Bioenergetics of Aquatic Animals

Albert Lucas

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Dedication

Albert Lucas died suddenly at his home in Brest shortly before the completion of the English version of this book. He had been not only an excellent teacher, but also a versatile and competent naturalist. An unselfish and wise man, he worked with several generations of students who are now involved with fundamental and applied fields of marine biology all over the world. This book will remain an enduring legacy to his students and all his friends.

Yves Le Gal
March, 1996
Deputy Director, Marine Biology Laboratory, College de France, Concarneau

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Translators' Note

This work has been translated in a way that is intended to retain, as far as possible, the accuracy, meaning and scientific content of all descriptions and explanations. Thus, where appropriate, slight adaptations of the original text have been made, in order to ensure that the English version reads as fluently as possible.

J.J.Watson
I.G.Priede

Preface

Since 1976, when I supervised the work of Meeransa Shafee (thesis obtained in 1980) and Peter Beninger (thesis obtained in 1982), my research activities have led me to reflect on the problems of bioenergetics. In addition, I was teaching, on the one hand, third-year students at the University of Western Brittany (UBO) from 1980 to 1988 and the University of Paris 6 from 1985 to 1988 and on the other hand, CINVESTAV students in Merida (Mexico) in 1987 and 1988. The reactions of the students allowed me to gradually improve my techniques of presenting bioenergetics.

Following these activities, I undertook to write a course which I envisaged would be published. The work was started in September 1990, after obtaining the agreement of the publishers, Masson, and the help of the Marine Biology Laboratory of UBO, and was completed in 1991. The first months of 1992 were devoted to editing and printing the definitive version.

My sincere thanks go to all the following people, for their help in achieving this book.

The Marine Biology Laboratory (UBO) and its director, M.Marcel Le Pennec, Mme Annie Corlay, Mlle Jocelyne L'Hostis and M.Alain Paimbeni for the illustrations; Alain Le Mercier, the Geography Laboratory (UBO) and its director, M.Jean-Claude Bodere; Mme Maryvonn Tosser and Mme Veronique Quere of the University Library (UBO). Also Peter Beninger (Moncton University, Canada), Jean-Pierre Bergeron (IFREMER-Nantes), Sigurd von Boletzky (CNRS, Banyuls), Mme Chantal Conand (UBO), Do Chi Tang (FAO, Rome), Jean Guillaume (INRA, St Pée/Nivelle), Maurice Heral (IFREMER, La Tremblade), S.J.Kaushik (INRA, St Pée/Nivelle), Yves Le Gal (Collège de France, Concarneau), Mlle Valérie Maxime (UBO), Jeanne Moal (IFREMER-Brest), Yvon Morizur (IFREMER-Brest), Mlle Michèle Regnault (CNRS, Roscoff).

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A.Lucas

Introduction

The meaning of the title of this work is probably self-evident, but it is perhaps useful to add some definitions and comments.

Bioenergetics This term is composed of two parts, 'bio' and 'energetics', which are examined in succession.

Bio is derived from the Greek word 'bios' meaning life. To define life, one can start by explaining what living organisms are. Living organisms are distinguished from inert matter by their structure and function. They possess an organization which has a hierarchical structure observed not only within a single cell (single-celled organisms) but also in groups of cells themselves organized in interdependent organs (multicellular organisms). This organization bestows on living organisms their capacity for assimilation and reproduction. Assimilation is a prerequisite for reproduction, therefore assimilation is the key characteristic of living organisms. This can be defined as the capacity of the organism to make its own substance, not only from inorganic matter (autotrophy) but also from organic matter (heterotrophy). When assimilation finally ceases, the death of the organism ensues. Thus, for the biologist, life is the state of an organism during active assimilation.

Energetics is the science that studies the different forms in which energy manifests itself; the term comes from the Greek 'energeia' and means force in action. This science originated in the 19th century as a result of practical problems posed by the invention of the steam engine. It was called, therefore, not energetics, but thermodynamics, because it was concerned with the conversion of heat to motion (Carnot, Joule, Kelvin, Clausius). If, originally, energetics had an applied character, it actually became one of the most theoretical of physical sciences (Prigogine, 1980 etc.). The branch of energetics concerned with living things came to be known as bioenergetics. We have seen how living things, whether autotrophic or heterotrophic, cannot subsist without resources obtained from their surroundings. Consequently, energetics can be defined as the quantification of the exchanges and transformations of energy and matter between living things and their environment.

Aquatic animals Animals are heterotrophic, multicellular organisms. In this way, they differ from other multicellular organisms such as green plants and from certain single-celled organisms such as the chemoautotrophic bacteria, which are capable of feeding themselves from inorganic matter (autotrophy). Another unique characteristic is the possession of well-developed functional systems (nerves and muscles) which enable locomotion in most animals, in other words the transformation of energy into movement. It can be seen, therefore, that animal bioenergetics is best characterized by comparison with plants and single-celled organisms.

The meaning given to 'aquatic animals' is highly variable, depending on the author. We intend to use the most restricted definition, because it is the only way to obtain a group of animals for which bioenergetics is homogeneous. Animals that live all their life, reproduce and respire in water will be considered as aquatic animals. This definition excludes marine mammals (cetaceans), birds and marine reptiles and the batrachians (frogs, toads and salamanders). Within our definition, therefore, are the fish (freshwater and marine), cyclostomes and a large number of invertebrates that are found mainly in the sea and sometimes in fresh water. Despite very different organizations, they are all integrally adapted to the aquatic medium.

Devotion of this study of bioenergetics to aquatic animals has not been a choice made by chance. There are two motivations. The first is practical: bioenergetics can provide answers to the problems of aquaculture in marine and fresh water. In the management of wild fish stocks, bioenergetics can provide quantitative data on production and allowable yields, for example when comparing different exploitation methods.

The second motivation concerns fundamental biology. Examination of animal energy balance helps us to understand the adaptations of different species leading to demographic equilibrium. It will be seen how, with aquatic animals, a large number of strategies have been realized, from the sessile microphage to the mobile predator.

1

Physical Concepts of Bioenergetics

> Nothing is created, neither in the operations of art, nor in those of nature and it can be proposed in principle, that in every operation, there is an equal quantity of matter before and after the operation/process, that the quality and quantity of the principles are the same and that there are only changes, modifications. (Lavoisier, 1792)

1.1 The basis of thermodynamics

Thermodynamics studies, not energy itself, but the exchanges of energy (and in certain cases the exchanges of matter) between a system and its environment. It is helpful to define these different terms.

1.1.1 Matter

Matter consists of elemental or particulate entities. According to Atkins (1987), thermodynamics is a 'soft physics', because it is not necessary to enter into the detail of the constituents of the atom. It will be considered that matter consists of atoms or molecules (groups of atoms) or of ions (atoms or molecules carrying an electric charge). These particles are very small, so that a small quantity of matter contains a vast number. Twelve grams of carbon contains 6.02×10^{23} atoms. This is Avogadro's number, which serves as the definition of the mole (Table 1.1).

Every particle possesses potential energy, derived from the interaction of its mass with surrounding gravitational fields and its electrical charge interacting with surrounding electromagnetic fields. Particles in motion also possess kinetic energy. The total energy of a particle is the sum of its potential energy and its kinetic energy. The energy of a quantity of matter is the sum of the total energies of all the particles of which it is made up, plus the energy of interactions between these particles. Each mole of chemical substance has a known energy which is designated its energetic value.

1.1.2 Exchanges of energy

Energy exchanges, whether exchanges of heat (measured by calorimetry) or other exchanges such as work are all measured in the same units. This can lead to confusion between the concepts of heat and work (Germain *et al.,* 1988).

Work is a system's organized exchange of energy with the exterior (Bok, 1989). Energy is transferred in the form of heat when the particles of the system move in a disorganized fashion. When work is supplied to a system, organized movement is communicated to its particles. Conversely, when a system supplies work, it tends to make the movement of the particles in its environment organized. An exchange of heat provokes disorganized movement of particles (Atkins, 1987).

1.1.3 Macroscopic systems

The laws of energetics were established for macroscopic systems. The following quotations provide some definitions.

> A system is defined as a collection of objects, inclined to interact with each other and with external objects, defined by a shared property, for example that of occupying a determined region in space. (Tonnelat, 1978)

> A macroscopic system…consists of a very large number N of particles or other kinds of subunits. We suppose that these particles consist of a portion of space separated from the exterior world by an imaginary geometric surface of the kind such that the numerical density, that is to say, N/volume, is finite. (Prigogine, 1972)

This last author gives examples: 1 cm^3 of gas at normal temperature and pressure would consist of 10^{20} molecules; the chromosome of a bacterium would be 10^6 molecules.

In this way, a macroscopic system (S), limited spatially, is surrounded by an

Table 1.1 Definition of the five basic SI units used in bioenergetics. The SI uses two other basic units: the intensity of electric current or ampere (A) and the intensity of light or candela (cd). All the other units (about 40) are derived from these seven units

A metre is the wavelength *in vacuo* of the orange radiation of the krypton-86 atom.
A kilogram is the mass of a platinum–iridium cylinder at BIPM, Paris. It is the only basic measure including a prefix (kilo), for historical reasons.
A second is the duration of 9 192 631 770 periods of the radiation corresponding to the transition between the two hyperfine levels of the ground state of the caesium-133 atom.
The kelvin is 1/273.16 of the thermodynamic temperature of the freezing point of water. The freezing point of water is 273.16 K, which corresponds to the Celsius temperature of 0.01 °C. Therefore degrees Celsius (t) can be translated into temperature in kelvin (T) as follows: $t = T - T_0$ or T_0 value + 273.15 K by definition.
A mole is the amount of substance that contains as many entities as there are atoms in 12 g of carbon 12. The entities must be specified: atoms, molecules, ions, electrons, other particles or groups of particles.

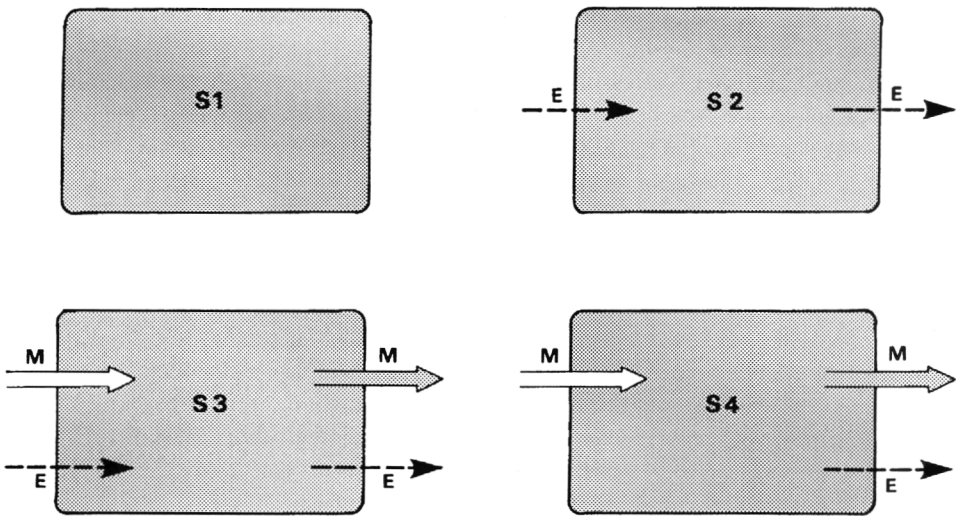


Figure 1.1 Representation of four macroscopic systems. The systems are shown in grey and their environment in white. E, energy exchanges; M, matter exchanges; S1, isolated system; S2, closed system; S3, open system using matter and energy (e.g. chlorophyllous organism); S4, open system using only matter (e.g. non-chlorophyllous organism).

'exterior world', again called environment (E). In the thermodynamic plan, the following cases can be distinguished.

- An *isolated system* exchanges neither matter nor energy with the environment. Such a model is purely conceptual but has theoretical applications.
- A *closed system* exchanges energy, but not matter, with the environment. For example, the world (ignoring meteorites received from space and satellites sent into space) is a closed system. A system that does not exchange heat is called adiabatic.
- An *open system* exchanges energy and matter with the environment—for example: a living organism. Different cases are presented in Figure 1.1. In general, for analysis, all systems can be divided into subsystems—for example, a biochemical reaction (closed system) within a living organism (open system).

1.2 First law of thermodynamics

1.2.1 The basic law

If the final state of a closed system is identical to the initial state after having exchanged heat and work with its environment, the process can be called a *cycle*. The first law of thermodynamics can be explained with reference to such a cycle.

In a closed system that has completed a cycle, the quantity of heat, *Q,* which it has given to the exterior (by convention -ve) is proportional to the work W received from the exterior (by convention +ve) or inversely. If W and Q are expressed in the same units, $W+Q=0$. The first law is commonly termed the principle of the conservation of energy. If an isolated

system or a closed system is considered as having completed a cycle, the quantity of energy remains constant. In other words, energy cannot be created or destroyed.

On the other hand, the expression $W+Q=0$ shows that heat and work are equivalent. The first principle is that of the equivalence of all forms of energy, for no matter what its form, energy is only exchanged through work or heat, which are equivalent.

1.2.2 Consequences of the first law

Hess's law

Let us assume that a closed system changes from an initial state A to a final state B, different from A. Two possible pathways from A to B and one pathway from B to A are considered, that is to say the return to the original state. From Figure 1.2, for the cycle: 1+3,

$$W_1+Q_1=-(W_3+Q_3)$$

and for the cycle: 2+3,

$$W_2+Q_2=-(W_3+Q_3)$$

therefore

$$W_1+Q_1=W_2+Q_2,$$

from which Hess's law is expressed: the global quantity of heat and work *(W+Q)* received or given by a closed system depends only on the initial state and the final state. In particular, in a chemical reaction, neither the speed of the reactions nor their number are taken into account.

Internal energy and enthalpy

Let U be the *internal energy* of a closed system in a given state. When, in the course of an acyclical transformation, the system passes from one state to another, the variations of internal energy provide quantities of exchanged heat and work:

$$\Delta U=\Delta W+\Delta Q.$$

When the system undergoing change is a chemical reaction, in most cases the only work provided is mechanical work against the exterior pressure. For a chemical reaction, therefore, two principal possibilities can be considered.

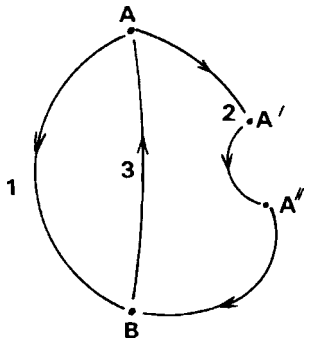


Figure 1.2 Illustration of Hess's law. (See text.)

If volume remains constant, as in the case of hermetically sealed calorimeters, no work is possible. In these conditions, the variation in internal energy during the course of the reaction is equal to the quantity of heat produced or received:

$$\Delta U=\Delta Q_v$$

where Q_v is the heat of reaction at constant volume.

By convention, a chemical reaction is:

- *exothermic* if $\Delta U<0$ (the system supplies heat to the exterior);
- *endothermic* if $\Delta U>0$ (the system receives heat from the exterior).

If pressure remains constant (for example at atmospheric pressure, where the reaction occurs in the atmosphere), the system will work against the external environment:

$$\Delta W=P\Delta V$$

where P is the external pressure andΔV is the variation in volume due to the chemical reaction. In this case:

$$\Delta U=\Delta Q_p-P\Delta V$$

therefore

$$\Delta U+P\Delta V=\Delta Q_p$$

where DQ_p is the heat of reaction at constant pressure. Because P is a constant, PDV is the change in the product PV, therefore:

$$\Delta(U+PV)=\Delta Q_p.$$

The magnitude of $U+PV$ designated by H is the *enthalpy* of the system. The change in enthalpy, $DH = DQ_p$, is the heat of the reaction, at constant pressure, of the chemical system considered.

For chemical reactions occurring between gases, the variations in volume are important, whereas for chemical reactions occurring between liquids or solids, volume changes are usually negligible. In consequence, in systems where only liquids and solids are involved, the change in enthalpy (ΔH) and the change in internal energy (ΔU) are approximately equal.

1.3 Second law of thermodynamics

1.3.1 The basic law

The second law (Atkins, 1984) gives direction to thermodynamic processes in time and forbids some processes which might otherwise be allowed under the first law. There are several equivalent ways of expressing the second law, from either the point of view of experimental observation or that of statistical thermodynamics.

Experimental observation

Historically, this approach was first used by Carnot, Kelvin and Clausius. From observations, two things can be noted.

1 It is impossible to achieve a transformation where the only result is the absorption of heat and its complete transformation into mechanical work. That is, no heat engine can be 100% efficient.
2 It is impossible to achieve a transformation where the only effect is the transfer of heat from a colder to a hotter body, i.e. a refrigerator can only remove heat at the expense of dissipation of heat elsewhere.

These two examples are evidence of thermodynamic asymmetry. In the first example, the asymmetry is in the conversion of heat to work, whereas in the second example it is evident that heat always flows spontaneously in the direction of decreasing temperature (Atkins, 1987).

To explain the second law in a single formula, the concept of *entropy* has been introduced. The second law can then be succinctly expressed as: for a closed system, entropy is either constant or increasing.

In the case of an isothermic reversible process, the change in entropy is defined as: change in entropy = (quantity of heat given to the system) × (absolute temperature of the system)$^{-1}$, i.e.

$$\Delta S=\Delta Q T^{-1}.$$

Where the system is heated, the amount of energy given to the system is positive and the change in entropy is positive. When the system gives heat to the environment, the amount of heat given to the system is negative and the change in entropy is negative. In the case of an irreversible process, entropy cannot increase, and therefore $\Delta S>0$.

Boltzmann's statistical interpretation

According to Boltzmann, the increase in entropy explains the increase in molecular disorder, measured in terms of probability, *P*. *P* (probability) and *S* (entropy) are linked by the formula:

$$S=\mathrm{k} \log P$$

where k is Boltzmann's constant: $k=1.3005\times10^{-23}$ J K^{-1}. The joule (J) is defined on page 14, and K (kelvin) is the unit of absolute temperature (Table 1.1).

1.3.2 Consequences of the law

Entropy and temperature

A result of the statistical definition of the second law is that at absolute zero, the entropy of a system is zero—for example, a crystal at 0 K (i.e. -273.16°C). In phenomenological thermodynamics, this rule is considered the third law of thermodynamics (Lehninger, 1978; Atkins, 1987).

Entropy increases in direct proportion to temperature; it is the same for molecular motion. At a given temperature, the entropy of a gas is higher than that of a liquid or a solid because, in the gaseous state, the molecular disorder is greater.

Free energy If a macroscopic system is considered at temperature *T,* the internal energy (free), called Helmholtz's free energy or the Helmholt function, is defined as follows:

$$F=U-TS$$

where U is internal energy, T is absolute temperature and S is entropy.

In transformations where the initial state and the final state are at the same temperature, the change ΔF in free energy can be described by the following equation:

$$\Delta F=\Delta U-T\Delta S.$$

At the same time, Gibbs free energy or free enthalpy G (also called the Gibbs function) is defined as follows:

$$G=H-TS$$

and the variation in free enthalpy

$$\Delta G=\Delta H-T\Delta S.$$

Because $H=U+PV$,

$$\Delta G=\Delta U+\Delta PV-T\Delta S.$$

If the reaction is isobaric,

$$\Delta G=\Delta U+P\Delta V-T\Delta S,$$

from which

$$\Delta U=\Delta G-P\Delta V+T\Delta S.$$

Thiss signifies that during a transformation in a closed isothermic and isobaric system, part of the internal energy remains convertible into work other than $P\Delta V$: that is the free enthalpy ΔG. In contrast, $T\Delta S$ is the energy dissipated in the form of heat.

In this way, the first law is followed exactly. Total energy (free energy + dissipated energy) remains constant, but the second law specifies that at each transformation, in an isolated or closed adiabatic system, part of the energy becomes unusable. In other words, at each transformation, there is quantitative constance (energy is conserved), but qualitative degradation (entropy increases).

Exergonic and endergonic processes

An isothermic and isobaric transformation that is accompanied by a decrease in free enthalpy is called *exergonic*. It can occur spontaneously (ΔG is negative). An isothermic transformation that is accompanied by an increase in free energy is called *endergonic* (ΔG is positive). It can occur only if associated with an exergonic reaction sufficient for total ΔG to be negative.

Variation in standard free enthalpy

In order to standardize the calculations of energy exchanges in the course of chemical reactions, a standard reference state has been arbitrarily chosen, which is described as follows:

- pressure of 1 atmosphere (101 325 Pa);

- temperature of 25°C;
- concentration of 1 mol dm^{-3} for solutions.

In this case, the standard variation of free enthalpy is denoted by $\Delta G°$. It is called ΔG' or $\Delta G°$' when the pH is also fixed at 7.

1.4 Present understanding of thermodynamics

1.4.1 Thermodynamics and open systems

All the examples used to demonstrate the first and second laws of thermodynamics are not only isolated but also closed systems. At this point we shall examine open systems and consequently living organisms. In the course of their ontogenetic development, living organisms acquire progressively more complex and organized structures and functions.

From this arises the question of whether open systems contravene the second law. The response of Prigogine (1972) is the following: 'the increase in entropy applies to the whole system, that is the living system and its environment…so the increase in entropy of the complete system is perfectly compatible with the decrease in entropy within the living system' and Atkins (1987) explains: 'order gushes out in one place because disorder localizes elsewhere'.

These answers, which reflect the general opinion, lead to the conclusion that the second law of thermodynamics does not apply to open systems. It can be applied, however, to the combined 'open system + environment'. In this way, the second law is true for the assembly of living organisms (biosphere) in association with their environment which, for the exchange of matter and energy, consists of the Sun, the Earth and the Moon. The assembly of the biosphere + Sun + Earth + Moon constitutes an isolated system in the thermodynamic sense. In this way, we cannot argue about an open system without integrating it within an isolated system, or ultimately, in a closed system accomplishing a cycle. But as stated by Prigogine (1972), 'this point of view does not tell us anything about the evolution of the living system itself'.

1.4.2 Dissipative structures and thermodynamics in general

In order to answer this last question, Prigogine and his collaborators developed generalized thermodynamics, which could be applied to open thermodynamic systems, receiving energy and matter from the outside world. If the external reserves of energy and matter are sufficiently large to remain in a permanent state, the open system may tend towards a constant state other than that of equilibrium. This is called the *stationary state of disequilibrium*. An open, macroscopic system, without equilibrium, is associated with *dissipative structures*.

A dissipative structure is one in which its formation results from dispersion. The classic example is that of *Bénard's instability*. This particularly simple example of hydrodynamics is described as follows. In the process described in Figure 1.3,

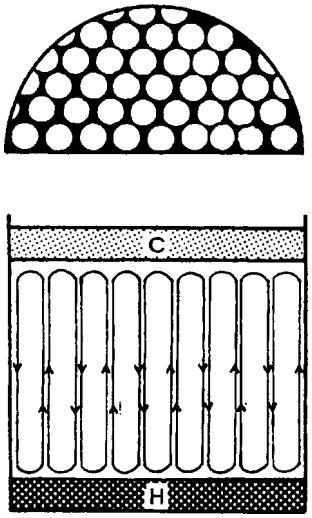


Figure 1.3 Device demonstrating Bénard's instability. Below: cross section of container. H, hot plate; C, cold plate. Convection currents within the liquid are indicated. Above: cross section of one-half of container seen from above, showing convection cells.

convection cells are formed in the liquid placed between a hot plate below and a cold plate above, when the difference in temperature between the two plates reaches a certain value. Below this value, the convection cells do not exist and the movements of the molecules are of a disorganized nature. The formation of convection cells is the establishment of a macroscopic structure not anticipated by Boltzmann's principle of order (Prigogine, 1972). Regarding these structures, Atkins (1987) makes two remarks. 'Firstly their appearance is accompanied by an increase in the speed of growth of the entropy of the Universe, because energy transfers more rapidly from the hot plate to the cold plate when it occurs in an organized way. Secondly, the most rapid increase in energy is accompanied by the formation of a structure…it is the transfer of energy which creates cellular structures; when the transfer ceases, the structures disappear.'

In this way, structures of this type are created and maintained thanks to exchanges of energy with the exterior world, in conditions of non-equilibrium. 'Boltzmann's principle of order, which gives a good description of states of equilibrium, is no longer applicable. The dissipative structures are associated with a completely different principle of order, which might be called order by fluctuation' (Prigogine, 1972).

Thus, when biological reactions are close to thermodynamic equilibrium, Boltzmann's principle of order dominates; in contrast, when they are far from thermodynamic equilibrium, chemical reactions comprising non-linear stages develop, giving rise to organized structures in time or space. Prigogine (1972) gives some examples.

- The enzymatic reaction of glycolysis consists of oscillations of constant period and amplitude starting from a stationary state of non-equilibrium. It therefore constitutes a temporal, dissipative structure. In the membranes of nerve cells, the state of polarization

(maintenance of ionic charges on each side) is a state 'without equilibrium', from which instability gives rise to a state of cyclical depolarization.
- In the same way, in the central nervous system, high-frequency rhythms can be analysed in terms of temporal dissipative structures.
- In certain amoebae (e.g. *Dictyostelium discoideum*), alternation of separate amoebae and aggregated amoebae in a multicellular body under the influence of cAMP, might be interpreted as a spatial dissipative structure.
- Interpretation of the phases of development of multicellular organisms could be related to spatial dissipative structures (localizations of cellular differentiations) and temporal dissipative structures (successions of organogenesis).

1.5 Application of thermodynamics to living organisms

The above references show the characteristics of living organisms, open systems in a state of non-equilibrium. The examples given explain particular situations but the explanation remains qualitative. There are still no quantitative laws for the analysis of exchanges of matter and energy which occur between living organisms and their environment.

In these conditions, it would not be surprising if the formulae and rules of bioenergetics depended entirely on classical thermodynamics. It is obvious that it is only a provisional arena, manifestly imperfect, which will one day be overtaken when usable formulae, from measurable data, will be proposed to bioenergeticians. In reality, in the absence of a suitable alternative, bioenergetics uses a pragmatic set of rules which are explained hereafter.

1.5.1 Application of Hess's law

Hess's law stipulates that only the difference between the initial and final states of a chemical reaction needs to be measured, regardless of which method is used. This allows the quantification of enzymatic reactions of living organisms by comparing them with combustion carried out, for example, in calorimeters. It follows that all combustible matter (essentially organic matter) can be quantified in a unit of energy. For example, it is accepted that the amount of energy obtained from the enzymatic breakdown of a mole of glucose into water and carbon dioxide is equal to the amount of energy obtained from the combustion of a mole of glucose in a calorimeter, which measures the amount of heat given out and therefore the amount of energy given out.

1.5.2 Variations in enthalpy

The second methodological comment concerns the conflict between the variation in internal energy (ΔU) and the variation in enthalpy (ΔH). In living organisms, most chemical reactions occur in the liquid phase. There is, therefore, during the course of a reaction, no notable difference in the volumes. In consequence ΔH and ΔU may be confused. To extend this observation, Helmholtz's variation in free energy (ΔF) may be confused with Gibbs free energy or change in free enthalpy (ΔG).

Energetic studies of chemical reactions require the calculation of change in standard free enthalpy $\Delta G°$. This relates in turn to the quantitative relationship which exists between the change in free enthalpy and the equilibrium constant,

$$\Delta G° = -RT \ln k_{eq}$$

where: R is the universal molar gas constant: 8.314 $Jmol^{-1}$ K^{-1}; T is the absolute temperature expressed in kelvin (K); the Naperian logarithm (natural log) can be transformed into decimal log ($\log_{10}$): In x=2.3 log x; and k_{eq} is the equilibrium constant of the reaction under consideration, which is:

$$A+B \Leftrightarrow C+D.$$

It can be deduced that $k_{eq}=[C]\,[D]/[A]\,[B]$ where [X] denotes the molar concentration of X. In practice, only reactions where ΔG is between +12.5 and -12.5 kJ mol^{-1} are reversible.

1.5.3 The entropy of living organisms

In studies of thermodynamics, not only energy (or power) is calculated, but also the entropy produced by the systems being studied. This is not the case in bioenergetics, because living organisms do not lend themselves to this type of calculation. In effect, as Brillouin (1959) wrote, 'to calculate the entropy of a system, it must be possible to create or destroy it in a reversible way. We cannot imagine a single reversible process by which a living organism could be created or killed; both birth and death are irreversible processes.'

This opposition between inert and living systems brings us to a better definition of living organisms. To the classical characteristics of the capacity for assimilation and reproduction, a certain number of others must be added.

- Each living organism, at the moment of its formation, receives genetic information consisting of a programme and the regulation of this programme. The progress of this programme translates, in the course of ontogenesis, into a capacity for self-organization (Atlan, 1985) which characterizes living organisms.
- Coupling and co-ordination exist at the cellular level (for example: biochemical coupling) and between one organ and another (remote coupling) and there is therefore regulation of function at all levels of organization (see for example, enzymatic regulation, section 2.1.2).
- From this results a structure and function of enormous complexity, the existence of which, in terms of probability, is highly improbable.

These characteristics are never found in inert matter; they are therefore unique to living organisms.

If the entropy of a system is described as having the outcome either of *maximum disorder* or of the *greatest probability,* note that the living organism is, on the one hand, opposed to entropy by its functional and structural complexity, because it does not evolve towards maximum disorder but towards biological order; on the other hand, the existence of these highly improbable structures is the result of evolution contrary to probability.

From where does this opposition to entropy come? Brillouin (1959), invoking information theory, considers that all information is a source of *negative entropy*. In this way, the living organism begins its development with a certain amount of negative entropy, which it utilizes using the genetic code. On the other hand, through the course of its existence, the living organism minimizes its production of entropy. In effect, Brillouin (1959, p. 103) writes, 'Prigogine indicated the following general principle: an open and stable system, maintained by irreversible processes, assumes a structure which corresponds to a minimum increase in entropy. This important point was rediscovered and discussed by M.Biot. This applies to living organisms.' This is also the opinion of Pascaud (1989), who writes: 'Some theoretical considerations (L.Onsager, I.Prigogine) show that the production of entropy by an irreversible thermodynamic system is minimum in the frame of a stationary state of flux of matter and energy. It is precisely the solution of renewal adopted by the living organism, an open system in stationary equilibrium of flux.'

In this way, the example of Bénard's instability (section 1.4.2), where the existence of 'structures' corresponds to an increase in entropy, would be viable for inert systems, but not for living systems.

In general, the conclusions shown are accepted by a large proportion of the scientific community. Nevertheless, the disagreement of Tonnelat (1978) must be voiced, on the concepts shown above, in particular his refusal to accept the relationship between information and negative entropy, as is evident from the following sentence (p. 202): 'In the state of our knowledge, recourse to information theory to evaluate the degree of organization of a biological system has instead turned attention, away from fundamental phenomena which are the origin of this organization, to abstract speculations.'

1.6 Physical measurements used in bioenergetics

1.6.1 Physical measurements and their units

Basic measurements

In the Système International d'Unités (SI), for measuring energy and its variations, it is necessary first to define five basic units:

- length measured in metres (m);
- mass measured in kilograms (kg);
- time measured in seconds (s);
- temperature measured in kelvin (K);
- quantity of a substance measured in moles (mol).

These units are defined in Table 1.1.

Derived measurements

Derived measurements that allow the definition of energy are:

- speed: m s^{-1};
- acceleration: m s^{-2};
- force, in newtons (N): m s^{-2}kg;

from which, energy in joules (J)=m^2 s^{-2} kg. The *joule* is the amount of work done when a force of one newton advances its point of application 1 metre in the direction of the force.

In scientific publications, it is recommended (or demanded) that SI units be used. Nevertheless, another unit of measure of energy is frequently encountered and defined experimentally, the calorie. The *calorie* (cal) is the amount of energy needed to raise the temperature of 1 g of water from 14.5°C to 15.5°C. The calorie is a certain amount of heat, therefore the joule is a certain amount of work. It has been established that 1 cal=4.186 J and that 1 J=0.238 cal. In the remainder of this work, only SI units are used and therefore all the energy quantities are expressed in joules.

Other measures, apart from energy, may also be used in bioenergetics.

- Variations in energy or enthalpy, such as ΔH, ΔG, ΔU are expressed in energy per mole: J mol^{-1}, which becomes m^2 s^{-2} kg mol^{-1}.
- Entropy S is expressed in energy per kelvin: J K^{-1}, which becomes m^2 s^{-2} kg K^{-1}.
- Variations in entropy ?S are expressed in energy per kelvin per mole: J $(K\ mol)^{-1}$, which becomes m^2 s^{-2} kg K^{-1} mol^{-1}.
- In exchanges of energy, time inevitably intervenes, leading to the notion of power. The unit of power is the *watt* (W), which corresponds to 1 joule per second: W=J s^{-1}, which becomes m^2 s^{-3} kg.
- When measuring respiration it is necessary to use pressure, which is measured in pascals: Pa=N m^{-2}=m^{-1} s^{-2} kg.

Multiples and submultiples

In working with measured values, multiples or submultiples of units of measure are used. These are formed by placing a prefix before the name of the unit. Table 1.2 gives the prefixes used in SI.

The multiples and submultiples of the kilogram and the metre and, it follows, the units of surface (m^2) and volume (m^3) are commonly used in biology. Nevertheless, the usage of the litre (L or l), another unit of volume, is strongly discouraged by the SI because of its definition: a litre is the volume occupied by 1 kg of water at its maximum density which corresponds to 0.99997 dm^3. The equation 1 L=1 dm^3 is therefore only an approximation because of the experimental definition of the litre.

The submultiples of the second are utilized according to the nomenclature of

Table 1.2 SI prefixes showing multiples and submultiples of units of measure on the left and right respectively

Factor	Prefix	Symbol	Factor	Prefix	Symbol
10^{18}	exa	E	10^{-1}	deci	d
10^{15}	peta	P	10^{-2}	centi	c
10^{12}	tera	T	10^{-3}	milli	m
10^{9}	giga	G	10^{-6}	micro	μ
10^{6}	mega	M	10^{-9}	nano	n
10^{3}	kilo	k	10^{-12}	pico	p
10^{2}	hecto	h	10^{-15}	femto	f
10^{1}	deca	da	10^{-18}	atto	a

Table 1.2. Nevertheless, for the multiples, the SI allows the minute (min) of 60 s, the hour (h), of 3600 s and the day (d), of 86 400 s.

Notes on nomenclature In general, biologists are in the habit of using the term *weight* when they wish to express *mass*. In actual fact, these 'weights' are expressed in kg (or multiples), therefore the weight is really a force: mass × g (gravity) which must therefore be expressed in newtons. As we know, gravity varies geographically. For a given mass, the weight is different from one latitude to another. Nevertheless, if, by convention, it is estimated that this difference is negligible, g becomes a constant. In this way, the value of a weight is very close to that of its corresponding mass. This shows that all the weights taken at the surface of the globe can be used as values of mass, without applying corrections. But this does not permit the substitution of the term weight for that of mass.

It has become general practice to use condensed expressions for weights. In this way, live weight, dry weight, and eviscerated weight indicate respectively the weight of the live animal, the weight of dry matter and the weight of the eviscerated animal. This method of expression is universally understood and used (dry weight = poids sec = peso seco). It can also be applied to mass. When mass applies to a whole organism which was living before its analysis, the tendency is to use the term biomass (B). On the contrary, if the mass applies to a chemical substance or a part of an organism, tissue or organ, the term mass *(M)* is retained (Table 1.3).

1.6.2 Transformations of physical measurements

Relative values

Relative values are the values obtained when evaluating a relationship between two numerical data sets. Whereas absolute values are unique to a given species, relative values acquire a more general character and allow direct comparisons between species. For example, the proportion of lipids in organic matter of different species of fish allows their division into lean fish and fatty fish.

The relationships used in biology belong to two mathematical categories. In the first case, the values of the numerator *(n)* and the denominator *(d)* belong to the same group. In the second case, *(n)* and *(d)* belong to two different groups.

Homogeneous relationships The values *(n)* and *(d)* must on the one hand be expressed in the same units and on the other hand be linked by a relationship. The

Table 1.3 Correct expressions of mass and abbreviations. (Bio)mass means 'mass or biomass'

Incorrect expressions	Correct expressions	Abbreviations
Dry weight	Dry (bio)mass	M_s or B_s
Ash-free dry weight	Ash-free dry (bio)mass	OM or OB
	or (bio)mass of organic matter	OM or OB
Live or wet weight	Live (bio)mass	M_v or B_v
Eviscerated weight	Eviscerated biomass	B_e

result of the relationship is therefore a quotient q. For example, when n is the mass of lipids (g) and d is the mass of organic matter (g), the quotient can be calculated:

n (in g)/d (in g)=q.

The quotient q can be expressed in written decimals or a decimal fraction, for example as a percentage (%).

The real relationship between lipid and organic matter is characterized by the fact that lipids are a part of organic matter. The theoretical limits of such a relationship are:

$n_{max}=d$ (all the organic matter is made of lipids) - in this case q=1; and
$n_{min}=0$ (the organic matter contains no lipids) - in this case q=0.

The values of q vary from 0 to 1 or from 0% to 100% and in this way 100% clearly expresses the maximum value that can be reached by the quotient.

Heterogeneous relationships The values *(n)* and *(d)* belong to two different groups if they are expressed in different units, for example, x and y. In this case the result of the relationship is not a quotient but a constant expressed as a function of these two units:

$n(\mathrm{x})/d(\mathrm{y})=a(\mathrm{x}, \mathrm{y}^{-1})$.

To omit these units constitutes a serious error, nevertheless frequently found in the biological literature. The values of *(n)* and *(d)* expressed in the same units belong to two different groups if no relationship exists between them, for example, when n is the mass of mineral material (g) and d is the mass of organic matter (g). The values of *(n)* and *(d)* are totally independent. The result of the relationship will be a number varying between extremely variable limits, of which the significance is purely empirical. If the result is expressed as a percentage, the value 100% is not necessarily a maximum.

Types of relationships Relative values can be divided into three: rate, index and efficiency.

The rate is the relationship between two numerical values indicating a gain, as a function of time. According to the units chosen, hourly, daily and annual rates are used, e.g. rate of growth of an organism. This is defined as the relationship between the growth in biomass *(B)* between times t_1 and t_2, and the biomass.

$$T_G = \frac{(B(t_2) - B(t_1))}{B(t_1)} = \frac{\Delta B(t_2 - t_1)}{B(t_1)}.$$

This rate is often expressed as a percentage, although it can exceed 100% (notably in young organisms) when the chosen unit of time is long. The rate can also be zero and even negative (loss of weight, for example).

Alternatively, the rate of growth can be calculated as:

$$V_G = \frac{\Delta B(t_2 - t_1)}{\Delta t}$$

where Δt is the chosen unit of time.

The index is the relationship between two numerical values indicating a state. It is established from two accurate and simultaneous measurements, e.g. condition factors. The correct indices are those where the two data sets are expressed in the same units. These indices are therefore without dimension and independent of time (section 2.4.1).

The efficiency is the relationship between two numerical values indicating an efficacy, for example the ratio of energy input to energy output. Efficiency can only be measured from data obtained over a certain period. If care is taken that the values of *(d)* and *(n)* are measured over the same period, in the same units, the relationship *n/d* is itself also a dimensionless number, e.g. energetic efficiencies (section 3.3.3).

Equivalents

Energetic values of organic matter In exchanges of matter between an animal and its environment, only organic matter is taken into account. To discover the energetic value of an organic substance, the evaluation can be direct or indirect.

Direct measure: this experimental measure is long and delicate, and is achieved by measuring the heat of combustion (enthalpy) of a given amount of the substance (1 g of dry matter for example) in a calorimeter.

Use of 'energetic equivalents': in this case, known energetic values for carbohydrates, proteins and lipids are used, provided that the elementary chemical composition of the substance being studied is known (respective percentages of carbohydrates, proteins and lipids).

These equivalents are as follows:

- for carbohydrates: 1 g=17.2 kJ according to all authors;
- for proteins: 1 g=23.9 kJ according to Ansell (1974);
  1 g=23.4 kJ according to Cho *et al.* (1982).

These equivalents have been calculated for aquatic animals: molluscs (Ansell) and fish (Cho *et al.*). Note that the value of conversion of a gram of protein is higher in aquatic animals than in terrestrial animals, because the cost of detoxification is lower as a result of the excretion of toxic ammonia into the water (Tacon and Cowey, 1985).

- for lipids: 1 g=39.8 kJ according to Ansell (1974);
  1 g=35.5 kJ according to Craig *et al.* (1978);
  1 g=33.0 kJ according to Beninger and Lucas (1984).

  The differences are due to the fact that Ansell analysed only neutral lipids while the other authors achieved more complete extractions of lipids which contained,aswell as neutral lipids, less calorific polar lipids. The equivalent is chosendepending on the type of extraction used.

If only the lipid content of a substance is known, a global equivalent of 20 kJ for 1 g can be adopted for the proteins and carbohydrates. Finally, there is the case where no biochemical analysis is done, but the global composition is known. For example, in the molluscs, the 'flesh' contains in the order of 20% lipids. In this case, the energetic value of 1 g of this tissue can be evaluated by adopting the equivalent of 20 kJ for 80% and the equivalent of 33 kJ for 20%. This gives 22.5 kJ for 1 g of flesh.

Oxycalorific coefficient Amongst the energy and matter exchanges between animals and their environment, there are respiratory exchanges. These involve oxygen

Table 1.4 Oxycalorific coefficients of different foods (see text)

Food	kJ $dm^{-3} O_2$	kJ $g^{-1} O_2$
Carbohydrates	21.1	14.8
Lipids	19.6	13.7
Proteins 1	19.1	13.4
Proteins 2	19.4	13.6
Herbivorous diet	20.4	14.3
Carnivorous diet	19.4	13.6

consumption and the expulsion of carbon dioxide and water. The expulsion of water cannot be measured (particularly in aquatic animals) and measurement of expelled carbon dioxide is difficult because of the solubility of carbon dioxide in water. It is for this reason that respiratory activity, which involves the degradation of organic matter, is most often measured by the consumption of oxygen.

In order to express a quantity of consumed oxygen in energetic units the *oxycalorific coefficient,* or heat coefficient of oxygen (Q_{ox}), can be used. This coefficient depends on the nature of the food used during respiration and, for proteins, it depends on the excreted products: ammonia (proteins 1), urea or uric acid (proteins 2). Table 1.4 gives the mean values given by Elliott and Davison (1975), Brafield and Llewellyn (1982) and Crisp (1984).

1.7 Comments and clarification

To clarify the physical basis of bioenergetics, a reminder of certain definitions is given as follows.

Energy is not created, it is transformed

When an aquaculturist has obtained a fish from a fertilized egg, he has obtained a potential energy greater than that he had originally, but he has not created energy, he has only transferred the chemical energy of the food to the fish. This idea of transformation or exchange of energy must be remembered when certain terms are loosely used. In this way, 'energetic production' means production by transfer of energy, and 'energetic budget' means balance of energy exchanges.

By referring to the quotation at the beginning of this chapter, we can say for energy what Lavoisier said for mass: energy cannot be created or lost, it can only be transformed or exchanged.

'The true nature of heat and work'

We borrow this quotation from Atkins (1987), who writes:

> At the start of the 19th century, it was thought that heat was an object, a fluid called "caloric". But today we know that this idea was false…on the contrary the two terms, heat and work designate methods of transfer of energy. It is a formidable intellectual leap, to have discovered

> that heat is not a form of energy: it is a particular method for transferring energy. This is equally true of work: to do work is to modify the energy of an object without bringing about differences in temperature.

In this way, the aquaculturist who has raised a fish has effected a transfer of energy in the form of chemical work. In effect, as we will see in the second chapter, enzymatic reactions of living organisms are isothermic, which is why Lehninger speaks of chemical work for biosynthesis.

If there are only two ways of exchanging energy (heat, work), there are many possible sources of energy, in other words, many forms of energy which are all equivalent, according to the first principle of thermodynamics.

The term 'exchange' implies the concept of time

The results of an exchange depend not only on the intensity of the exchange, but also its duration. Because the duration of the exchange must be taken into account, it must be expressed in joules per unit of time, which in SI units (section 1.6.1) corresponds to the watt. It would therefore be convenient to express the energy exchanges of living organisms in watts (or multiples), as is done for engines or power stations, for example. But engines and power stations are studied by physicists, accustomed to physical units, whereas living organisms are studied by biologists who prefer to measure energy in calories and time in hours, days or years: there is no question under these conditions of encountering the watt. We will return ultimately to these problems when considering energy balance of organisms and populations. For the immediate purpose, it is not necessary for biologists to imitate physicists on every point. Even physicists, or at least certain amongst them, have invented and used the kWh (kilowatt hour) which is a totally aberrant measure for expressing a quantity of energy. The kWh is only a joule twice transformed, the first time by division by time (seconds; section 1.6.1) and the second time by multiplication by time (hours). In this way, 1 kWh=3.6 MJ (megajoule, Table 1.2). When it is finally agreed that an amount of energy will always be expressed in joules (or multiples) and power in watts (or multiples), the expression of energetics will be much simpler.

Living organisms are open macroscopic systems

This implies that they exchange not only energy, but also matter, with their environment (section 1.1.3). The object of the exchanges is therefore the amount of 'matter and/or energy' which we will call 'matter-energy' for simplification. In heterotrophic organisms, only matter is taken from the environment (Figure 1.1) and matter and energy are returned to the environment (by the two methods of exchange, work and heat). In order for life to continue, these exchanges must be unceasing; they can therefore be characterized as fluxes. In this way, the state of a living organism is maintained by a flux of matter-energy exchanged with the environment. In fact the living organism does not exist by itself, it only exists as part of its environment. It is the same for its ontogeny. According to Atlan (1985), 'the execution of the genetic programme implies unending interactions not only between the genes and the rest of the cell but also between itself and the *environment*. This is also called *epigenetic development*.'

2

Cellular Bioenergetics

> Not only is the cell the basic unit of all living organisms, it is also a unit of convenient structure and function for observing the mechanism of conversions of energy in biology. (Lehninger, 1965)

The study of energetics at the cellular level involves characterizing cellular work, through analysis of the means, the necessary conditions and forms of this work together with methods of quantifying it.

