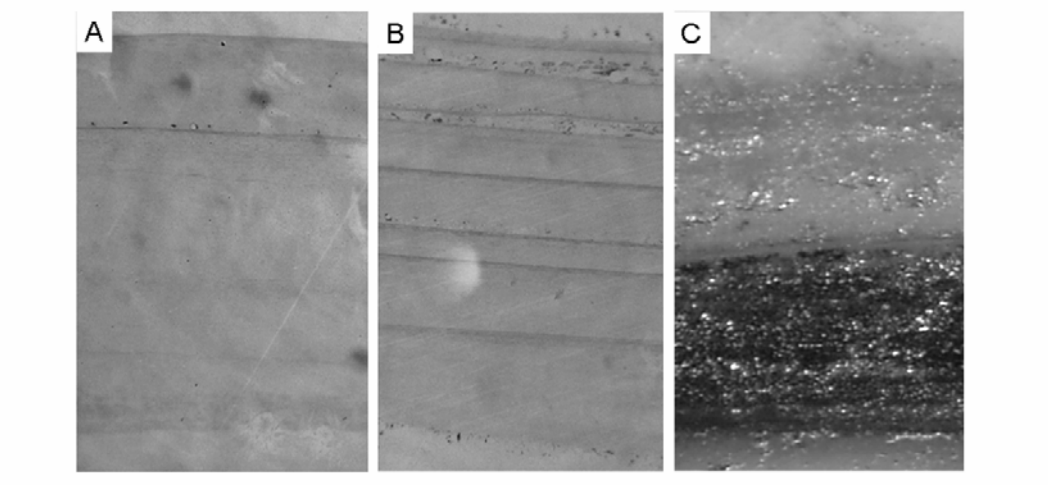


Figure 4. Optical microscopy image of transverse sections from the transparent (A), white (B) and black (C) regions of the tube.

X-ray spectra of the tube cross-sections revealed the most prevalent elements in all three sub-samples were: chlorine, osmium, sulfur and iron. Only weak signatures of other elements such as calcium, zinc and phosphorous were found (Figure 5). Chorine is an abundant element of the epoxy resin, and is therefore a tool to inversely highlight the presence of organic material on SEM images. Since all sub-samples used for XRA (not for SEM) were prepared with osmium, this element is present throughout the organic tube section. Sulfur is found mostly within the organic layers of the tube (Figures 5A iii, 5B iii, 5C iii). The signal for iron is the strongest of all the elements considered here, even for the translucent tube sub-sample. It is found throughout the tube section, both within and between the proteinaceous layers (Figures 5A ii, 5B ii, 5C ii).

3.2.2. Specific Characteristics of Tube Sub-samples

The XRA (spectra and image) analysis on the transparent tube sub-sample showed the tube to be comprised of two distinct bands (Figure 5A). The single spectra taken on the boundary between these two bands shows a strong iron signal and a smaller sulfide peak

(Figure 5A iv). Of particular note is the high concentration of iron in the band nearest to the tube's interior, and along a narrow strip (10µm thick) on the exterior surface (Figure 5A ii). Sulfur is comparatively much less abundant in tube sample (Figure 5A iii).

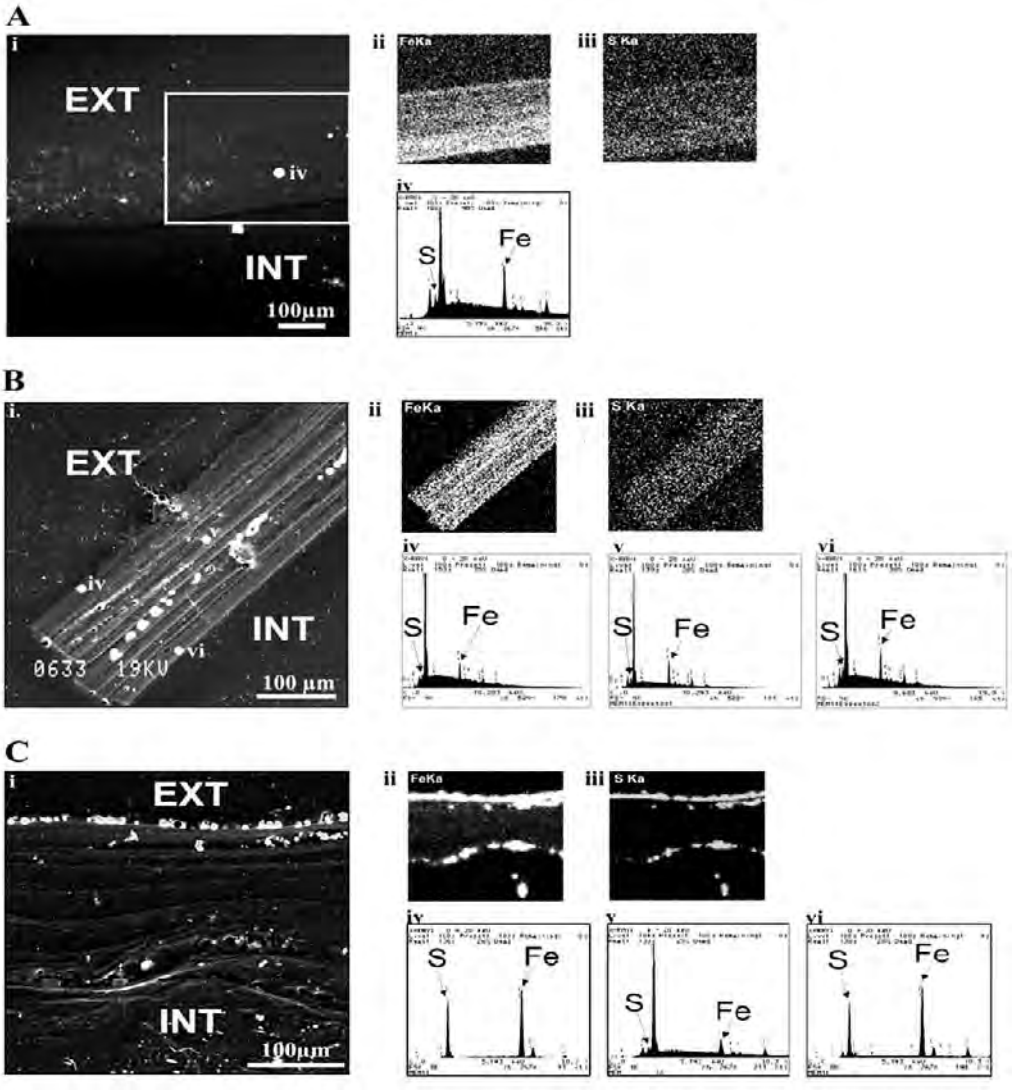


Figure 5. Composite figure of SEM images, XRA iron and sulfide mapping images and spectra of transverse sections from the transparent (A), white (B) and black (C) regions of the tube. XRA spectra locations correspond to the white dots on SEM images – elemental peaks are labeled.

In addition to the XRA images, three 'point-location' elemental spectra of the white tube sample were taken at the following locations: 1) the top edge of the tube wall, 2) an arbitrary central tube position, and 3) at the base of the tube wall. Analyzing the spectra enables a qualitative comparison of element abundance in the tube wall (Figure 5B iv, v, vi). The iron signal is quite stable in all three spectra, with no obvious iron gradient visible across the tube section, suggesting that the distribution of iron is fairly homogeneous. The sulfur signal is

slightly stronger in the spectrum taken from the external portion of the worm tube compared with the other two spectra taken from the middle portion of the tube and the interior. Overall, both the iron and sulfur signals are relatively homogeneous, with a slight increase in signal towards the tube's interior (Figures 5B ii, iii). However, it is clear that there is a much stronger iron signal in between the tube bands/layers (not targeted for point spectra analysis), where this metal appears to be concentrated (Figure 5B ii).

The cross-section of the black portion of the tube wall shows two very distinct discontinuous layers of highly dense material (Figure 5C i), surrounding the generally layered structure of the worm tube. XRA spectra on the tube cross-section reveal the most significant elements of these layers are again sulfur and iron (Figure 5C iv, vi). Both sulfur and iron are preferentially found in two main horizons, one at the tube's exterior surface, and one towards the tube's interior surface (Figure 5C ii and iii). There is no evident elemental layering in the main body of the tube section relating to the visible layered tube structure but this may be masked by the high signal intensity of the two surface layers. Point spectra analyses confirm the mapping data with a very strong signal for sulfur and iron found on the exterior and interior surface of the tube (Figure 5C iv and vi), whereas the central portion of the tube is comparatively depleted with reference to iron and sulfur signal (Figure 5C v).

3.3. Influence of Sulfide Diffusion on Chemical Conditions and Energy Budgets

The evolution of sulfide and oxygen concentrations over time strongly differs in our simulation between the white tube portion and the black tube portion (Figure 6A, B). In the former case, the sulfide concentration increases by almost of factor of 3 in 30 min while it decreases by about 2/3 in the later case as a result of its slow abiotic oxidation by oxygen. Accordingly, a decrease of O_2 is observed over time. In the white tube model, oxygen is reduced by about 80% of its initial value in 30 min. Whilst for the black tube model, less than half (41%) of its initial value is consumed in 30 min.

The energy available from sulfide oxidation also largely differs over time in the two cases (Figure 7). When sulfide is allowed to diffuse from the external medium, the available energy per volume unit, using oxygen as an electron acceptor, is maintained to a substantial level over time. It significantly increases (49%) in the first 7 minutes, and only progressively decreases afterward. Using nitrate as an alternative electron acceptor still increases the energy budget. The maximum increase (88 %) in the energy per volume unit is reached after 17 min. In comparison, for the black portion of the tube through which sulfide is not allowed to diffuse, the amount of energy available per volume unit continuously decreases over time as a result of the abiotic oxidation of sulfide by oxygen. Since oxygen is always in excess with respect to sulfide in this case, sulfide oxidation using nitrate is not considered as an alternative energy source.

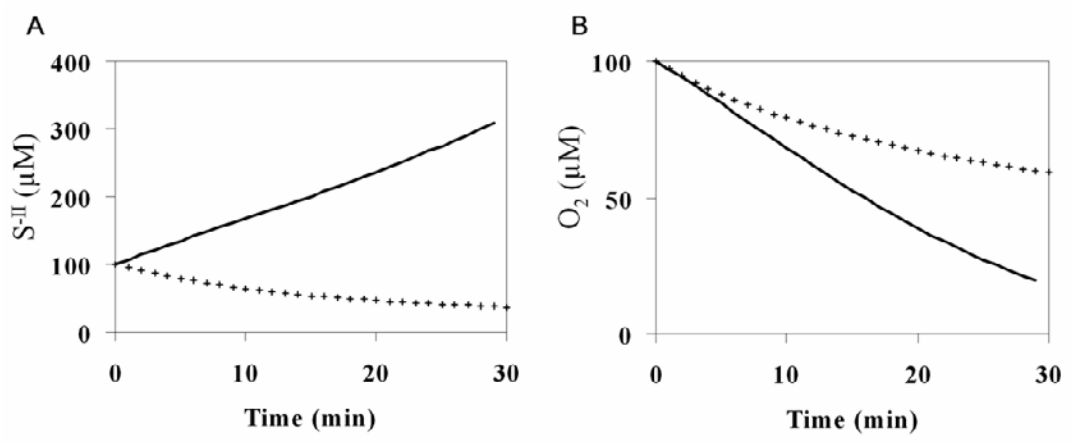


Figure 6. Predicted change in O_2 and sulfide concentrations with time after intermittent renewal of the internal medium. Initial conditions: Fe^{II} 10 µM, H_2S tot 100 µM, O_2 µM. A: White tube (plain line). B: Black tube (crosses).

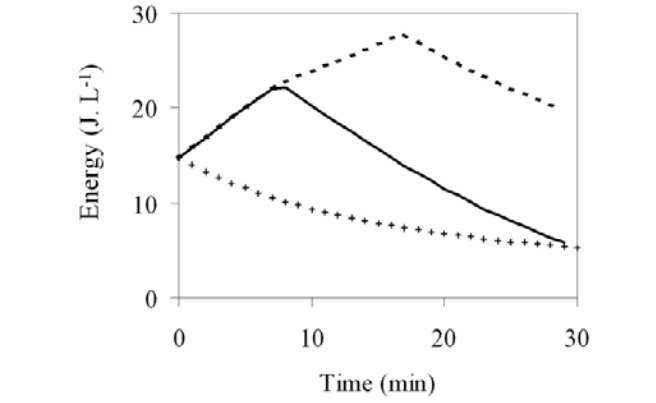


Figure 7. Estimate of the chemical energy per volume unit available from sulfide oxidation using oxygen alone (plain line) or both oxygen and nitrate (dotted line) in the white tube region, and using oxygen the black tube region (crosses).

4. DISCUSSION

4.1. Consequence of Tube Wall Permeability to Hydrogen Sulfide on the Microhabitat Chemistry

Previous studies emphasized the particularly high sulfide permeability of the basal part (‘root’) of tubes of siboglinid tubeworms from deep-sea sulfidic environments, as compared to their anterior part (Julian et al., 1998; Urcuyo et al. 2003). For *Lamellibrachia* sp., a symbiotic tubeworm from methane seep environments, H_2S uptake from deep layers of the sediment by diffusion through the root was identified as the main process sustaining a high sulfide supply rate for their thiotrophic endosymbionts. *Ridgeia piscesae*, another tubeworm species from North Pacific hydrothermal vents was similarly suggested to extract sulfide from

fluids circulating below the rocky seafloor by extending its root through cracks (Urcuyo et al. 2003). As compared to these 'root' tubes, the permeability of the most recent portions of the *A. pompejana* tube to hydrogen sulfide also appears substantial. The permeability of the white and transparent tube exceeds by a factor of 2.3 and 3.4, respectively, the mean permeability of the roots of *Ridgeia piscesae* (Table 2). The permeability of the white and transparent part of the *A. pompejana* tube only reaches 22% and 34%, respectively, of the mean value determined for the *Lamellibrachia* sp. 'root' tube, but when the thickest portion of this 'root' is considered (c.a. 0.15 mm thick), which is more relevant for such a comparison, the *A. pompejana* tube appears 2 to 3 times more permeable to H_2S than the *Lamellibrachia* sp. 'root' tube. If the higher temperature of this experimental study (32 °C instead of 20 °C in previous studies) may have favored sulfide diffusion through the wall, this factor alone is unlikely to explain the observed difference. This high permeability rather results in a remarkably high coefficient of diffusion of H_2S in the proteinaceous matrix forming the Alvinella tube wall (Table 3), almost reaching the remarkably high values achieved in the thinnest part of the *Lamellibrachia* sp. root tube (Julian et al. 1998).

Table 2. Tube wall H_2S permeability in different annelids from deep-sea sulfidic environments. Corresponding temperature and tube wall thickness are also reported.

Species	T (°C)	Wall thickness (mm)	H_2S permeability (10^5.cm.s^{-1})
Lamellibrachia sp. tube: trunk region[2]	20	0.05 to 0.8	3.7 ± 6.2.
Lamellibrachia sp. tube: root region[2]	20	0.03 to 0.15	41 ± 49
Ridgeia piscesae tube: root[1]		0.03 to 0.18	4.0 ± 2.3
*A. pompejana t*ube: transluscent region[3]	32	0.18 to 0.21	14 ± 3
A. pompejana tube: white region[3]	32	0.30 to 0.38	9.3 ± 1.2
A. pompejana tube: black region[3]	32	0.60 to 0.62	0.4 ± 0.5

[1] (Urcuyo et al. 2003).
[2] (Julian et al. 1999).
[3] This study.

As long as the tube of *A. pompejana* is devoid of mineral deposits, the diffusion of hydrogen sulfide through the tube wall appears to be particularly favored. The consequences of this property of the *Alvinella* tube wall on microhabitat conditions are important. From an initial sulfide content of 100µM inside the tube and an outside concentration of 1 mM, we estimated that H_2S diffusion through the white part of the tube wall would result in an increase in the sulfide concentration by 67 % inside the tube within 10 minutes, and that H_2S would reach up to 3 folds its initial level in 30 minutes. Here, sulfide diffusion is partly compensated by abiotic oxidation of sulfide catalyzed by Fe^{II}, assuming a concentration of 10 µM for this compound. A consequence of this process is the depletion of oxygen from the medium. In our simulation, the oxygen concentration appears to be reduced by 80 % in 30 min, twice the decrease estimated in the absence of sulfide diffusion for the mineralized portion of the tube.

4.2. Influence of Tube Ventilation on Energetic Budgets

There are two ways by which marine annelids renew the medium filling their tubes or burrows (Mill, 1978); continuously or intermittently. As discussed in Le Bris and Gaill (2007), the later hypothesis is supported from the brief in and out movements of the animal observed on videos, although a continuous component to the renewal of the medium cannot be discarded. Oxygen and nitrate should be transported inside the tube by advection from the seawater-dominated layer bathing tube openings, before being consumed abiotically or biologically. Conversely, the supply of sulfide to the tube microhabitat would only slightly rely on advection from this moderately sulfidic water, but is more likely sustained by diffusion of H_2S through the tube wall. Our modeling work shows that, in an intermittent renewal scenario, the diffusion of sulfide through the tube wall can substantially increase the energetic budget available for sulfide-oxidizing microbes inside the tube. The available energy per volume unit, using oxygen as an electron acceptor, would almost double within less than 10 minutes balancing its utilization by sulfide oxidizing microbes. The residence time of the medium inside the tube is therefore a key factor in the energy balance for the associated autotrophic microbial primary producers.

The main limitation to sulfide-oxidizing autotrophic carbon fixation in an intermittent renewal scenario is the availability of oxygen as an electron acceptor. According to this study, its fast depletion through abiotic oxidation of sulfide (and other electron donors not considered in the simulation) is likely to compete significantly with microbial uptake. Both processes should lead to the rapid depletion of oxygen in the tube, providing an explanation for Di Meo-Savoie et al. (2004) not detecting oxygen in tubes, whenever the seawater contribution to the medium was dominant. Nitrate does not react spontaneously with sulfide and should therefore remain available for microbes as an alternative electron donor. Nitrate use as an additional electron acceptor should increase the energy budget over the subsequent 20 minutes and maintain it at a substantial level after that. The maximum is reached after 17 minutes with an energy budget exceeding by 88 % of its initial value. The abundance of nitrate in the Alvinella pompejana tubes suggests that the availability of this compounds should not be limiting for chemoautotrophic carbon fixation. Although less energetically favorable on a molar basis, this pathway could significantly contribute to the chemosynthetic energy supply to the animal and its associated microbial community.

4.3. Sulfide and Electron Acceptor Supply during Successive Stages of Tube Growth

The posterior part of the tube (black portion) appears much less permeable to sulfide than its anterior part (transparent and white portions). The formation of relatively large sulfide crystals on the outer tube surface, similar to those forming the smoker wall, likely denote the exposure of this region of the tube to the hot fluids escaping the mineral substrate. Another remarkable point is that iron sulfide is formed on the internal surface of the tube, whereas no such mineral deposit is observed between layers. This process must therefore have occurred after the secretion of the last layer by the animal in this part of its tube. The integrity of the tube is here fully preserved, contrasting with the ‘fossilization’ stage described by Maginn et

al. (2002) where minerals formation between layers suggested the migration of hot fluids through a degraded tube wall.

On this basis a 3-stage evolution of the tube inner-environment with respect to sulfide and electron acceptors supply can be described. The animal has been observed secreting new (transparent) material at tube opening, progressively expanding its tube outwardly from the chimney wall. As described by Le Bris et al. (2005), this part of the tube lies in the seawater-dominated layer overlying the colony (Figure 8). Since the tube interior was also shown to be filled by this low sulfide medium, no diffusion should occur through the tube wall at this level. The ageing tube is later embedded in the assemblage of tubes and mucus forming a compact layer at the surface of the smoker. In this second stage the (white) tube wall is exposed to highly sulfidic fluids escaping the smoker wall, and as long as the inner medium is intermittently refreshed with the overlying water, sulfide exchange by diffusion can occur from the outer to the inner environment. In a last stage, while getting closer to the growing chimney wall, the tube become mineralized on its outer face exposed to hot fluids, as well as on its inner face, possibly due to the absence of ventilation in the oldest region of the tube. Its permeability to sulfide then strongly decreases, preventing its diffusion inside this region of the tube. This depicts a dynamic system, in which tube growth and mineralization would enable the achievement of a coarsely steady-state conditions for sulfide diffusion in the tube in a constantly changing environment under the influence of a high mineral accretion rate.

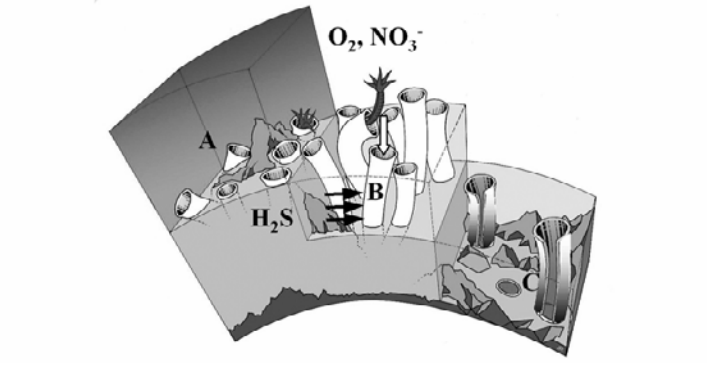


Figure 8. Scheme of an *Alvinella pompejana* colony showing the positioning of the transparent (A), white (B) and black (C) parts of the tube with respect to the smoker wall and the different pathways for nitrate, oxygen and sulfide transport in the tube.

5. CONCLUSION

Alvinella pompejana inhabits a hot and sulfide rich environment from which endosymbiotic associations relying on sulfide oxidizing symbionts, like the tubeworms *Riftia pachyptila* or *Ridgeia piscesae*, are excluded. The fast growth of its colonies should therefore be supported by another mechanism associating microbial primary producers to this animal species. The abundant microbial communities colonizing the interior of the *Alvinella pompejana* tube likely contributes to the efficiency of this association in colonizing chimney walls. This study depicts an original microenvironment from which sulfide and electron acceptors are supplied through separate ways to free-living or epibiotic chemoautotrophic

sulfide oxidizers. The properties of the tube wall should play a key role by allowing sulfide to diffuse from the fluids circulating outside the tubes to the inner medium. Within minutes, this diffusive sulfide flux should significantly contribute to increase the energy available for primary production, and would favor the use of nitrate as an alternative electron donor after complete oxygen consumption. These conditions are expected to be ultimately governed by the animal through tube wall secretion and ventilation, emphasizing the potential importance of mutualistic interaction between an annelid and chemosynthetic microbes in the colonization of this extreme environment.

ACKNOWLEDGEMENT

The authors would like to thank the chief scientist of the Biospeedo cruise, the captain and crew of the R/V Atalante and the Nautile operation group. Ph. Compère and G. Frébourg are also especially acknowledged for their support to L. Anderson in the microscopy work.

REFERENCES

Campbell, B. J.; Stein, J. L. & Cary, S. C. (2003). Evidence of chemolithoautotrophy in the bacterial community associated with Alvinella pompejana, a hydrothermal vent polychaete. *Applied Environmental Microbiology,* 69, 5070-5078.

Cary, S. C.; Cottrell, M. T.; Stein, J. L.; Camacho, F. & Desbruyères, D. (1997). Molecular identification and localization of filamentous symbiotic bacteria associated with the hydrothermal vent annelid Alvinella pompejana, *Applied Environmental Microbiology,* 63, 1124-1130.

Cary, S.C.; Shank, T. & Stein, J. (1998). Worms bask in extreme temperatures. *Nature,* 391, 545-546.

Chevaldonné, P. & Jollivet, D. (1993). Videoscopic study of deep-sea hydrothermal vent alvinellid polychaete populations: biomass estimation and behaviour. *Marine Ecology Progress Series,* 95, 251-262.

Di Meo-Savoie, C. A.; Luther, G. W. & Cary, S. C.. 2004. Physico-chemical characterization of the microhabitat of the epibionts associated with Alvinella pompejana, a hydrothermal vent annelid. *Geochimica Cosmochimica Acta,* 9, 2055-2066.

Gaill, F. & Hunt, S. (1986) Tubes of deep sea hydrothermal vent worms Riftia pachyptila (Vestimentifera) and Alvinella pompejana (Annelida). *Marine Ecology Progress Series,* 34, 267-274.

Gaill, F.; Desbruyères, D. & Laubier, L. (1988). Relationships between the "Pompeii worms" and their epibiotic bacteria, *Oceanologica Acta,* 8, 147-155.

Gaill, F. & Hunt, S. (1991). The biology of annelid worms from high temperature hydrothermal vent regions. *Reviews in Aquatic Sciences,* 4 (2-3), 107-137.

Haddad, A.; Camacho, F.; Durand, P. & Cary, S. C. (1995). Phylogenetic characterization of the epibiotic bacteria associated with the hydrothermal vent polychaete Alvinella pompejana, *Applied Environmental Microbiology,* 61, 1679-1687.

Hügler, M.; Wirsen, C. O.; Fuchs, G.; Taylor, C.D. and Sievert, S. M. (2005). Evidence for Autotrophic CO_2 Fixation via the Reductive Tricarboxylic Acid Cycle by Members of the ε Subdivision of Proteobacteria. *Journal of Bacteriology*, 3020–3027.

Jannasch, HW. Microbial interaction with hydrothermal fluids. In: Humphris SE, Zierenberg RA, Mullineaux LS, Thomson RE., editors. *Seafloor Hydrothermal Systems: Physical, Chemical, Biological and Geological Interactions.* Washington DC: AGU; 1995; 72-84.

Jeanthon, C. (2000). Molecular ecology of hydrothermal vent microbial communities. *Antonie van Leeuwenhoek*, 77, 117-133.

Johnson, K. S.; Childress, J.J.; Hessler, R.R.; Sakamoto-Arnold, C.L. & Beelher C.L. (1988). Chemical and biological interactions in the Rose Garden hydrothermal vent field, Galapagos spreading center. *Deep-Sea Research Part I*, 35, 1723-1744.

Julian, D.; Gaill, F.; Wood, E.; Arp, A. J. & Fisher, C. R. (1999). Roots as a site of hydrogen sulfide uptake in the hydrocarbon seep vestimentiferan Lamellibrachia sp. *The Journal of Experimental Biology*, 202, 2245-2257.

Le Bris, N.; Sarradin, P.M. & Caprais, J. C. (2003). Contrasted sulphide chemistries in the environment of 13°N EPR vent fauna. *Deep-Sea Research Part I*, 50, 737-747.

Le Bris, N.; Zbinden, M. & Gaill F. 2005. Processes controlling the physico-chemical micro-environments associated with Pompeii worms. *Deep-Sea Research Part I*, 52, 1071-1083.

Le Bris, N. & Gaill, F. (2007). How does the annelid Alvinella pompejana deal with an extreme hydrothermal environment? *Reviews in Environmental Science and Biotechnology*, 6, 197–221.

Luther, G. W.; Rozan, T. F.; Taillefert, M.; Nuzzio, D. B.; Di-Meo, C.; Shank, T. M.; Lutz, R. A. & Cary, S. C. (2001). Chemical speciation drives hydrothermal vent ecology. *Nature*, 410, 813-816

Maginn, E. J.; Little, C. T. S.; Herrington, R. J. & Mills, R. A. (2002). Sulphide mineralization in the deep-sea hydrothermal vent polychaete, Alvinella pompejana: implications for fossil preservation. *Marine Geology*, 181, 337-356.

Mill, P. J. (1978). *Physiology of Annelids*, New York, Academic Press.

Millero, F.J., 1996. *Chemical oceanography.* Boca Raton, New York, London, Tokyo, CRC Press..

Parkhust, D.L.; and Appelo, T. (1999). *User's guide to PHREEQC – a computer program for speciation, batch reaction, one-dimensional transport, and inverse geochemical modelling*. U.S. Geol. Survey, water-resource Invest.

Sarradin, P. M.; Caprais, J. C.; Briand, P.; Gaill, F.; Shillito, B.; & Desbruyères, D. (1998). Chemical and thermal description of the Genesis hydrothermal vent community environment (13°N, EPR). *Cahiers de Biologie Marine*, 159-167.

Taylor, C. D. & Wirsen, C. O. (1997). Microbiology and ecology of filamentous sulfur formation, *Science*, 277, 1483-1485.

Urcuyo, I. A.; Massoth, G. J.; Julian, D. & Fisher, C. R. (2003). Habitat, growth and physiological ecology of a basaltic community of Ridgeia piscesae from the Juan de Fuca Ridge. *Deep Sea Research Part I*, 50, 763-780.

Zhang, J Z & Millero, F J (1994). Kinetics of oxidation of hydrogen sulfide in natural waters. In: Alpers CN, Blowes, DW, editors. *Environmental Geochemistry of hydrogen sulfide in natural waters*, Washington DC: ACS, 393-411.

Zbinden, M.; Martinez, I; Guyot, F; Cambon-Bonavita, M-A; & Gaill, F. (2001). Zinc-iron sulphide mineralization in tubes of hydrothermal vent worms. *European Journal of Mineralogy*, 13, 653-658.

Zbinden, M.; Le Bris, N.; Compere, P.; Martinez, I.; Guyot, F. & Gaill, F. (2003). Mineralogical gradients associated with alvinellids at deep-sea hydrothermal vents. *Deep-Sea Research Part I*, 50, 269-280.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 177-192
ISBN: 978-1-60021-935-1

Chapter 5

ASSESSING BIOLOGICAL-PHYSICAL INTERACTION IN THE UPPER OCEAN FROM SPACE: ADVANTAGES AND PITFALLS

G. S. Karabashev
P. P. Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow, Russia

ABSTRACT

One important application of satellite ocean color observations is to advance the studies in biological-physical interaction (BPI) in the ocean. The satellite methods have to offer synopticity and space-time resolution of observations that are far beyond those of conventional techniques thus affording the capability to cover the BPI processes at local, regional, and global scales. As yet, this offer relies on comparative study of remotely sensed distributions of sea surface temperature (SST) as a physical feature of the marine environmentandchlorophyll concentration C from ocean color as a measure of phytoplankton abundance. As a rule, the ocean color bears no direct indication of chlorophyll but strongly depends on products of phytoplankton degradation that, supposedly, closely co-vary with chlorophyll at least in open ocean waters. This concept underlies present-day algorithms of retrieval of C from ocean color. Available literature on properties of optically significant admixtures of sea water gives grounds to expect that determinations of chlorophyll, based on this concept, tend to diminish actual differences in variability of living and "dead" matter in the marine environment which contradicts the goals of BPI research. Another drawback of "BPI-from-space" approach is in the fact that ocean color originates from the upper part of the euphotic layer of the ocean. Therefore, such an approach yields incomplete information at sites where phytoplankton collects in the seasonal pycnocline which usually belongs to the lower part of the euphotic layer. Observations of variability in chlorophyll concentration, CDOM fluorescence, and water temperature, induced by a subsurface intrusion, illustrate these circumstances. To summarize briefly, the ocean color methods can be regarded as a valuable support and reconnaissance tool of BPI research but in situ instrumentation and techniques are the

only means that provide data uniquely determined in physical nature which is of primary importance in the context of BPI.

INTRODUCTION

A challenging problem of biological-physical interaction (BPI) in the ocean has received the attention of oceanographers for many years. The BPI processes proceedunder highly variable environmental conditions characterized by an extremely broad range of spatial scales (10^{-3} – 10^{6} m) and time periods (10^{-1} – 10^{8} s). Investigations to understand these processes inherently imply, among others, the necessity to compare spatial distributions and time series of physical properties of marine environment and characteristics of populations of aquatic organisms. In contrast to continuously distributed physical properties, the population features describe discrete objects, some of which are able to spontaneously move in water which hampers direct comparisons of physics and biology in the context of BPI problem. Another difficulty was in the fact that marine biology techniques, based on specimen collection, were inconsistent with physical measurements in time- and space resolution.

A break-through occurred in the 1970s when first submersible fluorometers [Karabashev & Soloviev, 1973; Fruengel & Koch, 1976] made it possible to record chlorophyll fluorescence in water in the same way as a CTD probe records vertical profiles of water temperature and electric conductivity. The chlorophyll fluorescence intensity is a signal similar to that of a CTD probe in continuity and spatial resolution because 1) unicellular plants (phytoplankton), being the major carriers of chlorophyll and other photosynthetic pigments, are sufficiently abundant in ocean water and 2) the water volume of fluorescence origination in a typical fluorometer is sufficiently large. So, *in situ* fluorometry is able to cover at least the micro- and mesoscale variability with typical scales up to 10^{5} m when deployed from floating platforms. High sensitivity, lack of inertia, spectral selectivity, availability of hardware and other well-known advantages of fluorometry facilitated its introduction in marine research and combining with hydrophysical instrumentation that resulted in pioneer publications on BPI processes based on adequately collected data [Fasham, Pugh, 1976 and others].

Another approach has been initiated by Clarke and co-authors [1970]. They tested the feasibility of water constituents determination from spectra of solar radiation backscattered in the ocean surface layer and recorded with an airborne spectrometer. The backscattered water-leaving radiance increases with concentration of suspended matter and attenuates with content of light absorbers in water. The chlorophyll belongs to the latter exhibiting particularly strong absorption in the blue-violet range (peak at wavelength λ = 440 nm). So, it could be conceivable to observe a radiance minimum within this range and to use the deficit of radiance at the wavelengths of the minimum as a measure of chlorophyll content in water. However, no unambiguous spectral evidence of chlorophyll presence in water have been found during flights from the coastal zone to the Sargasso Sea periphery but it was discovered that "… the slopes of the spectra correlate quite closely with differences in chlorophyll concentration". The authors suggested that "…spectrophotometric procedures from aircraft (and perhaps from satellites) will be of great value in the rapid investigation of oceanic conditions, including conditions important for biological productivity". The idea was picked

up and developed so fast that the first "proof-of-concept" mission of satellite ocean color scanner CZCS (Coastal Zone Color Scanner, NASA) took place in 1978-1984 bringing a lot of information valuable for new insight of ocean life and for further development of the approach. Determination of chlorophyll concentration in water is one of the most demanded products of present-day ocean color scanners (OSC) such as SeaWiFS (Sea-viewing Wide Field-of-view Sensor), MODIS (Moderate-Resolution Imaging Spectroradiometer), and MERIS (MEdium Resolution Imaging Spectrometer Instrument).

Both MODIS and MERIS were added with a new capability to determine the chlorophyll not only from ocean color but also from chlorophyll fluorescence excited by solar radiation (so called natural fluorescence) [Abbot, Letelier, 1999]. This is the same physical phenomenon that is exploited in submersible fluorometers but the specificity of origination and excitation imparts some particular properties to the signal of natural fluorescence as a source of information on chlorophyll distribution in the ocean.

Thus, at present, chlorophyll concentration in water C is the only physical feature of living marine organisms, widely used as indication of phytoplankton abundance and can be assessed *in situ* and remotely. The sea surface temperature (*SST*) is the only fundamental physical characteristicof marine environment measurable both *in situ* and from space. At present, direct comparison of measured fields of C and *SST* is the only way to study BPI problem based on remote sensing. A more flexible approach is feasible when studying BPI from data of submersible instrumentation. The latter makes possible to involve much more informative set of fundamental physical properties of marine environment (water salinity S, water density σ_t,current velocity V etc.) and to add the C determinations with concurrently measured characteristics of photosynthetic activity of phytoplankton. The goal of the present commentary is to focus attention on nature of quantities and procedures relevant to BPI research.

THE NATURE OF REMOTELY SENSED CHLOROPHYLL

An estimate of the normalized water-leaving radiance spectrum $L_{WN}(\lambda)$ is a starting point of any procedure of chlorophyll determination from remote sensing data. $L_{WN}(\lambda)$ is computed from radiance recorded by an orbiting sensor that views the ocean through the terrestrial atmosphere. Here "normalized" means that 1) atmospheric contribution to the recorded radiance is eliminated and 2) the radiance is reduced to "normal" conditions, i.e., the Sun is at zenith and the sensor views water at nadir [Gordon et al., 1988]. In many studies spectral reflectance $R_{rs}(\lambda) = L_{WN}(\lambda)/ E_0(\lambda)$ is used instead of radiance ($E_0(\lambda)$ which is a downwelling irradiance just above the air-water interface). Being normalized, both quantities depend exclusively on concentration and composition of scatterers and absorbers of light in water. It was shown that

$$L_{WN}(\lambda) = \mathrm{f}\,[b_b(\lambda)/(a(\lambda) + b_b(\lambda))], \quad (1)$$

where $a(\lambda)$ is absorption coefficient and $b_b(\lambda)$ is backscattering coefficient of marine environment. According to present-day models of sea water optical properties,

$$b_b(\lambda) = b_{bw}(\lambda) + b_{bp}(\lambda), \quad (2)$$

$$a(\lambda) = a_w(\lambda) + a_{\mathrm{CDOM}}(\lambda) + a_{ch}(\lambda) + a_{cp}(\lambda), \quad (3)$$

$$a_{\mathrm{CDOM}}(\lambda) = a_{\mathrm{CDOM}}(\lambda_0) \exp[(-\gamma(\lambda - \lambda_0)] \quad (4)$$

where $b_{bw}(\lambda) \sim \lambda^{-4}$ accounts for backscattering due to density fluctuations of pure water and $b_{bp}(\lambda) \sim \lambda^{-n}$ (with $0 < |n| < 4$) is the backscattering coefficient of suspended particles,$a_w(\lambda)$ is absorption coefficient of pure water, $a_{\mathrm{CDOM}}(\lambda)$ is absorption coefficient of colored dissolved organic matter (CDOM) of natural origin, $a_{ch}(\lambda)$ is absorption coefficient of photosynthetic pigments (mainly chlorophyll *a*) of phytoplankton, and $a_{cp}(\lambda)$ is absorption coefficient of non-algal colored suspended particles.These coefficients depend on concentrations of respective substances in water which is the basis for determination of optically significant constituents of aquatic environment from data of *in situ* and remote observations. This presentation of optical properties of sea water is far from being exhaustive but appears helpful for brief qualitative description of main factors of L_{WN} origination.

