Modelling the physiological performance of *Daphnia* (Crustacea: Cladocera) under stress

A thesis submitted to the University of Stirling in partial fulfilment of the requirements for the degree of Doctor of Philosophy

by

António José Arsénia Nogueira

Institute of Aquaculture
University of Stirling
Scotland

- 1996 -

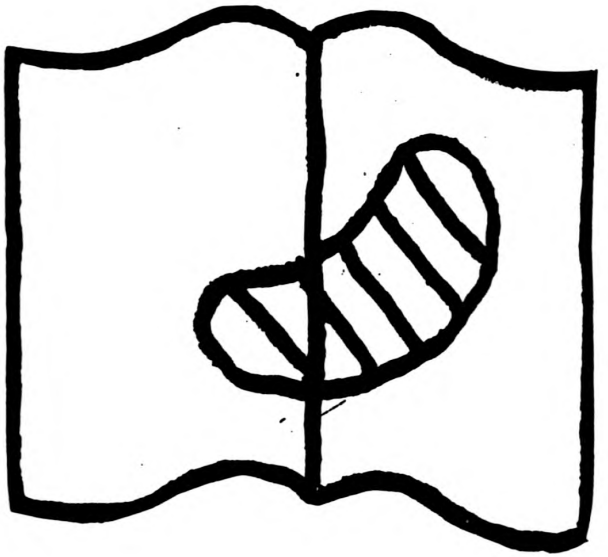


TABLE OF CONTENTS

LIST OF FIGURES

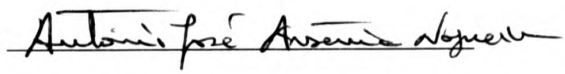
LIST OF TABLES

LIST OF EQUATIONS

DECLARATION

This thesis embodies the results of scientific experimental investigations carried out by A.J.A. Nogueira at the Institute of Aquaculture, University of Stirling, and at the Department of Zoology, University of Coimbra. The thesis has been composed independently by A.J.A. Nogueira and no part of this work has been submitted for other degree.

Stirling, July 24, 1996

Candidate: 

António José Arsénia Nogueira

ACKNOWLEDGEMENTS

Throughout the course of the work presented in this thesis I was given much help encouragement and advice by many people too numerous to mention, but to whom I want to extend my warmest thanks. I would, however, like to especially thank the following.

I would to express my deepest thanks to my supervisor Dr. Donald Baird for his guidance, encouragement and fruitful discussions about *Daphnia* physiology during the period of this study, and for critically reviewing this thesis.

I would also like to thank Professor Ronald Roberts (Head of the Institute) for providing facilities at the Institute of Aquaculture, University of Stirling.

My friend and colleague Professor Amadeu Soares strongly motivated me to start this project, and provided the facilities and supervision to the work carry out at the Department of Zoology, University of Coimbra, for which I am sincerely grateful.

I would like to thank Professor Vitor Madeira for his availability, help and precious hints during the biochemical analysis.

I would also like thank my good friend Dr. Malcolm Beveridge for is patience, and critic advises during the review of this thesis.

Susana, José Carlos and Paula thank very much for all your help during the long nights and never ending days looking at *Daphnia* during the experimental work.

This study was partly supported by grants from the British Council and JNICT through the "CIENCIA" and "PRAXIS XXI" programmes, for which I am deeply grateful.

The wider integrated framework of EC contracts under the "STEP" and "Environment" programmes provided the necessary conditions to execute this work.

Last but not least, I should also thank my wife Isabel for her love, encouragement and support especially during the particularly traumatic writing-up period.

ABSTRACT

A physiological model of an individual *Daphnia* was developed starting from first principles. The model was based on general life-cycle biology from which general allocation rules were developed. A simple feeding behaviour involving sensitivity to the presence of toxicants and no active food selection was implemented. The model addresses growth, survival, and reproduction under different environmental conditions, *e.g.* differing food levels and/or toxicant concentrations. Toxic effects are described on the basis of simple interactions with feeding and metabolic processes.

Model parameterisation was performed using available literature data together with data generated within this thesis. Values for almost all parameters used could determined from experimental data. Furthermore, parameters which could not be directly or indirectly estimated revealed themselves as insensitive.

Experimental work using pulsed food deprivation experiments on adolescent and adult instars was carried out to generate precise data on moulting, while generating information to test the allocation rules used in the model. Samples of individuals were collected at critical points during the experiments to judge if the biochemical composition of the samples was a good indicator of individual physiological state.

A full sensitivity analysis was carried out with the model which it was validated using data from differing food conditions and from different non-lethal cadmium

concentrations. During the validation process, the model revealed itself to be robust and able to simulate life-history performance under a range of environmental conditions.

The model is well-posed and robust, capturing the basic and essential properties of an individual and addressing its development from egg to adult. The model behaviour is consistent at both low and high food levels, whilst addressing the description of toxic effects in a realistic way. The model can be used as a valuable tool for teaching and experiment designing, by generating results for long term exposures to different environmental conditions within a short period of time.

1. GENERAL INTRODUCTION

"Read not to contradict and compute, nor to believe or taken for granted, nor to find talk and discourse, but to weigh and consider"

Francis Bacon (1561-1626)

1.1. WHY MODEL DAPHNIA ?

1.1.1. Ecological relevance

Science seeks the fundamental patterns in nature although they are inevitably manifest only as particular cases. In making a comparison between such cases it is necessary to separate the general from the specific, construct scientific theories, and understand the way in which the general interacts with the specific to create the phenomenon under study.

Biology consists of a hierarchy of theories, at different levels of generality (de Bernardi & Peters, 1987). Any taxonomic group of organisms will permit comparisons with general theories, although some taxa are more appropriate than others. *Daphnia* is an appropriate choice when dealing with studies on life-history theory since they:

- dominate the planktonic community of many lakes, can be easily sampled, and survive well in culture (Peters, 1987a);
- represent a group of intermediate size with enough species to represent a range of forms, physiologies, and ecologies but still small enough to maintain some homogeneity (de Bernardi & Peters, 1987);
- have a relatively short generation time, are inexpensive to culture since they are small enough to be cultured in large numbers, but large enough to be easily handled and monitored (Peters, 1987a);
- are key elements in freshwater trophic chains since they are among the dominant consumers of primary producers (phytoplankton) (Hebert, 1978) and represent an

important source of food for both invertebrate and vertebrate predators. Therefore effects on the structure of daphnid populations can have dramatic effects on the whole community (McCauley & Murdoch, 1987, 1990); and

- have been intensively studied from many points of view and have generated a large amount of literature (see Edmondson, 1987).

The comparison between general theories and available information shows that *Daphnia* can be used both to confirm general theories and to indicate anomalies (De Bernardi & Peters, 1987).

Daphnia are exemplar organisms that have been fundamental to the development of theories on predation (Holling, 1966), energy flow (Richman, 1958) population regulation (Slobodkin, 1954), competition (Frank, 1952, 1957) and evolution (Banta, 1939; Williams, 1966). Since *Daphnia* has been used at every level of biological investigation, it can be used as an ideal model to help understand the biology of other organisms generally.

1.1.2. Ecotoxicological relevance

Ecotoxicology is the science that studies the effects of toxic pollutants in the environment and its biota (Butler, 1978). *Daphnia* has been used as a test organism in aquatic toxicology (Anderson *et al.*, 1980), and an increasingly large number of studies use *Daphnia* to evaluate toxicity of new and existing substances and mixtures. Acute and chronic toxicity tests using *D. magna* are two of the techniques required to assess the potential impact of new chemicals in the freshwater aquatic environment (*e.g.* EC,

1979, 1986; OECD, 1981). Acute toxicity tests use large concentrations of toxicants, short-term exposure, food is usually absent, medium is not renewed and survival is usually the life-history trait used. On the other hand, chronic toxicity tests use low concentrations of toxicant, long-term exposure (usually over a complete life-cycle), food is added (usually in a daily basis), medium is renewed regularly, and the life-history traits used are usually growth and/or reproduction over the period of time of the test.

The ecological relevance of *D. magna* as important elements of standing water habitats combined with a short life cycle make it an ideal species for ecotoxicology. Moreover, *D. magna* is relatively sensitive to toxicants by comparison with other organisms (Buikema *et al.*, 1980; Adema & Vink, 1981; Baudo, 1987; Wong, 1987).

1.1.3. Appropriateness of *Daphnia* in mathematical modelling

Capturing the essence of the physiological dynamics of an individual can be an useful feature to model and interpret higher level phenomena (*e.g.* predator-prey interactions, population dynamics). For a long time *Daphnia* has been the preferred target of population modelling since among the freshwater herbivores it is one of the most efficient at converting algal energy in freshwater ecosystems (see Argentesi *et al.*, 1987).

Since the 1980's interest in *Daphnia* as an ideal organism for individual-based modelling has increased substantially (Gabriel, 1982; Paloheimo *et al.*, 1982;

Kooijman & Metz, 1984; Kooijman, 1986a, 1993; Gurney *et al.*, 1990; Hallam *et al.*, 1990a, b; Lassiter & Hallam, 1990; McCauley *et al.*, 1990). Some of these models were extended into structured population models (Kooijman & Metz, 1984; Nisbet *et al.*, 1989) while others have incorporated interactions with toxicants (lipophilic substances only) at population levels (Lassiter & Hallam, 1990; Hallam *et al.*, 1990b).

Considering both the ecological and ecotoxicological importance of *D. magna* it is important to establish a more generalised approach. This must describe the interaction between individuals and their environment, in particular with other classes of common toxicants, for a better understanding of population and community level effects. At the same time such models can be used to help understanding the potential ecological effects of toxicants with different modes of action of toxicants.