The means for cellular work are provided by the activity of enzymes, catalysts unique to living organisms and the energetic coupling of biochemical reactions. The conditions necessary for this work are the existence of sources of matter and energy which together assure the life of the cell. The sources of matter are represented by the organic compounds derived from the digestion of macro-molecules. Ultimately, the sources of energy in living organisms are either Hill's reaction (photolysis of water using energy from photons captured within the chloroplasts; i.e. the light phase of photosynthesis), or the oxidation of mineral substances (for example the oxidation of H_2S carried out by sulphur-oxidizing bacteria), or the degradation of organic matter in the presence (respiration) or absence (fermentation) of oxygen. As our study is limited to animals, only the last two cases will be examined. The different forms of cellular work are transport and concentration, chemical work or biosynthesis and mechanical work. We will ignore the minor forms such as the production of light or electricity. The measurement of cellular work cannot be carried out directly and consequently indirect tools that are characteristic indices of the metabolic activity of the cell are used.

2.1 The means of cellular work

As defined by Audigié and Zonszain (1988) 'organisms do not use heat; chemical energy represents the pivot of energetic transformations for living material'. In this context, the means of cellular work are on the one hand the existence of enzymes and on the other hand the generality of energetic couplings in chemical reactions.

2.1.1 Characteristics of enzymes

Properties of a catalyst

An *enzyme* is a biocatalyst, that is to say a biological catalyst which possesses firstly the general properties of a catalyst (Begue and Jayle, 1975):

- it decreases the energy necessary for the activation of a reaction;
- it therefore facilitates the reaction in a way that is thermodynamically possible, that is to say in a way which is exergonic;
- it accelerates the reaction in both directions when it is reversible;
- it does not modify the equilibrium of the reaction;
- it is intact at the end of the reaction.

An enzyme also possesses particular properties linked to its nature as a biocatalyst, which are as follows.

Enzyme kinetics

An enzymatic reaction is characterized by the fact that an enzyme-substrate complex is formed before the formation of the product (Figure 2.1). S is the substrate in its initial chemical composition, E is the enzyme and P is the product in its composition. The returning arrow indicates that the enzyme is restored intact after the reaction. The speed of the reaction is directly proportional to the concentration of ES, the enzyme-substrate complex. But this concentration depends on the product of the concentrations of E and S. Therefore, for a constant concentration of enzyme, the speed of the reaction will increase as a function of the concentration of the substrate. But this increase is subject to a saturation effect: above a certain concentration of substrate the speed will not increase any more; this is called V_{max} (Figure 2.2).

Michaelis' constant, K_M, which is defined as follows (Figure 2.1): $K_M=(k_2+k_3)/ k_1$, corresponds to the concentration of substrate for which the enzyme is semisaturated and therefore for which the speed of reaction observed $V=V_{max}/2$ (Figure 2.2).

K_M is a constant characteristic of an enzyme, and together with V_{max} determines the speed of the reaction. It also allows the evaluation of the affinity of the enzyme for the substrate. In effect, the lower the concentration of substrate required to reach $V_{max}/2$, the greater the affinity of the enzyme for its substrate.

In living organisms, the concentration of the substrate is generally in the order of K_M. Saturation of the substrate and the attainment of V_{max} are rare.

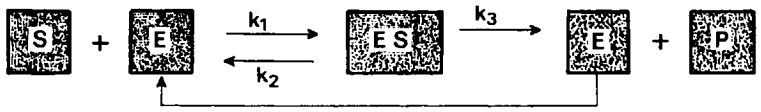


Figure 2.1 Diagram of an enzymatic reaction; k_1, k_2 and k_3 are the speeds of the different reactions. E, enzyme; S, substrate; P, product.

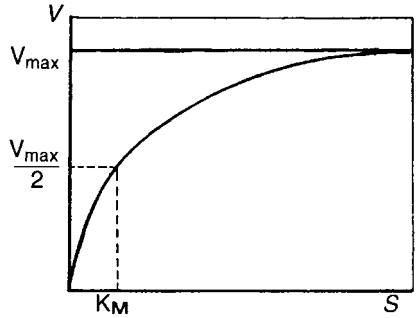


Figure 2.2 Definition of V_{max} and K_M in relation to speed of reaction *V* and substrate concentration (S).

Functional structure of enzymes

Enzymes are proteins with high molecular weights and complex structure, characterized by a precise geometry in which active sites are found, complementary to the substrates by their form and electronic structure. This last property allows repulsive van der Waals forces to be overcome. The shape allows stereospecific positioning of the substrate on the enzyme as shown in Figure 2.3.

Figure 2.3 gives a static view of enzyme function. From the information contained in enzymes, their function is in reality a dynamic process due, on the one hand, to the flexibility of the structure of the enzyme compared with the substrate and, on the other hand, to the phenomenon of cooperation between nucleophilic and electrophilic catalysis (*push-pull* phenomenon).

As for all proteins, enzymes are sensitive to pH (Figure 2.4) and temperature. Temperature gives rise to two phenomena: the activation of the reaction and the denaturing of the enzyme, both of which are increased with temperature (Figure 2.5). Therefore for each enzyme an optimal pH and an optimal temperature are defined, at which catalysis is most productive.

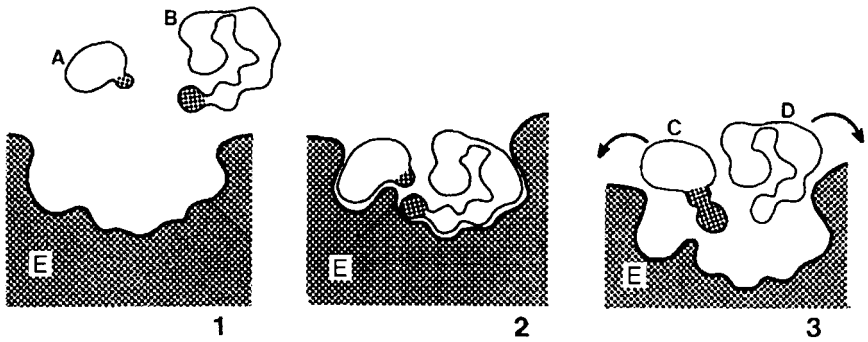


Figure 2.3 The role of hexokinase (E) In the formation of glucose-6-phosphate from ATP (B) and D-glucose (A). Part of the surface of the enzyme (E) shown in grey is the active site which allows the correct connection of the two molecules which must react. In (3), the two products, ADP (D) and glucose-6-phosphate (C), which have no affinity for the enzyme, leave the enzyme surface, which thus becomes ready for another reaction (after Théret *et al.,* 1983).

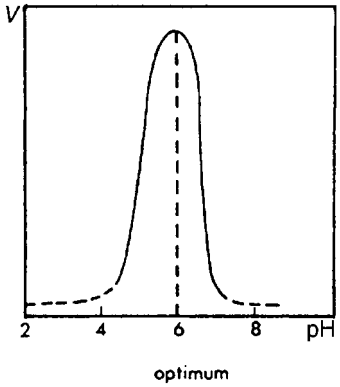


Figure 2.4 Variations in speed of enzymatic reaction as a function of pH: example shown is that of urease (after Weil, 1989).

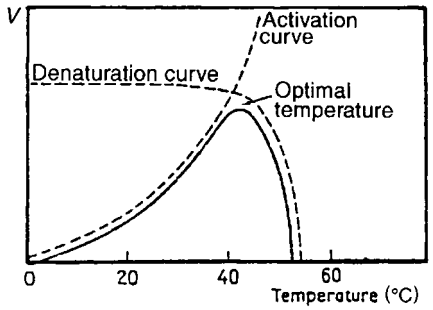


Figure 2.5 Influence of temperature on the rate of an enzymatic reaction (after Weil, 1989).

2.1.2 Enzymatic catalysis

Efficiency of enzymatic catalysis

The following example is borrowed from Bègue and Jayle (1975). A catalyst brings molecules into contact, orientated so that they must react so well that it is no longer necessary to increase the statistical probability of molecular collisions by heating the reactants. Thus, the decomposition of hydrogen peroxide:

$$H_2O_2 \text{ ----> } H_2O + \tfrac{1}{2}O_2$$

is slow at ambient temperature, because it requires 75 kJ to initiate the reaction per mole of hydrogen peroxide (34 g). If a primary catalyst of platinum sponge is provided, the activation energy, or amount of heat required, is no more than 50 kJ. If catalysis is achieved by an enzyme, catalase, the amount of heat required is no more than 8 kJ. This amount of heat is readily absorbed from the surrounding environment and the reaction is spontaneous. When a little hydrogen peroxide is placed on a wound, catalase is released from the damaged tissues: the formation of an abundant froth is observed which demonstrates the intense release of oxygen.

Using Arrhenius' formula, it can be calculated that the speed of decomposition of hydrogen peroxide is multiplied by 24 500 in the case of catalysis by platinum sponge and by 527×10^9 in the case of catalysis by catalase. This example demonstrates in a spectacular way the power of enzymatic catalysis. Figure 2.6 explains the mechanism of decrease in activation energy.

Regulation of enzymatic catalysis

Enzymatic regulation is one of the fundamental mechanisms of the *homeostatic regulation* of the internal environment, a concept put forward at the end of the 19th century by Claude Bernard (Rodwell, 1989).

Two main types of mechanism are put into play to regulate enzymatic catalysis, either modification of the concentration of enzyme or modification of the catalytic efficiency of the enzyme.

Regulation by modification of enzyme concentration The concentration of an enzyme is one of the factors influencing enzyme kinetics (section 2.1.1): the higher the concentration of an enzyme, the faster the speed of the reaction. It must be made clear that the concentration must always be referred to a limited space, because in the organism and in the cell, partitioning is very defined. Thus, in the cell there is the cytosolic compartment and the compartments of each cellular organelle, for example the mitochondrial compartment. All these compartments are limited by membranes which do not allow the passage of enzymes or newly formed metabolites. In order that the latter may pass from one compartment to another, they have to acquire a form permeable through the compartmental barrier (Rodwell, 1989).

The absolute quantity of an enzyme present in a cellular compartment is determined by its speed of synthesis k_s and its speed of degradation k_{deg}; in other words, the combination of k_s and k_{deg} determines the rate of *enzyme turnover*.

The biosynthesis of enzymes results from the information contained in DNA and transferred by mRNA. This biosynthesis can be blocked by the presence of certain metabolites, or activated by certain hormones.

Allosteric enzymes The kinetics of allosteric enzymes can generally be modified reversibly by substances with characteristics very different from those of the substrates. These natural products can act as inhibitors or activators. In a metabolic

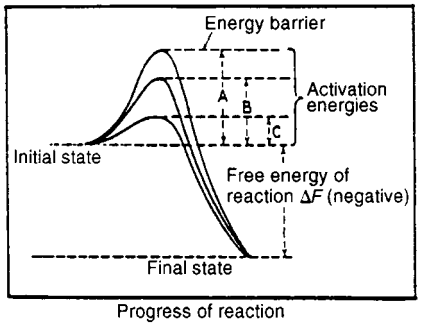


Figure 2.6 Activation energy required to trigger an exergonic reaction, in the absence of a catalyst (A), in the presence of a chemical catalyst (B) and in the presence of an appropriate enzyme(C) (after Weil, 1989).

pathway involving several enzymes, the end product is often an inhibitor of the first enzyme in the reaction chain.

The kinetics of allosteric enzymes are sigmoidal in nature. Figure 2.7 shows how these kinetics are modified by an inhibitor or an activator.

Regulation by metallic ions Metallic ions can play a regulatory role on more than a quarter of known enzymes. When the masses of substrate and metallic ion are nearly equal, the activity of the enzyme is maximal. An excess of metal or of substrate is inhibiting. The calcium ion is frequently used, not in its free form, but associated with specific proteins, notably calmodulin. The complex Ca^{2+} -calmodulin is involved in numerous cellular phenomena.

Regulation by phosphorylation Many enzymes exist in phosphorylated and non-phosphorylated forms. Phosphorylation is carried out by ATP:

enzyme+ATP—>enzyme-P+ADP.

Dephosphorylation is catalysed by a phosphoprotein, phosphatase:

enzyme-P+H_2O—>enzyme+P.

Enzymes that carry out phosphorylation are protein-kinases, which comprise a catalytic subunit, C, and a regulatory subunit, R, which fixes cyclic AMP. The fixation of cAMP detaches R from C which therefore becomes active:

RC (non-active)+cAMP—>R-cAMP+C (active).

cAMP is produced from the action of a membrane enzyme, adenylcyclase, on ATP:

ATP—->cAMP+pyrophosphate.

Adenylcyclase is normally inactive but can be activated by various peptide hormones (Kruh, 1989).

2.1.3 Energetic coupling

Energetic coupling linking endergonic reactions to exergonic reactions, is the means by which synthesis can occur in the living cell, the only condition being that the

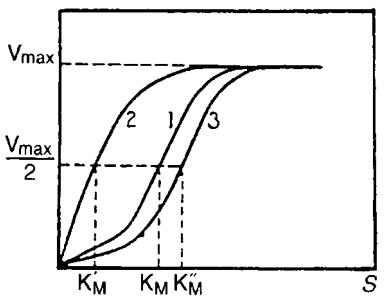


Figure 2.7 Kinetics curves for a reaction catalysed by an allosteric enzyme (1) by itself, (2) with activator, (3) with inhibitor. If the three curves rejoin at V_{max}, the values of K_M are very different. The reaction rates are also very different under normal conditions. S, substrate concentration (after Weil, 1989).

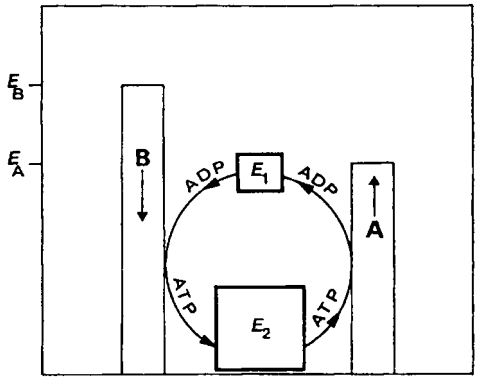


Figure 2.8 Diagram of free energy transfer from an exergonic reaction B to an endergonic reaction A. The amount of free energy E_B provided by B is greater than E_A, which is required for reaction A to occur. Energy passes from B to A due to ADP-ATP couples which temporarily store this energy, passing it from E_1 to E_2 when reaction B occurs and back to E_1 when reaction A occurs.

absolute value of the exergonic reaction must be greater than that of the endergonic reaction, so that the resultant of the two coupled reactions is exergonic.

To this thermodynamic condition (exergonic resultant) must be added a biochemical condition, because such coupling poses the problem of the simultaneous presence of two compounds in the same place in the cell. Coupling implies that transfer of energy from one reaction to the other is carried out by an intermediary compound common to both reactions, capable of temporary storage and the transfer of energy from one reaction to another: this is the case in the coupling of ATP/ADP (section 2.3.1). Figure 2.8 illustrates this process.

To illustrate this phenomenon, Audigié and Zonszain (1988) give the following example, where P_m denotes molecular phosphorus. The phosphorylation of glucose is an endergonic reaction, therefore not favoured thermodynamically:

glucose+P_m—>glucose-6-phosphate+H_2O $\Delta G'$=12.5 kJ mol^{-1}.

Coupling can also be envisaged with the hydrolysis of phosphoenolpyruvate or PEP, because this reaction is strongly exergonic:

PEP+H_2O—>pyruvate+Pm $\Delta G'$=-51.8 kJ mol^{-1}.

The transfer of energy between the two reactions occurs due to the coupling of ATP/ADP:

PEP+ADP—>pyruvate+ATP $\Delta G'$=-22.8 kJ $mol^{-1;}$
glucose+ATP—>glucose-6-phosphate+ADP $\Delta G'$=-16.5 kJ $mol^{-1.}$

2.2 The conditions of cellular work

2.2.1 Sources of energy

A fraction of the products of digestion of food which are fundamental organic substances, is in turn degraded. This degradation, which corresponds to an exergonic reaction, gives energy to the organism which is stored in the short term in the form of ATP and a certain

amount is dissipated in the form of heat into the environment. In animal cells, this source of energy from an exergonic reaction is obtained either from respiration in the presence of oxygen, or by fermentation in the absence of oxygen. Table 2.1 shows, for glucose, the transfer of energy achieved by respiration and Table 2.2 shows the transfers achieved by fermentation. At all times, an active animal cell either respires or ferments, accumulating energy in the form of ATP.

2.2.2 Sources of matter

Matter taken from the environment by living organisms is called food. In animals, food consists mainly of macromolecular organic matter. For this organic matter to be utilizable by the animal, it must first be subjected to moderate degradation which will

Table 2.1 Energetic characteristics of respiration

Physiological phenomenon	Aerobic respiration: anaerobic glycolysis + Krebs' cycle
Cellular location	Mitochondria
Initial substances	$C_6H_{12}O_6 + 6\ O_2$
Intermediate reactions	Approximately 70
Final substances	$6\ CO_2 + 6\ H_2O$
$\Delta G'$	$-2870\ kJ\ mol^{-1}$
Number of ATP	38

Yield: The 38 ATP formed correspond to 38×29.3 kJ or $1114\ kJ\ mol^{-1}$. The total combustion of glucose produces $2870\ kJ\ mol^{-1}$. The conversion efficiency of energy is therefore 38.8% – 'this yield is remarkable, but it must be noted that if the real cellular concentrations of ADP, P_i and ATP were take into account, an even higher efficiency would be obtained. In effect, this efficiency is close to 50% if one considers that a mean of 37.7 kJ is required to make a molecule of ATP under intracellular conditions' (Weil, 1989).

Table 2.2 Energetic characteristics of lactic fermentation

Physiological phenomenon	Lactic fermentation: anaerobic glycolysis
Cellular location	Cytoplasm
Initial substances	$C_6H_{12}O_6 + 6\ O_2$
Intermediate reactions	11
Final substance	$2\ C_3H_6O_3$
$\Delta G'$	$-218\ kJ\ mol^{-1}$
Number of ATP	2

Yield: 'The efficiency of the conversion of energy is $58.6 \times 100/218 = 27\%$ relating to the energy made available by the splitting of glucose into two molecules of lactic acid; but if the relationship is expressed in relation to the 2870 kJ liberated by crude combustion of glucose, the result is $58.6 \times 100/2870 = 2\%$ which is very poor compared with about 40% achieved by aerobic means. To carry out an identical amount of work, anaerobic cells must therefore consume much more (nearly 20 times) glucose per unit time than aerobic cells' (Weil, 1989).

make it into simple organic matter: this is called digestion. In this way, proteins or polypeptides are decomposed into amino acids, polysaccharides into monosaccharides and triacylglycerides into fatty acids and glycerol. Foods become *nutrients:* in effect, the nutrients, unlike foods, are 'directly assimilable substances which can be incorporated into cellular structures or used by energetic metabolism' (Rieutort, 1986).

In all animals, digestion is mediated by enzymes which act either in the cell that secreted them, or in a portion of the digestive tract. In the first case, the digestion is called *endocellular* (or intracellular): foods are absorbed by pinocytosis and subjected to digestion in the cytoplasm of a digestive cell (or absorbent cell). Endocellular digestion is carried out by lysosomal enzymes (glycosidases, acid phosphatases, peptidases) which are activated after fusion of the lysosomes with the digestive vacuoles (Rieutort, 1986). This type of digestion is exclusive to the sponges and platyhelminths.

In the second case, the digestion is *extracellular* and the cells which make the enzymes, often organized to form glands, are exocrine: the enzymes which they develop are secreted to the exterior and act in the lumen of the digestive tract. This type is found in fish and cephalopods (nevertheless, weak pinocytotic activity still persists).

In numerous aquatic animals, the two types of digestion coexist and play an equal role; this is the case in coelenterates, echinoderms, annelids, bivalves and gastropods.

Secretory cells are localized in the digestive apparatus; in contrast, absorbent cells can also be found in external epithelia, notably the gills.

2.3 Types of cellular work

NB: In the examples studied hereafter, we give the standard value of the change of free enthalpy for each chemical reaction. In biological reality, standard conditions rarely occur, in particular for temperature (25°C) and concentration (1 mol dm^{-3}). The values of ΔG' are therefore only indicative.

2.3.1 The work of transport and concentration

This form of cellular work, which often occurs unnoticed, was demonstrated by Lehninger (1965). It is essential in order that the two other forms of cellular work, biosynthesis (chemical work) and movement (mechanical work) can occur.

We shall distinguish the work of transport (of substances and energy) and the work of concentration (short-term and long-term storage) as follows.

Transport of substances

This type of transport, called *active transport,* involves work against osmotic pressure in order to concentrate, within compartments, substances which are essential to chemical reactions, for example ions such as K^+, Na^+, Ca^{2+} or soluble organic substances (for example glucose). This active transport, also known as a *pump,* requires energy. It can be accompanied by electrical phenomena (polarization and depolarization of membranes).

Transport of energy

Biological energy is transported by carriers of hydrogen ions (or of electrons) which are important in catabolism (respiration) as well as in anabolism (biosynthesis). Examples of such carriers are the cytochromes (a,b,c) and the coenzymes (or cosubstrates according to Weil, 1989), of which the most common is NAD (nicotinamide-adenine-dinucleotide) coupled to NADH:

$$NAD+2\ H^{+}+2e^{-} \longrightarrow NADH+H^{+}$$

Short-term storage

The short-term storage of energy is provided by phosphorylated compounds, which are capable of rapid release of part of the energy that they contain.

Adenosine triphosphate (ATP) According to Lehninger (1978), this was first isolated from muscle in 1930. Since then this compound has been found in all living cells (animal, plant, protistan) at a concentration varying between 10^{-3} and 5×10^{-3} mol dm^{-3} cellular water, or from 0.5 to 2.5 mg cm^{-3}.

Its chemical structure, as well as that of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are represented in Figure 2.9. From this diagram note that there are two types of phosphorylated bonds. The first concerns AMP, in which there is an ester bond in which hydrolysis is accompanied by a change in standard free enthalpy of $\Delta G'$=-12.5 kJ mol^{-1}. The two others are anhydride bonds in which, for each, hydrolysis is accompanied by a change in standard free enthalpy of $\Delta G'$=-29 kJ mol^{-1}. To denote this difference, in the last case, the link O-P is represented by the symbol ~, from Lipmann (1941), which indicates what is called a *high-energy bond*. This nomenclature, although established, leads to ambiguity because it is the energy liberated by hydrolysis and not the energy of the bond. Also certain authors prefer the expression *group transfer potential*.

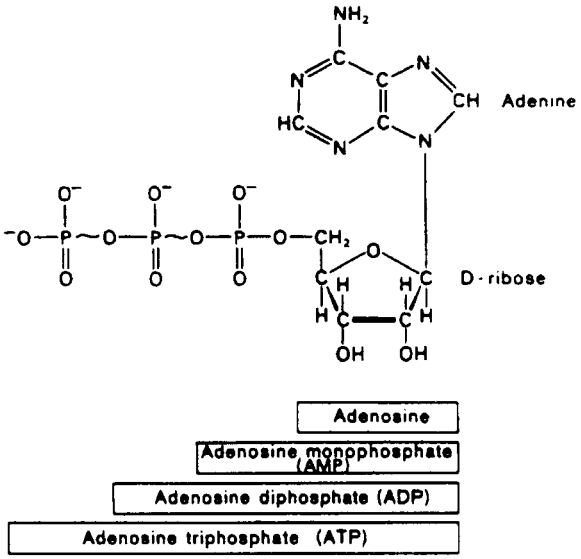


Figure 2.9 Structures of ATP, ADP and AMP. 'Energy-rich' links are designated by the symbol ~ (Lehninger, 1978).

In this way, due to the coupling of ATP/ADP, energy can be given up or stored according to the formulae:

$ATP+H_2O \rightarrow ADP+P_i$ $\quad \Delta G' = -29$ kJ mol^{-1};
$ADP+P_i \rightarrow ATP+H_2O$ $\quad \Delta G' = 29$ kJ mol^{-1}

where P_i denotes inorganic phosphorus. If energy is thought of in terms of currency, ATP is the means of payment in all chemical reactions in the cell and the means of temporary accumulation of potential energy, i.e. available for instant use. ATP therefore acts like a means of monetary exchange in the biological world. Faure (in Boitard *et al.,* 1991), regarded ATP as 'the universal form of free energy in living organisms'.

Other phosphorylated compounds Amongst the phosphorylated compounds most frequently present in the cells, two groups are distinguished, those which release more than 21 kJ mol^{-1} during hydrolysis and which are called 'high-energy compounds' and those which are considered 'low-energy compounds' (Table 2.3).

It is evident from Table 2.3 that ATP occupies an intermediate position. By virtue of this position, ATP, or more precisely the ATP/ADP couple, plays a key role in the transfer of energy between the higher-potential and lower-potential phosphate compounds. This is what Lehninger (1978) calls the 'central role of the ATP-ADP system' and which is illustrated in Figure 2.10.

Long-term storage

The long-term storage of energy is provided by substances which are collectively called *reserves*. They are the result of a polymerization and are usually large molecules, which cannot be rapidly used. This is the case in carbohydrate reserves (starch in plants; glycogen in animals) and lipid reserves (vegetable oils, animal fats).

Table 2.3 Variation in standard free enthalpy by hydrolysis of phosphate compounds present in living cells

Compounds	$\Delta G'$ (kJ mol^{-1})
High-energy compounds	
Phosphoenolpyruvate (PEP)	−51.9
1-3-Diphosphoglycerate	−49.4
Creatine-phosphate or phosphocreatine	−43.9
Acetyl-phosphate	−42.3
Adenosine triphosphate (ATP)	−29.0
Acetyl-coenzyme A (acetyl-CoA)	−26.4
Low-energy compounds	
Glucose-1-phosphate	−21.0
Fructose-6-phosphate	−15.8
Glucose-6-phosphate	−13.8
3-Phosphoglycerate	−12.8
Fructose-1-phosphate	−12.5
Glycerol-1-phosphate	−9.6

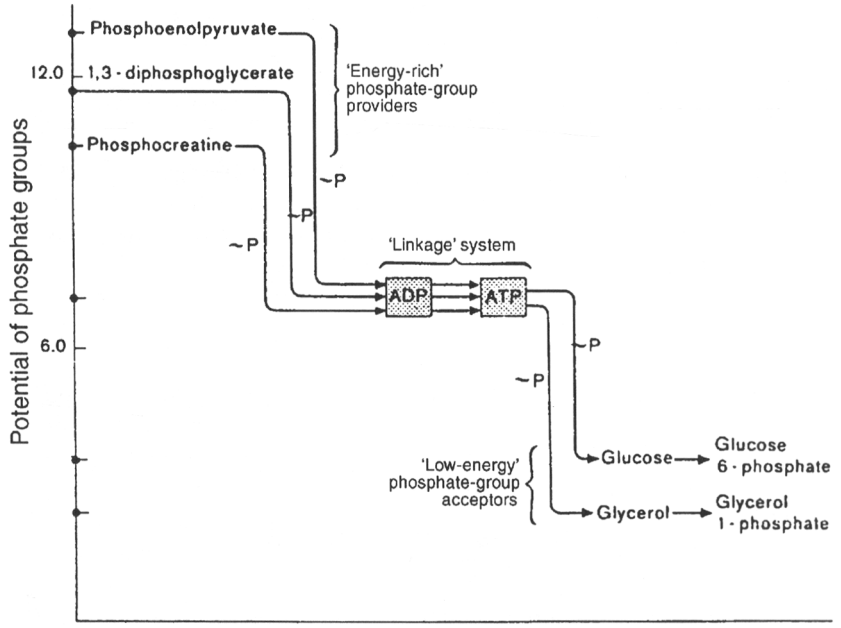


Figure 2.10 Transfer of phosphate groups from energy-rich donors to low-energy acceptors by the ATP-ADP system. The scale of potential of phosphate groups used here corresponds to the transformation $\Delta G' \times 10^{-3}$, the units being cal mol^{-1} (Lehninger, 1978).

2.3.2 Chemical work or biosynthesis

In the cell, endergonic synthetic reactions do not oppose exergonic degradation reactions. As Aubert *et al.* (1974) write, 'these two metabolisms are closely associated, not only in that one is the provider of chemical potential and the other the consumer of this same potential, but also in that the carbon bonds necessary for the synthesis of metabolites are taken from different points of the degradation pathway…. This integration of catabolism and anabolism in one single system is remarkable.'

In contrast, according to Lehninger (1978), the work of biosynthesis does not consist solely of the assembly of complex molecules of proteins, carbohydrates and lipids from simple precursors, but also assembling all these components to make subcellular structures such as the nucleus, mitochondria, endoplasmic reticulum etc. This concept is illustrated in Figure 2.11.

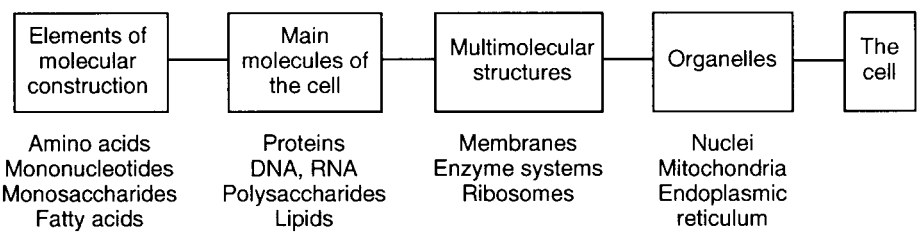


Figure 2.11 Principal stages of cellular biosynthesis in animals. Adapted from Lehninger (1978).

This can be illustrated by study of the biosynthesis of glycogen. Glycogen is a polysaccharide made up of branched chains; at the extremity of each chain, a molecule of glucose can attach itself, in this way increasing the mass of this macromolecule. Glycogen n becomes glycogen n+1. In order that this *glycosidic bond* can be established, it is necessary to activate the glucose, as this link does not occur spontaneously (equilibrium is not favourable).

A phosphorylation of glucose (which increases its energy content) occurs in two stages:

glucose+ATP—>glucose-6-phosphate+ADP; (1)
glucose-6-phosphate—>glucose-1-phosphate. (2)

Glucose activated in this way will form, together with UTP (uridine-triphosphate), uridine-diphosphoglucose (UDP-glucose), which can be considered as a transporter of glucose:

glucose-1-phosphate+UTP—>UDP-glucose+pyrophosphate. (3)

These reactions are completed by the following:

ATP+UDP—>ADP+UTP; (4)
pyrophosphate+H_2O—>2P. (5)

Finally, UDP-glucose transfers the glucose from its molecule to the existing glycogen:

UDP-glucose+glycogen n—>glycogen n+1+UDP. (6)

The overall result of the six reactions is:

glucose+2ATP+glycogen n—>glycogen n+1+2ADP+2P.

As Lehninger (1978) noticed, the sum of the reactions shows that the hydrolysis of two molecules of ATP is required, to assemble one glycosidic link of glycogen. As the energetic cost of this last operation is only 21 kJ mol^{-1}, the $\Delta G'$ of two ATP is 58 kJ mol^{-1}. There is therefore a strong exergonic resultant, which necessarily pushes the reaction in the direction of synthesis.

2.3.3 Mechanical work

Muscular contraction

In animals, the most spectacular mechanical work is that which provides muscular contraction and which results in movement. Movement is provoked by the contractile system of muscle which is made up of parallel myofibrils. The myofibril is the fundamental unit of muscle and is made of actomyosin. In the presence of Ca^{2+} ions, actomyosin can hydrolyse ATP into ADP + P_i, which results in contraction. There are three possible sources of the ATP necessary for contraction.

1 In aerobiosis, ATP comes from the respiration of the muscle using glucose derived from glycogen contained in the muscle cell or transported there by the blood.
2 In anaerobiosis when oxygen supply is insufficient, the muscle can break down glucose into lactic acid: ATP therefore comes from glycolysis.
3 Finally, ATP comes from the following reaction catalysed by creatine

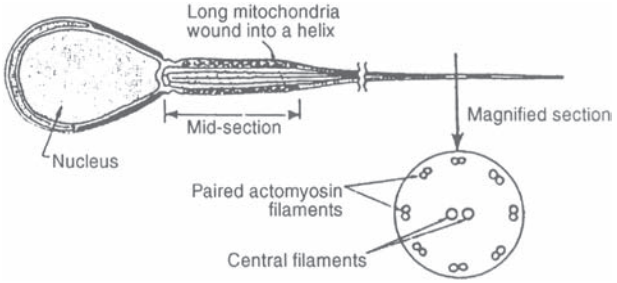


Figure 2.12 Section of a sperm cell. Note the proximity of the mitochondria (generators of ATP) and the fibrils of the flagella (consumers of ATP) (Lehninger, 1978).

phosphokinase:

$$\text{phosphocreatine} + \text{ADP} \Leftrightarrow \text{creatine} + \text{ATP}.$$

During muscle activity, *phosphocreatine* represents a phosphate reservoir rich in energy (Table 2.3). When muscle activity ceases, phosphocreatine is reconstituted from free creatine and ATP from respiration of the muscle cell. As this ATP is provided by mitochondria, their location in the cell is by no means random but follows a defined architecture. In striated muscle, mitochondria are adjacent to the striations and are arranged in a regular pattern. The structures that produce and utilize ATP are closely juxtaposed (Lehninger, 1978). In certain muscles, mitochondria are extremely abundant. For example, in Antarctic fish the red aerobic muscles are made up of myofibrils entirely surrounded by mitochondria the volume of which represents 30–60% of that of the muscle. This feature is interpreted as a compensation for the effects of the low temperatures on enzymatic reactions and diffusion rates (Johnston, 1989).

Movements of flagella and cilia

Flagella are all made up of eight double external fibrils which are contractile and two central fibrils which transmit stimuli. The contractile fibrils are made of actomyosin. In many spermatozoa, the flagellum is surrounded by mitochondria arranged helically around the intermediate section (Figure 2.12).

2.4 The evaluation of cellular work

A number of indices based on the analysis of potential energy of cells have been established. As the cell contains the basic structures common to all living organisms, certain indices must have a universal character. Some indices describe the status of short-term (ATP and adenylates) or long-term (glycogen) energy storage, others the intensity of cellular growth (aspartate transcarbamylase and nucleic acids) or state of fasting (nucleic acids).

2.4.1 ATP, adenylates and phosphorylated compounds

Preliminary remarks

The analysis carried out in the preceding section has made obvious the vital role played by ATP in energetic exchanges in the cell. It is the medium for accumulating energy from

exergonic reactions and a source of energy for endergonic reactions. It is therefore not surprising that cellular biochemists often use, rather than the joule, the number of ATPs available in the cell before or after metabolic reactions. A statement that the degradation of a mole of glucose provides 38 ATP from respiration and only 2 ATP from lactic fermentation, is more striking than to say that 2870 kJ mol^{-1} are released by respiration compared with 218 kJ mol^{-1} for lactic fermentation. Expression of metabolic results in numbers of ATP molecules rather than energy units is not trivial and can lead to confusion or error for two sets of reasons.

The first results from measurement, by chemical analysis, of only ATP and neglect of all other forms of short-term energy storage such as PEP and phosphocreatine, which are nevertheless abundant in certain cells.

The second consists of expression in 'ATP equivalents'. If this implies the integration of all other phosphorylated compounds by converting their energy value to an equivalent of ATP, the previous criticism is overcome and this provides a convenient measure of the total short-term energy store of the cell or organ in question. This method is therefore justifiable. On the contrary, if this implies the conversion into ATP equivalents of all energy contained in the cell or organ, including reserves (not rapidly utilizable) and structural proteins (never totally utilizable), an element of confusion is introduced which condemns such a practice.

Corresponding indices

The *adenylate index* or *adenylate energy charge* (AEC) was established by Atkinson and Walton in 1967 according to the following formula:

$$([ATP] + \tfrac{1}{2}[ADP]) / ([ATP] + [ADP] + [AMP]) = AEC.$$

According to Ivanovici (1980), the adenylate charge is a biochemical indicator of environmental 'stress'. In this formula, if all the adenylates are in the form of ATP, the value of the index is equal to 1, indicating a high energy potential.

According to Atkinson (1977), this index is characteristic of the regulatory ability of the cell. High values of the index, such as 0.9, for example, indicate that the animal is capable of regulation. In effect, this index can be maximized if AMP is eliminated. This can occur due to the action of *AMP deaminase,* which deaminates AMP and transforms it into inosine monophosphate (IMP). As this phenomenon occurs when the animal is alarmed, AMP deaminase is termed the *distress enzyme*.

This extremely sensitive index nevertheless poses a technical sampling problem: when an animal is captured it is likely to react with frequent and violent muscular contractions which considerably decrease the level of ATP before a sample can be obtained. Results can therefore be largely determined by the method of sampling (Lucas and Beninger, 1985).

Despite these difficulties, the AEC index is widely used (Le Gal, 1988). It has been measured, for example, for *Cardium,* a bivalve mollusc in the Sado Estuary in Portugal. In Figure 2.13, the results obtained show that the values of AEC give evidence of a recent modification to the environment, in this case the elevation of the temperature at power station outflows, which becomes stress-inducing in July, but not in March.

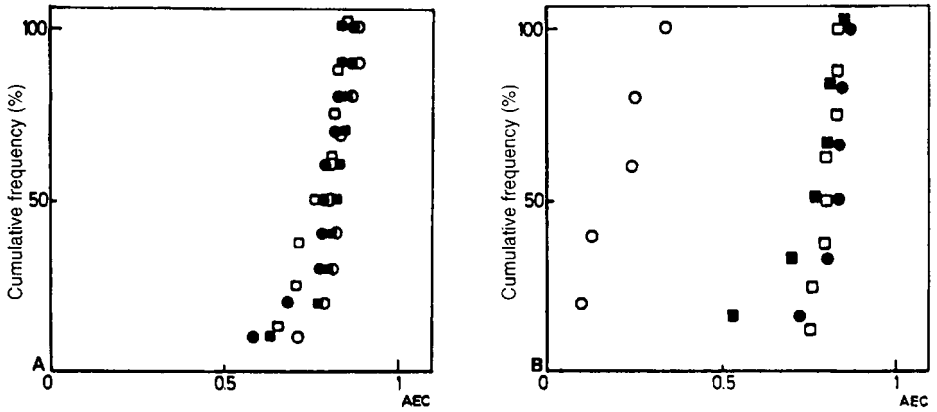


Figure 2.13 AEC values for *Cardium* sp. in March 1987 (left) and July 1986 (right) at four sampling stations in the Sado Estuary. Cumulative frequencies in percentages are shown on the *y*-axis. Each sampling station is represented by a different symbol. The station represented by an open circle is situated in the vicinity of a power station outflow. (Adapted from Picado *et al.* 1988.)

The phosphorylation potential is defined as follows:

$$\frac{[ATP]}{[ADP] \times [P_i]}.$$

According to Reed (1976), this index takes into account the state of the NAD-NADH system in the cytoplasm, the respiration rate and the oxidizing phosphorylations in the mitochondria.

Lavanchy's index was defined by Lavanchy *et al.* (1985) as follows:

$$\frac{[ATP] + [PC]}{[ATP] + [PC] + [P_i]}$$

where PC is phosphocreatine. Measurements of the concentrations of the three substances are made using the nuclear magnetic resonance (NMR) of phosphorus-31. This technique has enormous advantages because the animals are observed *in vivo* without special preparation and the spectra required for the calculations are obtained in a few minutes. According to Lavanchy *et al.* (1985), this index correlates well with the adenylate charge and phosphorylation potential.

The NMR technique and Lavanchy's index were used by Raffin and Thébault (1988) to study cold adaptation in the prawn *Palaemon serratus*. During cold periods, the prawns are less able to carry out muscular work. The results obtained show that this drop in muscular performance is due to a decrease in the available energy reserves and not to a diminution in efficiency of the mechanisms providing energy necessary for muscular work.

2.4.2 Glycogen

In many aquatic invertebrates, glycogen is the principal long-term energy reserve in both juveniles and adults. For this reason the index of glycogen/ash-free dry tissue has been

widely used, notably in the study of seasonal fluctuations of metabolism. For this purpose, the whole animal can be assayed, but it is more judicious to limit the samples to organs likely to accumulate glycogen—in bivalves, for example, the adductor muscle in pectinids, the mantle in mytilids and the digestive gland in ostreids (oysters).

2.4.3 Aspartate transcarbamylase (ATC)

ATC is an enzyme specific to the pathway of *de novo* biosynthesis of pyrimidine bases. Purine and pyrimidine bases are the fundamental units for the construction of nucleic acids; the growth of the latter is related to cellular division and protein synthesis, i.e. growth by cellular multiplication. It is therefore not surprising that this enzyme should be particularly active in fast-growing tissues. Thus, the values of ATC activity in an organ indicate the instantaneous growth rate of the organ. Bergeron and Alayse-Danet (1981) applied this concept to marine animals. They showed that in the scallop *Pecten maximus,* variations in ATC activity of the gonad and the mantle are correlated with the states of maturation of the gonad and the shell growth achieved by the mantle. Figure 2.14 shows the relationship between the specific activity of ATC and the mass of gonad in *Pecten maximus*. This species is hermaphroditic and has a gonad that is easily separated from the visceral mass, a feature that is rare in bivalves. The increase in mass of the gonad is a means of expressing the progress of gametogenesis. The initial phase corresponds to the multiplication of the germ calls, which explains the initial peak of ATC activity. The sensitivity of the method was demonstrated in several ways. For example, in *Pecten maximus,* a period of intense sexual maturation which lasted 4 days after spawning was linked to high ATC activity in the gonad, while at the same time the ATC activity of the mantle fell. This provides evidence for deviation from general metabolism over a very short time scale. Similarly, bass larvae showed falls in ATC

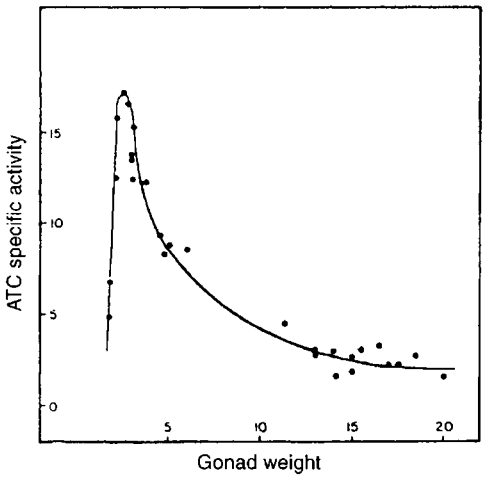


Figure 2.14 Change in specific activity of ATC during gonad growth (expressed in grams) in *Pecten maximus* (after Bergeron and Alayse-Danet, 1981).

activity when transferred between rearing tanks, which was due to the stress provoked by the transfers. This sensitivity implies that isolated values of this index should be interpreted with caution.

2.4.4 Nucleic acids

As stated in the previous section, nucleic acids, DNA and RNA, are the indicators of cellular growth. Measurements of these two nucleic acids must therefore be obtained from the metabolic activity of the tissues studied. According to Brafield (1985), the relationship between RNA/DNA is the most satisfactory indicator, not only for instantaneous growth rates but also for long-term growth rates. This view, widespread amongst biologists, runs counter to current observations showing simultaneous increases in DNA and RNA, notably in juvenile organisms where multiplication of nuclei and the synthesis of specific proteins go hand in hand. In this case, growth is intense, but the RNA/DNA ratio remains practically constant. This leads one to wonder what is represented by the division of RNA values by DNA values and why this ratio became so widely accepted as meaningful.

On the contrary, the ratios between DNA/biomass and RNA/biomass, where biomass can be expressed as dry matter, organic matter, live matter, amount of C etc., appear to be more logical. In effect, because the amount of DNA per nucleus in somatic tissues is constant for a given species, the amount of DNA per unit of biomass measures the number of cells. When the relative value of DNA increases, this expresses *cellular multiplication (hyperplasia)*. In contrast, the relative value of RNA is directly linked to protein synthesis and therefore, according to the expression of Luquet and Durand (1970), to *cellular growth (hypertrophy)*.

In the course of development, there can be alternation between growth due to hyperplasia and that due to hypertrophy. Such phenomena have been demonstrated in the shrimp *Crangon crangon* (Regnault and Luquet, 1974), as shown in Figure 2.15. In carrying out a simultaneous study of tissue and nucleic acid growth, Regnault and Luquet (1976) were able to explain the forms of growth as a function of feeding in *C. crangon*. Some young shrimps were raised for 4 months with either a normal or a

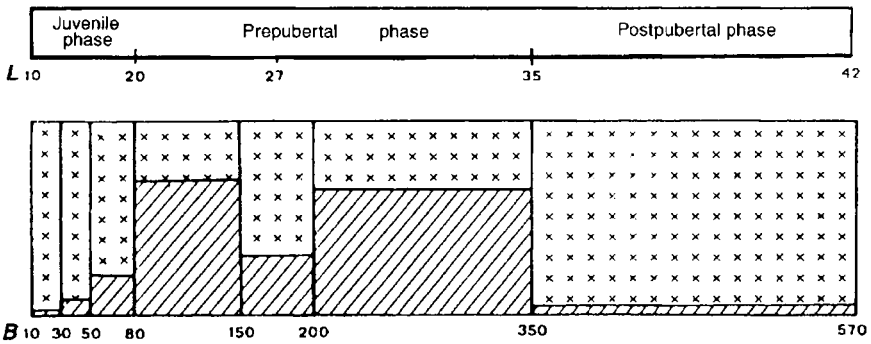


Figure 2.15 Relative proportions of hyperplasia (crosses) and hypertrophy (hatching) in the growth of *Crangon crangon*. *B,* biomass in mg; *L,* length in mm (after Regnault, 1977). Note: biomass scale is linear, but length scale is not.

semisynthetic diet. In the first case, the weight gain is due to cellular multiplication. In the second case, less rapid cellular growth was irregular: slowing down of hypertrophy in the first month, slowing down of hyperplasia in the second month, but resumption of hypertrophy alone in the third month. These results, which clarify the forms of growth, allow a better analysis of the validity of food rations.

In a study of larval and juvenile growth of the decapod *Hyas araneus,* Anger and Hirche (1990) obtained, with the help of different techniques, the simultaneous values for the following: weight of dry matter, proteins, C, N, DNA, RNA. A thorough analysis of the results led them to conclude that the values for nucleic acids cannot be used on the basis of usual hypotheses, when growth is associated with developmental phenomena.

As an alternative to preoccupation with growth, Bergeron *et al.* (1991) looked for a way of using the DNA/dry matter ratio as a *starvation index,* notably in fish larvae. In a fasted state, most other molecules disappear or decrease whilst the amount of DNA remains constant, which is translated into an increase in the ratio. Some preliminary experiments carried out on sole, *Solea solea,* showed that the values of this ratio depend upon the larval stage. At the symmetrical larva stage, the value of 30% corresponds to a threshold: in fed larvae, the index (approximately 25%) always remains less than 30% and in fasted larvae it reaches values of 40% or 50%.