The spectra of listed coefficients are shown in Figure 1 exclusive of $a_{cp}(\lambda)$ as the least studied and significant among them.As a rule, $b_{bp} >> b_{bw}$. When large particles of size $d >> \lambda$ control the backscattering, b_{bp} (λ) is flat, i.e., b_{bp} is independent of wavelength of solar radiation; if$d << \lambda$ (fine scatterers dominate the particle pool),b_{bp} (λ) is close to $b_{bw}(\lambda)$ in shape. Particle size distribution in the ocean widely varies in time and space but in any case there are no mechanisms of backscattering capable to produce extrema of water-leaving radiance in the visible.The strongest wavelength dependence ischaracteristic of $a_{\mathrm{CDOM}}(\lambda)$, usually $0{,}013 < \gamma < 0.017$ nm^{-1}. Absorption limits light penetration in water. $a_{\mathrm{CDOM}}(\lambda)$ and $a_w(\lambda)$ are monotonous functions of opposite spectral trend so that their sum exhibit a wide minimum (about 500 nm in Figure 1). Its position on the wavelength scale depends exclusively on the CDOM amount and property because $a_{\mathrm{CDOM}}(\lambda) << a_w(\lambda)$ in the red where light is strongly absorbed by pure water at constant rate all over the World Ocean. The wavelength range of absorption minimum is known as "transparency window" (TW) since solar radiation of such spectral composition penetrates water deeper than sunlight at shorter and longer wavelengths.

The autochthonous CDOM is a stable product of natural decomposition of marine organisms and, generally, is more abundant in surface ocean waters of higher biological productivity. This results in characteristic relationships between biological productivity and water-leaving radiance spectra of aquatic areas. Oligotrophic ones are poor in CDOM and their suspended matter is richer in fine particles so that $b_{bp}(\lambda)$ is close to $b_{bw}(\lambda)$ in shape. In this case TW falls in 470-490 nm interval and $L_{WN}(\lambda)$ is characterized by more or less monotonous growth with diminishing wavelength. Large areas of oceanic "deserts" on both sides of the equator exhibit such radiance spectra. In the eutrophic regions, the shorter is the wavelength, the stronger abundant CDOM attenuates the backscattered sunlight according to (5), so that TW shifts to 540–570 nm and L_{WN} peaks approximately in this spectral range. In waters of moderate bioproductivity, the L_{WN} peak falls in 490–540 nm range and its short-wavelength slope is not as steep as in the case of eutrophic waters.

$a_{ch}(\lambda)$ is the only coefficient exhibiting characteristic spectral structure as short- and longwave maxima of light absorption. When measured in living plankton cells, they are much smoother than in Figure 1. Absorption spectra of living phytoplankton cells can be particularly complicated due to various species of chlorophyll and other photosynthetic pigments. Chlorophyll *a* is a permanent member of pigment pool in a plant cell and, hence, at least the strongest short-wave absorption maximum of chlorophyll *a* is likely to manifest itself as a minimum in the blue-violet range of radiance spectrum in agreement with (1).

In some cases, such a pattern actually takes place. One of the most unambiguous chlorophyll signature on the spectrum of water-leaving radiance is displayed in Figure 2, curves *2* and *3* . This signature is particularly convincing owing 1) to reflectance maxima in the red where chlorophyll *a* fluorescence, excited at shorter wavelengths, adds together with the backscattered red solar radiationand 2) to inverse ratio of amplitudes of blue-violet minima and red maxima of reflectance (fluorescence intensity and light absorption grow with content of a fluorescent chlorophyll). The shape of spectrum *1* in Figure 2, deprived of measurable chlorophyll-related extrema, is quite common in waters of poor bioproductivity. Such extrema lack in radiance spectra of waters of higher trophicitytoo. It is universally recognized that chlorophyll in a living plant cell is "packed" in chloroplasts so that outer layers of the latter shade the inner ones which results in "underabsorption" of light by chlorophyll as compared to the absorption of the same amount of unpacked chlorophyll molecules. There are other mechanisms of light harvesting by photosynthetic pigments in a living plankton cell that reduce their contribution to shaping the spectra of water-leaving radiance.

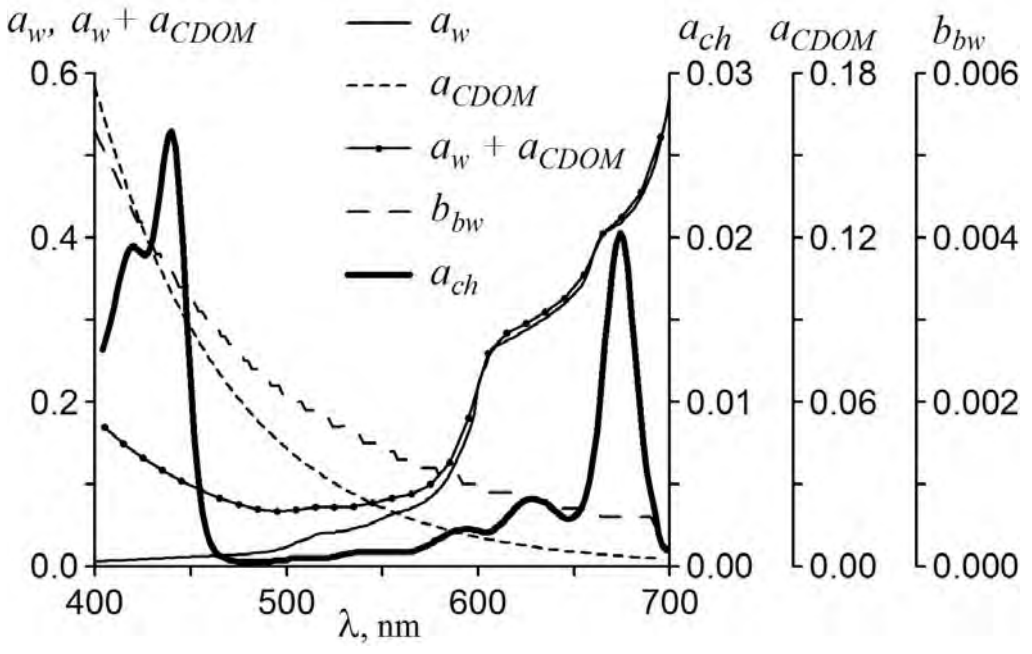


Figure 1. Spectra of absorption coefficient of pure water, a_w, m^{-1}; absorption coefficient of acetone solution of chlorophyll, a, m^{-1}; absorption coefficient of CDOM of moderately transparent sea water, $a_{CDOM}(\lambda)$, m^{-1}; backscattering coefficient of pure water, b_{bw}, m^{-1}.

The use of ratio of spectral-different radiances as a measure of chlorophyll content in marine environment regardless of chlorophyll manifestation in radiance spectra was justified by the idea: in the open ocean, all of the optically significant admixtures of the ocean water originate from phytoplankton (Case I waters [Morel, 1988]). Photosynthetic pigments, including chlorophyll, are essential part of unicellular plants. Since their abundance control the radiance ratio, the empirical relation between radiance ratio and chlorophyll content can be employed to retrieve the latter from the estimates of spectral water-leaving radiance. Decades of collective efforts of NASA team and associated researchers resulted in the SeaBAM data set of about 3000 synchronous determinations of radiance spectra and chlorophyll concentration collected all over the World Ocean. This set is a basis for development of algorithms aimed at retrieval of water properties, chlorophyll content inclusive, from the spectral radiance determinations [O'Reilly et al., 1998]. No universal approach has been found as yet for the Case II waters where optical-significant admixtures of marine origin co-exist with foreign colored and light scattering substances from different sources (continental run-off, Aeolian dust, resuspension of bottom sediments, pollution etc). Case II waters involve part of the shelf zone, inland seas, straits, open sea shallows, areas penetrated by river plumes and so on.

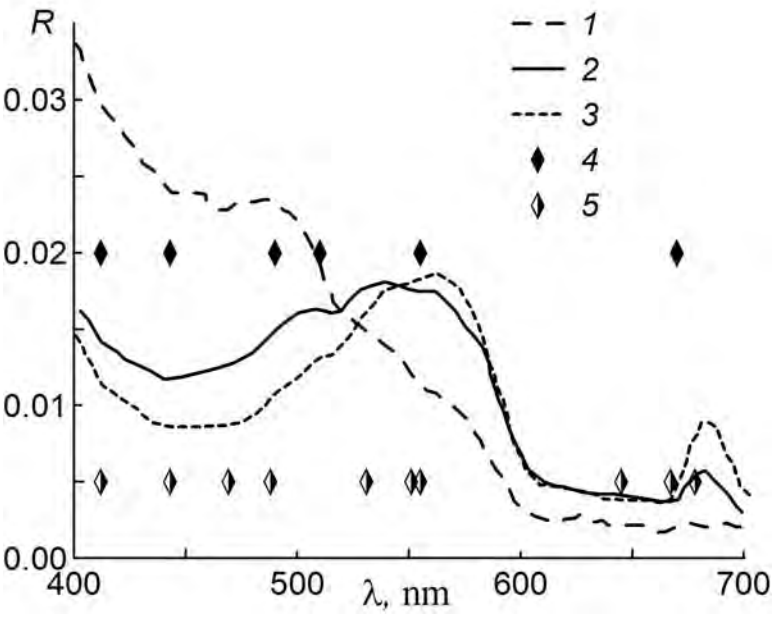


Figure 2.Dependence of reflectance R_{rs} on wavelength λ recorded with a floating spectroradiometer in November, 2003, in the zone of Malvinas/Brazil currents interaction outside (1) and within (2, 3) a body of chlorophyll-enriched waters[1]. Diamonds mark center wavelengths of spectral band of OCSs SeaWiFS (*4*) andMODIS (*5*).

Ten years of SeaWiFS operation plus observations of younger OCSs have brought enormous bulk of new information, opened new avenues of oceanographic research from

[1] The author is grateful for this unpublished experimental evidence to V.I. Burenkov and his team who collected the data during cruise 17 of R/V "Akademik Sergey Vavilov" in 2003.

space, and stimulated studies in mechanisms of interaction of solar radiation and sea water constituents. At the same time, a number of dark nooks remains in a temple of ocean color remote sensing.

To be universally valid, the concept of Case I waters requires one and the same residence time (RT) in water for phytoplankton, on the one hand, and, on the other, for CDOM molecules, suspended particles, and other products of phytoplankton degradation that shape $L_{WN}(\lambda)$. The phytoplankton RT falls in the range of one day (approximate rate of cell division) to two-three weeks (typical duration of phytoplankton blooms). It is much more difficult to assess the RT of products of living functions and phytoplankton degradation due to intricacy and multi-stage nature of involved processes.Using a submersible fluorometer, the author observed a two-fold increase in surface layer CDOM florescence intensity in three days after beginning of a phytoplankton bloom at site off Pacasmayo (Peru) that occurred on the background of local upwelling at steady wind and negligible convection (March 1974, 17th cruise of R/V "Akademik Kurchatov", unpublished). Origination of fluorescent material from phytoplankton cell exudates having RT of hours to days appears the most likely mechanism of CDOM fluorescence increase. Such substances are classed as labile marine dissolved organic carbon (DOC) according to Dafnerand Wangersky [2002]. These authors consider DOC as semi-labile if it turns over on a seasonal time scale, and report estimates of RT about 1300 years for the surface layer DOC and 6000 years for abyssal thickness DOC as refractory components in the oligotrophic gyre of the central North Pacific.

These RT estimates are valuable as landmarks but they cannot be directly attributed to CDOM. Examples of strong positive correlation between the absorption of the near UV radiation in sea water and fluorescence of the latter under UV excitation have been obtained by many researchers based on water sampling, *in situ* measurements, or remote sensing [Kalle, 1949; Brown, 1974; Karabashev & Zangalis, 1974; Hoge et al., 1993; Green & Bough, 1994 and others]).Further still, it was proposed to quantitatively assess the CDOM absorption from UV-excited fluorescence of natural sea water [Huge et al., 1993; Green & Bough, 1994]. However, the estimates of correlation between the same light absorption/fluorescence and concentration of DOC, or C_{orgi}, vary over wide limits in the active layer of the ocean being mostly close to zero [Stewart & Wetzel, 1981; Karabashev, 1987;]. This prompts to infer that the total DOM involves a fraction of the chromophore- and fluorophore-containing DOM that behaves in a specific manner.

Observations with submersible fluorometers revealed the following patterns [Karabashev & Soloviev, 1975; Karabashev, 1999]. In the oligotrophic ocean waters, the fluorescence intensity of DOM is minimal and quasi-constant in the upper mixed layer but enhances with depth in the pycnocline to a more or less permanent level. The surface fluorescence increases with bioproductivity so that surface maximum of CDOM fluorescence is typical of the eutrophic areas such as coastal upwellings where phytoplankton is abundant just below the air-water interface. This is consistent with the idea that "…labile and semi-labile pools play an important role in the DOC cycling in the surface waters, while refractory material is important in the deep and intermediate waters. In productive surface waters, as much as a third to a half of the DOC may be present as labile material, degraded biologically in hours to days". [Dafnerand Wangersky, 2002]. In oligotrophic aquatic areas, the phytoplankton accumulates in tens of meters below the water surface which makes unlikely that the much shallower remotely sensed layer of such areas contains labile or semi-labile DOC in meaningful quantities. Hence, the lower is bioproductivity of waters, the more likely is the

dominance of refractory CDOM in the light-absorbing DOC pool within a remotely sensed layer. If so, a chlorophyll distribution computed from a single image of an aquatic area on the basis of Case I water algorithm can be misleading in a sense that it describes behavior of degradation products of phytoplankton having nothing in common with phytoplankton at the instant the image has been recorded. Under stationary, low dispersion conditions the same algorithm can be useful since water constituents are independent of time. Long-period averaging of data of image series is another approach to obtain meaningful estimates of chlorophyll determinations from remote sensing of Case I waters. Better predictability of primary production from bio-optical models, achieved through data averaging over half-year or longer time intervals [Siegel et al., 2001], may be due, among others, to filtering out the effects of residence time of remotely sensed water constituents.

These effects impose a limitation on applicability of ocean color remote sensing to solving the BPI problems in the domain of mesoscale variability (kilometers to hundreds of kilometers in space and hours to weeks in time).Such an attempt has been already undertaken and demonstrated "… the global generality of previous local and regional findings that small-scale variability in ocean biology and physics occurs on comparable spatial length scales." [Doney et al., 2003]. Is this inference true or results from the fact that surface chlorophyll concentrations were "… calculated from the ratios of different water-leaving visible bands using ground-truthed empirical algorithms", based on the Case I water concept?The question is justified by the fact that this spatio-temporal domain involves physical forces of different nature and a lot of diverse non-linear reactions of the smallest and most abundant marine organismsto these forces (for instance, dependence of primary production on mixing, light conditions, water upwelling etc). The authors acknowledge the need in further work: "Two avenues to explore are cross-correlation studies of high spatial resolution sea surface height, sea surface temperature, and ocean color data and eddy resolving coupled biological-physical models." However, both avenues have chances to end up as impasses if BPI processes will be treated regardless of vertical extent.

IMPORTANCE OF VERTICAL EXTENT

The IR and microwave imagery of a water body originate from a surface film less than 1 mm thick, and is informative of events, occurring in the underlying layer, only if they manifest themselves just at the air-water interface. Advantage of the ocean color images is in the fact that water-leaving radiance originate from a layer that can be tens of meters thick. It was shown [Gordon and McCluney,1975] that 90% of L_{WN} originate from above the depth

$$Z = 1/ K_d, \tag{5}$$

where K_d is vertical attenuationcoefficient of solar radiation. Being one of the best studied optical features of marine environment, $K_d(\lambda)$ was used by N. Jerlov as a basis of the optical classification of ocean waters and fully considered in his monograph [1976]. $K_d(\lambda)$ is similar in shape to spectrum [aw(λ) + $a_{\mathrm{CDOM}}(\lambda)$] in Figure 1 so that maximal $Z_{TW} \approx 50$ m occurs in oligotrophic gyres on either side of the equator with minimal $K_d \approx 0{,}02$ m^{-1} at wavelengths of TW. In the red, $Z \leq 2$ m everywhere due to strong absorption of light by pure water. Z is the

most variable in the violet under the influence of inconstancy of CDOM. Hence, ability of OCSs to directly sense the objects and events below the ocean surface substantially depends on operational spectral band, optical properties of water, and thickness of the upper mixed layer Z_{mix}. Condition $Z_{mix} > Z_{TW}$ holds usually in the vast oligotrophic ocean provinces which prevents from immediate sensing of phytoplankton or products of its degradation that collect in the seasonal pycnocline below the mixed layer. Both $Z_{mix} < Z_{TW}$and $Z_{mix} > Z_{TW}$ occur in waters of higher bioproductivity.

Considernow spatial distributions of quantities, most closely related to those derived from estimates of radiance at the height of an orbiting sensor, but measured under conditions corresponding to Case I waters with the submersible fluorometer MZF [Karabashev, Khanaev, 1988] designed for synchronous recording of vertical profiles of water temperature T, fluorescence intensity F of CDOM and fluorescence intensity of chlorophyll. Fluorescencewas excited and measured in conventional spectral ranges (violet-blue and red for chlorophyll, near UV and blue–green for CDOM, respectively). A xenon strobe lamp emitted light pulses at a rate of 5 Hz to excite both fluorescences. The vertical profiles of chlorophyll concentration C were calculated based on chlorophyll fluorescence profile and standard determinations of chlorophyll *a* in water samplescollected concurrently with fluorometer operation. A temperature channel with awired sensor ensured resolution 0.03°C in temperature range 0–33°C. The instrument submersion depth was measured from 0 to 250 m at 0.25 m resolution. The output parameters of optical channels enabled to measure fluorescence intensity in linear scale in waters of diverse trophicity. The submersible part of the instrument with optics and digitized system of data collection and preprocessing was connected to onboardmodule with a single wire armored cable. Operational features of the instrument made it suitable for observations onboard a research vessel under the most severe weather conditions.

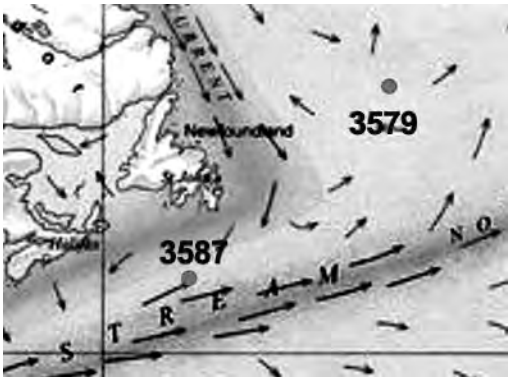


Figure 3. Position of stations 3579 and 3587 relative to the Labrador current (blue shade) and the Gulf Stream (magenta shade) as presented in the chart of the Atlantic mean surface currents in August. Sta 3579: 50°00′ N, 42°00′ W;September 8,1991;starting at 11:22GMT. Sta 3587: 43°06.0′ N, 54°38.3′ W; September 18, 1991;starting at 00:25 GMT.

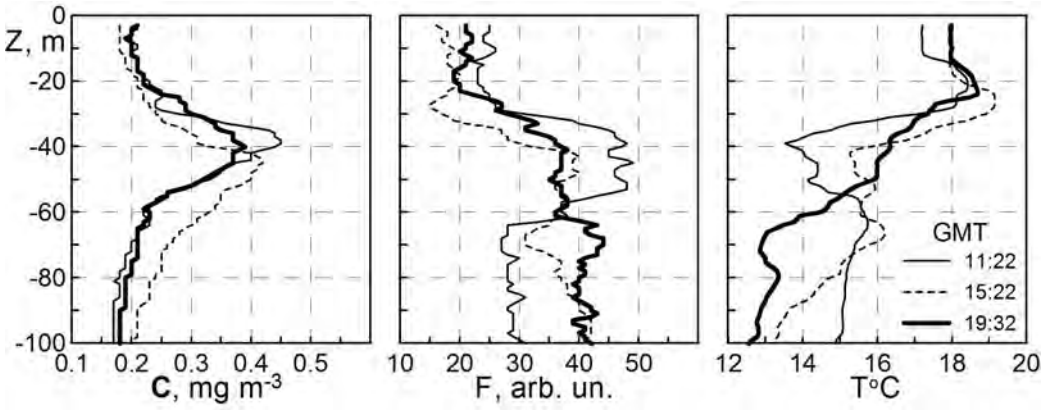


Figure 4. Vertical profiles of chlorophyll concentration C, fluorescence intensity F of CDOM, and water temperature T recorded at sta 3579 with the MZF fluorometer at 11:22, 15:22, and 19:32, GMT.

The Case I water example is dedicated tomanifestations of a subsurface intrusion discovered during the 23d cruise of the R/V *Vityaz* in September, 1991, in the western Atlantic ocean at stations 3579and 3587 (Figure 3).

At sta 3579, profiles of F and T for GMT 11:22 exhibited a disturbancefrom 35 to 55 m depth where water was cooler and fluoresced brighter relative to the upper and lower layers (Figure 4). Similar but weaker and somewhat deeper disturbance is evident in F and T profiles for GMT 15:22. There was no meaningful extrema in F and T profiles for GMT 19:32 except temperature minimum and F maximum of reduced amplitude at the depth of 70 m. On this background, the C profiles demonstrated better stability in shape and depth of their single peak that gravitated towards the upper part of the disturbance in F and T profiles.

Ten days later, similar disturbance of the profiles was found when starting works at sta 3587. It was decided to conduct serial observations of higher space-time resolution. Their results are shown in Figure 5. Four series of seven fluorometer castings each have been fulfilled at night when light harvesting ability of phytoplankton pigment system is unaffected by ambient light and linear dependence of the intensity of red fluorescence of phytoplankton on chlorophyll concentration in water appears the most probable. The series started at strong western wind that calmed down towards the end of observation period. The vessel driftedeastwards all the time, but because of its gradual deceleration the distances between castings dropped from initial 500 m of the first series to 110-130 m of the final one. In total, drift length made up 10-12 km. This should be kept in mind when considering data scatter in Figure 5.

The observations at sta 3587 revealed better reproducibility of T and F profiles for comparable time periods, greater amplitude of their disturbance, and slightly shallower C peaks in reference to patterns of sta 3579.Fairly low scatter of estimates of T, F and C in the upper mixed layer and below the 80 m depth allow us to consider stronger scatter of profiles at the depths of the disturbance as indication of actual spatio-temporal variability of the latter. To all appearance, the disturbance in T and F profiles was due to a subsurfaceintrusion of slope or/and Labrador waters that are the nearest suppliers of cold, CDOM-rich waters to sites of stas 3579 and 3587. However, exhaustive physical interpretation of patterns in Figure 4 and 5 is beyond the scope of the present study, especially because of exclusive complexity of

oceanography of the study area. Further consideration concerns the issues, relevant to BPI from space.

1) Mean water temperature, averaged over the upper 10 m thick layer for every series in Figure 5, changed as 17.8, 18.0, 17.6, 17.9 °C with standard deviation 0.18 °C. The latter is lower than errors of SST determination from satellite IR radiometers. So, it appears unlikely to recognize from space the intrusion-related patterns of temperature distribution at the air-water interface at environment conditions at sta 3587. The data of sta 3579 are too sparse to reach a definite conclusion.
2) Determinations of K_d at the same stations[2] have given $K_d(465) = 0.068$ m^{-1} and$K_d(525) = 0.084$ m^{-1} for sta 3579 and $K_d(465) = 0.043$ m^{-1} and$K_d(525) = 0.055$ m^{-1} for sta 3587. Substituting the lowest K_d in (6) yields $Z_{3579} \approx 15$ m and $Z_{3587} \approx 23$ m as estimates of thickness of the layer of water-leaving radiance origination. They may be slightly underestimated since waters under consideration are likely to belong to areas where TW falls in the range of 480 to 500 nm. Even if to assume overestimated $25 < Z_{TW} < 30$ m, none of the spectral channels of any OCS is able to reveal occurrence of described intrusion whose main body underlies this depth (Figure 4 and 5).
3) Being different in sign, the gross-structure of F profiles in Figure 4 and 5 "mimics" that of the temperature vertical distributions. In contrast, profiles of C keep their specific shape, and their peaks tend to occur within a narrow depth range regardless of perturbations in such environment properties as F and T. This is particularly evidentin Figure 4, profiles 11:22 and 19:32. As is known, ability to withstand the environment forcings of moderate strength is a basic property of living phytoplankton cells achievable be means of different mechanisms.Be it as it may, the patterns at stas 3579 and 3587 clearly point to the fact that using CDOM feature as a substitute for a physical characteristic of phytoplankton makes less evident the actual differences in variability of living and "dead" matter in marine environment which is unacceptable in the context of BPI research.

ALTERNATIVES TO RADIANCE BAND-RATIO METHODS

The spectra *2* and *3* in Figure 2 exemplify conditions for direct remote determination of chlorophyll from explicit manifestation of its absorption as the minimum of$R_{rs}(\lambda)$ centered at about 440 nm. To take advantage of this opportunity, it suffice to develop a technique for calculating the differencebetween $R_{rs}(\lambda)$ and a base curve at wavelengths of chlorophyll absorption band. The base curve is meant to be the spectrum of water-leaving radiance or reflectance of water with the same set of admixtures exclusive of chlorophyll. Unfortunately, this is not an easy matter to define the base curve from measurements because the present-day OCSs operate in the visible (Figure 2, symbols *4* and *5*) while the short-wavelength absorption band of chlorophyll extends to the UV range (Figure 1,$a_{ch}(400) >> 0$). In addition,

[2] K_d values have been adopted as given in a section of the cruise report prepared by Dr. B. Wozniak and his team from IO PAN, Sopot.

the L_{WN} at λ < 450 nm is often underestimated due to imperfections of atmospheric correction procedures. For these reasons, it seems more realistic to develop airborne and/or *in situ* UV-sensitive spectroradiometers to study origination of chlorophyll-indicative radiance spectra and their potential in BPI research.

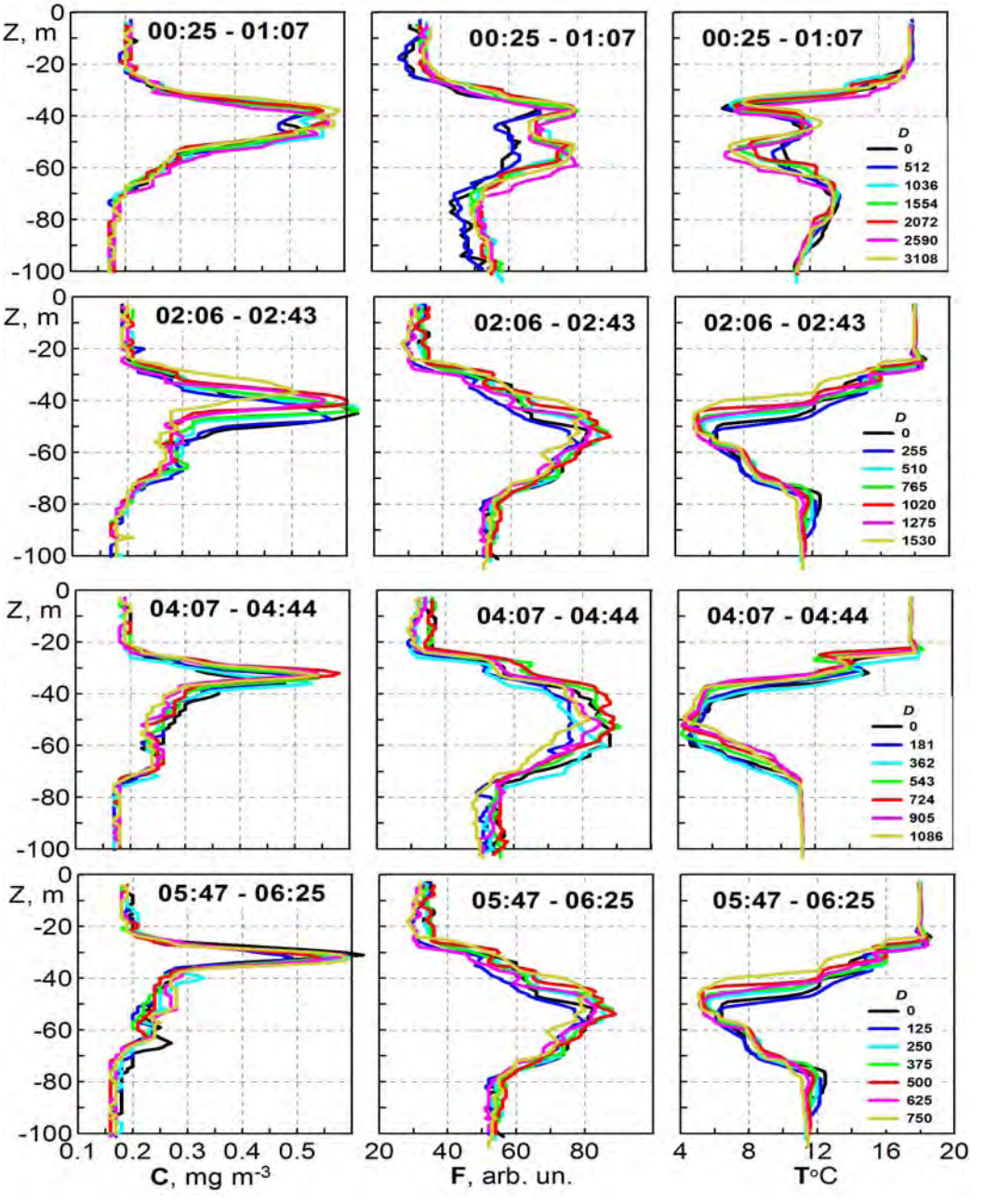


Figure 5. Vertical profiles of chlorophyll concentration, C, fluorescence of CDOM, F, and water temperature, T, plotted from data of series of repetitive castings of MZF fluorometer at sta 3589 (Figure 1). *D* is the distance between the starting point of a series and next castings.

Expression (1) and spectral functions (2)-(5) underlie the semi-empirical approach (SEA) to retrieval of chlorophyll from ocean color. In this case, the estimates of L_{WN} or R_{rs} are used to calculate empirical constants in expressions involved in a SEA model rather than to determine the pigment from the chlorophyll/band ratio regression. The SEA approach is advantageous in flexibility and, being sensitive to the physical meaning of involved quantities, makes it possible to account for dependences of chlorophyll optical properties on environment conditions [Carder et al., 2004]. If a SEA model is efficient, the right-hand part of (1) has to restore the shape of measured $L_{WN}(\lambda)$ in the left-hand part of (1). It is impossible to restore the shape of $L_{WN}(\lambda)$ such as spectra *2* and *3* in Figure 2, without substituting the spectral dependence of pigment absorption $a_{ch}(\lambda)$ into the relevant expressions. But $a_{ch}(\lambda)$ is needless if $L_{WN}(\lambda)$ carries no measurable indications of radiance minima caused by pigment absorption, as is the case of spectrum *1* in Figure 2 and of majority of water-leaving radiance spectra recorded in ocean areas of diverse trophicity. To exhaustively describe such spectra, it is suffice to know the features of light absorption by water and CDOM and light scattering by particles and water. Hence, it appears that SEA models, being applied to the most common$L_{WN}(\lambda)$ deprived of chlorophyll signature, borrow a fraction from CDOM absorption and ascribe it to photosynthetic pigments. If so, a SEA approach is as risky as a band-ratio method in the context of BPI.

In this respect, the natural fluorescence of chlorophyll (NFC) is a riskless choice since chlorophyll is the only sea water admixture that is able to emit light in the red. Therefore, it is relatively easy to separate NFC signal from the background when substantially weakened due to proper strong absorption of water. However, the latter reduces the thickness of NFC origination layer to 1,5-2 m which narrows diagnostic capabilities of NFC approach. In addition, the light harvesting ability of surface phytoplankton varies with light conditions. For instance, chlorophyll fluorescence of diatoms exhibits diurnal periodicity caused by self-shading due to coarsening of intracellular chlorophyll grains at bright solar illumination [Kiefer, 1973]. According to observations in tropical Pacific and Indian oceans, the diurnal periodicity is such that the upper part of vertical profiles of chlorophyll fluorescence intensity adheres to the cosine wave, that attenuates with depth, and, consequently, shallow maxima of fluorescence profiles turn out to be much sharper at noon as compared with those at midnight on the background of unchanged chlorophyll concentration[Karabashev, Soloviev, 1977; Karabashev, 1987]. This phenomenon was sometimes observed at moderate latitudes too. Other types of diurnal variability often co-exist with the light-induced diurnal periodicity of chlorophyll fluorescence, so the question of its contribution to NFC behavior still remains to be answered.

CONCLUSION

Undoubtedly, at present, many research teams are able to provide more examples of deficiency of indirect determinations of properties of marine biota in areas of highly variable oceanography classed as Case I waters in works of remote sensing domain. It will suffice to mention the regions of meandering western boundary currents, offshore periphery of large coastal upwellings, the contact zones of oceanic surface currents of different water properties, and the like. These are precisely the regions where BPI studies are the most promising both in

fundamental and practical aspects. By definition, the same is true concerning so called Case II waters. Hence, there are ocean provinces where particular attention should be given to residence time of sea water constituents relevant to applicability of products of present-day ocean color missions to BPI research. But the depth limit appears the most severe restriction of ocean color data in the context of BPI: it is impossible to imagine a successful phytoplanktonologist ignorant of processes in the lower half of the euphotic zone of the ocean.

Nevertheless, combined satellite observations of ocean color and hydrophysical characteristics are irreplaceable in synopticity and spatio-temporal resolution to an extent that they appear valid at least as a support and reconnaissance tool among the means of BPI experimental exploration. Obviously, *insitu* instrumentation constitutes a core of such means but a lot of efforts and inventiveness are needed to bring it to a level adequate to BPI requirements. It would be excellent to eliminate the monopoly of fluorescence as the only high-speed proxy of chlorophyll and to turn to the chlorophyll absorption whose concentration dependence is less influenced by environment conditions and physiological state of phytoplankton.

The present chapter is not, under any circumstances, to be regarded as a review of the BPI problem or, especially, of methods of chlorophyll determination from ocean color. Otherwise the reference list would have to be much longer. This commentary is meant only as a precautionary note addressed mainly to people without a background in experimental hydrooptics and/or ocean color remote sensing but interested in the application of their specific skills to the challenging problem of BPI as an inherently interdisciplinary research field.

REFERENCES

Abbott, M.R. &Letelier, R.M. (1999). Algorithm Theoretical Basis Document. Chlorophyll Fluorescence (MODIS Product Number 20). URL: *http://eospso.gsfc.nasa.gov/ftp_ATBD/REVIEW/MODIS/ATBD-MOD-22/atbd-mod-22.pdf*

Brown, M. (1974). Transmission spectroscopy investigations of natural waters. Re. Inst. Fysisk Oceanigrafi Univ. *Copenhagen,* No. 29, 21 pp.

Carder, K.L., Chen, F.R., Cannizzaro, J.P., Campbell, J.W., & Mitchell, B.G. (2004). Performance of the MODIS semi-analytical ocean color algorithm for chlorophyll-a. *Advances in Space Research,* vol. 33, 1152–1159.

Clarke, G. L., Ewing, G. C., & Lorenzen, C. J.(1970). Spectra of backscattered light from the sea obtained from aircraft as a measure of chlorophyll concentration. *Science,* vol. 167, No. 3921, 1119-1121.

Dafner, E.V. & Wangersky, P. J. (2002). A brief overview of modern directions in marine DOC studies. Part II– Recent progress in marine DOC studies. *J. Environ. Monit.*, vol. 4, 55–69.

Doney, S.C., Glover, D.M. & McCue, S.J. (2003). Mesoscale variability of Sea-viewing Wide Field-of-view Sensor (SeaWiFS) satellite ocean color: Global patterns and spatial scales. *Journal of Geophysical Research,* vol. 108, No. C2, 3024, doi:10.102912001J C000843.

Fasham, M.J. & Pugh, P.R. (1976). Observations on the horizontal coherence of chlorophyll "a" and temperature. *Deep-Sea Res.,* vol. 23, 539-550.

Fruengel, F. & Koch, C. (1976). Practical experience with the Variosense equipment in measuring chlorophyll concentration and fluorescent substances, like rhodamin, fluoresceine and some other substances. *IEEE Journ. of Oceanic Eng.,* OE1, No. 1, 21-32.

Gordon, H.R. & McCluney, W.R. (1975). Estimation of the depth of sunlight penetration in the sea for remote sensing. *Applied Optics, vol.* 14, No. 2, 413-416.

Gordon H.R., Brown O.B., Evans R.H., Brown J.W., Smith R.C., Baker K.S. & Clark D.K. (1988). A Semianalytic Radiance Model of Ocean Color. *Journal of Geophysical Research,* vol. 93. № D9, 10909-10924.

Green, S. A. & Bough, N. V. (1994). Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. *Limnology and Oceanography,* vol. 39, No. 8, 1903-1916.

Hoge, F.E., Vodacek, A. & Bough, N.V. (1993). Inherent optical properties of the ocean: Retrieval of the absorption coefficient of chromophoric dissolved organic matter from fluorescence measurements, *Limnology and Oceanography,* vol. 38, No. 7, 1394-1402.

Jerlov N.G., Marine Optics. Elsevier Oceanogr. Ser. 14. Amsterdam: Elsevier. 1976

Kalle, K. Fluoreszenz und Gelbstoff im Bottnischem und Finnischem Meerbusen. (1949).*Deutsche Hydrographische Zeitschrift,* Bd. 2, S. 117-124.

Karabashev, G.S. & Khanaev, S.A. (1988). Submersible multichannel fluorometer for marine ecological research. *Zhurnal Prikladnoy Spektroskopii,* vol. 48, No. 3, 515-518 (In Russian).

Karabashev, G.S. & Zangalis, K. P. (1974). Absorption of ultraviolet radiation and luminescence of substances dissolved in the sea water. Izvestiya Akademii Nauk SSSR, ser. *Fiziki atmosfery i okeana,* vol. 10, No. 7, 801-802 (In Russian).

Karabashev, G.S. (1999). On the potentialities of an objective classification of vertical profiles of DOM fluorescence in the active layer of the ocean. *Oceanology,*vol. 39, No. 5, 668-674.

Karabashev, G.S. Fluorescence in the ocean.Leningrad: Gidrometeoizdat; 1987 (in Russian).

Karabashev, G.S.,& Soloviev, A.N. (1973). Pulse-light submersible fluorometer IPF-70 for marine research. *Okeanologiya,* vol. 13, No. 2, 361-366 (In Russian).

Karabashev, G.S.,& Soloviev, A.N. (1975). Regularities of origination of vertical distributions of fluorescence intensity of dissolved organic matter in the active layer of the ocean. Trudy IO AN SSSR, vol. 102, 89-93 (In Russian).

Karabashev, G.S.,& Soloviev, A.N. (1977). Spatial and temporal variability of pigment fluorescence in living phytoplankton cells. *Polish Arch. Hydrobiol.,* vol. 24 Suppl., 201-213.

Kiefer, D.A. (1973). Chlorophyll a fluorescence in marine centric diatoms: response of chloroplasts to light and nutrient stress. *Marine Biology,* vol. 23, 39-46.

Morel, A. (1988). Optical modeling of the upper ocean in relation to its biogenous matter content (Case I waters). Journal of Geophysical Research, vol. 93, No. C9, 10749-10768.

O'Reilly, J. E., Maritorena, S., Mitchell, D. G., Siegel, D. A., Carder, K. L., Garver, S. A., Kahru, M. & McClain, C. (1998). Ocean color chlorophyll algorithms. *Journal of Geophysical Research,* vol. 103, No. C11, 24937-24953.