1.2. *DAPHNIA* BIOLOGY

1.2.1. Distribution

The genus *Daphnia* (Cladocera: Daphnidae) was given its name by O.F. Mueller in 1785 although pictorial representations of *Daphnia* were first published by Swammerdam in 1669 (Edmondson, 1987). *Daphnia* are tolerant to a wide range of alkalinities and to a wide range of salinities from freshwater to brackish water. Among those *Daphnia* species considered as large-bodied (Hrbácek, 1987), *D. magna* Straus and *D. pulex* Linnaeus emend. Leydig account for over 50% of the papers published in the last 20 years concerning the *Daphnia* genus.

Daphnids are the most ubiquitous herbivores in most lentic, highly productive systems of the holartic region (Brooks, 1959; Gulati, 1978; Hrbácek, 1987). *D. magna* is the largest species of *Daphnia* found throughout the temperate region of the northern hemisphere. It is present in ponds in Eurasia from England and North Africa to China and Manchuria (Leonhard & Lawrence, 1981). It can feed on bacteria growing in the superficial layer of sediment occurring in mesotrophic and eutrophic lakes, and particularly in natural fertilised habitats rich in organic matter (Hrbácek, 1987).

D. pulex occurs in rock pools, lakes and temporary pools partly shaded by vegetation, sometimes with *D. magna*. It is widely distributed from the arctic (Haney & Buchanan, 1987) to the temperate (Hrbácek, 1987) and tropical regions (Fernando *et al.*, 1987), occurring in North and South America, Greenland and Europe (Leonhard & Lawrence, 1981).

1.2.2. Life-cycle

The life-cycle of *Daphnia* has been subject to several reviews (*e.g.* Anderson, 1932; Anderson & Jenkins, 1942; Green, 1954, 1956; Hebert, 1978; Peters & Bernardi, 1987). In the natural environment *Daphnia* reproduce by cyclic parthenogenesis (Figure 1.1). Usually only females are produced from parthenogenetic eggs although some unfavourable environmental conditions can stimulate the production of males (Zaffagnini, 1987). Broods with males, during this period, can be single-sexed or have both sexes.

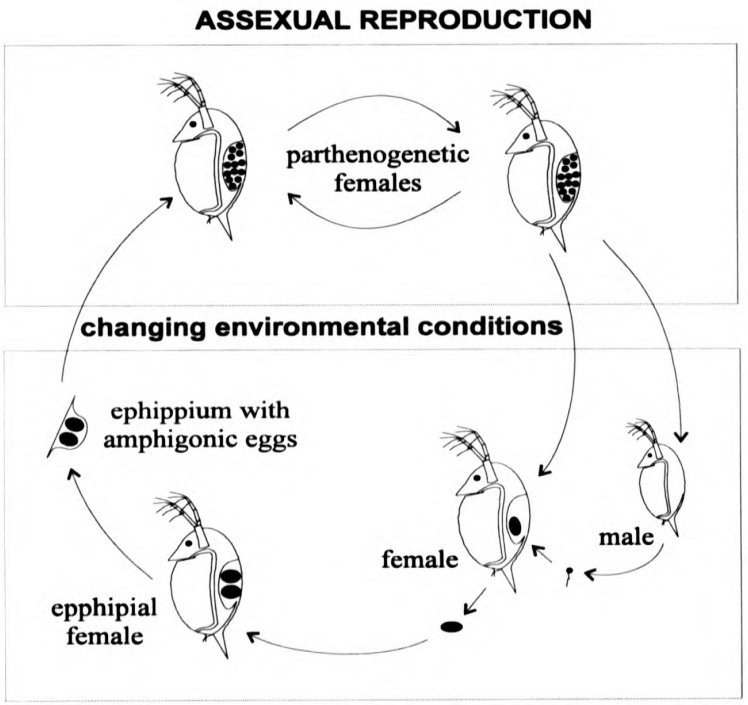


Figure 1.1 - Reproductive cycle of *Daphnia magna* (adapted from Zaffagnini, 1987, and Barnes *et al.*, 1990)

During sexual reproduction, females produce two haploid eggs that are fertilised by the male sperm. The fertilised amphigonic eggs are extruded into the brood chamber that is modified to form the ephippium. The development of the eggs is arrested at an early stage and the ephippium containing the eggs is shed with the maternal moult.

The ephippium containing the resting eggs hatches when conditions are favourable again, usually after winter, originating only females (Zaffagnini, 1987). *Daphnia* has a simple iteroparous life cycle (Sibly, 1989): under standard laboratory rearing conditions, *i.e.* optimal temperature, reasonably high food level, and constantly photoperiod, populations consist exclusively of parthenogenetic females genetically identical to the progenitors (Carvalho & Crisp, 1987; Hebert & Ward, 1972).

Daphnia, like all crustaceans, exhibits "saltatory" growth, *i.e.* individuals pass through a series of instars where increases in length can occur only at the beginning of the instar, immediately after moulting. Mature females release a variable number of eggs into the brood pouch where the embryonic development takes place while the eggs are carried. When the eggs hatch neonates are released as small, morphologically similar versions of their mothers, and are capable of independent feeding at this stage (Green, 1954).

In *Daphnia*, a reproductive event (see Figure 1.2) involves three intermoult periods designated as IM-1, IM-2, IM-3, *sensu* Bradley *et al.* (1991a):

- (IM-1): oogenesis and differentiation of oocytes from nurse cells take place in the first of these periods (in this period no significant change in mass of the oocytes occurs);
- (IM-2): oocytes are provisioned with maternal reserves for yolk formation. The major investment into eggs has been hypothesised to occur during the first third of this period (Bradley *et al.*, 1991a). This hypothesis was formulated from results of an experiment where individuals were deprived of food for increasing periods during the first third of the instar, resulting in proportionally smaller clutch sizes. A reduction of available resources allocated into reproduction, as a result of food deprivation, seems to be a more reasonable explanation for the reduction in fecundity. This agrees with another set of unpublished data where the same results were obtained when the individuals were deprived of food at the third of the instar (D.J. Baird, personal communication). Egg provisioning results in an increase in the volume of the ovaries, which become darker and protuberant. All these characteristics are easily observable, by eye and occur a few hours before ecdysis (McCauley *et al.*, 1990; personal observation).
- (IM-3) after moulting the female releases the eggs from both ovaries into the brood pouch, sequentially one at a time. From this point onward embryogenesis continues without any further maternal investment, other than potential costs of carrying the brood (*e.g.* increased predation risks, energy costs).

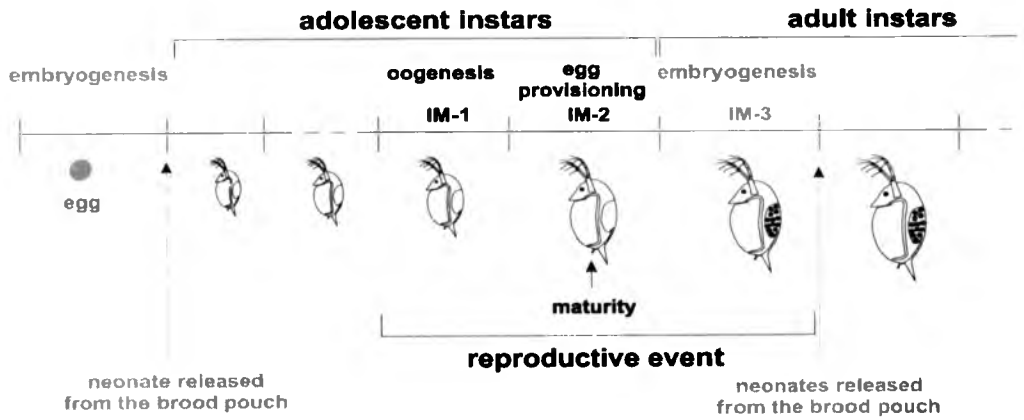


Figure 1.2 - Development of an individual *Daphnia* from egg to adult including a reproductive event (*sensu* Bradley *et al.*, 1991a): IM-1 oogenesis, IM-2 egg provisioning, IM-3 embryogenesis (oogenesis and egg provisioning (labelled in black) occur in the ovaries whilst embryogenesis (labelled in grey) takes place in the brood pouch of the mother).

In *Daphnia,* embryogenesis takes place inside the brood pouch of the mother for one instar (Zaffagnini, 1987; Threlkeld, 1987a). Fully developed neonates are released when the mother moults, at the end of IM-3, although neonates can start feeding even inside the brood pouch. After leaving the brood pouch, individuals grow over a variable number of adolescent instars until they reach maturity.

The number of adolescent instars varies among clones and across environments (Green, 1954, 1956; Lei & Armitage, 1980; Porter *et al.,* 1983; Stephenson & Watts, 1984; Taylor, 1985; Threlkeld, 1987a; Urabe, 1988). The adolescent period usually takes usually four instars under non-limiting food conditions, after which a maturation threshold is reached and females start producing eggs (Anderson, 1932; Anderson & Jenkins, 1942; Porcella *et al.,* 1969; Ebert, 1994a).

Here, maturity is defined as the developmental stage at which a female *Daphnia* is functionally able to produce, and provision eggs with matter reserves, *i.e.* when it is capable of allocating matter into reproduction. Thus, maturity is reached at some point in the last adolescent instar, *i.e.* IM2, before it starts producing and releasing eggs into the brood pouch.

Instar duration increases very rapidly during the adolescent period, levelling off when maturity is reached and growth rate slows down (Anderson, 1932; Anderson & Jenkins, 1942). Larger eggs tend to result in neonates of larger size (Guisande & Gliwicz, 1992). It has been shown that differential adolescent growth or even small differences in size at birth can lead to a variable number of pre-adult instars, associated with considerable

variation among other life-history traits (Anderson, 1932; Anderson & Jenkins, 1942; Green, 1956; Ebert, 1991). Thus, to analyse and interpret life-history traits (*e.g.* growth, reproduction and survival), it is necessary to understand the basic characteristics of individuals, particularly body length and mass, and their dependence on the environmental conditions.