3

Bioenergetics of Organisms: Concepts

> It is not energy *per se* that makes life go, but the flow of energy through the system.
> (Morowitz, 1968)

A living organism functions like an open system, receiving energy and matter from, and rejecting them into, its surroundings. An animal is a heterotroph which achieves its biosynthesis (anabolism) at the expense of energy obtained from organic matter which is consumed and then degraded (catabolism). Catabolism and anabolism collectively constitute metabolism.

3.1 Analysis of metabolism

The general schematic established by Duvigneaud (1974) can be applied to animals (Figure 3.1).

3.1.1 Food intake and its fate

A large number of feeding mechanisms exist in aquatic animals. For simplicity, we shall examine only the two extreme cases: macrophages which feed on discrete large prey items, discontinuous feeders; and microphages which feed on small nutritious particles, in general continuously.

The rate of consumption *(C)* is the amount of food which an organism takes from the environment per unit of time. From this must be subtracted the food that is not available for absorption by the study organism. The term *C* includes not only feeding but also wastage. The rate of ingestion *(I)* is the amount of food, per unit of time, that penetrates the digestive tract of an organism and remains there.

In macrophages such as reared fish, *C* can be easily determined: it is the amount of food provided per unit of time. *I* can be discovered by subtraction, by determining the proportion of food lost (F_1) so that $I=C-F_1$.

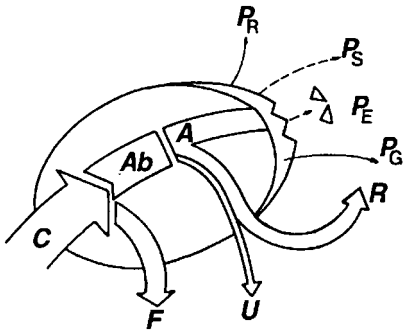


Figure 3.1 Diagram of the metabolism of an individual during an annual cycle. *C,* consumption; *Ab,* absorption; *A,* assimilation; *F,* faeces and pseudofaeces; *U,* urine; *R,* respiration; P_R, reproduction production (i.e. emitted products—see p. 53); P_S, secretion production; P_E, production of eliminated tissue; P_G, production of living tissue or increase in biomass or tissue growth (modified after Duvigneaud, 1974).

In microphages, neither consumption rates *(C)* nor ingestion rates *(I)* can be determined for animals *in situ*. It is necessary to transfer the animals to the laboratory and to place them in conditions as close as possible to those of their site of origin. For a given organism, the rates vary mainly as a function of the concentration of food particles, temperature and proportion of mineral particles. For each measurement, these variables must be well known and be maintained constant during the experimental analysis.

In microphages, a portion of the consumed particles is not ingested but rejected by the animal in the form of pseudofaeces (F_1). These are eliminated before or at the mouth and are more or less indistinguishable from faeces (F_2), which are eliminated through the anus. In certain cases, it is only the total $F_1+F_2=F$ which can be collected during an experiment. Knowing *F,* it is possible to discover the amount of food absorbed *(Ab)* per unit of time, on condition that the diet remains identical over a sufficient period of time (several hours or a few days) so that the faeces collected correspond well to the same type of diet. In this case:

$$C\text{-}F=Ab$$
or $$I\text{-}F_2=Ab.$$

Figure 3.2 gives an analytical illustration of the consumption and fate of food in a microphage (in this case a bivalve mollusc) and explains the formulae given previously.

In macrophages, as in microphages, absorbed food *Ab,* which has crossed the wall of the digestive tract, is found in the internal environment of the animal and therefore represents the gain in matter acquired by the animal during the time under consideration.

3.1.2 Absorbed matter and its fate

Nitrogenous excretion

Absorbed matter represented by *Ab* is not entirely metabolizable, because it is partly made up of amino acids. These, like all proteinaceous substances, produce

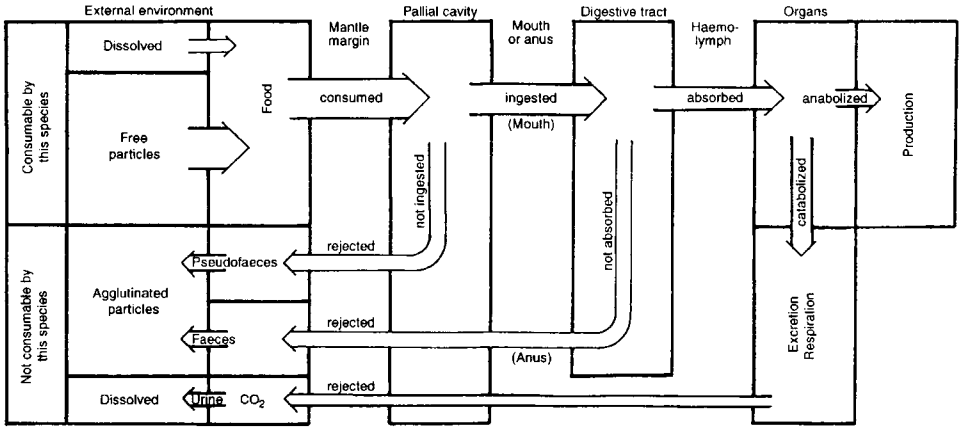


Figure 3.2 Diagram showing exchanges of food and metabolites between a bivalve and the external environment (modified from Lucas, 1982).

not only NH_3, which is very toxic, when they are catabolized, but also derived products (urea, uric acid) which are toxic to lesser degrees: all these products must therefore be eliminated from the organism and are the constituents of urine represented by *U*. In this way, the metabolizable part of *Ab* is *A=Ab-U*. The process of formation of urine and its elimination constitutes the basis of nitrogenous excretion.

In aquatic animals, ammonia, rapidly eliminated in the fluid environment, is the predominant means of excretion: these animals are *ammoniotelic*. Nevertheless, other nitrogenous products, such as amino acids, can be eliminated by animals under certain circumstances, notably during stress. In other cases, dissolved organic substances present in the environment can be absorbed by the organisms (notably through the gills). There is therefore exchange of dissolved organic matter between an organism and the environment. When the balance of these exchanges is to the advantage of the organism, it is called *osmotrophy* (dissolved organic substances therefore represent a net positive nutritive contribution). If osmotrophy occurs, the positive value will be added to *C*.

In contrast, when nitrogenous excretion prevails, the negative result is added to *F* as loss against food consumed. Hence the equation:

$C-(F+U)=A$ or $C-E=A$

where *A* represents metabolizable energy and *E* the excretions.

Catabolism: respiration and fermentation

Part of the matter represented by *A* will be catabolized, that is to say degraded (liberating energy), either by respiration or by fermentation. These two phenomena have been studied at the cellular level (Tables 2.1 and 2.2).

Respiration The respiration of an organism can be quantified by measuring oxygen consumption per unit of time under defined conditions of temperature, oxygen pressure in water, motor activity and feeding activity of the animal. Taking into consideration these last two factors, three types of measure are distinguished:

- R_s—standard or resting respiration rate (no feeding, no motor activity);
- R_f —frespiration rate after feeding;
- R_a —respiration rate during motor activity.

From the way in which these measures are defined, it is concluded that R_f is a result of R_s plus respiration due to feeding and that R_a is a result of R_s plus respiration due to motor activity. These values, the only ones that can be measured, cannot be added to establish R. For this, it is necessary to establish three components as shown in Figure 3.3:

- R_S —standard respiration rate;
- R_F —respiration rate due to intake of a meal;
- R_A—respiration rate due to motor activity.

In this case, the total respiration rate is the sum:

$$R=R_S+R_F+R_A.$$

In fact, R_F corresponds to the specific dynamic action (see below, p. 44) and R_A to *scope for activity* defined by Fry (1947) as the respiration rate during motor activity minus the standard respiration rate.

If we insist on the differences between the measures and components of R, that is to say in our nomenclature between R_f and R_F, R_a and R_A, it is because there are many imprecisions in this subject in the literature.

Other formulae exist, for example that of Calow (1985), who introduces the additional concept of *routine metabolism:* R_R (routinely active animal):

$$R=R_S+aR_{R\text{-}S}+bR_{R\text{-}S}+cR_{A\text{-}S}$$

where a,b,c are values expressing the fraction of time allowed to each 'metabolism'. Dall *et al.* (1990) argue that the term routine metabolism has not been used rigorously. For prawns or shrimps they propose the following definition: the respiration rate of individuals in close to natural conditions averaged over 24 hours.

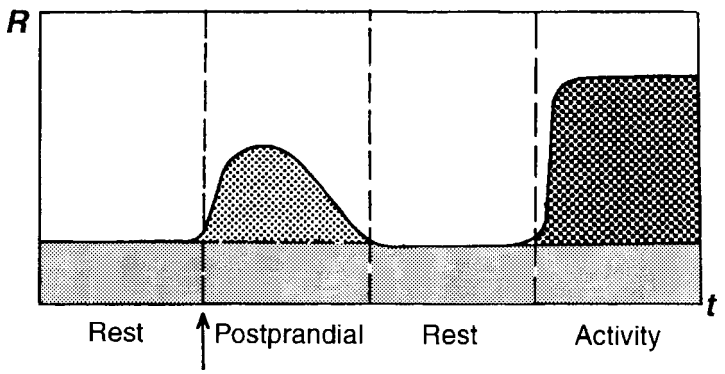


Figure 3.3 Diagram of the three components of respiration expressed in J h^{-1}. R, measured respiratory rate; t, time in hours, each sequence lasting n hours. Standard respiratory rate: R_S=light grey area divided by $4n$. Respiratory rate due to the intake of a meal: R_F=dark grey area divided by n. Respiratory rate due to motor activity: R_A=stippled area divided by n. The arrow indicates the point at which food is taken.

Such a definition would be suitable for other animals, because it takes into account possible circadian rhythms.

On the subject of R_F and specific dynamic action, a certain amount of confusion exists. This confusion arises from the diverse nomenclature that has been used: postprandial thermal effect, extra-heat, specific dynamic action (SDA), calorigenic effect, thermic effect, heat increment of feeding (HI), and dietary induced thermogenesis (DIT).

Kayser (1963) recalled that Lavoisier was the first to measure the difference between respiration rates of fasted, recently fed subjects and that Rubner in 1885 originated the idea of *specific dynamic action* in its application to the ingestion of proteins. In homeothermic animals, it has been shown that SDA represents 30% of the caloric content of a protein, 13% for a lipid and 5% for a carbohydrate. According to Jobling (1985), the origin of the activation of respiration would be due to the energy necessary for the digestion of ingested foods, the absorption of nutrients, the deamination of amino acids and the synthesis of the products of nitrogenous excretion. According to Knights (1985), it is difficult to discriminate between SDA and additional respiration due to excitement and activity with feeding. This could explain the variable results cited in the literature, in which SDA estimates range from 9% to 20% of the energy contained in the meal (Jobling, 1981).

SDA is considered as a tax, payable on conversion of food (Brody, 1964, p. 25), which is increased the richer the diet is in proteins; this can be expressed by the equation $R_F=mC$, where m is a coefficient varying from 0.05 (exclusively carbohydrates) to 0.3 (exclusively proteins). Presented in this way, SDA and tissue growth appear to be *competitors* because if SDA increases, the growth must decrease. In opposition to this conclusion, Jobling (1985) proposes considering SDA as an energetic expenditure associated with growth, according to the formula $R_F=rP$ where r is the energetic cost per unit of growth and P is production. From this approach, SDA and growth would be *interactive*.

In our opinion, in discussion of various formulae, reality is often forgotten. It is not because SDA is increased that growth is increased, it is because the diet contains lots of proteins, which are required by fish and cephalopods (section 5.1.1). This cause (the large proportion of proteins in the diet) has two separate effects: elevated growth and elevated SDA. If there were only proteins in the diet, SDA would be further increased, but not growth.

Fermentation Beside the abundant literature on respiration, contributions on fermentation in aquatic animals are few and often imprecise. This relates to the difficulty in measuring the intensity of fermentations. Nevertheless, it is known that this mode of catabolism exists in certain invertebrates such as bivalve molluscs which can, under anoxic conditions, degrade carbohydrates into succinates or to lactates. According to Hammen (1980), in certain bivalves *(Crassostrea, Mytilus),* fermentation can take place in the aerobic state, alongside respiration. This assertion is based on the results of calorimetry and respirometry (section 4.1.2). Nevertheless Famme *et al.* (1981), while experimenting on *Mytilus edulis,* invalidated Hammen's conclusions.

Generally, the intensity of fermentative phenomena is usually ignored and the majority of authors have neglected the energetic contribution of fermentation. This lack of information, however, may not be as serious as one might think: the majority of animals are organisms that 'repay their oxygen debt' (e.g. section 5.3.3), i.e. as shown in Figure 3.4. This means that energy gained from fermentation will ultimately become apparent oxygen consumption after the period of anoxia.

It should be noted with respect to this how delicate measurement of oxygen consumption can be. If, before this measurement, the animal was subjected to some trauma leading to a suspension of normal activity, it might, during the measurement, be in a phase of oxygen debt payment, which obviously would not reflect its usual metabolism.

Anabolism

Anabolism, which corresponds to biosynthesis, is designated by *P* (production) in energetic balance sheets.

Measurements of anabolism It must be made clear that what is measured in classical methods is the *apparent anabolism,* which corresponds to the *apparent biosynthesis*. In every living organism, there is constant renewal of molecular structures within cells and cells within tissues (Pascaud, 1989). Cellular renewal is taken into consideration in the production balance sheet in the form P_E (section 4.3.2). On the other hand, molecular renewal occurs unnoticed by the global assessments carried out on organisms. To detect it, methods of isotopic labelling must be used. For example, the amount of proteins retained by an organism per unit of time (apparent protein synthesis) only represents part of the amount of proteins synthesized per unit of time (actual protein synthesis) taking account of their continuous degradation. Figure 3.5 illustrates this phenomenon, as well as the use of amino acids as an energy source.

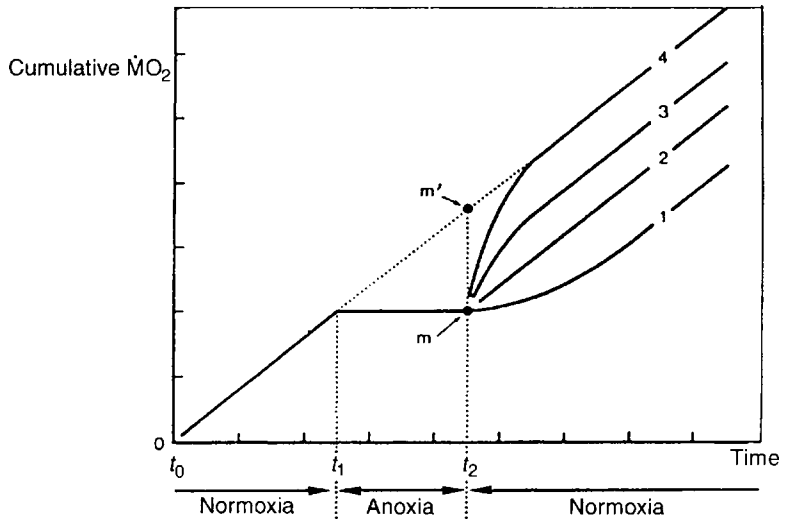


Figure 3.4 The reactions of an organism subjected successively to normoxia (from t_0 to t_1), anoxia (from t_1 to t_2) and normoxia (from t_2) allows different types to be distinguished: types 1 and 2, which do not repay their oxygen debt at all and regain their oxygen consumption rate quite rapidly; type 3, which partially pays its oxygen debt; and type *4,* which fully repays its oxygen debt. $\dot{M}O_2$, cumulative oxygen consumption; m, value of $\dot{M}O_2$ at time t_2; m', value of $\dot{M}O_2$ at time t_2 if no anoxia has occurred (after Dejours, 1975).

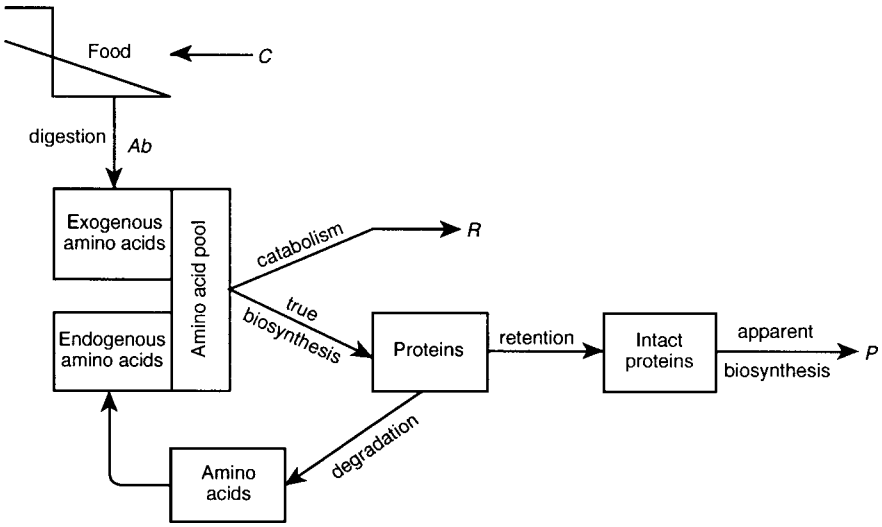


Figure 3.5 Schematic showing the use of amino acids and proteins in an organism over a given period *(t). Ab,* absorption; *C,* consumption; *P,* production; *R,* respiration. From the pool of amino acids, some are catabolized and others are used in protein synthesis. During the time *(t),* some of these proteins remain in the cell where they were synthesized, and others are broken down into amino acids. In this way real and apparent biosynthesis can be distinguished, the latter being equal to *P* over the time period *(t).*

Fauconneau (1980) used labelled leucine to measure the rate of protein turnover in various organs of the rainbow trout: it was high in the liver, gills and digestive tract, and weak in the muscles. Generally, molecular renewal is less intense in fish than in mammals (Fauconneau, 1980).

The overall production is relatively complex and depends on the organism studied. Nevertheless, two types of production can be distinguished.

Tissue production This corresponds partly to growth in biomass (P_G) and partly to eliminated tissues (P_E). Tissues may be eliminated either in a violent way (e.g. an appendage discarded by autotomy in a crustacean, or a bivalve siphon eaten by a fish; P_{E1}), or continuously, corresponding to tissue renewal (e.g. desquamation; P_{E2}). It should be noted that the desquamation of the digestive tract is integrated into the total faeces. In effect, faeces have two origins: exogenous faeces, ingested foods not absorbed; and endogenous faeces, cells of the digestive apparatus eliminated by desquamation. Tissue renewal occurs in all organs, the speed of renewal depending on the organ (verified using biochemicals).

The elimination of sexual products also corresponds to an elimination of living cells (spermatozoids or oocytes), but because of the importance of the reproductive process, it is usually classed separately (P_R).

Production of inert substances Almost all secretions (P_S) are made up of inert substances, generally proteinaceous and/or carbohydrate in nature. Some of these substances remain on or in the animal and are called *residues* (e.g. the carapace of crustaceans, which is not eliminated except at moults, or the shell and byssus of molluscs). Because of their

accumulation in one place, they are very easy to measure over a given period of time. Residual secretions are designated by P_{S1}.

Other secretions, on the contrary, are continually eliminated into the external surroundings, gradually at the rate at which they are produced. These are designated by P_{S2}. The example most widely dispersed by aquatic species is mucus (section 4.3.2).

3.2 Energetic equilibrium equations

There are several ways of establishing an energy balance of a living organism. We shall examine three, each of which has its own logic and coherence. In all energy balance equations, the values are expressed in joules per unit of time, which can vary from a second to a year, according to the aim and the conditions of the work carried out.

3.2.1 Energy balance sheet

A simple way of establishing the budget is to proceed as for a financial budget, balancing gains and losses of matter-energy between the organism and its environment. In this case the components of the assessment, expressed in energetic units per unit of time, are divided as follows:

Gains
- Food consumed *(C)*

Losses
- Excreta, i.e. non-utilizable part of food *(F+U)*
- Eliminated tissues (P_E)
- Shed reproductive products (P_R)
- Non-residual secretions, e.g. mucus (P_{S2})
- Respiration *(R)*

Balance
- Tissues formed (P_G)
- Residual tissues, e.g. shell (P_{S1})

This assessment allows the establishment of the equation:

$$C-(F+U+P_E+P_R+P_{S2}+R)=P_G+P_{S1}.$$

3.2.2 Metabolic energy balance

Another approach is to carry out an analysis based on the physiology of the living organism and to clearly distinguish the part of *C* which is metabolizable *(A)* from that which is not *(F+U)*. *A* in turn can be divided into two parts: firstly, anabolism *(P)* and secondly catabolism *(R),* within which can be distinguished that which comes under maintenance and that which comes under activity.

Approach based on the duality: anabolism and catabolism

Certain elements of the balance sheet result from an analysis of the environment: that which is taken up by the animal *(C)* and that which is rejected, unused *(F+U)*. Others result from an analysis of what happens inside the animal (*P* and *R*). A first equation can therefore be written expressing this dichotomy:

$$C-(F+U)=P+R \quad \text{or} \quad C-E=P+R$$

where *C* is consumption, *F* is faeces, *U* is urine, *P* is production, *R* is respiration and *E* is excreta. This is the fundamental equation of bioenergetics. Certain terms can be broken down, thus:

$$E=F_1+F_2+U \quad \text{and} \quad F=F_1+F_2$$

where F_1 is pseudofaeces or food given but not ingested, and F_2 is faeces or part of food ingested but not absorbed.

From this we deduce:

$C-F_1=I$ where *I* is food ingested;
$C-F=Ab$ where *Ab* is food absorbed;
$C-(F+U)=A$ where *A* is metabolizable food.

As we have seen before, respiration can be broken down as follows:

$$R=R_S+R_F+R_A.$$

Production *P* can be broken down as follows:

$$P=P_G+P_R+P_S+P_E$$

where P_G is tissue production or growth, P_R is the production of eliminated reproductive products, P_S is the production of secretory products, and P_E is the production of tissues eliminated either violently (through predation), or continuously, by use, which requires compensatory cellular renewal.

Figure 3.6 illustrates what is described in the preceding equations.

Approach based on the duality: maintenance and activity

A distinction must be made between upkeep or maintenance (which is therefore obligatory) and various activities, such as growth, reproduction and movement (which can be temporarily suppressed without affecting survival of the animal).

If the daily rates of consumption Q_C and growth Q_G are considered, both expressed in percentages of biomass of the animal, it can be seen that the maintenance ration Q_{CM} is the ration that corresponds to zero growth rate (Figure 3.7).

If we return to the metabolic equilibrium equations, maintenance consists of, without modification, R_S, P_S and P_E, to which is added R_F, corresponding to the ration Q_{CM}, and R_A, corresponding to the energetic expenditure of movements required to acquire the ration Q_{CM}, as shown in Figure 3.8.

The notion of maintenance, initially defined for mobile species (notably fish), was extended to sessile species by Bayne and his collaborators. From their work on *Mytilus edulis,* Hawkins and Bayne (1985) and Widdows and Hawkins (1989) contributed to the widespread use of the idea of maintenance. In addition, Bayne and Hawkins (1990), by expressing maintenance not in terms of ration, but as metabolizable energy of the ration, allow new physiological interpretations.

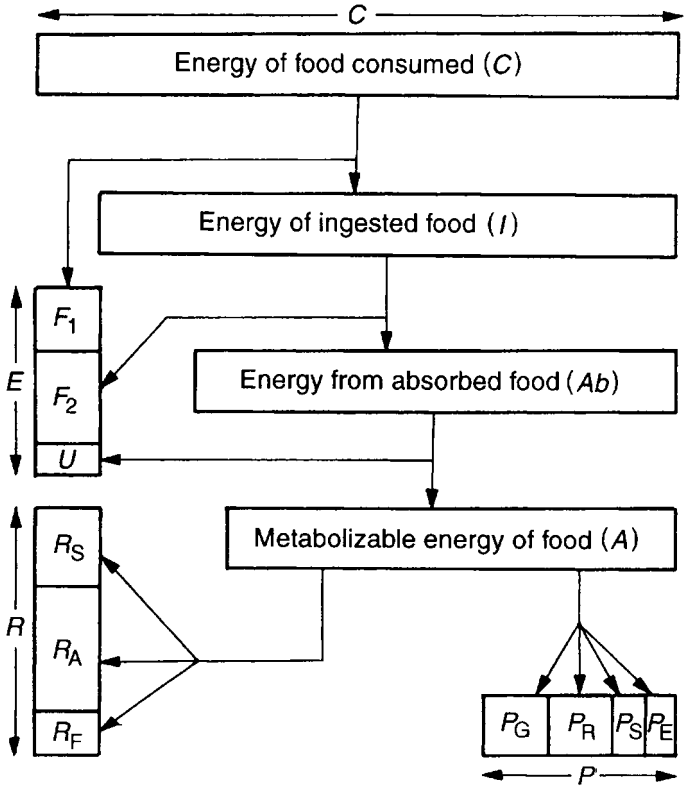


Figure 3.6 Diagram showing the partitioning of food energy during metabolism. Relative values established over a year give a good understanding of P_G and P_R. An energy budget for a shorter period (month or day) could give a zero value for P_R and a zero or negative value for P_G. The lengths of the boxes are proportional to the amount of energy, so it can be seen that $C=E+R+P$, that $A=P+R$ and that $A=C-E$.

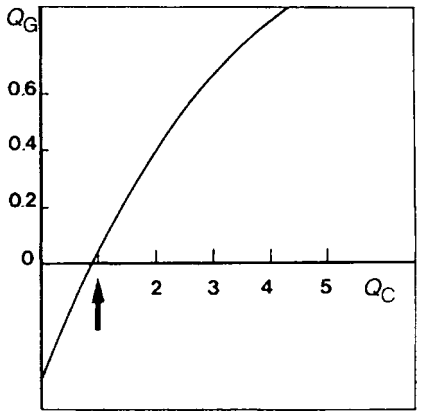


Figure 3.7 Relationship between daily growth rates Q_G and consumption Q_C, both expressed in percentage of biomass of the organism being studied. The arrow indicates the maintenance value (Q_{CM}) of Q_C correspond ing to Q_G=0.

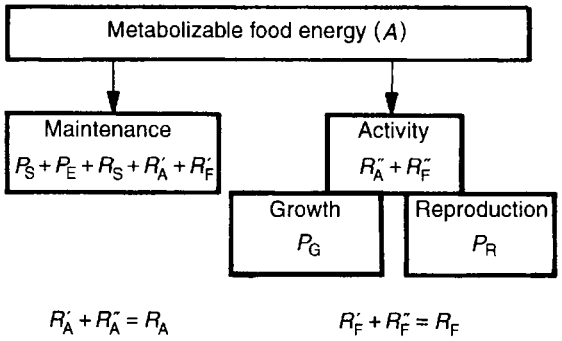


Figure 3.8 The energetic costs of maintenance and activity.

3.2.3 Ethological energetic balance

Another way of estimating energy balance is to consider the energetic cost of different essential functions of the animal, from an ethological view, e.g. reproduction, nutrition, defence etc.

Cost of reproduction

According to Wootton (1985), the cost of reproduction consists of three facets: those of primary sexual products, i.e. emitted oocytes or sperm and all the substances which accompany them (corresponding to P_R); those of secondary sexual characteristics, usually different in the two sexes and particularly developed in the male (this corresponds to a part of P_G); and that of reproductive behaviour, which is expressed in diverse manifestations such as courtship display, nest building, territorial defence, care of young, migrations to spawning sites, and so on. All these activities form part of catabolism (R_A) and decrease proportionally the amount of available energy *(A)*. This approach allows a better appreciation of the part played by each sex in reproduction. For example, in the case of the three-spined stickleback, *Gasterosteus aculeatus* (Wootton, 1985), there is a considerable difference between the P_R of females and males, in favour of the females. But the male expends more on secondary sexual characteristics (elaboration of colouring) and above all in behaviours, such as courtship display, territorial defence and nest building. For nest building, as well as the energetic expense of necessary movements, the male must secrete a certain amount of mucus to stick the nest together. In this way, taking total costs into account, despite having a much smaller P_R than that of the female, the male, as a consequence of his behavioural activities, expends almost as much energy as the female.

Cost of feeding and defence

Finding food also requires energy expenditure, for example migration to find feeding areas, or pursuit of prey. The same reasoning is valid for finding safety, such as flight from predators, and migration to find protected areas (shelter, for example). Thus the energy cost of migration can be attributed to defence, feeding or reproduction, depending on the functional objectives of the animal.

Such interpretations are not always obvious and sometimes tend to be anthropomorphic. This does not detract from an innovative approach which can reveal unsuspected aspects, like the reproductive cost according to sex. The stickleback is a revealing example.

3.3 Some misconceptions to avoid

No matter what type of balance sheet is adopted, it must be applied rigorously, without changing the logic or the meaning of the terms. It may be thought that such recommendations would be self-evident. However, it must be emphasized that, despite the clarity of the definitions, confusion is too often perpetuated (including in classic textbooks) regarding the subject of the components of metabolism.

3.3.1 The concept of production

At the organism level, we have defined production as the result of anabolic activities during a certain period of time. This idea is justified by the fact that when observers study an organism, they are looking at the inside view: it is normal that they call all that is produced, production (what will become the structure and the destiny of the product). It is known that the products are either tissues (P_G, P_R, P_E) or inert substances (P_S). It is also known that certain products are conserved (P_G and P_{S1}) or eliminated (P_R, P_E and P_{S2}). Although this might appear unnecessary, it does not appear superfluous, in this case, to use the term *anabolic production*.

When they study a population, observers do not place themselves inside each organism that makes up the population: they must consider the population as a whole and therefore have a more external view. For a given period of time, they will therefore call the difference in biomass between the start and finish of the considered period, production. This indicates that they take into account only the retained production by the organisms that make up the population (i.e. P_G and eventually P_{S1}). In this case, it is not superfluous to specify that the production in question is *demographic production*.

In passing from the scale of the individual to that of the population, the term 'production' has changed its meaning. In the former, it corresponds to a physiological interpretation, in the latter an ecological one. In these conditions, it is not surprising to note that production by organisms is correctly interpreted by physiologists in terms of anabolic production.

Ecologists, on the contrary, accustomed to the study of populations, have applied to organisms, more or less explicitly, the idea of demographic production, which has led them to confuse production P with tissue growth P_G. This mistake was first committed by Crisp (1971, 1984), who writes (1984) 'Production *(P)*. That part of the assimilated food or energy that is retained and incorporated in the biomass of the organism, but excluding the reproductive bodies released from the organism. This may also be regarded simply as "growth".' Crisp's successors continued this confusion between P and P_G, notably in the idea of *scope for growth,* defined as follows: S_C=*A*-*R;* when it is known that *A*-*R*=*P,* in other words, ambiguously, scope for 'growth' is none other than *P,* as emphasized by Lucas and Beninger (1985).

3.3.2 The concept of catabolism and entropy

On the subject of catabolism, two errors are frequent. The first is not to employ this term and to replace it with 'metabolism', i.e. to confuse a part with the whole. For example, Brafield and Llewellyn (1982, p. 50) write, like many authors, 'the respiratory rate, or metabolic rate'. The second is to consider that the only role of catabolism (and therefore respiration) is to dissipate energy as heat. For example, the previous authors maintain 'energy lost as heat is represented by R (respiration) in an energy budget'. Such pieces of writing ignore catabolism and misrepresent the role of respiration. We repeat that catabolism is the only function which allows, in animals, the acquisition of energy. In this operation (see Tables 2.1 and 2.2), aerobic catabolism (respiration) provides better yields than anaerobic catabolism (fermentation). In the two cases, potential energy is dissipated as heat, but some of it is also transformed into molecules of ATP, without which biosynthesis could not occur.

Certain authors do not resist making a further blunder by declaring 'this energy degraded into irretrievable heat is called entropy' (Rosnay, 1977, p. 24). It is without doubt not superfluous to specify that entropy is not energy. In effect (section 1.3), in a chemical reaction, the energy dissipated in the form of heat is $T\Delta S$, T being the absolute temperature, ΔS the variation in entropy. Energy is expressed in joules, J, entropy in joules per kelvin, JK^{-1} (section 1.6.1).

3.3.3 The concept of tissue growth

A common mistake is to designate P_G 'somatic growth'. P_G represents tissue growth, therefore also that of the gonad. In effect, P_R is only the energy represented by the *emitted products* of reproduction. As long as the gametes are inside the animal, they participate in the development of P_G. This is logical, because sometimes unshed gametes are used by the organism, as reserves, to make new somatic tissues. Moreover, in the gonad, somatic tissues exist which show that possible distinctions between somatic tissue and gonad tissue are not straightforward.

Figure 3.9 clarifies these repeated confusions. In this schematic, the areas and the letters represent masses (variables of state); the arrows and the numbers express exchanges of energy (variables of flux). Hatched areas: gametes and products of reproduction. Stippled areas: somatic tissues.

Examination of this diagram shows us that:

P=1; P_G=2+3+4; P_S+P_E=5; P_R=6

3.3.4 The concept of secretion

Bioenergeticians very often confuse secretion and excretion: they do not see the difference between a product of anabolism (secretion) and excreta, i.e. the non-metabolizable part of food (excretion). In this way, Crisp (1984) writes, 'Excreta *(E)*. That part of the consumption that is absorbed and later passed out of the body as secreted material, as, for example, in the urine. Many organisms produce a number of other exudates such as milk, mucus, shed

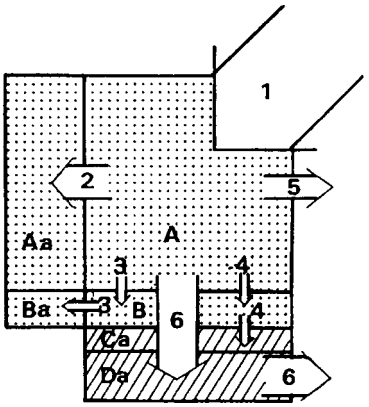
cuticle, nematocysts etc….' Amongst Crisp's examples, milk and nematocysts correspond to P_E, mucus and cuticle to P_S. This mistake is found in many publications, even recent ones. For example, in a synthesis by Bayne and Newell (1983) under the title 'energy loss due to excretion', mucus is the first substance mentioned.

3.4 Time frame of energy budgets

The time frame of an energy budget, i.e. the time elapsing between two calculations, can be chosen on the initiative of the researcher. In making such choices, researchers must take into account the aim being pursued and the feasibility of measurements during the given time. We use the term 'time frame' rather than period because the latter is not applicable to non-cyclical time intervals. In effect, 'the period of a cyclical phenomenon is the relatively constant interval of time, separating two identical states of the same given physiological, biological or morphological variation' (Brusle, 1969).

3.4.1 Long- and medium-time-frame energy budgets

All the classic energy budgets fall within this category. Two cases present themselves: either the time frame follows a natural rhythm, or it is independent of it.



A	tissues of all organs except gonad;
Aa	annual growth of these tissues;
B	somatic tissues of the gonad;
Ba	annual growth of these tissues;
Ca	unshed gametes and products of reproduction;
Da	shed gametes and products of reproduction;
1	non-respiratory energy acquired in 1 year;
2 and 3	energy used for somatic tissue growth;
4	energy corresponding to unshed gametes and products of reproduction (eventually reused by somatic tissues);
5	energy of secreted products and eliminated tissues;
6	energy of effectively shed gametes and products of reproduction (reproductive effort).

Figure 3.9 Diagram showing an organism's use of anabolic energy acquired over a year. (See text.)

Time frames corresponding to a natural rhythm

Table 3.1 describes four natural rhythms, two of which apply to tides. For species living in fresh water or in non-tidal seas, there are therefore only two natural rhythms: the year and the day.

The annual rhythm is characterized by progress through the seasons where, in temperate countries, two factors dominate: daylength (photoperiod) and temperature. Added to these is rainfall, the effects of which on fresh, estuarine and coastal waters are far from negligible. In the equatorial zone the variations in photoperiod and temperature are considerably lessened; because of the monsoon phenomenon, rainfall becomes the preponderant factor (alternating dry and wet season).

The circadian or diurnal rhythm is characterized by the alternation of day and night. In circadian rhythms, light appears to be the most efficient *synchronizer*. A synchronizer is an environmental factor that varies cyclically, to which living organisms coordinate their own activities. For example, the existence of a circadian rhythm has been demonstrated in the enzymatic activity in the shrimp *Penaeus kerathurus* (Van Wormhoudt *et al.,* 1972).

Tidal rhythm corresponds to the ebb and flow which takes place two times per lunar day (24 h 48 min) and causes each time a period of immersion in the intertidal zone. The semisynodic or half-lunar-month rhythm is related to the size of the tides and the alternation between neap and spring tides. Every 15 days there occur strong spring tides, which are characterized by the lowest immersion at the intertidal level on the ebb and by the highest high tides on the flow. This fact is exploited by a Californian fish, the grumion *Leuresthes tenuis,* which allows itself to be carried onshore on the highest tides in order to place its spawn in the humid sand where it remains and develops. At the next spring tide the waves wash away the sand and release the alevins which are now able to swim (Dajoz, 1971).

In bioenergetics, annual energy budgets are most often calculated from animals that live for several years. Daily energy budgets are used either for short-term experiments or for studying short stages of development, e.g. the larval stage of bivalves, which usually lasts about 20 days.

Table 3.1 Characterization of time periods linked to geophysical or biological rhythms

Interval	Frequency	Geophysical rhythms	Biological rhythms
Less than 0.5 h	High frequency		Physiological rhythms e.g. cardiac rhythm
Between 0.5 h and 2.5 days	Medium frequency		Ecological rhythms
Less than 24 h		Lunar half-day: 12 h 24 min	Tidal rhythm (sea)
Approx. 24 h		Solar day: 24 h	Diel rhythm; circadian rhythm
Greater than 24 h			
Greater than 2.5 days	Low frequency		Ecological rhythms
		Half lunar month: 14.76 days	Semisynodic rhythm
		Solar year: 365.25 days	Annual rhythm

Time frames not corresponding to a natural rhythm

The most frequently occurring time frames in this category are monthly time frames. Unlike the lunar month, which influences the amplitude of the tide, the solar month (30.44 days, rounded to 30 days) only represents an arbitrary division of the year and cannot be considered as a 'period of a cyclical phenomenon'; in fact, monthly time frames are brought in to show evidence of the seasonal variations of the annual cycle. In using the fortnight or 10 days, certain authors aim to be more precise in their description.

Traditionally, all measurements of long-term energy budgets are expressed in joules per year, month or day, according to the time frame chosen by the experimenter. Nevertheless, if, as we said in the conclusion to Chapter 2, SI units are used, the results of energy budgets are expressed in watts (or multiples). This way of showing what makes up the energy budget of an organism is none other than calculation of the *power* of that organism.

3.4.2 Short-term energy budgets

Short-term energy budgets are essentially confined to respiratory activity and, contrary to long-term energy budgets, they are usually expressed in watts. From the two respiratory extremes, R_A (*active respiratory rate,* considered as maximal) and R_s (*standard respiratory rate,* considered as minimal), the two following ideas have been established.

The *metabolic scope* is defined as the difference between R_A and R_s: $MS=R_A-R_s$. The metabolic scope is a power expressed in watts, because R_A and R_s are expressed in these units.

The *metabolic power index* is on the contrary a dimensionless number as a result of its definition:

$$S=(R-R_s)/(R_A-R_s).$$

The index ranges from 0 to 1. In effect, if R (measured respiratory rate) is equal to R_s, $S=0$; if R is equal to R_A, $S=1$, but in general the value of S is in the middle. In effect, an animal cannot live permanently either in a state of maximal or minimal activity, because in these two extreme states, for physiological reasons, the mortality rate is considerably increased. Figure 3.10 combines these factors.

The choice of the frequency of measurements remains. Priede (1985) has examined this problem. According to him, for fish, fluctuations in oxygen uptake in the environment are regulated on a time base of 1 minute. The metabolic power index can therefore be estimated every minute. Successive measurements can last between 0.5 h and 1 h to obtain a real appreciation of the fluctuations in respiratory rate. In Figure 3.10, it can therefore be considered that the interval between two points representing the value of S is of the order of 1 minute.

To complete this review of the time frame of energy budgets, we would like to insist on the fact that the long- and medium-term energy budgets do not conflict with short-term energy budgets. Whatever the time frame, every energy budget can be calculated in watts (or multiples) and for every energy budget, values for metabolic scope can be established.

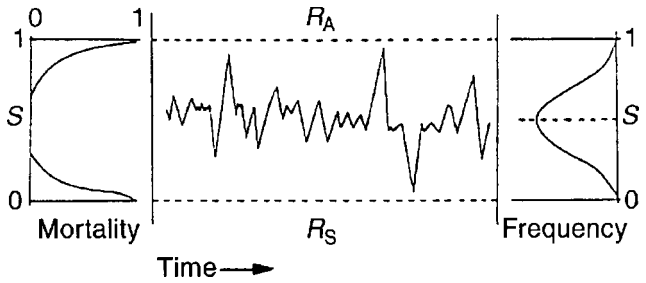


Figure 3.10 Diagram showing the temporal fluctuations in respiratory rate *(R)* between R_A and R_S. The scale of the index of metabolic power varies between 0 and 1 (see text). On the left, the diagram shows that the closer *S* approaches its limits (0 and 1), the more the mortality rate of the animals studied increases. Fortunately these values are rarely encountered (see right side of diagram). (After Priede, 1985.)

3.5 Indices and efficiencies

3.5.1 Energetic efficiencies

From long-term energy budgets, the efficiencies shown in Table 3.2 are commonly used. The gross growth efficiency is sometimes called *gross food conversion efficiency*. Brown (1957) has, moreover, proposed a *net food conversion efficiency* defined as follows: $P_G/(C\text{-}C_M)$, where C_M is the food consumed, used for maintenance. This distinction is adopted by certain authors, e.g. Klaoudatos and Apostolopoulos (1986). Some other authors such as O'Dor and Wells (1987) do not make the distinction and call P_G/C *conversion efficiency*. This terminology can lead to confusion, as there exists in applied biology, a practical measure, the *conversion rate,* defined as follows: the relationship between the amount of dry food (M_s) and gain in biomass (M_v), i.e. C (as M_s)/P_G (as M_v).

Table 3.2 Energetic efficiencies derived from metabolic measurements

Assimilation efficiency	$\frac{A}{C} = \frac{P+R}{C} = \frac{C-E}{C} = \frac{P+R}{P+R+E}$
Gross production efficiency	$\frac{P}{C} = \frac{P_G+P_R+P_S+P_E}{C} = \frac{P}{P+R+E}$
Net production efficiency	$\frac{P}{A} = \frac{P_G+P_R+P_S+P_E}{A} = \frac{P}{P+R}$
Gross growth efficiency	$\frac{P_G}{C} = \frac{P_G}{P+R+E} = \frac{P_G}{P_G+P_R+P_S+P_E+R+E}$
Net growth efficiency	$\frac{P_G}{A} = \frac{P_G}{P+R} = \frac{P_G}{P_G+P_R+P_S+P_E+R}$

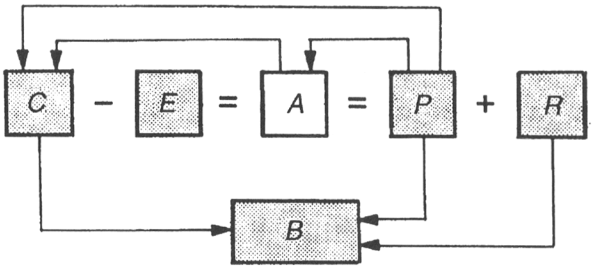


Figure 3.11 Measurable (grey) or deducible (white) components in a complete energy budget. The main relationships of biological interest are indicated by the arrows (origins indicating numerators, points the denominators).

3.5.2 Energetic indices related to biomass

The opportunity will arise later (section 5.4) to show the importance of relationships such as *P/B, R/B, C/B.* This reminds us that it is not enough to know the values of the different variables of an energy budget, but that one must also know the biomass of the organism involved. There is every interest in measuring this biomass *(B)* in the form of ash-free dry weight, i.e. organic matter (M_o). Figure 3.11 summarizes these factors.

3.5.3 The unexpected effect of negative values of P_G

When, in monthly energy budgets, P_G is negative, the net growth efficiency P_G/A gives uninterpretable values, as they can vary between 0 and infinity (Shafee and Lucas, 1982). To resolve this problem, Lucas and Shafee (1983) proposed that metabolizable energy be considered in its totality (A_T) and could come either from foods, therefore from the exterior (A_E) or from reserves, therefore from the interior of the organism (A_I):

$$A_T = A_E + A_I.$$

A_I is zero when P_G is positive or zero. Using this approach (Lucas and Shafee, 1983), the ratio P_G/A_T ranges from -1 to +1.

For other efficiencies such as the net production efficiency P/A, the gross growth efficiency P_G/C, or the gross production efficiency P/C, the extension of the process which consists of considering negative P_G values as positive sources of energy is not satisfactory. These three efficiencies are only, by definition, applicable to annual energy budgets. In this way, net growth efficiency P_G/A is differentiated from the others because it can be used as a good index of physiological condition, evidence of the seasonal variations in metabolism (Lucas and Beninger, 1985).

3.6 Modelling of energy budgets

In global energy budgets, the principal problems arising are, on the one hand, the acquisition of matter and, on the other hand, the partition of acquired matter, notably the allocation of

matter for growth and the allocation of matter for reproduction. These three problems will be examined hereafter by describing certain models, as well as the supporting fundamental ideas.

3.6.1 Acquisition of matter

In the acquisition of matter, two chronologically differentiated functions can be distinguished:

- ingestion *(I)* which can be confused with consumption *(C)* if pseudofaeces are treated as faeces $F=F_1+F_2$ (section 3.1.1);
- digestion, which leads to the absorption *(Ab)* of ingested substances.

The transition from *I* (or *C*) to *Ab* has been modelled by Sibly (1981), who used the following fundamental ideas.

The digestibility of a food is defined according to two indices: *apparent digestibility* and *true digestibility*. The index of apparent digestibility is defined as:

$$D_a=(I-F)/I$$

where *I* is the amount ingested and *F* is the amount defecated. This value of *F* consists of faeces of both exogenous and endogenous origin (section 3.1.2).

To obtain the real value of the digestibility of a food, only faeces of exogenous (or food) origin (F_{ex}) need to be taken into account. This is the true digestibility index, defined as follows:

$$D_V=(I-F_{ex})/I=[I-(F-F_{en})]/I=Ab/I.$$

The *retention time* is the inclusive time between the start of digestion and the instant of effective absorption of the foods. This retention time is shorter (and more difficult to measure) than the *transit time* which occurs between ingestion of food and defecation of non-ingested residues.