Siegel, D.A., Westberry, T.K., O'Brien, M.C., Nelson, N.B., Michaels, A.F., Morrison, J.R., Scott, A., Caporelli, E.A., Sorensen, J.C., Maritorena, S., Garver, S.A., Brody, E.A., Ubante, J. & Hammer, M.A. (2001). Bio-Optical Modeling of Primary Production on Regional Scales: The Bermuda BioOptics Project. *Deep-Sea Research Part II: Topical Studies in Oceanography*, vol. 48, No. 8-9, 1865-1896.

Stewart, A. J. & Wetzel, R.G. (1981). Asymmetrical relationships between absorbance, fluorescence, and dissolved organic carbon. Limnol. *Oceanogr.*, vol. 26, No. 3, 590-597.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 193-206
ISBN: 978-1-60021-935-1

Chapter 6

PHYSIOLGICAL DIVERSITY IN WIDELY DISTRIBUTED MICROZOOPLANKTON: DIGESTION IN THE CILIATE *EUPLOTES VANNUS*

John R. Dolan[1*] and D. Wayne Coats[2]
[1]Marine Microbial Ecology, Laboratoire d'Océanographie, Université Paris6 CNRS UMR 7093, Station Zoologique, B.P. 28, 06230 Villefranche-sur-Mer, France
[2]Smithsonian Environmental Research Center, P. O. Box 28, Edgewater MD, USA

ABSTRACT

Many planktonic microbes exhibit nearly global distributions. Apparent adaptability to wide ranges of environmental conditions in cosmopolitan species could involve physiological diversity among individuals. However, variability between individuals among microorganisms is rarely considered. We examined individual variability in the common marine ciliate *Euplotes vannus*. Digestion was followed in individual cells subjected to different levels of ambient food concentration. Average digestion times were not significantly different because, in the absence of food or in the presence of super-saturating food concentrations, there was a large variability between individuals. Some ciliates completed food vacuole processing under 1 h while others took as long as 17 h. The range of digestion times, which increased under extreme conditions, may correspond to range of growth rates or growth potentials among individuals.

* dolan@obs-vlfr.fr

INTRODUCTION

Limits of Averages

Variation between individuals is a component of biological diversity which has received very little attention; commonly attempts are made in to minimize, rather than characterize, individual variability (Spicer & Gaston 1999). Establishing and comparing "averages" is the standard means of describing populations or species. This is true despite the fact that employing averages has familiar pitfalls such as masking distinct differences within a group and thus representing a descriptor of little value. Obviously, averages are acknowledged to be mathematical representations, not literal descriptions, but the possibility that averages may be unrealistic representations can be easy to neglect. Consider for example, estimating population reproductive rates with averages and a hypothetical human population with an "average" birth rate of 2.5 children per female. Let the number 2.5 represents the average of two sets of individuals (equal in abundance and mortality rates) but with different fertilities, say one with 1 child and the other with 4 children per female. Not only does t the "average" female does exist in this population but more importantly, if any factors differentially influence the two distinct sets of individuals, it would be impossible to predict the net effect using a single arithmetic average value of fertility. Thus, consideration of average rates may be not only unrealistic in the sense the "average does not exist", but can lead to erroneous conclusions. It is then of some significant interest to consider physiological diversity within a population. This has long been recognized and most recently underlined by Spicer & Gaston (1999). For any particular species, its capability to colonize new habitats, or react to shifts in climatic conditions, or adapt to changes in resources are the types of topics difficult if not impossible to address without some knowledge of the physiological diversity found within the species in terms of diversity among individuals.

With regard to planktonic organisms, physiological diversity between individuals is very rarely assessed. Firstly, as most planktonic organisms are microscopic observations of individual microorganisms are techniquely difficult. Secondly, they live in time and space scales very distinct from those with which we are familiar. Perhaps though the most important reason is that individual variability among microbes are simply not considered to be of much importance. Microbes and most planktonic organisms are characterized by very large population sizes, and as large sample or population size is often equated with 'normality' of distribution, average values are thought to be good descriptors.

Here we report on physiological diversity in the planktonic protist ciliate *Euplotes vannus*. Data on the variability of digestion with feeding conditions, ambient food levels, was gathered. The experiments were not designed originally to establish variability or ranges of rates, but rather to produce averages. We were able to examine physiological diversity only because we followed individual ciliates. However, we show the inadequacy of using averages to describe a physiological rate in the ciliate. Below, we place planktonic ciliates in their ecological rôle and briefly describe the nature of previous studies on *Euplotes vannus*, on which a great many studies have been conducted but very few of which documented differences between individuals.

Ecological Role of Plankton Ciliates and Euplotes Vannus

In the plankton, primary production is carried out by organisms occupying 3 different size-classes: 1) picoplankton-size organisms (0.2 - 2.0 µm in size) autotrophic prokaryotes such as *Synechococcus* and *Prochlorococcus* and small eukaryotic flagellates, 2) nanoplankton-sized organisms (2.0 - 20 µm) mainly eukaryotic flagellates and small diatoms, and 3) microplankton-size organisms (20 -200 µm in size) primarily large dinoflagellates and diatoms. Microzooplankton, composed of planktonic ciliates and heterotrophic dinoflagellates between the sizes of 20 and 200 microns, are the consumers of much of this primary production. Calbet & Landry (2004) estimated that a surprisingly constant 70 % of total planktonic primary production is consumed by ciliates and dinoflagellates across marine systems. This consistency and magnitude of microzooplankton grazing has been challenged (Dolan & McKeon 2005) but there is little doubt that microzooplankton are important in plankton food webs. Microzooplankton, are the link between nanoplankton, and to a lesser extent pico-plankton, and the higher trophic levels which prey on microzooplankton in the form of copepods, larval fish, etc. Among the ciliate component of the microzooplankton, distinct trophic guilds (groups of ciliate species whose diets appeared specialized to some degree) can de distinguished (Dolan 1991). Ciliates whose prey are mainly pico-plankton sized prey are microphagous, those feeding on larger prey are macrophagous and ciliates capable of feeding on other ciliates are predatory. *Euplotes vannus* feeds ciliates as well as other prey types and so is considered a predatory ciliate.

Euplotes Vannus

We isolated *Euplotes vannus* from the Chesapeake Bay. However, the ciliate is very widely distributed, as are many species of the microzooplankton. Euplotes are described as "marine cosmopolitan" and indeed *Euplotes vannus* is found in a wide range of ecosystems from the Chesapeake Bay, the origin of the cultures used in the present work (Dolan & Coats 1991a), to the coastal waters of Denmark (Fenchel 2004), Japan, Italy and California (e.g., Dini & Nyberg 1999). In common with most species of *Euplotes*, while often found in the plankton, it is ordinarily found among the benthic ciliates. In size it is about 100 microns in length. *Euplotes vannus* appears to feed upon algae, other ciliates as well as bacteria. It can be among the ciliates which colonize marine snow particles (Artolozaga et al. 1997).

Euplotes vannus has been the subject of many investigations. The species was used in some of the first studies on nutrient regeneration by protists (Johannes 1965; Gast & Hörstman 1983). The relationships between food type and food concentration and population growth have been investigated (Capriuolo et al. 1988; Dini & Nyberg 1999; Dolan & Coats 1991a). Selective feeding has been examined (Premke & Arndt 2000; Lewitus et al. 2006; Scott et al. 2003). Behavioral experiments have been conducted with *Euplotes vannus* (Tomaru et al. 2001; Fenchel 2004). Taken as a typical coastal ciliate, it has been subjected to several toxicology studies (Stebbing et al. 1990; Coppellotti 1988; Ricci et al. 1997; Xu et al. 2004; Mori et al. 2003). Many membrane physiology investigations have focused on *Euplotes vannus* (Stock et al. 1977; Kruppel & Wissing 1996; Kruppel et al. 1995; Lueken et al. 1996). Molecular and phylogenetic data have become available in recent years (Petroni et al. 2003; Shang et al. 2002; Cheng & Song 2002; Petroni et al. 2002). While diversity, including

physiological, between strains or mating types has been considered (Walton et al. 1995; Jones & Gates 1994; Caprette & Gates 1994) physiological diversity between individuals, to our knowledge, has not been addressed.

Use of Digestion Data

The interest in examining digestion in ciliates may appear obscure but it is potentially very useful in the estimation of feeding in natural populations. Estimating feeding rates in field populations is generally problematic with regard to planktonic organisms. One manner, possible in principle, is using 'gut contents' combined with digestion rate data. The ingestion rate, assuming steady-state conditions, equals food contents multiplied by digestion rate. The interest in the approach lies in the possibility of combining experimentally derived digestion rate data with estimates of average food contents from *in situ* or natural populations. Food content data can often be obtained relatively easily by analyzing freshly caught animals with little manipulation. This approach has been applied to benthic ciliates (Goulder 1972, 1973; Fenchel 1975), planktonic ciliates (Kopylov & Tumantseva 1987; Dolan & Coats 1991b) and heterotrophic nanoflagellates feeding on Synechococcus (Dolan & Simek 1999). Thus digestion rates, and their variability with ambient food levels and food type have been studied in ciliates fed algae and other ciliates (Capriuolo & Degan 1991; Dolan & Coats 1991a, Dolan & Simek 1997) and have concluded that digestion rate is insensitive to ambient food concentrations and food type. However, these studies sampled populations with time and did not attempt to asses individual variability.

Significance of Physiological Diversity

We report the results of a set of experiments designed to determine the effects of ambient food levels on digestion in the ciliate *Euplotes vannus*. The experimental design of following individual cells is a design rarely employed with regard to microbes and allowed us to assess physiological diversity in *Euplotes vannus*. Our results show that a population of *Euplotes vannus* contains individuals with distinct reactions to a given set of conditions. We followed digestion of labeled prey by monitoring individual ciliates digesting prey under different conditions of ambient food levels. Comparison of average values suggested no significant effect of ambient food levels on digestion rates. However, examination of the distribution of digestion times among individual ciliates showed that when food was abundant, individual *Euplotes vannus* cells reacted in different fashions- including notably rapid or prolonged digestion. The different durations of digestions among individuals likely correspond with different growth rates. Thus, *Euplotes vannus* populations are composed of individuals which will probably exhibit distinct growth responses when exposed to high prey concentrations. This hypothesis would not have been supported based on the evidence of no differences in digestion with ambient food level, a conclusion based on population averages.

METHODS AND MATERIALS

Isolation, cultivation and generation of growth curves of *Euplotes vannus* and its food organism, the bactivorous scuticociliate *Metanophrys* sp. are given in detail in Dolan & Coats (1991a). For each of the 4 experiments, log-phase cultures of *Euplotes vannus* and *Metanoprys* were used. Prey ciliates (*Metanophrys* sp.) were labeled with fluorescent microspheres and fed to *Euplotes vannus* as described in Dolan & Coats (1991a). *Euplotes vannus* were washed free of labeled *Metanophrys* and individual cells were micro-pipetted into, and isolated in, 1 ml well plates. Ciliates in the well plates were examined using an inverted microscope equipped with epifluorescence permiting visual verification of ciliate cell contents. For each experiment, a set of 30 individual *Euplotes vannus* containing *Metanophrys* labeled with fluorescent microspheres (Figure 1) assembled. Elapsed time from separating *Euplotes vannus* from labeled *Metanophyrys* to composing a set of 30 Euplotes, each isolated in an individual well of a well plate, was about 20 minutes.

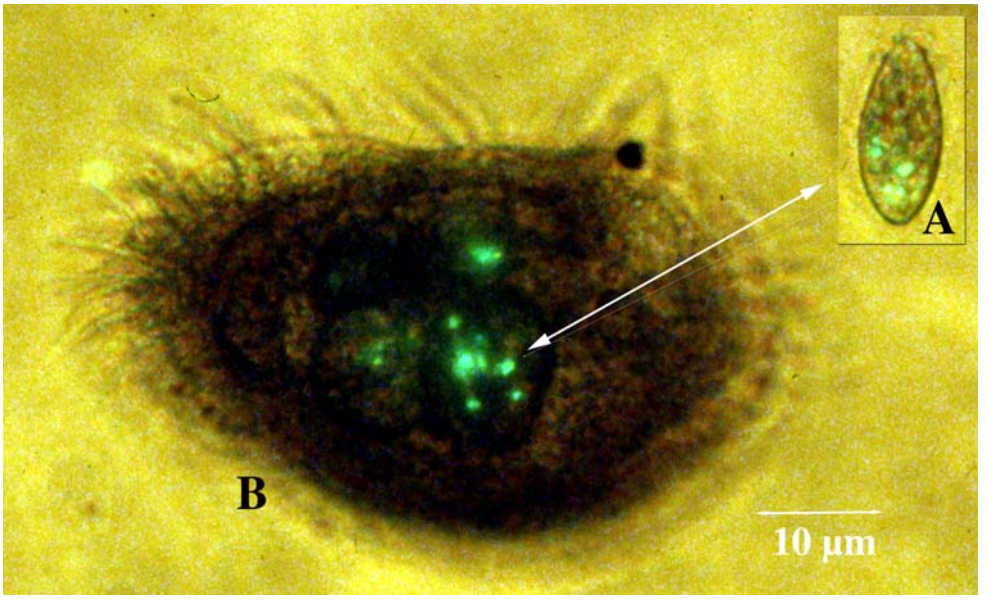


Figure 1. Photomicrographs of a *Metanophrys* (A) labeled with fluorescent microspheres and a *Euplotes vannus* containing several labeled Metanophyrys. The photomicrographs were obtained by using transmitted phase-contrast and epifluorescence illumination.

For each experiment, individual *Euplotes vannus* were examined at 30 minute intervals until the fluorescent microspheres contained within the labeled prey were expelled, marking the end of prey digestion. We thus obtained a digestion time for each *Euplotes* cell. All cultures, labeling and observations were conducted at 20° C in a temperature-controlled chamber.

In the first experiment *Euplotes vannus* were monitored in wells containing culture medium filtered free of food organisms. Thus the ciliates were digesting prey while being starved. In the second, third and fourth experiments, the solutions in the well plates contained, respectively, 100,1000 and 10,000 *Metanophyrys* ml^{-1}. In the latter 3 experiments, *Euplotes*

vannus cells were transferred to fresh prey solutions approximately every 2 hours. It should be noted that feeding rates reach a maximum in *Euplotes vannus* preying on *Metanophrys* saturates at a food concentration of about 100 *Metanophrys* ml^{-1} (Dolan & Coats 1991). Thus, the 1000 and 10000 prey concentrations represented super-saturated food concentrations for *Euplotes vannus*.

RESULTS

Digestion time, in terms of averages, while appearing to increase with ambient food level, were not significantly different using a t-test comparing results of the 4 experiments due to the relatively large standard deviations (Figure 2). At 20° C, the 'average' Euplotes digesting *Metanophrys* while held in prey-free water and therefore not feeding, digested its food vacuole contents and expelled the inert fluorescent microsphere which labeled its food item, in about 5 hours. The 'average' value for Euplotes digesting in the presence of 100 prey per ml was also about 5 hours. Population averages for ciliates held digesting in the presence of high concentrations of food increased to about 10 h but with a considerable amount of variability. Considering the averages alone and their associated standard deviations, no effect of ambient food level on the digestion time of *Euplotes vannus* can be shown. Thus, digestion time appeared fixed and not responsive to the availability of food.

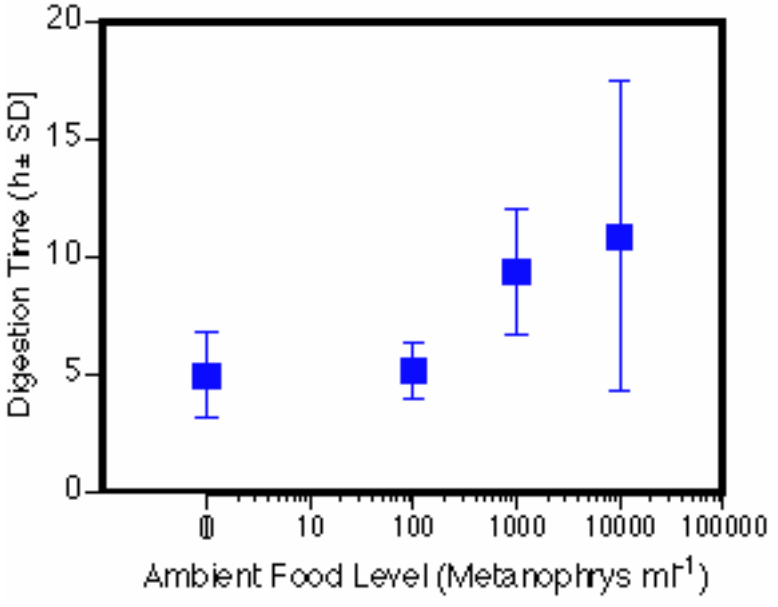


Figure 2. Average digestion times (±standard deviations) in Euplotes vannus digesting Metanophrys as a function of ambient concentrations of Metanophrys. Note that wityh the relatively large standard deviations, no significant differences in digestion times based on a comparison means could be shown.

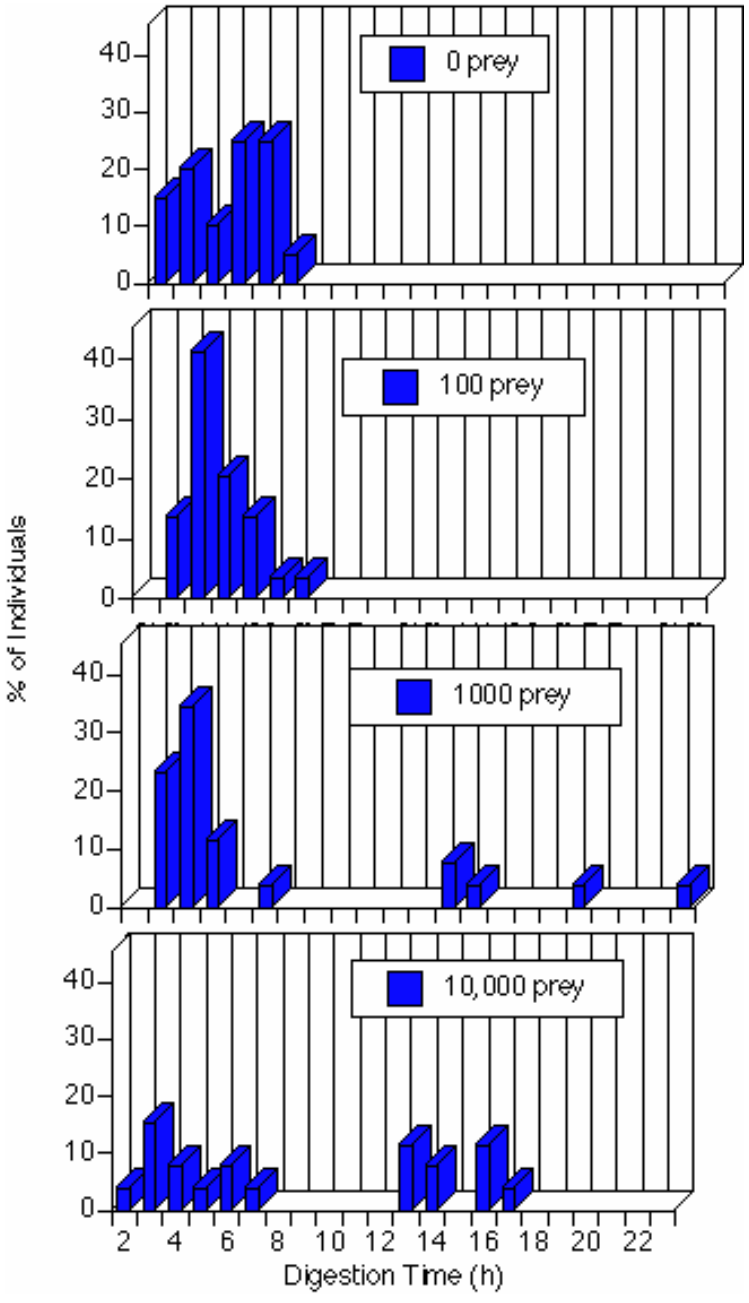


Figure 3. Distribution of digestion times in the 4 experiments. Note that rapid digestion times (1 - 2 h) were detected only in experiments with *Euplotes* digesting will being starved (top panel) or while digesting in the presence of super-abundant prey. Long digestion times (> 9 h) were observed in presence of high food concentrations.

However, distributions of digestion times, in contrast to average values, showed distinct differences with ambient food levels (Figure 3). In non-feeding cells, digestion times appeared to show a bi-modal distribution. Approximately equal and relatively large proportions (about 30 %)of individuals ciliates were rapid digesters (≤ 2 h) and others slow

digesters (≥ 6h). The small numbers of cells used precludes a rigorous test of distribution. In *Euplotes vannus* held in prey concentrations of 100 prey per ml, the distribution of digestion times approached normality with a modal digestion time of about 3 h accounting for about 45 % of the individuals. Despite the differences in distributions between digestion in starving and ciliates feeding in 100 prey ml-1, average digestion times for the entire populations were very similar (Figure 3). With high prey concentrations of 1000 or 10,000 prey ml^{-1}, some *Euplotes vannus* showed digestion times similar to ciliates held in lower food concentrations of 2 or 3 h, while others showed very long digestion times, approaching 24 h. At 1000 prey ml-1, there was a relatively high proportion, about 60% of 'fast digesters' , showing digestion times ≤ 2 h). Thus, the existence of two distinct populations, or individuals showing distinct digestion durations, were apparent in *Euplotes vannus* when subjected to super-abundant prey levels and perhaps starved (held in food-free water).

DISCUSSION

Physiological Diversity

The manner in which we gathered data on digestion in *Euplotes vannus*, following individual ciliates, allowed us to see the physiological diversity among individual ciliates. In experiments sampling with time an entire population (e.g., Capriuolo & Degnan 1991; Dolan & Coats 1991a,b) this information can not be extracted. To return to our example in the "Introduction" concerning rates birth rates in a human population, - net rates of change can be estimated by sampling a population over time and simply noting shifts in abundances, but important demographic details will be masked. Our experimental data showed that the ciliate population was not homogenous but rather it appears that ciliates can react in 2 distinct fashions to the presence of super-saturating food concentrations and perhaps a lack of food.

Euplotes vannus digesting prey in a solution free of food showed a dispersed population, relative to those processing food in the presence of 100 prey ml^{-1}, a concentration supporting exponential population growth (Dolan & Coats 1991a). Under very high food concentrations, 1000 or 10,000 *Metanophrys* ml^{-1}, the tendency was towards a dispersal of digestion times within a population was exaggerated. Thus, under perhaps unusual circumstances of no food or very abundant food, the some individual ciliates tended to either process food rapidly or very slowly. Presumably the distinct digestion times recorded would correspond with different digestion efficiencies, biomass production and different rates of cell division. Before considering if there are indeed different patterns employed by different individuals, or rather in some individuals the digestion process simply malfunctions when challenged, it is worth briefly reviewing food vacuole formation in ciliates. The following account is based on studies of food vacuole formation and membrane processing in *Paramecium* (Allen & Fok 1993; Fok & Allen 1990,1993; Fok & Schockley 1985; Fok et al. 1982), Kaneshiro et al. (1992), Plattner & Kissmehl 2003; Ramoino (1996).

One of the characteristics which distinguish most ciliates from members of many other protist groups is the existence of a differentiated mouth region where food vacuole formation begins (e.g., Hausmann & Radek 1993). Thus, in contrast to amboeba, in ciliates the food vacuole membrane is formed from disk-shaped vesicles in the mouth region, rather than

simply from the plasma membrane. In addition to discodial vesicles, acidosomes, at about 1 µm in dia considerably larger than discodial vesicles, are also found at the mouth region. As the food vacuole forms from the discoidal vesicles, the acidosomes 'dock' but do not fuse with the forming vacuole. Acidosomes, as the name implies are low pH vesicles. When the food vacuole reaches a certain size, or yet some other unknown cellular trigger is reached, the vacuole is pinched off from the mouth region and is transported to the interior of the cell. The acidosomes then fuse with the vacuole and the original vacuole membrane is retrieved with an end result of the nearly entire replacement of the membrane with that derived from the acidosomes. The food vacuole, once formed of acidosome membrane is then associated with docking of lysosomes. These membrane bound sacs contain acidic hydrolyses. It is possible that a second membrane replacement occurs with the fusion of lysosomes. The lysomes appear to simultaneously fuse with the vacuole and actual digestion begins. After a short time (in *Paramecium* 20 minutes) another change in membrane characteristics occurs. Lysosome membrane appears to be removed from the vacuole. At this point, the food vacuole may be 'defecation competent' or another change in membrane properties may be necessary before binding at the cytoproct region is possible. The vacuole is transported to the cytoproct region and at this point is acid phosphatase negative. The cytoproct opens and the undigested matter is expelled but the vacuole membrane remains inside the cell. Membrane from the food vacuole is tubularized and the tubules released into the cell. From this scenario it is clear that membrane supply and flow is primordial in the formation and processing of food vacuoles. Most if not all membranes appear to be recycled. Thus, the original food vacuole membrane formed from the discodial vesicles are themselves derived, at least in part, from re-cycled food vacuole membrane transported back to the mouth region after indigested matter is expelled from the cell. Likewise, acidosomes, based on antigen results, seem to have a heterogeneous origin but recycling of membrane is suspected to occur.

The complex sequence of events based on studies of *Paramecium* may or may not characterize most ciliates. Food vacuole processing has been examined in few species in any detail and there is at least one report of a different pattern. *Pseudomicrothorax dubius* feeds on filamentous blue-green algae and in this ciliate, lysosomes fuse with food vacuole as it is formed in the mouth region (Peck & Hausmann 1980). The processing of food vacuoles in *Euplotes vannus*, if strictly sequential and following that known from *Paramecium*, can be short-circuited as we observed a few instances in which prey-containing food vacuoles merged. Indeed, a breakdown in the sequence is an explanation for long digestion times.

In the scenario based on *Paramecium*, a food vacuole will remain in the cell if it is not first fused with acidosomes and the membrane replaced and/or not subsequently fused with lysosomes. The longest digestion times in *Euplotes vannus* were observed in cells held in solutions of 1,000 prey ml^{-1} (Figure 3). In circumstances of over-abundant prey one may hypothesize an imbalance between the formation of early stage food vacuoles and the supply of acidosomes and lysosomes. For example, some of the membrane generally used for the fabrication of lysosomes or acidosomes was perhaps monopolized in the form of early stage food vacuoles. It is difficult to explain the retention of ingested matter in the presence of abundant food as a useful strategy. Therefore, it seems most likely that in some individuals there was a breakdown in coordination among the distinct components of the digestion process. What is noteworthy is the fact that long digestion times characterized only a portion of the ciliates subjected to high food concentrations while digesting.

Our data show that a treatment effect of ambient food level can be discerned on a portion of the ciliates, a portion hidden in the averages, a comparison of which suggested no significant differences among treatments. We conclude then perhaps re-stating the obvious (but often ignored or under-appreciated): individuals vary. In the ciliate *Euplotes vannus*, we found considerable variability in terms of patterns of digestion found in even the small populations examined. Such an apparent physiological diversity may in part explain its wide distribution.

Using Food Contents and Digestion Rates to Estimate Ingestion Rates

Our results indicate that while average population rates of digestion appeared insensitive to ambient food levels, parts of the ciliate populations displayed long digestion times. The portion of the ciliate population thus effected by food level could be substantial, with nearly 40% of individuals showing long (> 12h digestion rates (Figure 3) but the phenomenon masked by individual to individual variability. If long digestion times are associated with inefficient exploitation of food, a population feeding under conditions of high prey availability will possibly have a lower average growth rate. However, as we noted, a substantial portion of the populations showed very rapid digestion times perhaps with corresponding generation times.

CONCLUSION

Previous studies have underlined the differences between strains of the same ciliate species or clones derived from a single strain, largely in terms of growth rates or temperature responses (e.g., Wiesse & Montagnes 1998; Weisse & Rammer 2006). Differences between individuals, while suspected to exist, have received very little, if any, attention even with regard to basic activities of ecological interest such as feeding rates (Weisse 2002). However, differences between individual cells can exist, as we have shown. Some *Euplotes vannus* showed digestion times in the presence of over-abundant prey similar to those found in simply optimal prey concentrations, others showed faster digestion or slower digestion rates. In the absence of food, some individuals slowed while other increased digestion times. Such physiological diversity among individuals is likely in part an explanation for the wide geographic distribution of *Euplotes vannus*.

ACKNOWLEDGEMENTS

Financial support for this work was originally provided by the National Science Foundation (USA) through grant OCE 8800076. Motivation to re-examine old data was provoked by Spicer & Gaston's book "Physiolgical Diversity and Its Ecological Implications" and the French ANR Biodiversity project AQUAPARADOX. Financial support was also provided by the Marplan project, part of the MARBEF Network of Excellence (Contract No. GOCE-CT-2003-505446). This is contribution MPS-07100 of MARBEF.

REFERENCES

Allen, R.D., Fok, A.K. 1993. Endosomal membrane traffic of ciliates. Advances in Cell and Molecular Biology of Membranes, vol 2B, *Membrane Traffic in Protozoa*, pp 283-309.

Artolozaga, I., Santamaría, E., López, A., Ayo, B., Iriberri, J. 1997. Succession of bacterivorous protists on laboratory-made marine snow. *Journal of Plankton Research*, 19:1429-1440.

Calbet, A., Landry, M.R. 2004. Phytoplankton growth, microzooplankton grazing and carbon cycling in marine systems. *Limnology and Oceanography*, 49:51-57.

Caprette, C.L., Gates, M.A. 1994. Quantitative analysis of interbreeding in populations of vannus-morphotype *Euplotes*, with special attention to the nominal species *Euplotes vannus* and *Euplotes crassus*. *Journal of Eukaryotic Microbiology*, 41: 316-324.

Capriulo, G.M.,, Schreiner, R.A., Dexter, B.L. 1988. Differential growth of *Euplotes vannus* fed fragmented versus unfragmented chains of *Skeletonema costatum*. *Marine Ecology Progress Series*, 47:205-209.

Capriulo, G. M. and C. Degnan. 1991. Effect of food concentration on digestion and vacuole passage time in the heterotrichous marine ciliate *Fabrea salina. Marine Biolology*, 110:199-202.

Chen Z.G., Song W.B. 2002. Phylogenetic positions of *Aspidisca steini* and *Euplotes vannus* within the order euplotida (Hypotrichia : Ciliophora) inferred from complete small subunit ribosomal RNA gene sequences. *Acta Protozoologica*, 41:1-9.

Coppellotti O. 1988. Sensitivity to copper in a ciliate as a possible component of biological monitoring in the Lagoon of Venice. *Archives of Environmental Contamination and Toxicology*, 35:417-425.

Dini, F., Nyberg, D. 1999. Growth rates of marine ciliates on diverse organisms reveal ecological specializations within morphospecies. *Microbial Ecology*, 37:13-22.

Dolan, J.R. 1991. Guilds of ciliate microzooplankton in the Chesapeake Bay. *Estuarine Coastal and Shelf Science*, 33:137-152.

Dolan, J.R., Coats, D.W. 1991a. A study of feeding in predacious ciliates using prey ciliates labeled with fluorescent microspheres. *Journal of Plankton Research*, 13:609-627.

Dolan, J.R., Coats, D.W. 1991b. Preliminary prey digestion in a predacious estuarine ciliate and the use of digestion data to estimate ingestion. *Limnology and Oceanography*, 36:558-565.

Dolan, J.R., Simek, K. 1997. Processing of ingested matter in *Strombidium sulcatum*, a marine ciliate (Oligotrichida). *Limnology and Oceanography*, 43:393-397.

Dolan, J.R., Simek, K. 1998. Ingestion and digestion of an autotrophic picoplankter, *Synechococcus*, by a heterotrophic nanoflagellate, *Bodo saltans*. *Limnology and Oceanography*, 43:1740-1746.

Dolan, J.R., Simek, K. 1999. Diel periodicity in *Synechococcus* populations and grazing by heterotrophic nanoflagellates: analysis of food vacuole contents. *Limnology and Oceanography*, 44:1565-1570.

Dolan, J.R., McKeon, K. 2005. The reliability of grazing rate estimates from dilution experiments: have we over-estimated rates of organic carbon consumption by microzooplankton? *Ocean Science*, 1:1-7.

Fenchel, T. 1975. The quantitative importance of benthic microfauna of an arctic tundra pond. *Hydrobiologia*, 46:445-464.

Fenchel, T. 2004. Orientation in two dimensions: chemosensory motile behaviour of *Euplotes vannus*. *European Journal of Protistology*, 40:49-55.

Fok, A.K., Allen, R.D. 1990. The phagosome-lysosome membrane system and its regulation in *Paramecium*. *International Review of Cytology*, 123:61-94.

Fok, A.K., Allen, R.D. 1993. Membrane flow in the digestive cycle of *Paramecium*. Advances in Cell and Molecular Biology of Membranes, vol 2B, *Membrane Traffic in Protozoa*, pp 311-337.

Fok, A.G., Lee, Y., Allen, R.D. 1982. The correlation of digestive vacuole pH and size with the digestive cycle in *Paramecium caudatum*. *Journal of Protozoology*, 29:409-414.

Fok, A.G., Schockley, B.U. 1985. Processing of digestive vacuoles in *Tetrahymena* and the effects of dichloroisoproternol. *Journal of Protozoology*, 32:6-9.

Gast, V., Horstmann, U. 1983. N-remineralization of phyto- and bacterioplankton by the marine ciliate *Euplotes vannus*. *Marine Ecology Progress Series*, 13:55-60.

Goulder, R. 1972. Grazing by the ciliated protozoon *Loxodes magnus* on the alga *Scenedesmus* in a eutrophic pond. *Oikos*, 23:109-115.

Goulder, R. 1973. Observations over 24 hour on the quantity of algae inside grazing ciliated protozoa. *Oecologia*, 13:117-182.

Hausmann, K., Radek, R. 1993. A comparative survey on phagosome formation in protozoa. Advances in Cell and Molecular Biology of Membranes, vol 2B, *Membrane Traffic in Protozoa*, pp 259-282.

Johannes, R.E. 1965. Influence of marine protozoa on nutrient regeneration. *Limnology and Oceanography*, 10:434-442.

Jones, T.C., Gates, M.A. 1994. A morphometric study of euryhalanity in marine populations of the ciliate genus *Euplotes*. *Journal of Eukaryotic Microbiology*, 41: 303-316.

Kaneshiro, E.S., Reuter, S.F., Quattrone, F.J., Morris, R.E. 1992. Sustained food vacuole formation by axenic *Paramecium tetraurelia* and the inhibition of membrane recycling by alcian blue. *Journal of Protozoology*, 39:713-718.

Kopylov, A.I., Tumantseva, N.I.1987. Analysis of the contents of tintinnid food vacuoles and evaluation of their contribution to the consumption of phytoplankton production off the Peru coast. *Oceanology*, 27:343-347.

Kruppel T., Wissing, F. 1996. Characterisation of the voltage-activated calcium current in the marine ciliate *Euplotes vannus*. *Cell Calcium*, 19: 229-241.

Kruppel, T., Rabe, H., Dummler, B., Lueken, W. 1995. The Depolarizing mechanoreceptor potential and Ca/Mg receptor current of the marine ciliate *Euplotes vannus*. *Journal of Comparative physiology A- Sensory Neural and Behavioral Physiology*, 177:511-517.

Lewitus, A.J., Wetz, M.S., Willis, B.M., Burkholder, J.M., Parrow, M.W., Glasgow, H.B. 2006. Grazing activity of *Pfiesteria piscicida* (Dinophyceae) and susceptibility to ciliate predation vary with toxicity status. Harmful Algae, 5:427-434.

Lueken W., Ricci N., Kruppel T. 1996. Rhythmic spontaneous depolarizations determine a slow-and-fast rhythm in walking of the marine hypotrich *Euplotes vannus*. *European Journal of Protistology*, 32:47-54.

Mori, G., Erra F., Cionini K. 2003. Sublethal doses of heavy metals and Slow-Down pattern of *Euplotes crassus* (Ciliophora, Hypotrichia): a behavioural bioassay. *Italian Journal of Zoology*, 70:23-30.

Peck, R.K., Hausmann, K. 1980. Primary lysosomes of the ciliate *Pseudomicrothorax dubius*: cytochemical identification and role in phgocytosis. *Journal of Protozoology,* 32:501-508.

Petroni, G. Rosati, C. Vannini, L. Modeo, F., Dini, F. Verni, B. 2003. In situ identification by fluorescently labeled oligonucleotide probes of morphologically similar, closely related ciliate species. *Microbial Ecology,* 45:156-162.

Petroni G., Dini F., Verni F. 2002. A molecular approach to the tangled intrageneric relationships underlying phylogeny in *Euplotes* (Ciliophora, Spirotrichea). *Molecular Phylogenetics and Evolution*, 22:118-130.

Petroni, G., Rosati G., Vannini C., 2003. In situ identification by fluorescently labeled oligonucleotide probes of morphologically similar, closely related ciliate species. *Microbial Ecology*, 45:156-162.

Plattner, H., Kissmehl, R. 2003. Molecular aspects of membrane trafficking in *Paramecium*. *International Review of Cytology*, 232:185-216.

Premke, K., Arndt, H. 2000. Predation on heterotrophic flagellates by protists: Food selectivity determined using a live-staining technique. *Archiv für Hydrobiologie,* 150:17-28.

Ramoino, P. 1996. Membrane supply and food vacuole formation in *Paramecium primaurelia*. *Archiv für Protistenkunde,* 147:323-329.

Ricci N., Luvera G., Cacciatori M., Banchetti R., Lueken W. 1997. The effects of 2 µM Hg^{++} on the ethogram of *Euplotes vannus* (Ciliata, Hypotrichida). *European Journal of Protistlogy*, 33:63-71.

Lueken W, Ricci N, Kruppel T. 1996. Rhythmic spontaneous depolarizations determine a slow and fast rhythm in walking of the marine hypotrich ciliate *Euplotes vannus*. *European Journal of Protistology*, 32: 47-57.

Scott, G., Gransden, S.G., Lewitus, A.J. 2003. Grazing of two euplotid ciliates on the heterotrophic dinoflagellates *Pfiesteria piscicida* and *Cryptoperidiniopsis* sp. *Aquatic Microbial Ecology*, 33:303-308.

Shang H.M., Chen Z.G., Song W.B. 2002. Species separation among seven *Euplotes* spp. (Protozoa : Ciliophora : Hypotrichida) using PCR/RFLP analysis of nuclear ribosomal DNA. *Journal of Zoology* 258:375-379.

Spicer, J.I., Gaston, K.J. 1999. *Physiological Diversity and Its Ecological Implications.,* London: Blackwell Science, 241pp. Book.