1.3. THESIS AIMS AND OUTLINE

Previous *Daphnia* models have tried to capture general properties of individuals to allow the development of structured population models. Despite the importance of *Daphnia* as a test organism, little has been done on modelling the interaction between toxic stress and individual physiology, *e.g.* the effects of toxicants on feeding, growth, reproduction. Some of the approaches based on individuals, although good enough to be incorporated at population level, are based on poor descriptions of the performance of individuals. This thesis will address these issues by:

- **reviewing existing literature in modelling physiological processes of an individual *Daphnia* (Chapter 2).** Individual-based models will be generically introduced and linked to models of individuals. Existing *Daphnia* models of individuals will be reviewed and compared, with particular emphasis on the underlying assumptions and formulations involved in modelling the physiology of an individual. The suitability and performance of the models will be evaluated based on their ability to describe dynamically key physiological parameters of an individual: growth, reproduction and survival.

- **generating and parameterising a *Daphnia* model describing the physiology of an individual under stress, *i.e.* chemical stress and food deprivation (Chapter 3).** A dynamic model of an individual *Daphnia* will be proposed, and the rationale behind it will be discussed in detail. A general framework will be defined using a conceptual formulation of the interactions between the main physiological processes. Feeding will be the key process, dynamically dependent on the interactions with other physiological and environmental parameters. The model will be parameterised using available data in the literature and requirements for further experimental data will be justified.
- **carrying out the experimental work necessary to generate information to the model (Chapter 4).** A new experimental design will be presented to evaluate the effects of short periods of food deprivation during an instar. This aims to complement the limited information available in the literature on the physiological performance of an individual under pulsed feeding conditions in terms of growth and reproduction.
- **calibrating and validating the model using existing data (Chapter 5).** Sensitivity analysis of the model using the final set of parameters will be presented. Model simulations using realistic ranges for the most sensitive parameters will be analysed. Experimental data generated by other researchers, or obtained during the course this work, will be used to calibrate and validate the model.
- **discussing model applications and general suitability (Chapter 6).** The performance of the model will be addressed in an integrated and critical

perspective. The present needs of the model will be established and future improvements will be discussed.

This thesis will establish, parameterise and validate an individual model for *D. magna*. The model validation will be based on a single laboratory genotype: clone F (Baird *et al.*, 1991b) for which there is extensive and consistent physiological and ecotoxicological information available.

2. INDIVIDUAL DAPHNIA MODELS

"Life is short, Art long, Opportunity fleeting, Experience treacherous, Judgement difficult"

Hippocrates, Aphorisms

2.1. INTRODUCTION

Reductionism is an approach to science that assumes that the properties of a system can be derived from the properties of its components (Lomnicki, 1992). This concept has been widely applied in ecology at the population level (Sinko & Streifer, 1969; Lominicki, 1988) and at the ecosystem level (May, 1973; Lomnicki, 1992; Metz & de Roos, 1992), *e.g.* in predator-prey systems. The Lotka-Volterra predator-prey equations (Peschel & Mende, 1986), for example, are not phenomenological descriptions of the predator-prey system but rather they attempt to derive its properties from a very simplified description of the properties of their elements (individuals). More recently, the individual-based approach has been used as the starting point for size-structured population models of *Daphnia* (Kooijman, 1986a, b; Nisbet *et al.*, 1989; Lassiter & Hallam, 1990; Hallam *et al.*, 1990a). These so-called individual-based *Daphnia* models describe the population state based on a detailed description of the physiological state of its component individuals (DeAngelis & Gross, 1992).

In deterministic systems, the behaviour of the system must be determined in advance since predicted values are computed exactly. The results generated depend only on the starting conditions that ensure reproducibility of results. Alternatively, in stochastic or probabilistic systems the behaviour of the system is determined according to some probability distribution function. Thus, different results can be generated for the same starting conditions (Jeffers, 1978). Metz & de Roos (1992) consider two types of state in individual-based models:

- the state of an individual (*i*-state) can be defined as the information needed to specify the response of the individual to its environment (*e.g.* age, size, instar, reproductive status, hunger). Thus, the *i*-state contains the information necessary to predict the behaviour of the organism.
- the population state (*p*-state) depends on the reproduction, mortality, and migration of individuals moving through their life-cycles. Thus, it should be possible to derive the *p*-state from the dynamics of the *i*-state, since a *p*-state variable is equivalent to the distribution of all individuals with the same *i*-state, *i.e.* individuals in the same instar (Caswell & John, 1992).

The state-of-the-art in individual-based modelling was published recently following a symposium held in Knoxville in 1990 that gathered the main specialists in the field (DeAngelis & Gross, 1992). Individual-based models can be very useful by enhancing our understanding of the system under study while promising to be highly testable since mechanisms at an individual level are incorporated into the model. The knowledge obtained from individuals can help in incorporating description of mechanisms and processes determining vital rates, since populations are made up of classes of individuals. Nonetheless, individual-based models need to be tested to be validated (Murdoch *et al.*, 1992).

Models of individual organisms try to systematically describe the way in which many *i*-state physiological and ecological variables, such as ingestion, growth and reproduction, depend on body size, and/or environmental factors. Body size relationships have for some time been discussed and applied in a much broader way (Peters, 1983; Schmidt-

Nielsen, 1984) to predict ecological parameters, such as mass transfer efficiencies in food webs.

The aims of this chapter are to review the most recent models of individual *Daphnia*, compare the approaches used to model different physiological components of the organism, and to assess their general suitability in describing an individual *Daphnia* life-cycle under stressed conditions (*e.g.* fluctuating food levels).

2.2. GENERAL FEATURES OF *DAPHNIA* MODELS

One of the first individual *Daphnia* models was published by Paloheimo *et al.* (1982) based on experimental results for *D. pulex*. This model characterises the individual by mass (body weight) only. Although it describes feeding, assimilation, growth and reproduction in some detail, it cannot be applied to conditions outside the experimentally defined boundaries within which it was constructed. Thus, it cannot be used to describe life under conditions of scarce or non-existent food resources .

Kooijman & Metz (1984) present a detailed framework for an individual-based model of *Daphnia* that was used as the baseline for an age-structured population model of *Daphnia*. Growth was described by coupling a type 2 functional response for feeding (Holling, 1966) with the von Bertalanffy growth law (von Bertalanffy, 1938). Subsequent versions of the model (Kooijman, 1986a, b) incorporate energy reserves as a key compartment. Kooijman's model is essentially a volumetric model where energy is used as currency. Volume and wet weight are assumed equivalent, and length is

derived from wet weight. All processes are scaled with body volume. Ingested materials are converted to energy that is treated as interchangeably equivalent to matter. Since its first publication (Kooijman & Metz, 1984), the model has evolved into a theory of "Dynamic Energy Budgets", or DEB theory, and has been extended to all organisms at all levels of organisation (Kooijman, 1993). Kooijman describes mortality under toxic stress based only on survival probability functions, independent of the physiological state of the organism (Kooijman & Metz, 1984; Kooijman, 1993). This approach can be dangerous since the susceptibility to stress, and consequently the probability of death, increases when the nutritional state of the organism is poor. For example, after reproducing, an organism will be more vulnerable since stored material was partially depleted for egg provisioning. Consequently, an organism under stress has more difficulty in fulfilling the energetic demands of metabolic processes (*e.g.* respiration, tissue repair).

Some of the ideas in Kooijman's approach have been used as the basis for the development of more recent *Daphnia* models (McCauley *et al.*, 1990; Gurney *et al.*, 1990; Hallam *et al.*, 1990a). In these models, growth is a dynamic function of food level, and a reserves component makes survival possible for short periods under food deprivation conditions.

Yet another model (Gurney *et al.*, 1990) considers reproduction in *Daphnia* as being food dependent, while survival depends only on body reserves. Since partitioning of food resources occurs only between reproduction and growth-plus-maintenance the concept of weight-for-length is introduced. Weight-for-length includes structural mass

and reserves, and is defined as the optimal weight for an individual of a given length. It is not clear which fraction of the weight-for-length corresponds to reserves, although the model allows the organism to lose weight below their weight-for-length. Since no minimum levels are established, a starved organism will be considered dead only when its mass drops to zero, which is clearly unsatisfactory from a biological point of view.

Thieme (1988) uses the initial Kooijman/Metz model to mathematically evaluate the well-posedness of the model equations (*i.e.* showing that there are solutions for the mathematical equations that are unique and depend continuously on the model parameters). Three alternative sub-models were tested based on the assumptions:

1. initially formulated by Kooijman & Metz (1984), *i.e.* synchronised investment in growth and reproduction in the adolescent phase, with continuous investment in reproduction since birth, but not synchronised in adults with growth stopping before reproduction. According to Kooijman, reproductive investment during the adolescent phase is directed to maturation, *i.e.* development of the gonads (Kooijman & Metz, 1984)
2. used by Gurney, McCauley, Murdoch and Nisbet in the development of their model (McCauley *et al.*, 1990; Gurney *et al.*, 1990), *i.e.* maturation was not separated from growth, and all energy not needed for maintenance was channelled into growth. At the end of the adolescent phase, when a size-for-maturity was reached, the energy investment was increasingly diverted from growth to reproduction. Thus, growth rate decreases exponentially while the initial reproductive rate remains unaffected.

3. that maturation and growth are separate but synchronised which has the conceptual consequence of organisms reproducing only when they grow. The incorporation of this slight change does not affect the growth rate but alters the reproduction rate. (It is worth pointing out here that in the Kooijman/Metz, model growth in length and reproduction are continuous rather than discrete processes).