If we examine the curves $f(T_1)$ and $f(T_2)$ in Figure 3.12, note that the acquisition of matter by digestion consists of three phases:

- the first is the handling time of the food which requires a supply of energy: $f(T)$ is negative;
- the second corresponds to the intensive absorption of nutrients: $f(T)$ is positive with a steep slope;
- in the third, absorption decreases because of the small amount of nutrients in relation to indigestible substances: $f(T)$ is positive with a shallow slope.

The amount of energy obtained from 1 g of food is a function of time: $f(T)$. The amount of food entering the digestive tract per unit of time is *r:* it is the rate of ingestion and is expressed in g s^{-1}. From which:

mass ingested: $W_i=rT$ whence $r=Wi/T$ (1)
rate of energy gain: $rf(T)$ (2)

or again:

$$Wi\, f(T)/T. \quad (3)$$

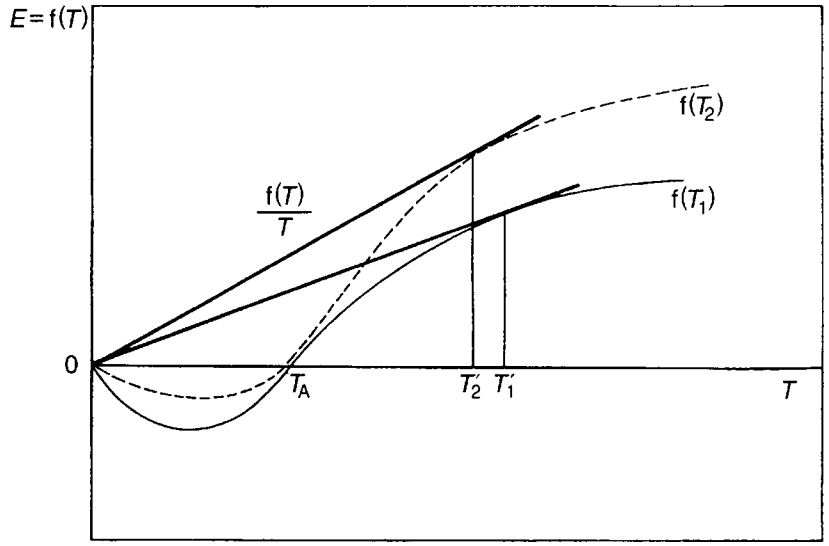


Figure 3.12 Sibly's model of digestion. Two curves, $f(T_1)$ and $f(T_2)$, corresponding to different foods, are shown. On each curve three phases are recognized: handling of food— $f(T)$ negative; intensive absorption—$f(T)$ positive with a steep slope; residual absorption—$f(T)$ positive with shallow slope. The tangent $f(T)/T$ allows calculation of the optimal digestion time: T'_1 for $f(T_1)$ and T'_2 for $f(T_2)$. E, energy obtained (calculated per 1 g of food); T, time (in seconds); T_A, duration of food handling. The curve $f(T_2)$ shows a higher rate of energy acquisition than $f(T_1)$ for two reasons: less energy expended in food handling and steeper slope in the active absorption phase. Original presentation, inspired by Sibly (1981).

PREDICTIONS

Prediction 1 To maximize the rate of obtaining energy, the animal must maximize T if r is fixed (Equation 2). In this case, when the rate of ingestion is limited, digestion must last longer.

Prediction 2 For a given ingested mass, the best strategy is to maximize $f(T)/T$ (Equation 3). This applies particularly when the capacity of the digestive cavity is limited in volume. The optimal retention time T' can be calculated graphically (Figure 3.12).

Prediction 3 Animals feeding on food of mediocre energy value must have a larger digestive cavity, all other things being equal. In effect, for poor digestibility, $f(T)$ is lower and $f(T)/T$ is also lower (Figure 3.12).

EXAMPLE

To illustrate prediction 1, Lawrence (1987) cites the case of a sea urchin (Table 3.3) which, as its ration is decreased, increases its absorption efficiency by longer retention time of the bolus. This compensation is nevertheless insufficient to maintain the quantity of food absorbed.

In the previous analysis, the *digestibility* corresponds to the number of joules provided by 1 g of food, while the *rate of obtaining energy* is the number of joules provided by 1 g of food per unit of time (here the second: s).

Table 3.3 Effect of food ration value on absorption rate and amount of food absorbed in *Strongylocentrotus intermedius* fed on *Laminaria japonica* (after Fuji, 1967, in Lawrence, 1987); ind, individual

Food ration (mg ind^{-1} day^{-1})	Absorption rate (%)	Amount absorbed (mg ind^{-1} day^{-1})
205	66	135
137	72	99
50	80	40
32	82	26

Sibly's model also, therefore, allows an analysis of digestibility. Thus the graph shows that the digestibility of a food depends, not only on its chemical composition (the richer the food, the steeper the slope of f*(T)*), but also the cost of breaking down the food in the digestive tract (the more difficult it is to break down, the more energy is expended). In Figure 3.12, these two considerations are unfavourable to f(T_1) and favourable to f(T_2).

3.6.2 Partition of acquired matter between growth and reproduction

If it is accepted that P_S and P_E are constants, which is frequently the case, the remaining metabolizable energy $A'=A-(P_S+P_E)$ will be partitioned between P_G and P_R. In other words, that which is used for reproduction, is not used for tissue growth. P_R is not a permanent activity, neither during a lifetime, nor (generally) in the course of a year. During a lifetime, there is always a juvenile phase of development which corresponds to an absence of reproductive activity. It is brief in animals with a short life span, which are generally *semelparous* (i.e. they have only one reproductive phase in their life). It is longer in animals with a long life span, which are generally *iteroparous* (i.e. they reproduce several times during their lifetimes). The pectinids, bivalve molluscs, are good examples because the gonad is separate from the visceral mass, which is rarely the case in bivalves.

Argopecten irradians (bay scallop) is a species native to the north-east coasts of the United States, which reproduces at the end of summer or in autumn. Growth is very rapid, the animal reaching 6 cm at 10 months. It reproduces at age about 1 year and dies some time after, without reproducing a second time (Gutsell, 1930).

Chlamys islandica, studied on the Norwegian coast close to Trömso, reproduces in June or August. Sexual maturation is delayed and gradual: it appears in about 5% of individuals at 3 years, 30% at 4 years, 70% at 5 years and all individuals are mature at 6 years and more. This clam lives about 15 years (Vahl, 1981). During the course of a year, reproduction can be continuous or discontinuous. There is therefore a period of reproduction which can consist of one or many releases of gametes, while the remaining time corresponds to sexual rest periods.

In *Argopecten purpuratus,* reproduction is continuous throughout the year, in Peru (Wolff, 1988) and in the north of Chile (Disalvo *et al.*, 1984).

In *Pecten maximus,* differences between populations have been observed. The populations of Brest and St Brieuc (Brittany), despite being on the same latitude, have different reproductive behaviours. In St Brieuc, maturation is synchronized at the beginning of March; the release of gametes is also synchronized, taking place in July, and, according to the year, a second release can occur at the end of August. In Brest, there are mature individuals throughout the year and partial, non-synchronized releases of gametes occur from May to September (Paulet *et al.,* 1988).

There is therefore considerable variation between species and also between populations of the same species, which increases the difficulties in establishing a general model.

As we have seen, sexual maturation does not appear at the start of development; it is a separate function, which will occur depending on the age or the size of the organism considered. To simplify, we shall not try to discover if it is age or size that activates the maturity of the animal. Let us consider Figure 3.13, the abscissa B representing the mass of the animal, the ordinate P' representing utilizable energy when maintenance has been assured. If we accept that P_S and P_E are inevitably expended in maintenance,

$$P' = P_G + P_R.$$

If P_R=0, P' is used only for P_G, which has the effect of increasing the mass of the organism to maximum. Each of the three parabolas represents either individuals of different ages, or individuals of the same age on different dietary regimes.

As long as P_R is zero, B_{max} is reached. If all or part of P' is used for reproduction, B is reduced by the same amount. According to Sebens (1979), this change in metabolism must occur when P' is at its maximum, i.e. when there is the maximum amount of energy available to proceed with the division between P_R and P_G.

Let us consider, for example, parabola (3). If, at point P'_{3max}, part of the remaining energy is passed on to reproduction as represented in Figure 3.13, an organism of mass B_3 is obtained (and not B_{3max}).

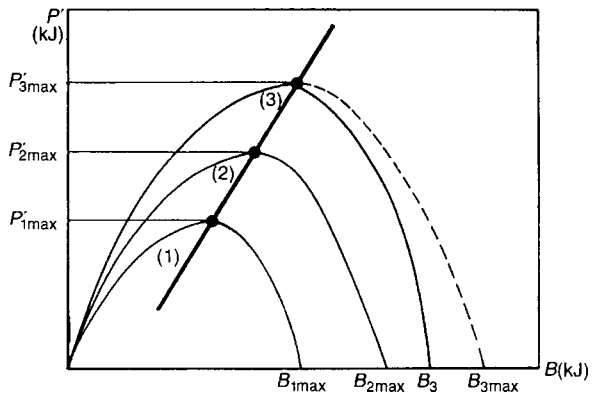


Figure 3.13 Relationship between available energy P' (expressed in kJ) and the biomass of organism B (converted into kJ). Curves (1), (2) and (3) represent three successive states of the organism. In curve (3), a portion of P' is used for reproduction; consequently, the organism reaches weight B_3 and not B_{3max}. (See text.) Original presentation from a model presented by Calow (1981).

3.6.3 Growth modelling

Numerous growth models have been established. We do not deem it necessary to present them here because this can be left to more specialist texts. However, it is interesting to review the origin of von Bertalanffy's model for two reasons. On the one hand it is the most widely used model in biology and on the other hand it was based on bioenergetic arguments. Von Bertalanffy (1938) states in the introduction to his work that 'growth is the measurable increase of an organic systems (sic), produced by its assimilation of materials obtained from its environment'. That is, the organism is considered as an open system receiving a flow of organic matter from its environment. Consequently, von Bertalanffy thought it legitimate to model on a stationary chemical system

a —><— b

where substance a is continually introduced and substance b continually removed. If E is the amount of material a introduced into the system per unit of time and k the rate of transformation of a into b, per unit of time, the concentration of a increases according to the equation

dm/dt=E-km.

Assuming that organisms can be considered as stationary chemical states, von Bertalanffy assumes two hypotheses:

- the *construction constant* per unit of time represented by H is proportional to the absorptive surface of the organism (Rubner's law);
- the *destruction constant* per unit of time represented by K is proportional to the mass of the organism.

In the case of isometric growth, mass is related to volume and can be deduced from linear dimensions.

In fact, von Bertalanffy's two equations are written in the form

$$L_t = L_\infty \left[1 - e^{-k(t - t_0)}\right]$$

and

$$M_t = M_\infty \left[1 - e^{-k(t - t_0)}\right]^3$$

where L_t is length at time t, L_∞ is as explained below, k is an instantaneous rate, t_0 is time at age zero (or at hatching, etc.), M_t is mass at time t, and M_∞ is as explained below.

To illustrate his theory, as well as examples of growth in the rat, von Bertalanffy modelled growth of length and mass of a cyprinodont fish, the guppy *Poecilia reticulata*. The results of this are given in Figure 3.14. The author justifies choosing this species because it is viviparous, therefore the young resemble the parents, which justifies the hypothesis of isometric growth. The choice of *Poecilia* is equally justified by the fact that this species shows very pronounced sexual dimorphism, the female reaching twice the length of the male, and by the fact that the growth of this fish in warm water (24°C in experiments) is very fast. The differences in growth between the different growth trials are interpreted by von Bertalanffy as a reflection of genetic differences.

Von Bertalanffy's growth model has been used in an acceptable way for a considerable number of species. Its validity can therefore not be called into

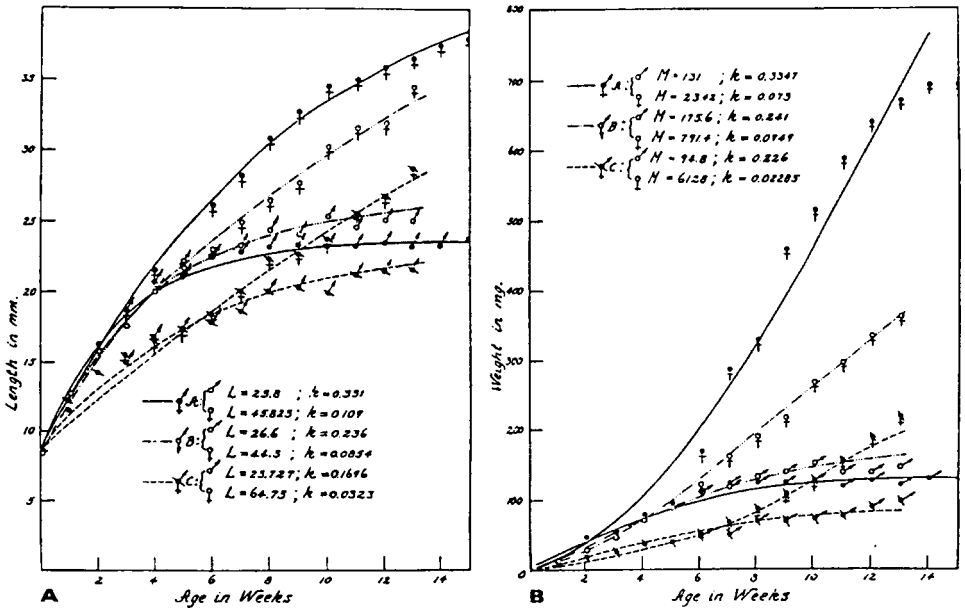


Figure 3.14 Growth in length (A) and weight (B) of three groups of guppies, *Poecilia reticulata (= Lebistes reticulatus).* The sexes are distinguishable after 3 weeks, allowing the separation of males and females. In panel (A), L=L_∞; in panel (B), M=M_∞ (reproduced from von Bertalanffy, 1938).

question. Nevertheless, this model has aroused lively controversies, on the one hand on the interpretation of size limits L_∞ or M_∞, and on the other hand on the concept of 'construction' proportional to the 'absorptive surface' of the organism.

Certain authors have taken L_∞ (or M_∞) to be the maximum size that can be attained by the species studied. This biological interpretation, which is found many times in disaccord with observations, has been mostly opposed by scientists for whom L_∞ is only a mathematical prop devoid of any biological significance (Laurec and Le Guen, 1981). This view is also that of von Bertalanffy, who wrote (1938, p. 192) that L_∞ is none other than 'the final value to which the growth curve asymptotically approaches'.

Rubner's rule used by von Bertalanffy has also aroused innumerable controversies. What von Bertalanffy understood by 'construction' is not synonymous with anabolism as might be believed; in effect, he says (1938, p. 184), 'construction of building materials is proportional to some surface'. He specifies later (p. 187), 'it is found that species possessing a rich development of intestinal (i.e. resorbing) surfaces grow much larger than such with poorer' and '…metabolism (O_2 consumption) is a surface function in most animals (especially fishes and mammals)'.

In this way, two types of surfaces are involved: that of the intestine (for absorption) and that of the respiratory apparatus (for respiration). Constructive metabolism, H, is expressed in this way:

$$H=Ab+R.$$

His ideas on 'destructive metabolism', *K,* are not explicit. By deduction, the following equation can be proposed:

$$K=U+P_E+P_R+P_S.$$

All these functions occur in proportion to the mass of the body (renewal of tissue, for example) or to the mass of organs which carry out this or that function (excretion of urine, exocrine secretions, sexual products), but it is the same for respiration which takes place within every cell, that is to say throughout the body mass (section 5.3.3). Certainly transport of oxygen is required, but does not this transport depend as much on the capacity of the circulatory system as on the capacity of the surfaces of the gills or lungs? We shall stop our comments there to avoid entering controversy. Our goal was only to return to sources of ideas to clarify what is considered to be von Bertalanff's metabolic interpretation of growth.

4

Bioenergetics of Organisms: Methods

> In water direct measurements of heat loss and excretory losses are virtually impossible, so energy budgets for aquatic animals are always approximations.
>
> (O'Dor and Wells, 1987)

The aim of this chapter is to analyse the main techniques which can be utilized to establish energy budgets of organisms and to compare their performances. It is therefore essentially a reflection on methodological approaches and not a practical account of a series of technical protocols.

As all components of the energy budget, with the exception of respiration, are evaluated using masses of organic matter, one section will be devoted to this subject. Another will be confined to calorimetry, a technique unique to energetics. Afterwards, physiological methods based on experiments and ecological methods based on statistical treatment of data obtained *in situ* will be examined. Lastly, we shall study mixed methods which seek to combine the advantages of the previous two methods.

4.1 The processing of organic matter

In an energy budget, all the components except respiration *(R)* are expressed as values of biomass, where only organic matter is taken into account. The ash-free dry mass (OM) must therefore be calculated (Table 1.3). For this two processes are necessary: the elimination of water and the measuring out of ashes.

4.1.1 Elimination of water

The problem of drying or desiccation of tissues and other substances is fundamental. The most frequently used method is the use of an oven at 100°C. But consequences of this method are the melting of lipids, which pour out onto the surfaces of the receptacle, and evaporation of certain volatile fatty acids. Freeze-drying has the disadvantages that it does not remove all the water and it can only be used for small amounts of tissue or substance.

Drying using a desiccant is the most reliable way. It is preferable to use silica gel. To avoid a lengthy operation (up to 20 h), it is advisable to work in a vacuum (Beninger, 1982; Ivell, 1983).

The last method is the most exact and should be used when great precision is required. Freeze-drying results in a constant overestimate and therefore allows comparisons and calculation of relative values. To obtain exact absolute values, it is necessary to multiply the results obtained by a correction factor (corresponding to the proportion of water remaining in the freeze-dried material). This correction factor can be calculated by drying using a desiccant. Oven-drying is to be avoided, because of the loss of fatty matter (Beninger, 1982).

4.1.2 Measurement of ashes

To measure ashes, the usual method consists of destroying the organic matter by burning, weighing the ashes and therefore establishing the values of OM by difference.

For tissues or products low in ashes, Grove *et al.* (1961) advise incineration for 24 h in an oven at 550°C. For calcified tissues or substances, the use of the oven is more delicate, because if the temperature is too high, it results in decomposition of calcium carbonate and release of CO_2. This decomposition occurs from 460°C and, at 550°C, the overestimation of organic matter corresponds to a multiplication by 2.5 (Goulletquer and Wolowicz, 1989). Also, in the case of calcified matter, burning must be carried out in a regulated oven at 450°C±5°C for 36–48 h. A lower temperature means there is a risk of incomplete destruction of the organic matter.

For calcified matter, an alternative to burning is elimination of calcium carbonate by decalcification or chemical extraction. Chemical extraction can be carried out using EDTA at pH 7.4 or various acids such as a sulpho-perchloric mixture (sulphuric acid 35% vol., perchloric acid 65% vol.) or 10% trichloroacetic acid (Pujol *et al.,* 1970). When decalcification is complete, the solution is filtered and the remaining material is rinsed in distilled water, then dried and weighed. A subsample is transferred to the oven to estimate the amount of ashes remaining. This method tends to underestimate the quantity of organic matter (Figure 4.1) but has the advantage of allowing subsequent chemical analysis of the organic matter obtained.

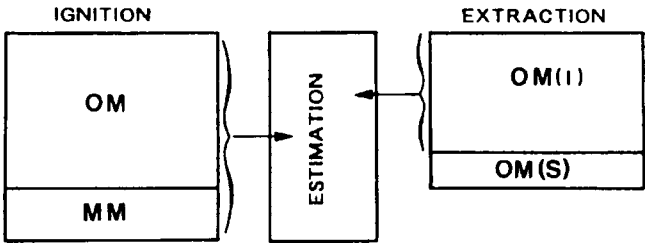


Figure 4.1 Diagram showing possible errors in the estimation of organic matter (OM) in a calcified product. Using burning, not only is the organic matter destroyed, but some of the calcium carbonate is lost as CO_2 (MM). This leads to over estimation of OM. Using extraction, the soluble part of OM is not taken into account, leading to underestimation of the total amount of OM. MM, inorganic matter; OM(I), insoluble organic matter; OM(S), soluble organic matter.

As we have seen (section 1.5.1), the masses of organic matter (OM) are converted into units of energy, either by direct measurement using calorimetry, or by the use of energetic equivalents. The latter have been examined in section 1.6.2 and calorimetry will be treated in section 4.2.

4.1.3 Elemental analysis

The chromatographic CHN analyser is currently used in ecology and, in certain cases, its use appears in bioenergetics. The advantage of this technique is the speed of processing, which allows multiplication of samples and in this way the refinement of the study of a phenomenon. The disadvantage, in bioenergetics, is the destruction of the organic matter, which leads to obtaining only relative values expressed either in carbon or in nitrogen.

By this method, Anger (1990) established 'budgets' for C and N which follow one another during the course of larval development of various crabs. Using relative values of carbon for respiration *(R),* nitrogen for excretion *(U),* nitrogen and carbon for ingestion *(I),* growth (P_G) and secretions (P_s), he was able to calculate assimilation, growth and production efficiencies. An example based on these principles is analysed in section 5.1.3.

4.2 Calorimetry

4.2.1 Preliminary remarks

Calorimeters are a means of measuring release or absorption of heat resulting from chemical reactions or metabolic activity of living organisms. More specifically, bioenergetic procedures carried out in calorimeters are of two types: either measurements of energy released by the destruction of various substances (usually organic matter), or measurements of heat released per unit of time by living organisms as a function of their state and activity.

According to this concept, a calorimeter measures either changes in internal energy ΔU or variations in enthalpy ΔH, at the time of chemical reactions. As we saw in section 1.2.2, ΔU is equal to the heat of reaction at constant volume (Q_v), ΔH is equal to the heat of reaction at constant pressure (Q_p). The use of thermocouples has allowed improvement of the precision of calorimeters. A thermocouple is a metallic circuit made up of two metals joined by two welded junctions (Figure 4.2). A difference in temperature between the two junctions creates an electromotive force *(E)* directly proportional to the temperature gradient:

$$E=K(T_h-T_c).$$

This is the Peltier-Seebeck effect.

By using *differential* calorimeters, a large number of causes of error are eliminated. This technique consists of using two identical or non-identical containers, from which temperature differences can be detected by a thermocouple (Figure 4.4).

A calorimeter that attains great precision and which can, therefore, measure small quantities of heat or work on small quantities of material is called a *microcalorimeter.*

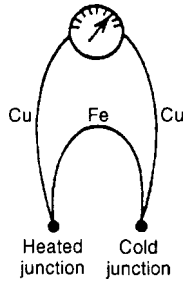


Figure 4.2 Diagram of a thermocouple (Tonnelat, 1968). Cu, copper; Fe, iron.

4.2.2 Adiabatic calorimeters

Adiabatic calorimeters do not exchange heat as they are surrounded by an insulated container. They are called heat-retention calorimeters. The simplest of the adiabatic calorimeters is that of Berthelot (Figure 4.3). During a chemical reaction, a thermometer allows the assessment of the heat of reaction. This type of calorimeter measures variations in internal heat, as it operates at constant volume (Q_v).

Berthelot's calorimeter has been modified in different ways. One of these models, represented in Figure 4.4, is an isothermic calorimeter. It consists of a hermetically sealed container R_1, corresponding to the internal part of Berthelot's calorimeter. To avoid all loss of heat by R_1, container R_2 connected to R_1 by a thermocouple is heated by a resistance, in such a way that there is no temperature difference between R_1 and R_2. The wall E is insulated to avoid all loss of heat by container R_2.

Adiabatic calorimeters can achieve good performances, such as the microcalorimeter of MacEwan and Anderson (1955), which was designed to measure the heat of combustion of biological material weighing about 50 mg.

4.2.3 Heat-flux calorimeters

In these calorimeters, exchanges of heat between the container and the external wall are not suppressed as in adiabatic ones, but on the contrary are favoured (Figure 4.5). They are also called heat-diffusion calorimeters. In effect, it is the flux (f) of heat which is measured: $f=p(T_{int}-T_{ext})$ where p represents the conductivity of the

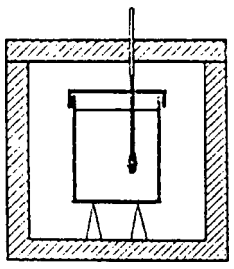


Figure 4.3 Berthelot's calorimeter (Tonnelat, 1968).

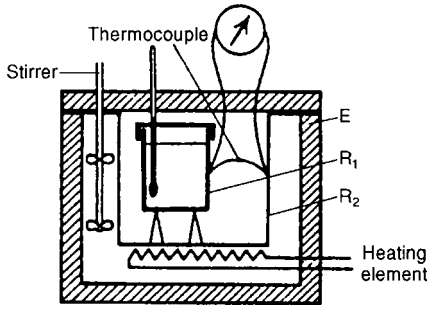


Figure 4.4 Adiabatic, isothermic calorimeter with differential setting (after Tonnelat, 1968). See text.

medium which separates the two containers. A set of thermocouples between these provides a good measurement of heat flux as a function of time. The Tian-Calvet microcalorimeter allows measurement of heat released, either by a chemical reaction, or by the catabolic activities of living organisms. Nevertheless, Calvet and Prat (1958) only give measurements carried out on non-aquatic animals or plants. According to Senez (1973), this type of calorimeter measures Q_p.

A more recent type of microcalorimeter is fully described by Phillipson (1964) and has been widely used by biologists. This apparatus consists of a small-capacity bomb calorimeter (5–100 mg of dry matter) in which combustion is carried out by electrical ignition. Eight thermocouples, which link a ring of copper surrounding the bomb to an aluminium base, are connected to a potentiometer. According to Phillipson (1964), his apparatus 'has a precision better than 2.5% with biological materials'.

4.2.4 Aquarium calorimeters

If one refers to the quotation at the beginning of this chapter, there would be no calorimetric measurement possible for aquatic animals. This statement appears to be

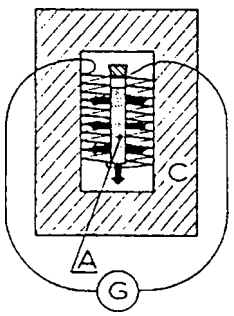


Figure 4.5 Tian-Calvet microcalorimeter. Most of the heat produced is conducted into the outer container by thermocouples which surround the internal container. The electromotive force of the thermoelectric battery made up of thermocouples is measured and is proportional to the calorific power (Js^{-1}). A, internal container where the reaction occurs; C, external container with good heat conductivity; G, galvanometer (reproduced from Calvet and Prat, 1958).

refuted by the existence of aquarium calorimeters, but as we have seen, this type of apparatus is rare, imprecise and can only be used for small animals. They belong to one or other of the two types previously studied, as is shown by the following two examples.

The aquarium calorimeter of Lowe (1978) consists of a 9 cm-long tube with a volume of 100 cm^3, thermally isolated by a double wall separated by a vacuum. In this tube, water circulates with a flow of 4.3 $cm^3\ min^{-1}$. Temperature is measured at the inlet and outlet of the tube. It is therefore a heat-diffusion calorimeter where water plays the role of heat diffuser. The stickleback was used in experiments in this apparatus by Lowe (1978). A similar type of apparatus, only smaller, is described by Brafield and Llewellyn (1982).

The differential adiabatic microcalorimeter of Hammen (1979, 1980), to which a respirometer is connected, simultaneously measures oxygen consumption and heat released from small marine animals. The methodological interest of coupling heat released and oxygen consumed, is to verify whether oxygen consumption covers all the catabolic activities (section 3.1.2). Hammen's apparatus consists of a 280 cm^3 Thermos flask (Dewar flask surrounded by thermal insulation). The vessel contains oxygen-saturated sea water and the animals; it is closed by a rubber stopper through which run the probes of a quartz thermometer (precision 0.0001°C) and a polarographic oxygen meter. Another, identically instrumented Thermos flask, but without animals, acts as a reference.

A full explanation of simultaneous measurements of oxygen consumption and direct calorimetry can be found in a study by Gnaiger (1983).

4.3 Experimental methods

We shall study in succession the equipment used and the physiological functions measured.

4.3.1 Experimental aquaria

Installations without water circulation

This is the most simple model. The basic unit consists of a tank containing water, with volume and shape suitable for the project. The set-up is linked to different measuring devices and can consist of two or more similar tanks, one of which acts as a control. An example is the set-up used by Parsons (1990) for studying a shark. The aquarium is annular and measures 113 cm×240 cm×40 cm. A polarographic electrode measures the oxygen tension. The experiment is interrupted at 50% saturation, which nevertheless allows measurements to be carried out for 11 h at a stretch. By using a stopwatch and a scale on the base of the aquarium, it was possible to calculate swimming speed, knowing that the sharks always move along the outside edge.

Installations with recycled water circulation

A circuit is established in such a way that water passes through similar experimental chambers,

arranged in parallel. Measuring devices are connected to successive chambers in turn, according to a predefined sequence.

The problem is to maintain a constant water quality throughout the experiment. It is therefore necessary that the principal characteristics of water (temperature, oxygenation, salinity, pH, contents of ammonia, nitrites, nitrates and urea, concentrations of mineral and organic particles, dissolved organic matter content, density of bacteria) be relatively constant and that they not exceed certain critical values. In this list of water quality parameters, the values of the first four can be voluntarily fixed by the experimenter, while the contents of nitrogenous products, organic matter and bacterial density can only be recorded.

Nitrogenous products come either from animals' urine, mainly consisting of ammonia, or from the bacterial degradation of organic matter: faeces, unused food. This degradation also results in the formation of ammonia. An equilibrium exists between the molecular form (NH_3) and the ionized form, ammonium (NH_4^+). According to Spotte (1970), this equilibrium is a function of temperature and pH. The higher the values of these two parameters, the more the molecular, un-ionized form is favoured. For example, at pH 7 and at 10°C there is 0.3% NH_3, whereas at pH 9.5 and at 20°C, there is 62.1%.

Under the action of specialized bacteria *(Nitrosomonas),* ammoniacal compounds are oxidized into nitrites (NO_2) which in turn are transformed by other bacteria *(Nitrobacter)* into nitrates (NO_3).

The toxicity of nitrogenous products decreases in the order: NH_3, NH_4^+, NO_2, NO_3. According to Terver (1979), the maximum acceptable quantities in an aquarium are: for NH_3, 0.01 mg l^{-1}; for NH_4^+ and NO_2, 0.1 mg l^{-1}; for NO_3, 100 mg l^{-1}. A newly installed circuit contains many intermediate products of nitrification until the nitrifying bacteria settle in, a process which requires several days or some weeks. Organic matter, particulate or dissolved, must be eliminated, because of the toxic products released on its decomposition. Particulate organic matter can be removed manually, using a suction hose for example. According to Alayse (1979), bubble filters are a means of eliminating organic matter, especially proteins (Figure 4.6).

The bacterial flora plays a beneficial role in nitrification and must therefore be protected. This specific microflora usually settles on various supports, notably on those made of calcium carbonate (such as shells). Nevertheless, among the bacteria which circulate in sea water, some, e.g. *Vibrio,* can be pathogenic to experimental animals and may reach contaminating levels. It is therefore appropriate to limit their proliferation. Alayse (1979) advises the partial sterilization of the circulating water by ultraviolet rays and advises against the use of antibiotics. Certain authors recommend ozone.

To illustrate these different recommendations, a model circuit with recycled water is described in Figure 4.7. A large amount of technical documentation on closed recirculation systems can be found in Maurel (1984).

Installations with non-recycled circulating water

In systems located at the edge of the sea, river or lake, the water is rarely pumped directly because of the sudden variations in its quality from tidal effects or meteorological conditions. Usually, a reservoir is placed between the natural environment and the experimental apparatus. With this type of installation, rapid variations in water quality can be avoided.

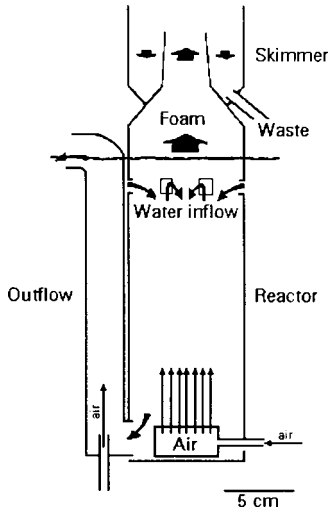


Figure 4.6 Diagram of a bubble filter. The apparatus has three parts: the skimmer, where bubbles are expelled; the reactor, where water to be purified enters at the top and air at the bottom; and the outflow pipe, which allows the renewal of water to be filtered (after Alayse, 1979).

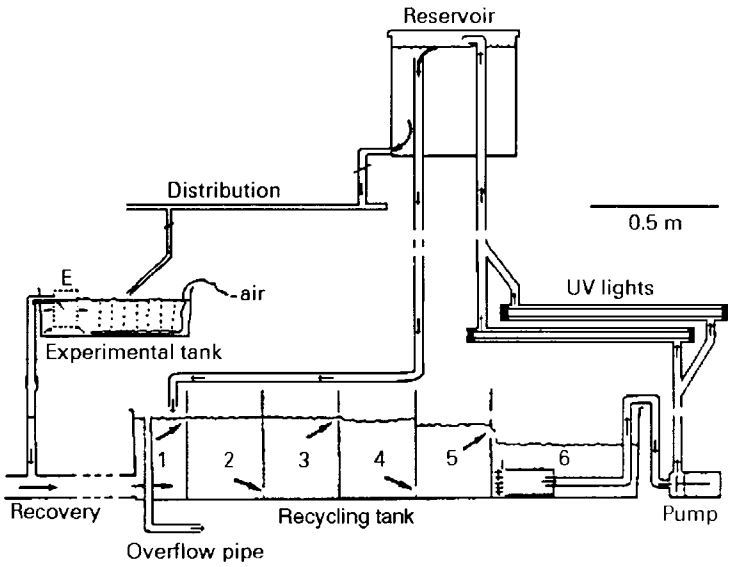


Figure 4.7 Diagram of a sea water recycling circuit. A single experimental tank is shown: it is possible to have several in parallel. The recycling tank consists of (1) skimmers, (2 and 3) pieces of ringed plastic which favour clarification, (4 and 5) calcareous debris which regulates pH and acts as a support for nitrifying bacteria, (6) green algae which lower the nitrate concentration. E, bubble filter (after Alayse, 1979).

These variations happen regularly as a result of seasonal effects, or can result from chance events (phytoplankton blooms,
eutrophication, red tides or other forms of pollution). Local conditions are therefore determining factors and must be known. However, this influence of natural conditions can also be put to good use. For instance, at the IFREMER Laboratory in La Tremblade, water from the reservoir can be used either freshly pumped with simple filtration through a 250 µm mesh, or several days later, after being left to settle (fewer mineral particles, but more phytoplankton, which has multiplied). In the first case, the conditions are close to those of the Seudre Estuary; in the second, the conditions are similar to those of an oyster bed (section 7.2.1).

4.3.2 Measurement of functions

Consumption of food by microphages

In microphages, measuring consumption *(C)* is difficult, because the retention rate is a function of the concentration of the food particles. Thus, despite final corrections, measurements made in a closed system, where the particle concentration decreases progressively as a result of consumption by the experimental animal, do not give an exact value for *C*. Only set-ups that maintain constant particle concentration can be used to resolve this problem (Figure 4.8).

Oxygen consumption

Although some respirometers have been conceived that could be used *in situ* (Svoboda and Ott, 1983), measurements of oxygen consumption are most often made in the laboratory. There are two kinds of measurement.

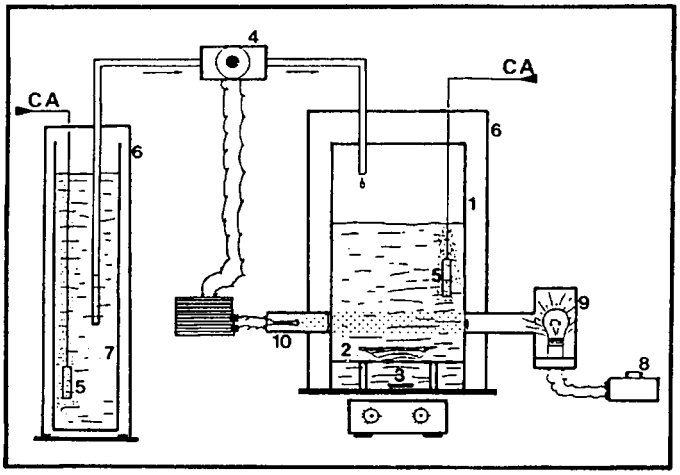


Figure 4.8 Device allowing the measurement of phytoplankton consumption at a given concentration. By means of a photoelectric cell and an electronic relay, when the concentration decreases noticeably, a certain amount of phytoplankton is added to the experimental chamber. CA, compressed air; 1, clear acrylic wall; 2, perforated support; 3, magnetic flea; 4, peristaltic pump; 5, air diffuser; 6, opaque cylinder; 7, graduated cylinder containing concentrated suspension of phytoplankton; 8, rheostat; 9, bulb; 10, photoelectric cell (after Suprapto, 1986).

In a *closed respirometer,* the oxygen pressure decreases progressively as a result of the respiration of the animal. This method is not accurate as the oxygen consumption depends on the partial pressure of oxygen in the water.

The *circulating water respirometer* has a constant oxygen content at the entrance to the respiratory chamber, which avoids the disadvantage of the previous method. Nevertheless this method is more delicate, particularly in that (1) the water flow must be constant and precisely measured, and (2) the difference between the results measured at the entrance and exit of the respiratory chamber must be sufficiently great to be measurable.

For swimming species, as well as respiratory chambers, *swimming tunnels* are used. These consist of a wide tube, usually made of clear acrylic, in which water flows at a rate that can be regulated and measured. Fish, as well as cephalopods, swim against a current. While maintaining position, they are expending energy and therefore R_A can be measured. When the flow stops, measurement of R_S is possible. The transparency of the tunnel allows multiple observations. This kind of apparatus was originally used by Brett (1964) for fish (salmonids) and by O'Dor (1982) for cephalopods (squid).

Two methods are currently used to measure oxygen: either chemical analysis using the *Winkler method,* which gives results in oxygen concentration (C_{O2}) or the polarographic oxygen sensor which gives results in oxygen pressure (P_{O2}). Wide coverage of the subject can be found in the multi-author work edited by Gnaiger and Forster (1983).

Henry's equation allows the conversion of P_{O2} to C_{O2}:

$$C_{O2}=aP_{O2}$$

where a is the solubility coefficient of oxygen in water. This coefficient, given in tables, varies in relation to temperature and salinity. Knowing the flow rate Q, expressed in $l\,h^{-1}$, the oxygen consumption in moles per gram animal and per hour can be calculated:

$$MO_2 \text{ mol } g^{-1}\ h^{-1}=a \text{ mol } l^{-1} \text{ torr}^{-1}\ (P_{O2e}-P_{O2s}) \text{ torr } Q\ l\ h^{-1}/M(g)$$

where M represents the mass of the respiring animal. This mass is traditionally expressed as dry weight (M_s) for invertebrates and wet weight for fish (M_v).

In order to convert moles to volume of oxygen, the formula $PV=nRT$ is applied, where: P is partial pressure of O_2 (torr); V is volume of oxygen (l); n is number of moles; R is the gas constant, 62.4×10^3 torr K^{-1} mol^{-1}; and T is absolute temperature in water (K). Note that 760 torr=1 atm=101 325 Pa.

In order to measure small oxygen consumptions *(microrespirometry),* precision devices are used at constant temperature. Three types can be described.

Warburg's apparatus consists of a small respirometry chamber containing a potash container which absorbs released CO_2. The decrease in pressure is measured using a manometer connected to the chamber.

The *cartesian diver* is even more sensitive and allows the measurement of consumptions between 0.1 and 0.01 $mm^3\ h^{-1}$, the minimum being 0.00002 $mm^3\ h^{-1}$ (Kayser, 1963). The respirometry chamber consists of a cartesian diver which, to start with, has the same density as water. This density increases as a result of the absorption of oxygen by the living organism and CO_2 by the potash. By lowering the pressure at the surface of the liquid, the cartesian diver is allowed to regain its initial position. This technique is particularly useful for larvae of, for example, gastropods (Erickson, 1984).

In *Grunbaum's apparatus,* where the respirometry chamber contains 2 cm^3 of sea water, CO_2 is also absorbed by potash and the decrease in volume is noted from the displacement of a liquid index in a capillary tube (Grunbaum *et al.,* 1955). This apparatus has been used notably by Vernberg and Costlow (1966) on crab larvae and by MacInnes and Thurberg (1973) on oyster larvae.

Nitrogenous excretion

It is necessary to treat the products of nitrogenous excretion separately, because of their dissolved state and hence the impossibility of separating them from the animal's environment. Unlike for terrestrial animals, from which it is easy to collect all urine, only an approximation can be made for aquatic animals, by measuring changes in nitrogenous products in their surroundings.

In aquatic animals, nitrogenous excretion is mainly in the form of ammonia. Thus, ammonia represents 60–90% of nitrogenous excretion in the bivalves (Bayne and Newell, 1983) and the same is found in the teleosts (Brafield, 1985). As we have seen previously, ammonia appears either in molecular (NH_3) or ionized (NH_4^+) form, but analytical methods do not usually differentiate between them and measure the total ammoniacal nitrogen.

As well as NH_3 and NH_4^+, other inorganic substances are involved which, classically, are not taken into account in energy balances. Most bioenergeticians consider them to be outwith the balance. Nevertheless, some authors have established the 'energetic value' of ammonia. For example, Brafield (1985) estimated it to be 349.9 kJ mol^{-1} or 20.5 J mg^{-1}.

In contrast, urea and uric acid are synthesized internally by the animal and therefore correspond to an energy expenditure, which must be taken into account. The proportions of urea and uric acid in the urine can change seasonally, so it is necessary to carry out several analyses throughout the year.

The presence of amino acids in urine is proven, but it usually corresponds to states of stress which are usually temporary. As we have said previously, the excretion of amino acids is counterbalanced by their absorption: it is therefore a matter of establishing a net balance resolution in either a gain (nutrition) or a deficit (excretion).

Other experimental measurements

In experimental tanks, other measurements can be made, of which we shall give some examples.

Faeces production rate (F_2) as a function of food type can be determined by using a faecal marker in the food ration being studied. This marker must be insoluble, non-toxic and indigestible in order to be found in the faeces, where it can be recognized, for example by its colour. This is the case for chromium oxide (Cr_2O_3), by far the most widely used marker. Talbot (1985) gives examples of other faecal markers for aquatic animals.

Rate of mucus production (P_{S2}) remains a delicate task, all the more because production increases in animals in a state of stress. Nevertheless, Kideys and Hartnoll (1991) have given a full account of a protocol applicable to molluscs and have shown the intensity of this secretion (27% of ingested energy in the whelk).

Rate of cellular renewal (P_{E2}) is determined by an autoradiographic or biochemical method, either using a marked thymidine precursor, which reveals nucleic synthesis and therefore cellular mitoses, or using an amino acid which reveals protein synthesis, or even

by marking characteristic molecules in the cell (Pascaud, 1989). Few results are known for aquatic animals, as most effort in this area has been directed towards mammals. It can be noted that the renewal rate varies greatly between tissues: zero in cardiac and nerve tissue, low in the liver (400 days in the rat), fast in the integuments and digestive tract, particularly in the intestine (3 days in the dog).

Reproductive production (P_R) can be determined experimentally for a single emission of gametes. In effect, methods based on induced spawnings are especially suitable for fish and molluscs. To estimate P_R it is ensured that the spawning is not disturbed, males and females are separated and spawning products are collected using fine filters, and the energy balance is estimated from several spawnings. The results obtained from this method are generally underestimates, either as a result of the loss of soluble substances which accompany the gametes or through loss of part of the products during handling.

For example, Wilde and Berghuis (1978) studied the spawning of *Macoma baltica* in the laboratory, as the only method of determining P_R for this species is from induced spawning. The authors use thermal change, going from 5°C to 12°C in 5 minutes. Individuals that start spawning are isolated. Spawned eggs and sperm are collected using Millipore 0.15 μm mesh filters, rinsed in distilled water, then dried in a desiccator. The individuals that spawned are marked and used in further experiments on following days: in this way the gonad is finally completely, or nearly completely, empty after spawning a series of egg batches.

4.4 Ecological methods

4.4.1 Measurement of retained production

When production is cumulative, either living (tissue) or inert (secretion), the amount produced is the difference between the values measured at the end Q_{tn+1} and the start Q_{tn} of a certain period. In general, to find out these values, it is necessary to kill the animal. Therefore a batch of standard animals, maintained *in situ,* is worked on and for each sample a certain number of animals are taken to obtain a mean value. It therefore acts as a good ecological measurement.

Tissue production

In order to find out the difference in tissue mass between two samples, it is always necessary to kill the animal. In certain cases (annelids, for example), the whole animal is dehydrated and ash content measured: the value of OM corresponding to P_G is therefore obtained. In other cases (molluscs, for example), the flesh is separated from the shell in a way that avoids decalcification.

If the value of Q_{tn+1} is less than that of Q_{tn}, P_G is negative. This situation arises from winter weight loss or spawning of gametes.

Secretion production

Certain secretions, for example mouthparts, annelid spines and bristles, cephalopod beaks, gastropod opercula and bivalve byssus, are made of organic matter. However, the secretions are most often calcified. This is the case for certain opercula and byssus, like that of the

bivalve *Anomia* (Pujol *et al.,* 1970) and many others: calciferous sponge spicules, anthozoan skeletons, bryozoan zoecium, brachiopod and mollusc shells, crustacean carapaces, echinoderm tests, etc.

4.4.2 Measurement of eliminated production

Reproductive products

The cost of reproduction (P_R) is most often measured using ecological methods. The principle is as follows. Two samples are taken from individuals from a natural/wild or captive population, one before and one after reproduction. In order that P_R can be estimated from the difference between the two values, it is absolutely necessary that, in the population being studied, the emission of gametes be synchronized. If there is great individual variability in the progression of reproductive activity, this method cannot be applied—for example, the population of *Pecten maximus* in Brest Harbour (Paulet *et al.,* 1988).

When spawnings are synchronized, there are two ways of evaluating P_R, according to the species. Some have well-defined gonads which can be separated from the rest of the organs and weighed on their own. The difference in their mass before and after spawning allows the value of P_R to be calculated. Other species have gonads that cannot be separated from the other organs (e.g. gastropods, bivalves, annelids); in this case the tissues are weighed together and it is supposed that the mass of the other tissues remains constant, before and after the emission of gametes. The difference in weight before and after spawning therefore represents the weight of spawned reproductive products.