Stebbing, A.R.D., Soria, S., Burt, G.R., Cleary, J.J. 1990. Water quality bioassays in two Bermudan Harbours using the ciliate *Euplotes vannus*, in relation to tributyltin distribution. *Journal of Experimental Marine Biology and Ecology, 1*38:159-166.

Stock C., Kruppel T., Lueken W., Key, G. 1997. Congruence of electrical properties in two Antarctic and two middle-latitude marine species of *Euplotes* (Ciliata, Hypotrichida). *Journal of Eukaryotic Microbiology* 44:427-433.

Tomaru A, Matsuoka T, Kogure K. 2001. Photoreaction of a colorless marine ciliate, *Euplotes vannus*. *Archiv für Hydrobiologie* 152:647-660.

Walton, B.M., Gates, M.A., Kloss, A., Fisher, J. 1995. Intraspecific variability in the thermal-dependance of locomotion, population-growth, and mating in the ciliated protist *Euplotes vannus*. *Physiological Zoology*, 68:98-113.

Weisse, T. 2002. The significance of inter- and intraspecific variation in bacterivorous and herbivorous protists. *Antonie van Leeuwenhoek*, 81:327-341.

Weisse, T., Montagnes, D.J.S. 1998. Effect of temperature on inter- and intraspecific isolates of *Urotricha* (Prostomatida, Ciliophora). *Aquatic Microbial Ecology*, 15:285-291.

Weisse, T., Rammer, S. 2006. Pronounced ecophysiological clonal differences of two common freshwater ciliates, *Coleps spetai* (Prostomatida) and *Rimostrombidium lacustris* (Oligotrichida), challenge the morphospecies concept. *Journal of Plankton Research*, 28:55-63.

Xu, H.L, Song W.B., Warren A., 2004. An investigation of the tolerance to ammonia of the marine ciliate *Euplotes vannus* (Protozoa, Ciliophora). *Hydrobiologia*, 519:189-195.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 207-216
ISBN: 978-1-60021-935-1

Chapter 7

INEFFICIENT SI UPTAKE KINETICS BY NATURAL PHYTOPLANKTON ASSEMBLAGES IN OCEANIC AND PLUMEWATERS OF THE WESTERN ATLANTIC OCEAN

Rebecca F. Shipe
Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 621 Charles Young Drive South, Los Angeles, CA 90405, USA

ABSTRACT

Diatoms are important primary producers in global oceans and dominate in extensive areas of the western Atlantic ocean that are influenced by the low salinity, high dissolved silicon (dSi) waters of the Amazon River plume. Experiments were conducted to compare the Si uptake kinetics of diatoms in plume influenced waters and in nearby oceanic waters. At 5 oceanic stations, salinities ranged from 35.69 to 36.27, dSi concentrations ranged from 0.9 to 2.0 μM and biogenic silica (bSi) concentrations ranged from 0.02 to 0.07 μmol l^{-1}. At 6 plumewater stations, salinities ranged from 31.08 to 33.76, dSi concentrations ranged from 2.3 to 8.7 μM and biogenic silica (bSi) concentrations ranged from 2.3 to 8.7 μmol $l^{-}1$. Si uptake kinetics were measured in multiple bottle incubations with dSi additions ranging from 1.25 to 40 μM. At oceanic stations, uptake kinetics generally fit the Michaelis-Menten function, with half saturation constants (Ks) between 13 and 25 μM and maximum biomass-specific uptake rates of 0.26 to 0.50 hr^{-1}. At two plumewater stations with high biogenic silica concentrations, uptake increased linearly with substrate concentration. At the remaining 4 plumewater stations, uptake kinetics fit the Michaelis-Menten function, and Ks values were 68.5 to 90.6 μM. In both environments, dSi concentrations were present at high concentrations relative to other macronutrients. We suggest that the observed inefficient uptake kinetics in both environments is an adaptation that allows diatom populations to be Si limited, which has been shown to be an adaptive advantage in phytoplankton competition.

INTRODUCTION

The Amazon River contributes 18% of the river water discharged into the world oceans (Oltman, 1968) and can produce 300 km diameter plumes in the western Atlantic Ocean (Muller-Karger et al., 1988). These plumewaters are the source of a large fraction of the riverine dissolved Si to the global oceans (Livingstone, 1963) and diatoms are often the dominant primary producers in the estuary (Milliman and Boyle, 1975) and further offshore along the continental shelf (DeMaster et al., 1986, DeMaster et al., 1996). Further, diatoms have global significance as a source of primary production in the oceans (Nelson et al., 1995). Since the majority of diatom taxa require dissolved Si (dSi) to construct a biogenic silica (bSi) cell wall (Lewin, 1962), the availability of dSi limits the rate at which it is taken up in a variety of systems (e.g. Nelson and Dortch, 1996, Brzezinski and Nelson, 1996, Leynaert et al., 2001). Further, dSi availability has been indicated in the limitation of new production rates (Dugdale and Wilkerson, 1998) and even primary productivity rates (Kristiansen et al., 2001, Shipe et al., 2007).

Uptake of Si in laboratory studies and natural assemblages generally follows the Michelis-Menten model of enzyme kinetics (e.g. Paasche, 1973, Nelson and Dortch, 1996). In this rectangular hyperbolic function, the biomass specific uptake rate, V, is determined according to the equation $V = (V_{max} \times [dSi])/(Ks + [dSi])$, where [dSi] is the dSi concentration, V_{max} is the maximum biomass specific uptake rate and Ks is the half saturation constant, or concentration at which the uptake rate is half of V_{max} (e.g. Goering, et al., 1973, Harrison et al., 1976). Growth rates of diatoms are also regulated by similar saturation kinetics following Monod (1942), but the half saturation constant for growth is often less than that for uptake, as cells can grow with reduced Si content.

In eutrophic systems, Ks is normally lower than ambient dSi concentration (review by Martin-Jézéquel et al., 2000) such that cells are growing at or near their maximum uptake rate until the end of blooms, when dSi concentrations are depleted. Cells adapted to oligotrophic waters would be expected to have more efficient uptake kinetics (i.e. half saturation constants higher than ambient dSi concentrations). Accordingly, efficient uptake kinetics have been shown in Gulf Stream warm core rings where Ks ranged from 0.5 to 0.9 μM (Nelson and Brzezinski, 1990) and in laboratory cultures of diatoms in which Ks ranged from 0.8 to 2.3 μM (Nelson et al., 1976). In contrast, half saturation constants of natural assemblages at two locations in the Equatorial Pacific were 1.57 and 2.42 μM, approximately the same as ambient dSi concentrations (Leynaert et al., 2001). Even more inefficient uptake kinetics occur in the North Atlantic gyre, where Si uptake rates increased linearly with dSi additions (Brzezinski and Nelson, 1996). Previously reported work in the western Atlantic Ocean suggests substrate limited Si uptake rates during both high and low influence of plumewaters; estimates of Ks from two bottle enrichment experiments were 8.5 M at oligotrophic stations and 10.9μM at plumewater stations (Shipe et al., 2007).

In this paper, we present detailed descriptions and discussion of the kinetics of Si uptake in plumewater and oceanic waters in the western North Atlantic Ocean. Extensive areas of the normally oligotrophic tropical waters in this region are seasonally influenced by river outflow (Muller-Karger et al., 1988), which is greatest in the spring and summer (Nittrouer et al., 1991). Amazon waters have extremely high dSi concentrations relative to N and P nutrients; sourcewaters have 144 μM dSi, 16 μM nitrate and 0.7 μM phosphate (DeMaster and Pope,

1996). However, both oceanic and plumewaters contain a high Si:N nutrient ratio (DeMaster and Pope, 1996) relative to the Si:N ≈ 1 of most diatoms (e.g. Brzezinski, 1985).

METHODS

The kinetics of Si uptake was measured in natural phytoplankton assemblages at 11 stations between 4°-12° N and 42°-58°W in the western Atlantic Ocean. Five oceanic stations were sampled in January and February 2001 and 6 stations with a range of plumewater influence were sampled in July and August 2001 (Figure 1). Sampling of water properties is described by Shipe et al., (2006) and each station was evaluated as either "oceanic" or "plumewater" based on statistical analysis of 8 relevant environmental variables including salinity, nutrient concentrations, Si pools, phytoplankton biomass and productivity. Water samples for the kinetic experiments and dSi analysis were collected in Niskin sampling bottles at the depth of 50% of surface irradiance.

For Si uptake experiments, water was divided into 140 ml aliquots in polycarbonate bottles and sodium metasilicate was used to raise the dSi concentrations to 1.25, 2.5, 3.75, 5, 10, 20 and 40 μM above ambient concentrations. Si uptake rates were determined using tracer additions of 7.8 x 10^{-5} μCi ^{32}Si per ml of seawater (specific activity 23,000 Bq (μg Si)$^{-1}$). Samples were kept on deck in flowing seawater incubators at natural light and temperature conditions. After four hours, samples were filtered onto 0.6 μm polycarbonate filters and returned to the shore-based laboratory. Samples were dissolved in 2.5M HF and activities were counted in Packard Ultima Gold XR scintillation cocktail on a Beckman LS 6000 liquid scintillation counter. In order to determine biomass-specific uptake rates, bSi concentrations were determined on samples from the same depth, as previously reported (Shipe et al., 2006). Si uptake rates were calculated using the logarithmic model of Brzezinski and Phillips (1997).

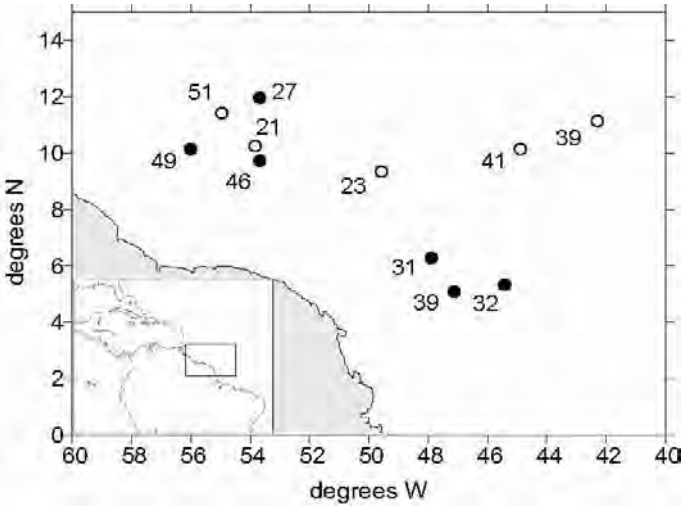


Figure 1. Map of station locations and station numbers of kinetic uptake experiments in the western Atlantic Ocean in 2001. Open symbols indicate oligotrophic stations occupied in January-February and solid symbols indicate stations occupied under a range of plumewater influence in July-August.

Table 1. Summary of conditions at 5 oceanic (first 5 listed) stations and 6 plumewater (last 6) stations at which uptake experiments were conducted in the western Atlantic Ocean

Station	Date	sample depth (m)	mld (m)	salinity	dSi (μM)	bSi (μmol l^{-1})	Si uptake (μmol l^{-1} h^{-1})
23	2 Feb 01	5	82	35.99	0.9	0.02	0.0003
21	31 Jan 01	13	96	36.27	1.4	0.02	0.0003
41	11 Feb 01	10	63	36.03	1.7	0.04	0.0004
51	18 Feb 01	17	70	35.82	1.8	0.04	0.0002
39	10 Feb 01	14	103	35.69	2.0	0.07	0.0004
49	10 Aug 01	5	8	33.76	2.3	0.20	0.0020
46	8 Aug 01	5	8	32.39	2.9	4.20	0.0021
32	31 Jul 01	4	8	33.07	3.1	0.32	0.0016
31	30 Jul 01	4	15	32.99	4.1	0.32	0.0022
27	27 Jul 01	3	14	31.08	5.4	2.06	0.0064
39	4 Aug 01	2	9	32.04	8.7	0.12	0.0052

Included are the depth of 50% of surface irradiance, where water was collected for experiments, mixed layer depth and salinity (given as practical salinity units), *in situ* dissolved silicon, biogenic silica concentrations and Si uptake rates at that depth.

DSi concentrations were determined following the standard colorimetric method of Strickland and Parsons (1972). Samples were immediately filtered through 0.6 μm polycarbonate membranes to remove particles and the filtrate was stored in a shipboard refrigerator for up to three days prior to manual analysis onboard ship.

Biomass-specific uptake rates as a function of dSi concentrations were fit to a rectangular hyperbola and to a line. For uptake kinetics that had a more statistically significant fit to a rectangular hyperbola, Ks and V_{max} were determined from this regression. For kinetics that had more statistically significant fit to a line, slopes were determined with a least squares regression.

RESULTS

Oceanic and plumewater stations were characterized by quite different physical, chemical and biological properties. Near-surface (1m) salinities at the oceanic stations for the experiments ranged from 35.82 to 36.27 and dSi concentrations at the sample depth ranged from 0.9 to 2.0 μM (Table 1). At plumewater stations, near-surface salinities ranged from 31.08 to 33.76 and dSi concentrations at the sample depth ranged from 2.3 to 8.7 μM. Diatom biomass was consistently low at oceanic stations; bSi concentrations ranged from 0.02 to 0.07 μmol l^{-1} (Table 1). At plumewater stations, bSi concentrations ranged widely, from 0.20 to 4.2 μmol l^{-1}. *In situ* Si uptake rates were also consistently low in the oceanic water samples, ranging from 0.0002 to 0.0004 μmol l^{-1} h^{-1} whereas they were an order of magnitude higher at plumewater stations, ranging from 0.0016 to 0.0064 μmol l^{-1} h^{-1}.

Si uptake kinetic curves indicated severe substrate limitation at all stations. At 4 of 5 oceanic stations, Si uptake kinetics approximately followed the Michaelis-Menten function,

with a hyperbolic increase in uptake rates with increasing dSi concentrations (Figure 2). Estimated half saturation constants (Ks) for silica uptake varied from 13.0 to 25.2 μM and maximum biomass-specific uptake rates (V_{max}) were 0.26-0.55 h^{-1}. At one oceanic station, uptake rates increased linearly with dSi concentration to a maximum rate of 0.35 h^{-1}. These rates correspond to doublings of Si content in 0.5 to 2.7 hours. At the 6 plumewater stations, Si uptake rates showed even more dramatic responses to increasing dSi concentrations (Figure 3). Rates increased linearly with dSi concentrations at the two stations with the highest diatom biomass as indicated by bSi content. At the remaining 4 stations, Si uptake kinetics fit the Michaelis-Menten function. Estimated Ks from these hyperbolic curves ranged between 68.5 and 90.6 μM. V_{max} at plumewater stations ranged from 0.06 to 1.4 h^{-1}); these rates correspond to maximum doubling times of 0.5 to 12 hours.

The initial slopes of uptake kinetic curves (in units of h^{-1} μM^{-1}) indicate the efficiency of nutrient uptake at low nutrient concentrations. Higher slopes would indicate more efficient uptake with increasing nutrient concentrations. On average, slopes were much higher at oceanic stations, but varied widely between 1.9 and 19.2 x 10^3 h^{-1} μM^{-1}. However, there was a significant inverse relationship between dSi and the slopes at all stations, suggesting greater uptake efficiency at the oligotrophic stations (Figure 4).

Table 2. Summary of Si uptake kinetic parameters from experiments in oceanic waters and plumewaters in the western Atlantic Ocean at stations as in Table 1

Station	Ks (μM)	V_{max} (h^{-1})	Slope (10^{-3} h^{-1} μM^{-1})	r	% of bSi
23	13.0	0.50	19.2	0.93	31
21	14.8	0.55	1.9	0.97	25
41	-	≥ 0.35	8.5	0.99	13
51	17.0	0.30	8.8	0.96	36
39	25.2	0.26	5.2	0.99	14
49	74.1	1.32	8.9	0.99	7
46	-	≥ 0.25	5.6	0.98	18
32	90.6	0.41	0.3	0.92	11
31	75.2	0.50	3.3	0.99	54
27	-	≥ 0.06	1.6	0.98	21
39	68.5	1.40	1.0	0.95	45

Included are the estimated half saturation constant (Ks) and maximum uptake rate (V_{max}), initial slope of the Si uptake kinetic curve and correlation coefficient for the hyperbola or line fit. Also given is the percentage of bSi content that was taken up during the 4 hour incubation at the highest experimental Si uptake rate measured. The initial slopes of hyperbolic functions are estimated as 0.5 V_{max}/Ks.

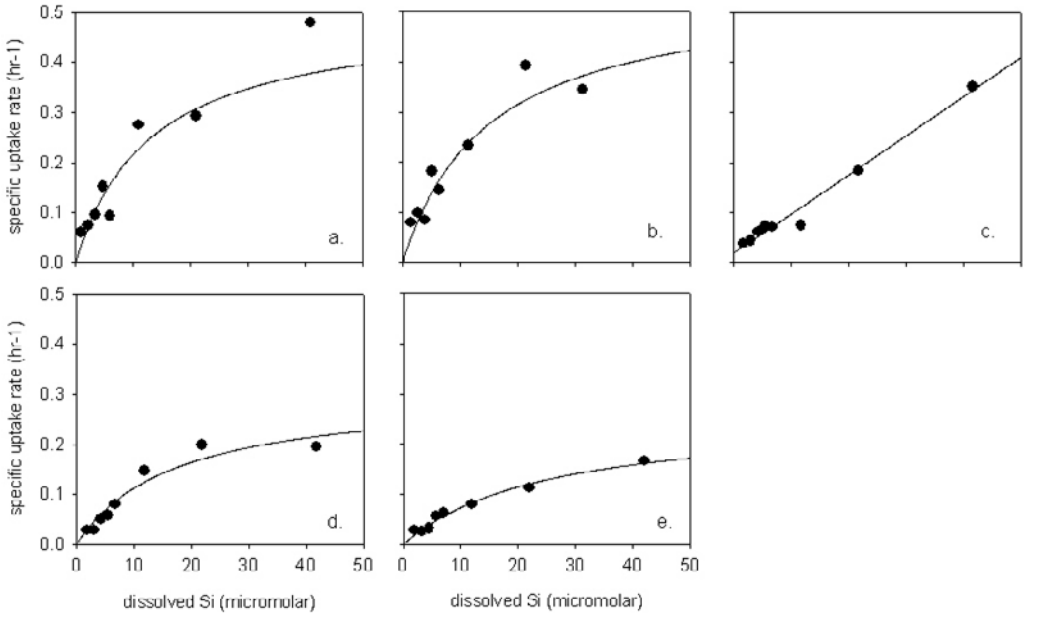


Figure 2. Si uptake kinetics determined experimentally in oceanic waters collected at station number (a.) 23 (b.) 21 (c.) 41 (d.) 51 and (e.) 39. Symbols represent results of bottle experiments and lines indicate rectangular hyperbolic (a, b, d, e) or linear (c) regressions.

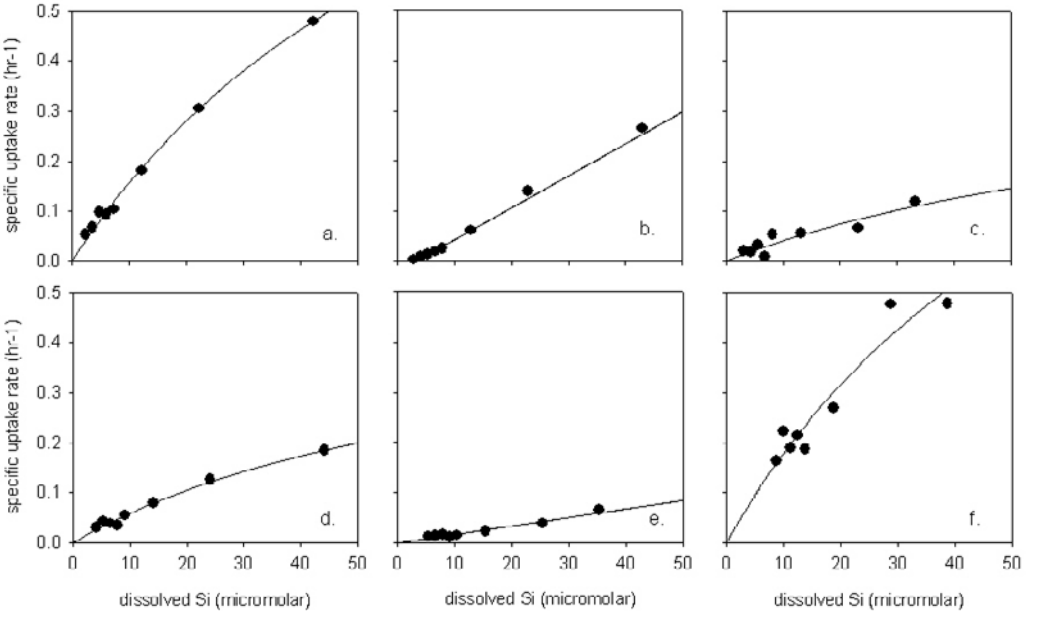


Figure 3. Si uptake kinetics determined experimentally in river plume influenced waters collected at station number (a.) 49 (b.) 46 (c.) 32 (d.) 31 (e.) 27 and (f.) 39. Symbols represent results of bottle experiments and lines indicate rectangular hyperbolic (a, c, d, f) or linear (b, e) regressions.

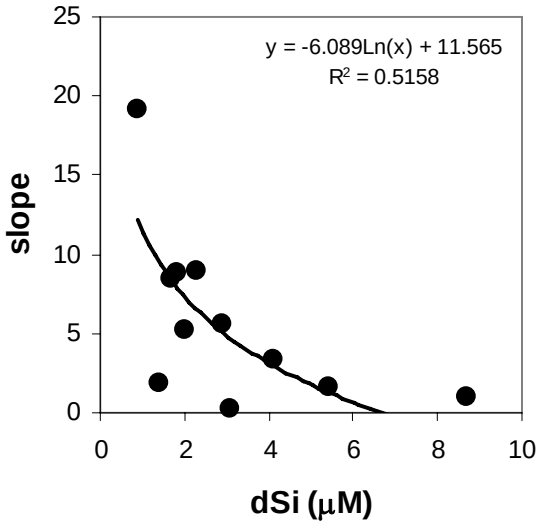


Figure 4. Dependence of the initial slope of Si uptake kinetic curve on *in situ* dissolved silicon concentrations at 11 stations in the western Atlantic Ocean. Data indicate a statistically significant logarithmic increase in the initial slope of the Si uptake kinetic curve with decreasing dissolved silicon concentrations.

DISCUSSION

Results indicated extremely inefficient uptake kinetics at all oceanic and plumewater stations. Hyperbolic or linear uptake with increasing experimental dSi concentrations indicate that *in situ* ambient dSi concentrations limit uptake rates to less than 1% of V_{max} (or <1% of the uptake rate measured at the highest experimental dSi concentration). This extent of substrate limitation was unexpected as other macronutrient concentrations in this region were <1 μM (D. Capone, pers comm). In contrast, Si uptake is rarely substrate limited in the Mississippi River plume (Nelson and Dortch, 1996). However, substrate limitation of Si uptake in natural assemblages at high dSi concentrations have been previously observed in the Gulf of California (at 3.3 and 3.6 μM dSi; Azam and Chisholm, 1976), the Southern Ocean (at 5.6 to 6.6 μM dSi; Nelson and Tréguer, 1992), and offshore of central California in the Monterey Bay (at 2.4 to 27.8 μM dSi; Brzezinski et al., 1997). Yet, Ks of the magnitude reported here have only been observed in isolated plasma membrane vesicles from diatoms (Bhattacharyya and Volcani, 1980).

Possible physiological mechanisms to account for the observed inefficient Si uptake kinetics include luxury uptake and the storage of Si intracellularly and/or a "shift up" in Si metabolism. Diatoms are known to store Si in intracellular pools that can contain up to 56% of the total cellular Si content (review by Martin-Jézéquel et al., 2000). At the rates that Si was taken up during the study, it is possible that the cells could store the dSi in internal pools; at the maximum uptake rates measured, the quantity of dSi taken up was generally less than 20 % of total cellular bSi content, and was at highest 54 % of total bSi content. However, the experiments may have stopped just short of the limit of the cells' capacity to store Si; longer incubations or higher dSi concentrations may have shown more clear saturation kinetics.

The term “shift up” refers to an empirical phenomenon whereby cells that are exposed to high nutrient concentrations have higher maximum uptake rates than those in low nutrient concentrations. For example, *Skeletonema costatum* has two physiological states with two sets of kinetic constants for “fast-adapted’ and ‘slow-adapted” cells (Harrison et al., 1976). *S. costatum* was a common component of the microplankton assemblage in the samples observed in this study, and it is possible that their exposure to high dSi concentrations from the Amazon plume resulted in a “fast-adapted” population.

The Si limitation observed in this study and others may not be a disadvantage but rather may be an adaptive advantage for the diatoms. Smetacek (1985) suggests that the increased sinking rate of Si limited cells may allow the cells to sink into nutrient rich deep waters where they may seed future blooms during resuspension or upwelling events. This process is feasible in the Amazon estuary and inner shelf where resuspension of sediments and nutrients support diatom blooms (DeMaster et al., 1996). Another hypothesis suggested by Brzezinski and Nelson (1996) is that diatoms can recover more quickly from Si limitation than N limitation; this theory that has been supported by laboratory experiments (DeLaRocha and Passow, 2004). Thus, it is possible that the diatom community of the western Atlantic Ocean has the most inefficient uptake kinetics described to date in order to ensure Si limitation under conditions where Si is generally present in very high ratios relative to other macronutrients.

ACKNOWLEDGEMENTS

The authors thank Doug Capone, Edward Carpenter and Ajit Subramaniam for leadership during cruises. Michael Neumann, Matthew Floge and Troy Gunderson provided technical support at sea.

REFERENCES

Azam F, Chisholm SW (1976) Silicic acid uptake and incorporation by natural marine phytoplankton assemblages. *Limnology and Oceanography* 21:427-433.

Bhattacharyya P, Volcani BE (1980) Sodium-dependent silicate transport in the apochlorotic marine diatom Nitzschia alba. *Proceedings of the National Academy of Science* USA. 77:6386-6390.

Brzezinski MA (1985) The Si:C:N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables. *Journal of Phycology* 21:347-357.

Brzezinski MA, Nelson DM (1996) Chronic substrate limitation of silicic acid uptake rates in the western Sargasso Sea. *Deep-Sea Research II* 43:437-453.

Brzezinski MA, Phillips DR (1997) Evaluation of 32Si as a tracer for measuring silica production rates in marine waters. *Limnology and Oceanography* 42:856-865.

Brzezinski MA, Phillips DR, Chavez FP, Friederich GE, Dugdale RC (1997) Silica production in the Monterey, California, upwelling system. *Limnology and Oceanography* 42:1694-1705.

DeLaRocha CL, Passow U (2002) Recovery of *Thalassiosira weisflogii* from nitrogen and silicon starvation. *Limnology and Oceanography* 49:245-255.

DeMaster DJ, Kuehl SA, Nittrouer CA (1986) Effects of suspended sediments on geochemical processes near the mouth of the Amazon River: Examination of biological silica uptake and the fate of particle-reactive elements. *Continental Shelf Research* 6:107-125.

DeMaster DJ, Pope RH (1996) Nutrient dynamics in Amazon shelf waters: Results from AMASSEDS. *Continental Shelf Research* 16:263-289.

DeMaster DJ, Smith WO, Nelson DM, Aller JY (1996) Biogeochemical processes in Amazon shelf waters: Chemical distributions and uptake rates of silicon, carbon and nitrogen. *Continental Shelf Research* 16:617-643.

Dugdale RC, Wilkerson FP (1998) Silicate regulation of new production in the equatorial Pacific upwelling. *Nature* 391:270.

Goering JJ, Nelson DM, Carter JA (1973) Silicic acid uptake by natural populations of marine phytoplankton. *Deep-Sea Research* 20:777-789.

Harrison PJ, Conway HL, Dugdale RC (1976) Marine diatoms grown in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady-state growth kinetics of *Skeletonema costatum*. *Marine Biology* 33:177-186.

Kristiansen S, Farbrot T, Naustvol L-J (2001) Spring bloom nutrient dynamics in the Oslofjord. *Marine Ecology Progress Series* 219:41-49.

Lewin JC (1962) Silicification. In: Lewin RA (ed) *Physiology and Biochemistry of Algae.* Academic Press, New York, p 445-455.

Leynaert A, Tréguer P, Lancelot C, Rodier M (2001) Silicon limitation of biogenic silica production in the Equatorial Pacific. *Deep-Sea Research* I 48:639-660.

Livingstone DA (1963) Chemical composition of rivers and lakes. *U.S. Geological Survey, Prof. Paper* 440G:63 pp.

Martin- Jézéquel V, Hildebrand M, Brzezinski MA (2000) Silicon metabolism in diatoms: Implications for growth. *Journal of Phycology* 36:821-840.

Milliman JD, Boyle E (1975) Biological uptake of dissolved silica in the Amazon River Estuary. *Science* 189:995-997.

Monod J (1942) *Recherches sur la croissance des cultures bacteriennes.* 2nd ed., Vol. Herman, Paris.

Muller-Karger FE, McClain CR, Richardson PL (1988) The dispersal of the Amazon's water. *Nature* 333:56-59.

Nelson DM, Goering SS, Kilham SS, Guillard RRL (1976) Kinetics of silicic acid uptake and rates of silica dissolution in the marine diatom *Thalassiosira pseudonana*. *Journal of Phycology* 12:246-252.

Nelson DM, Brzezinski MA (1990) Kinetics of silicic acid uptake by natural diatom assemblages in two Gulf Stream warm-core rings. *Marine Ecology Progress Series* 62:283-292.

Nelson DM, Dortch Q (1996) Silicic acid depletion and silicon limitation in the plume of the Mississippi River: evidence from kinetic studies in spring and summer. *Marine Ecology* Progress Series 136:163-178.

Nelson DM, Treguer P (1992) Role of silicon as a limiting nutrient to Antarctic diatoms: evidence from kinetic studies in the Ross Sea ice-edge zone. *Marine Ecology Progress Series* 80:255-264.

Nittrouer CA, DeMaster DJ, Figueiredo AG, Rine JM (1991) AmasSeds: An Interdisciplinary Investigation of a Complex Coastal Environment. *Oceanography:*3-7.

Oltman RE (1968) Reconnaissance investigations of the discharge and water quality of the Amazon River. U.S. geological Survey, *Circular* 552:16 pp.

Paasche E (1973) Silicon and the ecology of marine diatoms II. Silicate-uptake kinetics of five diatom species. *Marine Biology* 19:262-269.

Shipe RF, Curtaz J, Subramaniam A, Carpenter EJ, Capone DG (2006) Diatom biomass and productivity in oceanic and plumewaters of the eastern tropical Atlantic Ocean. *Deep-Sea Research* I 53:1320-1334.

Shipe RF, Carpenter EJ, Govil S, Capone DG (2007) Limitation of phytoplankton production by Si and N in the western Atlantic Ocean. *Marine Ecology Progress Series* 338:33-45.

Smetacek VS (1985) Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. *Marine Biology* 84:239-251.

Strickland JDH, Parsons TR (1972) *A Practical Handbook of Seawater Analysis*, Vol 167. Bull. Fish. Res. Board Can., Ottawa.

In: Biological Oceanography Research Trends
Editor: Lea P. Mertens, pp. 217-267
ISBN: 978-1-60021-935-1

Chapter 8

FRONTIERS AND TECHNOLOGICAL ADVANCES IN MICROBIAL PROCESSES AND CARBON CYCLING IN THE OCEAN

Nianzhi Jiao[1,*], Chuanlun Zhang[2,3], Feng Chen[4], Jinjun Kan[5] and Fan Zhang[1]

[1]National Key Laboratory for Marine Environmental Sciences, University of Xiamen, Xiamen, Fujian 361005, China;
[2]The Third Institute of Oceanography, State Oceanic Administration, Xiamen, Fujian 361005, China
[3]Department of Marine Sciences, University of Georgia, Athens GA 30602 USA
[4]Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Baltimore, Maryland 21202, USA;
[5]Department of Earth Sciences, University of Southern California, Los Angeles, CA 90089-0656, USA

ABSTRACT

This chapter discusses recent progress in microbial oceanography with emphasis on microbial processes and mechanisms related to carbon cycling in the ocean, including the newly recognized microbial light utilization in the surface ocean, archaeal carbon fixation and methane oxidation in the deep ocean and sediment, as well as lysis of host organisms by viroplankton and its influence on carbon cycling in the water column. Key functional groups of microorganisms include *Prochlorococcus* which possess unique photosynthesis pigments, the divinyl chlrophylls; Aerobic anoxygenic phototrophic bacteria (AAPB) which possess bacterial chlorophyll *a*; Rhodopsin containing proteobacteria (PR); Nonthermophilic crenarchaeota, which use ammonia as a major energy source for autotrophic growth; and the ANME groups of archaea which oxidize methane

* Corresponding author. Tel.: +86592 2187869; fax: +86592 2187869. E-mail address: jiao@xmu.edu.cn (N. Jiao).

for energy. Recent findings have challenged to the conventional concepts and theories. To face these challenges we propose novel models based on an understanding of newly discovered microbial processes. For carbon cycling in the surface ocean, a conceptual model is proposed based on light bio-utilization where bacteriochlorophyll *a* induced anoxygenic phototrophy and proteiorhodopsin based proton pump are included. For carbon sequestration below the surface ocean, the concept of "non-sinking biological pump" is proposed, which is in contrast to the well known sinking flux based biological pump. Furthermore, a putative model for the co-evolution of early life and Earth is proposed based on an understanding of the ecological and molecular features of *Prochlorococcus* and AAPB in the present oceans. In addition to scientific frontiers, relevant state-of-the-art techniques in current microbial oceanography are also presented, including atomic force microscopy that bridges microorganisms to environments through analysis of their cell surfaces; flow cytometry based determination of bacterial membrane potential that provides continuous monitoring of cellular physiological response to environmental changes; time series observation based infrared epifluorescence microscopy (TIREM) for accurate enumeration of bacteriochlorophyll containing microorganisms; meta-genomics and proteomics as tools providing insights into microbial community composition and functions; and microbial lipid biomarkers and isotope signatures to trace carbon flow at the molecular level.

The ocean is the largest ecosystem on the Earth. Microorganisms are the most abundant organisms in the ocean, one liter of seawater contains as many of them as the total human population of the planet. Microorganisms are indispensable for mankind's survival on Earth. In the early stages of the Earth history, cyanobacteria, one of the major autotrophic groups of microorganisms, produced oxygen through photosynthesis and transformed the atmosphere from a reducing state to an oxic state, which supports modern forms of life including human beings. Microorganisms are known to play a key role in carbon cycling in the ocean and consequently affect the global climate. While autotrophic microbes affect the carbon cycle by CO_2 fixation, heterotrophic bacteria are the major driving force for oxidation of organic matter into CO_2, which is often coupled to cycling of nitrogen, sulfur, iron, and other redox-sensitive elements in the ocean. Viruses are known to interact with other microorganisms as either consumers of their hosts or carriers of genetically transferred elements.

Marine microorganisms are extremely diverse and versatile. A large proportion of the microorganisms living in the sea have yet to be cultivated. With application of newly emerged technology, scientists have just begun to explore the unusual microbial activities in the marine environment. This chapter focuses on the following newly discovered microbial processes and mechanisms related to carbon cycling in the ocean, and the relevant state-of-the-art techniques involved.

1. Newly recognized microbial light-utilization in the euphotic zone and its contribution to carbon cycling in the ocean
2. Primary production and carbon cycling in the deep sea: archaeal carbon fixation and methane oxidation
3. Virus processes and their influence on ocean carbon cycling and microbial evolution
4. Ocean carbon sequestration mechanisms: the particulate organic carbon (POC) based "sinking biological pump" v.s. the dissolved organic carbon (DOC) based "non-sinking biological pump"

5. Looking back into the ancient oceans from the modern microbial scenarios
6. State-of-the-art techniques in current microbial oceanography

1. NEWLY RECOGNIZED MICROBIAL LIGHT UTILIZATION IN THE EUPHOTIC ZONE AND ITS CONTRIBUTION TO CARBON CYCLING IN THE OCEAN

Photosynthesis in the sea is thought to be induced primarily by chlorophylls. However recent studies have revealed that light-harvesting pigments are not just confined to normal chlorophylls. Other pigments also contribute substantially to light bio-utilization in the sea, such as divinyl-chlorophyll in *Prochlorococcus*, which is extremely abundant in tropical and subtropical oceans, and bacterial chlorophyll in aerobic anoxygenic phototrophic bacteria (AAPB), which are newly recognized as ubiquitous in all marine environments (Kolber et al., 2001; Béjà et al., 2002; Jiao et al., 2007). Furthermore, a breakthrough in the environmental genomics of marine microbes has revealed the wide existence of proteorhodopsin (PR)-bearing bacteria that account for 13% of the total microorganisms in the surface water of the ocean (Sabehi et al., 2005). These new findings shed light on the non-chlorophyll dependent pathways of light utilization and contrast with the well-known chlorophyll-dependent photosynthesis; the new paths are not to be ignored in energy metabolism and carbon cycling in the ocean.

1.1. Divinyl Chlorophyll-Induced Photosynthesis in *Prochlorococcus*

Prochlorococcus, whose major photosynthetic pigment is divinyl-chlorophyll, are the smallest (0.6-0.7μm) photosynthetic organisms known in the world. The absorption maximum of divinyl-chlorophyll shifts 8-10 nm to the red end of the spectrum compared with normal chlorophyll, which helps the cells to utilize dim light efficiently at the bottom of the euphotic zone. *Prochlorococcus* numerically dominate the marine autotrophs between 40°N (maximum 60°N) and 40°S (generally 10^5cells/mL in oceanic water). They are also an important contributor to biomass and primary production in the major oceans. In the Sargasso Sea, 30% of chlorophyll and 25% of primary production are provided by *Prochlorococcus* annually (Georicke and Welschmeyer, 1993). In the North Pacific, *Prochlorococcus* contribute 41% of total chlorophyll (Seki et al., 2001) and 35% of depth-integrated carbon biomass for the upper 200m water column (Campbell et al., 1997). In the central equatorial Pacific, *Prochlorococcus* contribute 27 to 41% of the total photosynthetic carbon (Binder et al., 1996). In the Mediterranean Sea, 31% of the bulk chlorophyll is contributed by divinyl-chlorophyll (Buck et al., 1996). In the Northwest Pacific marginal seas *Prochlorococcus* contribute 5%-30% of total photosynthetic carbon biomass (Jiao et al., 2002, 2005). Moreover, the growth rate of *Prochlorococcus* is very high in natural water as indicated by cell cycle analysis. This is remarkable given the fact that populations of *Prochlorococcus* divide once a day (1 div/d) based on field observation (Vaulot et al., 1995). Some studies even revealed an ultradian growth of 1.4 div/d in the Arabian Sea (Shalapyonok et al., 1998). With ubiquitous distribution, high abundance and biomass, and high growth rate, *Prochlorococcus* are key contributors to global carbon cycling.