The second sub-model (McCauley *et al.*, 1990; Gurney *et al.*, 1990) was found to be superior to the other two, since it was simpler and more consistent, and heuristically and mathematically was proven to be well posed (Thieme, 1988).

The model published by Hallam *et al.* (1990a) is a mass model (*i.e.* the organism is defined by its mass). Like Kooijman (1986a), a mass-to-energy integrator is used to account for general maintenance costs. The organism's body mass is divided into protein-plus-carbohydrate ("structure") and lipid ("storage"). These compartments are composed of two fractions: a labile fraction that can be converted into energy to cover energy demands or used to provision eggs, and a non-labile fraction that accounts for the cellular mass of the organism. Thus, increments in the non-labile fraction are equivalent to growth. Using this approach, the authors hoped to capture in a more realistic way the physiological dynamics of *Daphnia*. It is well known that the lipid composition and metabolism of an organism determines its susceptibility to hydrophobic chemicals. Thus, the separation of lipid dynamics allowed for incorporation, at the population level, of the toxic effects of hydrophobic components

(Hallam *et al.*, 1990b; Lassiter & Hallam, 1990). However, this approach reduces the generality of the model.

2.3. FEEDING

Feeding is one of the best documented features of *Daphnia* biology. There is good agreement about the shape of the mathematical functions relating feeding rate with food concentration and body length. Food concentration relationships for filter-feeders are commonly described using a type 2 functional response (Holling, 1959, 1965, 1966). Michaelis-Menten kinetics are usually used to describe ingestion as a function of food concentration, since this hyperbolic response is generally accepted as the one that best describes the food intake of most invertebrates (Holling, 1966):

$$Intake = I_{max}\left(\frac{F}{F + F_h}\right) \tag{2.1}$$

where:

	F	-	food concentration
	F_h	-	half saturation constant
	I_{max}	-	maximum ingestion rate

Effects of body size on ingestion are normally expressed as power functions, for which the parameters can be easily estimated by log-transformation of dependent and independent variables. The shape of the ingestion function (*Intake*) is defined by food

concentration, while maximum ingestion rate (I_{max}) varies with body length, according to the universally used power curve:

$$I_{max} = \eta L^{\delta} \qquad (2.2)$$

where:
- η - constant
- δ - scaling factor related to body shape
- L - body length of the organism

Measurements of maximum feeding rates in *D. pulex* seem to indicate that feeding rate is proportionally higher in smaller daphnids than in larger ones (Lynch *et al.*, 1986). Using these experimental data, McCauley *et al.* (1990) added a modifier function to (2.2), to describe this apparent depression of feeding in adults. Feeding has been modelled consistently, using the same basic type of functional response, because of the large body of published information. A review on this subject has been published by Lampert (1987).

The direct measurement of assimilation (amount of digested resources entering the blood) is technically very difficult and the results can be highly variable. Nevertheless, there has been some work on the evaluation of assimilation rate as a function of food concentration using radiotracer techniques (*e.g.* Lampert, 1977; Porter *et al.*, 1982; Lampert, 1986). The simplest strategy for modelling assimilation assumes that assimilation efficiency is constant and independent of animal size or food level, and that assimilated material is a linear function of ingested material (Kooijman & Metz,

1984; Kooijman, 1986a; Hallam *et al.*, 1990a; McCauley *et al.*, 1990). Assimilation efficiency has also been modelled dynamically as a decreasing polynomial function of food concentration, although no biological explanation was given (Paloheimo *et al.*, 1982).

2.4. MAINTENANCE

Maintenance, *sensu lato*, can be defined as the price the organism has to pay to grow, reproduce, and survive. Maintenance is a very important component in individual-based modelling, representing an important and unrecoverable amount of matter that is continuously lost during the life cycle. Maintenance in *Daphnia* is generally expressed in terms of respiratory costs, including activity costs (usually measured indirectly through oxygen consumption), and moulting costs (mass to build a new carapace and energetic losses associated with this approach).

2.4.1. Respiration

Respiration is usually expressed as the amount of oxygen consumed by an individual per unit of mass per unit of time, since oxygen consumption can be easily measured. There are three main sources of oxygen consumption: overhead costs of maintaining a certain body mass (including tissue turnover resulting from disruption and synthesis of cellular components), costs associated with feeding (mainly filtering activity, digestive and absorptive costs), and activity costs.

It is commonly assumed that respiratory, or metabolic costs (R) scale with body mass (W) through a power relationship (Peters, 1983):

$$R = \varphi W^{\theta} \tag{2.3}$$

where: φ - respiration coefficient

θ - body mass scaling factor

Experimental values for θ of 0.75-1.0 have been experimentally measured in *Daphnia* (Richman, 1958; Buikema, 1972; Lampert, 1986; Glazier, 1991; Perrin *et al.*, 1992). *Daphnia* spends most of the time swimming in the water column while collecting food particles from the surrounding environment. The physical forces associated with swimming are important for small aquatic organisms since they represent an appreciable fraction of maintenance costs (Gerritsen, 1984; Hallam *et al.*, 1990a). Models of individuals generally account for respiration-plus-activity costs under maintenance (Paloheimo *et al.*, 1982; Gabriel, 1982; Kooijman, 1986a, b, 1993; McCauley *et al.*, 1990; Gurney *et al.*, 1990), while others (Hallam *et al.*, 1990a) have modelled activity separately, as a function of body volume and surface area, using the mathematical formulation of Gerritsen (1984). Modelling activity separately does not necessarily add more realism to the model since similar results can be obtained modelling the overall maintenance costs using equation (2.3) with θ close to 1.0 (McCauley *et al.*, 1990).

In *Daphnia*, respiration rate is highly dependent on food level (Lampert, 1986; Perrin *et al.*, 1992). Nevertheless, none of the existing models account for this important addition to respiratory costs. The enhancement of respiration due to feeding is usually referred as "Specific Dynamic Action", SDA, or the "calorigenic effect of food" (Klekowski & Duncan, 1975).

2.4.2. Moulting

Growth in length in crustaceans is not a continuous process. Although increase in mass is generally continuous, crustaceans can increase in length only when they moult. The moulting process can be considered as crucial, since size increases, reproduction, and survival all depend on successful moulting. Crustaceans moult by releasing the old carapace (exoskeleton or *exuvium*) and replacing it with a new one. The new carapace develops beneath the existing one during the intermoult period. Calcium is removed from the old carapace and incorporated in the new one before shedding of the carapace begins (Green, 1963). Volumetric expansion of the body occurs as a result of water swelling following moulting. Mature females of crustaceans with an external brood pouch (*e.g. Daphnia magna*) release eggs into the brood pouch before the new carapace hardens (Green, 1963).

The construction of a new carapace therefore requires matter to be allocated and energy is lost during this process. The mass of the *exuvium* was found to be related by a power relationship to the dry mass of the individual (Waddell, 1993). The carapace represents among 4% of the dry mass of adolescents to as much as 15% of the dry mass of adults

of *D. pulex* (Lynch *et al.*, 1986). Models use different approaches to account for moulting costs. These may be described as:

- explicit, where it is assumed that this is a continuous investment in a new carapace and it is included in the general maintenance costs (Paloheimo *et al.*, 1982; Kooijman, 1986a, b; McCauley *et al.*, 1990);
- implicit, where moulting is treated as a discrete event in which the new carapace is "instantaneously" provisioned from the reserves pool (Hallam *et al.*, 1990a).

Instar duration is variable between different species of *Daphnia* (Bottrell, 1975). Even within the same species, instar duration varies during the life-time (Anderson, 1932; Porcella *et al.*, 1969) and as a function of environmental conditions, such as day length (Buikema, 1973) and temperature (Porcella *et al.*, 1969; Bottrell, 1975; Goss & Bunting, 1983). Nevertheless, existing models assume a fixed instar duration regardless of developmental stage and nutritional state of the organism. The instar duration of a reproducing adult has usually been assumed, *e.g.* 2 days (Gurney *et al.*, 1990) or 3 days for *D. pulex* (Paloheimo *et al.*, 1982), and 4 days for *D. magna* (Hallam *et al.*, 1990a). However, Kooijman's model does not require instar duration to be specified because of his assumptions of continuous investment in growth and reproduction (Kooijman, 1986a, b).

2.5. ALLOCATION RULES

Partitioning of resources is crucial in any compartmental model. Interactions between different compartments determine the physiological performance of the organism in terms of growth, reproduction and survival. The existence of a reserves compartment from which resources can be obtained in the absence of food is essential to describe individual performance under starvation. All existing individual *Daphnia* models save one consider a reserves compartment (Figure 2.1), the exception being that of Paloheimo *et al.* (1982), in which maintenance costs are supported by feeding.