To ensure that samples are taken at the right time, i.e. 'just before' and 'just after' spawning, the phases of reproduction must be closely followed. When certain indices indicate that spawning is imminent, the first sample is taken and a number of days later a second, to verify the state of the gonads of the individuals sampled. This procedure, which is strictly the only possible method, is rarely followed. Most often, authors content themselves with taking monthly samples and base their calculations on changes in tissue weight: this always leads to an underestimate of P_R. In fact, when the tissue mass is at its highest, the gonad is not necessarily at its heaviest; conversely, when the tissue mass is lowest, the gonad is not inevitably empty as it can start to mature or accumulate reserves.

Eliminated tissue

The evaluation of violently eliminated tissue (P_{E2}) is carried out using ecological methods. For example, if we consider the predation on bivalve siphons by fish, there are two complementary approaches: the study of the bivalves or of the fish. Hodgson (1982a,b) examined the effect of predation on a bivalve with a long siphon, *Scrobicularia plana*. The amputation had only a small effect on the animal, which closed its valves for only 14–15 seconds. The cut siphon remained functional and the animal repaired it, its original length being attained in about 4 days. When 50% of the siphon was taken by the predator, this represented a loss of 0.2 kJ but required 0.6 kJ for regeneration. The author does not indicate the frequency of this predation.

4.5 Differences between the two measures

4.5.1 Time and measurements

Frequency of measurements

Before examining the differences, regarding time, between the two methods (physiological and ecological), it must be noted that the frequencies of measurements are chosen in relation to the developmental stages of the organisms (Table 4.1). These stages vary from one zoological group to another; the example in the table is that of a bivalve and on average this can be applied to many marine invertebrates. Thus, for the perizygote stage, which corresponds to the preparation and formation of the zygote, Epel (1978) gave the sequence of events observed in the sea urchin. The unit of measure is the second, but the scale is logarithmic, because it extends from 1 to 6000 s and the first events are more frequent and numerous (e.g. ionic exchanges) than the last (e.g. cell division).

To return to the comparison of the ecological and physiological methods: it is noted that the frequency of measurements hardly differs from one to the other. Indeed, when a sample is taken *in situ,* experiments are carried out in the laboratory at the same time. If the frequency of the measurements is the same, the same number of data, according to an identical rhythm, will be obtained. However, they do not have equal significance, as we shall see hereafter.

Duration of measurements

The duration of a measurement is of a different order in ecological and physiological methods. In the former, the entire time period between two successive measurements is taken into account, whereas in the latter the period is limited to that of the measurement. Thus, for growth experiments, the result obtained from two consecutive measurements corresponds to the period of a month, if samples are taken monthly. In contrast, for respiration, the results correspond to the duration of the experiment, which is most often about an hour.

The consequence of this temporal heterogeneity in the information is the inequality of the precision of the measurements, which can cause bias in the results. In order to reduce this bias, certain rules must be followed.

When P_G and P_{S2} are measured monthly (monthly energy balances, indicators of seasonal changes in metabolism), the values of *C, F, U* and *R* which have been

Table 4.1 Developmental stages and frequency of measurements in a bivalve (e.g. a mussel). The nomenclature for the stages is that of Lucas (1984)

Developmental stage	Interval between measurements
Perizygotic	Seconds, then minutes
Embryonic	Hours
Larval	Days
Postlarval	Days
Juvenile	Weeks
Adult	Months

Table 4.2 Conditions for measurement of metabolic parameters for a microphagous species. The symbol * indicates that the animal dies after the measurement

Parameter	Site	Duration	Climatic factors
P_G	*In situ**	Months	Uncontrolled, variable
P_{S2}	*In situ**	Months	Uncontrolled, variable
P_R	*In situ**	Several days	Uncontrolled, variable
P_R	Laboratory	Several hours	Controlled, variable
C	Laboratory	Hours	Controlled, constant
F	Laboratory	Several hours	Controlled, constant
U	Laboratory	Hours	Controlled, constant
R	Laboratory	Hours	Controlled, constant

established for 1 h can only be multiplied by (30×24)=720 if it is certain that these phenomena are constant and that, in particular, they are not subject to circadian changes, of diel (24 h) or tidal (24 h 50 min) origin. Where this is not the case, a weighting is imposed.

For annual energy balances, P_G and P_E are measured at the same time in two consecutive years. However, the hourly values of *C, F, U* and *R* cannot always be multiplied by (365×24)=8760 because it is well known that there are seasonal differences in these values. To be more careful, it would be best to measure them every month. Finally, to discover the precise number and intensity of spawnings of gametes (P_R), observation is required at least once or twice a week during the reproductive period.

4.5.2 Conditions of measurement

As indicated by Goulletquer *et al.* (1989, p. 105), 'if, between two samples, the animal in the natural environment integrates fluctuations in its surroundings, measurements in the laboratory remain instantaneous'. Not only are these measurements of a short time scale, but also the climatic factors are constant and even arbitrary, even if the experimenter attempts to reproduce the natural conditions as well as possible. In the ecological method, the animal is killed at the end of the measurement, which is not the case with the physiological method. Table 4.2 summarizes the differences between the two methods.

4.6 Mixed methods

In research to improve the validity of measurements, different avenues have been explored. One of these consists of carrying out biochemical analyses without killing the animal. Another route involves no longer carrying out experiments in the artificial surroundings of the laboratory, but in the natural environment. Appro-aches of this kind have grown in number

and some of them have been successful. In other cases, the aim is to verify results obtained in the laboratory using those obtained *in situ.* All these methods have the shared characteristic of bringing together ecological and physiological methods and it is for this reason that they are called *mixed*.

4.6.1 Quantitative analysis on live animals

Magnetic resonance spectrometry or nuclear magnetic resonance (NMR) is a nuclear physics method which allows the quantification of certain chemical substances without disturbing the animal's metabolism. Gadian (1984) established the basis of this non-destructive and non-invasive method. The physical bases and principal biological applications can be found in Authier *et al.* (1990). Since 1973, results have been obtained by measuring the spin of certain nuclei of biological interest: ^{31}P, ^{13}C, ^{23}Na and more recently, ^{1}H. We gave an example in section 2.4.1, where ^{31}P was used (Lavanchy's index). For small animals, NMR is certainly the method of choice.

4.6.2 Experimental measurements in situ

To illustrate the diversity of experimental measurements carried out *in situ,* three examples are given, two on oysters, one on cephalopods.

The device for recording oyster valve movements conceived by His (1970) comprises elements sufficiently simple and robust to be used at sea. On an oyster previously fixed to a stable support, the upper valve is linked by a nylon thread to a mechanical recording apparatus. All contractions of the adductor muscle are transmitted to the valve and are registered on the calibrated paper of the recorder: short contractions correspond to the expulsion of faeces and pseudofaeces; stepped contractions preceding closure over a long period imply the cessation of respiration, feeding and excretion. Several oysters can be recorded simultaneously. The duration of the experiment is usually a week. A thermograph records water temperatures in parallel.

Particle traps can be adapted for the collection *in situ* of oyster faeces and pseudofaeces, notably in raised cultures (oyster tables). A model developed by Sornin is shown in Figure 4.9. One trap is placed underneath a certain number of oysters for 24 h, another underneath an empty area. The latter collects sediments naturally settling out. The quantities of biodeposits are calculated by difference.

To determine the energy expenditure of swimming of cephalopods, Webber and O'Dor (1986) used an ultrasound sensor transmitter. In this case, the sensor detects the pressure in the mantle cavity of a squid, *Illex illecebrosus*. The apparatus was placed as shown in Figure 4.10 and did not bother the animal at all. The first trials were made in a swimming tunnel, but according to O'Dor and Shadwick (1989) the experiment can be carried out on a free animal. The same technique was applied to nautilus, *Nautilus pompilius,* where the apparatus is fixed to the shell. To avoid all disturbance, the equipment has the same density as that of the animal (O'Dor *et al.,* 1990).

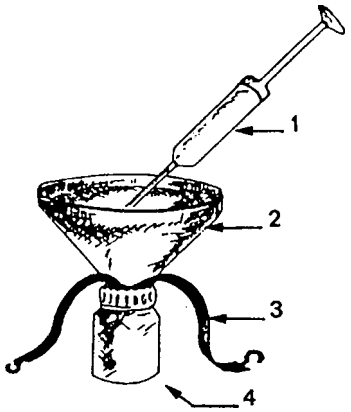


Figure 4.9 Particle trap at the moment of sampling. 1, sampling syringe; 2, container; 3, elastic fixing strap; 4, reservoir (modified after Sornin *et al.,* 1983).

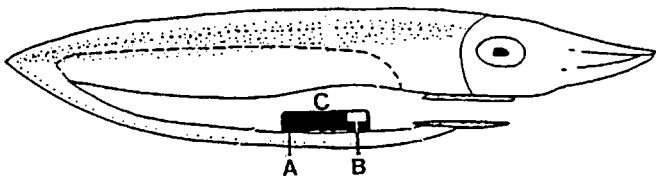


Figure 4.10 Catch-release ultrasound pressure transmitter (C) placed in the pallial cavity of a squid. A, fixing needle; B, cannula transmitting the external hydrostatic pressure (modified from O'Dor and Shadwick, 1989).

4.6.3 Parallel measurements

'The validity of extending experimental results obtained in artificial conditions in the laboratory to much more complex situations encountered by species in the natural environment is one of the central problems of much ecophysiological research.' This is the opinion of Truchot and Duhamel-Jouve (1980), who completed results obtained on the shore crab, *Carcinus maenas,* in the laboratory with *in situ* observations. This intertidal species lives in pools where the changes in oxygen concentration are subjected to a diel rhythm (high during the day, low at night) inverse to the changes in CO_2. The animal maintains aerobic metabolism by adjusting the flow of water across the gill chambers (gill ventilation). After having demonstrated, in the controlled conditions of the laboratory, that the gill ventilation rate depends only on P_{O2}, and not on P_{CO2}, the authors asked themselves if, in the unceasingly variable conditions of the intertidal environment, the regulation would also only obey this factor. Observations *in situ* confirmed this explanation. Why not work directly in the natural environment? The authors respond: 'the conclusions which could be drawn were to a large extent based on the results obtained in the laboratory, where the most effective methods could be used'.

5

Bioenergetics of Organisms: Analysis of Results

> Far from being static, energy budgets change both with immediate environmental conditions and with age. (Pianka, 1981)

The formulae in Chapter 3 were developed for a living organism, i.e. for an individual. When the results are arrived at, it is noted that the 'individual' energy budgets are not obtained from individuals taken at random, but from individuals representative either of different classes of a natural population or of different batches of a reared population. This representative individual is termed a standard animal and is precisely defined in a statistical way. In the results given hereafter, it is therefore understood that all the values given have been calculated statistically, from measurements carried out on a large number of individuals.

In this chapter, our goal is not to establish an exhaustive summary of results obtained from different species of aquatic animals, but to analyse selected examples which show how bioenergeticians have resolved certain cases.

Finally, with reference to Pianka's quotation, we would not want to present the results as statistical data (and definitively accepted) but rather as data influenced by environmental and intrinsic conditions, notably the age of the animals studied. These two themes will appear in the rest of this chapter.

Two kinds of results will be presented: those which describe energy budgets and those which show the changes in energy budgets, either as a function of biomass or under the influence of different factors.

With regard to energy budgets, we shall distinguish two scales, which we propose to call *macrobioenergetic* and *microbioenergetic*. In macrobioenergetics, global energy budgets are established, based on equations which describe all fluxes of matter or energy through the system being considered. In microbioenergetics, energy budgets are established which are partial or limited, either in their field of investigation, or in their duration.

5.1 Macrobioenergetics

The energy budgets are grouped according to the modes of feeding and movement of the animals studied. To this end, we recall some definitions in referring to Peres (1976).

Macrophages are animals that capture prey that are large in comparison to their own size. The inverse is true for *microphages*. *Sedentary* species make only rare or weak movements. *Errant* or *mobile* species move actively relative to currents, either by swimming or by a combination of swimming and walking along the bottom. *Pelagic* animals live in deep water, *benthic* ones on the bottom, *demersal* ones near the bottom. In the pelagic environment, *plankton,* which is passive in relation to the movements of water masses (except for vertical migrations), is distinguished from *nekton,* which is capable of active swimming.

5.1.1 Mobile macrophages

Fish, cephalopods and crustaceans are the three predominant zoological groups amongst the mobile macrophages. Examples will be limited to the first two groups.

Although bioenergetics is limited to quantitative exchanges of matter and energy, it must be remembered that mobile macrophages have increased requirements for proteins in their diet. According to Tacon and Cowey (1985), in about twenty species studied (not only carnivores, but also herbivores and omnivores), the dietary protein requirement expressed in per cent of biomass varied from 35% to 55%. But these numbers only have a relative value when it is known, as shown by Ogino (1980), that the protein requirement decreases as the ration increases. In effect, Ogino established for juvenile trout and carp that when the daily ration (expressed in per cent of biomass) increased from 2% to 4%, the protein requirement decreased from 60% to 30%.

Energy budgets of reared fish

Long-term energy budgets established not over a year, but for shorter periods, in relation to rearing rates, particularly interest aquaculturists. The examples examined relate to two groups of fish which are cultured intensively, salmonids and tilapia.

Salmonid energy budget The example given in Figure 5.1 shows an energy budget for a rainbow trout, as a function of a type of food ration. When an immature animal is involved, it can be estimated that $P=P_G$. The efficiencies can therefore be calculated as follows:

assimilation efficiency A/C=73%
gross efficiency $P/C=P_G/C$=29%
net efficiency $P/A=P_G/A$=40%.

Tilapia energy budget Table 5.1 gives the energy budgets established experimentally over 30 days at 27°C for six rearing groups of tilapia of the species *Sarotherodon mossambicus* by Musisi (1984, in Brafield, 1985). The interesting fact about these energy budgets is that everything is measured, including consumption. In the original experiment, respiration was measured using two methods. The results were close and so only one series of values is shown. Note that the energy budgets from different experiments are not strictly equal to 100%, which is a result of the difficulty of taking measurements, even when the greatest care is taken.

Table 5.1 Results of 30-day rearing experiments on *Sarotherodon mossambicus* (modified from Brafield, 1985)

Experiment number	C (kJ)	P (kJ)	R (kJ)	U (kJ)	F (kJ)	$(P+R+F+U)/C$ (%)
1	427.9	56.8	294.5	35.6	50.7	102.3
2	585.9	34.2	444.0	40.1	109.3	107.1
3	553.4	71.1	247.3	52.1	103.2	85.6
4	633.5	60.8	218.5	36.0	152.5	73.8
5	654.1	82.1	388.0	34.1	178.6	104.4
6	540.7	38.5	393.4	30.4	122.1	108.1
Mean	566	57	331	38	119	

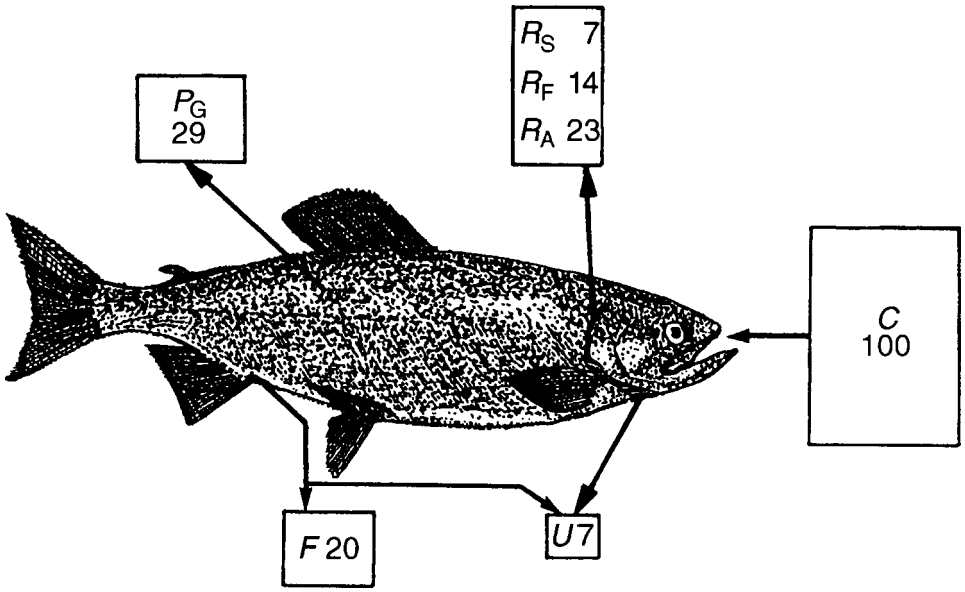


Figure 5.1 Energy budget for a farmed rainbow trout, *Oncorhynchus mykiss*. Figure produced from numerical data obtained by Alavoine (1981).

In this table, mean values have been established in order to calculate efficiencies:

assimilation efficiency A/C=68.5%
net production efficiency P/A=14.7%
gross production efficiency P/C=10%.

Production efficiencies are not as high as in the preceding example, but it must be remembered that in this experiment part of C was wasted, whereas in the previous case, it was assumed that all the energy in C was ingested.

The two examples just given concern reared fish. Only the production efficiencies could be calculated because the detailed values of P, established from growth in weight, were not given. Nevertheless, it is known that P_S and P_E are low in fish and can be considered to be

negligible, and that P_R is zero as the individuals are not reproducing. Consequently $P=P_G$. In this particular case, the values for growth efficiency would be very close to those for production efficiency.

Pelagic fish energy budgets: the tunas

The tunas are characterized by their adaptation to a pelagic lifestyle. In physiological terms, three particular examples can be cited (Kitchell *et al.,* 1978):

- the requirement to swim continuously because of the absence of a swim bladder and respiratory pump;
- the existence of a higher internal than ambient temperature as a result of the retention of metabolic heat obtained from special circulatory devices;
- a respiratory rate which appears to be independent both of ambient temperature and of the allometric effect of weight.

The measurement of energy budgets for tunas is made difficult by two phenomena:

- the difficulty of maintaining them in captivity, even for a short period, to measure food consumption, respiration, urine and faeces production. Only a few species have been observed experimentally, for example the skipjack tuna, *Katsuwonus pelamis;*
- their large size which makes it difficult to measure dry weight and chemical composition, data necessary for the calculation of bioenergetics in relation to biomass. For this reason, various empirical formulae have been developed.

Thus, 'metabolic biomass' (B_{met}) is calculated from the total biomass (B_f) as follows: $B_{met}=B_f^{0.8}$ (Sharp and Vlymen, 1978).

The proportion of lipids in wet tissues (M_v) is calculated in the following way: % of lipids in M_v= [weight of lipids] [4 (M_s - weight of lipids)]$^{-1}$. In this case, dry matter and lipid weights were calculated from a minimum sample size (Dotson, 1978).

With regard to respiration, because tunas have to swim constantly, Magnuson (1973) introduced the concept of *minimum speed,* V_{100}, the speed at which lift is just equal to body weight. *Net catabolism* (R_{net}) is the respiratory expenditure corresponding to standard respiration (R_S) plus movement at the minimum speed. So:

$$R_{net}=R_S+R_A\ (V_{100}).$$

However, the net catabolism of a fish in captivity (from which measurements are made) is about half, or even a third of that of an equivalent fish in the wild. Therefore, for fish living in the wild, the energetic consequences of double or triple net catabolism levels must be considered. The results obtained by Kitchell *et al.* (1978) for *Katsuwonus pelamis* are shown in Table 5.2. In this particular case, the components of the energy budget were measured over 1 day, from immature animals (therefore P_R=0) with a biomass of 1 kg, maintained at 24°C. From these measurements per day, the classic efficiencies can be calculated. As P is represented by P_G, P_R is zero and P_S and P_E are negligible, growth and production efficiencies are combined (Table 5.3). These calculations of efficiencies show that, irrespective of the kind of food ration given, assimilation efficiencies are nearly constant; however, growth and production efficiencies decrease greatly as ration increases. These efficiencies are only about half those found for tilapias and salmonids, which are adapted to rearing conditions and do not have to swim continuously.

Table 5.2 Energy budgets for three dietary regimes in the skipjack tuna, *Katsuwonus* pelamis, expressed in kJ day^{-1} after data from Kitchell *et al.* (1978). (See text.) Levels 1, 2, 3 are increasing rations

Diet	C/B (%)	C	F	U	R_{net}	R_F	P_G
Level 1	7.3	238	38	12	121	38	29
Level 2	13.1	414	63	21	238	63	29
Level 3	19.0	598	88	29	364	88	29

Table 5.3 Efficiencies (%) established for *Katsuwonus pelamis* from the data in Table 5.2

Efficiency	Level 1	Level 2	Level 3
$P / C = P_G / C$	12.2	7.0	4.8
$P / A = P_G / A$	15.4	8.7	6.0
A / C	78.9	79.7	80.4

Cephalopod energy budgets

General remarks The dimensions of cephalopods are very variable, from 1 g in the pygmy cuttlefish *Idiosepius* to 1 tonne in the giant squid *Architeuthis* (Boletzky, 1989). Although they are macrophagous carnivores, they differ in their lifestyles and bioenergetics (Table 5.4). In this table, it can be observed that group 2 lies between the two others, that the relative daily ration increases from group 1 to 3 while the growth efficiency decreases. To emphasize these differences, we shall study the bioenergetics of a benthic species and describe those of oceanic pelagic species in comparison to high seas fishes.

Benthic cephalopod The life cycle of the common octopus, *Octopus vulgaris,* in the Mediterranean has three phases: summer growth when the water temperature is about 20°C, winter maintenance in deep water at about 15°C and spring reproduction, after which the animal dies (O'Dor and Wells, 1987). The summer growth has been mainly studied. The results of rearing during this period are given in Table 5.5.

From these data can be calculated the assimilation efficiency 94%, gross growth efficiency 51%, net growth efficiency 54%. These efficiencies are remarkable and result from the excellent assimilation of food which is mainly used for tissue growth. However, these results are from rearing experiments carried out during the summer period, which is particularly favourable for growth. This does not detract from the fact that a 500 g *Octopus vulgaris* (Tables 5.2, 5.3) and a 1 kg *Katsuwonus pelamis* both attain the same production power of 370 mW, in other words the production per unit weight in the octopus is twice that of the skipjack. This confirms other data of O'Dor and Wells (1987), who concluded that a 250 g octopus at the start of the summer can reach 1 kg in 100 days and that during its lifetime, a 1 kg octopus has only consumed 2.5 to 3 kg of prey.

Table 5.4 Bioenergetic data obtained in captivity for cephalopods from three ecological classes. Group 1 : benthic species *(Octopus joubini, O. vulgaris, O. dofleini, Eledone cirrhosa)*. Group 2: coastal pelagic species *(Sepia officinalis)*. Group 3: deep-sea pelagic species *(Loligo opalescens, Illex illecebrosus)*. *B,* biomass; *C,* daily ration; P_G, daily growth. Values are expressed in percentages (modified from O'Dor and Wells, 1987)

Group	C / B	P_G / B	P_G / C
1	3.7	1.9	51
2	7.5	3.2	43
3	9.7	2.6	27

Table 5.5 Bioenergetic data (kJ day $^{-1}$) for *Octopus vulgaris* with a biomass of 500 g (from data of O'Dor and Wells, 1987)

C	E	A	R	P_G
63	4	59	27	32

Oceanic pelagic cephalopods Bioenergetic data on squid are incomplete because these animals of the high seas cannot be kept in good condition in captivity for any length of time. Their feeding and locomotory activities in the wild are poorly understood. Nevertheless, some observations have been made which allowed O'Dor and Webber (1986) to say that squid have the same lifestyle but not the same pace of life as oceanic fish.

The factors common to the two species are that they live in the deep sea, are carnivorous macrophages (and therefore competitors or antagonists), generally live in shoals and carry out vertical and/or horizontal migrations. The similarities end there.

They differ in their mode of swimming; squid use jet propulsion whereas pelagic fish use undulation of the body. Swimming by jet propulsion is disadvantageous as it is discontinuous. The most efficient squid uses twice the amount of energy to swim at half the speed of an average pelagic fish (O'Dor and Webber, 1986).

Respiration is very intense in the squids; even without pigment cells, they are, for temperature and size, the aquatic poikilotherms with the highest rate of oxygen consumption. The reason for this is the movements of the mantle which circulate water over the gills and, indirectly, activate the circulation of haemolymph. To support this high rate of catabolism, consumption is high in rearing tanks (Table 5.4) but appears to be more variable in the natural environment. A very effective chromatic camouflage allows squid to capture prey (mainly crustaceans) more successfully and to avoid predators (fish). Like the fish, their food ration contains a high proportion of proteins, for which their assimilation rate is 96%. Lipids are stored in the digestive gland, but only represent 5% of the biomass, compared with between 10% and 25% in fish. Glycogen reserves are similarly low: 0.4% of the biomass. Thus squid have almost no reserves. In fact, notably for sudden movements, they use their body proteins, aided by a high concentration of free amino acids and catabolizing enzymes in their tissues (O'Dor and Webber, 1986).

Juvenile squid grow very quickly (Table 5.4). They live for a short time, usually not more than 2 years. They are semelparous and have high reproductive effort: the mature gonads can represent 33% of the biomass (O'Dor and Wells, 1987). They very often undertake long migrations to reach their spawning grounds.

In summary, squid are bioenergetically very different from pelagic fish: the food which they capture, essentially protein, is quickly utilized by intense respiration and fast growth. They have almost no reserves. When reproduction commences, growth ceases. They only reproduce once and die soon afterwards (O'Dor and Webber, 1986).

5.1.2 Sedentary microphages

Most sedentary microphages are invertebrates. Many are *sessile* and *suspension feeders* (e.g. tube-dwelling annelid worms), others are *burrowing detritivores* (e.g. pectinarid polychaete worms) or *sediment feeders* (e.g. holothurians) and lastly the *grazers* are mobile, but move very little (e.g. limpets) (Peres, 1976).

General characteristics

The metabolic characteristics of sedentary microphages are very different from those of mobile macrophages. For clarification, let us examine the oyster, *Crassostrea gigas*. This species is sessile and therefore has no locomotory activity. It closes its valves during emersions in the intertidal zone, but it can also do this in the subtidal zone (section 4.6.2). When the valves are shut, both feeding and respiration cease. In fact, closure of the valves provokes a metabolic shut-down. It remains to be known whether the animal pays an oxygen debt and to what extent, and if a similar phenomenon occurs in feeding. When the valves are open, feeding is continuous and regular; there are therefore no true 'meals' and SDA does not exist. It is sufficient to measure a single value of R, corresponding to routine metabolism (section 3.1.2).

All the examples of macrophages we have analysed concern juveniles. This is not the case for the invertebrates, in which sexual maturity is generally reached in less than a year. Also the production of residual secretions is common. Thus P is no longer equivalent to P_G.

For many microphages, the measurement of food ration is difficult to achieve (section 4.3.2). Authors very often give up trying to evaluate C. The same reasoning is valid for E.

Thus, two approaches exist. The first, despite its difficulties, consists of measuring C and E without ignoring measurements of P and R. There are therefore two methods of measuring A, according to the formula: $C\text{-}E=A=P+R$. The second approach is not to measure C and E, but to calculate A from P and R. Results from these two methods are given later.

To illustrate these energy budgets, we have developed a method of illustration which we term 'biproportional', with histograms of components expressed as percentages of A and C (Figure 5.2). The use of two kinds of percentage in the same diagram is not redundant, because both gross (C) and net (A) efficiency values are shown. Also, the percentages in relation to A allow direct comparison with energy budgets where only A has been calculated (C and E unknown). In the latter, the diagram can only be monoproportional (Figure 5.3).

Energetics of two suspension feeders

The Japanese scallop Fuji and Hashizume (1974) established full energy budgets for three age classes of *Patinopecten yessoensis,* the Japanese scallop. The study was made simultaneously on individuals dredged from the bottom (natural population) and individuals reared at the same site but in submerged cages (experimental population). Figure 5.2 shows the energy budgets for animals between 1 and 3 years old. The following remarks can be made, on analysis of these results.

Patinopecten assimilates better when it is 1 year old than 3 years old, because *E* is lower both in absolute and relative value. The assimilation efficiency *A/C* confirms

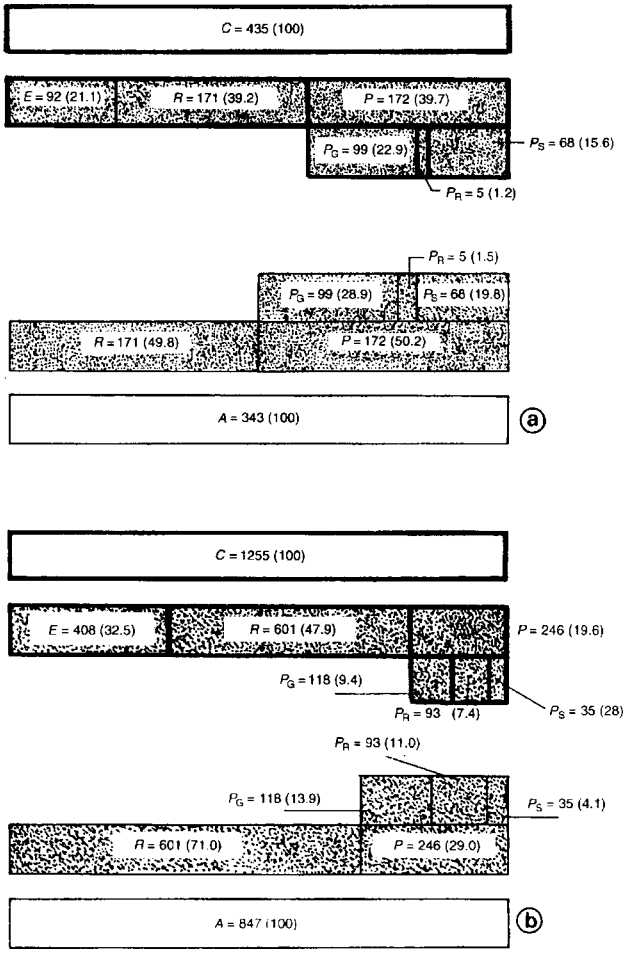


Figure 5.2 Biproportional diagram of the energy budget of *Patinopecten yessoensis*. Age classes of 1 year (a) and 3 years (b). Figure produced from numerical data of Fuji and Hashizume (1974). In white, estimated values; in grey, measured values. Thick-lined histograms, percentages calculated in relation to *C;* thin-lined histograms, percentages calculated in relation to *A*. In the rectangles, absolute values are expressed in kJ ind^{-1} $year^{-1}$. The numbers in parentheses are percentages. The areas of the rectangles are proportional to these percentages. *A,* assimilation; *C,* consumption; *E,* excreta; *P,* production; P_G, tissue growth; P_R, reproduction; P_S, secretions; *R,* respiration.

this: it is 78.8% at 1 year, 67.5% at 3 years. At 1 year old, the production P of *Patinopecten* is slightly higher than the cost of respiration R, but at 3 years old, P is much lower than R, which is shown by the lower production efficiency at 3 years than at 1 year. The same applies to P_G and the growth efficiency (Figure 5.2). In contrast, the values for reproduction are higher at 3 years than at 1 year, both in absolute and in relative terms. Thus $P_R/A = 13.9\%$ at 3 years, compared with 1.5% at 1 year.

The Brest Harbour queen scallop Shafee and Lucas (1982) established the individual energy budget for five age classes of another pectinid, *Chlamys varia*. Figure 5.3 gives the results for the 1 year and 3 year age classes studied for the period April 1977 to March 1978. Although only values of A, P and R are known, the monoproportional representation allows immediate comparison with *P. yessoensis*.

As in the previous case, note that the production efficiency is higher in the younger animals: 33.3% at 1 year and only 10.4% at 3 years, and that the proportion of reproduction is higher at 3 years (2.1 kJ ind^{-1} year^{-1}) than at 1 year (0.04 kJ ind^{-1} year^{-1}) (the latter value was so small it could not be shown in the figure). The similarities end there, however.

There is a considerable difference between the absolute values obtained for the two species: assimilation is 12 to 17 times higher in *Patinopecten* than in *Chlamys,* even at the same age (1 and 3 years). This disparity is related to size differences between the two species, the height of the shell being 3–4 times larger in *Patinopecten.* The net growth efficiencies for *P. yessoensis* are much larger, at 1 and 3 years, than those for *C. varia* (28.9% and 13.9% respectively on one side; 25.0% and 5.6% on the other). The situation is the same for production efficiency (Figures 5.2 and 5.3).

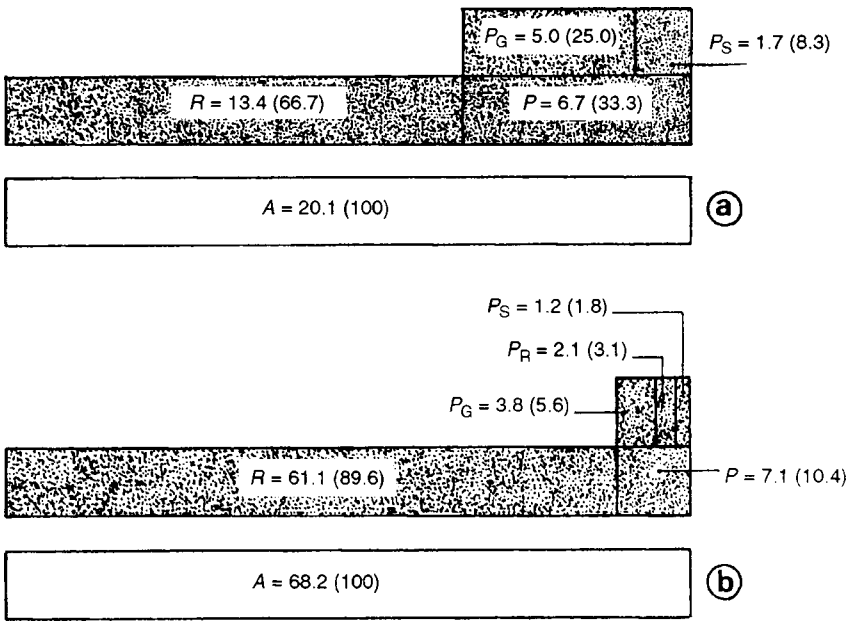


Figure 5.3 Diagram of the energy budget of *Chlamys varia*. Age classes of 1 year (a) and 3 years (b). (Figure produced from data of Shafee and Lucas, 1982). Conventions as in Figure 5.2.

5.1.3 Planktonic predators

The abundance of zooplankton in seas and lakes prompts the study of these animals which are consumers of production, either at the primary or at the secondary production level.

The example chosen is a *gelatinous predator,* the hydromedusa *Cladonema californicum,* studied by Costello (1991). This coastal species, which does not grow larger than 3 mm, swims little and often remains attached to various substrates, including algae. It was chosen as it is easy to rear: it feeds readily on brine shrimp *Anemia* nauplii. The experiment lasted for 29 days. The metabolic budget was established in terms of dry matter of C and N. Energetic units are not used.

Two phases can be distinguished in the experimental cycle. Between 1 and 2.5 mm: high growth rate and $P_G > R+U$. Between 2.5 and 3 mm: fall in growth rate and $P_G < R+U$. The successive values for growth yields illustrate this phenomenon (Table 5.6).

Gelatinous predators constitute an ecologically characteristic group, having high growth rates, short generation times, high fecundity and food consumption dependent on prey density (Costello, 1991). As a result of these characteristics, they are adapted to respond rapidly to zooplankton stock increases. The jellyfish studied has these characteristics, but less intensely than some other, large, planktonic predators such as the ctenophores. Thus, the maximum specific growth rate is 0.26 for *C. californicum* whilst it reaches 0.78 in the ctenophores; egg production is also lower in *C. californicum*. Lastly, this species can utilize its body proteins during several weeks of fasting, whilst populations of ctenophores collapse when prey is scarce. The group of gelatinous predators is not physiologically homogeneous.

5.1.4 Symbiotic species

Symbiotic species are numerous in the aquatic environment and accommodate micro-organisms with which they have trophic relationships.

Enterosymbiotic species contain micro-organisms, which participate in digestion, in their digestive tract. This phenomenon, which is well known in ruminants, exists in certain herbivorous fish (Fishelson *et al.,* 1985; Rimmer and Wiebe, 1987; Clements, 1991).

Endosymbiotic species contain either monocellular or filamentous algae or bacteria in their tissues. They abound in coral reefs, the depths of the oceans, in both hypoxic and anoxic environments. The systematic groups most involved are the

Table 5.6 Values of gross (GGE) and net (NGE) growth efficiencies as a function of diameter in the jellyfish *Cladonema californicum* (after data from Costello, 1991)

	Diameter (mm)		
	1	2.5	3
GGE (%)	30	45	20
NGE (%)	50	60	30

coelenterates, sponges, turbellarians, polychaetes, bivalves and pogonophorans. We shall look at associations with algae. For associations with bacteria, one should consult Laubier and Desbruyères (1984) and Fiala-Médioni and Felbeck (1990).

The presence of monocellular endozoic algae in aquatic invertebrates has been known for more than a century. It is only recently that a clear idea has emerged of the trophic relationships between the symbionts and host species. In fact, the role of zooxanthellae (in the marine environment) and zoochlorellae (in fresh water) has been the subject of controversy for a long time. In 1931, Gardiner explained that corals regularly consume their symbionts, while Yonge and Nicholls (1931) were convinced that madreporan corals do not obtain any nutrition from zooxanthellae.

Frendenthal (1962) concluded that all zooxanthellae belong to the same species, *Symbiodinium microadriaticum,* a dinoflagellate. Algae can be eliminated by the madreporans, normally in small quantities, but sometimes on a massive scale (the phenomenon of bleaching of coral).

Corals are colonial actinozoans with a calcareous skeleton; in the branched forms, a polyp which secretes either a perforated or compact skeleton is found at the end of each protuberance (Figure 5.4). When alive, the perforated type of skeleton contains more tissue than the unperforated type. Thus, according to Davies (1991), the skeleton of *Pocillopora damicornis* (unperforated) only contains 1.8% tissue whereas those (perforated) of *Montipora verrucosa* and *Porites lobata* contain 4.5% and 4.4% respectively.

This brief description of the structure of corals allows us to understand that the association between coral and zooxanthellae consists of three energetic compartments: the assembly of zooxanthellae, all the animal tissues, and the skeleton, which is an inert secretion of calcium carbonate and organic matter, mainly proteins. As in bivalve shells, this organic matter is not recyclable (section 5.4.1). Figure 5.5 explains in detail the exchanges which take place between the three compartments (the fourth being the natural environment) as a result of 11 processes. As well as these exchanges, partial exchanges between the respiration of the coral and the photosynthesis of the zooxanthellae can occur (Muscatine, 1990). Thus, some of the oxygen released by photosynthesis is used by the corals and, in return, some of the respiratory CO_2 is used in photosynthesis.

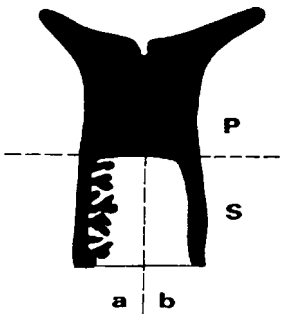


Figure 5.4 Diagram showing an actinozoan with a calcareous skeleton. In black, living tissue; in white, inert substances (organic + calcareous framework). The polyp (P) is above and the skeleton (S) below. In (a), perforated skeleton with canals containing tissue; in (b), solid skeleton, with tissue only on the surface.

The principal characteristics of this association are summarized in a review by Archituv and Dubinsky (1990). Firstly, the ratio of the respective biomasses is 1:20, algae:corals, which is remarkable when we consider that in a completely different ecosystem, the ratio is a minimum of 10:1, plants: animals. Comparing two colonies of *Stylophora pistillata,* one adapted to bright light (HL) and the other to low light (LL), it was concluded that 95% (HL) and 91% (LL) of assimilated carbon was transferred to the coral. This only allowed a low rate of renewal of zooxanthellae by division: in 53 (HL) or 73 days (LL). The HL colony derived all its carbon requirements from this association, whereas the LL colony obtained only 37% of its requirements.

Sometimes the carbon input of an animal exceeds its requirements. This is the case for *Pocillopora eydouxi,* which receives 90% of photosynthetic metabolites, representing 213% of its energetic requirements. The animal therefore has to get rid of this excess, which it does by producing mucus. In other cases, secretion of mucus occurs because the supply of vegetable matter is not in equilibrium: there is too much carbon and not enough nitrogen. Thus the animal is not lacking in energy, but in nitrogenous matter. It can obtain this in three ways (Figure 5.5), by digestion of the zooxanthellae (which occurs sometimes), or by capturing plankton or particles, or by absorption of organic substances, notably amino acids. Thus, symbiotic algae provide a significant amount of their photosynthetic products (an average of 87.4% for seven species of coral studied) but about 50% of this supply is not used by the animal, the excess being eliminated in the form of mucus. Nevertheless, this process is very different from what happens in aphids, contrary to the opinion of Davies (1984). In aphids, which parasitize plants by sucking their sap, a large proportion of the carbohydrates ingested are not absorbed but excreted via the anus in the form of faeces: this food always remains outwith the interior metabolism of the animal and

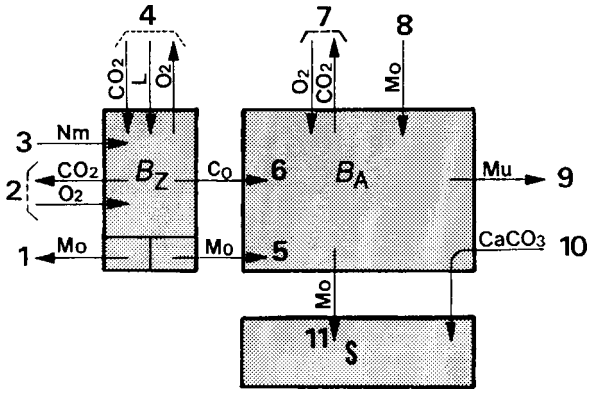


Figure 5.5 Diagram showing exchanges of energy and matter in the three metabolic compartments which constitute the association between coral and zooxanthellae. B_A, biomass of animal tissue (in black in Figure 5.4); B_Z; biomass of zooxanthellae; *S,* weight of inert skeletal matter (in white in Figure 5.4). Exchanges: in addition to the chemical formulae: C_o, organic carbon; L, light energy; Mo, organic matter; Mu, mucus; Nm, inorganic nitrogen. Functions: 1, elimination of zooxanthellae; *2,* respiration; 3, absorption of inorganic nitrogen; 4, chlorophyllous assimilation; 5, absorption of zooxanthellae; 6, translocation of organic carbon; 7, respiration; 8, heterotrophic feeding; 9, mucus secretion; 10, absorption and translocation of calcium carbonate; 11, translocation of organic matter.

does not participate in its metabolism. In corals, in which monocellular algae are within the tissues, excess carbohydrate originates from the internal environment and can only be eliminated in a secretion (hence by anabolism).

5.2 Microbioenergetics

Microbioenergetics is used either for the analysis of a phenomenon with a short time scale or for analysis of a particular physiological activity.

5.2.1 Energy budgets for temporary phenomena

Amongst the energy budgets within a limited time frame, most are concerned with *critical phases* of development or temporary environmental stress (appearance of red tides, for example). In both cases, the animal faces metabolic difficulties for a short period of time. Precise energetic analyses allow a better understanding of these difficulties and, eventually, better protection for the animal.

Bioenergetics of a critical phase in a cirripede

A critical phase of development is defined as a period of time during which, for a given cohort, the mortality rate rises significantly and suddenly, unrelated to any direct environmental cause. This is the case for metamorphosis in marine invertebrates, especially when the animal transfers from a pelagic to a benthic lifestyle. We shall examine the crustacean cirripede *Balanus balanoides,* studied by Lucas *et al.* (1979) by way of example.

The authors used two complementary methods: proximal biochemical analysis and oxygen consumption. The cost of metamorphosis was expressed in three ways: materials, morphogenesis and exploration. *Cypris* larvae can explore the substrate for 3–4 weeks before attaching themselves. All energy expenditures are calculated per larva over the period of metamorphosis. Originally expressed in 10^{-2} calories by the authors, these have been converted into millijoules.

For morphogenesis, the results are the mean of the values found by the two methods: 117 mJ using the biochemical method and 134 mJ using respiration, giving a mean of 125 mJ. The cost of materials (biochemical method) is 285 mJ and that of exploration (respiration) is 209 mJ. Lipid reserves form the source of energy for exploration: after 3 or 4 weeks these reserves are spent.

Bioenergetics of critical phases in bivalves

Bioenergetic studies have been carried out on bivalves during critical phases of early development. The two critical phases are characterized by the fact that the organism does not feed; to survive these periods successfully it must live on its reserves for a certain time.

The first critical phase is during development, from the egg, of the veliger larva D equipped with feeding and digestive organs and therefore capable of feeding from the external environment. This phase has been studied by Whyte *et al.* (1990) in the pectinid *Crassadoma gigantea*. The reserves used during this period, which lasts 2–3 days depending on temperature, are initially contained in the egg and are therefore dependent on parental metabolism. During embryogenesis, the matter necessary for development utilizes 9.8% of

carbohydrates, 43.5% of proteins and 46.7% of lipids. The formation of the shell, which does not appear until the end of the period studied, requires 64.4% of energy expenditure. The RNA/DNA ratio declines from 18.6 in the egg to 2.2 in the larva D.

The second critical phase occurs at the moment of metamorphosis when the 'eyed larva' attaches and transforms into a postlarva. Holland and Spencer (1973) found that in the flat oyster, *Ostrea edulis,* the energy required for metamorphosis comes mainly from neutral lipids.This opinion has been confirmed by several authors and a colorimetric test (Sudan black) has been perfected for eyed larvae to find out what proportion of a cohort is truly competent, i.e. rich in lipid globules. Nevertheless, in 1979, Bartlett found that proteins are the principal source of energy in the Pacific oyster, *Crassostrea gigas*. Proteins also represented 62% of energy sources in an experiment on *Ostrea edulis* (Rodriguez *et al.,* 1990). Avoiding reopening the controversy, they consider that there is no selective utilization of biochemical reserves and that the larva takes energy from any available source.