1.2. Bacterial Chlorophyll Induced Phototrophy in Aerobic Anoxygenic Phototrophic Bacteria (AAPB)

AAPB are an important group of heterotrophic bacteria capable of harvesting light energy, and appear to have a particular role in the ocean's carbon cycling (Kolber et al., 2001). The percentage of AAPB in total bacteria (AAPB%) is a key to evaluating the ecological significance of AAPB in marine ecosystems. Since the bacterial chlorophyll a (BChl. a)-based phototrophic function in AAPB is a supplement to their normal heterotrophic diet of DOC (Beatty, 2002; Suyama et al., 2002; Koblizek et al., 2003), it is thus expected to make AAPB more competent when starved of organic carbon for respiration, and provides AAPB with a selective advantage in oligotrophic environments (Kolber et al., 2000; Kolber et al., 2001; Beatty, 2002). A most recent global ocean survey revealed that AAPB are able to maintain high diversity but low abundance in oligotrophic waters, and become more abundant but less diverse in eutrophic waters (Jiao et al., 2007). The oligotrophic ocean (such as the Western North Pacific Gyre) may maintain diverse AAPB species but not necessarily high abundance as previously expected (Kolber et al., 2000; Beatty, 2002). Abundance of AAPB is very variable ranging from 10^2 to 10^4 cells/L. In surface water, AAPB account for 4% of total bacteria in the Indian Ocean, 1.6% in the Atlantic Ocean, and 1.1 % in the Pacific Ocean. In certain eutrophic environments, however, AAPB can make up 20% of total bacteria (Jiao et al., 2007). Since the cell volume of AAPB on average is usually twofold-fourfold that of the other heterotrophic bacteria (Yurkov and Beatty, 1998; Sieracki et al., 2006;), AAPB are easier to be grazed or settle easily out of the euphotic zone through vertical flux. AAPB are thus an important functional member of the community and may play a particular role in carbon cycling in the upper ocean ecosystem. Meanwhile, AAPB are capable of generating chemical energy (ATP) from sunlight for cell metabolism and can essentially save consumption of organic carbon for their respiration. If the amount of saved organic carbon is significant, it will change the current understanding of carbon cycling in the ocean.

1.3. Phototrophy through Proton-Pumping in Rhodopsin-Containing Bacteria

Oxygenic eukaryotic algae, prokaryotic cyanobacteria and anoxygenic photosynthetic bacteria obtain photo-energy by light-harvesting pigments such as normal chlorophyll, divinyl chlorophyll, and bacterial chlorophyll (Fenchel, 2001). Recent studies of environmental genomes have revealed that PR can directly use light energy to transport protons through the plasma inner-membrane so that a proton gradient potential can build up for ATP production (Béjà et al., 2000).

PRs are commonly found in α- and γ-proteobacteria and in CytophagaFlavobacterium-Bacteriodes (CFB), which are among the most abundant bacterial groups in seawater (Venter et al., 2004). In addition, some archaea contain ion-pump rhodopsins such as the proton-pump (bacteriorhodopsin, BR) and chloride pump (halorhodopsin, HR) ones. Both BR and HR have maximum absorption wavelength (λ_{max}) at about 570 nm. Driven by light, BR transports H^+ out of the membrane and establishes an electronic gradient potential that can be used by ATP synthase to synthesize ATP for cell metabolism; while HR can transport Cl^- in through the

membrane under light so as to sustain the balance of pH in the cell, thereby acclimatizing the cell to extreme environments.

Different types of PR proteins are able to adsorb different wavelengths of light at different water depths (Béjà et al., 2001). The spectroscopic characteristics of PR split the known active proteins of PR into three groups, namely green-light PR (GPR), blue-light PR (BPR) and blue-green-light PR (B-G-PR). GPR with the absorption peak (λ_{max}) of 525 nm is found in the illuminated surface water of the Pacific Ocean, Mediterranean Sea, Red Sea and China seas (Jiao et al., 2006). BPR and B-G-PR have λ_{max} at 490 and 515 nm, respectively, and are found in more oligotrophic or deeper water (Man et al., 2003; Wang et al., 2003; Man Aharonovich et al., 2004; Jiao et al., unpublished).

PR-bearing bacteria may account for up to 13% of the total microorganisms in surface waters (Sabehi et al., 2005). Given the wide distribution, high diversity and great abundance of PR bacteria, the estimated photosynthetic flux, calculated from the cellular PR content of 2.4×10^4 PR molecules in the case of SAR86 (Béjà et al., 2001) and photoreceptor quantum conversion efficiency (Michel and Oesterhelt, 1980; Tsutomu and Koichi, 1992), would be high and cannot be ignored in any model of energy flow or carbon cycling. It has been estimated that approximately 25,000 PR protein molecules occupy one cell of *Pelagibacter ubique*, making up 20% of the inner superficial area of the cell (Giovannoni et al., 2005). Such estimates will become more accurate as more strains are isolated and cultured, and further specialized techniques become applicable to this field. In any case, light energy utilized through the PR pathway is expected to play a significant role in the marine ecosystem.

1.4. Light Bio-Utilization and Carbon Cycling in Surface Oceans

The above novel discoveries have changed the conventional concept of biological utilization of light energy in the sea and are challenging the assessment of primary production based on photosynthetic pigments either through *in situ* measurements or remote sensing observations. We hereby propose a conceptual model of bio-utilization of light and carbon cycling in surface oceans based on our knowledge of the literature and our own data (Figure 1). In this model, light energy utilization pathways are divided into three categories: Path I, chlorophyll-dependent oxygenic photosynthesis comprised of photosystem I and photosystem II, through which light is captured and transformed into chemical energy and meanwhile carbon dioxide is fixed to organic carbon. This photosynthesis is carried out by marine algae/phytoplankton (with normal chlorophyll) and cyanobacteria (with normal chlorophyll or divinyl chlorophyll). Path II, bacteriochlorophyll-dependent anoxygenic photosynthesis by aerobic and anaerobic anoxygenic photosynthetic bacteria, which use light for chemical energy but carbon fixation due to lack of photosystem II. Path III is PR-dependant light utilization. Lacking photosystem I and II, PR-containing bacteria directly trap light by PR protein and use the proton-pump mechanism to produce ATP. These three pathways constitute the major mechanisms of light bio-utilization in the sea and drive the energy flow and carbon cycling in different ways: Path I directly leads to the fixation of carbon dioxide, whereas Paths II and III produce ATP to reduce the consumption of organic carbon and contribute indirectly to carbon cycling in the ocean (Figure 1).

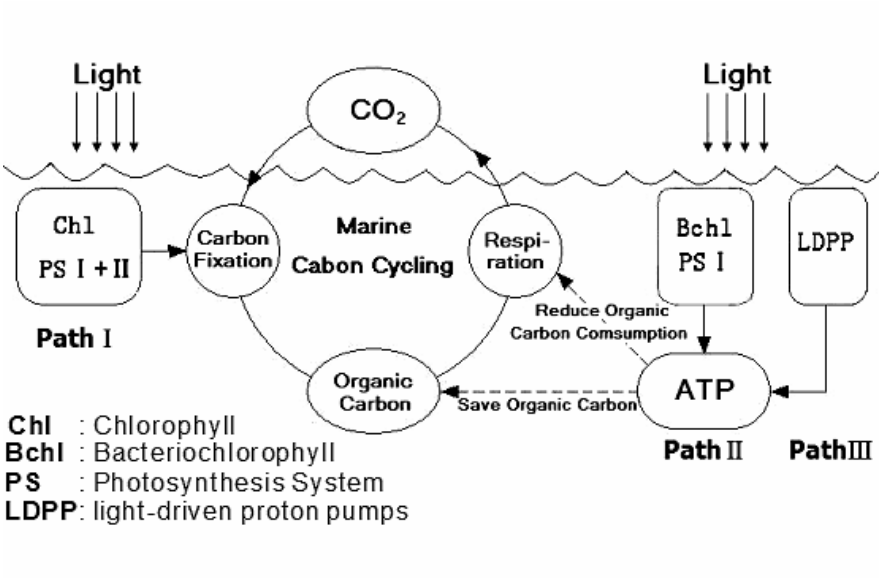


Figure 1. A model of light bio-utilization and carbon cycling in the surface ocean (modified from Jiao et al., 2006).

2. PRIMARY PRODUCTION AND CARBON CYCLING IN THE DEEP SEA: ARCHAEAL CARBON FIXATION AND METHANE OXIDATION

The discovery of nonthermophilic archaea (crenarchaeota and euryarchaeota) in the open ocean and coastal water using culture-independent molecular technologies was reported in two seminal papers in the early 1990's (DeLong, 1992; Fuhrman et al., 1992), and this corrected our previous biased view that crenarcheota only existed in extreme environments such as hydrothermal vents or terrestrial thermal springs. Since then, research on nonthermophilic archaea has been intensely pursued intensely and it is now clear that these organisms occur widely in low temperature marine and terrestrial environments (see reviews by DeLong, 1998; Schleper et al., 2005). Overall, in marine water columns, nonthermophilic archaea are dominated by Group I crenarchaeota and Group II euryarchaeota. In marine sediments, not only Group I crenarchaeota and Group II euryarchaeota are present but also a variety of other archaeal species, including the Marine Benthic Group crenarchaeota, Miscellaneous Crenarchaeotal Group, and an unidentified euryarchaeota group (Vetriani et al., 1999; Inagaki et al., 2006; Biddle et al., 2006). In addition, anaerobic methane-oxidizing archaea (ANME groups) widely occur in association with gas hydrates, cold seeps, and organic rich thermal sediments (e.g., Boetius et al., 2000; Orphan et al., 2001; Mills et al., 2003; Knittel et al., 2005) as well as in anoxic water bodies like the Black Sea and Cariaco Basin (Madrid et al., 2001; Durisch-Kaiser et al., 2005; Schubert et al., 2006). This section reviews recent observations of the occurrence and diversity, abundance, metabolic pathways, and contributions to carbon and nitrogen cycles of nonthermophilic archaea in the deep oceans and marine sediments.

2.1. Occurrence and Diversity of Nonthermophilic Archaea in Water Column and Marine Sediments

The first detection of PCR-based archaeal 16S rRNA genes in the marine plankton included a few sequences that are closely related to known hyperthermophilic species (Furman et al., 1992; DeLong, 1992). This observation was initially uncertain as some of these archaea were questioned as being allochthonous thermophiles transported from a putative hydrothermal vent. Soon after, however, DeLong and co-workers discovered high abundance of archaea in cold aerobic Arctic water (DeLong et al., 1994) and also a crenarchaeal symbiont in a cold marine sponge (Preston et al., 1996), crystallizing the fact that the newly discovered archaea are native to the low-temperature marine environment. Since then, a large number of novel archaeal phylotypes have been detected in almost every niche of the marine biotope, ranging from surface to abyssal depths of the Pacific and Atlantic Oceans as well as in deep marine sediments (see review by Schelper et al., 2005). So far, the vast majority of planktonic archaea fall into the group I. 1A crenarchaeota and group II euryarchaeota. It has been observed that group I. 1A crenarcheota generally increase in abundance at greater depths (>200 m); whereas, the group II euryarchaeota are predominant in the photic zone (< 200 m) but also are found in significant abundance in deeper waters (Massana et al., 1997; Karner et al., 2001; Herndl et al., 2005; DeLong et al., 2006; Teira et al., 2006).

Nonthermophilic archaea in marine sediments are mostly reported from cold-seep and gas hydrate-related environments where methane serves as an abundant energy source fueling the anaerobic oxidation of methane (AOM) coupled to sulfate reduction (Hinrichs et al., 1999; Orphan et al., 2001). In diffusion prone settings of deep sea marine sediments, where methane and sulfate fluxes are low, the AOM process is identified in a sediment horizon, namely the sulfate-methane transition zone, into which both methane and sulfate diffuse (Biddle et al., 2006).

In cold seeps and gas hydrates, the archaeal community is dominated by the uncultured ANME groups of euryarchaeota that are known to mediate the AOM (*e.g.*, Inagaki et al., 2003; Biddle et al., 2006; Inagaki et al., 2006; Kendall et al., 2007). In particular, ANME-1 and ANME-2 phylotypes are most abundant although their relative distributions varies from location to location. For example, ANME- 1 is predominant in the Black Sea, whereas ANME-2 is predominant at Hydrate Ridge (Knittel et al., 2005; Nauhaus et al., 2005). In the Gulf of Mexico, both ANME-1 and ANME-2 are conspicuous (Lanoil et al., 2001; Mills et al., 2003; Lloyd et al., 2006; Reed et al., 2006; Pi et al., in rivision). In the diffusion prone underlying the highly productive surface water off the coast of Peru, however, the sedimentary archaeal communities are dominated by the Marine Benthic Group B and the Miscellaneous Crenarchaeotal Group, whereas methanotrophic archaea such as ANME-1 and ANME-2 are not detected (Biddle et al., 2006). Other non-cold seep or non-gas hydrate deep-sea marine sediments are also dominated by nonthermophilic crenarchaeota and nonANME groups of euryarchaeota (e.g., Vetriani et al., 1999; Inagaki et al., 2003; Lanoil et al.,2005; Wang et al., 2005; Inagaki et al., 2006). In particular, marine benthic group B and Thermoplasmatales appear to be cosmopolitan in normal marine sediments (Orphan et al., 2001; Teske, 2006).

2.2. Abundance of Nonthermophilic Archaea in the Water Column and Marine Sediments

The observation of very large numbers of nonthermophilic archaea in the open ocean adds another dimension to the importance of these organisms in the marine ecosystem. An early estimate of the abundance of planktonic archaea was made in temperate coastal marine waters and polar seas, which ranged from about 10 to 30% of the total prokaryotic biomass (DeLong, 1992). In 2001, a major study was undertaken in the Pacific Ocean, which demonstrated the dominance of archaea in the mesopelagic zone of this water body (Karner et al., 2001). Monthly sampling within a 12-month period showed that pelagic crenarchaeota comprised a large fraction of the total marine picoplankton below 150 m (the bottom of the euphotic zone) and increased with depth, reaching about 40% of the total DNA-containing picoplankton detected using DAPI staining and fluorescent isothiocyanate multiple labeled probes (Karner et al., 2001). Estimates based on this study indicate that the world's oceans contain approximately 1.3 x 1028 archaeal cells, which is of the same order of magnitude as bacteria (3.1 x 1028 cells) (Karner et al., 2001). Another comprehensive study in the Atlantic Ocean, demonstrated the consistently higher abundance of archaea than bacteria below the 100-m depth, using an improved catalyzed reporter deposition-fluorescence in situ hybridization method and specific oligonucleotide probes (Herndl et al., 2005). Estimates of the archaeal production in the mesopelagic and bathypelagic North Atlantic showed 13-27% contributions to the total prokaryotic production in the oxygen minimum layer, 41-84% in Labrador sea water, and 10-20% in the North Atlantic deep water (Herndl et al., 2005).

While group I crenarchaeota commonly comprise the majority of deep water planktonic archaea, it was observed in one instance that a deeply branching crenarchaeal group related to a hot spring clade (pSL 12) was significantly more abundant than the group I crenarchaeota below the euphotic zone in the North Pacific subtropical gyre (Mincer et al., 2007), suggesting that certain environmental conditions may stimulate the growth of multiple species of crenarchaeota. Another observation involves the unusual abundance of group I crenarchaeota in the suboxic layers of the Black Sea, which makes up to 98% of the total archaeal 16S rRNA gene copies (Coolen et al., 2007), suggesting that these archaeal organisms may be microaerophilic and may be able to tolerant suboxic conditions.

Estimates of archaea in marine sediments are complicated by the heterogeneous distribution of these organisms in the porous medium. Geochemical proxies, however, have indicated that 90% of the methane produced in the marine sediments is consumed before it reaches the water column (Valentine and Reeburgh, 2000), suggesting that a large population of methane-oxidizing archaea exists in the sediments and plays a significant role in controlling the flux of methane into the atmosphere. Other studies have estimated that the abundance of archaea in marine sediments varies between 0.01% and 30% of total biomass with high values often occurring near the seafloor (Vetriani et al., 1999; Inakagi et al., 2006).

2.3. Metabolic Pathways of Nonthermophilic Archaea in the Water Column and Marine Sediments

The physiology and biochemistry of nonthermophilic archaea have also begun to be elucidated as a result of developments in environmental genomics (Venter et al., 2004; Schleper et al., 2005; Hallam et al., 2006a) and the genomic analysis of a single crenarchaeal phylotype Cenarchaeum symbiosium in a marine sponge (Preston et al., 1996; Hallam et al., 2006b). The recently isolated pure culture of the mesophilic crenarchaeon, Nitrosopumilus maritimus, adds new insight into the physiology of non-thermophilic crenarchaeota (Könneke et al., 2005). Mounting evidence indicates that some of the nonthermophilic crenarchaeota use ammonia as a major energy source for autotrophic growth (Francis et al., 2005; Könneke et al., 2005; Nicol and Schleper, 2006; Wuchter et al., 2006). Other molecular and geochemical studies show that nonthermophilic crenarchaeota can take up amino acids and organic carbon, indicating that some of them are heterotrophs or mixotrophs (Biddle et al., 2006; Herndl et al., 2005; Ingalls et al., 2006; Ouverney et al., 2000; Teira et al., 2006). One recent study showed that some of the benthic crenarchaeota may oxidize methane using it as an energy source and other organic carbon as the carbon source (Biddle et al., 2006).

On the other hand, ANME groups of the euryarchaeota oxidize methane using a reverse methanogenesis pathway (Boetius et al., 2000; Hallam et al., 2003, 2004; Valentine, 2000). While no isolate is available of any of the ANME group members for detailed biochemical studies, genomic analysis of the AOM community in Eel River Basin sediment could distinguish the ANME-1 group from the ANME-2 group based on G+C content and depth of the whole genome shotgun coverage, Importantly, Hallam et al., (2004) demonstrated that nearly all the genes typically associated with methane production are present in the ANME-1 group, except a step encoded by the mer gene. It was proposed that the loss of mcr activity in ANME-1 could promote AOM by increasing the activation barrier for conversion of methylene- H4MPT to methyl-H4MPT. Overall, the association of most of the genes for methanogenesis in the ANME groups strongly supports the hypothesis that AOM proceeds as a reversed pathway of methane production.

The Group II euryarchaeota are known to contain light-harvesting proteins (PRs), suggesting that these novel euryarchaeota may perform light-dependent cellular energy metabolism (DeLong et al., 2006; Frigaard et al., 2006).

2.4. Contribution of Nonthermophilic Archaea to Carbon and Nitrogen Cycles in the Deep Ocean

A picture is emerging that the majority of the nonthermophilic archaea in the deep ocean are chemoautotrophs and may use ammonia as a major energy source. Herndl et al., (2005) presented leucine-incorporation evidence that the actively growing archaea in the oxygenated water column, which numerically dominate the prokaryotic community in the North Atlantic below a depth of 100 m to 2435-2790 m in the North Atlantic Deep Water, use bicarbonate or CO_2 as a carbon source. Independently, Ingalls et al., (2006) quantified archaeal autotrophy in the subtropical North Pacific gyre using natural radiocarbon and demonstrated, based on an isotopic mass balance model, that 83% of total archaeal metabolism is autotrophy that fixes

^{14}C-depleted dissolved inorganic carbon in the deep water; whereas, the remaining metabolism may contribute to heterotrophic consumption of the ^{14}C-enriched modern organic carbon. Based on the depth-integrated mean C fixation rate (0.014 fmol C archaeon^{-1} day^{-1}) and on the assumption that the global ocean contains approximately 1.3 x 10^{28} cells of archaea, Herndl et al., (2005) derived a global inorganic carbon fixation rate of 6.55 x 10^{13} mol C year^{-1}. This global carbon fixation rate is compared with that derived from archaeal nitrification, which is estimated to be 3.3 x 10^{13} mol C year^{-1} (assuming all of the ammonium would be oxidized by crenarchaeota fixing one carbon atom for every 10 nitrogen molecules oxidized) (Wuchter et al., 2006). The agreement between these estimated rates suggest that archaeal nitrification coupled to inorganic carbon fixation may contribute profoundly to the biogeochemical cycles of carbon and nitrogen in the global ocean (Figure 2).

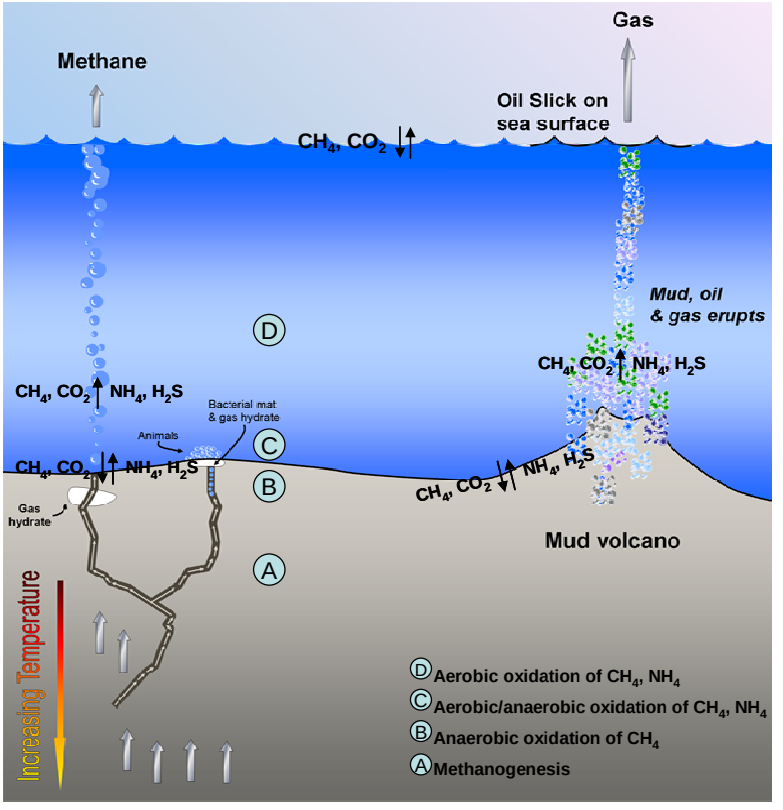


Fig. 2. A model of carbon, nitrogen, and sulfur cycling in the deep ocean and deep-sea marine sediments affected by gas hydrates or cold seeps (Modified from Whelan et al., 2005).

Methane formation by methanogens in the dark ocean is also a major sink of CO_2; however, its contribution to carbon fixation is two orders of magnitude lower (~8 x 10^{11} mol C year^{-1}) than that from crenarchaeota in the global ocean (Herndl et al., 2005). In marine sediments, methanogens are the sole source of biogenic methane below the sulfate reduction zone; whereas within the sulfate reduction zone, methane-oxidizing archaea in consortia with sulfate-reducing bacteria are mainly responsible for methane consumption (Figure 2). Although heterotrophic crenarchaeota are known to perform oxidation of methane (Biddle et al., 2006), their relative contribution to methane consumption is unknown. In either case, CO_2

from anaerobic methane oxidation is largely sequestrated into carbonate minerals that constitute a major fraction of the marine sediment or form carbonate mounds above the ocean floor. This has been observed frequently in the Gulf of Mexico (Formolo et al., 2004; Sassen et al., 2004) as well as other methane-rich marine sediments (e.g., Aloisi et al., 2002), where gas hydrates and cold seeps are found abundantly. Globally, a huge amount of methane (> 10 trillion tons; Zimmer, 2001) may be sequestrated in the hydrate structure in marine sediment or hydrate mounds, which are known as an attractive alternative energy resource beyond petroleum hydrocarbons and coals.

3. VIRUS PROCESSES AND THEIR INFLUENCE ON OCEAN CARBON CYCLING AND THE EVOLUTION OF LIFE IN MARINE ENVIRONMENTS

Discovery of high abundance of virus-like particles (VLPs) in seawater in the late 1980's (Bergh et al., 1989; Proctor and Fuhrman, 1990) raised many new concerns regarding the ecological roles of marine viruses. Viruses are now believed to have significant impacts on biomass, production, population structure and genetic diversity of microorganisms living in aquatic environments (Fuhrman, 1999; Fuhrman and Suttle, 1993; Suttle, 1994; Wommack and Colwell, 2000). The majority of marine viruses (or virioplankton) are the bacteriophages that infect bacterioplankton (Fuhrman, 1999; Wommack and Colwell, 2000). Meanwhile, many new types of viruses have been isolated from eukaryotic microalgae (Suttle, 2005), suggesting that algal viruses are an important part of phytoplankton life cycles.

3.1. Enumeration of VLPs in Aquatic Environments

The concentration of VLPs in different aquatic environments has been measured using different methods including transmission electron microscopy, epifluorescence microscopy and flow cytometry (FCM). In most cases, the viral abundance in the aquatic environments ranges from 10^6 to 10^7 VLPs/mL, which is approximately 10 times more than the bacterial cell concentration. The two most common fluorescent dyes used for enumerating viral particles are SYBR Green I (Noble et al., 1998) and SYBR Gold (Chen et al., 2001). SYBR Gold has been demonstrated to be a bright and stable stain for counting viruses and bacteria in aquatic environments (Chen et al., 2001, Shibata et al., 2006). SYBR Gold stains both DNA and RNA. Viruses or bacteria stained with SYBR Gold emit yellow-green fluorescence (with blue-green light excitation) that can be enumerated under epifluorescence microscopy (Figure 3). The detailed protocols for staining VLPs using SYBR Gold can be acquired from the website (http://www.umbi.umd.edu/%7Ecomb/faculty/chen/chen.html). SYBR Green I and SBYR Gold were first coupled with FCM to enumerate VLPs in different types of water samples (Marie et al., 1999; Chen et al., 2001). Counting VLPs by FCM is rapid and reproducible. And so FCM has become a powerful tool to investigate viral abundance in aquatic environments.

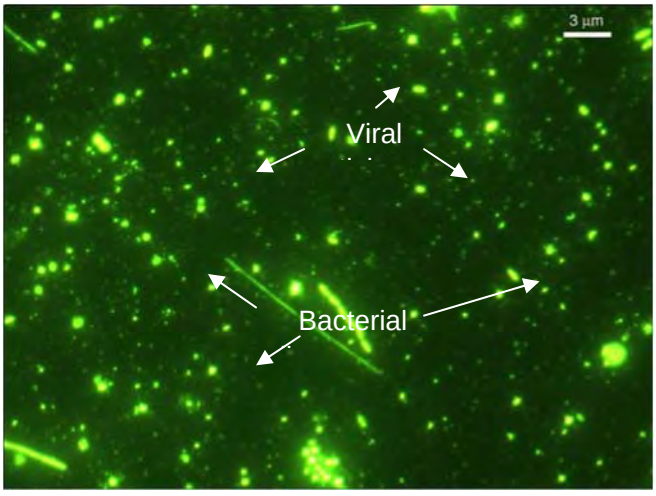


Figure 3. A microscopic view of viral like particles, VLPs (small arrows) and bacterial cells (large arrows) in an estuarine water sample (Chesapeake Bay) stained by SYBR Gold. There were about 10^7 VLPs/mL and 10^6 bacterial cells/mL in this sample.

3.2. Viruses That Are Known to Infect Major Primary Producers

Despite the fact that bacterial viruses have been isolated from seawater since the 1950's, only recently have various types of viruses responsible for most of the carbon fixation in the ocean been isolated from marine environments. These viruses infect marine Synechococcus (Suttle 1993; Waterbury 1993; Wilson 1993; Lu et al., 2001), *Prochlorococcus* (Sullivan et al., 2003; Sullivan et al., 2005), and other phytoplankton (i.e. diatoms and dinoflagellates) (see Suttle 2005 for review). It has long been known that picocyanobacteria including Synechococcus and *Prochlorococcus* could contribute the majority of the ocean's primary production. The distribution of picocyanobacterial viruses (cyanophages) had been found to be synchronized with picocyanobacteria over time and space (Waterbury 1993; Suttle and Chan 1994). Cyanophage sequences make up 1-10% of bacterial community genomes in the photic zone of the open oceans, suggesting that the picocyanobacteria are subject to frequent viral infection in nature (DeLong et al., 2006). This is consistent with an earlier study showing that a portion of unicellular cyanobacteria in natural seawater contains matured phages (Proctor and Fuhrman 1990). The close relationship between cyanobacteria and cyanophage was also found in estuarine ecosystems such as Chesapeake Bay (Chen et al., unpublished data). A recent study indicated that *Synechococcus* living in an estuary are infected by host-specific cyanophages while broad-host-range cyanophages are more common for *Synechococcus* living in the open ocean (Wang and Chen, in press). A highly specific phage-host relationship was also noticed in *Prochlorococcus* adapted to the high light environment (Sullivan et al., 2003). These studies suggested that the distribution of viral genotypes could be influenced by their specific hosts being adapted to different environments rather than uniformly distributed in the ocean. A recent survey of viral community metagenomics in the ocean suggests that viral diversity is greatly influenced by geographic locations (Angly et al., 2006).

3.3. "Symbiotic" Relationship between Virus and Host

Although there are about one million "free-living" virioplankton in a drop of water, a large portion of marine viruses (phages) are hidden inside host cells forming a symbiotic relationship with their hosts. These phages integrate their genomic DNA into bacterial chromosome and co-exist with their host cells without killing them. A bacterium that carries phage (or prophage) DNA is called a lysogenic bacterium or lysogen. Lysogenic bacteria are capable of spontaneous lysis due to the release of the prophage from the bacterial chromosome. Lysogenic and lytic cycles are the two major forms of interaction between bacteriophage and bacteria. Earlier studies have shown that about 40% of heterotrophic bacteria isolated from marine environments contain an inducible prophage (Jiang and Paul, 1996). The traditional method for determining a lysogen is to monitor the growth of bacteria (OD600) treated with mitomycin C or UV light. Our recent studies showed that the traditional method could overlook the lysogenic potential among marine heterotrophic bacteria. By monitoring production of viral particles using epifluorescence microscopy or FCM, we estimated that about 80% of bacterial strains (~30 strains tested) isolated from marine sources produced VLPs (Chen et al., unpublished data). The concentration of VLPs produced by these bacteria ranged from 10^7 to 10^9 per mL, and varied with bacterial strains, growth stages, culture media, and concentration of mitomycin C. A significant portion of bacterial isolates released VLPs spontaneously regardless of the treatment with mitomycin. Spontaneous release of bacteriophages has been reported in some bacterial systems, but has not been recognized widely among marine bacteria. Our results suggest that the proportion of marine lysogens could be much higher than we thought, particularly when auto-induction of the prophage is considered. Direct counting methods coupled with SYBR Gold are sensitive enough to enumerate VLPs from cultured bacteria, even with low phage yield. Despite the great lysogenic potential among heterotrophic bacteria, the mechanism of lysogenic induction and their impact on global nutrient and carbon cycles in the natural aquatic environment still remains largely unexplored.

The prevalence of lysogeny in the marine environment has been an issue for debate. Although it has been reported that a high proportion of marine samples contained prophages inducible by mitomycin C and UV light (Cochran et al., 1998a, b), other researchers found that lysogeny is not an important source of phage production or bacterial mortality in coastal waters (Wilcox and Fuhrman, 1994; Weinbauer and Suttle, 1996). Induction of lysogeny only caused 3% of the total bacterial mortality in seawater (Weinbauer and Suttle, 1996).

To explain such a discrepancy, further analysis of bacterial population composition is necessary. In the marine environment, free-living prokaryotes, such as the SAR11 group, *Synechococcus* and *Prochlorococcus* together could build up more than 80% of the bacterial community. They are either autotrophic or have a minimum requirement for nutrients and dissolved organic matter (DOM). Their genome sizes (typically less than 3 Mb) are relatively small compared to other heterotrophic bacteria (> 4 Mb) that prefer to grow under rich DOM. Genome sequences of *Pelagibacter ubique* (SAR11), *Synechococcus* and *Prochlorococcus* revealed no intact prophage on their chromosome (Rocap et al., 2003; Dufresne et al., 2003; Palenik et al., 2003; Giovannoni et al., 2005; Palenik et al., 2006). On the other hand, many cultured heterotrophic bacteria contain intact genome of prophage(s) (Canchaya et al., 2003; Brussow et al., 2004, Chen et al., 2006). It is likely that lytic infection is more common among the free-living bacterial populations with relatively small genome sizes, while lysogenic infection is more important to the bacteria with relatively large genomes. In natural

aquatic environments, the free-living bacteria that build up the the majority of bacterial community are not lysogenic. When the whole bacterial community is induced for lysogenic production, only the minor bacterial populations (with large genome size) are induced, resulting in a low percentage of detectable lysogeny in natural waters. High frequency of prophage induction found in isolated bacteria is due to the fact that we are testing a different group of bacteria. Most of the hetertrophic bacteria were isolated from freshwater or seawater using the rich culture media. Although these "easy-to-grow" bacteria can be very diverse, they may not be dominant populations in nature. A high proportion of these cultivated bacteria occupy specific ecological niches (e.g. particle associated, biofilm forming, or algae associated). They tend to have a larger genome size than free-living bacteria. Having a large genome increases the capacity for carrying additional genes, and hence provides the cells with more flexibility to cope with environmental changes. Genome sequences from marine bacteria are increasing rapidly in GenBank. According to the latest in silico survey, a high proportion of heterotrophic bacteria with large genome contain intact prophage(s) (Chen, unpublished data). For example, a marine roseobacter, Silicibacter sp TM1040 contains three inducible prophages and one genetic transfer agent (GTA) (Chen et al., 2006). Recently, the complete GTA structure was found in nearly all the genome s of marine roseobacters (Lang and Beatty, 2007). Phage, prophage and GTA form the genetic transfer element and can contribute greatly to bacterial diversification and genomic evolution (Weinbauer and Rassoulzadegan., 2004; Chen et al., 2006; Lang and Beatty, 2007).

3.4. Viral Lysis and C and N Cycling

Bacterioplankton and phytoplankton grow constantly and maintain high production in the aquatic ecosystem. On the other hand, the total number of microbial cells in the water system is relatively stable at a given time. What mechanisms are responsible for keeping the microbial abundance in balance? For a long time, it was believed that zooplankton grazing was the sole mechanism responsible for removing bacterial cells. The high abundance and production of viral particles discovered in the 1990's challenged this paradigm. It has been estimated that about 10-20% of heterotrophic bacteria and 10% of cyanobacteria are destroyed daily (Fuhrman and Suttle, 1993, Suttle, 1994). Both viral lysis and grazing are now considered as equally important for removing microbial cells and keeping them in balance in the aquatic ecosystem. Moreover, destruction of microbial cells (bacteria and microalgae) by viral lysis releases a significant amount of cellular materials which is an important source of organic carbon and nitrogen, and nutrients and trace elements in the aquatic microbial food web (Proctor and Fuhrman, 1990; Fuhrman and Suttle, 1993; Thingstad et al., 1993; Gobler et al., 1997; Wilhelm and Suttle; 1999, Fuhrman 1999). About 1 μg/L of DOC per bacterial generation could be released due to viral lysis (Proctor and Fuhrman, 1990). Wilhelm et al., (1998) reported that the release of DOC by viral lysis in the nearshore (0.7-5.2 μg·L-1·d-1) was higher than that in the offshore (0.1-0.6μg·L-1·d-1). Although viral lysis only releases a small fraction of the total DOM pool on a daily basis, it could constitute a significant part of rapidly cycling carbon and nitrogen on the global scale. The release of DOC caused by lytic infection of marine viruses will divert or short-circuit the flow of photosyntheically fixed organic carbon in marine food webs (Wilhelm and Suttle,

1999) (Figure 4). Nearly 25% of the organic carbon in the sea is regenerated within viral loop instead of transferring to high food chain (Wilhelm and Suttle, 1999).

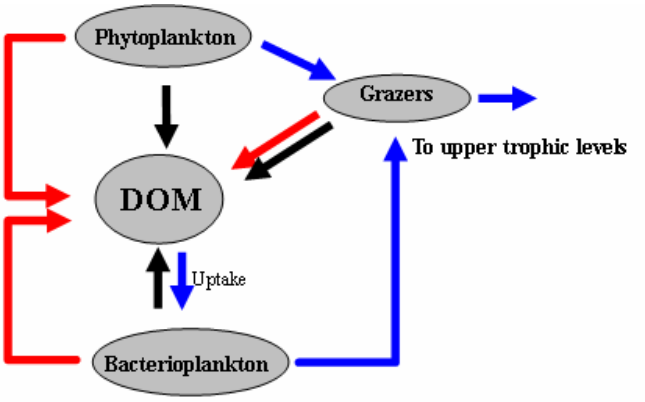


Figure 4. The "Viral Loop" in marine planktonic food web. Viral lysis of bacterioplankton, phytoplankton and zooplankton liberates dissolved organic matter from cells into the DOM pool (shown in red). Grazing or uptake of DOM is shown in blue. Excretion from microbial cells (black) also contributes to the DOM pool.

4. Ocean Carbon Sequestration Mechanisms: Sinking Versus Non-Sinking Biological Pumps

Particulate organic carbon (POC) formed by organisms in the euphotic zone can sink out of the surface layer and partially sink down to the deep sea, thus preventing the carbon from being returned into the atmosphere for hundreds of years (Feely et al., 2001). This process is primarily driven by biota and is thus called a "biological pump" (Figure 5). In contrast, dissolved organic carbon (DOC) is not sinkable, and thus used to be considered as a zero contribution to the "biological pump". Microorganisms were even once thought to be insignificant contributors to the "sinking biological pump" due to their small size. However, recent studies have revealed that neither DOC nor microorganisms are negligible in terms of carbon sequestration of atmospheric CO_2 by the ocean. Labile DOC (LDOC) can be picked up by heterotrophic bacteria and then transported to upper trophic levels through the "microbial loop" (bacteria→ flagellates→ciliates→metazoan) (Azam et al., 1983), and consequently forming sinking POC again. Pico-sized planktons can also form significant sinking flux because of their "aggregation mechanisms" (Richardson et al., 2007).

In contrast to the well known sinking biological pump, we propose here the concept of "non-sinking biological pump" based on an understanding of the role of refractory DOC (RDOC), which is resistant to biological decomposition. Usually, DOC can be generated from exudation, grazing, egesting, death processes, especially enzymatic decomposition and viral lysis processes. Contrasting to the sinking property of POC, DOC does not sink. The fate of DOC is either being uptaken by heterotrophic processes or staying in the water until being

further decomposed by other processes such as UV radiochemical transformation (Mopper et al., 1991; Miller and Moran, 1997).