In those models which consider a reserves compartment, maintenance resources come directly (Hallam *et al.*, 1990a; McCauley *et al.*, 1990, Gurney *et al.*, 1990; Bradley *et al.*, 1991b) or indirectly (Kooijman, 1986a, b) from the reserves compartment. Maintenance is always assumed to have priority over all other processes. Thus, the ability to survive depends on the organism being able to fulfil its maintenance requirements. It is always assumed that resources in the blood are in equilibrium with reserves, when this compartment is present. After entering the blood ingested materials are:

- partitioned between growth and reproduction after supplying maintenance needs (Paloheimo *et al.*, 1982)
- allocated directly from the blood to the reserves compartment (Kooijman, 1986a, b)
- partitioned between reserves and growth (Hallam *et al.*, 1990a)

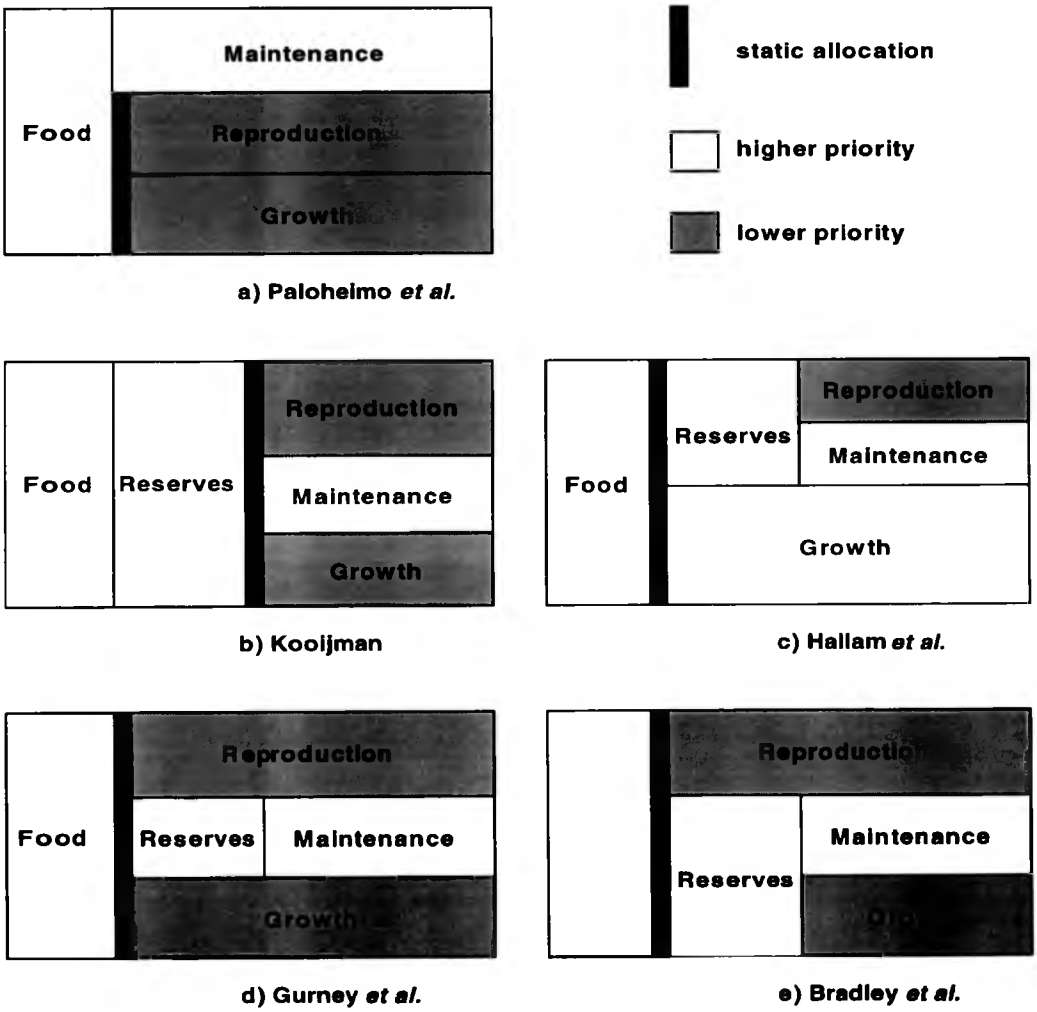


Figure 2.1 - Allocation models for *Daphnia*: a) Paloheimo *et al.* (1982); b) Kooijman (1986a); c) Hallam *et al.* (1990a); d) Gurney *et al.* (1990); e) Bradley *et al.* (1991b). A static allocation between two compartments implies a pre-defined allocation of available resources.

- partitioned between reproduction, and growth-plus-reserves (McCauley *et al.*, 1990; Gurney *et al.*, 1990)
- partitioned between reproduction and growth-plus-maintenance (Bradley *et al.*, 1991b).

Allocation priorities between growth and reproduction determine the performance of the organism under different environmental conditions (*e.g.* starvation, toxic stress). The source of resources for provisioning of eggs is also a determining factor since it will determine whether or not reproduction continues in the absence of food intake.

2.6. GROWTH

The process of growth in living organisms is difficult to strictly define. Growth is generally defined as the increase in size and mass of the body and its parts, *i.e.* a quantitative change in body size/mass. Davenport at the end of last century, and Morgan in the beginning of this century, both emphasised that "... organic growth is increase in volume .." (Needham, 1964). Growth seems to depend upon a number of associated processes such as cell division and cell movement, which by themselves contribute nothing to synthesis and increase in size. At the same time, a variable and often large proportion of size increase is independent of the synthesis of biological materials (Needham, 1964). An increase in volume is sometimes merely due to an increase in the amount of water, salts, fats or other stored materials. Although some of these can be significant components of living systems, there is a difficulty in deciding which are significant components. Keeping these considerations in mind, growth can be defined

as "the addition of materials to that which is already organised into a living pattern" (Young, 1950 *in* Needham, 1964). This definition allows the separation of growth from an increase in mass due to an increase in stored material (*e.g.* fatty acids, glycogen).

Growth in *Daphnia* can be defined as the increase in structurally organised materials (structural mass). Thus, *Daphnia* needs resources to grow. In the absence of food, there are two opposing points of view which state that:

- growth will continue at the expenses of reserves as long as the organism has enough materials to cover maintenance demands (Kooijman, 1986a, b; Bradley *et al.*, 1991b).
- growth stops if the organism does not feed (Paloheimo *et al.*, 1982; Hallam *et al.*, 1990a; McCauley *et al.*, 1990). This approach considers that matter used for structural growth comes directly from assimilated food.

Kooijman's model (Kooijman, 1986a, b, 1993) is fully dependent on reserves levels. In this model, reserves utilisation is based on two assumptions:

- by default, a constant fraction of utilised reserves is invested in growth-plus-maintenance while the remainder is invested in reproduction, according to the so-called kappa-rule, a resource allocation rule. If this fraction is insufficient to meet maintenance costs then the fraction is increased until maintenance demands are met. Thus, when reserve levels are low, growth stops before reproduction.
- the kinetics of reserves utilisation is assumed to be age-dependent at constant food levels.

Organism growth is defined according to the von Bertalanffy growth curve by Kooijman's model, as a result of the kappa-rule (Kooijman, 1986a, b). It is difficult to understand what happens to the reproductive investment of adolescents since the kappa-rule implies a continuous investment in reproduction. According to Kooijman, this investment is directed to maturation of the gonads in the adolescent phase (Kooijman, 1986a, b; Thieme, 1988). However, this would imply a perfect synchronisation of the two processes in adolescence, *i.e.* growth stops if, and only if maturation of the gonads stops.

Hallam *et al.* (1990a) consider that assimilated materials are shared between growth and reserves at fixed rates. According to McCauley *et al.* (1990) daphnids invest all assimilated resources in growth-plus-maintenance until they are big enough to start reproducing. In the first adult instar, individuals start to allocate material to reproduction. The partitioning between reproduction and growth-plus-reserves is described by an exponential decay curve, varying inversely with length. If the amount of assimilated material is insufficient to meet maintenance requirements, or if the organism is under its weight-for-length (*i.e.* at a low reserves level), then assimilation will be directed to growth-plus-maintenance. Balancing the levels of allocation to structure and reserves has a higher priority than reproduction.

2.7. REPRODUCTION AND SURVIVAL

Kooijman (1990a, b) has produced the only model to consider continuous investment in reproduction from birth by assuming a fixed partitioning of resources between reproduction and growth-plus-maintenance. The model assumes that during the adolescent stage, reproductive investment is directed to the development of gonads. At the end of the adolescent period the gonads are fully grown and the organism starts producing eggs continuously, and it is not necessary to define instar duration. This mechanism allows the organism to continue reproducting, even in the absence of food, as long as reserves are available (Kooijman, 1986a). As a consequence, in the absence of food, the organism quickly runs out of reserves. McCauley *et al.* (1990) estimated that, using Kooijman's model, a 2 mm *D. pulex* would survive for less than 12 hours. This value is in fact much lower than has been observed for *Daphnia*, *i.e.* between 2 to 10 days (Table 2.1).

Table 2.1 - Observed survival of different *Daphnia* species under starvation at 20°C

Specie	Size (mm)	Survival (d)	Notes	Source
D. parvula	-	2-4	neonates	Tessier & Consolati, 1989
D. galeata	-	2-3	neonates	Tessier *et al.*, 1983
D. galeata		3-5	neonates	Tessier & Consolati, 1989
D. galeata	-	4-6	adults 3rd brood	Tessier *et al.*, 1983
D. pulex	-	5 *	adults	Richman, 1958
D. magna	0.8	3-6	neonates	Tessier *et al.*, 1983
D. magna	0.8	6-7	neonates	Glazier, 1992
D. magna	-	4 *	adults 1st brood	Threlkeld, 1976
D. magna	1.8-4.0	4-9	-	Kooijman, 1986a
D. magna	4.7	6-10	adults 3rd brood	Tessier *et al.*, 1983

* average value

Other models treat reproductive investment as a continuous and irreversible process (*e.g.* Gurney *et al.*, 1990; Kooijman, 1986a). An individual can reproduce even if it is 'under-weight', *i.e.* its weight is lower than its weight-for-length (see above and also Gurney *et al.*, 1990), as long as mass has already been channelled into eggs during the instar. According to this approach, a starved individual will prioritise restoration of its weight-for-length over investment in reproduction. However, it is difficult to understand the process of resistance to starvation using the model of Gurney *et al.* (1990) since we do not know the exact fraction of an individual is weight which can be invested in maintenance. As a consequence, although the model has performed reasonably well against the data set used to validate it, its performance at low food levels is poor in predicting growth and reproduction (E. McCauley, personal communication).