Temporarily unfavourable climatic conditions

Extreme temperatures (either in summer or in winter) frequently provoke precarious physiological states. We shall examine the response of a mobile species to unfavourable conditions. In mobile species, the metabolic power index (section 3.4.2) allows the evaluation of the physiological capacity of the organism to respire sufficiently in order to meet, simultaneously, the requirements of R_S, R_A and R_F. As R_S is obviously obligatory, there remains therefore the permanent conflict between R_A and R_F i.e. locomotion and specific dynamic action (SDA) (Priede, 1985). The conflict becomes crucial if the animal has a low respiratory power/capacity, in which case, according to Priede, when the animal swims at maximum speed, it cannot carry out any digestion. Conversely, while digesting, it can only move very slowly. This precarious state is illustrated in Figure 5.6 and involves brown trout, *Salmo trutta,* living in a Scottish loch in summer. The temperature of 15°C is an upper threshold for this species. Observations on these trout showed that they stay close to the bottom and remain

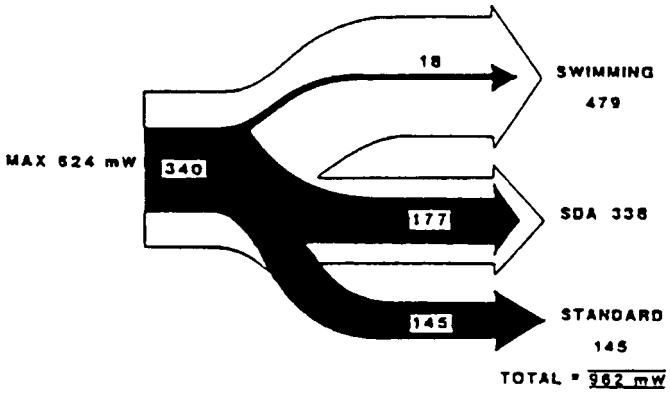


Figure 5.6 Brown trout, *Salmo trutta* (500 g), living in a Scottish loch at 15°C during summer. The white arrows indicate the theoretical respiratory power (expressed in mW) which can be attained by these trout. The actual power attained for the three respiratory states is shown in black. The width of the arrows is proportional to the calculated powers. Note that respiratory expenditure for swimming is greatly reduced in summer (after Priede, 1985).

immobile for most of the time. Laboratory measurements allowed calculation of the power available for R_S, R_A and R_F. Note that outwith the summer period, these trout regain a wider respiratory capacity, which allows more activity. Therefore, for the trout, there exists in summer a critical period linked to temperature.

5.2.2 Analysis of a physiological activity

The activity studied as an example is swimming, the means of locomotion in water. Only swimming in fish is considered.

General features of swimming in fish

The principal characteristics of swimming in fish are shown in Table 5.7. For the many fish which move as a result of undulations of the body, it is the *myomeres* or *myotomes* which contract. In each myomere are found lateral red muscle (which also exists as deep muscle in the tunas) which occupies about 10% volume (20% in the tunas) and white muscles which make up the remainder of the volume. The characteristics and roles of these two kinds of muscle are shown in Table 5.8. In some fish, such as the carp, there are pink muscles which, by structure and physiology, are intermediate between red and white muscle (Blake, 1983).

Slow swimming is effected by red muscle activity only and causes an increase in oxygen consumption: R_A increases. During sustained swimming, i.e. that lasting more than 2 minutes, if the swimming speed is not too high, only the aerobic muscles are used. If a certain threshold of sustained swimming is reached, or a critical swimming speed is reached, oxygen consumption is not the only source of

Table 5.7 Fish swimming types. Classification of Breder (1926)

Type	Swimming method	Body shape	Example
Anguilliform	Whole-body undulations	Long and thin	Eels
Subcarangiform	Undulations of posterior half of body	Fusiform	Salmonids
Carangiform	Undulations of posterior third of body	Fusiform	Clupeids
Thunniform	Undulations of caudal peduncle	Fusiform	Tunas
Ostraciiform	Fin movement only	Variable	Sea horses

Table 5.8 Characteristics and roles of white and red muscles in fishes. After data from Blake (1983), Bone (1978) and Johnston (1989)

Red muscles	White muscles
Slow contractions	Fast contractions
Do not tire quickly	Tire quickly
Aerobic metabolism	Anaerobic metabolism
Many capillaries	Few capillaries
Rich in myoglobin	Many myofibrils
Many mitochondria	Few mitochondria
Thin fibres	Thick fibres

energy because the anaerobic white muscles are also activated to reach maximum swimming speed (U_{max}).

After a bout of high-speed swimming, the animal pays its oxygen debt, during a recovery period (Figure 3.4). To discover the true cost of swimming, the payment of this debt must be included in the energy expenditure. Critical swimming speed (U_{crit}) is defined as the speed at which anaerobic processes commence. Below this critical swimming speed, metabolism is solely aerobic.

The following relationship exists between respiration *(R)* and swimming speed *(U):*

$R(U)$=a cU^b

where a is the standard respiration rate when U=0. *R(U),* the respiration measured, can be replaced by $R_t(U)$, total respiration including oxygen debt.

The relationships between biomass and speed on the one hand, and biomass and respiration on the other hand, are allometric:

U=aB^b and R=a'$B^{b'}$.

The cost of transport is the energy cost per unit of mass swimming over one unit of distance. It can be expressed in two ways: gross cost, $R_t(U)/U$, and net cost, $(R_t(U)-R_s)/U$, where $R_t(U)$ and R_s are expressed in joules per unit of time and weight and U in distance per unit time. The cost of locomotion is therefore expressed in units of length (metres) and weight (grams). The cost of locomotion is usually higher in small animals than in large ones.

The speed for which the gross cost of locomotion is minimal is called the optimal speed (U_{opt}). Finally, when species that swim continuously (tunas and other scombrids, and sharks) are quiescent they tend to adopt what is called a voluntary speed (U_{vol}). For certain authors (Weihs, 1977), $U_{vol}=U_{opt}$.

Speed is measured normally (m s^{-1}) but many authors relate this to absolute size and have adopted the idea of body length per second (bl.s^{-1}).

An estimate of the distance that can be covered by an animal by the expenditure of energy equivalent to a 25% reduction in body weight is called the *energetic range*. The energetic range increases in proportion to body weight.

Examples

The tunas, which have to swim constantly (section 5.1.2) have a respiratory rate 3–4 times higher than that of the salmon. Their critical speed is 3.5 bl.s^{-1} and they can reach maximum swimming speeds of 10 bl.s^{-1} (Priede, 1985).

Another species which swims constantly, the shark *Sphyrna tiburo* from Florida, has been studied by Parsons (1990). Working at 25°C on individuals between 34 cm (95 g) and 95 cm (4.6 kg), he established allometric relationships between routine respiration rate, cost of locomotion, energetic range and biomass (M_v). From these equations, it is found that, for a 1 kg individual, the respiration rate is 80 kJ kg^{-1} day^{-1}, cost of locomotion 2.9 Jg^{-1} $km^{-}1$ and energetic range, 500 km. Finally, the relationship between optimal speed and body length was found to be: U_{opt}=4.9 $L^{0.496}$ with r=0.972, where U is measured in cm s^{-1} and L, the length, in cm; r is a correlation coefficient. Thus, for a 70 cm individual, U_{opt}=40 cm s^{-1}.

The basking shark, *Cetorhinus maximus,* feeds on plankton which it collects by filtration. Its speed is ruled by plankton density: the lower the density, the faster it must swim to

collect sufficient food. In winter, however, when plankton is scarce, it cannot cope and drops to deep water where it remains in an inactive state or torpor (Priede, 1985).

The plaice, *Pleuronectes platessa,* which has no swim bladder and a high density, incurs a high cost when it leaves the bottom (posture effect). It also has no other solution than to continue swimming at the critical speed and then to rest again on the bottom, invisible to predators by virtue of its ability to change colour to match the background (Priede, 1985).

Kaufmann (1990) studied in detail the energetics of swimming in two species of cyprinids in the Danube, *Chalcalburnus chalcoides* and *Rutilus rutilus,* at larval and postlarval stages. Tissue growth is intense during their first 3 months of life, when they grow from 1.5 mg to 1 g, an increase of 3 orders of magnitude. At the same time, they undergo morphological transformations which totally change their mode of swimming (progressive appearance of fins and change from anguilliform to subcarangiform swimming) and transformation of respiration from cutaneous uptake through a mixed intermediate period to gills. The respiration rate is twice that of the adults, routine swimming speed reaches 6–8 $bl.s^{-1}$ and maximum swimming speed 25 $bl.s^{-1}$.

5.3 Correlations between metabolism and biomass

The preceding results (sections 5.1 and 5.2) have shown that the values of the different components of metabolism in one species vary from one age class to another, which leads to the supposition that, as the weight of the organism increases with age, there should be a relationship between the components of the energy budget and the weight of the organism being studied. We shall look successively at the relationships between weight and *P, C* and *R*. Traditionally, biomass is the term used and the ratios $P/\bar{B}$, $C/\bar{B}$ and $R/\bar{B}$ are calculated, where $\bar{B}$ is the mean weight during the observation period. Lastly, we shall examine separately 'reproductive effort', because of the wide range of ways in which it is expressed (section 5.4).

5.3.1 $P/\bar{B}$ ratio

In ecology, calculating $P/\bar{B}$ is a classic way of expressing a relative value for production. A certain number of remarks can be made, not only on its characteristics, but also on its values.

Characteristics

P is most often simply P_G, because most studies are carried out on population levels for which P_R, and usually P_E and P_S, are not included in the calculations (section 3.2.2).

The two variables involved are not of the same nature. Frontier and Pichot-Viale (1991) stated that 'biomass…is a variable of state; its production or consumption are variables of flux'. In fact, all the components of an energy budget are variables of flux: they only have values that are functions of time.

The duration of the budget is usually 1 year. During this time, the biomass changes; it is therefore necessary to measure it several times to obtain an almost exact mean value. The 'ratio' cannot be a dimensionless number, as the numerator P_G is a quantity of matter produced in the course of a year, therefore a weight per unit of time $(M\,t^{-1})$ while B is only a weight (M). The number must retain t^{-1}, a fact which certain authors neglect.

On the other hand, by dividing production by biomass, one is attempting to obtain a relative figure for production, by reducing it to a unit of mass, either kilograms or grams, depending on the size of the animal. If this productivity is production per unit weight, P_G/B represents productivity per unit time.

The inverse ratio, *B/P,* which has a time dimension, is called biomass renewal time or, more usually, *turnover* (Frontier and Pichot-Viale, 1991). The higher the productivity the faster the turnover. Small animals are more productive than large ones.

P/B values

Two rules have been established:

1 Annual productivity is higher in short-lived species than in long-lived ones.
2 Annual productivity of a species decreases proportionally as its biomass increases.

The first rule has been confirmed by Robertson (1979) for the marine macrobenthos, by compilation of data from 19 bivalves, 14 gastropods and 16 other species (polychaetes, crustaceans and echinoderms). From these 49 species he established the following regression, where *L* is the *life span* expressed in years:

$$\log_{10} P/B = 0.660\ (\pm 0.089) - (0.726 \pm 0.147)\ \log_{10} L,\ r = -0.8350.$$

This implies that where $L = 1$, 2 or 4, annual production is about 4.6, 2.7 or 1.6 respectively. The major problem in this kind of exercise is determining life span sufficiently accurately.

The second rule does not apply to larval stages or even, according to some authors, to any juvenile animals. Thus this rule is only valid above a certain minimum size, e.g. 10 mm in the mussel *Mytilus edulis* (Theisen, 1973).

The decrease in productivity in proportion to the increase in biomass is due partly to decreased feeding efficiency and partly to increased maintenance costs (Vahl, 1973). According to Bernard (1983), the pumping rate of bivalves becomes less and less efficient as the body grows. This argument is a specific illustration of the first reason considered by Vahl (1973).

P_S is easy to measure for secretion of deposits such as bivalve shells. The proportion of organic matter in relation to total shell weight varies according to species and age. Price *et al.* (1976) noted that this proportion varied between 1.4% and 21.4%, with a mean of 4.5%, for 14 species of bivalve. Cameron *et al.* (1979) found values of 2.3% to 3.8% for three freshwater bivalves. According to Price *et al.* (1976), the proportion of organic matter in the shell is higher for younger than older individuals. It is, however, more interesting to compare the energy accumulated in the shell with that in the tissues, as shown in Table 5.9.

Note that there is great variability (from 1.4% to 58.8%) in the energetic ratio shell/tissue, but that for most species, the proportion is far from being negligible.

Ecologists studying this problem have remarked that this organic matter in the shell is not recyclable, thus forming an *ecological cul-de-sac*. The same is true for coral skeletons (section 5.1.4). Taking a wider view at the ecosystem level, Frontier and Pichot-Viale (1991) defined *secondary auxiliary energy* as 'that part of the energy assimilated by animals available for growth and reproduction that is given over to the construction and maintenance of structures which allow survival'.

Table 5.9 Energy content (E_s) of some bivalve shells, expressed as a percentage of the energy content of the tissues *(E)*. Table constructed using data from Newell (1982). *L,* length; M_s, secreted mass (dry matter); E_s, secreted energy; M_v, biomass; *E,* energy

	Shell			Tissues		
Species (references)	*L* (mm)	M_s (g)	E_s (kJ)	M_v (g)	*E* (kJ)	E_s / *E* (%)
Scrobicularia plana (Hughes, 1970)	50	3.2	0.3	0.9	18.8	1.4
Crassostrea virginica (Dame, 1972)		5.7	1.2	0.1	3.2	37.4
Mytilus edulis (Jorgensen, 1976)	35–40	1.3	4.3	0.3		20.0
Geukensia demissa (Jorgensen, 1976)	60	9.3	5.4	1.1		43.6
Ostrea edulis (Rodhouse, 1978)	100	46.4	3.6	78.9		58.8

5.3.2 C/$\bar{B}$ratio

The relationship between consumption and biomass of a species is allometric: $C=a\bar{B}^b$ where b<1. For example, for *Mytilaster lineatus* $C=0.79\ \bar{B}^{0.55}$ and for *Teredo navalis* $C=0.45\bar{B}^{0.66}$ (Tsikhon-Lucanina, 1976).

Consumption per unit weight *C/B* also decreases in proportion to increasing biomass. Winter (1970) proposes the following allometric relationship:

$C/\bar{B}=aW^{b-1}$ or $\log C/\bar{B}=(b-1)\log \bar{B}+\log a$.

Applying this to *Crassostrea virginica,* Epifiano and Ewart (1977) found $C/\bar{B}=0.001B^{-0.41}$.

5.3.3 R/$\bar{B}$ ratio

For aquatic animals, Zeuthen (1953) carried out exhaustive work on oxygen consumption in relation to biomass for different species and for different development stages of the same species. He showed that, in both cases, oxygen consumption *(R)* and biomass *(B)* are linked by an allometric equation of the kind $Y=aX^b$.

In the interspecific study, using a double logarithmic scale (for *R* and for $\bar{B}$), he obtained straight lines with different slopes depending on the size of the organisms: 0.7 for unicellular organisms, 0.95 for small metazoans (e.g. larvae) and 0.8 for larger poikilotherms and homoiotherms.

In the developmental study he showed, particularly for *Mytilus, Artemi*a and *Asterias,* that the slopes vary successively within the following limits: 0.75–0.80, then 0.95–1.0 and lastly 0.6–0.3 (Figure 5.7). He concluded that during ontogeny, a species reproduces the phenomena observed between species and even intensifies them.

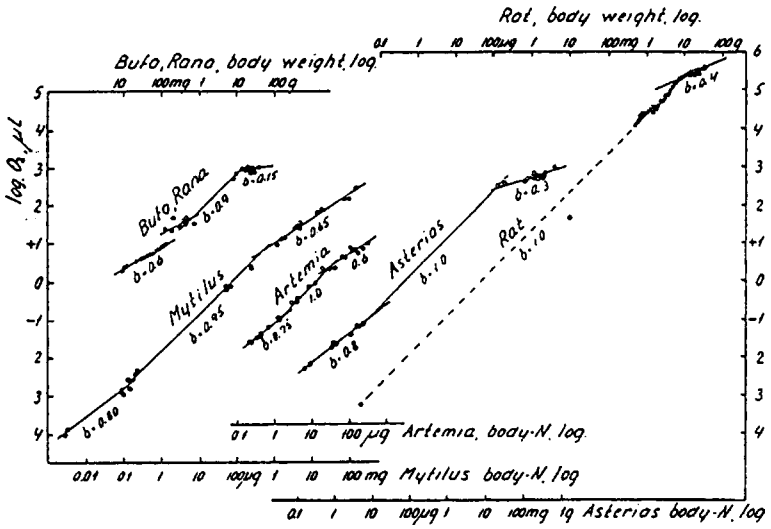


Figure 5.7 Intraspecific comparison of oxygen consumption as a function of developmental stage. Note: all masses relating to the rat should be multiplied by 10. The masses of the three aquatic animals are expressed as nitrogen content. Reproduced from Zeuthen (1953).

Since the work of Zeuthen, several authors have studied the $R/\bar{B}$ ratio. An example is that of Bernard (1983), who distinguished between species with high oxygen consumption in which the influence of biomass is very strong (group a in Figure 5.8), and those with low oxygen consumption, on which biomass has little effect (group b in Figure 5.8).

To complete this review of the relationships between the components of the energy budget and biomass, the review of Banse (1979) on net growth efficiency $P_G/(P+R)$ should be mentioned, where the author notes that the values for this efficiency are not in proportion to specific biomass (defined as the biomass at sexual

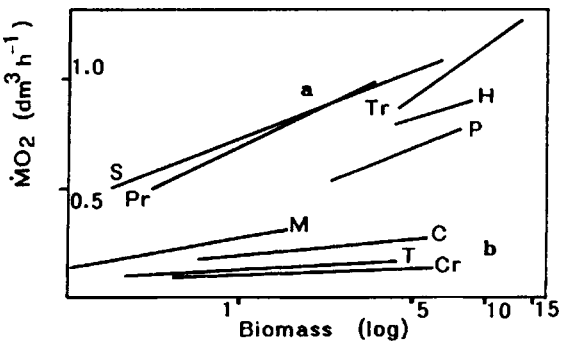


Figure 5.8 Respiration (measured by pumping rate) as a function of biomass in some bivalves. Semi-logarithmic scale. C, *Clinocardium nuttallii;* Cr, Crassostrea gigas; H, *Hinnites giganteus;* M, *Mytilus edulis;* P, *Patinopecten caurinus;* Pr, *Protothaca staminea;* S, *Saxidomus giganteus;* T, *Tapes phillipinarum;* Tr, *Tresus capax* (after Bernard, 1983). See text for explanation of groups a, b.

maturity of the species being considered) even though this dependency has been shown for *P* and for *R*.

5.3.4 Allometric metabolic relationships: limits of validity

Many allometric relationships have been cited in the preceding pages: they have been given as they are, the only precaution being taken to define the species to which they relate. Many authors proceed in this way but, in so doing, they forget that the data obtained for one species at a certain stage of development in a certain geographical area cannot be generalized for all stages of development or all geographical areas occupied by the species.

For example, for the American oyster *Crassostrea virginica,* a eurythermic species with a distribution stretching from the Maritime Provinces of Canada to the Gulf of Mexico, one would concede that the metabolic data would not be the same for populations in Canada, which live under the ice for a good part of the year, as for the populations in Mexico, which live in the mangrove. It is therefore necessary to define spatial limits on the validity of a study.

It is also necessary to define age or size limits for which the allometric relationship is valid. Admittedly, if the author does not specify the stage of development, it is understood that the relationships established do not apply to embryonic, larval or postlarval stages. However, at the point during its development when the individual becomes an imago, with the definitive morphology of the species into which it is developing, then it might be considered correct to use the allometric equation. This, however, would be a mistake, as juvenile and adult metabolisms differ, not only for reproduction (Figures 5.2 and 5.3) but also for other metabolic characteristics: the *C/B* and *R/B* ratios are usually different for juveniles and adults (see Figure 5.9 for an example).

Studying the energy budgets of the young-of-the-year and that of adults in the yellow perch *Perca flavescens,* a planktonic feeder, Post (1990) found that if certain parameters of respiration and consumption were identical, others were markedly different. He concluded that it is inappropriate to extrapolate metabolic allometry from adults to juveniles: this is demonstrated in Figure 5.9.

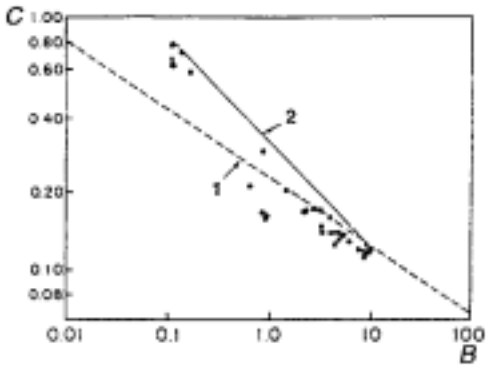


Figure 5.9 Allometry between maximal consumption and biomass in *Perca flavescens*. Curve 1, model for adults after Kitchell *et al.* (1977). Curve 2, model for juveniles established from data obtained from Lake St George (Ontario). The 33 points represent the data *in situ*. *C,* consumption in g g^{-1} day^{-1}; *B,* biomass in g (modified from Post, 1990).

5.4 Reproductive effort

5.4.1 Definitions

The portion of energy allowed for reproduction in an energy budget is universally called *reproductive effort*. However, there are an astonishing number of ways of expressing reproductive effort (RE). This no doubt stems from the original definition of reproductive effort given by Williams (1966) where the cost of reproduction was evaluated according to ethological concepts (section 3.2.1). Later, Hirshfield and Tinkle (1975) gave the term a metabolic definition: that proportion of the energy budget of an organism used for reproduction. It therefore involves P_R, which we have defined. Thus, reproductive effort can be expressed by the ratio $P_R/\bar{B}$, but this is not the only possible relationship (Table 5.10).

The examples in Table 5.10 show many ways of expressing reproductive effort. The differences are due partly to the adaptation required to the animal's anatomy and partly to the goal pursued: either a global budget or a precise study of one spawning, especially for females. We shall not discuss these specific considerations and only envisage global budgets, established over a year (Figure 3.6), including both sexes.

Because reproductive effort is a ratio, it does not matter very much if M_v, M_s, organic carbon or Ce is used provided the same units are used in the denominator and numerator. Translating the different formulae according to our nomenclature, we find:

(1) extending the observations over a year and taking males into account:
RE=P_R/C
(2) RE=$P_R/(A\text{-}R)$=P_R/P
(3) RE=P_R/B
(4) RE=P_R/A
(5) RE=$P_R/\bar{B}$
(6) RE=P_R/P

Table 5.10 Various methods for the calculation of reproductive effort. Abbreviations: Ce, energy content; Org. carb., organic carbon; W_l, weight of living material; N, numerator; D, denominator

Authors	Animals	Definition of reproductive effort	Ref.[a]
Wootton (1985)	Fish	N: Ce of eggs in a spawning batch D: Ce of food between spawnings	1
Russel-Hunter and Romano (1979)	Molluscs	N: Ce of annual spawnings D: annual non-respired energy assimilation	2
		N: Org. carb. in annual spawnings D: Org. carb. of a female before spawning	3
Parry (1982)	Molluscs	N: Ce of annual spawnings D: annual assimilable energy	4
		N: Ce of annual spawnings D: annual minimum Ce of somatic tissues	3
Conand (1989)	Echinoderms	N: W_l of annual gonad production D: W_l of annual somatic + gonad production	6

[a] Equation reference number.

Table 5.11 Reproductive effort, expressed as a percentage, of four limpets (gastropods), after Parry (1982)

Species	$P_R / \bar{B}$ (year^{-1})	P_R / A	P_R / P
Patella peroni	219	26.6	75.6
Patelloidea alticostata	102	10.3	63.3
Cellana tramoserica	102	12.1	73.2
Notoacmea petterdi	78	11.5	74.0

Thus, despite differences in their expression, (2) and (6) are identical (but measured differently). The same is true for (3) and (5).

In summary, P_R can be related to C, A and P. P_R/C corresponds to P_G/C and can be called gross reproductive efficiency; P_R/A corresponds to P_G/A and can be called net reproductive efficiency. The ratio P_R/P is more limited and corresponds to the antagonism between P_R and P_G (as P_S and P_E are most often negligible). Lastly, as there is $P_G/\bar{B}$, there is also $P_R/\bar{B}$. The remarks made in section 5.3.1 are also valid for $P_R/\bar{B}$, which is not a true index.

The values of RE depend on the formula used. For example, Parry (1982) calculated values for four different species of limpet in Australia (Table 5.11).

An intraspecific, usually increasing, allometric relationship exists between P_R and $\bar{B}$, which indicates that, per unit weight, larger individuals devote more to reproduction than smaller ones. This may be an age-related development.

5.5 Factors influencing energy budgets

5.5.1 Principal factors

As for all biological phenomena, two kinds of factors act on the metabolism: internal (or intrinsic) factors and external (or extrinsic) factors.

Internal factors are by their very nature extremely diverse, because of the differences in structure between systematic groups. For example, in the case of fish, according to Matty and Lone (1985), the following endocrine glands are implicated in fish metabolism: the thyroid, the gonads, chromaffin cells, interrenal tissue, the endocrine pancreas and the pituitary gland.

Metabolism is not only influenced by hormones and transmitter substances, but also parasites, infectious diseases, stress agents and the general physiological status of the animal in relation to genetics, age, reproduction and so on.

Amongst specific hormonal (or neuroendocrine) actions which are of particular interest to the aquaculturist are the factors influencing growth, sexual development, spawning and metamorphosis.

The important external factors are: food, for all animals; temperature, for poikilotherms; oxygen concentration and salinity, for all aquatic poikilotherms; and turbidity, for all aquatic microphages. Lastly, for those species that can survive it, emersion is also a factor which influences metabolism.

All the factors cited have a direct influence on the efficiency of aquaculture and some of them will be studied in Chapter 7. Nevertheless, we shall carry out at this stage a basic analysis of temperature and its effect on metabolism.

5.5.2 The effects of temperature

In order to carry out an effective analysis of an external factor which acts on the physiology of a species not only by simple presence-absence, but as a function of its intensity, it is vital to establish an *ecophysiological diagram*. This diagram shows the response of the animal considered for all values of the factor at which it can survive. This concept will be applied to the temperature factor.

The concept of thermobiological amplitude

For living organisms, there is an optimum temperature and upper and lower lethal temperatures. The difference between the lower lethal temperature and upper lethal temperature constitutes the *thermobiological amplitude*. The amplitude is large in *eurytherms* and small in *stenotherms*.

Not all temperatures contained within the thermal amplitude have the same effects on metabolism. An analysis of these differences can be found Figure in 5.10. The movement criteria adopted for distinguishing torpor from coma vary according to species. This can be the movement of appendages in crustaceans or ciliary movements in many marine invertebrates. In some cases it is not possible to distinguish between torpor and coma.

Q_{10}, degree days and the normal curve

The concept of Q_{10} was established to show that, in the poikilotherms, at least within certain limits, an increase in temperature causes an increase in metabolism. If respiration is taken as the means of evaluating metabolic activity, Q_{10} is the relationship between the values of oxygen consumption at a temperature of t+10°C and at a temperature t: $Q_{10}=MO_2^{t+10}/MO_2^{t}$. When the observations are made at temperatures that do not differ by 10°C, Van't Hoff's formula is applied:

$$Q_{10}=[MO_2t_2/MO_2t_1]/[10/(t_2-t_1)].$$

Most values of Q_{10} lie between 2 and 3. However, conditions of measurement should be specified. It is particularly important to indicate the temperature range over which Q_{10} was calculated. Thus, if we take the example of Figure 5.10, it is obvious that the Q_{10} (0°C-10°C) is of the same order as the Q_{10} (10°C-20°C) whereas the Q_{10} (20°C-30°C) is completely different as it becomes less than 1.

In many studies carried out on the duration of biological phenomena (number of days to reach metamorphosis, time required to reach a certain size), *degree days* are used. In order to do this, the number of degrees Celsius away from a biological zero is multiplied by the duration of the phenomenon in days. The biological zero, which differs between species, is the temperature at which the biological phenomenon being studied ceases. Although this idea has no scientific significance, it has certain uses. For certain authors, the number of degree days would be constant for a given biological phenomenon. Thus, Pionetti (1984) gave the following formula for the

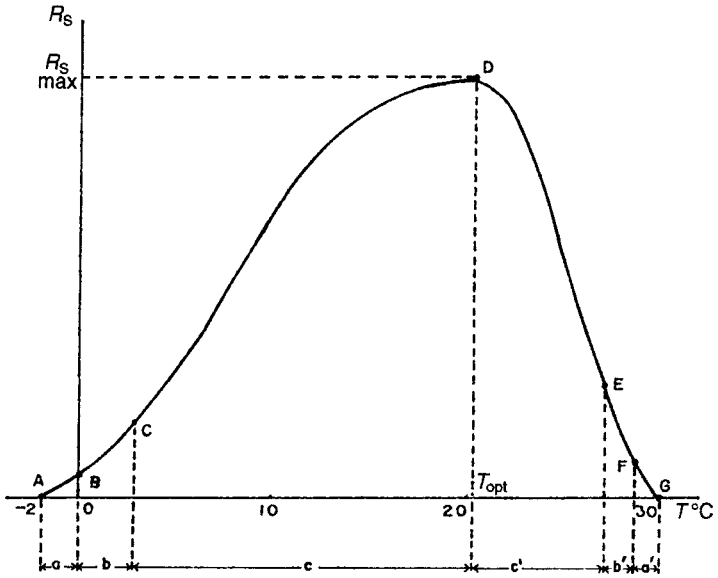


Figure 5.10 Thermophysiological profile of a fictitious species. This species is eurythermic due to the wide range (32°C) of temperatures in which it can live (lethal temperatures, -2°C and 30°C). On the ordinate, metabolic intensity is measured by standard respiration (R_S). On the abscissa, temperature is expressed in degrees Celsius. Various physiological states are distinguished on the thermophysiological curve: CD and DE, activity (coordinated movements possible); BC and EF, torpor (uncoordinated movements possible); A and G, death (zero oxygen consumption). On the abscissa, temperature ranges associated with these states can be defined: a and a', stages of psychro-coma and thermo-coma; b and b', psychro-torpor and thermo-torpor; c and c', periods of infra- and supra-optimal activity, other than at the optimal temperature (in this case, 21°C) (modified from Lucas, 1991).

incubation of four species of Sparidae: $H(T\text{-}7.5)=540$. In this case, time is measured in hours, the biological zero is 7.5°C and the degree hours are 540. One only needs to refer to Figure 5.10 to understand that the practical idea of degree days is only usable in narrow temperature ranges and it would also be necessary to define a 'biological maximum' for each species.

The *normal curve* proposed by Krogh (1914) merits the same criticisms. This empirical curve (Figure 5.11) established between 5°C and 30°C, is obviously not suitable for stenotherms and in all probability not for all eurytherms.

Direct influence of temperature on metabolism

With reference to the thermobiological diagram (Figure 5.10), it shows that increasing temperature is only favourable during the ascending phase of the curve. Experimenters usually only work over a narrow range of temperatures below the optimum. This is the case, for example, for an experiment carried out on the rainbow trout (Cho *et al.,* 1982). The growth curves of three batches of trout raised at different temperatures, but under identical environmental conditions and feeding regimes, are shown in Figure 5.12. The rainbow trout is a cold water stenotherm. For this reason the temperature range is only 5°C. Also, 15°C must be

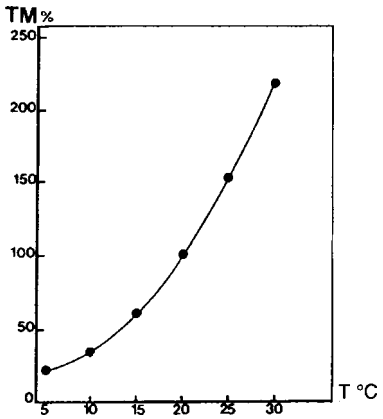


Figure 5.11 Krogh's standard curve. On the ordinate: metabolic rate (per cent); on the abscissa: temperature (degrees Celsius).

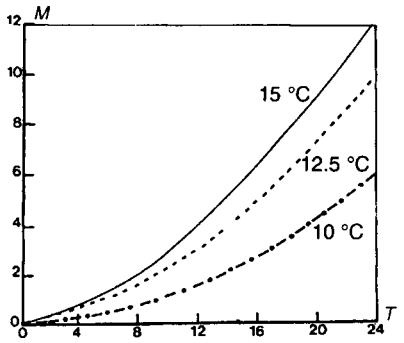


Figure 5.12 Influence of temperature on the growth of rainbow trout. *M,* biomass of 100 fish (in kg); *T,* time in weeks (after Cho *et al.,* 1982).

close to the optimal temperature, because the weight gain is greater between 10°C and 12.5°C than between 12.5°C and 15°C. Despite the fact that food supply was constant, the simple effect of temperature is remarkable: a batch of 100 fish at 15°C reached 4 kg after 12 weeks, whereas it took 20 weeks to reach the same weight at 10°C.

Thermal acclimation

Living organisms are unlike machines, which can function immediately and optimally, at any temperature. When there is a noticeable temperature change, the animal requires several weeks or days to adapt to the new thermal conditions. During this time of adaptation or *acclimation,* structural and biochemical changes occur in the animal, as shown in the following examples.

When swimming speed in the carp increases, there is successive mobilization of red, then pink, then white muscles (section 5.2.2). When carp adapted to 15°C are placed, some in water at 20°C, some at 10°C, the former resort to using their white muscles when swimming

speed reaches 46 cms^{-1}, the latter when it reaches 26 cms^{-1}. After several weeks, this difference is reduced, as the carp adapted to 10°C have increased their percentage of red muscle (Johnston *et al.,* 1990).

The haemolymph of teleost fish freezes at -0.8°C (De Vries, 1988), but in certain Antarctic species, the freezing point of the haemolymph is lowered to -2.7°C. This adaptation is achieved either by the elevation of the salinity of the haemolymph or more especially because of the presence of *antifreezes,* in the form of peptides (AFP) and glycopeptides (AFGP). It is interesting to note that AFP and AFGP are present all year round in the haemolymph of the Antarctic species, but only in the winter in North Atlantic species (Johnston, 1990). The factor triggering the presence of antifreezes in the haemolymph is either the drop in temperature, for example the threshold is 0°C in the Atlantic cod, *Gadus morhua,* or photoperiod. Thus, in *Microgadus tomcod,* antifreezes appear in November when the sea starts to cool and day length decreases (Johnston, 1990). Other examples of thermal acclimation are given by Le Gal (1988).

5.6 Summary of the energetics of organisms

Following three chapters devoted to the bioenergetics of organisms, it is useful to summarize the main points.

Energy budgets can be calculated according to different criteria. Three of these which were examined are budgetary, ethological and metabolic. We opted for the last because of the solidity of the biological bases on which they rest. This does not signify rejection of the two others. However, it was stated that mixing the three criteria (for example mixing of budgetary and metabolic) is to be avoided, as it leads to confusion.

Using the symbols we have adopted, the basic equation for an energy budget is written: $C\text{-}E=A=P+R$. Several comments are required.

A is the *metabolizable* energy of the food. *M* is used to express the weight of an organism and for a long time, *assimilation* was a term used, henceforth considered imprecise and abandoned. Nevertheless it still occurs in certain expressions, such as assimilation efficiency *C/A*.

Some biologists are reticent about adopting a broad definition for consumption *C*. Here, *C* also includes not only wasted food (in rearing) but also food which is seized and then rejected by the mouth or any other capturing organ. The broad definition which we have adopted allows the estimation of the true cost of feeding in the energy budget. In fact, the precise definition of *C* is unimportant, as it is compensated for by a proportional value of F_1. Thus, the value for ingestion does not change because $I=C\text{-}F_1$.

$P+R$ represent anabolism and catabolism respectively, according to the clear distinction of Claude Bernard. It is regrettable that, more and more often, authors use the term metabolism instead of catabolism, under the pretext that respiration is the most widely used indicator of metabolism.

In all energy budgets, as noted in this chapter, values of *P* are underestimated, notably because P_S and P_E are not usually measured, with the exception of P_{S1}. This leads to the calculation of incomplete budgets, whilst giving the impression that they are global budgets. To remedy this, values for P_E and P_{S2}, expressed as percentages of *B,* should firstly be established for a representative species of a zoological group. These could serve as the basis for estimating these two elements, for all species in the group. This would only be an

approximation, but the results would be more realistic than those obtained when P_{S2} and P_E are completely ignored.

Another error is the calculation of incomplete budgets which do not allow the calculation of A. This is the case when E is known and C is not, or conversely, or when P is known but R is not, or conversely. There are also the budgets which are only complete by virtue of expedients such as the use of results published on the same species, as if these published values are definitive and universal in character and as though there is no effect of intrinsic and extrinsic factors, which can differ greatly between experiments. Such studies are useless.

The combustion calorimeter and the *in vivo* calorimeter represent the two methods specific to energetics, where one of the means of energy exchange, heat, is measured directly. Microcalorimeters which work for aquatic animals are a recent development. These apparatuses, coupled to microrespirometers, allow the precise measurement of the intensity of catabolic activity in relation to many factors. Technological advances in this area are becoming more and more necessary for progress in the study of physiological phenomena.

By taking into account cellular renewal expressed in P_{E2} while most bioenergeticians ignore it, we wanted to establish a clear distinction between variables of *flux* (components of the metabolic budget) and variables of *state* (biomass). However, the existence of molecular renewal, notably for structural proteins (section 3.1.2, p. 45 and Figure 3.5) and functional proteins such as enzymes (section 2.1.2, p. 25), shows that biomass is itself subject to a unique matter-energy flux. However, because of the regulation of molecular renewal, biomass appears stable and can be treated as such in macrobioenergetics. In high-precision energetic evaluations, however, the cost of molecular renewal should not be ignored.

Eight global energy budgets were described, but for the exception of the two pectinids, it was not possible to present them in the same way and therefore to draw comparisons. This is a result of the diverse protocols and techniques used by different authors. We have composed summary tables, indicating the values of the components of metabolism. The ways of conceiving, measuring and expressing these values are so diverse that it is doubtful if the tables are of any value.

During the course of these chapters, we have justified our choices. They are based on rigorous metabolic analysis and on the use of standard SI physical units. Thus, in bioenergetics, progress is not only technological, but also conceptual.

6

Population Bioenergetics

> Variables of state and variables of flux must always be very clearly distinguished.
> (Frontier and Pichot-Viale, 1991)

In the preceding chapters we have discussed individuals representative of either different classes (age or size) of a natural population or different batches in a reared population. Thus, in the two cases (nature and aquaculture) we have used the same term *population:* this should be justified. After describing the static variables of state of a population, we shall describe their kinetics. Lastly, we shall tackle the analysis of methods of study of the principal components of an energy budget, or variables of flux, in a population.

6.1 Population characteristics

6.1.1 General characteristics

In biology, the concept of a population is defined differently according to the aim of the studies being carried out: genetics, ecology, ethology, dynamics. We shall give a quick presentation of these points of view.

For the geneticist, 'the population is a genetic community, as it has a common genetic base, a gene pool, distributed amongst its members, but whose distribution is constantly modified by exchanges between lineages. Such an assembly of sexual, interbreeding organisms is usually called a *Mendelian population,* as distinct from an assemblage that reproduces asexually, parthenogenically or by self-fertilisation' (Binder, 1967).

For the ecologist, the population is 'an assemblage of individuals of the same species occupying the same ecosystem…. If it is true that populations are the fundamental building blocks of ecosystems, they cannot constitute a functional unit that can be studied in isolation. From the functional point of view, the smallest subject an ecologist can study is what is termed the population-environment system' (Barbault, 1981).

For the ethologist, 'the definition of a population is essentially social, in the widest sense, even if the network of inter-individual interactions which define the structure of a population are largely influenced by environmental factors and may in turn accidentally be decisive. Where there is a social structure, there is a population' (Legay and Debouzie, 1985).

In the study of population dynamics, a population 'is the assemblage of individuals living in a defined ecosystem and possessing common characters which are transmissible by heredity…. A population is an open bioenergetic system which constantly exchanges energy with its environment' (Laurec and Le Guen, 1981).

The above ideas can be assembled to obtain the widest possible definition: a biological population is an assemblage made up of individuals, which live in a defined space and which have unique genetic exchange between them, and continually exchange matter and energy with their environment. This definition is applicable to natural populations. Is it also applicable to reared populations?

Whether rearing is carried out in an open (oysters) or confined system (salmonids), there is always energy exchange with the environment, through acquisition of food and excretion. The definition of space is much easier for reared populations than for natural populations. A farm constitutes an assemblage of individuals, but usually, as a result of numerous gradings based on size or weight, this structure is reduced to very homogeneous batches in the sense 'of uniformity of individual characteristics' according to the expression of Laurec and Le Guen (1981).

With regard to the genetic criteria used to define a population, there are large differences, according to the farming methods used. When *seed* (spat of molluscs, alevins of fish or postlarvae of crustaceans) for aquaculture are taken from the natural environment, genetic mixing has already taken place. Thus, when oyster spat settles on collectors, the population is truly *panmictic* because the gametes are randomly distributed in the natural environment, from a large number of parents each producing a large number of gametes. In contrast, in hatcheries for molluscs, fish and crustaceans, mixing is no longer observed because only a limited number of parents (usually selected) are used to provide a large number of offspring.

Wilkins (1975), noting the low number of individuals used for the induced fertilization in bivalves, feared that the impoverishment of the gene pool would lead to genetic drift in the output from hatcheries. Studies by Gosling (1982) and Lucas *et al.* (1983) have shown these fears to have no foundation.

To summarize, farms constitute populations which are distinguished from natural populations by their lack of spatial diversity, both demographic and genetic, but this does not mean that they cannot be studied using the same methods.

6.1.2 Spatial structures

Limits and homogeneity of space

Spatial limits are well defined for farmed populations, even if rearing is carried out in the natural environment. Thus, for the oyster farmer the area of his concessions is known and, within each concession, the size of the livestock and the quantity of seed of the year can be determined.

Delimitation is more difficult and less precise for natural populations. In the benthic environment, limits can be defined for sessile or burrowing species in a restricted area, but much less well for mobile species, especially if they migrate (e.g. crayfish). In the pelagic environment, limits are difficult to define because of the vertical and horizontal migrations carried out by the planktonic or nektonic populations which live there.

Within a defined space, an ecological inventory is necessary to assess homogeneity or heterogeneity which might affect the metabolism of the species being studied. Where there is spatial heterogeneity, it is necessary to work on subassemblages. Research is occasionally carried out at such heterogeneous sites to discover the effect *in situ* of a factor on a species' metabolism.

Example of a homogeneous space Shafee (1980) confined his study of the sessile species, *Chlamys varia,* to the Lanvéoc bank in Brest Harbour. This 360 km^2 area is homogeneous in terms of *bathymetry* (-6 to -9m), *substrate*—consisting of calcareous debris (dead calcareous alga, *Lithothamnium calcareum)* and old mollusc shells—and *its fauna:* dominated by *Chlamys varia* along with some other bivalves, gastropods (including *Crepidula fornicata),* echinoderms (urchins) and crustaceans (hermit crabs) and in terms of its *climatic factors* (temperature varies between 17°C and 8°C through the year and salinity fluctuates around 34.7‰).

Examples of heterogeneous spaces Such spaces might be a study site consisting of two different bathymetric levels, one intertidal (with periodic emersion), the other subtidal (without emersion); a mosaic of rocky and sandy areas; wet patches on beaches; a thermal plume from an industrial outflow, etc.

Types of distributions Knowledge of distribution characteristics is essential for determining the abundance of a population. When sampling is carried out randomly in a uniform space, three types of spatial distributions are found (Barbault, 1981). If the individuals fall into a *random distribution,* then the frequency of samples with 0, 1, 2,…*n* individuals follows a Poisson series. In this case the mean number x per quadrat is equal or close to s^2, the variance. When $s^2 > x$, this corresponds to a *clustered distribution.* When $s^2 = 0$ the distribution is *uniform.* These three types of distribution are shown in Figure 6.1.

6.1.3 Demographic structures

We shall confine ourselves here to an explanation of classical methods. For fuller information, the reader could consult works devoted to biological populations, e.g. Barbault (1981).

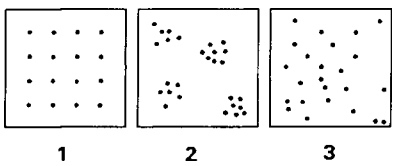


Figure 6.1 Spatial distribution patterns of individuals on a surface: 1, uniform; 2, clustered; 3, random (after Barbault, 1981).

Age classes and cohorts

In general, a population consists of animals of different ages. Many different methods of ageing can be utilized. Thus, an *age class* is associated with each year. As date of birth is not usually known exactly, a convention is used: the age group to which an animal belongs is defined by reference to the number of January the firsts it has lived through (Laurec and Le Guen, 1981). Thus, an animal born in August appears in age class 0 until 31 December. On 1 January it passes into age class 1. This convention is widely applied in fisheries, but not always for unexploited species. By convention, an animal changes its age class every year, either on 1 January or on some other predefined date.

According to Laurec and Le Guen (1981), a *cohort* consists of the assembly of animals in a population that were born in the same year. This cohort is characterized by calendar year of birth. It is this precise and simple system which we have adopted. Nevertheless, a certain amount of confusion surrounds the definition of a cohort. Thus, for Dajoz (1974) a cohort consists of 'a group of individuals which have lived simultaneously through the same significant event but are not necessarily the same age'. It is this definition which is used in human demography. Finally, for some ecologists, a cohort is made up of individuals of the 'same age', so that if there are two reproductive periods in one year (spring and autumn for example), there are two cohorts for one year. To resolve this confusion, Laurec and Le Guen (1981) propose to give the cohort the sense of an annual cohort and to call assemblies that are more limited in time, *microcohorts* or *minicohorts,* according to the scale.

Age pyramids

A pyramid-shaped figure showing age classes of a population can be used to assess the demographic composition of a population. It takes the form of a double horizontal histogram which gives a representation of the population by sex and age. The figure can have a tapered outline, hence the name pyramid (Pressat, 1979).

Widely used in human demography, age pyramids have the advantage of giving an instant view of the demographic equilibrium. Growing populations have an age pyramid with a wide base. A bell-shaped curve is indicative of a decreasing population (Dajoz, 1974). A war or an epidemic can have different effects on the numbers in certain cohorts and may affect the sexes in different ways.

In animal biology, the sex of individuals is rarely taken into account; a single histogram is therefore produced, with the youngest cohorts at the base and the oldest at the peak. The numbers in each cohort form the basis of population dynamics, especially for the calculation of total mortality.