Compared with LDOC, the turnover time of RDOC is very long, approximately 4000-6000 years (Williams and Druffel, 1987; Bauer et al., 1992). Although RDOC does not sink, its long residence time in the water column functions in the same way as the sinking biological pump in terms of carbon sequestration of atmospheric CO_2 in the sea. As RDOC is either produced from biota or left by bacteria, such processes can be called a "non-sinking biological pump" (Figure 5).

The DOC pool in the ocean is estimated to be 700 Gt (Nogawa and Tanoue, 2003), the second largest carbon reservoir in the ocean and approximately equivalent to the carbon stock in atmospheric CO_2 (~750 Gt) or terrestrial biomass (~600 Gt) (Hedges, 1992). Approximately 650 Gt of marine DOC is RDOC (Nogawa and Tanoue, 2003) accounting for the majority of DOC in the ocean. Therefore, the non-sinking biological pump is one of the keys to an understanding of the carbon sink of the ocean. DOC accumulation in the ocean from ancient time to the present has demonstrated its significance in affecting carbon cycling in the ocean.

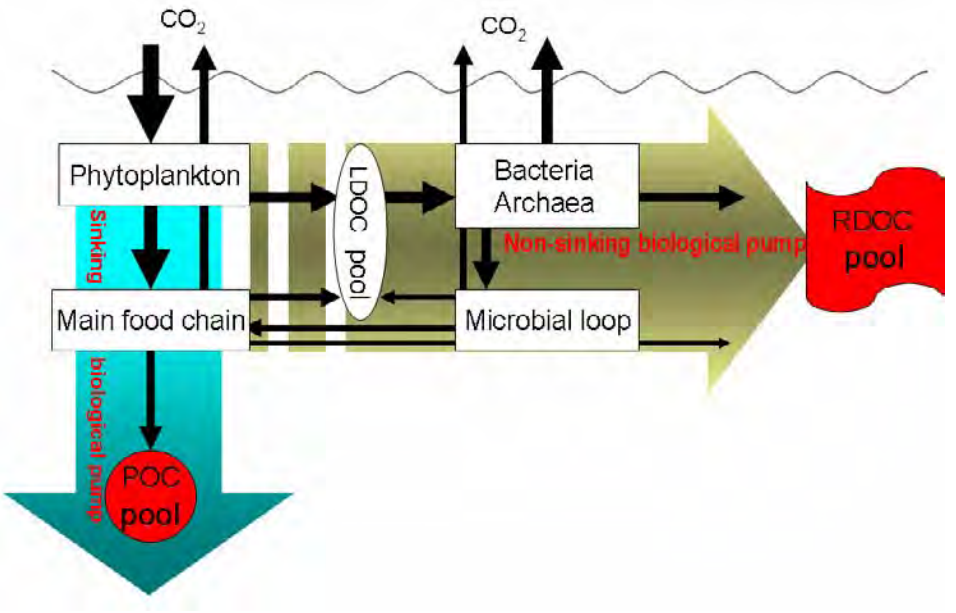


Figure 5. A diagram of "Non-sinking biological pump" versus "Sinking biological pump" (Modified from Jiao et al., 2006).

5. Looking Back into the Ancient Oceans from the Modern Microbial Ecological Scenarios

Microorganisms are usually ignored in deep sea paleogeological records because of their "non-sinking" property. However, this traditional perception may not be true. As mentioned above, pico-sized microorganisms do sink through "aggregation" or "trophic transformation"

mechanisms (Richardson et al., 2007). Although rarely fossilized in the sediments or rocks, microorganisms do leave molecular tracers in the geological records. A deeper understanding of microorganisms in today's ocean will help us to better probe the paleomicrobial ecology in the geological past. The ecological patterns of *Prochlorococcus* or AAPB in the present oceans may inspire us to imagine what kind of habitats they might have had in the ancient. For today's *Prochlorococcus*, the favorite environmental conditions would be high temperature, low light, and low nutrients. Their molecular features of *Prochlorococcus* include the unique photosynthesis pigment (divinyl-chlorophyll a) and the lack of nitrate reductase encoding genes. Examining the history of Earth, one will find that such an environment might have occurred in ancient time when the planet was covered with thick greenhouse gasses (e.g., CO_2, CH_4) and surface temperature was high and irradiation low. There was obviously no nitrate in the ocean due to the reduced atmosphere at that time. Could that have been the time when *Prochlorococcus* arose?

Most recently, unusual patterns were observed for the diversity and abundance of AAPB: the abundance of AAPB increases with increasing chlorophyll concentration, whereas the diversity of AAPB is higher in oligotrophic oceans than in eutrophic waters (Jiao et al., 2007). Such a phenomenon is likely to be due to different mechanisms underlying the abundance and the diversity of AAPB. While the abundance of AAPB is primarily driven by the availability of suitable carbon sources, the diversity of AAPB appears to be related to co-evolution in a changing environment in the ocean. It has been proposed that all proteobacteria descended from a common purple photosynthetic bacterial ancestor (Xiong et al., 2000). During evolution, some of them may have lost their photosynthetic genes, while others may retained these genes (Beatty, 2002). Those proteobacteria with photosynthetic genes and adapted to aerobic environments might have become AAPB. The unique metabolic features of AAPB include the use of few carbon sources and a moderate requirement for light and oxygen (Nogawa and Tanoue, 2003). AAPB are also closely related to cyanobacteria in phylogeny (Jiao et al., 2007). These observations are provocative evidence that AAPB could be an important life component in the Earth's early history, mimicking the long reign of cyanobacteria in the Mesoproterozoic when oceans were primarily anoxic below the moderately oxic surface water (Anbar and Knoll, 2002; Kerr, 2002). During the Mesoproterozoic period, DOC in the surface oceans was contributed mainly by cyanobacteria (Bralower et al., 2002; Dumitrescu and Brassell, 2003) and was much less complex compared to the DOC composition of today's ocean. With increasing organic matters and oxygen in the ocean later on, particularly after the emergence and evolution of plants, some original AAPB species consequently might have lost their photosynthetic genes and become non-AAPB, or have been eliminated through natural selection. The heterotrophic non-AAPB out competed AAPB for utilizing diverse DOC in today's oceans. The photosynthetic function in AAPB primarily provides the cell with an energy source but not a carbon source (Yurkov and Beatty, 1998; Kolber et al., 2000; Kolber et al., 2001). An external carbon source is ultimately critical for growth of AAPB. Meanwhile, non-AAPB are more versatile in carbon utilization. Thus, the diversification of DOC species during evolution would favor non-AAPB rather than AAPB. As demonstrated by a scenario in the present ocean (Jiao et al., 2007), in coastal waters, river runoffs transport terrestrial organic matters into the sea, resulting in high abundance and high diversity of carbon source. The AAPB in such environments may have become more dependent on DOC than on light, and only those that have adapted to such conditions remain AAPB. AAPB species could more readily loose their photosynthetic genes

and autotrophic life style, resulting in lower genetic diversity in eutrophic coastal areas than oligotrophic waters.

The above observations in *Prochlorococcus* and AAPB demonstrate scenarios along environmental gradients in the present ocean and may provide model organisms for exploring the co-evolution of life and Earth. Based on the understanding of the current molecular ecological features of *Prochlorococcus* and AAPB, a putative time chart of such co-evolution is proposed (Figure 6).

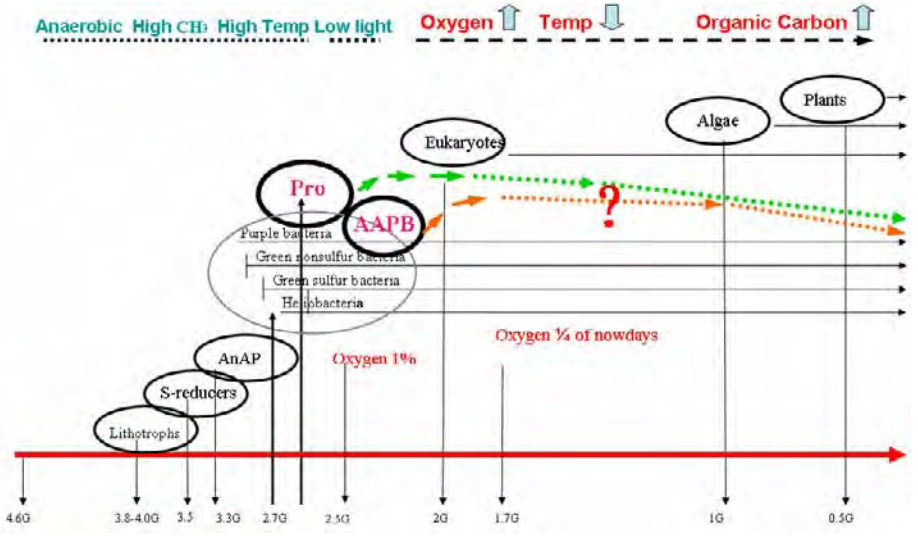


Figure 6 A putative chart of co-evolution of life and the earth as seen from current molecular ecological processes of *Prochlorococcus* and AAPB (Jiao, 2007). (Red arrow indicates time axes, yr)

6. STATE-OF-THE-ART TECHNIQUES IN CURRENT MICROBIAL OCEANOGRAPHY

6.1. Linking Microorganisms to Marine Environments through the Observation of Cell Surfaces with Atomic Force Microscopy

Microscopic observation is a fundamental and critical approach in the study of microbial processes. Epi-fluorescence microscopy and electron microscopy have been successfully applied in aquatic microbiology (Beveridge and Graham, 1991; Noble et al., 1998). However, all the observations involve treatments such as chemical fixation or staining, which may undermine cell intactness and the validity of surface structure (Mozes et al., 1991). However, the direct observation of the microorganisms in aqueous solution remained unfeasible. Thus, there was clearly a need for in situ nondestructive observation of cells at high resolution. Atomic Force Microscopy (AFM) invented in the mid-1980s (Binnig et al., 1986) is a new approach to examining microorganisms for information beyond what we can see with conventional microscopy. AFM offers not only three-dimensional images of the target cell in real time under living conditions (Kasas et al., 1993), but also quantitative and qualitative surface physical information (McElfresh et al., 2004). Furthermore, AFM provides

information concerning physiological variations related to changing environmental conditions (Müller et al., 1999; Dufrêne, 2002, 2004). Therefore, AFM provides a link between microbiology and oceanography through the observation of cell surfaces.

Instrumentation

Normally in AFM, a laser beam is focused on the free end of the cantilever, and the position of the reflected beam in the form of a light spot is detected by a position-sensitive detector designated as a photodiode. The photodiode detects change in the reflected laser spot and conveys this information to AFM controller. The controller receives this signal then generates a voltage signal accordingly to a piezoelectric scanner, which makes retracting or extending movement to keep the relative distance between the tip and the sample constant. Therefore, the tip acts like a finger keeping a constant force pressing against the surface and the AFM operator can adjust the cantilever deflection in real time to reach an appropriate interaction force. So finally a closed-loop feedback system is formed to give a high resolution 3-D topography of the target cell.

According to the ways in which AFM tip trace the sample surface, AFM imaging modes can be divided into two categories: contact mode and tapping mode. The most widely used imaging mode is the contact mode. In contact mode, the instrument generates height and deflection images. The height image can provide quantitative height measurements, and allows accurate measurement of surface roughness or thickness of the bio-film layers. The deflection image does not indicate true height topography; but it provides more elaborate surface details than the height signal due to its high response frequency. Besides this, force measurement is a distinct feature in the contact mode. This force information is valuable to assess tip-sample separation distance and the binding events between complementary bio-molecules at the interface (Butt et al., 1995).

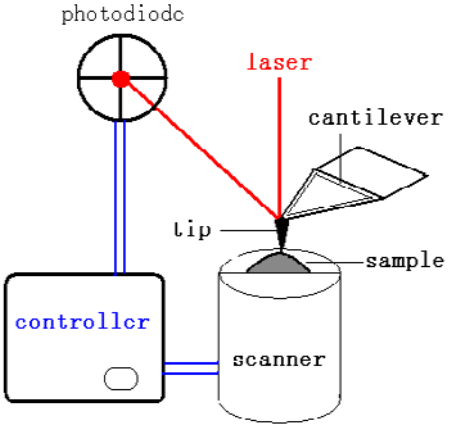


Fig. 7. General Principal of AFM

In the tapping mode, the tip is vibrating, driven by an appropriate frequency generated by the probe holder. Instead of cantilever deflection, the amplitude of the cantilever is kept constant during scanning. So in the tapping mode, AFM tips do not keep constant contact

with the target surface, and consequently there is basically no lateral force exerted by the sharp tip which may compromise the integrity of soft cell surfaces, thus circumventing the major drawback in the contact mode. Besides surface topography information, tapping mode AFM simultaneously collects the signal from timing of mechanical vibration data designated as the phase signal. Data from phase images can be interpreted as a map of energy dissipated by an interaction force imposed on the surface at the corresponding point of the sample, allowing researchers to examine mechanical as well as electrical or magnetic changes on the surface.

Meanwhile, AFM technique faces some limitations in practice. The shapes of cells are normally elliptical, which means that although the top of the cell may be flat, and suitable for AFM tip scanning, the edge of the cell always declines dramatically in height. Therefore, when imaging the edges of cells, ascribe to the normally four-sided pyramid geometry of probes, it is quite possible that not the curvature but the sides of the tip make contact with the edges of the cells, resulting in image artifacts on the cell edge (Velegol et al., 2003). To eliminate this artifact, side wall angle of the tip should be considered in practice.

The other problem for single cell imaging arises in fixing the cells in a steady position so as to resist the lateral force exerted by the AFM tip during scanning. A membrane pore trap may work for spherical shaped cells, but for those cells in cylindrical cells, this problem still remains (Gad and Ikai, 1995; Doktycz et al., 2003).

Application of AFM in Aquatic Sciences

Linkage between cell surface and environment. The non-destructive high resolution of AFM gives the potential to monitor cell surface information under different environmental conditions. The molecular architecture of photosynthetic membrane has been intensely studied (Scheuring et al., 2003; Bahatyrova et al., 2004). The photosynthetic membrane plays a central role in cell responses to a changing environment, but the underlying mechanism remains elusive. A comparative study on the membrane proteins of photosynthetic bacteria was conducted, focused on the modulation of the light harvest complex and reaction center architecture during chromatic adaptation. The selected aquatic anaerobic photoheterotrophic bacteria *Rhodospirillum photometricum* was cultured in high- and low-light illumination. In the AFM images, the light harvest complex and the reaction center can be easily distinguished (Bahatyrova et al., 2004). AFM images demonstrate that core complexes in membranes subject to high light are distributed homogeneously with LH-LH contact and LH2-core contact. This architecture makes LH2 easier to pass the photons they capture to the core complex. While in low light the membrane displays uneven distribution. There is justification for this phenomenon. Generally, the ratio of LH2 to core complex may show positive correlation with light availability during cell growth, based on the assumption that more antennae pigments are necessary under low irradiance than high irradiance. (Scheuring et al., 2005).

As the edifice of the solar-driven ecosystem, photosynthetic organisms have a fundamental impact on the whole food chain. Anoxygenic photosynthetic bacteria are considered to be the prototype of photosynthesis carriers. So the relevant studies have great significance in unveiling evolutionary processes in photosynthesis. The research brings forward a promising model for the assembly of the anoxygenic photosynthetic apparatus. It is a step towards elucidating how photosynthetic membranes rearrange to utilize light with higher efficiency at molecular resolution. (Scheuring et al., 2005).

This typical case incorporates AFM techniques to resolve a complicated scientific issue in aquatic photosynthesis and convinces us that this technique has great potential to unveil the relationship between dividing functional surface structures and outer environmental signal such as temperature, salinity and even carbon sources.

Other research by Gaboriaud et al. (2005) connects environmental changes to microbial surface nano-mechanical properties. In this case, the cell wall of gram-negative bacteria in fluid was investigated at pH 4 and pH 10. The results indicated that the turgor pressure was about twice as high at pH 4 than at pH 10, and the thickness of the cell wall increased from 60 nm to 140 nm and softened as the pH rose from 4 to 10 (Gaboriaud et al., 2005). The authors proposed that higher intermolecular repulsive force may account for the expansion of cells in raising the pH situation, especially when the periplasimic space is enlarged by higher electrostatic repulsive forces between the inside of the outer membrane and the outside of the cytoplasmic membrane (Gaboriaud et al., 2005). Although cells in the natural environment may not undergo such a dramatic change in pH value, the research revealed the tendency of the surface feature to respond to change in pH. The expansion of the cell wall can result in cascading consequences in the surface chemical and physical features such as an increase in the permeability of the cell walls, which may cause higher probability of cell lysis. This can be useful in antibiotic studies or anti-biofouling applications. The results from this study underpin the view that AFM force curves can be applied to assess the influence of environmental factors imposed on microbial surface and the mechanism at bacterium–aqueous-solution interfaces. Moreover, a liquid medium results in no capillary force between the tip and the sample, which means no further scraping damage to the soft surfaces during imaging. This advantage enables reliable continuous visualization of surface change in situ along environmental gradients designed in the experiment.

Binding events in aquatic system

AFM technique can be far more than an imaging tool as more and more emphasis is laid on the interaction between tip and target surface molecule recognition (Hinterdorfer et al., 1996; Rief et al., 1997; Müller et al., 1999; Razatos et al., 1998). Adhesive force on the cell surface is crucial in understanding the intricacies of interfacial phenomena such as bacterial attachment to organic-rich particles or phytoplankton cells (Fletcher et al., 1996; Emerson and Camesano, 2004; Ivanova et al., 2006) and biofilm formation (Auerbach et al., 2000).

Adhesion between cells is a complicated processes. When a cell approaches a surface, it is required that the cell overcomes the unspecific repulsive force before contacting the surface. After cells contact a surface, corresponding specific macro-molecular forces such as surface antigen-antibody binding or exposed carbohydrate interaction may begin to function. So the information from a force curve can reveal the physiological processes in cell adhesion. In a regular force-distance curve measurement, AFM tips are controlled to approach the surface, and the instrument can record cantilever deflection and calculate adhesive force (Xu et al., 1994; Rotsch and Radmacher, 1997; Fang et al., 2000).

However, simply using the AFM tip to touch the cell surface, has given researchers little insight into the interaction force in a real situation, especially those surface molecule recognition binding events that happen at the cell-to-cell interface. To tackle this problem, the tips of AFM probes have been modified with bio-molecules to touch the target surface. The experiment conducted by Touhami et al. (2003) used tips coated with oligoglucose to contact the live yeast cells under flocculating and non-flocculating conditions. The specific adhesion

force was only detected under flocculating condition, indicating that lectin-carbohydrate interaction might be involved in the flocculation of yeast cells (Touhami et al., 2003).

In recent years, another novel form of probe called biological-active-force probe was introduced the study of microbial cells study and their interaction with the living habitat (Lower et al., 2000). This technique has been successfully applied in bacteria-mineral interface study (Lower et al., 2001). In the marine ecosystem, bacteria and micro-algae are considered to be major colonizers of most submerged surfaces. The extracellular production substance (EPS) which the marine species release to the environment may facilitate their attachment to inanimate surfaces or the biofilm formation in the aquatic environment (Vandevevere and Kirchman, 1993). Research by Arce et al. (2004) used the tips functionalized with live diatom cells to touch hydrophobic and hydrophilic material surfaces, and they recorded the force curve versus distance when the cell was detached from the surface (Arce et al., 2004). The experiment provided useful information for the design of the anti-fouling material.

The increasing investigations which focused on the surface force-distance curve between biomolecules, with the aid of AFM, has resulted in the accumulation of much data on cell surface physical properties. The surface macromolecular force combined with the exterior structure and composition may explain the binding events in the aquatic system.

Prospectus. The marine environment is extremely corrosive, and the great variation in environmental factors including temperature, salinity, pressure, alkalinity, transparency and nutrient availability lead to a great bio-diversity of the marine ecosystems. The contribution of microorganisms to this high diversity can not be ignored. Due to their long history since the origin of life on earth, microbial cells have successfully developed in the course of evolution under harsh environmental pressure. And for these organisms, surface is the only channel for cells to communicate with other cells and it is the only interface with the diverse environments. So the information delivered to the cell surface is the outcome of the potential physiological process adapting to the environment (Dufrêne, 2004). These chemical and physical properties are essential for understanding the events which happened in the aquatic ecosystem. The reports mentioned above have proved the capability of AFM technique for single cell surface study virtually *in situ*.

There are ever-lasting incidents taking place every minute in aquatic microcosm, exploring such events in microcosmic require substantial application and development of AFM technique in aquatic research to fathom these unsolved questions.

1. Gram negative bacteria occupy a large fraction of the aquatic environment. Secretion of membrane vesicle (MV) from cell walls during growth is a distinct feature of gram negative bacteria which differs from their gram-positive counterparts. MV structure is normally a bilayered sphere 50 to 250 nm in diameter and composed of mainly outer membrane proteins, LPS and phospholipids (Beveridge et al., 1997). The MV exterior is negatively charged, which means that these anionic groups can interact with cations in the environment as potential sites in the natural environments. There is speculation that MVs separated from bacterial walls can lyse other bacteria species when those bacteria are suffering from limited nutrition pressure (Beveridge, 1999). The AFM technique provides a chance to manipulate MVs from a bacterial culture to touch the animate particle surface or other cultured strains. The outcome may indicate the

possible species-specific binding event mediated by MV and allow us to extrapolate the ecological implication.

2. Viruses are also important players in marine ecosystems. Due to their tiny size, and their relationship with the host, viruses are normally studied based at the population level (Wommack and Colwell, 2000). AFM has sufficient resolution to monitor the delicate structure on a virus particle in fluid during studies of surface dynamic (Kuznetsov et al., 2001). Besides, when coated by viruses, the AFM tip can touch living cells or inanimate surfaces for a comparative study of the interaction relationship between different components in the aquatic eco-system.

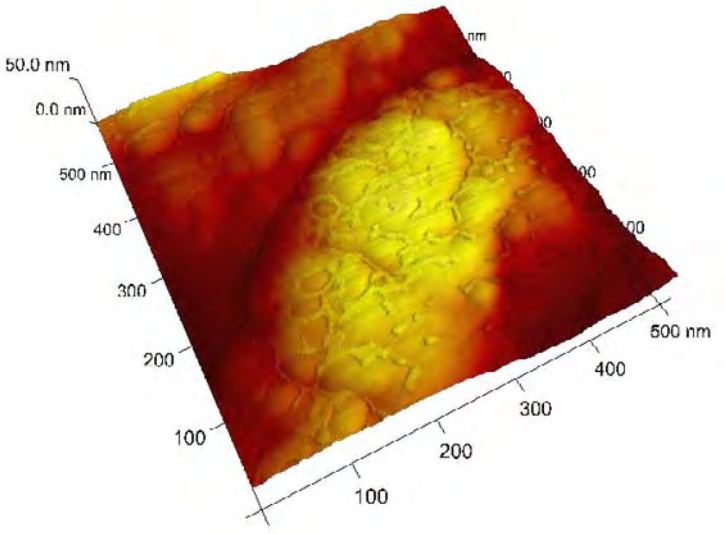


Figure 8. Surface height image of the phototrophic bacterium DSMZ6997 *Erythrobacter longus* with tapping mode.

In summary, the high resolution and non-destructive liquid imaging mode make AFM a useful imaging tool for water research at the microcosmic scale. The research objects can be marine microorganisms including phytoplankton (Higgins et al., 2003), bacteria (Camesano et al., 2000; Pelling et al., 2005), spores (Chada et al., 2003) and viruses (Kuznetsov et al., 2005), such as nutrient substances like dissolved organic molecules (Santschi et al., 1998; Lead et al., 2005). Meanwhile, AFM presents information on structure-function relationships, which enhance our knowledge concerning the processes which the contributors conduct in the microbial loop, allowing us to take a closer look at their success in mediating mass transfer and energy flow in nature. Furthermore, the scientific issues such as how microbial cells work in their living condition and how they communicate with their counterparts and other organisms can be addressed.

6.2. Continuous Monitoring of Cellular Physiological Response to Environmental Changes - Determination of Membrane Potential by Flow Cytometry

Marine microbial ecologists are often puzzled by deviation of bacterial production from bacterial abundance in field observations, since bacterial production does not necessarily increase with increasing bacterial abundance. As a matter of fact, a considerable portion of bacteria are alive but not physiologically active, and only the active cells can metabolize efficiently (Roszack and Colwell, 1987; Sherr et al., 1999). Bacterial cells in nature are in different physiological states: some of them are very active and "hardworking" for production, while some others are just "sleeping". The sleeping cells are easily included in bacterial counts either by microscopy or flowcytometry and yet are not contributing effectively to bacterial production. Discrimination of bacteria at different physiological states would be of crucial importance for a better understanding of functional structure and thus the role of microbial processes in carbon cycling in the sea.

To date, only a few techniques have been developed to measure the physiological states of bacterial cells, such as "PI-" or "CTC-" protocols. These methods are capable of discriminating a whole bacterial community into 2 groups, either dead cells by propidium iodide (PI) (Williams et al., 1998) or respiratory active or non-respiratory cells by 5-cyano-2,3-ditoyltetrazolium chloride (CTC) (Rodriguez etal 1992; McFeters et al., 1995; Sherr et al., 1999). In the "CTC-PI protocol", CTC-positive cells are regarded as "alive cells" and PI-positive cells as "dead cells". Unfortunately, there is a group of neutral cells accounting for a great portion of total bacteria (20-70%, in the case of the East China Sea) falling outside either the "alive" or the "dead" categories; they are neither CTC-positive nor PI-positive. These cells are not really dead, but are not active enough to be detected by CTC, raising the question of how to determine the physiological status of such cells.

Membrane potential (MP) plays a key role in cell physiological processes and therefore could serve as a better parameter for measurement of physiological activity, since MP is a part of the generation of proton motive force, and is closely involved in ATP generation, substance transport, bacterial chemotaxis and survival at low pH (Novo et al., 1999). Furthermore, some permeases involved in substance transport are most active with the presence of MP. Finally, since bacteria have no mitochondria, MP also reflects membrane integrity and energy status as well as viability of the cells (Müller et al., 2000).

FCM is a powerful tool for single cell analysis. The combination of FCM and suitable fluorescence probes has been proved to be a useful approach for MP measurement in bacteria (Novo et al., 1999; Novo et al., 2000). The probes in MP estimation by FCM are mostly fluorescent cationic or anionic lipophilic probes (so called slow dye) whose distribution mechanism is dependent on cell MP (Nernst distribution: $[C+]i/[C+]o = e^{F\Delta\Psi / RT}$, where $\Delta\Psi$ is the membrane potential, R the gas constant (8.314510J/mol K), T the temperature (K), F the Faraday constant (9.6485309×10^4 C/mol), [C+]i the concentration of C inside the cell, and [C+]o the concentration of C outside the cell). Using $DiOC_6(3)$ (3,3'-dihexyloxacarbocyanine iodide) as the MP probe, a FCM-based ratiometric technique (red fluorescence to green fluorescence) protocol for continuous measurements of MP of the marine bacteria is established (Jiao et al., 2004). Examination by batch culture and continuous

culture validated the protocol for monitoring the above so-called "neutral cells" and quantification of their abundance. Compared with previous similar work, the protocol developed by Jiao et al. (2004) is characterized by 1) lower dye concentration (10 µM), which is 1/3 of the dye concentration required in the previous $DiOC_2(3)$ ratiometric protocol (Novo et al., 1999), thus saving the dye and reducing the toxicity to the cells, and 2) longer staining time (20-30 min at room temperature) allowing the operator to have enough time to handle the sample and measure MP on a flow cytometer .

Based on the experimental data obtained from batch cultures and a strictly controlled continuous culture reactor, Jiao et al., (2004) proposed a MP model illustrating the general characteristics of the MP curve and mechanisms (Figure 9). In this model, T is incubation time, MP_{max} is the maximum MP value of the curve, T_{max} (=T1-T0) is the time to reach the MP_{max}; K_{mp} is the slope of the exponential phase of the MP curve. S_{max} is the minimum concentration (threshold) of a particular substrate required for the test organism to reach its MP_{max}. With these MP parameters, one can show the differences among species, or characterize a given species by checking the responses in MP parameters to changes of environmental conditions. For different species, if incubation conditions are the same, MP_{max}, K_{mp}, and T_{max} would be different between species and can be considered as species-specific features. For a single species, the MP_{max}, K_{mp}, and T_{max} are functions of substrate and incubation conditions. If the environmental conditions are the same, S_{max} is also a species-specific feature. Increase of the substrate availability beyond Smax will not change the MP_{max}. The time that the MP_{max} can last (T2-T1) is a function of extra substrate beyond the S_{max} in such case. After the MP_{max}, MP will fall to the lowest value (close to that in the stationary phase) as the populations reach their maximum abundance (as indicated by the dashed lines between a and A, b and B, and c and C). The above "bacterial production and abundance inconsistency puzzle" is then well explained by the model. e.g., in the plateau stage of the population kinetic curves, although the abundance of the population is very high, the MP values are very low, showing a scenario of high bacterial abundance but low bacterial production in the field.

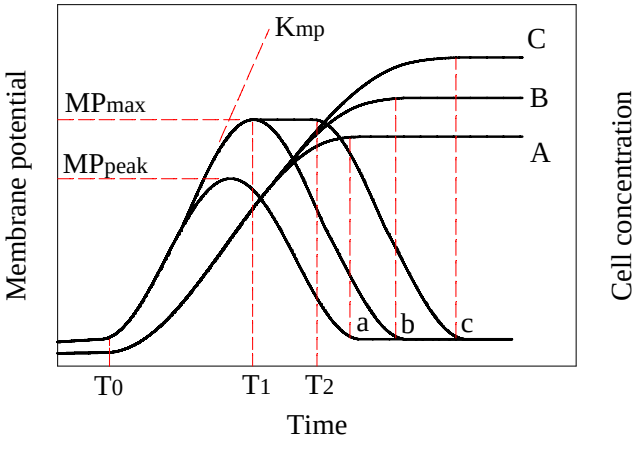


Figure 9. A model of the microbial membrane potential theory and applications (Jiao et al., 2004)

6.3. A New Approach for Accurate Enumeration of Bacteriochlorophyll-Containing Microbes in the Marine Environment - Time Series Observation Based Infrared Epifluorescence Microscopy (TIREM)

Bacteriochlorophyll *a* containing microbes (BCM) are unique in the marine ecosystem. Accurate determination of their abundance is critical for the understanding of their role in energy flow and carbon cycling in the ocean. For example, a newly recognized functional group of BCM, the AAPB have been reported to be critical for carbon cycling in the ocean (Yurkov and Beatty, 1998; Kolber et al., 2000; Kolber et al., 2001; Jiao at al., 2007). However, quantification of AAPB is difficult and different approaches have reached different conclusions about the distribution patterns of AAPB in marine environments. The most convenient approach to date for enumeration of AAPB is epifluorescence microscopy, in which infrared fluorescence from BChl a is used as the diagnostic signal for determination of AAPB (Kolber et al., 2001; Schwalbach and Fuhrman, 2005). Unfortunately, this approach has been proven to involve significant errors (30%-fold) due to the presence of cyanobacteria (Zhang and Jiao 2004; Schwalbach and Fuhrman, 2005), as cyanobacteria can emit infrared fluorescence when excited under the AAPB microscope settings and their fluorescence can be of the same order of magnitude as AAPB in field samples. Theoretically, cyanobacteria can be subtracted from the infrared positive counts; practically, however, it is rather difficult to count the cells accurately, especially for *Prochlorococcus*. Most recently, Jiao et al. (2006) established a "Time series observation based InfraRed Epifluorescence Microscopy (TIREM)" approach which is capable of accurate enumeration of BCM.

The TIREM protocol features time series observation, auto-imaging and digital analysis. In principle, the correct count of BCM can be obtained by subtracting the cyanobacterial count from the total infrared positive count. The tricky point is that *Prochlorococcus*, the most abundant cyanobacteria, are readily visible in infrared images but not visible in the initial cyanobacteiral images because their emission signals are masked by brighter fluorescence from larger cells such as *Synechococcus* coexisting in seawater samples. *Prochlorococcus* become gradually visible when the fluorescence from *Synechococcus* cells decay to a certain extent after a period of exposure to excitation light. Therefore the plateau count of the cyanobacterial cells in the time series observed cyanobacterial images rather than the initial count as previously believed, represent the correct count for the total cyanobacteria (*Synechococcus* plus *Prochlorococcus* cells).

In practice, an aliquot of 20 mL seawater is fixed for 15 min with 2% polyformaldehyde (PFA) immediately after sampling, and then stained with DAPI for 30 minutes in dark. Cells are filtered onto 0.2 μm-pore-size black polycarbonate membranes (Whatman) for microscopic observation. Cells with infrared fluorescence are viewed with an infrared-sensitive charge-coupled device (CCD) camera (e.g., SPOT Diagnostic Instruments, Inc.) fitted on an epifluorescence microscope (e.g., Carl Zeiss Light Microscopy AXIOSKOP 40) using the following filter set (Chroma Technology Corp): excitation 350 to 550 nm, emission LP 850 nm, beam splitter 650 nm (Kolber et al., 2001; Zhang and Jiao, 2004) (IRsettings). DAPI-stained cells are used as reference and are viewed at excitation 330 to 390 nm, emission 440 to 490 nm, beam splitter 400 to 430 nm (Zeiss Filter set 02) (Kolber et al., 2001; Zhang and Jiao, 2004) (DAPI-settings). Cyanobacterial cells are viewed at excitation BP 546±12 nm, emission LP 590 nm (Li and Wood, 1988), beam splitter FT 580 nm (Zeiss Filter set 15) (Cyano-settings). DAPI-stained images (DAPI-image), Cyanobacterial images

(Cyano-image) and infrared images (IR-image) are acquired for each microscopic view field using a × 100 oil immersion objective. All images are acquired using automatic exposure with a proper gain limit. Time series observations are conducted on DAPI-, Cyano- and IR-images for 10-15 minutes from the beginning of the exposure. Image analysis is conducted to obtain three dynamic curves of IR-, Cyano- and DAPI-counts from each microscopic view field. The accurate estimation of AAP B is calculated from the formula: [AAPB counts] = [plateau count of infrared positive cells] – [plateau count of cyanobacterial cells] (Jiao et al., 2006) (Figure 10). At least thirty microscopic fields of each sample should be viewed, and the mean of each microscopic field is multiplied by appropriate factors to yield the cell concentrations in the original samples.

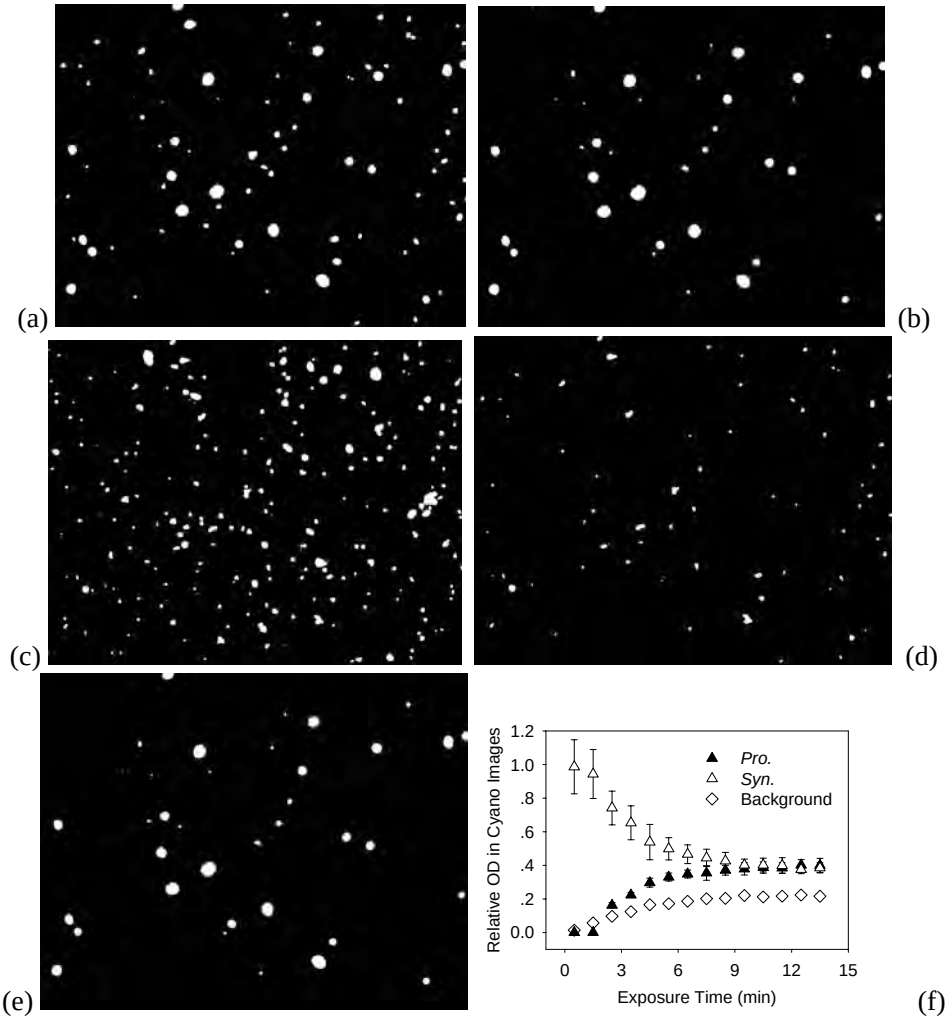


Figure 10. Imaging processes of the TIREM outputs of a field sample from the China Sea. a, IR-image for total IR positives; b, Cyano-image for total cyanobacterial positives; c, DAPI-image for total microbial positives; d, output of Boolean AND [(a), (c)] for IR cell counts; e, output of Boolean AND [(a) , (b)] for cyanobacterial cell counts. BCM counts = [d] – [e] f. Time course of the relative optical density (OD) of the fluorescence of *Synechococcus* and *Prochlorococcus* and the background in the CCD cyanoimages, show why time series observation is necessary (Jiao et al., 2006).

In classical EM protocols, rapid observation is necessary to avoid quenching of fluorescence and thus reduce the negative errors. This belief turned out not to be true. As described above, time series observations are actually the key to obtaining reliable data. Furthermore, time series observation might be necessary for IR-images or DAPI images whenever the case is applicable (when two or more groups of cells present in the same sample, but with different density of cellular fluorescence). This is a conceptual change to the traditional theory related to fluorescence microscopic observation.

6.4. New Insights into Microbial Community Composition and Functions through Metagenomics and Proteomics

Bacteria are metabolically diverse. Aquatic bacteria play pivotal roles in natural environmental processes and thus provide a large untapped resource for the discovery of novel metabolism, enzymes and pathways. The fact that only a small fraction of bacterial populations can be cultivated poses a great challenge to the understanding of the in situactivities and metabolism of natural bacterial assemblages. Recently, community-based genomics and proteomics have been exploited to study microbial functions in natural environments.