2.8. CONCLUSIONS

In order to successfully describe the physiology of an individual under non-lethal stress, an individual model must, in my opinion, conform to the following assumptions:

i. instar duration should be a dynamic process,

ii. the model must incorporate a reserves compartment,

iii. it must be possible to generate realistic outputs (growth, fecundity, and time to death), and

iv. the model must perform consistently at both 'high' and 'low' food levels.

All existing models fail assumption (i), which is critical especially under fluctuating food levels (*e.g.* serial transfer culture conditions). All models fulfil assumption (ii) except that of Paloheimo *et al.* (1982). Model outputs always conformed reasonably well with the experimental data used for validation (assumption iii). Hallam *et al.* (1990a) did not present experimental data to validate the model and the magnitude of body weights simulated by the model is completely unrealistic (*e.g.* while the total mass of an individual *Daphnia* is less than 1 mg (Glazier, 1992) simulated values from the model are an order of magnitude higher). Population inferences cannot be drawn from the performance of Hallam's model unless the some parameters are redefined more carefully. Finally, none of the existing models deals realistically with low food levels (assumption iv), and this has particular relevance for predicting effects of toxic chemicals, as shall be discussed later.

3. MODEL STRUCTURE

"Its is vain to do with more what can be done with fewer"

William of Occam (early 14th century)

3.1. ENERGETIC MODELS OF PHYSIOLOGICAL SYSTEMS

3.1.1. Introduction to thermodynamics

In physical terms, energy is the ability to do mechanical work. Energy is an intangible and conserved property of a system, originally derived by physicists to describe certain phenomena of the external world. Thermodynamics is a mathematically-based physical science centred upon the concept of energy. The principles of thermodynamics were originally derived from physical and chemical systems but they can be applied equally well to biological systems. A system is considered to be some arbitrary and convenient macroscopic portion of matter selected for study and including the surroundings or environment (Wilson, 1972). Thermodynamic systems can be of three types (Prigogine, 1962 *in* Wilson, 1972):

- isolated systems, which exchange neither matter nor energy with their surroundings;
- closed systems, which may exchange energy with their surroundings but which do not exchange matter; and
- open systems, in which both energy and matter may be exchanged with their surrounding environment.

Living systems are open systems constantly exchanging both matter and energy with their environments. A very important property of open systems is their ability to reach a steady state when the inflow and outflow of matter and energy are balanced. It will be helpful to examine the laws governing the transference and transformation of energy in systems generally, before considering the mechanism by which the energy is produced

and transferred in biological systems. Such laws are the basis of thermodynamics (Wilson, 1972):

- First Law or law of conservation of energy. This law states that the amount of energy in a system and its surroundings neither increases nor decreases although transformations of energy from one form to another can occur. The internal energy of a system is a state function whose value changes but only on the initial and final states of the system.
- Second Law or entropy law. This law, unlike the first, is concerned with the directionality of energetic changes. It has been demonstrated that while energy can be completely converted into an equivalent amount of heat, heat cannot be completely converted back to an equivalent amount of work. Spontaneous processes are thermodynamically irreversible since they release heat that is lost to the environment and is not fully recoverable. The reverse process will not occur unless energy is supplied to the system from some external source. The heat release from spontaneous processes contributes to increase the degree of disorder or randomness in a system. Entropy is the thermodynamic function used to measure the disorder of a system. The entropy of all systems, including biological systems, tends to increase continuously. However, living organisms are highly organised systems, *i.e.* systems with fairly low levels of entropy. It has been proposed (Schrödinger, 1977) that living organisms achieve this by living on 'negative entropy', since the entropy of simple substances is higher than that of more the structured substances on which they feed on (*e.g.* glucose *versus* glycogen). Moreover they are able to maintain themselves at a high

level of orderliness (*i.e.* a low level of entropy) by radiating excess metabolic heat to their surroundings.

3.1.2. Physiological approach to bioenergetics

Here I deal with individuals as biological systems which are highly structured and organised. The maintenance of this organisation, and the increase in complexity of these systems, requires the expenditure of energy. As mentioned above, living organisms are a good example of open systems constantly exchanging matter (resources) and energy (heat) with their surroundings.

The energetics associated with biological systems can be balanced using general equations. Biological systems can be either organisms, populations, single trophic levels or even whole ecosystems (Klekowski & Duncan, 1975). The theoretical background used to establish the equations for bioenergetics is based on the following assumptions, that:

- the first law of thermodynamics is applicable to biological systems,
- matter and energy are functionally equivalent,
- all forms of energy, apart from heat, are inter-convertible under conditions of constant pressure and temperature,
- energy is always transformed (energy can not be created or destroyed), and
- reactions only occur spontaneously if there is some degradation loss of energy into heat production (Philipson, 1975).

Two overlapping concepts are commonly used in physiological ecology as equivalents: resources and energy. Resources *sensu lato* refers both to matter itself and its potential energy content. In physiological terms, resources comprise a large variety of resources (*e.g.* proteins, carbohydrates, lipids, aminoacids, carbon, nitrogen) from which energy can be obtained. Energy is the driving force for physiological processes; however, sometimes matter can be rather more limiting than energy, *e.g.* glycogen and calcium salts are needed to build the carapace of crustaceans (Wilson, 1972; Calow & Townsend, 1981).

Energy budgets are easier to compile than matter budgets, since energy associated with biological components (*e.g.* biological tissues) can be easily quantified. Furthermore it can be argued that energy is a more general measure of resources since all compounds, organic or inorganic, are convertible to potential energy. The advantages of the energy budget approach have been highlighted by several works on physiological modelling of individuals (*e.g.* Gabriel, 1982; Kooijman & Metz, 1984; Kooijman, 1986a, b, 1993; Kooijman *et al.*, 1987; Hallam *et al.*, 1990a). However, some view matter budgets as biologically more realistic in physiological modelling (*e.g.* McCauley *et al.*, 1990; Gurney *et al.*, 1990), since not all potential energy can be converted into biologically useful energy.

The release of resources from food to provision the energetic demand of physiological processes involves a complex series of biochemical reactions. The interaction between the main physiological processes can be summarised through the bioenergetic balance of an individual (Figure 3.1). Acquired food items (C) enter the gut where they are

digested. The undigested fraction of the food is released back to the environment as faecal waste (**F**), while the digested fraction after being hydrolysed in the gut into sugars, fatty acids and glycerol, and aminoacids is then absorbed into the blood or lymph. Aminoacids have to be de-aminated before they can be utilised as fuel for respiratory processes. Therefore nitrogenous products toxic to the organism, like urea, uric acid, ammonia, creatinine and other nitrogenous metabolites (**U**) are produced and excreted (Klekowski & Duncan, 1975). Although sometimes it is difficult to measure "urine" independently of the faecal waste, the nitrogenous fraction of excreta can give important information about the rate of protein utilisation as a respiratory substrate. Metabolisable energy is usually referred to as assimilation (**A**) and is composed of two fractions:

- production (**P**), includes investment on growth, repair of tissues, and storage of reserves usable, for example, to provision eggs, sperm or embryos; and,
- energy required for maintenance of life (**R**), *i.e.* to carry out various activities: mechanical work, chemical synthesis, active transport of resources and conversion of food into active metabolites (Specific Dynamic Action).

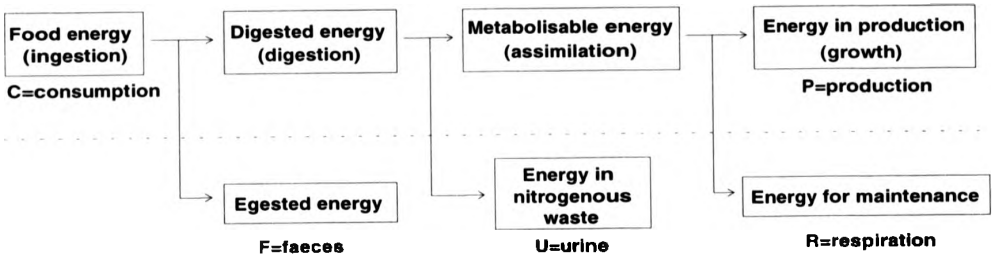


Figure 3.1 - Fate of energy in an organism (modified from Klekowski & Duncan, 1975). Boxes bellow the dashed line represent losses.

All these processes are dynamic, *i.e.* their rates change with external conditions, age, or state of development. The final balance between the different compartments can be represented by

$$\mathbf{C = P + R + U + F}$$

while assimilation (**A**) represents the physiologically useful energy, which is equal to acquired food minus the excreta, *i.e.*

$$\mathbf{A = C - F - U}$$

The assimilated fraction is used to supply fuel to all internal processes including respiration, growth and reproduction.

3.1.3. Scope for growth

Growth is one of the most important components of the matter and energy balance of an organism. Bioenergetic approaches consider growth equivalent to increase in mass. The concept of scope for growth (**SfG**), or net growth efficiency, was introduced to bioenergetics by Warren & Davis (1967) as the difference between assimilation and respiration, *i.e.*, **A-R**.

Techniques to measure scope for growth were developed as methods to investigate the effects of environmental parameters on biological production. Much of the work with scope for growth has been carried out using marine invertebrates including molluscs (*e.g.* Jorgensen, 1990), echinoderms, cnidarians and copepods (see Maltby, 1992). The freshwater crustacean *Gammarus pulex* have also been intensively used in experiment carried out by researchers at Sheffield University (*e.g.* Naylor *et al.*, 1989; Maltby *et al.*,

1990), to assess the effects on scope for growth of different classes of toxicants ranging from metals to organics (Maltby, 1992). According to Jorgensen (1990), scope for growth is only a sensitive measure of growth for conditions below the optimal, but becomes a poor indicator of growth rates as conditions approach the optimum.