Age-size keys

A detailed analysis of the methods of determining age as a function of size can be found in Daget and Le Guen (1975a).

Age determination of an individual

This is carried out using marking. Daget and Le Guen (1975a) distinguish between natural and experimental marking.

Experimental marking involves giving an individual a unique mark. The size of the individual is noted at the time of marking. When it is recaptured alive, the growth rate over the intervening time period can be established. Again a mark must be found that is suitable for the species being studied: one that does not bother the animal, does not provoke necrosis or irritation and remains legible after several years. For fish, an exhaustive description of marks used can be found in Laird and Scott (1978). For sedentary species, a variation of this technique involves placing individuals of the same age together in a cage or defined space (collective marking).

Natural marking arises in the hard parts of the body, which show bands that are related to growth. It is necessary that these marks be legible and not blurred by phenomena other than that which is being studied, annual growth. Natural growth markers are found in most systematic groups. The best known are: for fish, scales, otoliths and many bony structures; for bivalves, the shell and ligament. Two examples follow.

In the clam *Venus verrucosa,* a bivalve with a thick shell, slow growth and life span of 15–20 years, statistical analysis of size frequency gives no result. In order

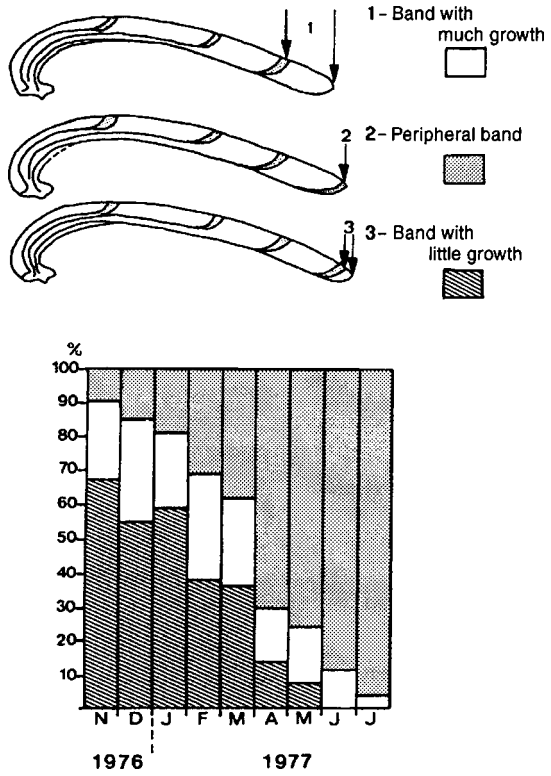


Figure 6.2 Position of the distal band from November 1976 to July 1977 in Venus *verrucosa* in the western English Channel (Rade de Brest, Brittany and Baie de Granville, Normandy). Above, shell section showing three characteristic positions of the distal band. Below, a histogram based on 742 observations, indicating the monthly percentage of individuals corresponding to the three categories. From this it is concluded that the growth lag occurs between November and February-March and that from April there is a significant resumption of growth (Djabali and Yahiaoui, 1978).

to establish an age-size key, the authors had to read ligament striations (Le Gall, 1969) or shell bands (Djabali and Yahiaoui, 1978; Berthou, 1983). The latter proved annual periodicity of bands indicating cessation of growth, by studying the monthly position of the distal band (Figure 6.2).

Deniel (1990) studied the growth of 10 species of flatfish in the Bay of Douarnenez (Brittany). He established an age-size key for each species, based on the analysis of otoliths which show alternation between an opaque (autumn-winter) and a clear (spring-summer) area. For sufficiently thin otoliths, counting of the bands was carried out directly using a binocular microscope; the burning method of Möller-Christensen (1964) was used for thicker otoliths.

Age determination using statistics

This can be carried out in populations where reproduction is synchronous and occurs over a short time period. As a result, the generations are well separated and this is shown by distinct modes in size-frequency histograms. Polymodal analysis of such histograms allows recognition of successive cohorts and calculation of their size (Figure 6.3).

When successive modes are indistinct or the histograms appear asymmetric, the hypothesis of *hidden modes* can be invoked to apply the method of successive maxima, as explained in Figure 6.4.

From age-size keys, demographic structures can be established, in other words the relative numbers of each cohort making up the population at a given time. In order to convert relative into absolute values, it is necessary to know the population density.

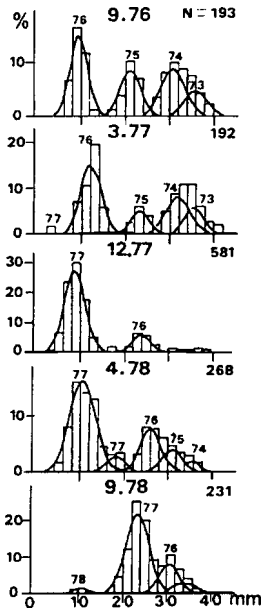


Figure 6.3 Length-frequency histogram of the bivalve *Donax trunculus* in Douarnenez Bay (Brittany) from September 1976 to September 1978. N, number of individuals. In this population the juveniles occupy different areas from those of the adults: they may be underrepresented in samples, e.g. September 1978 (reproduced from Guillou, 1982). Note differing vertical scales.

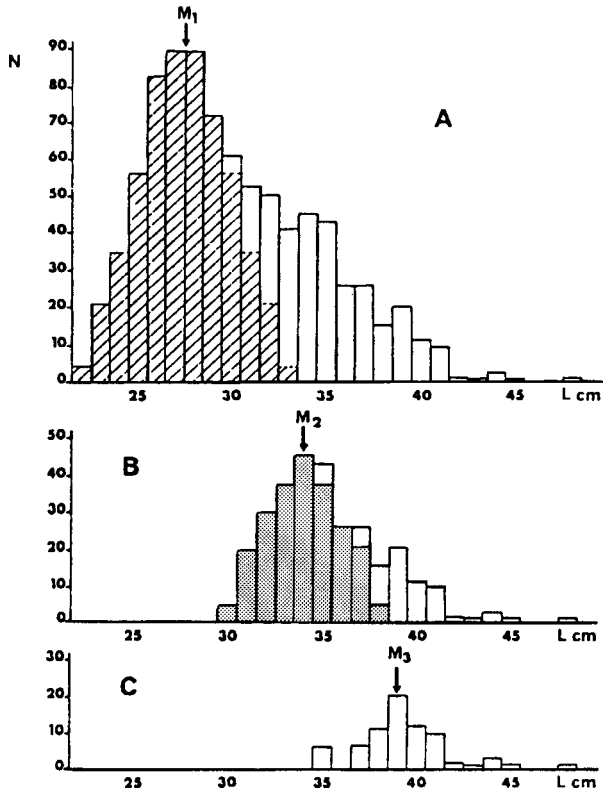


Figure 6.4 Examples of the application of the successive maxima method. (A) Complete sample (858 fish *Pseudolithus elongatus* landed at Pointe Noire, 17 March 1965; data of Daget and Le Guen, 1975a); from mode M_1, the right-hand part of the distribution, which includes individuals from the 1st age group (hatched), is reconstructed by symmetry. (B) Once the individuals from the 1st age group are removed, a new mode M_2 is evident, from which the group of individuals constituting the 2nd age group can be identified (stippled). (C) The individuals remaining constitute a 3rd age group with mode M_3=39 cm (reproduced from Barbault, 1981).

Estimation of population density

Density is the number of individuals per unit surface or volume. To simplify matters we shall only look at species distributed on a surface i.e. benthic species.

As Legay and Debouzie (1985, p. 45) noted, 'the term density should only be used for the interior of a space which is sufficiently homogeneous for the mean number of individuals per unit surface to be meaningful'. This takes us back to section 6.1.2, regarding the homogeneity of space and the distribution of individuals. If the distribution is not homogeneous, subsectors (or strata) are created, within which there is constant density. For example, Shafee (1980), working on the population of *Chlamys varia* on the Lanvéoc Bank, read the growth bands of the shell to establish a size-age relationship. Thereafter he carried out routine dredging to establish demographic structure and population density. The area covered by the dredging was determined from the size of the dredge and the length of each tow. To evaluate the efficiency of the dredge, preliminary studies were carried out, in which

divers collected all the clams remaining after a dredge had passed over the bottom. This enabled results to be corrected to give relatively accurate estimates of not only the structure but also the density of the population. By these means, it was possible to follow recruitment and mortality rate through the course of a year.

6.2 Demographic kinetics

According to Barbault (1981, p. 30), '*population dynamics* involves defining the action of diverse environmental factors on demographic variables' while *demographic kinetics* is outwith environmental conditions. Frontier and Pichot-Viale (1991, p. 120) clarify this distinction. Kinetics describes changes in abundance and structure with time, dynamics gives the explanation of these changes. In effect, 'in physics… kinetics is the study of movements of bodies and dynamics is the explanation of these movements by the "forces" applied'. Hereafter we shall study the development of demographic structures with time, first at the cohort and then at the population level.

6.2.1 Development of a cohort

In order to follow the events which influence the demography of a cohort, a *double classification* is used, namely the time elapsed since the origin of the cohort (expressed in years) and the number of individuals in each cohort for each year. This information can then be tabulated for all cohorts in a population in what Pressat (1961) termed a Lexis diagram.

Figure 6.5 shows the advantage of this method of representation. To follow a given cohort, the diagram is read across a diagonal. In this figure, the mean values for five cohorts are to be found at the right of the diagram. Thus a *mean cohort* is obtained,

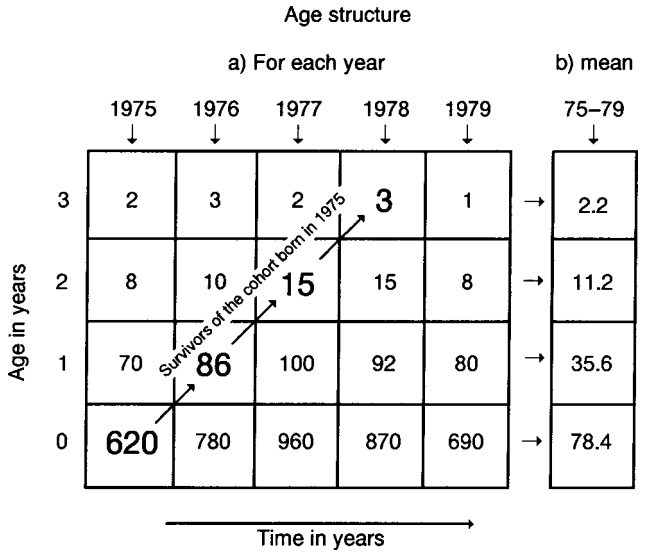


Figure 6.5 Lexis diagram of an imaginary population followed over a period of 5 years (modified from Barbault, 1981).

which is less influenced by fluctuations in external factors. From such values, the survival curve of a cohort can be obtained, which is representative of the species.

Survival tables can be established from Lexis diagrams: the most widely used are the *longitudinal table* or *age-specific table* and the *transversal table* or *time-specific table*. Tables 6.1 and 6.2 were produced using the data in Figure 6.5. Daget and Le Guen (1975b) noted that instead of quotients q_x, it is preferable to calculate instantaneous coefficients of mortality, which have the advantage of being additive and leading to integrals which can be calculated when incorporated into production equations. In proposing the hypothesis that the number of individuals disappearing per unit time is proportional to the number of individuals remaining and to a factor Z which is the instantaneous coefficient of mortality, we have: $dN/dt=-ZN$ or $dN/N=-Zdt$. If the total number at time $t=0$ is N_0, the number of individuals remaining at time t is N_t and we have: $N_t=N_0\ e^{-Zt}$. Knowing N_0 and Z, this relationship allows calculation of the number of individuals surviving at any time.

The most representative survival curves can be assigned to four groups (Figure 6.6).

- Group 1: strongly convex survival curve. All individuals have about the same life span and die almost simultaneously.
- Group 2: strongly concave curve. Mortality mainly affects the young.
- Group 3: weakly sigmoidal survival curve. A constant fraction of the population dies at each age.

Table 6.1 Longitudinal survival table of the cohort born in 1975: x is age in years; n_x number of individuals at age x; l_x, the number of individuals surviving to age x, out of 1000 individuals; d_x, the number of individuals disappearing between x and $x+1$; q_x, the mortality quotient between x and $x+1$, also called age-specific mortality rate; e_x, the mean probability of living to age x (data from Barbault, 1981)

x	n_x	l_x	d_x	q_x	e_x
0	620	1000			0.668
			861	0.861	
1	86	139			0.709
			115	0.827	
2	15	24			0.708
			19	0.792	
3	3	5			0.5

Table 6.2 Transverse survival table for 1975–1976 (conventions as in Table 6.1)

x	Age structure (mean for 1975 and 76)	d_x between 1975 and 76	q_x between 1975 and 76
0	700	620 – 86 = 534	534/620 = 0.861
1	78	70 – 10 = 60	60/70 = 0.857
2	9	8 – 3 = 5	5/8 = 0.625
3	2.5	2 – 0 = 2	2/2 = 1

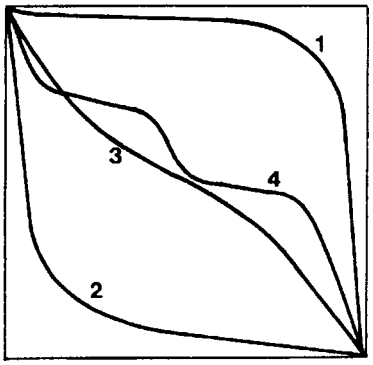


Figure 6.6 Main types of cohort survival curve. On the vertical axis: number of survivors per 1000 individuals. On the horizontal axis: age expressed as a percentage of the mean life length of a cohort. See text (modified from Dajoz, 1974).

- Group 4: curve with several inflexion points. Irregular survival rate.

6.2.2 Growth of a population

Several kinds of population growth exist. However, two of these are of particular theoretical interest. The first is the population with *exponential growth* in numbers or geometric growth, characterized by *r,* a constant *instantaneous growth rate* called the *maximum intrinsic growth coefficient* (Figure 6.7). The second is the population with *logistic growth* which, in the formulation of Verhulst (1845), is limited in numbers as a function of environmental constraints; this size limit of numbers *(N)* is expressed by the letter *K* (Figure 6.7).

Geometric growth is observed in the case of organisms colonizing an *a priori* unlimited environment; logistic growth occurs in a population in a naturally limited environment.

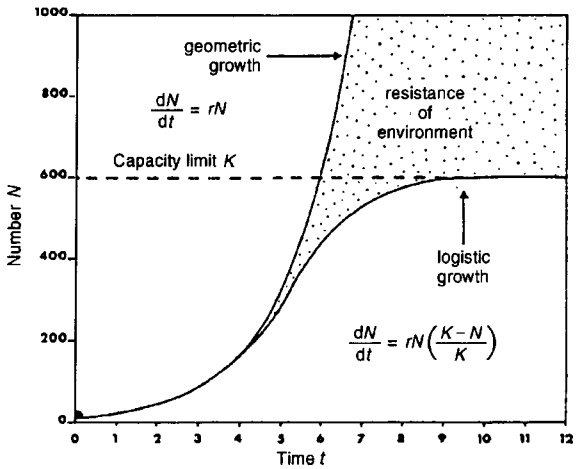


Figure 6.7 Exponential and logistic growth. The equations for the two curves are given in the figure (modified from Barbault, 1981).

6.3 Demographic production

In starting on this subject, we enter the domain of population dynamics. According to the distinction previously established (section 5.3.1), we take into account a variable of flux, production, the values of which are influenced by 'various environmental factors' (Barbault, 1981).

6.3.1 Different concepts of production

When production is talked about in demographic terms, it always involves tissue production, i.e. P_G. The values of P_R, P_S and P_E are not taken into consideration in order to evaluate 'demographic production' as was explained in section 3.2.2.

Demographic production can be expressed in two different ways (Crisp, 1971). The first approach (P_1) is to add together the growth of all the members of the population over the period studied and to ignore the fate of this biomass. The second approach (P_2) is to ignore growth and to consider the fate of the biomass produced during the study period. Conan *et al.* (1976) give these two approaches an ecological interpretation. P_1 is an evaluation of the balance of accumulated energy provided by lower trophic levels. P_2 is an evaluation of the energy remaining, corresponding to the amount of tissue provided to higher trophic levels.

The P_1 type of approach is the only one possible for the bioenergetic study of an isolated organism. Application of bioenergetics to a population in an ecological context, however, broadens the scope of considerations, leading to concepts such as production type P_2. In order to complete this analysis we propose a strictly bioenergetic interpretation of P_1 and P_2 for a population considered as an open thermodynamic system, exchanging energy and matter with its environment (Figure 6.8).

The expression P_3 results from another approach which is neither metabolic nor ecological, but of a socio-economic nature. P_3 is effectively a way of expressing production, from which humans profit, per unit time. For the human economy it is a yield obtained from fishing a natural population, or more exactly a stock of living animals, the size of which is bounded by a minimum and a maximum (section 7.1.1).

6.3.2 Production in one or more cohorts

This particular case occurs when the population being studied is lacking in natural recruitment and is made up of one or several identifiable cohorts. Some examples follow.

In aquaculture, young individuals are transferred into a confined environment. For example young mullet are captured in an estuary and transferred to a pond or lake where they do not reproduce but grow well. The aquaculture of sedentary species, such as oysters and mussels, is based on the segregation of annual cohorts which are penned in different zones, depending on their age.

By carrying out mass marking, individuals from one particular cohort can be distinguished from others. This is what happens in Japan where young abalone are released into the wild to grow at least expense. As the apex of the shell has a particular coloration acquired from the food given in the hatchery, they cannot be confused with abalone born in the wild.

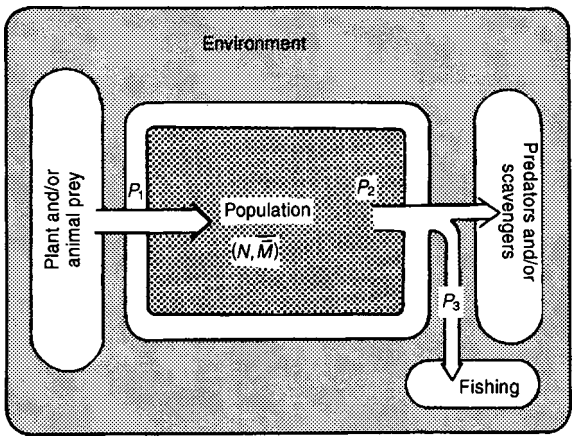


Figure 6.8 A population, an open thermodynamic system (dotted), exchanges energy and matter with its environment (shaded). The methods P_1 and P_2 allow the measurement of demographic production using two approaches. P_1 is the amount of energy-matter that passes, per unit time, from the environment into the population. P_2 is the amount of energy-matter that passes, per unit time, from the population into the environment. If the population is in equilibrium (numbers and biomass are constant), $P_1=P_2$. P_3 represents the portion of P_2 taken by human fishing per unit of time. If the population is not exploited, P_3 does not exist and all energy-matter, P_2 is used by predators and scavengers. The arrows correspond to variables of flux and the dotted area to variables of state.

Calculation of the production of such a population can be carried out arithmetically or graphically.

Arithmetic method

We shall examine successively the demographic productions P_1 and P_2, as defined in section 6.3.1. To calculate production, the increment summation method (ISM) is applied, as advocated by Crisp (1984). If the following data are given: N_t, the number in a cohort at time t, and M_t, the mean individual weight at time t, where t=1, 2,…n and where n is the number of measurement dates, the *accumulated production,* P_1,at time t becomes:

$$P_2(t)=[(N_t+N_{t+1})/2][M_{t+1}-M_t]$$

From this the annual production of a cohort is:

$$P_1(\text{annual}) = \sum_0^1 P_1(t).$$

This method of calculation with several differences in notation was developed and illustrated by Lamotte (1973): see Figure 6.9.

Output production, P_2, can be evaluated in a similar way:

$$P_2(t)=[(M_t+M_{t+1})/2][N_{t+1}-N_t]$$

$$\text{from which } P_2(\text{annual}) = \sum_0^1 P_2(t).$$

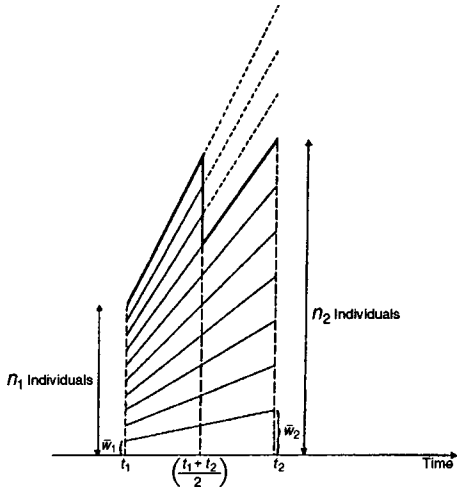


Figure 6.9 Diagram showing how the formula allowing calculation of the production of a cohort between times t_1 and t_2 is established; $\bar{W}_1$ and $\bar{W}_2$ are the mean individual weights at times t_1 and t_2; n_1 and n_2 are the numbers at t_1 and t_2. From this the production between t_1 and t_2 is: $P=[(n_2+n_1)/2]\,[\bar{W}_2-\bar{W}_1]$ (adapted from Lamotte, 1973).

In order for these calculations to be valid, the time between measurements must be short enough that growth and mortality rates can be assumed to be constant.

Brey (1990) noted that the ISM has a major flaw: the variability of production is not estimated. To fill this gap, he proposed the non-parametric statistical 'bootstrap' method.

Graphical method

In a cohort, while time passes, the number N of individuals decreases and the mean individual weight increases, except possibly in the case of weight loss during hibernation. Figure 6.6 showed various survival curves. We shall retain curve 2 because of its common occurrence in the aquatic environment, notably in the invertebrates. Figure 6.10 shows the survival and mean individual weight of an imaginary cohort over the course of a year.

From this can be calculated a graph of the production of a cohort (Figure 6.11), which is the basis of the dynamics of exploited populations. If at time t_i, the number N_i is multiplied by the mean individual weight $\bar{M}$, the production of the cohort is obtained. This production increases until it is appropriate to call it critical production (P_{cr}), at a critical age (t_{cr}). Thereafter it decreases when the growth in weight no longer compensates for loss of numbers.

6.3.3 Production of a population

At the population level, *recruitment* must be taken into account, i.e. the arrival of young individuals in a population. Forms of recruitment vary widely between species and the term has a unique meaning for fisheries (section 7.1.2).

When recruitment and development of cohorts are broadly similar from one year to the next (stable population), production of all cohorts together can be carried out from measurements from one year only. Successive cohorts present in the population at one time

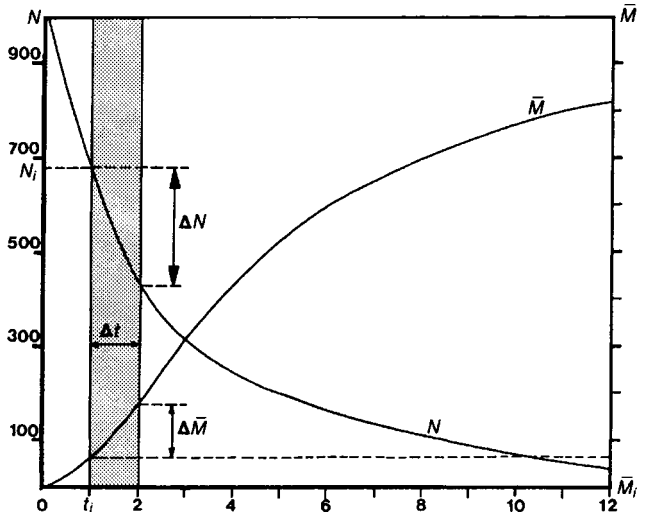


Figure 6.10 Theoretical curves showing the development, with time, of the numbers *(N)* out of 1000 individuals and the mean individual weight $(\bar{M})$ of a cohort. On the abscissa, time in months. Over a period of time (in this case, 1 month), the mean weight increases by $\Delta\bar{M}$ and the number of individuals decreases by ΔN.

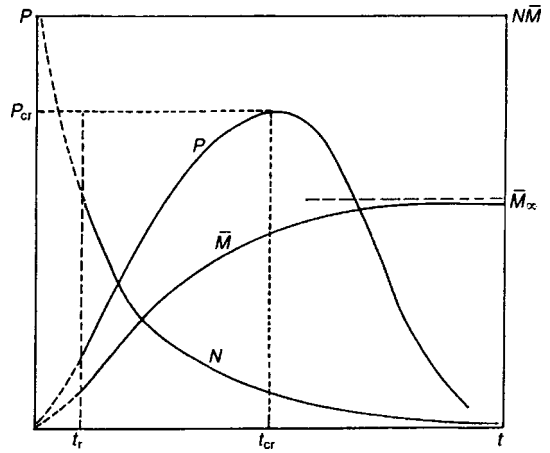


Figure 6.11 Development with time of the production of a cohort. On the ordinate: *P,* production; P_{cr}, critical (or maximal) production; *N,* numbers, $\bar{M}$ mean individual weight; $\bar{M}_\infty$ (often called W_∞) is the asymptotic weight. On the abscissa, time *t* is expressed in years; t_r, age at recruitment; t_{cr}, critical age.

are regarded as representative of cohorts in successive years. The population production, at time t_i, is therefore equal to the sum of the calculated productions of a cohort at time t_i, t_{i+1}, t_{i+2},…t_{i+n}, *n* being the number of cohorts present in the population and time being expressed in years.

For example, Shafee (1980) calculated P_1 and P_2 using the formulae of Crisp (1971). He considered five age groups from 0 to 4, to represent five cohorts. The original results were expressed in kcal per 100 m^2. We have converted them into

kJ m^{-2}. The author studied the population over 2 years in order to assess interannual fluctuations.

The results obtained from the two methods (Table 6.3) differ by 2% to 5% in relation to the mean for all age groups, except group 0. In the latter, in 1976 as well as in 1977, the differences are considerable. How can this be explained? According to Conan *et al.* (1976), during the recruitment period in a natural population the changes from $\bar{M}_t$ to $\bar{M}_{t+1}$ due to metabolic processes are masked by the arrival of young, small individuals. Thus, $\bar{M}_{t-1}$-$\bar{M}_t$ is an underestimate, whence the values of P_1 are markedly lower than those of P_2.

NB: All the ratios (e.g. $P/\bar{B}$), correlations and efficiencies established for the production of organisms (Chapter 5) can also be applied to populations.

6.3.4 Interannual variations in production

Apart from pathological infections, variations in production of a population from one year to the next are essentially due, in temperate countries, to the amount of food available and temperature. When these two factors have high values, production is increased. In many cases, especially in herbivores and microphages, the amount of food is itself affected by temperature. Thus, knowledge of temperature values throughout the year is essential for analysis of the results.

Water temperatures are not always known, while air temperatures can be obtained from the very tight observation network of the Meteorological Services. A precise relationship exists between air temperature and that of continental or coastal waters. This correlation was shown for the entire Marennes-Oléron basin by Héral *et al.* (1986).

Thus knowledge of temperatures throughout the year where a population has been studied allows the results to be put into perspective. To this end, the mean of temperatures observed over 35 years is taken as a reference. The temperatures recorded during the study year are compared and any deviations from the mean, either upwards or downwards, are noted (Figure 6.12).

Table 6.3 Production calculated using two methods, over 2 years, of a population of the queen scallop, *Chlamys varia,* at Lanvéoc, in Brest Harbour (Brittany). Values are in kJ m^{-2} (recalculated from Shafee, 1980)

Age groups (years)	1976		1977	
	P_1	P_2	P_1	P_2
0	3.1	4.5	2.4	4.4
1	6.7	6.4	6.5	6.2
2	5.4	5.2	2.4	2.6
3	2.9	2.8	2.4	2.1
4	0.9	0.8	1.0	1.1
Total	19.0	19.7	14.7	16.4

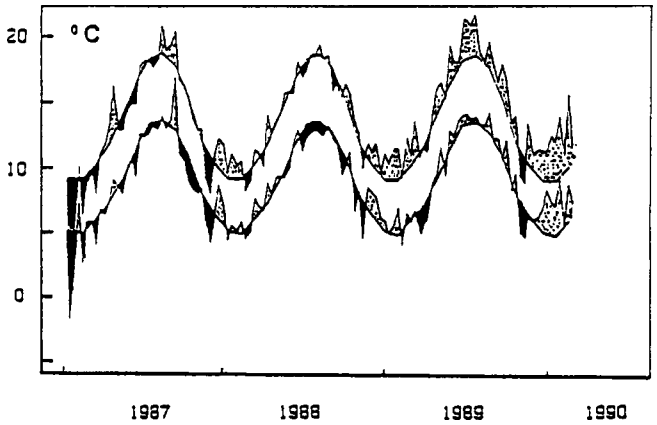


Figure 6.12 Change in maximum and minimum aerial temperatures taken every 10 days from 1987 to 1990 in relation to mean curves established for the past 35 years at the semaphore on l'île de Batz (Brittany). Periods when the temperature is lower than the mean are shown in black and those where it is higher, in grey. Note that 1987 is lower than the mean, 1988 is close to it and 1989 above the mean (Guillou and Tartu, 1991).

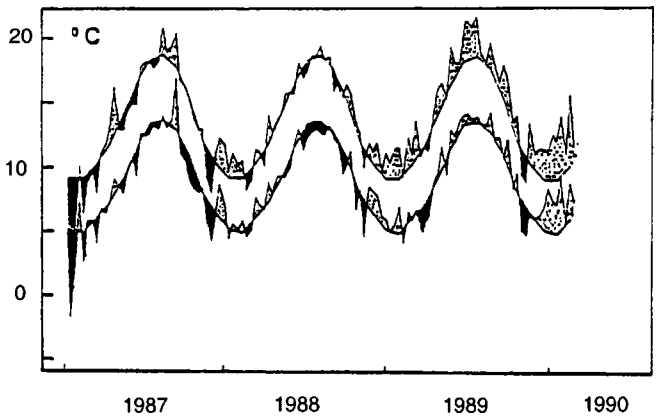


Figure 6.13 Population of *Cerastoderma edule* (Bivalvia) at Saint-Paul-de-Léon. Density (number per m^2) of young recruits (R) of 1 to 2.5 mm inclusive and overall density of the class GO in which the size is between 1 and 15 mm inclusive, resulting from recruitment in 1987, 1988 and 1989. Note that recruitment is higher in 1989 than in 1988, which itself is higher than in 1987. There is therefore a positive correlation between temperature and recruitment (Guillou and Tartu, 1991).

Guillou and Tartu (1991) studied recruitment in a population of cockles, *Cerastoderma edule,* at Saint-Pol-de-Léon (near l'île de Batz) in relation to temperature records for the years 1987 to 1990. The warmer the year, the higher the intensity of recruitment (Figure 6.13).

6.4 Other elements of the energy budget

As for production, all the elements of an energy budget can be calculated at the population level when the demographic structure and population density, and values of *C, E, R,* P_R etc. obtained from representative individuals from each cohort in the population, are known.

Results for E and P_R will be examined because of the ecological importance of these two fluxes.

6.4.1 Excreta and biodeposits

Excreta are made up of pseudofaeces, faeces and urine (section 3.1) $E=F_1+F_2+U$. In natural aquatic environments, urine is dilute and is not taken into account.

Biodeposits are particulate excreta which are deposited on the substrate. They are mixed with the sedimentation of the seston (mineral and organic). Also, when measuring biodeposition using a particle trap (Figure 4.11 and section 4.6.1), the weight of sestonic sedimentation must also be measured and subtracted from the weight of the mixture of biodeposits + sestonic sediments.

In intensive aquaculture, biodeposits are harmful. Thus, below fish-rearing cages in the sea (e.g. salmonids), oyster culture rafts (notably in Japan) or raised shellfish cultures, it is often noted that these deposits with high organic content are the centre of hydrogen-sulphide-releasing fermentations. The more intense the culture and the slower the water currents, the higher the toxic effect. This is the case, for example, in the Inland Sea of Japan, where according to Mariojouls and Kusuki (1987), suspended oyster cultures attain a density of 270 kg m^{-2}. Under these extreme conditions, the periodic moving of the rafts is insufficient; instead the bottom must be turned over by a harrow to encourage oxygenation and mineralization of the organic detritus.

The intensity of biodeposition varies between species, seasons and sites. Table 6.4 gives an example. In February, organic biodeposits are at their highest: they are equivalent, in one day, to the body weight of *Mytilus edulis*. For *Crassostrea gigas* in the bay of Hiroshima, Mariojouls and Kusuki (1987) found in June a value of 200 mg day^{-1} per g of dry flesh, i.e. a mean value comparable to the results of Deslous-Paoli *et al.* (1987).

These values are not only of significance for cultured populations but also all benthic populations. Figure 6.14 illustrates this phenomenon in a population of filter feeders.

Table 6.4 Weight of organic matter (OM) of biodeposits of three species of molluscs in the Marennes-Oléron basin. The values of OM are expressed in mg day^{-1} per gram dry flesh (data from Deslous-Paoli *et al.,* 1987)

Species	July	February
Mytilus edulis	67	1080
Crassostrea gigas	74	537
Crepidula fornicata	23	81

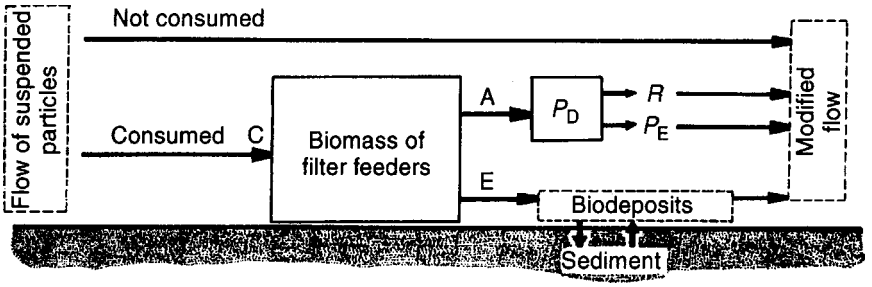


Figure 6.14 The flux of suspended particles passing over a population of filter feeders is modified. The particles consumed (C) are either assimilated (A) or eliminated (E), constituting biodeposits (faeces + pseudofaeces). Some of the biodeposits are carried away by the current and some combine with the sediment. Assimilation allows development of biomass (P_D), respiration *(R)* and eliminated production (P_E).

6.4.2 Reproduction

In demography, reproductive activity can be expressed in two ways: either by studying the number of births or eggs (fecundity) or by studying the energetic cost of reproduction (reproductive effort).

Fecundity

The *gross fecundity rate* is the relationship between the number of births (or eggs), *n,* taking place during a period of time (usually a year) and the mean size, *N,* of the population during this period.

The *age-specific fecundity rate* (Barbault, 1981) is the mean number of females, m_x, produced by a female of age *x*. In order to calculate this rate, a *fecundity table* is used, modelled after the longitudinal survival tables (Table 6.1) to which one column is added for m_x and another for the product $l_x m_x$. The values of m_x are obtained from the mean number of spawnings per year and from the *rate of female maturity* at age *x*. From this we obtain the *net rate of reproduction* R_0:

$$R_0 = \sum_{pr}^{dr} l_x m_x$$

where *pr* is age at first reproduction and *dr* age at last reproduction. When R_0=1 the reproductive rate will maintain constant population size.

At age *x* a female has a reproductive expectation V_x which has two elements: m_x, which is the immediate reproductive value, and V'_x, which is the future reproductive expectation or *residual reproductive value* (RRV).

$$V_x = m_x + V'_x$$

where

$$V'_x = \sum_{x}^{dr} \frac{l_{x+1}}{l_x} m_{x+1}.$$

The reproductive values can be calculated precisely in species with low fecundity and most studies are carried out on mammals, birds, reptiles, insects. When the number of eggs reaches thousands or millions, as is the case for most aquatic animals, the results are more uncertain. Despite these difficulties, such studies have been carried out on some species of marine molluscs and echinoderms (Vahl, 1981).

Reproductive effort

Reproductive effort (RE) in organisms has been defined and illustrated in section 5.3.5. The reproductive effort of a population is derived from data for individual, representative organisms from each age class of the population being studied. Thus, the values given in Table 5.11 for some patellids by Parry (1982) were in fact calculated for four populations representing the four species studied.

Reproductive effort defined as P_R/A has been utilized to establish the concept of demographic strategy, notably by Williams (1966). According to this concept, an organism uses up a limited amount of energy which it must divide between three functions: maintenance, growth and reproduction. Reproductive effort expended by an individual at age x is a function of V'_x (Williams, 1966). This hypothesis is based on certain observations. Thus, the higher m_x, the greater the risks of growth retardation or cessation. If it is assumed that the allocation of energy to maintenance is constant, when the portion for reproduction increases, that for growth decreases by the same amount. In other respects, at the demographic level, intense reproduction increases the risk of mortality and therefore causes a decrease in number of reproductive individuals (Calow and Woolhead, 1977).

Study of real cases of aquatic species sheds a different light on these theoretical considerations. Two examples follow.

In the queen scallop, *Chlamys islandica,* which lives for about 20–25 years, reaching maturity at 5 years, Vahl (1981) was unable to find a relationship between RE and RRV until the age of 13 years.

The case of the threespine stickleback, *Gasterosteus aculeatus,* studied by Wootton (1985), is different. The stickleback is effectively a semelparous species which reproduces at 1 year old, the spawning being divided into several successive batches. According to Wootton (1985), fecundity in the stickleback is a function of body weight, which is usually the case in teleosts. More exactly, the number of eggs per batch is determined by the biomass of the female when maturation begins. Consequently, if females receiving a higher food ration are compared with those on a reduced ration, it is noted that the former have a higher growth rate and that their fecundity is slightly improved by the addition of some extra batches. On the contrary, females on the reduced ration proceed with the development of their eggs, by using their body reserves, notably from the liver. It follows that their biomass decreases greatly and by definition their reproductive effort is higher than that of the well-fed females.

r and K selection

The concept of demographic strategy (Williams, 1966) has been expanded by MacArthur and Wilson (1967), who introduced the idea of *r* selection for expanding populations in which reproductive effort is intense, as opposed to *K* selection. Referring to Figure 6.7, note that the *K* value represents the size limit (or

Table 6.5 Characteristics of *r* and *K* strategies (Pianka, 1970)

Characteristics	*r* Selection	*K* Selection
Environmental conditions	Variable, unpredictable	Constant, predictable
Numbers	Variable, less than *K*	Stationary, close to *K*
Mortality	Irregular, sometimes catastrophic, density-independent	Regular, density-dependent
Reproduction	Precocious, high RE, semelparity	Late, low RE, iteroparity
Development	Fast, no protection of young, adults small and short-lived	Slow, protection of young, adults large and long-lived

maximum density) of the population in the environment considered. The characteristics of *r* and *K* strategies have been listed by Pianka (1970) and are shown in Table 6.5.

Table 6.5 only cites extreme cases, while it is very obvious that all sorts of intermediate or non-conforming situations exist between the two strategies. This has been shown with regard to cephalopods (Boletzky, 1986). In particular the dichotomy between semelparity and iteroparity is an oversimplification (Table 6.6).

Boletzky (1986) showed also that certain cephalopods, considered as *r* strategists, nevertheless have *K*-type characteristics, for example incubation and ovoviviparity which ensure protection until the juvenile stage.

After becoming extremely fashionable, the *r-K* strategy theory was widely questioned. Thus Barbault (1987) denounced 'the error which spread throughout the seventies was in tacking on *r-K* terminology to any simple difference arising between populations or species'. He recommended restricting the use of this terminology to certain cases: *K* selection tends to produce high population densities and causes growth in size of the population to equilibrium. He also added that demographic strategy is not limited only to *r* and *K* selection and that other causes can be invoked: climatic constraints, habitat stability, predation, requirement for dispersion etc.

Table 6.6 Frequencies of reproductive activities characterizing a species

Production of young	Single reproductive season	Several reproductive seasons
One batch per season	Monoseasonal Uniparous	Multiseasonal Uniparous
Several batches per season	Monoseasonal Iteroparous	Multiseasonal Iteroparous

6.5 Comments on ecosystem energetics

6.5.1 General considerations

It might be tempting to believe that after passing from the cell to the organism, and from organism to population, the next step would be to the ecosystem. Such a step is insurmountable and this is why.

The graduation cell-organism-population is based on results all obtained following analysis of metabolism. The key formula of bioenergetics: $C\text{-}E=A=P+R$ applies equally well to a cell, an organism or a population. In other words, throughout all chapters we have studied aquatic animals, either individually (the organism), or at the level of their structural unit (the cell) or at the level of their demographic unit (the population) —three approaches to one subject.

An aquatic ecosystem is not made up only of aquatic living organisms. It also has a non-living part and it is this which forms its foundation. On the one hand, geomorphology imposes limits and means of exchange between neighbouring ecosystems. On the other hand, the characteristics of water masses, their dynamics, are influenced by winds and tides and their physico-chemical composition is the result of the combined effects of climate and the activity of the living organisms which live there. Thus *ecosystem energetics* cannot be considered without 'bioenergetics'. With regard to the living organisms in the ecosystem, they are not confined to some populations of animals (the subject of this work), but include also plant populations which are influenced mainly by sunlight and certain mineral salts, and bacterial populations influenced by organic matter and temperature.

Consequently, to study the energetics of an ecosystem, a *synthetic process* has to be adopted, in order to follow the evolution of major flows of matter and energy. This process is the opposite of that which we have followed hitherto. The same is true for methods: there are those in 'synecology' which can be applied to ecosystems.

6.5.2 Study of an example

To clarify this opposition, we shall study the real example of the Marennes-Oléron basin. In this shellfish culture area, three main kinds of biotope can be distinguished (Figure 6.15). The first site is a zone of oyster beds (section 7.2.1) located on either side of the Seudre estuary and to a lesser extent in the Charente. Site 2 is in a tidal convergence zone, where the shore emerges at low tide. Zone 3 is the subtidal area of the estuary, always immersed. In the study of the Marennes-Oléron basin, two kinds of research into energetics can be envisaged, one looking at the ecosystem and the other at the populations of this ecosystem.

In the former, the entire basin would have to be taken into consideration, as would the sites of exchanges with neighbouring ecosystems. The channels of Antioche and Maumusson make up the marine domain; the river domain consists of the Charente and the Seudre. In these exchanges there are not only the water masses with their particular physico-chemical characteristics, but also the transported material. This seston consists of inert particles and living planktonic organisms (Pérès, 1976). In passing from populations of fixed benthic algae to bacterial colonies, to sessile or burrowing animals, the composition of these fluxes will be modified (Figure 6.14) in relation not only to benthic populations, but also to the zones previously distin-

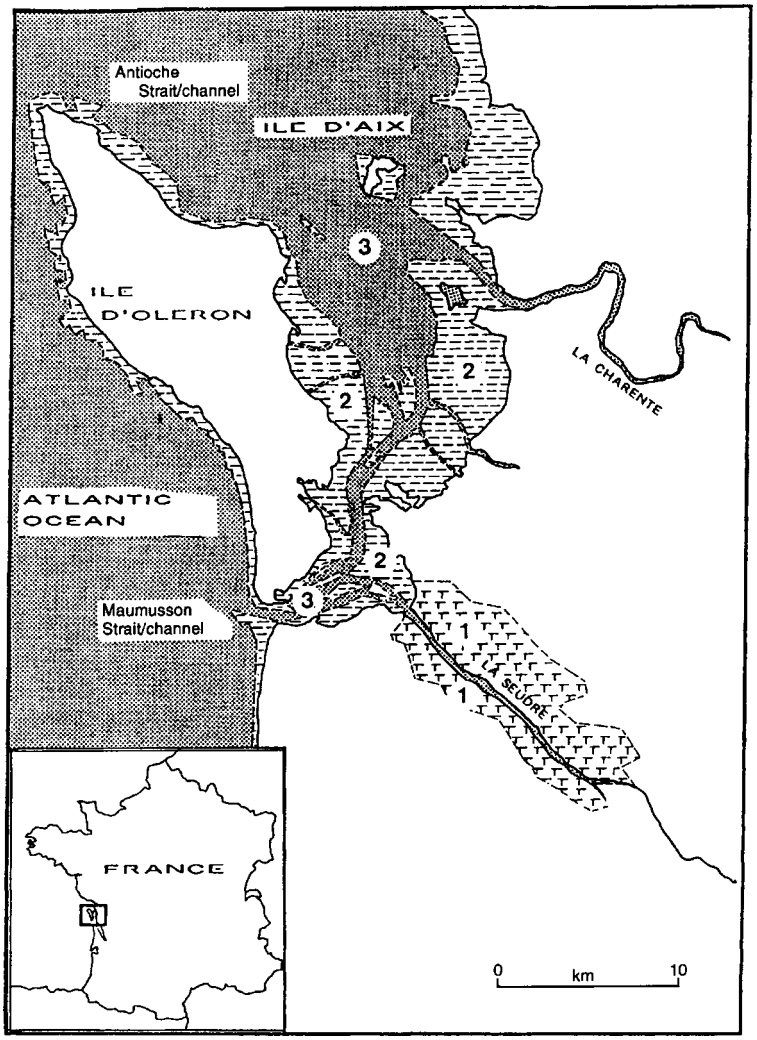


Figure 6.15 Ecosystem of the shellfish culture area at Marennes-Oléron. 1, Oyster beds; 2, intertidal zone; 3, subtidal zone. Map courtesy IFREMER-La Tremblade.

guished (oyster beds, shore, subtidal). The complexity of the problem is evident. It can only be resolved by a multidisciplinary team working over several years.

Even if study of the whole ecosystem is dismissed as unfeasible, the bioenergetician can take part in specific studies of natural or cultivated populations (section 7.2.1). As remarked by Barbault (1989), populations cannot be isolated, as they fit into an environment with which they have a direct relationship and on which they are dependent: the true functional unit is the *population-environment system*. This parallels the concept, in bioenergetics, of an *open system*. This process is illustrated by Figure 6.16. The advantage of such studies is the speed with which results are obtained. By working simultaneously on different age classes of populations, results are obtained at the end of a year.