Community genomics (or metagenomics) is the analysis of the collective microbial genomes contained in an environmental sample (for a review see Riesenfeld et al., 2004). Whole genome shotgun sequence data from the Sargasso Sea yielded 1.0 billion base pairs of nonredundant environmental sequences and 1,184 16S rRNA gene fragments (Venter et al., 2004). Assuming 97% similarity as a cutoff, Venter et al. concluded that 1,800 genomic species of bacteria and 145 new phylotypes were present in the samples. This study alone contributed about 1.2 millions new genes and translated proteins to the public database. In the North Pacific Subtropical Gyre, DeLong et al. (2006) constructed seven genomic libraries along a depth continuum from 10m to 4000m and in total about 64 Mbp of assembled DNA sequences were obtained. Most recently, the global ocean sampling effort contributed another 7.7 million sequence reads (or 6.3 billion bp DNA sequences) to the database (Rusch et al., 2007), and unveiled new types of functional proteins (i.e. RuBisCO and UV repair genes) at the ocean's surface (Yooseph et al., 2007). The CAMERA database (Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis) (http://camera.calit2.net) will be beneficial to many aquatic microbial ecologists.

Metagenomics not only provides new information concerning into microbial diversity but also new insights into the potential functions of microbes living in nature. Novel metabolic activities of the not-yet-cultivated microbes can be discovered through metagenomics. For example, using metagenomics it was found that an uncultured Gammaproteobacteria, SAR86 group contains a PR gene, which was only seen in archaea previously (Béjà et al., 2000a). The discovery of the PR gene in SAR86 revealed a novel photosynthetic pathway in aquatic bacteria. Subsequent studies showed that the PR gene is widely distributed in many marine Proteobacteria and spectrally tuned at different water depths for various light sources (Béjà et al., 2001; Venter et al., 2004), indicating the ecological adaptation of this novel type of marine phototroph. In addition, discovery of an ammonia monooxygenase in archaeal genomic scaffolds suggests that oceanic nitrification is not solely mediated by eubacteria (Venter et al., 2004). Bioinformatical analysis of metagenomic data obtained from different

microbial ecosystems demonstrated that predicted functional gene expressions are clustered according to their environments (Tringe et al., 2005), suggesting the potential niche-adaptation of these microbes. Although metagenomics is a powerful tool to uncover potential new functional genes, it does not provide information on whether these genes are expressed or active under a particular environmental condition.

The proteome was defined as "the total protein complement able to be encoded by a given genome" (Wasinger et al., 1995). Proteomics is a technique that systematically documents and analyzes the proteins expressed in biological samples. Community proteomics (metaproteomics) studies the protein expression in natural microbial assemblages. The goal of proteomics is to study the changes in protein expression, modification, and interaction on a large scale with a view to understand global, integrated processes at the protein level (Blackstock and Weir, 1999). These changes are likely due to biological perturbations (Anderson and Anderson, 1998) and effects of environmental conditions (Shepard et al.,2000).

As stated in the 'central dogma' of molecular biology, biological activities can be characterized at three different levels: DNA, RNA and protein. Protein is the final product of gene expression. Nearly all the cellular activities are performed by enzymes that are made up of individual proteins. It is generally believed that cellular and biological functions can be better interpreted at the protein level than the DNA or RNA level. Furthermore, extensive studies on yeast and mammalian cells demonstrated that protein expression does not always directly correlate to mRNA expression (Pradet-Balade et al., 2001). Therefore, studying gene function at the protein level has great potential for understanding the actual biological activities.

Proteomic analysis includes the 'classic' two-dimensional gel electrophoresis (2DGE)- based approach and the non-gel-based approach. Each approach has its own strengths and weaknesses. 2DGE was first introduced about 30 years ago (O'Farrell 1975) and is still extensively applied in current studies. In late the 1990s, non-gel based proteomic techniques began to emerge, including surface enhanced laser desorption/ionization (SELDI) and liquid chromatography-mass spectrometry (LC-MS). Benefiting from these new technologies, protein samples can be depleted or concentrated, pre-fractionated or de-complexified before downstream analysis. The non-gel based methods are particularly useful when dealing with complex or limited amounts of sample. However, non-gel-based approaches cannot compete with 2DGE-based proteomics with regard to protein quantification, because intensity and size of protein spots on 2D gels provide more accurate estimation of the level of gene expression than that inferred from MS spectra. In addition, 'proteome fingerprints' can be obtained by visual observations of proteome distribution patterns among different species or the same species under different environmental stresses. Therefore, old-fashioned 2DGE-based proteomics still holds its merits in current proteomic studies.

In 2DGE, proteins are separated based on their isoelectric point (pI) and molecular weight (MW). In the first dimension, high voltage power enforces the individual protein species to migrate until they reach their neutral pH point (pI). Proteins with the same pI are further separated based on their MW in the second dimension (Fichmann and Westermeier, 1999). Protein spots of interest can be excised from the gel and characterized via Edman N-terminal sequencing or MS.

Recently, several groups of researchers have applied community proteomics to investigate functional gene expression in various microbial communities.The first application of microbial metaproteomics was used to decipher the metabolic details of the enhanced

biological phosphorus removal process in activated sludge wastewater treatment (Wilmes and Bond, 2004). Strong expression of proteins involved in phosphorus removal was evident as revealed by metaproteomics (Wilmes and Bond, 2004). More recently, community proteomics was applied to investigate the biofilm community associated with acid mine drainage (AMD) (Ram et al., 2005). Metaproteomic analysis of the AMD biofilm greatly benefited from its existing metagenomic database (Tyson et al., 2004). A total of 2033 individual proteins excised from 2DGE were positively identified. Proteins linked to environmental challenges including chaperone, thioredoxins and peroxiredoxins were found to be abundant in the total proteomes. In addition, a large portion of proteins could not be assigned to particular functional categories suggesting possible novel gene products (Ram et al., 2005). A study on DOM from lake water and forest soils demonstrated that proteomic fingerprints can be used to describe presence and activity of organisms in an ecosystem (Schulze et al., 2005). More proteins (78%) originating from bacteria were found in lake DOM than in forest soil (50%), and the number of identified proteins and taxonomic groups significantly varied in winter and summer(Schulze et al., 2005). In the marine environment, 2DGE-based community proteomics was first developed and applied to explore the protein expression patterns of picoplankton from Chesapeake Bay (Kan et al., 2005). Typically, a few hundred of protein spots were detected on a 2-D gel after picoplankton (0.2-3 j.tm) were collected from 40 liters of water (Figure 11). Although highly reproducible 2-D gel patterns can be achieved, complex microbial communities in the aquatic environment pose a great challenge to community proteomics. Identification of environmental proteins based on mass spectrometry often encounter two main problems: 1) insufficient protein loading; and 2) lack of corresponding metagenomic database. It was found that the metaproteomic patterns of estuarine microbial communities were greatly influenced by the microbe composition(Kan and Chen, unpublished).

Functional proteins can be more diverse than species diversity in nature. Soil and aquatic samples contain diverse microbial species. It has been estimated that there are 160 taxa of bacteria in one milliliter of seawater, and 6,400-38,000 taxa of bacteria in one gram of soil (Curtis et al., 2002). Assuming that the average genome size of environmental bacteria is ~3 Mb and 1 kb of sequence encodes one gene, one can expect to observe 4.8× 105 expressed proteins in 1 mL of seawater (Wilmes and Bond, 2006). This number could be significantly higher if considering diverse protein conformations (at least 10 times the gene number) resulting from transcriptional or translational modifications. This is beyond the resolution of current proteomic tools and consequently, metaproteomics of water or soil samples can only resolve a minute fraction of the highly expressed (abundant) proteins.

Microbial community genomes recovered from marine environments still contain numerous sequences without known function (Venter et al., 2004; DeLong et al., 2006, Rusch et al., 2007; Yooseph et al., 2007). Metagenomic sequences provide a useful database for the identifications of environmental proteomes. Future studies on metaproteomics will link the protein identification to their source and ecological roles, and thus improve our understanding of the functional pathways of environmental microbes. Comparative metaproteomics is an approach to understand how microbial processes are regulated by various environmental parameters such as light, salinity or nutrients. Finally, identification of microbial proteins in situ may allow us to uncovering important or novel biogeochemical functions.

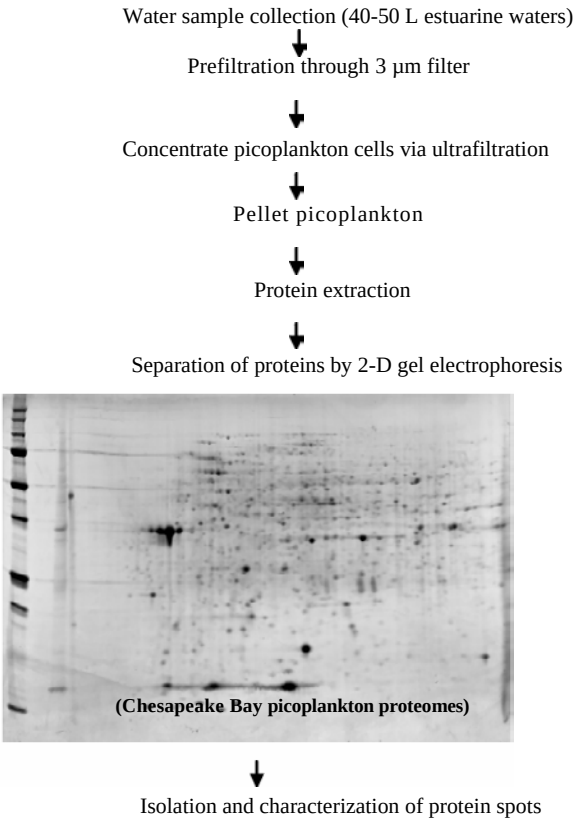


Figure 11. Flow chart showing the major procedures involved in community based proteomics. Chesapeake Bay metaproteomes contained proteins from pI 4–8 with apparent molecular masses between 10–80 kDa (Kan et al., 2005). For open ocean water, the sample volume should be increased according to the microbial cell counts.

6.5. Microbial Lipid Biomarkers and Isotope Signatures – Tracing Carbon Flow at the Molecular Level

Advances in lipid biogeochemistry

Lipid biomarkers derived from cell membranes provide quantitative information about microbial community structure without the need for culturing and isolation, and are one of the most useful biochemical measures of *in situ* interactions between microbial species and the chemical or physical environments in which they live (Ringelberg et al., 1989). Bacteria commonly have ester-linked lipids and some species contain additional non-phytanyl ether lipids (Langworthy et al., 1983; Huber et al., 1992). Archaea, on the other hand, have phytanyl (isoprenoidal) ether lipids, which can be distinguished from non-isoprenoidal ether lipids in bacterial species (Langworthy et al., 1983; Pancost et al., 2001; Pancost and Damste, 2003; Weijers et al., 2006). Thus, analysis of lipid structures can provide indisputable distinctions between the two domains.

Distinctions are also possible among different groups or species of archaea and bacteria (see the reviews by Pancost and Damste, 2003; Brocks and Pearson, 2005). Among bacteria, for

example, cyanobacteria contain unique hopanoids (Summon et al., 1996), planctomycetes have ladderanes (lipids containing up to five linearly fused cyclobutane moieties with cis ring junctions; Damste et al., 2002a), and sulfate-reducing bacteria are characterized by iso- and anteiso-branched fatty acids (Wakeham et al., 1995; Zhang et al., 2002). Among the archaea, *Methanococcus jannaschii* contains an unusual macrocyclic glycerol diether 2,3-di-O-phytanyl-sn-glycerol (Comita and Gagosian, 1983) and *Methanopyrus kandleri* contains a unique 2,3-di-O-geranylgeranyl-sn-glycerol (Hafenbradl et al., 1993). Hedrick et al., (1991) showed that the patterns of ether lipids clearly differentiated between three species of methanogens chosen from Methanobacteriales, Methanococcales, and Methanomicrobiales.

Early studies of archaeal lipids, however, were hampered by the tedious cleavage procedure for GC and GC-MS analysis. Recent advances in LC-MS have allowed rapid detection of archaeal lipids, particularly the large molecules of glycerol dialkyl glycerol tetraethers (GDGTs) from diverse environments (Hopmans et al., 2000; Schouten et al., 2000). Among the GDGTs is a unique biomarker, called crenarchaeol, which contains four cyclopentyl rings and one cyclohexyl ring (structure II, Figure 12). Crenarchaeol has been recognized as an abundant biomarker for planktonic crenarchaeota in the open ocean and in marine sediments (e.g., Schouten et al., 2000, 2002; Pearson et al., 2001; Damste et al., 2002b; Zhang et al., 2003), and recently has been observed to occur widely in terrestrial hot springs (Pearson et al., 2004; Zhang et al., 2006). Furthermore, radio-carbon analysis of individual archaeal lipids including crenarchaeol revealed that the majority of nonthermophilic crenarchaeota in the deep ocean grow lithoautotrophically and may play an important role in global carbon (Ingalls et al., 2006).

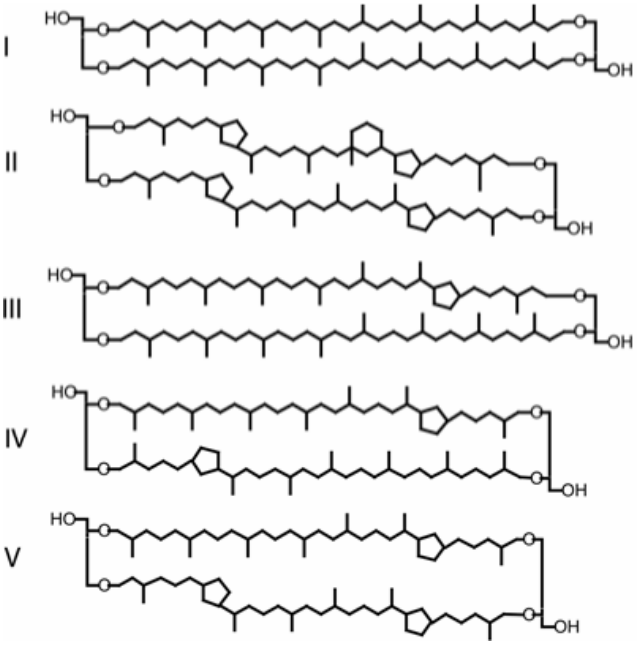


Figure 12. Representative structures of glycerol dialkyl glycerol tetraethers (GDGTs) identified from intact archaeal lipids. I = GDGT-0 (no ring structure), II = crenarchaeol (four cyclopentyl rings and one cyclohexyl ring), III = GDGT-1 (one cyclopentyl ring), IV = GDGT-2 (two cyclopentyl rings), V = GDGT-3 (three cyclopentyl rings), and II' = crenarchaeol regio-isomer, not shown. Drawings are modified from Damste et al. (2002a) and Schouten et al. (2002).

Advances in stable isotope biogeochemistry

Stable carbon isotopes remain one of the best geochemical tools for identifying biological signatures in natural environments. In particular, recent developments in compound-specific isotope ratio mass spectrometry allow us to trace the carbon flow at the molecular level along autotrophic and heterotrophic pathways (e.g., Hayes et al., 1990; Macko et al., 1994; Jahnke et al., 1995; Hinrichs et al., 1999; Pancost et al., 1998; 1999; Zhang, 2002; Figure 13). For example, bacterial lipids enriched in ^{13}C can be attributed to photosynthetic green sulfur bacteria (Jahnke et al., 2001; Glaeser and Overmann 2003; House et al., 2003; Zhang et al., 2004) and compounds depleted in ^{13}C can be attributed to cyanobacteria or methane-oxidizing bacteria (Freeman et al., 1990; Summons et al., 1994, 1996).

Stable isotope analysis of archaeal lipids, on the other hand, has helped us identify the carbon substrate for the growth of archaea in natural environments. For example, in marine sediments associated with cold seeps and gas hydrates, the $\delta^{13}C$ values of certain archaeal lipids (e.g., archaeol, sn-2 (sn-3) hydroxyarchaeol) were extremely low indicating the utilization of methane (the most ^{13}C-depleted organic carbon) by archaea (Hinrichs et al., 1999; Pancost et al., 2001; Zhang et al., 2002; and a review by Pancost and Damste et al., 2003). Similarly, certain biomarkers from sulfate-reducing bacteria (e.g., i1 5, ai15, i1 7, ai17) also bear ^{13}C-depleted isotopic signatures derived from methane (Figure 13). Such evidence helped delineate an important pathway of AOM by the consortia of archaea and sulfate-reducing bacteria (Boetius et al., 2000; Orphan et al., 2001a, b).

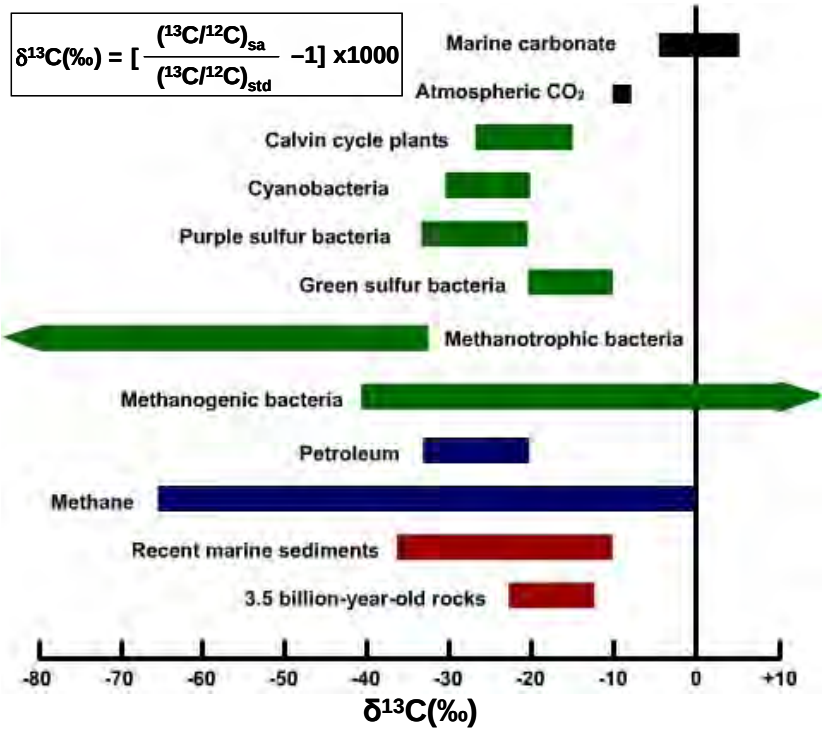


Figure 13. Stable carbon isotopes in different substances (Modified from Madigan, M.T. and Martinko, J.M. 2006, Brock Biology of Microorganisms, 11th edition, Prentice Hall, Upper Saddle River, NJ.).

Direct links between microbial populations and ongoing biogeochemical processes in the natural environment can also be understood based on the incorporation of ^{13}C-labeled substrates into the lipid biomarkers. This technique can significantly expand the application of lipid biochemistry and stable isotope geochemistry in ecological studies because lipid biomarkers can be labeled and identified from microbial populations that are actively metabolizing the labeled substrate (Boschker et al., 1998). For example, based on the incorporation of ^{13}C-labeled acetate into phospholipid fatty acids, Boschker et al. (1998) found that the ongoing sulfate reduction in some estuarine and brackish sediments was performed by sulfate-reducing bacteria similar to the Gram-positive *Desulfotomaculum acetoxidans* and not by a population of the more widely studied Gram-negative *Desulfobacter* spp.

Genome-enabled isotope biogeochemistry - a new frontier at the interface between geosciences and life sciences.

With recent advances in environmental genomics, proteomics and metabolomics, biogeochemistry is on the verge of a new revolution at the beginning of the 21st century. In particular, stable isotopes can be intimately integrated with the tri-omics to address profound biogeochemical questions, which were intractable only a few years ago. For example, genomics can now enable the identification of the species and population abundances of dominant microorganisms responsible for isotope bio-signatures in natural environments; proteomics can enable the identification and quantification of the enzymes and their activities affecting isotope variations by different microorganisms; and metabolomics can provide insight into isotope dynamics and carbon-flow pathways among substrates, cellular components, and products of microbial activities. On the other hand, stable-isotope biogeochemistry can provide valuable and necessary information complementing discoveries in genomics, proteomics and metabolomics. For example, the use of stable carbon-isotopes of lipid biomarkers can allow the determination of the pathways of CO_2 fixation by different autotrophs. The isotropic composition of other life-essential elements including nitrogen, oxygen, hydrogen and sulfur, can also provide fundamental information about the pathways and mechanisms of biogeochemical cycling of these elements in natural or contaminated ecosystems. From a biological perspective, stable isotopes of these life-essential elements are unique environmental tracers that can be used for real-time monitoring and coupling of complex biological processes occurring at the molecular level. For example, ^{13}C-labelled DNA or RNA can lead to species-specific identification of active microbial populations responsible for a known biogeochemical process in the natural environment (Radajewski et al., 2003; Friedrich, 2006). As interdisciplinary research becomes the mainstream of science, we expect that new discoveries in ocean sciences will continue to emerge following advances in various technologies.

ACKNOWLEDGEMENTS

The authors thank A. Hu, Y. Zeng and B. Wei for their assistance during the preparation of the manuscript, Prof. I. J Hodgkiss MBE for his polishing the language. This work was supported by the projects 2007CB815904, 2006BAC11B04, 704029; 2003DF000040; 40632013; 40576063 and UGA, UMIB projects.

REFERENCES

Aloisi, G., Bouloubassi, I., Heijs, S. K., Pancost, R. D., Pierre, C., Damste, J. S. S., Gottschal, J. C., Forney, L. J., & Rouchy, J. M. (2002). CH_4-consuming microorganisms and the formation of carbonate crusts at cold seeps. *Earth and Planetary Science Letters*, 203, 195-203.

Anbar, A. D. & Knoll, A. H. (2002). Proterozoic ocean chemistry and evolution: A bioinorganic bridge? *Science*, 297, 1137-1142.

Anderson, N. L. & Anderson, N. G. (1998). Proteome and proteomics: New technologies, new concepts, and new words. *Electrophoresis*, 19, 1853-1861.

Angly, F. E., Felts, B., Breitbart, M., Salamon, P., Edwards, R. A., Carlson, C., Chan, A. M., Haynes, M., Kelley, S., Liu, H., Mahaffy, J. M., Mueller, J. E., Nulton, J., Olson, R., Parsons, R., Rayhawk, S., Suttle, C. A., & Rohwer, F. (2006). The marine viromes of four oceanic regions. *Plos Biology*, 4, 2121-2131.

Arce, F. T., Avci, R., Beech, I. B., Cooksey, K. E., & Wigglesworth-Cooksey, B. (2004). live bioprobe for studying diatom-surface interactions. *Biophysical Journal*, 87, 4284-4297.

Auerbach, I. D., Sorensen, C., Hansma, H. G., & Holden, P. A. (2000). Physical morphology and surface properties of unsaturated *Pseudomonas putida* biofilms. *Journal of Bacteriology*, 182, 3809-3815.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., & Tingstad, F. (1983). The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*, 10, 257-263.

Bahatyrova, S., Frese, R. N., Siebert, C. A., Olsen, J. D., van der Werf, K. O., van Grondelle, R., Niederman, R. A., Bullough, P. A., & Hunter, C. N. (2004). The native architecture of a photosynthetic membrane. *Nature*, 430, 1058-1062.

Bauer, J. E., Williams, P. M., & Druffel, E. R. M. (1992). ^{14}C activity of dissolved organic carbon fractions in the northcentral Pacific and Sargasso Sea. *Nature*, 357, 667-670.

Beatty, J. T. (2002). On the natural selection and evolution of the aerobic phototrophic bacteria. *Photosynthesis Research*, 73, 109-114.

Béjà, O., Aravind, L., Koonin, E. V., Suzuki, M. T., Hadd, A., Nguyen, L. P., Jovanovich, S., Gates, C. M., Feldman, R. A., Spudich, J. L., Spudich, E. N., & DeLong, E. F. (2000). Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science*, 289, 1902-1906.

Béjà, O., Spudich, E. N., Spudich, J. L., Leclerc, M., & DeLong, E. F. (2001). Proteorhodopsin phototrophy in the ocean. *Nature*, 411, 786-789.

Béjà, O., Suzuki, M. T., Heidelberg, J. F., Nelson, W. C., Preston, C. M., Hamada, T., Eisen, J. A., Fraser, C. M., & DeLong, E. F. (2002). Unsuspected diversity among marine aerobic anoxygenic phototrophs. *Nature*, 415, 630-633.

Bergh, O., Borsheim, K. Y., Bratbak, G., & Heldal, M. (1989). High abundance of viruses found in aquatic environments. Nature, 340, 467–468.

Beveridge, T. J. & Graham, L. L. (1991). Surface layers of bacteria. *Microbiology and Molecular Biology Reviews*, 55, 684-705.

Beveridge, T. J., Makin, S. A., Kadurugamuwa, J. L., & Li, Z. (1997). Interactions between biofilms and the environment. *FEMS Microbiology Reviews*, 20, 291-303,.

Beveridge, T. J. (1999). Structures of Gram-Negative Cell Walls and Their Derived Membrane Vesicles. *Journal of Bacteriology*, 181, 4725-4733.

Biddle, J. F., Lipp, J. S., Lever, M. A., Lloyd, K. G., Sorensen, K. B., Anderson, R., Fredricks, H. F., Elvert, M., Kelly, T. J., Schrag, D. P., Sogin, M. L., Brenchley, J. E., Teske, A., House, C. H., & Hinrichs, K. U. (2006). Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 3846-3851.

Binder, B. J., Chisholm, S. W., Olson, R. J., Frankel, S. L., & Worden, A. Z. (1996). Dynamics of picophytoplankton, ultraphytoplankton and bacteria in the central equatorial Pacific. *Deep-Sea Research II*, 43, 907-931.

Binnig, G., Quate, C. F., & Gerber, C. (1986). Atomic force microscope. *Physical Review Letters*, 56, 930 - 933.

Blackstock, W. P. & Weir, M. P. (1999). Proteomics: quantitative and physical mapping of cellular proteins. *Trends in Biotechnology*, 17, 121-127.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B. B., Witte, U., & Pfannkuche, O. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, 407, 623-626.

Boschker, H. T. S., Nold, S. C., Wellsbury, P., Bos, D., de Graaf, W., Pel, R., Parkes, R. J., & Cappenberg, T. E. (1998). Direct linking of microbial populations to specific biogeochemical processes by C-13-labelling of biomarkers. *Nature*, 392, 801-805.

Bralower, T. J., Silva, I. P., & Malone, M. J. (2002). New evidence for abrupt climate change in the Cretaceous and Paleogene: An Ocean Drilling Program expedition to Shatsky rise, northwest Pacific. *GSA Today*, 12, 4-10.

Brocks, J. J. & Pearson, A. (2005) Building the biomarker tree of life. In *Molecular Geomicrobiology*. Chantilly: Mineralogical Soc America, pp. 233-258.

Brussow, H., Canchaya, C., & Hardt, W. D. (2004). Phages and the evolution of bacterial pathogens: From genomic rearrangements to lysogenic conversion. *Microbiology and Molecular Biology Reviews*, 68, 560-602.

Buck, K. R., Chavez, F. P., & Campbell, L. (1996). Basin-wide distributions of living carbon components and the inverted trophic pyramid of the central gyre of the north Atlantic Ocean, summer 1993. *Aquatic Microbial Ecology*, 10, 283-298.

Butt, H. J., Jaschke, M., & Ducker, W. (1995). Measuring surface forces in aqueous electrolyte solution with the atomic force microscope. *Bioelectrochemistry and Bioenergetics*, 38, 191-201.

Camesano, T. A., Natan, M. J., & Logan, B. E. (2000). Observation of changes in bacterial cell morphology: using tapping mode atomic force microscopy. *Langmuir*, 16, 4563-4572.

Campbell, L., Liu, H. B., Nolla, H. A., & Vaulot, D. (1997). Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991-1994 ENSO event. *Deep-Sea Research Part I-Oceanographic Research Papers*, 44, 167-&.

Canchaya, C., Proux, C., Fournous, G., Bruttin, A., & Brussow, H. (2003). Prophage genomics. *Microbiology and Molecular Biology Reviews*, 67, 473-473.

Chada, V. G. R., Sanstad, E. A., Wang, R., & Driks, A. (2003). Morphogenesis of *Bacillus* spore surfaces. *Journal of Bacteriology*, 185, 6255-6261.

Chen, F., Lu, J. R., Binder, B. J., Liu, Y. C., & Hodson, R. E. (2001). Application of digital image analysis and flow cytometry to enumerate marine viruses stained with SYBR gold. *Applied and Environmental Microbiology*, 67, 539-545.

Chen, F., Wang, K., Kan, J. J., Suzuki, M. T., & Wommack, K. E. (2006). Diverse and unique picocyanobacteria in Chesapeake Bay, revealed by 16S-23S rRNA internal transcribed Spacer sequences. *Applied and Environmental Microbiology*, 72, 2239-2243.

Chen, F., Wang, K., Stewart, J., & Belas, R. (2006). Induction of multiple prophages from a marine bacterium: a genomic approach. *Applied and Environmental Microbiology*, 72, 4995-5001.

Cochran, P. K., Kellogg, C. A., & Paul, J. H. (1998). Prophage induction of indigenous marine lysogenic bacteria by environmental pollutants. *Marine Ecology-Progress Series*, 164, 125-133.

Cochran, P. K. & Paul, J. H. (1998). Seasonal abundance of lysogenic bacteria in a subtropical estuary. *Applied and Environmental Microbiology*, 64, 2308-2312.

Comita, P. B. & Gagosian, R. B. (1983). Membrane lipid from deep sea hydrothermal vent methanogen: A new macrocyclic glycerol diether. *Science*, 222, 1329-1331.

Coolen, M. J. L., Abbas, B., van Bleijswijk, J., Hopmans, E. C., Kuypers, M. M. M., Wakeham, S. G., & Damste, J. S. S. (2007). Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids. *Environmental Microbiology*, 9, 1001-1016.

Curtis, T. P., Sloan, W. T., & Scannell, J. W. (2002). Estimating prokaryotic diversity and its limits. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 10494-10499.

Damste, J. S. S., Rijpstra, W. I. C., Hopmans, E. C., Prahl, F. G., Wakeham, S. G., & Schouten, S. (2002). Distribution of membrane lipids of planktonic Crenarchaeota in the Arabian seat. *Applied and Environmental Microbiology*, 68, 2997-3002.

Damste, J. S. S., Strous, M., Rijpstra, W. I. C., Hopmans, E. C., Geenevasen, J. A. J., van Duin, A. C. T., van Niftrik, L. A., & Jetten, M. S. M. (2002). Linearly concatenated cyclobutane lipids form a dense bacterial membrane. *Nature*, 419, 708-712.

Delong, E. F. (1992). Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 5685-5689.

DeLong, E. F., Wu, K. Y., Prezelin, B. B., & Jovine, R. V. (1994). High abundance of Archaea in Antarctic marine picoplankton. *Nature*, 371, 695-697.

DeLong, E. F., King, L. L., Massana, R., Cittone, H., Murray, A., Schleper, C., & Wakeham, S. G. (1998). Dibiphytanyl ether lipids in nonthermophilic crenarchaeotes. *Applied and Environmental Microbiology*, 64, 1133-1138.

DeLong, E. F., Preston, C. M., Mincer, T., Rich, V., Hallam, S. J., Frigaard, N. U., Martinez, A., Sullivan, M. B., Edwards, R., Brito, B. R., Chisholm, S. W., & Karl, D. M. (2006). Community genomics among stratified microbial assemblages in the ocean's interior. *Science*, 311, 496-503.

Doktycz, M. J., Sullivan, C. J., Hoyt, P. R., Pelletier, D. A., Wu, S., & Allison, D. P. (2003). AFM imaging of bacteria in liquid media immobilized on gelatin coated mica surfaces. *Ultramicroscopy*, 97, 209-216.

Dufrêne, Y. F. (2002). Atomic Force Microscopy, a Powerful Tool in Microbiology. *Journal of Bacteriology*, 184, 5205-5213.

Dufrêne, Y. F. (2004). Using nanotechniques to explore microbial surfaces. *Nature*, 2, 451-460.

Dufrêne, Y. F. (2004). Refining our perception of bacterial surfaces with the atomic force microscope. *Journal of Bacteriology*, 186, 3283-3285.

Dufresne, A., Salanoubat, M., Partensky, F., Artiguenave, F., Axmann, I. M., Barbe, V., Duprat, S., Galperin, M. Y., Koonin, E. V., Le Gall, F., Makarova, K. S., Ostrowski, M., Oztas, S., Robert, C., Rogozin, I. B., Scanlan, D. J., de Marsac, N. T., Weissenbach, J., Wincker, P., Wolf, Y. I., & Hess, W. R. (2003). Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 10020-10025.

Dumitrescu, M. & Brassell, S.C. (2003) Biomarkers confirm cyanobacteria as a major source of planktonic organic matter during the Early Aptian. Geological Society of America (GSA) Seattle Annual Meeting (November 2-5, 2003, Abstracts with Programs, pp. 391). URL: *http://gsa.confex.com/gsa/2003AM/finalprogram/abstract_67241.htm*.

Durisch-Kaiser, E., Klauser, L., Wehrli, B., & Schubert, C. (2005). Evidence of intense archaeal and bacterial methanotrophic activity in the black sea water column. *Applied and Environmental Microbiology*, 71, 8099-8106.

Emerson, R. J. & Camesano, T. A. (2004). Nanoscale investigation of pathogenic microbial adhesion to a biomaterial. *Applied and Environmental Microbiology*, 70, 6012-6022.

Fang, H. H. P., Chan, K. Y., & Xu, L. C. (2000). Quantification of bacterial adhesion forces using atomic force microscopy (AFM). *Journal of Microbiological Methods*, 40, 89-97.

Feely, R. A., Sabine, C. L., Takahashi, T., & Wanninkhof, R. (2001). Uptake and Storage of carbon dioxide in the Ocean: the Global CO2 Survey. *Oceanography*, 14, 18-32.

Fenchel, T. (2001). Ecology - Marine bugs and carbon flow. Science, 292, 2444-2445.

Fichmann, J. & Westermeier, R. (1999) *2-D proteome analysis protocols*. New Jersey: Humana Press.

Fletcher, M., 1996. Bacterial attachment in aquatic environments: a diversity of surfaces and adhesion strategies. In: Fletcher, M. (Eds.), *Bacterial Adhesion: Molecular and Ecological Diversity* (pp. 1–24), New York: John Wiley & Sons.

Formolo, M. J., Lyons, T. W., Zhang, C. L., Kelley, C., Sassen, R., Horita, J., & Cole, D. R. (2004). Quantifying carbon sources in the formation of authigenic carbonates at gas hydrate sites in the Gulf of Mexico. *Chemical Geology*, 205, 253-264.

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., & Oakley, B. B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 14683-14688.

Freeman, K. H., Hayes, J. M., Trendel, J. M., & Albrecht, P. (1990). Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. *Nature*, 343, 254-256.

Friedrich, M. W. (2006). Stable-isotope probing of DNA: insights into the function of uncultivated microorganisms from isotopically labeled metagenomes. *Current Opinion in Biotechnology*, 17, 59-66.

Frigaard, N. U., Martinez, A., Mincer, T. J., & DeLong, E. F. (2006). Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. *Nature*, 439, 847-850.

Fuhrman, J. A., McCallum, K., & Davis, A. A. (1992). Novel major archaebacterial group from marine plankton. *Nature*, 356, 148-149.

Fuhrman, J. A. & Suttle, C. A. (1993). Viruses in marine planktonic systems. *Oceanography*, 6, 50-62.

Fuhrman, J. A. (1999). Marine viruses and their biogeochemical and ecological effects. *Nature*, 399, 541-548.

Gaboriaud, F., Bailet, S., Dague, E., & Jorand, F. (2005). Surface Structure and Nanomechanical Properties of *Shewanella putrefaciens* Bacteria at Two pH values (4 and 10) Determined by Atomic Force Microscopy. *Journal of Bacteriology*, 187, 3864-3868.

Gad, M. & Ikai, A. (1995). Method for immobilizing microbial cells on gel surface for dynamic AFM studies. *Biophysical Journal*, 69, 2226-2233.

Georicke, R. & Welschmeyer, N. A. (1993). The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. *Deep-Sea Research*, 2283-2294.

Giovannoni, S. J., Bibbs, L., Cho, J. C., Stapels, M. D., Desiderio, R., Vergin, K. L., Rappe, M. S., Laney, S., Wilhelm, L. J., Tripp, H. J., Mathur, E. J., & Barofsky, D. F. (2005). Proteorhodopsin in the ubiquitous marine bacterium SAR11. *Nature*, 438, 82-85.

Giovannoni, S. J., Tripp, H. J., Givan, S., Podar, M., Vergin, K. L., Baptista, D., Bibbs, L., Eads, J., Richardson, T. H., Noordewier, M., Rappe, M. S., Short, J. M., Carrington, J. C., & Mathur, E. J. (2005). Genome streamlining in a cosmopolitan oceanic bacterium. *Science*, 309, 1242-1245.

Glaeser, J. & Overmann, J. (2003). Characterization and in situ carbon metabolism of phototrophic consortia. *Applied and Environmental Microbiology*, 69, 3739-3750.

Gobler, C. J., Hutchins, D. A., Fisher, N. S., Cosper, E. M., & Sanudo-Wilhelmy, S. A. (1997). Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. *Limnology and Oceanography*, 42, 1492-1504.

Hafenbradl, D., Keller, M., Thiericke, R., & Stetter, K. O. (1993). A novel unsaturated archaeal ether core lipid from the hyperthermophile *Methanopyrus kandleri*. *Systematics and Applied Microbiology*, 16, 165-169.

Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., & DeLong, E. F. (2003). Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing archaea. *Applied and Environmental Microbiology*, 69, 5483-5491.

Hallam, S. J., Putnam, N., Preston, C. M., Detter, J. C., Rokhsar, D., Richardson, P. M., & DeLong, E. F. (2004). Reverse methanogenesis: Testing the hypothesis with environmental genomics. *Science*, 305, 1457-1462.

Hallam, S. J., Konstantinidis, K. T., Putnam, N., Schleper, C., Watanabe, Y., Sugahara, J., Preston, C., de la Torre, J., Richardson, P. M., & DeLong, E. F. (2006). Genomic analysis of the uncultivated marine crenarchaeote Cenarchaeum symbiosum. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 18296-18301.

Hallam, S. J., Mincer, T. J., Schleper, C., Preston, C. M., Roberts, K., Richardson, P. M., & DeLong, E. F. (2006). Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *Plos Biology*, 4, 520-536.

Hayes, J. M., Freeman, K. H., Popp, B. N., & Hoham, C. H. (1990). Compound-specific isotopic analyses: A novel tool for reconstruction of ancient biogeochemical processes. *Organic Geochemistry*, 16, 1115-1128.