Physiological approaches to the energetics of individuals involve the creation of structured, simplified formulations: an individual is considered as a system where the inflow (food acquisition) must be balanced with the outflows (excreta, production). This formulation is the simplest approach to a model of an individual. Some energetics-based models have extended this idea to provide more detail concerning growth and reproduction in *Daphnia* (Paloheimo *et al.*, 1982; Gabriel, 1982).

3.2. RATIONALE FOR A PHYSIOLOGICAL DAPHNIA MODEL

3.2.1. Pseudo bond graphs

Detailed modelling of the physiological performance of an organism implies a biological understanding of the interactions between the main physiological processes. The representation of a system using pseudo-bond graph models, common in systems engineering (Oster *et al.*, 1973), has already been applied to outline physiological problems (Barreto *et al.*, 1984; Karnopp & Azarbaijani, 1981) since process interactions become clearly obvious with this type of representation.

Pseudo bond graphs describe a system using sets of sources, sinks, junctions, valves and resistances. The currency in the system can be energy, matter, or whatever unit seems most appropriate. However mass is preferable in modelling individuals since it is closest to the real situation. Mass flows from sources into sinks across valves and/or junctions. Valves can be either uni- or bi-directional, but they always offer some resistance to flow. Thus, flow through a valve is always associated with mass/energy losses. Flow across junctions is conservative, *i.e.* no losses of matter/energy occur while the flow is diverted into two or more sinks.

3.2.2. Physiological dynamics on *Daphnia*

The physiological processes, of an individual *Daphnia,* were grouped under five general categories and treated as a set of interacting compartments in a pseudo bond-graph model (Figure 3.2). Blood (haemolymph) is treated as a temporary repository for assimilated materials (junction). The flow of resources is associated, as mentioned above, with losses originating from different biochemical processes (Lehninger, 1973), comprising:

- absorption of resources through the gut wall,
- turnover of resources (e.g. proteins *versus* amino acids, polysaccharides *versus* monosaccharides, lipids *versus* fatty acids, DNA and RNA *versus* nucleotides), and
- resource oxidation to produce ATP (respiratory cycles).

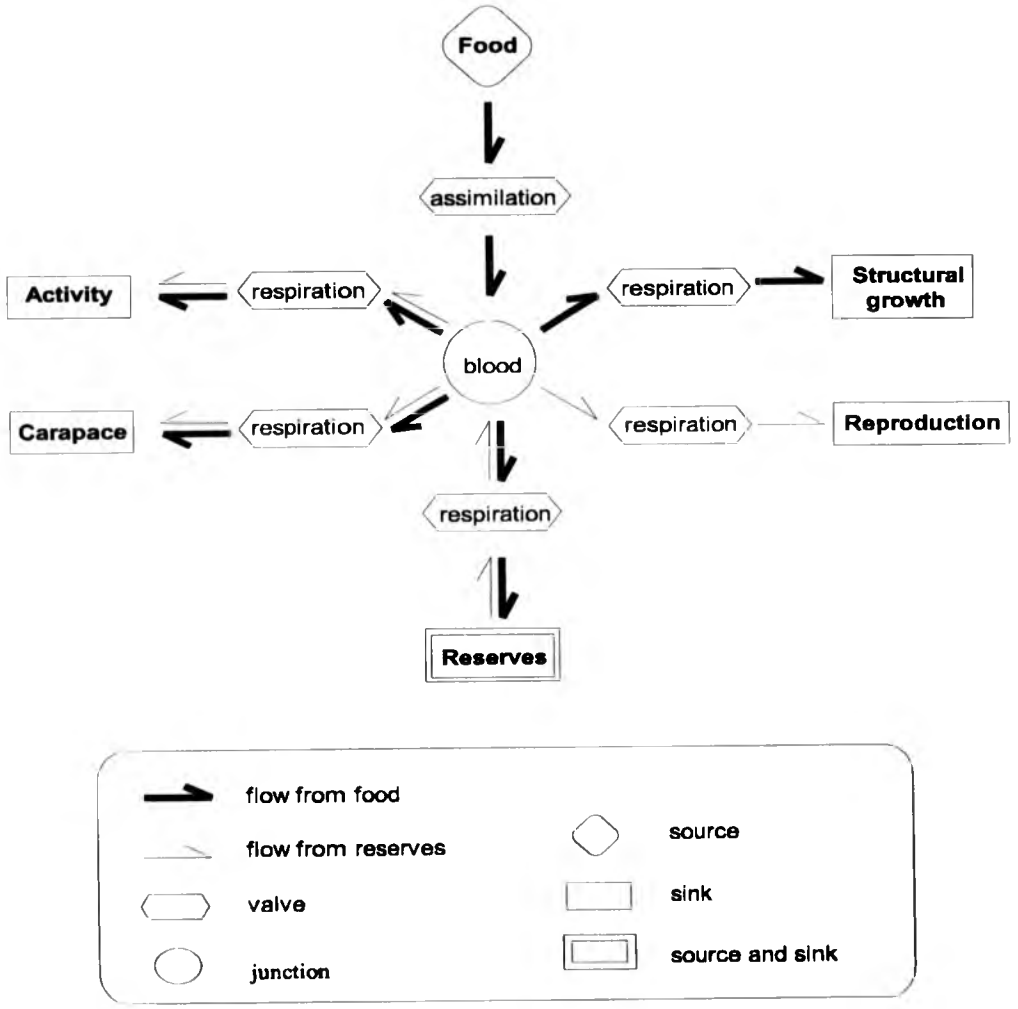


Figure 3.2 - Simplified pseudo-bond graph of the proposed model of an individual *Daphnia*.

Resources collected from the environment (feeding, digestion and assimilation) flow into sinks before being used for specific tasks (*e.g.* general metabolism, increases in cellular mass, reproduction). Three categories of sink were defined according to their role in the organism:

- volatile sinks in which resource flows are used to provide energy for physiological processes becoming irreversibly lost during this process (*e.g.* activity, respiration);
- static sinks, in which mass flow is used to supply 'building blocks' to the organism, which become unavailable to other processes after allocation (*e.g.* carapace, structural growth, reproduction); and
- dynamic sinks, in which mass flow is bi-directional, *i.e.* the sink can act both as sink and source (*e.g.* reserves).

Mass allocation to specific compartments or processes is critical since it will determine growth, reproduction, and survival of the individual, and hence its fitness (see below). Two conceptually different pathways of mass flow are considered in the individual:

- the food path (thick arrows) that supplies mass required by all processes in the organism under optimum food levels, except for reproduction, and
- the reserves path (thin arrows) that supplies mass for all processes, except structural growth, under sub-optimum food conditions (e.g. starving organism). This approach implies that investment in structural growth is dependent on feeding levels.

The existence of two different flow pathways in the individual does not imply different physical pathways, instead underlines specific allocation rules under conditions of food deprivation. Thus, growth occurs only in the presence of food, while reproduction depends on the level of stored reserves. Vital processes such as activity, metabolism and moulting always have priority over other processes (since they ensure survival), and can derive resources from both food and reserves. For an individual *Daphnia* the investment in reproduction, in the absence of food, would be an evolutionary wasteful use of resources if it compromises the survival of the individual.

3.2.3. Individual fitness

All individuals carry genetic information that they can transfer totally, in parthenogenetic individuals only, to future generations. The fitness of an individual refers to the relative contribution, of its genotype to the gene pool of future generations (Calow & Townsend, 1981). One approach to quantify fitness in evolutionary ecology has been to treat it as mathematically as equivalent to the intrinsic age of increase, r, used in the so called Euler-Lotka equation (Sibly & Calow, 1986):

$$1 = \sum_{x=1}^{\infty} e^{-rx} \, l_x \, m_x \qquad (3.1)$$

where
- r - intrinsic rate of increase
- x - age
- l_x - survival of individuals of age x
- m_x - fecundity of individuals of age x.

An individual can therefore maximise its fitness by minimising maturation time and by maximising survival and/or fecundity. However these minimisation/maximisation processes may involve trade-offs between the different components.

Daphnia magna has a short developmental time (approximately 5 days under abundant food conditions) before reaching maturity. During this time the individuals increase in size, and when maturity is reached they are capable of producing a clutch of eggs every 3 to 4 days while continuing to increase in size. Thus, allocation priorities in an individual *Daphnia* should be the result of trade-offs between maturation time, fecundity and survival. Consequently, a model of an individual should implement an allocation strategy that maximises the fitness of the individual, while considering the constraints imposed by trade-offs for limited resources.

3.2.4. Resource acquisition

The processes of feeding and assimilation in filter-feeding zooplankton have been intensively studied in recent decades. The feeding rate of a filter feeder can be considered to be a function of particle size and density. It has been hypothesised that daphnids collect particles at a rate proportional to the rate of encounter until a maximum feeding rate is reached, this maximum rate being determined by mechanical processing constraints (McMahon & Rigler, 1965; Holling, 1965, 1966). Selective foraging behaviour has been documented for some filter-feeding zooplankton, *e.g.* calanoid copepods (Kerfoot & Kirk, 1991; Bern, 1990; DeMott, 1986, 1988) and some species of rotifers that can select food items, rejecting low energy particles such as

suspended clay (Kirk & Gilbert, 1990; Kirk, 1991a, b). However, most daphnids are considered to be generalist feeders, *i.e.* non-selective feeders (DeMott, 1982, 1985, 1986, 1988; McCabe & O´Brien, 1983; Kirk, 1991a, b; Kerfoot & Kirk, 1991) with some exceptions such as *Bosmina* (Starkweather & Bogdan, 1980; Bern, 1990; DeMott, 1982, 1985) and *Diaphanosoma* (Bern, 1990; Kerfoot & Kirk, 1991), and *Chydorus* (DeMott, 1985). It has been suggested that the organoleptic properties of the particles can be used by some filter feeders to select particles with higher nutritional value (DeMott, 1986, 1988) and to avoid those that are noxious (Porter, 1977).

a) Functional feeding responses

The most comprehensive analysis of the factors influencing rates of resource utilisation is presented by Holling (1959, 1965, 1966). The change in feeding rate that occurs as a result of changes in the density of food items was called by Holling as a "functional feeding response". In general, organisms show one of three types of functional feeding responses (Figure 3.3) (Holling, 1959):

- type I responses are characteristic of animals which consume food at a rate proportional to the rate of encounter (*e.g.* some filter feeders), up to a saturation level at which there is no longer any change in the feeding rate with increasing food density.