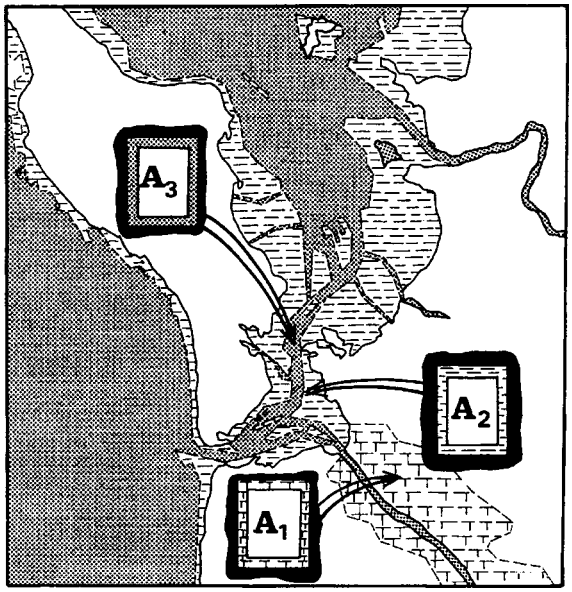


Figure 6.16 Representation, for an animal species A, of the population-environment systems in the three site types (1, 2, 3) in the Marennes-Oléron basin. Outside the immediate environment of each population, the collective ecosystem is shown by a black box.

We have explained thes reasons for rejecting the study of the energetics of ecosystems. This does not mean that we are ignoring their existence. On the contrary, we know that all organisms are part of a population and that all populations exist within the body of an ecosystem. Thus we propose the study of an open population-environment system as the ultimate limit of bioenergetics. In the environment other populations have direct relationships with the population being studied, not only those which constitute the food of the species being considered. Thus, as Morowitz (1968) wrote, 'A one-species ecological system is never found. The carbon cycle requires at least one primary producer and a method of returning carbon to the CO_2 pool', which led him to conclude 'sustained life under present-day conditions is a property of an ecological system rather than a single organism or species'. This is also our conclusion.

7

The Scope of Bioenergetics Applications

> A well-conducted biological study should have as its starting point the theme of energy and its transformations. (Lehninger, 1978)

In preceding chapters we described the goals of bioenergetics and the methods used in order to attain them. Due to the universal characteristic of the energetic unit, bioenergetics allows the interpretation of physiological or demographic phenomena to be simplified and the sensible comparison of species. Thus, bioenergetics brings new understanding of the metabolism of organisms and population dynamics. This is a fundamental role which justifies studies of energetics at cell, organism and population levels. Bioenergetics is also used in related applied or pure sciences.

7.1 Applied sciences in the aquatic environment

The two areas where the bioenergetics of aquatic animals can be brought in are fisheries and aquaculture.

7.1.1 Fisheries

Basic concepts

Fisheries is a gathering activity in the natural environment, taking place in seas, lakes or rivers. In the sea, there are deep sea and coastal fisheries. Study of fisheries consists of a descriptive facet (species fished and method of capture) and a theoretical and quantitative facet which involves the dynamics of the populations studied and the influence of fishing methods on catches.

A marine population has particular characteristics as, due to the characteristics of the fishing gear, not all age classes can be caught. Specific nomenclature has also been established. *Recruitment* is the process whereby the youngest fraction of the population integrates for the first time into the assembly of 'accessible' individuals (Laurec and Le Guen, 1981). *Discharge* [French *réforme*] (continuing the military analogy) is the inverse

process to recruitment, i.e. the exclusion, for whatever reason, of certain individuals from the exploitable population. This covers emigration, fisheries gear avoidance by certain fish, lack of interest from the fisherman in certain categories of individual etc. The *fish stock* is the assembly of exploitable individuals. *Fishing effort,* applied to a stock of aquatic animals, is a measure of all the methods of capture used by fishers on the stock, over a defined period of time. This definition implies that the number of fishing vessels, their characteristics, fishing gear, level of activity and human capabilities in play etc. are taken into account (Laurec and Le Guen, 1981). Fishing effort is expressed using a variety of simple measures such as number of hooks submerged or of pots placed over a given time; in these examples, the unit of effort is the hook or the pot. The *catch per unit effort* (CPUE) is the relationship between catch and effort for a given period.

To obtain a good understanding of the dynamics of a marine population, estimates must be made of age of recruitment, age at first capture, maximum age at capture and age of discharge. This allows the definition of exploited and exploitable phases. These definitions are illustrated in Figure 7.1.

Management of exploited populations

Fisheries science studies the management of exploited populations of aquatic species. In forming the hypothesis of stability of the environment, models have been established which allow calculation of the annual catch that maintains the stock at its optimum level. Let us consider for example Schaefer's model (Figure 7.2).

Let f be the fishing effort per unit time and B the biomass of the stock. In a state of equilibrium, a decreasing linear relationship exists between f and B, represented by the straight line. If Y is the yield per unit effort, note that it increases with f up to a value Y_{max} beyond which it decreases as shown by the parabola.

The role of fisheries biologists is to calculate, for a given stock, the optimal fishing effort corresponding to Y_{max} or, for prudence, corresponding to the ascending phase of Y close to Y_{max}. More details about Schaefer's model can be found in Daget (1989) and information about other models in Daget and Le Guen (1975b), Laurec and Le Guen (1981), Pitcher and Hart (1982) and Cushing (1988).

Applications

Many fish, crustacean and molluscan stocks have been studied and modelled with a view to rational exploitation, from different models. This has resulted in the production of fisheries regulations: limitation of fishing effort (by means of licences and quotas),

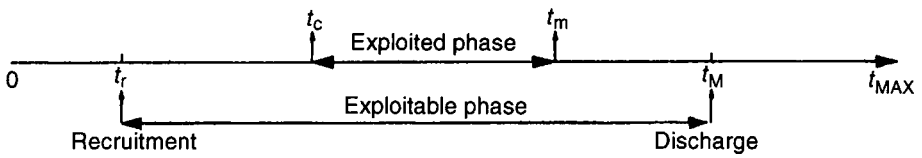


Figure 7.1 Fishing exploitation of animals as a function of their age. t_r, age at recruitment; t_c, age at first capture; t_m, maximum age in catches; t_M, discharge age; t_{MAX}, overall longevity. In the figure all the ages chosen are different. Most often, $t_m=t_M=t_{MAX}$ (modified from Laurec and Le Guen, 1981).

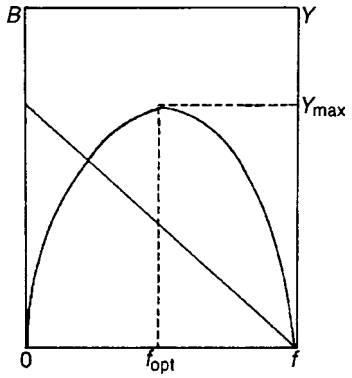


Figure 7.2 Schaefer's model. *B,* biomass; *f,* fishing effort; *Y,* yield (see text).

fixing minimum catch size, closure of the fishery during certain periods, definition of fishing gear (for example mesh size of nets) and the banning of certain gear.

In most studies of population dynamics of exploited species, size-age keys are usually utilized before calculation of the biomass, but results are not expressed in energetic equivalents. For practical reasons, it is in fact necessary to express regulations in terms of length of an available animal and production in tonnes landed, but the use of energetic equivalents is certainly indicated when it is desirable to establish yields.

This was done by Leach (1976), not only for fisheries but also for numerous agricultural activities. He established an *energetic yield,* E_r, defined as the relationship between energy acquired (production of the system) and energy expended (in investment and functioning of the system) during a year. According to this author, values of E_r are greater than 1 for most agricultural activities, notably in tropical areas. The lowest values are those found in fisheries. Thus, for the British fishing fleet, E_r=0.05. Although it is strictly outwith the field of bioenergetics, this kind of exercise allows comparison of different types of fishery with each other or different aquaculture units. The difficulty arises in correctly assessing energy expenditure, because of economic complexities.

Production can simply be expressed in watts (or multiples). This allows comparison between fisheries and, taking relative areas into account, yields per hectare. It might be agreed that this has already been done by utilizing annual tonnage and that consequently expression in power (watts) is redundant. But how can a tonne of algae be related to a tonne of tuna, or a tonne of winkles to a tonne of octopus? The proportions of water and skeletal material vary and can bias the results. If organic matter content is converted to joules, comparisons are possible in universal energetic units.

Nevertheless, most scientists involved in fish stock management have not included energetics measurements in their theoretical or applied studies. Further discussion is therefore unnecessary.

7.1.2 Aquaculture

Aquaculture can be defined as culture or raising of aquatic plant or animal species where humans intervene for all or part of their developmental cycle. Obviously, the cultivated species are used by humans, most often for food, but sometimes for other means, e.g. pearl oysters.

Animal aquaculture has diversified enormously since the 1970s, especially in the marine environment. There is also a large number of kinds of aquaculture. From five criteria, Table 7.1 defines 10 kinds of aquaculture which themselves contain intermediate situations.

The bioenergetic criterion was highlighted by Laubier (1986) 'from the energetic point of view, there is production aquaculture and transformation aquaculture'. In production aquaculture, the animals raised use only natural food, which is the case for all microphages, notably marine molluscs. In transformation aquaculture, humans provide food for the animals, which transform it. This is the case for fish and crustaceans. An intermediate situation consists of acting on natural production by human intervention, for example, the addition of fertilizers in a semifarmed environment in order to intensify phytoplankton production, or the addition of algal cultures to modify the composition of the phytoplankton.

The life cycle is controlled to a greater or lesser extent. It is not necessarily profitable to acquire control of the whole life cycle. Thus, the initial life cycle of molluscs is controlled (conditioning of broodstock, larval rearing, metamorphosis, postlarval rearing) but for certain species such as the Pacific oyster, *Crassostrea gigas,* production of juveniles or spat in hatcheries and nurseries is more costly than the collection of spat from the wild. In contrast, for the Manila clam, *Tapes philippinarum,* capture is irrelevant and the only way is complete culture. Rearing can start at any intermediate stage between egg and juvenile. For example, rearing of conches, *Strombus gigas,* depends on spawn collected from the wild and starts with hatching, i.e. the veliger larva stage.

Demographic criteria depend both on environment and on species. Certain species cannot survive intensive rearing, at least at certain stages of development. As for the development of sites for rearing, this is totally different for intensive or extensive aquaculture. Between these two extreme types are intermediate situations, hence described as semi-intensive (or semiextensive) aquaculture.

Table 7.1 Classification of different types of aquaculture using five criteria. All kinds of intermediate situations exist, between those categories described (see text)

Criterion	Aquaculture type	Characteristics
Bioenergetics	Production	Natural food
	Transformation	Human-made food
Life cycle	Partial	Incomplete cycle, juvenile to adult
	Complete	Complete cycle, egg to adult
Demography	Extensive	Low densities
	Intensive	High densities
Location	Open environment	Natural site
	Closed environment	Human-made site
Salinity	Freshwater	Salinity less than 0.5‰
	Marine	Salinity close to 35‰

The siting of a farm in an open or confined environment has decisive consequences on the possibilities for control. In an oyster farm in deep water, control over external factors is impossible, the most that can be done being some antipredator measures. In contrast, in a hatchery, all external factors can be controlled and the characteristics of the rearing water modified and adapted as required. There are innumerable culture methods with many varied possibilities, between the hatchery and the open sea.

Salinity is the ecological factor that allows differentiation between marine and freshwater farms. Fish culture controlling the entire life cycle of some freshwater species has been carried out for a century, while complete rearing of marine fish has only recently been achieved for some species (sea bass, *Dicentrarchus labrax,* gilthead sea bream, *Sparus auratus*). This stems from, among other reasons, the fact that the quality of the environment is more difficult to maintain in the sea than in fresh water. Certain cultivated species, such as some salmonids and sturgeon, are anadromous, i.e. part of their life cycle occurs in fresh water and part in sea water.

It has been demonstrated that aquaculture has a great variety of forms. We shall see hereafter in which cases bioenergetics may be applied, by limiting ourselves to the two most developed animal aquaculture activities: molluscan aquaculture and fish farming.

7.2 Production in molluscan aquaculture

Molluscan aquaculture has a long tradition and has been revived since the 1960s when the production of spat from hatcheries was added to that collected from the wild. This revival succeeded due to the enterprising spirit of the molluscan aquaculturists and the contribution of fundamental biological studies of cultivated molluscs. Bioenergetics has not been neglected in this effort, as we shall see from some examples.

7.2.1 Cultures in the natural environment

Bivalve culture in claires

Claires are oyster-rearing beds characteristic of maritime marshes on the Atlantic coast of France between the Gironde and the Vilaine. They are most abundant along the Seudre and Charente estuaries, where they cover 35000 ha (Goulletquer *et al.,* 1988).

Oyster beds are shallow (about 50 cm of water) rectangular basins between 300 and 500 m^2, with a clay bottom covered in mud, which flood with sea water at each high tide. These basins were originally hollowed out and prepared for the collection of sea salt. However, for more than a century, salt marshes have been progressively converted for the culture, or more precisely the finishing, of Pacific oysters, hence the name 'claire' (Henry, 1980).

As a result of the relative decline of traditional oyster farming, some claires have been used, since 1980, for the culture of the Manila clam, *Tapes philippinarum*. This species has been found to be more productive in oyster beds than *Crassostrea gigas,* but has shown growth checks (arrests) at the end of spring. To investigate this phenomenon, Goulletquer *et al.* (1988) decided to carry out an in-depth bioenergetics study of this clam and of the energetics of this clearly defined ecosystem. An oyster bed of 576 m^2, with a volume of 345 m^3, containing a population of 1-year-old Japanese clams at a density of 85 individuals per m^2, was studied from November 1982 to August 1983.

Each month, the following parameters were measured for 20 individual clams: shell weight, dry matter weight, proteins, carbohydrates, lipids, ash and energetic value, determined by Phillipson microcalorimetry. The energetic value of the organic matter of the shell was established by equivalence (21.05 J mg^{-1}). In order to follow reproduction, smears were taken as well as histological sections. Respiration was evaluated from laboratory measurements.

Every fortnight, environmental measurements were made of temperature, salinity, dissolved oxygen, turbidity, ammonia, chlorophyll *a,* phaeopigments, proteins, lipids, certain carbohydrates, amino acids and a count of heterotrophic bacteria. The energetic values of proteins, lipids and carbohydrates were calculated by equivalence. Samples of the water-sediment interface were analysed each month according to the same protocol. This collection of measurements showed that the density of 85 individuals per m^2, which represents a biomass of 1 kgm^{-2}, is too high in relation to the trophic capacity of an oyster bed, which is a semiclosed environment with limited input from the exterior. If the reasons for negative values of P_G in December are properly understood, the May phenomenon can only be explained by permanent competition between phytoplanktonic cell multiplication and their consumption by the clams. This phenomenon limits or removes the phytoplankton blooms, which are favourable to clam tissue production. At the same time, gamete maturation and emission are retarded. By limiting the density of clams to 400 gm^{-2} an individual gain is obtained (P_G of 7 g $year^{-1}$ instead of 4 g $year^{-1}$) but this requires increasing the culture surface by a factor of 2.5.

To summarize, bioenergetic analysis gives the molluscan aquaculturist the information required to choose the most productive density in relation to the surface area of beds available.

Management of an oyster culture lagoon

A similar, but simpler, process was followed by Shafee and Lucas (1989) on the aquaculture potential of the Oualidia Lagoon in Morocco (Figure 7.3). Various measurements were made over 16 months, partly on cultivated oysters, *Crassostrea gigas,* partly on the characteristics of the water at different points in the lagoon. The chlorophyll *a* content was considered as an indicator of consumption *(C),* which was confirmed by the corresponding growth values (P_G). Taking into account the

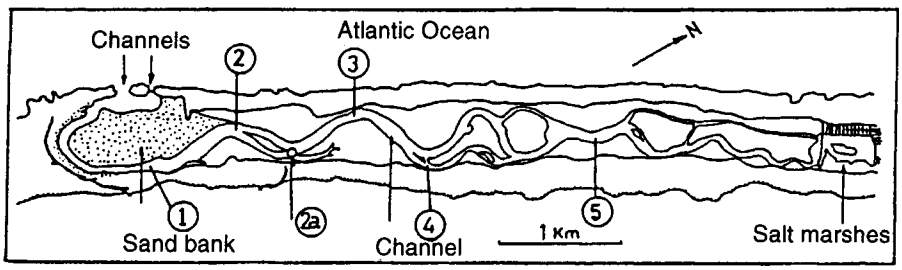


Figure 7.3 Oualidia Lagoon (Morocco) at low tide. At high tide, the interior of the lagoon is entirely covered in water (tidal range of 2 m). Numbers indicate the measuring stations (modified from Shafee and Lucas, 1989).

chlorophyll *a* values as a function of seasons and sites (lower values in downstream than upstream water), a strategy of controlled oyster growth was established in relation to sites and emersion times of the cultures.

Modelling oyster production in the natural environment

From data collected from three oyster-farming sites in England, Askew (1978) established a production and financial revenue model for the oysters *Crassostrea gigas* and *Ostrea edulis,* as a function of individual biomass at the start of culturing. The process consisted of three stages where he established:

- the instantaneous annual growth rate as a function of individual biomass and the same relationship for the instantaneous mortality rate over 30 days;
- a seasonal adjustment of the previous results;
- a relationship between individual biomass and price.

From these data, all presented in graphical form, he produced tables in which, as a function of time expressed in months, he predicted individual biomass, number of survivors and financial value of the stock, for each species at each oyster farm.

7.2.2 Nursery culture

In aquaculture, nurseries are installations set up for the growing on or raising of small juvenile or postlarval-stage individuals. These animals are of the order of 1 or several millimetres and are subjected to high levels of predation in the natural environment, which is not the case in the nursery.

Two types of nursery, land- and sea-based, can be distinguished, the running costs of which differ (Lucas and Gérard, 1982). We shall only consider here the land-based nurseries which were very popular in the 1970s. There are few variants to this type of installation, as *upwelling columns* have been universally adopted by the professionals because of their practical advantages and good yields (Figure 7.4). The

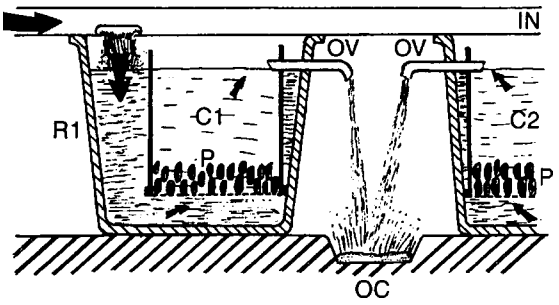


Figure 7.4 Diagram of ascending current cylinders in a land-based nursery. The cylinders (C1 and C2) are made of rigid plastic with mesh material at the base and in this case contain juvenile clams (P). Sea water rich in phytoplankton circulates in an inflow pipe (IN) and is diverted to a series of long reservoirs or raceways, seen in transverse section (R1 etc.). Each raceway contains about ten cylinders which have overflow pipes (OV) at the same level, which ensures equal flow in each one. OC, outflow channel, common to raceways R1 and R2 (modified after Lucas and Gérard, 1982).

differences arise around the food source, which consists of phytoplankton. The source can be natural, for example a lagoon with high primary production, or artificial, using monospecific cultures of algae in tanks, as used in the hatcheries.

Between these two source types, there are all kinds of intermediates, for example cultures from coarsely filtered or centrifuged sea water (thus allowing the phytoplankton through). When the sources are natural, toxic algae can develop, for example *Phaeocystis pouchettii* (Rodhouse *et al.,* 1981). Whatever the source, the main difficulty in nurseries is ensuring an adequate supply of phytoplankton at all times.

Some fundamental studies have been carried out in experimental nurseries. They deal mainly with production as a function of various factors: species raised, food quality and quantity, installation type, temperature, turbidity etc. An abundance of data have been produced from this work. Direct comparisons are difficult, because of the many experimental protocols and diverse ways of expressing the results.

7.3 Bioenergetics in fish farming

The recent progress in fish farming, in fresh water and then in the sea, is above all due to two fundamental disciplines: physiology (including nutrition) and genetics. Bioenergetics has also played a part in this progress: one only needs to refer to the journal *Aquaculture,* where there have never been more articles based on energetics. The work of Balphour Hepher entitled *Nutrition of Pond Fishes,* a general survey of recent knowledge (1988) on fish farming in fresh water, also demonstrates the role of bioenergetics with numerous references to this subject.

7.3.1 Production models

In order to attain a production model for a cultivated species, it is necessary to know simultaneously the state of the stock, i.e. the mean growth rate (in biomass) and the often considerable variations in this growth rate, as well as the number of survivors.

These characteristics of a reared stock lead to two kinds of parameter: those of the rearing water and those which are unique to the stock. In hatcheries and widely in nurseries, all the factors affecting environment and stock are controllable. In contrast, where older animals are being reared in tanks or floating cages, only the stock factors are controllable and the factors acting on the water depend to a large extent on the natural environment. The farmer cannot alter temperature, oxygenation (apart from, in ponds, increasing the flow), salinity or turbidity etc. These are chance parameters (Querellou, 1984). In contrast, the factors of the stock depend only on the rearer: these are management parameters. This is the case for feeding (quality, quantity, rate of supply), rearing density, linked to a strategy of grading (criteria and frequency) and to the capacity of the installation (volume and flow). There is also the policy of disease control (methods, frequency) which has direct consequences on mortality.

The aim of predictive production models is therefore to forecast the status of a stock at a given time. All these models take into account either temperature or ration, or both at the same time. Even if temperature cannot be regulated, it can be predicted from local climatic records (Figure 6.13). It is these predictions which are used in the model. There follows an analysis of some growth models.

According to Querellou (1984), von Bertalanffy's model (section 4.4.3) 'is of little practical use in aquaculture'. Other growth models have been examined, such as that of Iwama and Tautz (1981) which does not take ration into account but assumes it to be optimal. The general equation is:

$$W_t^b = W_0^b + G_s t \quad (1)$$

where W is weight, G_s the growth slope, t is time in days and b the weight exponent. According to these authors, for salmonids, there is a linear relationship between G_s and temperature T, in the range of temperatures between 3.8°C and 12.8°C: $G_s = T/1000$. Elsewhere, b=1/3 whence the expression of equation (1) applied to salmonids becomes:

$$W_t^{1/3} = W_0^{1/3} (T/1000)t. \quad (2)$$

Using examples, Iwama and Tautz (1981) showed that this model can be used in three ways.

1 It allows the prediction of mean weight for fish of a known initial weight after a known number of days rearing and predicted temperatures.
2 It allows the prediction of the time required to obtain fish of weight W_t, where W_0 and T are known.
3 It allows the prediction of the temperature required to obtain fish of a certain weight W_t, where W_0 is known and t is fixed in advance.

According to Querellou (1984), a practical model, very similar to that of Iwama and Tautz (1981), has been established by the Fish Culture Information System for Hatcheries, the FISH, as follows:

$$W = (W_0^{0.3333} + 0.0101741\ t)^{3.0003}. \quad (3)$$

Returning to equation (2) with T=10°C effectively gives:

$$W = (W_0^{0.33} + 0.010\ t)3.$$

In France, a growth model (CEMAGREF) for trout based on 4500 degree days has been established on quite similar principles.

The models which we have just examined have been established on energetic considerations and are very useful to the aquaculturist in production management. In the examples studied, ration appears as a fixed parameter, defined as optimal. The only variables are temperature, length of rearing and original size of the fish. It therefore remains to examine ration, over which the aquaculturist evidently has control.

7.3.2 Food rations

Utilization of food rations

In the majority of fish farms, the final product is generally a juvenile and reproduction is not taken into account. Hence in a population that constitutes one batch of fish, the energy from metabolizable food is shared only between maintenance and growth as reproductive effort is nil (section 6.4.2).

The concept of maintenance has been defined in section 3.2.1. The rate of growth (Q_G) was shown graphically in Figure 3.7 as a function of food ration consumption (Q_C). Q_G/Q_C was indicated not only for values close to maintenance (Q_{CM} where Q_G=0) but for all possible

values of Q_C. Figure 7.5 shows the classic representation of this relationship after Brett and Groves (1979) in order to clearly demonstrate the role of bioenergetics in aquaculture.

Such a curve effectively allows the farmer to calculate the ration necessary for a certain biomass of fish so as to obtain a certain growth rate. Q_{COPT}, the optimal ration, gives the optimum growth rate (Q_{OPT}) i.e. maximum growth efficiency. But it is not only the optimal rate, biologically defined, which is of use to the farmer. For example, if he needs to be sure of sales in a short period of time, he will be interested in increasing the ration in order to obtain a growth rate close to Q_{GMAX}. According to the situation, the aquaculturist can use this graph to control the growth rate by supplying a ration which achieves his objectives. Obviously this curve is only valid for the species, age class and temperatures that have been taken into consideration.

Besides these curves, feeding charts are used, which are based on the same principles. Four types of table are found in Hepher (1988); their characteristics are given in Table 7.2. Commercial fish feed manufacturers normally supply such tables as a guide to using their products.

In more fundamental studies, tables and curves are given jointly. For example, Huisman (1976) established rations required for rainbow trout raised at 15°C and carp raised at 23°C to attain various growth rates and oxygen consumptions, from calculation of Q_{CM}.

Finally, in certain cases, an equation is recommended to fish farmers, for example the following, empirical relationship established by Hepher (1988) for the carp *Cyprinus carpio* raised in Israel, in which the growth rate is 45%.

$$Q_A=[0.07\ W^{0.8}+0.176\ W^{0.66}\ (0.905+0.001\ 89\ W)/0.45]\ 4.186$$

where Q_A is the energetic value of the daily metabolizable food, expressed in kJ (in kcal in the original formula), and W denotes weight of fish. A relationship between Q_A and Q_C can be established as a function of the characteristics of the food.

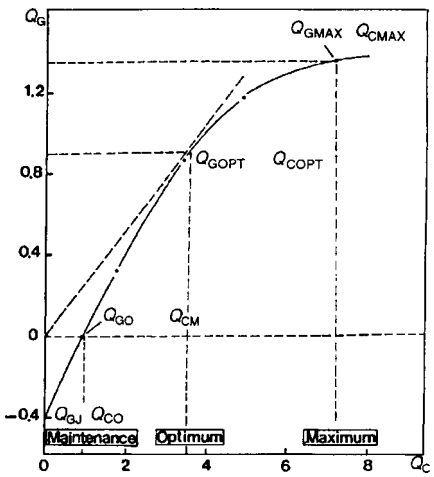


Figure 7.5 Interaction between food ration and growth in Alaskan red salmon alevins, *Oncorhynchus nerka,* raised at 10°C. Curve established by Brett and Groves (1979). Q_C and Q_G, daily rates of consumption and growth expressed as percentage of biomass of the organism being studied. J, juvenile; M, maintenance; OPT, optimal; MAX, maximal (modified after Hepher, 1988).

Table 7.2 Example of feeding tables used in aquaculture (after documents provided by Hepher, 1988)

Expression of daily ration	Parameter 1	Parameter 2	Species
% of body mass	Temperature	Size class (mass)	Rainbow trout
g ind^{-1} and % proteins	Body mass	Density per ha	Carp
g ind^{-1}	Body mass		Tilapia
% of body mass	Body mass	Temperature	Catfish

These examples demonstrate that many studies have allowed the establishment of precise relationships between food rations and growth rates for many species, size classes, temperatures etc. All the data are expressed in percentage body weight of the fish. This means that the fish farmer has to know the fish weight at all times and proceed with grading when the difference between largest and smallest within a batch becomes too great.

Fundamental studies on diet composition

Fundamental studies of food rations are very desirable because on a real fish farm, a large part of the production cost of fish flesh is due to the cost of the feed. The diet of a farmed fish effectively consists of a high proportion of proteins and in addition, an important part of the costly proteins are in the form of flour or fish meal (Fauconneau, 1980).

The food ration must be responsive to the needs of the animal. Quantitative needs are established with the aid of energetics and qualitative needs using nutritional studies: protein/lipid/carbohydrate equilibrium, amino acids and essential fatty acids, trace elements, vitamins and various physiological factors, for example the squid factor which stimulates growth in prawns (*Penaeus* sp.) by inducing cellular hypertrophy (Guillaume *et al.,* 1989).

A strategy for the formulation of synthetic feeds, based on energetics, has been established by Guillaume (1990). Taking into account the fact that the protein requirements vary inversely with the energetic value of the food (section 5.1.1), he advocated studies of the sparing effect of proteins, by increasing the proportion of lipids and, to a lesser extent, carbohydrates. However, this effect is specific and required studies to be carried out, species by species. For example, in the rainbow trout, *Oncorhynchus mykiss,* the optimal protein fraction can be lowered from 45–50% to 38% when the lipids are increased from 2% to 18%. He proposed a four-point programme. Set an energetic level of feeding (as a function of biomass); establish the minimum level of proteins and the maximum level of lipids and carbohydrates. This same concern about the energetic value of the ration is also found in Cho and Kaushik (1990).

7.3.3 Stress and its effects

Stress is defined as 'the physiological response of an organism to a stressor' or 'the collective non-specific physiological reactions of an organism against some kind of attack' (Aldrin, 1985). The responses of the attacked organism are successively the alarm reaction, the stage

of resistance (corresponding to a new adaptive equilibrium) and finally either exhaustion (or upset of equilibrium) ending in death, or recuperation (or resumption of initial activity).

Stress is a vital reaction in wild animals for fight or flight, but it has lost any adaptive meaning for the captive animal, which can neither fight nor flee (Aldrin, 1985). Under farmed conditions there are numerous causes of stress, some short-term (grading, transferring and handling), others permanent or long-term (overpopulation, excess organic matter or urine, variations in oxygen tension, thermal shocks etc.).

The primary and secondary effects of stress on an organism are summarized in Figure 7.6. The tertiary effects are lowered resistance to disease, risk of salt-water imbalance or energetic disequilibrium. The third is of interest in this context.

In a stressed animal, the energetic balance is not in equilibrium for two reasons. Firstly, the energetic gain decreases because food consumption and evacuation are disturbed in the stressed state (Talbot, 1985) which is characterized in particular by loss of appetite. For example, handling provokes the cessation of consumption for three consecutive days in the brown trout *Salmo trutta* (Pickering *et al.,* 1982).

Secondly, energy expenditure increases, in the form either of agitation (rapid swimming, aggressive reactions, intense respiration; Knights, 1985) or of excessive utilization of reserves, notably liver glycogen, provoking blood hyperglycaemia (Schreck, 1981).

Thus, in an animal in a stressed state, there is an acknowledged energetic imbalance which, in the farm, can cause slowed growth, increased mortality and eventually cessation of reproduction (Billard *et al.,* 1981). Monitoring of the

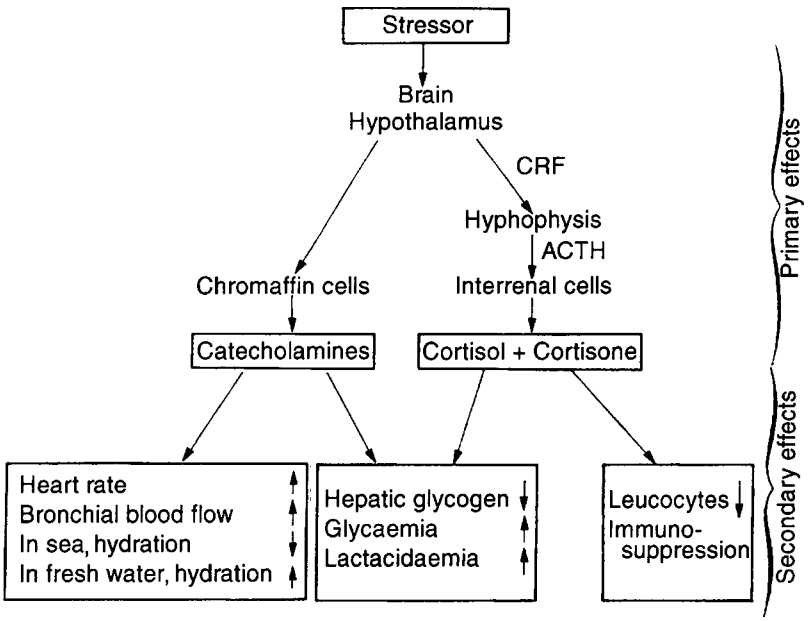


Figure 7.6 Physiological effects of a stressor on a teleost fish. ACTH, adrenocorticotrophic hormone; CRF, corticotrophin releasing factor. Arrows pointing upwards indicate an increase, those pointing downwards, a decrease. Modified from Aldrin (1985) taken from Mazeaud *et al.* (1977).

bioenergetic balance of livestock (growth rate and various efficiencies) can be a means of discovering states of stress which are sometimes ignored by farmers (Knights, 1985).

7.4 Bioenergetics and fundamental sciences

7.4.1 General considerations

Certain fundamental sciences frequently resort to the methods of bioenergetics in order to resolve problems they encounter. This is true in particular in ecology and physiology. Examples in this volume have shown that physiological and ecological concepts do not always agree, for instance on the subject of production.

In ecology, the International Biological Programme (IBP) developed, in the 1960s, bioenergetic concepts of biological production in all its forms, from bacterium to animal and from forest to sea, and encouraged energetic analyses of ecosystems. This programme has given rise to the emergence of ecological energeticists in numerous countries. These specialists very quickly came to speak the same language, due to the publication, in the 1970s, of about 25 works, often reedited in the 1980s, entitled 'IBP Handbooks', of which more than half are concerned with bioenergetics.

The breakthrough of bioenergetics in physiology has not benefited from a special programme but has nevertheless developed steadily, especially since the 1980s. Amongst the scientific journals reporting this kind of study, it is appropriate to note the *Journal of Experimental Biology,* where many studies of the cost of locomotion, including swimming, can be found.

Lastly, ecophysiology has also benefited from energetics in acquiring rigour and precision. Leaving generalities aside, we shall analyse an example.

7.4.2 Study of an example, the snow crab

The snow crab, *Chionoecetes opilio,* is among the most exploited species of crab in the world but is confined to cold waters of below 5°C. Foyle *et al.* (1989) sought reasons for this thermal limitation, by studying adult males. Firstly they wanted to discover if respiratory activity was implicated, which they found was not the case, as the routine rate was maintained up to 18°C with a Q_{10} of 2.2. Feeding and locomotion of these animals were then studied, allowing energy budgets in relation to temperature to be constructed.

Ingestion of food (squid, prawn and mussel) *(I)* as well as the corresponding faeces *(F)* were measured. Taking into account the small amount of urine (ammoniac), the authors were able to calculate *(A)*. The three components R_S, R_A and R_F were calculated following locomotor and feeding activity. R_S increased greatly with temperature, while R_A and R_F decreased. From these results it was possible to calculate production *P.* According to these authors, at this stage, where there is no moult occurring, the only tissue production P_G would be that of the gonad, which is not important, because the value of *P* is derived by difference. The results are shown in Table 7.3.

The negative results between 1°C and 0°C perplexed Foyle *et al.* (1989) as they

Table 7.3 Energy budgets for the snow crab (adult males) at different temperatures. *T,* temperature in degrees Celsius; *A,* metabolizable energy; *R,* catabolism; P_G, tissue production (anabolism). *A, R* and P_G are expressed in kJ kg^{-1} day^{-1}. Data obtained from graphs produced by Foyle *et al.* (1989)

T	*A*	*R*	P_G
0	3.3	5.0	−1.7
1	5.4	5.4	0
5	8.4	5.8	2.6
7	6.7	6.7	0
15	0	12.5	−12.5

had found crabs present and active in the water at 0°C. In fact a window of activity is noted between 0°C and 5°C and bioenergetic analysis predicts a positive budget between 1°C and 7°C. There is therefore a slight discrepancy which can be explained either by the stage of the intermoult cycle or by differences in metabolism between aquarium and natural environment.

Thus the general biology of the species as well as the thermal limits can be understood by recourse to bioenergetics.

Conclusion

In order to conclude this work, we shall leave aside the particular case of aquatic animals to consider bioenergetics in general.

Bioenergetics is based on the use of universal units of energy. It is a simple application of thermodynamic methods allowing the quantification of exchanges of matter and energy between the environment and the open macroscopic system.

This universality of bioenergetics is threatened in three ways: by the multiplicity of living systems which it studies, by the differences in scale in the studies undertaken and by the diversity of disciplines which make use of it.

Systematics, which is involved with structures, has described the multiplicity of living organisms. It has also placed them in hierarchies, allocating the taxa to different levels. Is it possible to arrive at similar, less diverse groups, but using energetic criteria? We shall attempt to do this. The first criterion appears to be, as in systematics, the distinction between unicellular and multicellular organisms. This structural difference corresponds to a fundamental functional difference: production results in multiplication of individuals in the former as opposed to the increase in tissue biomass in the latter. The second criterion is quite obviously autotrophy. In the multicellular organisms, to which we shall limit ourselves from now on, autotrophy is chlorophyllous. It uses light energy to acquire chemical energy and transform inorganic matter into organic matter. A third criterion, that of locomotion, as a permanent or only temporary characteristic (for those which revert to a sessile or parasitic lifestyle) allows characterization of animals. The last criterion is that of an aquatic lifestyle. Life on our planet had its origins in water and water has remained the main component of all biological systems (Morowitz, 1968). Living organisms that have remained in the original environment have avoided many of the problems of managing water balance. In the terrestrial environment, neither plants nor animals can replace water with any other substance and their survival is based on water economy. This adaptation, which can be interpreted as a fight against desiccation, has required the development of specific mechanisms, themselves energy consumers.

Thus with three criteria we can distinguish six groups of multicellular organism: animals, chlorophyll-containing plants, and heterotrophic plants, either aquatic or terrestrial. Other criteria of energetic interest can be used to establish subgroups: for example, aerobic or not,

sexual reproduction or not, homoiothermic or not, direct development or not, as well as colonial, symbiotic or parasitic habit/lifestyles. Classification of organisms using energetic criteria also leads to multiplicity of models, which inevitably results in a multiplicity of methods of study.

We have shown (Chapter 5) the differences in scale in bioenergetic studies. This demonstrates the dynamic nature of this science and shows its capacity to establish global budgets (macrobioenergetics) as well as budgets limited temporally or functionally (microbioenergetics). When microbioenergetics brings useful pieces of information to macrobioenergetics, the cohesion between the two disciplines is reinforced. For example, specific studies which allow the evaluation of eliminated products (P_{S2}) such as mucus (section 4.3.2) or tissue renewal (P_{E2}) in different organs, contribute to a better understanding of global budgets.

Microbioenergetics may become increasingly specialized, thus distancing itself from bioenergetics. This is the case, for example, in certain studies of swimming (section 5.2.2) which specialize in the details of hydrodynamics; also in cellular microbioenergetics, which is a specialized area of molecular biology. From then on, contact with macrobioenergetics is broken and results in the disintegration of what was originally a coherent field of study.

The risk of disintegration intensifies in proportion to the diversity of the disciplines that utilize bioenergetics. Certainly the diversity of its uses demonstrates its intrinsic richness. It is like a connecting thread between the fundamental sciences, such as ecology, physiology, ecophysiology, ethology, pathology and even the theory of evolution (Maddox, 1991). Bioenergetics is also a constant reference point for applied sciences such as dietetics and hygiene, agriculture, aquaculture and fisheries. However, the extension of bioenergetics into so many disciplines has the risk of leading to specific interpretations. For example, the cost of reproduction is expressed by different energetic values in physiology (section 5.3.5), ecology (section 6.4.2) and ethology (section 3.2.3).

To summarize, the unity of bioenergetics is doubly threatened. On one hand, very diverse modes of study have been imposed on bioenergetics as a result of the diversity of the structure and function of living organisms, leading in turn to multiplicity of modes of expression. On the other hand, there is the risk of the disintegration of bioenergetics either by its many specializations (internal danger) or by the utilization of bioenergetics by a multitude of different fundamental and applied sciences (external danger).

In order to safeguard unity, it is important to be rigorous in bioenergetic interpretations, to strive for clear definitions, univocal concepts and true and universal expression of results. This is what we have attempted to do throughout this work.

Appendix: Symbols

Symbols adopted in this work for the establishment of energy budgets

Note: All symbols represent fluxes expressed in quantities of energy (or matter, transformed into its energy equivalent) per unit time: $Q_E\ t^{-1}$. This represents power. In bioenergetics, the units of time vary between a second and a year, according to experimental conditions and type of budget. The use of powers expressed in watts ($J\ s^{-1}$) is advised.

A	metabolizable flux; assimilation
Ab	absorption
C	consumption
E	excretion
F	faeces and pseudofaeces
F_1	pseudofaeces
F_2	faeces
I	ingestion
P	production
P_E	eliminated tissues
P_{E1}	violently eliminated tissues
P_{E2}	tissue renewal
P_G	tissue production
P_R	reproductive products
P_S	secreted products
P_{S1}	remaining (residual) secretions
P_{S2}	eliminated secretions
R	respiration
R_A	respiration due to activity
R_F	postprandial respiration
R_S	standard respiration
U	urine

Relationships between symbols

$$A = C - E = P + R$$
$$Ab = C - F$$
$$C = E + P + R$$
$$E = C - (R + P)$$
$$F = C - (U + R + P)$$
$$F_1 = C - (F_2 + U + R + P)$$
$$F_2 = C - (F_1 + U + R + P)$$
$$I = C - F_1$$
$$P = C - (E + R)$$
$$P_E = P - (P_G + P_R + P_S) = P_{E1} + P_{E2}$$
$$P_G = P - (P_E + P_R + P_S)$$
$$P_R = P - (P_E + P_G + P_S)$$
$$P_S = P(P_E + P_G + P_R) = P_{S1} + P_{S2}$$
$$R = C - (E + P)$$
$$R_A = R - (R_F + R_S)$$
$$R_F = R - (R_A + R_S)$$
$$R_S = R - (R_A + R_F)$$
$$U = E - F = E - (F_1 + F_2)$$

Symbols in the English edition

The following is a list of symbols used in the English edition, together with the page (in parentheses) where each is first mentioned. This list is not comprehensive but includes those which are used repeatedly or may be confused. For full definitions see the text.

A	metabolizable energy; assimilation (Figure 3.1)
A^E	metabolizable energy from exterior (i.e. food) (56)
A^I	metabolizable energy from interior (i.e. reserves) (57)
A_T	total metabolizable energy (57)
Ab	absorption (Figure 3.1)
$\bar{B}$	mean weight (98)
B	biomass (16)
B_e	eviscerated biomass (Table 1.3)
B_f	total biomass (85)
B_{met}	metabolic biomass (85)
B_s	dry biomass (Table 1.3)
B_v	live biomass (Table 1.3)
C_{O2}	oxygen concentration (74)
C	consumption (Figure 3.1)
C_M	consumed food used for maintenance (56)
D_a	apparent digestibility (58)
D_V	true digestibility (58)
E	electromotive force (67)
E	energy (free) (Figure 2.8)
E	energy content of tissues (Table 5.9)
E	excretion (42)
E_r	energetic yield of fisheries, agriculture etc. (135)
E_s	secreted energy (e.g. energy content of shell) (Table 5.9)
F	Helmholtz's free energy (8)
F	faeces (Figure 3.1)
F_1	pseudofaeces; proportion of food lost upon consumption (i.e. not ingested) (41)
F_2	faeces; proportion of food ingested but not absorbed (41)
g	gravity (16)
G	Gibbs free energy or free enthalpy (8)
G_s	growth slope (141)
H	constructive metabolism (63)
H	enthalpy (7)
I	ingestion (40)
k	von Bertalanffy parameter: instantaneous rate (62)
k_{deg}	speed of degradation (25)
k_{eq}	equilibrium constant (12)
k_s	speed of synthesis (25)
k_1 etc.	speed of reaction (Figure 2.1)
K_M	Michaelis' constant (22)
K	destructive metabolism (64)
K	in logistic growth, the maximum number of individuals that the environment can sustain (119)
L_∞	von Bertalanffy parameter: asymptotic length (62)
L	length (Figure 2.15)
L	life span (99)
M_∞	von Bertalanffy parameter: asymptotic mass (62)
MO_2	oxygen consumption (Figure 3.4)

M	mass (16)
M_o	organic matter; ash-free dry weight; ash-free dry biomass (56)
M_s	dry mass (Table 1.3)
M_s	secreted mass (Table 5.9)
M_v	live mass (Table 1.3)
MS	metabolic scope (55)
OB,OM	organic matter (ash-free dry biomass) (Table 1.3)
P	external pressure (constant) (6)
P_i	inorganic phosphorus (Table 2.1)
P_m	molecular phosphorus (27)
P	partial pressure of O_2 (74)
P	probability (8)
P	production (44)
P_E	production of eliminated tissue (Figure 3.1)
P_{E1}	violently eliminated tissues (46)
P_{E2}	tissue renewal (46)
P_G	production of living tissue (including unshed gametes); increase in biomass; tissue growth (Figure 3.1)
P_{O2}	oxygen pressure (74)
P_R	production of shed reproductive products (Figure 3.1)
P_S	production of secreted products (Figure 3.1)
P_{S1}	residual secretions, e.g. shell (47)
P_{S2}	eliminated secretions, e.g. mucus (47)
Q_{10}	relationship between values of O_2 consumption at two temperatures differing by 10°C (105)
Q	flow rate (74)
Q	quantity of heat (5)
Q_A	energetic value of daily metabolizable food (142)
Q_C	daily rate of consumption (as per cent of biomass of the animal) (48)
Q_{CM}	maintenance ration (48)
Q_{COPT}	optimal ration (142)
Q_G	daily rate of growth (as per cent of biomass of the animal) (48)
Q_{ox}	oxycalorific coefficient (19)
Q_{OPT}	optimal growth rate (142)
r	correlation coefficient (97)
r	ingestion rate (58)
r	instantaneous growth rate, 'maximum intrinsic growth coefficient' in exponential growth (119)
RE	reproductive effort (103)
R	universal molar gas constant (12)
R	respiration (Figure 3.1)
R_a	respiration rate during motor activity (43)
R_A	respiration rate due to motor activity (43)
R_f	respiration rate after feeding (43)
R_F	respiration rate due to intake of a meal (43)
R_{net}	net catabolism (85)
R_o	net rate of reproduction (127)
R_R	routine metabolism (43)
R_S	standard respiration rate (43)
S	entropy (8)
S	metabolic power index (55)
S	substrate concentration (22)
S_C	scope for growth (51)
t_0	von Bertalanffy parameter: time at age zero, hatching etc. (62)
t	temperature in °C (Table 1.1)
t	time (17)
T	temperature (8)

T	time (Figure 3.12)
T_G	rate of growth (17)
U	internal energy of a closed system (6)
U	swimming speed (97)
U	urine (Figure 3.1)
V_{max}	maximum speed of reaction (22)
V	volume (6)
V	speed of reaction (22)
V_G	rate of growth (17)
V_x	reproductive expectation at age x (127)
V_{100}	minimum swimming speed (85)
W	quantity of work (5)
W	weight (141)
Wi	mass ingested (58)
Z	instantaneous coefficient of mortality (118)

α	solubility coefficient of oxygen in water (74)

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