Hedges, J. I. (1992). Global biogeochemical cycles: progress and problems. *Marine Chemistry*, 39, 67-93.

Hedrick, D. B., Gucker, J. B., & White, D. C. (1991). Archaebacterial ether lipid diversity analyzed by supercritical fluid chromatography: integration with a bacterial lipid protocol. *Journal of Lipid Research*, 32, 659-666.

Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., & Pernthaler, J. (2005). Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. *Applied and Environmental Microbiology*, 71, 2303-2309.

Higgins, M. J., Sader, J. E., Mulvaney, P., & Wetherbee, R. (2003). Probing the surface of living diatoms with atomic force microscopy: the nanostructure and nanomechanical properties of the mucilage layer. *Journal of Phycology*, 39, 722-734.

Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., & DeLong, E. F. (1999). Methane-consuming archaebacteria in marine sediments. *Nature*, 398, 802-805.

Hinterdorfer, P., Baumgartner, W., Gruber, H. J., Schilcher, K., & Schindler, H. (1996). Detection and localization of individual antibody-antigen recognition events by atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 3477-3481.

Hopmans, E. C., Schouten, S., Pancost, R. D., van der Meer, M. T. J., & Damste, J. S. S. (2000). Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, 14, 585-589.

House, C. H., Schopf, J. W., & Stetter, K. O. (2003). Carbon isotopic fractionation by Archaeans and other thermophilic prokaryotes. *Organic Geochemistry*, 34, 345-356.

Huber, R., Wilharm , T., Huber, D., Trincone, A., Burgraf, S., Konig, H., Rachel, R., Rochinger, I., Fricke, H., & Stetter, K. O. (1992). *Aquifex pyrophilus* gen. nov. sp. nov., represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacteria. *Systematics and Applied Microbiology*, 15, 340-351.

Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K., Nealson, K. H., & Horikoshi, K. (2003). Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Applied and Environmental Microbiology*, 69, 7224-7235.

Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., Nealson, K. H., Horikoshi, K., D'Hondt, S., & Jorgensen, B. B. (2006). Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments, on the Pacific Ocean Margin. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 2815-2820.

Ingalls, A. E., Shah, S. R., Hansman, R. L., Aluwihare, L. I., Santos, G. M., Druffel, E. R. M., & Pearson, A. (2006). Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 6442-6447.

Ivanova, E. P., Alexeeva, Y. V., Pham, D. K., Wright, J. P., & Nicolaul, D. V. (2006). ATP level variations in heterotrophic bacteria during attachment on hydrophilic and hydrophobic surfaces. *International Microbiology*, 9, 37-46.

Jahnke, L. L., Summons, R. E., Dowling, L. M., & Zahiralis, K. D. (1995). Identification of methanotrophic lipid biomarkers in cold-seep mussel gills-Chemical and isotopic analysis. *Applied and Environmental Microbiology*, 61, 576-582.

Jahnke, L. L., Eder, W., Huber, R., Hope, J. M., Hinrichs, K. U., Hayes, J. M., Des Marais, D. J., Cady, S. L., & Summons, R. E. (2001). Signature lipids and stable carbon isotope analyses of octopus spring hyperthermophilic communities compared with those of Aquificales representatives. *Applied and Environmental Microbiology*, 67, 5179-5189.

Jiang, S. C. & Paul, J. H. (1996). Occurrence of lysogenic bacteria in marine microbial communities as determined by prophage induction. *Marine Ecology Progress Series*, 142, 27-28.

Jean, W., Lorraine, E., Lawrence, C., Steven, L., Harry, R. (2005). Surface and subsurface manifestations of gas movement through a N-S stransect of the Gulf of Mexico, *Marine and Petroleum Geology*, 22, 479-497.

Jiao, N. Z., Yang, Y. H., Koshikawa, H., & Watanabe, M. (2002). Influence of hydrographic conditions on picoplankton distribution in the East China Sea. *Aquatic Microbial Ecology*, 30, 37-48.

Jiao, N. Z., Sieracki, M. E., Zhang, Y., & Du, H. L. (2003). Aerobic anoxygenic phototrophic bacteria and their roles in marine ecosystems. *Chinese Science Bulletin*, 48, 1064-1068.

Jiao, N. Z., Yang, Y. J., & Luo, T. W. (2004). Membrane potential based characterization by flow cytometry of physiological states in an aerobic anoxygenic phototrophic bacterium. *Aquatic Microbial Ecology*, 37, 149-158.

Jiao, N. Z., Yang, Y. H., Hong, N., Ma, Y., Harada, S., Koshikawa, H., & Watanabe, M. (2005). Dynamics of autotrophic picoplankton and heterotrophic bacteria in the East China Sea. *Continental Shelf Research*, 25, 1265-1279.

Jiao, N. Z., Feng, F. Y., & Wei, B. (2006). Proteorhodopsin - A new path for biological utilization of light energy in the sea. *Chinese Science Bulletin*, 51, 889-896.

Jiao, N. Z., Zhang, Y., & Chen, Y. (2006). Time series observation based InfraRed Epifluorescence Microscopic (TIREM) approach for accurate enumeration of bacteriochlorophyll-containing microbes in marine environments. *Journal of Microbiological Methods*, 65, 442-452.

Jiao N. Z. (2006). *Marine Microbial Ecology*. Beijing, China: Science Press.

Jiao, N. Z., Yang, Y. H., Zeng, Y. H., Hong, N., Liu, R. L. Chen, F., & Wang, P. X. (2007). Distinct Distribution Pattern of Abundance and Diversity of Aerobic Anoxygenic Phototrophic Bacteria in the Global Ocean. *Environmental Microbiology*, 9(12):3091-3099.

Kan, J., Hanson, T. E., Ginter, J. M., Wang, K., & Chen, F. (2005). Metaproteomic analysis of Chesapeake Bay microbial communities. *Saline Systems*, 1, 7.

Karner, M. B., DeLong, E. F., & Karl, D. M. (2001). Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature*, 409, 507-510.

Kasas, S., Gotzos, V., & Celio, M. R. (1993). Obeservation of living cells using the atomic force microscope. *Biophysical Journal*, 64, 539-544.

Kendall, M. M., Wardlaw, G. D., Tang, C. F., Bonin, A. S., Liu, Y. T., & Valentine, D. L. (2007). Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of *Methanogenium boonei* sp nov. *Applied and Environmental Microbiology*, 73, 407-414.

Kerr, R. A. (2002). Evolution - Could poor nutrition have held life back? *Science*, 297, 1104-1105.

Knittel, K., Losekann, T., Boetius, A., Kort, R., & Amann, R. (2005). Diversity and distribution of methanotrophic archaea at cold seeps. *Applied and Environmental Microbiology*, 71, 467-479.

Koblizek, M., Béjà, O., Bidigare, R. R., Christensen, S., Benitez-Nelson, B., Vetriani, C., Kolber, M. K., Falkowski, P. G., & Kolber, Z. S. (2003). Isolation and characterization of *Erythrobacter* sp strains from the upper ocean. *Archives of Microbiology*, 180, 327-338.

Kolber, Z. S., Van Dover, C. L., Niederman, R. A., & Falkowski, P. G. (2000). Bacterial photosynthesis in surface waters of the open ocean. *Nature*, 407, 177-179.

Kolber, Z. S., Plumley, F. G., Lang, A. S., Beatty, J. T., Blankenship, R. E., VanDover, C. L., Vetriani, C., Koblizek, M., Rathgeber, C., & Falkowski, P. G. (2001). Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. *Science*, 292, 2492-2495.

Könneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B., & Stahl, D. A. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature*, 437, 543-546.

Kuznetsov, Y. G., Malkin, A. J., Lucas, R. W., Plomp, M., & McPherson, A. (2001). Imaging of viruses by atomic force microscopy. *Journal of General Virology*, 82, 2025-2034.

Kuznetsov, Y. G., Gurnon, J. R., Etten, J. L., & McPherson, A. (2005). Atomic force microscopy investigation of a chlorella virus, PBCV-1. *Journal of Structural Biology*, 149, 256-263.

Lang, A. S. & Beatty, J. T. (2007). Importance of widespread gene transfer agent genes in alpha-proteobacteria. *Trends in Microbiology*, 15, 54-62.

Langworthy, T. A., Holzer, G., Zeikus, J. G., & Tornabene, T. G. (1983). Iso-and anteiso-branched glycerol diethers of the thermophilic anaerobes *Thermo- desulfotobacterium commune*. *Systematics and Applied Microbiology*, 4, 1-17.

Lanoil, B. D., Sassen, R., La Duc, M. T., Sweet, S. T., & Nealson, K. H. (2001). Bacteria and Archaea physically associated with Gulf of Mexico gas hydrates. *Applied and Environmental Microbiology*, 67, 5143-5153.

Lanoil, B. D., La Duc, M. T., Wright, M., Kastner, M., Nealson, K. H., & Bartlett, D. (2005). Archaeal diversity in ODP legacy borehole 892b and associated seawater and sediments of the Cascadia Margin. *FEMS Microbiology Ecology*, 54, 167-177.

Lead, J. R., Muirhead, D., & Gibson, C. T. (2005). Characterization of freshwater natural aquatic colloids by atomic force microscopy (AFM). *Environmental Science & Technology*, 39, 6930 -6936.

Li, W. K. W. & Wood, A. M. (1988). Vertical distribution of North Atlantic ultraphytoplankton: analysis by flow cytometry and epifluorescence microscopy. *Deep-Sea Research*, 35, 1615-1638.

Lloyd, K. G., Lapham, L., & Teske, A. (2006). Anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Applied and Environmental Microbiology*, 72, 7218-7230.

Lower, S. K., Tadanier, C. J., & Hochella, M. F. (2000). Measuring interfacial and adhesion forces between bacteria and mineral surfaces with biological force microscopy. *Geochimica Et Cosmochimica Acta*, 64, 3133-3139.

Lower, S. K., Hochella, M. F., & Beveridge, T. J. (2001). Bacterial Recognition of Mineral Surfaces: Nanoscale Interactions Between *Shewanella* and α-FeOOH. *Science*, 292, 1360 - 1363.

Lu, J., Chen, F., & Hodson, R. E. (2001). Distribution, isolation, host specificity, and diversity of cyanophages infecting marine *Synechococcus* spp. in river estuaries. *Applied and Environmental Microbiology*, 67, 3285-3290.

Macko, S. A., Engel, M. H., & Qian, Y. (1994). Early diagenesis and organic matter preservation—A molecular stable carbon isotope perspective. *Chemical Geology*, 114, 365-379.

Madigan M. T., & Martinko J. M. (2006). *Brock Biology of Microorganisms* (11th Ed.). New Jersey: Prentice Hall.

Madrid, V. M., Taylor, G. T., Scranton, M. I., & Chistoserdov, A. Y. (2001). Phylogenetic diversity of bacterial and archaeal communities in the anoxic zone of the Cariaco Basin. *Applied and Environmental Microbiology*, 67, 1663-1674.

Man, D. L., Wang, W. W., Sabehi, G., Aravind, L., Post, A. F., Massana, R., Spudich, E. N., Spudich, J. L., & Béjà, O. (2003). Diversification and spectral tuning in marine proteorhodopsins. *Embo Journal*, 22, 1725-1731.

Man-Aharonovich, D., Sabehi, G., Sineshchekov, O. A., Spudich, E. N., Spudich, J. L., & Béjà, O. (2004). Characterization of RS29, a blue-green proteorhodopsin variant from the Red Sea. *Photochemical & Photobiological Sciences*, 3, 459-462.

Marie, D., Brussaard, C. P. D., Thyrhaug, R., Bratbak, G., & Vaulot, D. (1999). Enumeration of marine viruses in culture and natural samples by flow cytometry. *Applied and Environmental Microbiology*, 65, 45-52.

Massana, R., Murray, A. E., Preston, C. M., & DeLong, E. F. (1997). Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Applied and Environmental Microbiology*, 63, 50-56.

McElfresh, M., Baesu, E., Balhorn, R., Belak, J., & Allen, M. J. (2002). Combining constitutive materials modeling with atomic force microscopy to understand the mechanical properties of living cells. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 6493-6497.

McFeters, G. A., Yu, F. P., Pyle, B. H., & Stewart, P. S. (1995). Physiological assessment of bacteria using fluorochromes. *Journal of Microbiological Methods*, 21, 1-13.

Michel, H. & Oesterhelt, D. (1980). Electrochemical proton gradient across the cell membrane of *Halobacterium halobium*: effect of N,N9-dicyclohexylcarbodiimide, relation to intracellular adenosine triphosphate, adenosine diphosphate, and phosphate concentration, and influenceof the potassium gradient. *Biochemistry*, 19, 4607~4614.

Miller, W. & Moran, M. A. (1997). Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. *Limnology and Oceanography*, 42, 1317-1324.

Mills, H. J., Hodges, C., Wilson, K., MacDonald, I. R., & Sobecky, P. A. (2003). Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. *FEMS Microbiology Ecology*, 46, 39-52.

Mincer, T. J., Church, M. J., Taylor, L. T., Preston, C., Kar, D. M., & DeLong, E. F. (2007). Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. *Environmental Microbiology*, 9, 1162-1175.

Mopper, K., Zhou, X., Kieber, R. J., Kieber, D. J., Sikorski, R. J., & Jones, R. D. (1991). Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. *Nature*, 353, 60-62.

Mozes, N., Handley, P. S., Busscher, H. J., & Rouxhet, P. G. (1991). *Microbial cell surface analysis: structural and physicochemical methods*. New York: VCH Publishers.

Müller, D. J., Baumeister, W., & Engel, A. (1999). Controlled unzipping of a bacterial surface layer with atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 13170-13174.

Müller, S., Ullrich, S., Lösche, A., Loffhagen, N., & Babel, W. (2000). Flow cytometric techniques to characterise physiological states of *Acinetobacter calcoaceticus*. *Journal of Microbiological Methods*, 25, 79-86.

Nauhaus, K., Treude, T., Boetius, A., & Kruger, M. (2005). Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. *Environmental Microbiology*, 7, 98-106.

Nicol, G. W. & Schleper, C. (2006). Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology*, 14, 207-212.

Noble, R. T. & Fuhrman, J. A. (1998). Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology*, 14, 113-118.

Nogawa, H. & Tanoue, E. (2003). Dissolved organic matter in oceanic waters. *Journal of Oceanography*, 59, 129-147.

Novo, D., Perlmutter, N. G., Hunt, R. H., & Shapiro, H. M. (1999). Accurate flow cytometric membrane potential measurement in bacteria using diethyloxacarbocyanine and a ratiometric technique. *Cytometry*, 35, 55-63.

Novo, D. J., Perlmutter, N. G., Hunt, R. H., & Shapiro, H. M. (2000). Multiparameter flow cytometric analysis of antibiotic effects on membrane potential, membrane permeability, and bacterial counts of Staphylococcus aureus and Micrococcus luteus. *Antimicrobial Agents and Chemotherapy*, 44, 827-834.

O'Farrell, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *Journal of Biological Chemistry*, 250, 4007-4021.

Orphan, V. J., Hinrichs, K. U., Ussler, W., Paull, C. K., Taylor, L. T., Sylva, S. P., Hayes, J. M., & Delong, E. F. (2001). Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Applied and Environmental Microbiology*, 67, 1922-1934.

Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D., & DeLong, E. F. (2001). Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, 293, 484-487.

Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D., & DeLong, E. F. (2002). Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 7663-7668.

Ouverney, C. C. & Fuhrman, J. A. (2000). Marine planktonic Archaea take up amino acids. *Applied and Environmental Microbiology*, 66, 4829-4833.

Palenik, B., Brahamsha, B., Larimer, F. W., Land, M., Hauser, L., Chain, P., Lamerdin, J., Regala, W., Allen, E. E., McCarren, J., Paulsen, I., Dufresne, A., Partensky, F., Webb, E. A., & Waterbury, J. (2003). The genome of a motile marine *Synechococcus*. *Nature*, 424, 1037-1042.

Palenik, B., Ren, Q. H., Dupont, C. L., Myers, G. S., Heidelberg, J. F., Badger, J. H., Madupu, R., Nelson, W. C., Brinkac, L. M., Dodson, R. J., Durkin, A. S., Daugherty, S. C., Sullivan, S. A., Khouri, H., Mohamoud, Y., Halpin, R., & Paulsen, I. T. (2006). Genome sequence of *Synechococcus* CC9311: Insights into adaptation to a coastal environment. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 13555-13559.

Pancost, R. D., Freeman, K. H., Patzkowsky, M. E., Wavrek, D. A., & Collister, J. W. (1998). Molecular indicators of redox and marine photoautotroph composition in the late Middle Ordovician of Iowa, USA. *Organic Geochemistry*, 29, 1649-1662.

Pancost, R. D., Freeman, K. H., & Wakeham, S. G. (1999). Controls on the carbon-isotope compositions of compounds in Peru surface waters. *Organic Geochemistry*, 30, 319-340.

Pancost, R. D., Bouloubassi, I., Aloisi, G., & Damste, J. S. S. (2001). Three series of non-isoprenoidal dialkyl glycerol diethers in cold-seep carbonate crusts. *Organic Geochemistry*, 32, 695-707.

Pancost, R. D., Hopmans, E. C., & Damste, J. S. S. (2001). Archaeal lipids in Mediterranean cold seeps: Molecular proxies for anaerobic methane oxidation. *Geochimica Et Cosmochimica Acta*, 65, 1611-1627.

Pancost, R. D. & Damste, J. S. S. (2003). Carbon isotopic compositions of prokaryotic lipids as tracers of carbon cycling in diverse settings. *Chemical Geology*, 195, 29-58.

Pearson, A., McNichol, A. P., Benitez-Nelson, B. C., Hayes, J. M., & Eglinton, T. I. (2001). Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific Delta C-14 analysis. *Geochimica Et Cosmochimica Acta*, 65, 3123-3137.

Pearson, A., Huang, Z., Ingalls, A. E., Romanek, C. S., Wiegel, J., Freeman, K. H., Smittenberg, R. H., & Zhang, C. L. (2004). Nonmarine crenarchaeol in Nevada hot springs. *Applied and Environmental Microbiology*, 70, 5229-5237.

Pelling, A. E., Li, Y., Shi, W., & Gimzewski, J. K. (2005). Nanoscale visualization and characterization of Myxococcus xanthus cells with atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 6484-6489.

Pi Y., Ye Q., Jiang H., Pearson A., Li S., Noakes J., Culp R., Dong H., & Zhang C. L. (2007). Lipids and phylogenetic characterization of Archaea associated with gas hydrates in the Gulf of Mexico. *FEMS Microbiology Ecology*, In revision.

Pradet-Balade, B., Boulme, F., Beug, H., Mullner, E. W., & Garcia-Sanz, J. A. (2001). Translation control: bridging the gap between genomics and proteomics? *Trends in Biochemical Sciences*, 26, 225-229.

Preston, C. M., Wu, K. Y., Molinsky, T. F., & DeLong, E. F. (1996). A psychrophilic crenarchaeote inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 6241-6246.

Proctor, L. M. & Fuhrman, J. A. (1990). Viral mortality of marine bacteria and cyanobacteria. *Nature*, 343, 60–62.

Radajewski, S., McDonald, I. R., & Murrell, J. C. (2003). Stable-isotope probing of nucleic acids: a window to the function of uncultured microorganisms. *Current Opinion in Biotechnology*, 14, 296-302.

Ram, R. J., VerBerkmoes, N. C., Thelen, M. P., Tyson, G. W., Baker, B. J., Blake, R. C., Shah, M., Hettich, R. L., & Banfield, J. F. (2005). Community proteomics of a natural microbial biofilm. *Science*, 308, 1915-1920.

Razatos, A., Ong, Y. L., Sharma, M. M., & Georgiou, G. (1998). Molecular determinants of bacterial adhesion monitored by atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 11059-11064.

Reed, A. J., Lutz, R. A., & Vetriani, C. (2006). Vertical distribution and diversity of bacteria and archaea in sulfide and methane-rich cold seep sediments located at the base of the Florida Escarpment. *Extremophiles*, 10, 199-211.

Richardson, T. L., & Jackson, G. A. (2007). Small phytoplankton and carbon export from the surface ocean. *Science*, 315, 838-840.

Rief, M., Oesterhelt, F., Heymann, B., & Gaub, H. E. (1997). Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science*, 275, 1295 - 1297.

Ringelberg, D. B., Davis, J. D., Smith, G. A., Pfiffner, S. M., Nichols, P. D., Nickels, J. S., Henson, J. M., Wilson, J. T., Yates, M., Kampbell, D. H., Read, H. W., Stocksdale, T. T., & White, D. C. (1989). Validation of signature polarlipid fatty acid biomarkers for alkane-utilizing bacteria in soils and subsurface aquifer materials. *FEMS Microbiology Ecology*, 62, 39-50.

Rocap, G., Larimer, F. W., Lamerdin, J., Malfatti, S., Chain, P., Ahlgren, N. A., Arellano, A., Coleman, M., Hauser, L., Hess, W. R., Johnson, Z. I., Land, M., Lindell, D., Post, A. F., Regala, W., Shah, M., Shaw, S. L., Steglich, C., Sullivan, M. B., Ting, C. S., Tolonen, A., Webb, E. A., Zinser, E. R., & Chisholm, S. W. (2003). Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature*, 424, 1042-1047.

Rodriguez, G. G., Phipps, D., Ishiguro, K., & Ridgway, H. F. (1992). Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Applied and Environmental Microbiology*, 58, 1801-1808.

Roszack, D. B. & Colwell, R. R. (1987). Survival strategies of bacteria in the natural environment. *Microbiology and Molecular Biology Reviews*, 51, 365-379.

Rotsch, C. & Radmacher, M. (1997). Mapping local electrostatic forces with the atomic force microscope. *Langmuir*, 13, 2825-2832.

Rusch, D. B., Halpern, A. L., Sutton, G., Heidelberg, K. B., Williamson, S., Yooseph, S., Wu, D. Y., Eisen, J. A., Hoffman, J. M., Remington, K., Beeson, K., Tran, B., Smith, H., Baden-Tillson, H., Stewart, C., Thorpe, J., Freeman, J., Andrews-Pfannkoch, C., Venter, J. E., Li, K., Kravitz, S., Heidelberg, J. F., Utterback, T., Rogers, Y. H., Falcon, L. I., Souza, V., Bonilla-Rosso, G., Eguiarte, L. E., Karl, D. M., Sathyendranath, S., Platt, T., Bermingham, E., Gallardo, V., Tamayo-Castillo, G., Ferrari, M. R., Strausberg, R. L., Nealson, K., Friedman, R., Frazier, M., & Venter, J. C. (2007). The Sorcerer II Global Ocean Sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. *Plos Biology*, 5, 398-431.

Sabehi, G., Loy, A., Jung, K. H., Partha, R., Spudich, J. L., Isaacson, T., Hirschberg, J., Wagner, M., & Béjà, O. (2005). New insights into metabolic properties of marine bacteria encoding proteorhodopsins. *Plos Biology*, 3, 1409-1417.

Santschi, P. H., Balnois, E., Wilkinson, K. J., Zhang, J., & Buffle, J. (1998). Fibrillar polysaccharides in marine macromolecular organic matter as imaged by atomic force microscopy and transmission electron microscopy. *Limnology and Oceanography*, 43, 896-908.

Sassen, R., Roberts, H. H., Carney, R., Milkov, A. V., DeFreitas, D. A., Lanoil, B., & Zhang, C. L. (2004). Free hydrocarbon gas, gas hydrate, and authigenic minerals in

chemosynthetic communities of the northern Gulf of Mexico continental slope: relation to microbial processes. *Chemical Geology*, 205, 195-217.

Scheuring, S., Seguin, J., Marco, S., Levy, D., Robert, B., & Rigaud, J. L. (2003). Nanodissection and high-resolution imaging of the *Rhodopseudomonas viridis* photosynthetic core complex in native membranes by AFM. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 1690-1693.

Scheuring, S. & Sturgis, J. N. (2005). Chromatic Adaptation of Photosynthetic Membranes. *Science*, 309, 484-487.

Schleper, C., Jurgens, G., & Jonuscheit, M. (2005). Genomic studies of uncultivated archaea. *Nature Reviews Microbiology*, 3, 479-488.

Schouten, S., Hopmans, E. C., Pancost, R. D., & Damste, J. S. S. (2000). Widespread occurrence of structurally diverse tetraether membrane lipids: Evidence for the ubiquitous presence of low-temperature relatives of hyperthermophiles. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 14421-14426.

Schouten, S., Hopmans, E. C., Schefuss, E., & Damste, J. S. S. (2002). Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures? *Earth and Planetary Science Letters*, 204, 265-274.

Schubert, C. J., Coolen, M. J. L., Neretin, L. N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E. C., Damste, J. S. S., Wakeham, S., & Kuypers, M. M. M. (2006). Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, 8, 1844-1856.

Schulze, W. X., Gleixner, G., Kaiser, K., Guggenberger, G., Mann, M., & Schulze, E. D. (2005). A proteomic fingerprint of dissolved organic carbon and of soil particles. *Oecologia*, 142, 335-343.

Schwalbach, M. S. & Fuhrman, J. A. (2005). Wide-ranging abundances of aerobic anoxygenic phototrophic bacteria in the world ocean revealed by epifluorescence microscopy and quantitative PCR. *Limnology and Oceanography*, 50, 620-628.

Seki, M. P., Polovina, J. J., Brainard, R. E., Bidigare, R. R., Leonard, C. L., & Foley, D. G. (2001). Biological enhancement at cyclonic eddies tracked with GOES thermal imagery in Hawaiian waters. *Geophysical Research Letters*, 28, 1583-1586.

Shalapyonok, A., Olson, R. J., & Shalapyonok, L. S. (1998). Ultradian growth in *Prochlorococcus* spp. *Applied and Environmental Microbiology*, 64, 1066-1069.

Shepard, J. L., Olsson, B., Tedengren, M., & Bradley, B. P. (2000). Protein expression signatures identified in Mytilus edulis exposed to PCBs, copper and salinity stress. *Marine Environmental Research*, 50, 337-340.

Sherr, B. F., A., d. G. P., & Sherr, E. B. (1999). Estimating abundance and single-cell characteristics of respiring bacteria via the redox dye, CTC. *Aquatic Microbial Ecology*, 18, 117-131.

Shibata, A., Goto, Y., Saito, H., Kikuchi, T., Toda, T., & Taguchi, S. (2006). Comparison of SYBR Green I and SYBR Gold stains for enumerating bacteria and viruses by epifluorescence microscopy. *Aquatic Microbial Ecology*, 43, 223-231.

Sieracki, M. E., Gilg, I. C., Thier, E. C., Poulton, N. J., & Goericke, R. (2006). Distribution of planktonic aerobic anoxygenic photoheterotrophic bacteria in the northwest Atlantic. *Limnology and Oceanography*, 51, 38-46.

Sullivan, M. B., Waterbury, J. B., & Chisholm, S. W. (2003). Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. *Nature*, 424, 1047-1051.

Sullivan, M. B., Coleman, M. L., Weigele, P., Rohwer, F., & Chisholm, S. W. (2005). Three *Prochlorococcus* cyanophage genomes: Signature features and ecological interpretations. *Plos Biology*, 3, 790-806.

Summons, R. E., Jane, L. L., & Roksandic, Z. (1994). Carbon isotopic fractionation in lipids from metamorphic bacteria: Relevance for interpretation of the geochemical record of biomarkers. *Geochimica Et Cosmochimica Acta*, 58, 2853-2863.

Summons R. E., Jahnke L. L., & Simoneit R. T. (1996). Lipid biomarkers for bacterial ecosystems: Studies of cultured organisms, hydrothermal environments and ancient sediments. In G. R. Bock & J. A. Goode (Eds.), *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)* (pp. 174-194). New York: John Wiley & Sons.

Suttle, C. A., Chan, A. M., & Cottrell, M. T. (1990). Infection of phytoplankton by viruses and reduction of primary productivity. *Nature*, 347, 467-469.

Suttle, C. A., & Chan, A. M. (1993). Marine cyanophages infecting oceanic and coastal strains of *Synechococcus*: abundance, morphology, cross-reactivity and growth characteristics. *Marine Ecology Progress Series*, 92, 99-109.

Suttle, C. A. a. C. A. M. (1993). Marine cyanophages infecting oceanic and coastal strains of Synechococcus: abundance, morphology, cross-reactivity and growth characteristics. *Marine Ecology Progress Series*, 92, 99-109.

Suttle, C. A. (1994). The significance of viruses to mortality in aquatic microbial communities. *Microbial Ecology*, 28, 237-243.

Suttle, C. A., & Chan, A. M. (1994). Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Applied and Environmental Microbiology*, 60, 3167-3174.

Suttle, C. A. (2005). Viruses in the sea. *Nature*, 437, 356-361.

Suyama, T., Shigematsu, T., Suzuki, T., Tokiwa, Y., Kanagawa, T., Nagashima, K. V. P., & Hanada, S. (2002). Photosynthetic apparatus in *Roseateles depolymerans* 61A is transcriptionally induced by carbon limitation. *Applied and Environmental Microbiology*, 68, 1665-1673.

Teira, E., Lebaron, P., van Aken, H., & Herndl, G. J. (2006). Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. *Limnology and Oceanography*, 51, 2131-2144.

Teske, A. P. (2006). Microbial communities of deep marine subsurface sediments: Molecular and cultivation surveys. *Geomicrobiology Journal*, 23, 357-368.

Thingstad, T. F., Heldal, M., Bratbak, G., & Dundas, I. (1993). Are viruses important partners in pelagic food webs. *Trends in Ecology and Evolution*, 8, 209-213.

Touhami, A., Hoffmann, B., Vasella, A., Denis, F. A., & Dufrêne, Y. F. (2003). Aggregation of yeast cells: direct measurement of discrete lectin–carbohydrate interactions *Microbiology*, 149, 2873-2878.

Tringe, S. G., von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., Podar, M., Short, J. M., Mathur, E. J., Detter, J. C., Bork, P., Hugenholtz, P., & Rubin, E. M. (2005). Comparative metagenomics of microbial communities. *Science*, 308, 554-557.

Tsutomu, M. & Koichi, K. (1992). Quantum conversion and image detection by a bacteriorhodopsin-based artificial photoreceptor. *Science*, 255, 342~344.

Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., & Banfield, J. F. (2004). Community

structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428, 37-43.

Valentine, D. L. & Reeburgh, W. S. (2000). New perspectives on anaerobic methane oxidation. *Environmental Microbiology*, 2, 477-484.

Vandevivere, P. & Kirchman, D. L. (1993). Attachment stimulates synthesis by a bacterium exopolysaccharide synthesis by a bacterium. *Applied and Environmental Microbiology*, 59, 3280-3286.

Vaulot, D., Marie, D., Olson, R. J., & Chisholm, S. W. (1995). Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the Equatorial Pacific Ocean. *Science*, 8, 1480-1482.

Velegol, S. B., Pardi, S., Li, X., Velegol, D., & Logan, B. E. (2003). AFM Imaging Artifacts due to Bacterial Cell Height and AFM Tip Geometry. *Langmuir*, 19, 851-857.

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D. Y., Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M. W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkoch, C., Rogers, Y. H., & Smith, H. O. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, 304, 66-74.

Vetriani, C., Jannasch, H. W., MacGregor, B. J., Stahl, D. A., & Reysenbach, A. L. (1999). Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments. *Applied and Environmental Microbiology*, 65, 4375-4384.

Wakeham, S. G. (1995). Lipid biomarkers for heterotrophic alteration of suspended particulate organic matter in oxygenated and anoxic water columns of the ocean. *Deep-Sea Research Part I-Oceanographic Research Papers*, 42, 1749-1771.

Whelan, J., Eglinton, L., Cathles III, L., Losh, S., Roberts, H. (2005). Surface and subsurface manifestations of gas movement through a N–S transect of the Gulf of Mexico. *Marine and Petroleum Geology* 22, 479–497.

Wang, W. W., Sineshchekov, O. A., Spudich, E. N., & Spudich, J. L. (2003). Spectroscopic and photochemical characterization of a deep ocean proteorhodopsin. *Journal of Biological Chemistry*, 278, 33985-33991.

Wang, P., Xiao, X., & Wang, F. P. (2005). Phylogenetic analysis of Archaea in the deep-sea sediments of west Pacific Warm Pool. *Extremophiles*, 9, 209-217.

Wang, K. & F., Chen. (2007). Prevalence of highly host-specific cyanophages in the estuarine environment. *Environmental Microbiology*, In press.

Wasinger, V. C., Cordwell, S. J., Cerpa Poljak, A., Yan, J. X., Gooley, A. A., wilkins, M. R., Duncan, M. W., Harris, R., Williams, K. L., & Humphery-Smith, I. (1995). Progress with gene product mapping of Moli cutes: Mycoplasma genitalium. *Electrophoresis*, 16, 1090-1094.

Waterbury, J. B. & Valois, F. W. (1993). Resistance to co-occuring phages enables marine *Synechococcus* communities to coexist wth cyanophages abundant in seawater. *Applied and Environmental Microbiology*, 59, 3393-3399.

Weijers, J. W. H., Schouten, S., Hopmans, E. C., Geenevasen, J. A. J., David, O. R. P., Coleman, J. M., Pancost, R. D., & Sinninghe Damste, J. S. (2006). Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. *Environmental Microbiology*, 8, 648-657.

Weinbauer, M. G. & Suttle, C. A. (1996). Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. *Applied and Environmental Microbiology*, 62, 4374–4380.

Weinbauer, M. G. & Rassoulzadegan, F. (2004). Are viruses driving microbial diversification and diversity? *Environmental Microbiology*, 6, 1-11.

Wilcox, R. M. & Fuhrman, J. A. (1994). Bacterial viruses in coastal seawater: lytic rather than lysogenic production. *Marine Ecology Progress Series*, 114, 35-45.

Wilhelm, S. W., Weinbauer, M. G., Suttle, C. A., & Jeffrey, W. H. (1998). The role of sunlight in the removal and repair of viruses in the sea. *Limnology and Oceanography*, 43, 586-592.

Wilhelm, S. W. & Suttle, C. A. (1999). Viruses and Nutrient Cycles in the Sea - Viruses play critical roles in the structure and function of aquatic food webs. *Bioscience*, 49, 781-788.

Williams, P. M. & Druffel, E. R. M. (1987). Radiocarbon in dissolved organic matter in the central North Pacific Ocean. *Nature*, 330, 246-248.

Williams, S. C., Hong, Y., Danavall, D. C. A., Howard-Jones, M. H., Gibson, D., Frischer, M. E., & Verity, P. G. (1998). Distinguishing between living and nonliving bacteria: Evaluation of the vital stain propidium iodide and its combined use with molecular probes in aquatic samples. *Journal of Microbiological Methods*, 32, 225-236.

Wilmes, P. & Bond, P. L. (2004). The application of two-dimensional polyacrylamide gel electrophoresis and downstream analyses to a mixed community of prokaryotic microorganisms. *Environmental Microbiology*, 6, 911-920.

Wilmes, P. & Bond, P. L. (2006). Metaproteomics: studying functional gene expression in microbial ecosystems. *Trends in Microbiology*, 14, 92-97.

Wilson, W. H., Joint, I. R., Carr, N. G., & Mann, N. H. (1993). Isolation and molecular characterization of five marine cyanophages propagated on *Synechococcus* sp. strain WH7803. *Applied and Environmental Microbiology*, 59, 3736-3742.

Wommack, K. E. & Colwell, R. R. (2000). Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews*, 64, 69-114.

Wuchter, C., Abbas, B., Coolen, M. J. L., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G. J., Middelburg, J. J., Schouten, S., & Damste, J. S. S. (2006). Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 12317-12322.

Xiong, J., Fischer, W. M., Inoue, K., Nakahara, M., & Bauer, C. E. (2000). Molecular evidence for the early evolution of photosynthesis. *Science*, 289, 1724-1730.

Xu, W., Mulhern, P. J., Blackford, B. L., Jericho, M. H., & Templeton, I. (1994). A new atomic force microscopy technique for the measurement of the elastic properties of biological materials. *Scanning Microscopy*, 8, 499-506.

Yooseph, S., Sutton, G., Rusch, D. B., Halpern, A. L., Williamson, S. J., Remington, K., Eisen, J. A., Heidelberg, K. B., Manning, G., Li, W. Z., Jaroszewski, L., Cieplak, P., Miller, C. S., Li, H. Y., Mashiyama, S. T., Joachimiak, M. P., van Belle, C., Chandonia, J. M., Soergel, D. A., Zhai, Y. F., Natarajan, K., Lee, S., Raphael, B. J., Bafna, V., Friedman, R., Brenner, S. E., Godzik, A., Eisenberg, D., Dixon, J. E., Taylor, S. S., Strausberg, R. L., Frazier, M., & Venter, J. C. (2007). The Sorcerer II Global Ocean Sampling expedition: Expanding the universe of protein families. *Plos Biology*, 5, 432-466.

Yurkov, V. V. & Beatty, J. T. (1998). Aerobic anoxygenic phototrophic bacteria. *Microbiology and Molecular Biology Reviews*, 62, 695-724.

Zhang, C. L. L. (2002). Stable carbon isotopes of lipid biomarkers: analysis of metabolites and metabolic fates of environmental microorganisms. *Current Opinion in Biotechnology*, 13, 25-30.

Zhang, C. L. L., Li, Y. L., Wall, J. D., Larsen, L., Sassen, R., Huang, Y. S., Wang, Y., Peacock, A., White, D. C., Horita, J., & Cole, D. R. (2002). Lipid and carbon isotopic evidence of methane-oxidizing and sulfate-reducing bacteria in association with gas hydrates from the Gulf of Mexico. *Geology*, 30, 239-242.

Zhang, C. L., Pancost, R. D., Sassen, R., Qian, Y., & Macko, S. A. (2003). Archaeal lipid biomarkers and isotopic evidence of anaerobic methane oxidation associated with gas hydrates in the Gulf of Mexico. *Organic Geochemistry*, 34, 827-836.

Zhang, C. L., Fouke, B. W., Bonheyo, G. T., Peacock, A. D., White, D. C., Huang, Y. S., & Romanek, C. S. (2004). Lipid biomarkers and carbon-isotopes of modern travertine deposits (Yellowstone National P ark, USA): Implications for biogeochemical dynamics in hot-spring systems. *Geochimica Et Cosmochimica Acta*, 68, 3157-3169.

Zhang, Y. & Jiao, N. Z. (2004). Method for quantification of aerobic anoxygenic phototrophic bacteria. *Chinese Science Bulletin*, 49, 597-599.

Zhang, C. L., Pearson, A., Li, Y. L., Mills, G., & Wiegel, J. (2006). Thermophilic temperature optimum for crenarchaeol synthesis and its implication for archaeal evolution. *Applied and Environmental Microbiology*, 72, 4419-4422.

Zimmer, C. (2001). Biogeochemistry - 'Inconceivable' bugs eat methane on the ocean floor. *Science*, 293, 418-419.

INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z