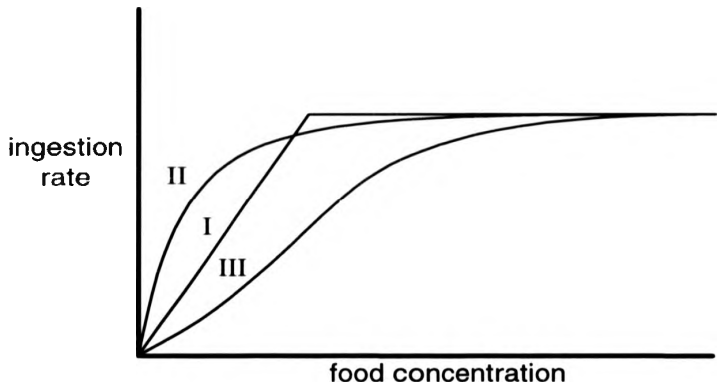


Figure 3.3 - Functional responses for the relationship between food concentration and ingestion rate (adapted from Holling, 1959).

- type II responses are characteristic of organisms that take a certain time to capture, process and ingest food items. Feeding rate increases with food concentration at a continuously decreasing rate until a plateau is reached. Since most invertebrates follow this kind of functional response it has been referred to as "the invertebrate curve" (Holling, 1966).
- type III responses are characteristic of organisms showing some sort of learning behaviour about food items they usually collect (*e.g.* calanoid copepods, which use taste as an indicator of the nutritional value of food items). This implies that if a preferred food item is scarce it will be eaten proportionally less (Sibly & Calow, 1986).

Although some studies suggest the use of a type I response for *Daphnia* (Rigler, 1961; McMahon & Rigler, 1963), a type II model has been preferred by others (*e.g.* Lampert, 1977; Porter *et al.*, 1982, 1983; Kooijman & Metz, 1984). Type II models are biologically more meaningful since the rates of the suite of feeding behaviours change smoothly around the "incipient limiting concentration" (McMahon & Rigler, 1963), *i.e.* food concentration at which feeding rate becomes maximum.

The type II feeding response considers digestive constraints on feeding (Penry & Jumars, 1987) and it can be derived mathematically from analogies to chemical reactors (Real, 1977; Penry & Jumars, 1987). The type II model can be easily deduced from the predator-prey interactions between *Daphnia* and its algal food, using an enzyme-predator analogy (Real, 1977):

$$A + C \underset{k2}{\overset{k1}{\rightleftarrows}} X \xrightarrow{k3} P + C \qquad (3.2)$$

where:

A	-	prey (algae)
C	-	predator (*Daphnia*)
X	-	intermediate state associated with capture and handling
k1, k2, k3	-	rate constants associated with transformations into designated states
P	-	predator production

Considering the conservation constraint

$$C_0 = C + X$$

the solution of the kinetics equations for the interactions at steady-state gives the well known Michaelis-Menten equation of enzyme kinetics

$$f = \frac{dP}{dt} = k_3 X = F\frac{A}{G + A},$$

where $F = k_3 C_0$ and $G = (k_2 + k_3) / k_1$. This equation predicts that prey consumption is hyperbolic with the change in prey concentration.

b) Foraging strategies

Organisms need to search for, collect, and process food items to obtain enough resources to survive, grow and reproduce. Optimal foraging theory, a way of conceptualising resource acquisition by animals, conceives the world as being divided into patches, each containing food. This theory is concerned with the order in which the patches should be visited and the time that must be spent in each, to maximise the net rate of energy intake.

According to the theory of optimal foraging an organism should exploit first patches which can supply energy at a rate equal or superior to the average of the overall environment. The organism should remain in a patch when the net intake rate in the patch falls below the average of the environment. Thus, if a forager feeding selectively in high energy patches encounters a poor patch it should only feed there if the initial yield is greater than the overall rate at which it had previously been obtaining energy (Townsend & Hughes, 1981; Sibly & Calow, 1986).

Although optimal foraging theory has already been tested for several species of vertebrates (see Townsend & Hughes, 1981; Sibly & Calow, 1986) little has been done with small zooplankton, in particular filter feeders. Experimental testing would be very difficult since experimental conditions are never completely identical and dependency between two or more parameters increases the likelihood of bias (Peters & Downing, 1984). However, mechanistic models of the feeding process offer an alternative (*e.g.* Lam & Frost, 1976; Lehman, 1976): these two models, although based on different premises, yield approximately to the same predictions for selective feeders (type III

feeding responses). However, the predictions of foraging and energy optimisation models may not be applicable to continuous non-selective filter feeders as *Daphnia* which do not allocate time and energy to discrete periods of search, capture, handling and rest (Porter *et al.*, 1983).

Energy optimisation models for filter feeders (Lam & Frost, 1976; Lehman, 1976) predict a reduction in filtering activity (usually indicated by a drop in appendage beat rate) at both low and high food concentrations. Lehman (1976) considers that this foraging model is also valid for Cladocera, although there is almost no evidence to support this theory at low food levels (Muck & Lampert, 1980), except from an isolated observation of reduction in the appendage beat rates of *Daphnia rosea* (Burns, 1968). Studies relating fitness with the functional response showed that *Daphnia* does not follow optimal foraging theory, and that a type II curve was the most appropriate to describe feeding since:

- *Daphnia* shows no orientation to or tendency to remain in patches of algae (Porter *et al.*, 1982, 1983),
- *Daphnia* is a generalist filter feeder which maintains a constant filtering rate well below food levels encountered in nature, *i.e.* there is no feeding threshold in *Daphnia* (Porter *et al.*, 1982),
- above the incipient limiting concentration the observed constant mandible beat rates imply constant ingestion rates (McMahon & Rigler, 1963; Burns, 1968; Starkweather, 1978), and

- carapace gape offers a means of regulating the volume of water filtered (Gliwicz & Siedlar, 1980) but no effect of food quality was observed (Porter *et al.*, 1982).

c) Dynamic of food particles

Laboratory cultures of *Daphnia* are normally fed on a daily basis. Algal particles are usually uniformly distributed in the culture vessel when the animals are transferred, and cultures are left undisturbed until the next transfer or feeding period. Food particles with a density close to that of water will remain in suspension (*e.g. Selenastrum* species have two large vacuoles that decrease their density). However, algae more dense than water will sink rapidly, sometimes within a few hours (Burns, 1969; Enserink *et al.*, 1990), accumulating at the bottom of the culture vessel if it is left undisturbed (Arnold, 1971). *Daphnia* feeds preferentially from the water column, although it can browse at the bottom of the vessel when food is scarce in the water column (McMahon & Rigler, 1963; Burns, 1969; Horton *et al.*, 1979; personal observation).

Animal-transfer together with foraging are the main disturbance factors in long term *Daphnia* cultures associated with food particles deposited at the bottom of the culture vessels. Thus, it can be considered that the instantaneous availability of food (3.3) will be associated with the period of time the cultures remain undisturbed:

$$F_{avail} = Food \frac{t_S}{t_S + t_f} \quad (3.3)$$

where: $Food$ - food concentration in culture

t_S - settling time for half of the particles

t_f - time the cultures remain undisturbed.

This instantaneous measure of food availability (F_{avail}) does not imply that sedimented food particles are not available. Instead, as food concentration in the water column is reduced, more particles become available either through browsing or by resuspension of particles due to individuals swimming near the bottom of the culture vessels.

d) Feeding apparatus

The feeding apparatus of daphnids has been widely studied in several species. Daphnids possess highly specialised thoracic appendages for the collection of food particles, that are probably the most advanced apparatus within the Branchiopoda (Lampert, 1987). The components of the feeding apparatus are small, and morphologically complex. Five trunk limbs, with movements that in some cases are very rapid and difficult to observe and interpret, are partly hidden within the carapace forming a suction and pressure pump (Fryer, 1987).

The feeding process involves the abstraction of particles from the water by specialised filters (setal fans forming meshes) on the third and fourth pair of limbs (Korínek & Machácek, 1980; Geller & Müller, 1981; Arruda, 1983; Gophen & Geller, 1984;

Brendelberger, 1985; Brendelberger & Geller, 1985; DeMott, 1985; Koza & Korínek, 1985; Fryer, 1987). Nonetheless, it has been claimed that the flow regime around the filtering setae produces very low Reynolds numbers (viscous flow) resulting in little or no flow around the setules of the putative filters (Gerritsen & Porter, 1982). The filtering combs on limbs 3 and 4 would behave like paddles while limbs 1 and 2 could be the primary collection surfaces for food particles (Gerritsen & Porter, 1982). This suggestion was initially supported by Ganf & Shiel (1985a, b) while categorically rejected by Brendelberger & Geller (1985) and Bendelberger (1985). More recent estimations of the drag forces based on the theory of slender bodies in a viscous fluid have confirmed the filtering function of these limbs (Geller & Knisely, 1988).

The large variety and positioning of setae on limbs 1 and 2 indicates an active self-cleaning apparatus that sweeps particles upwards and dorsally from filtering combs, along the food grove, towards the mouthparts (Geller & Müller, 1981; Ganf & Shiel, 1985b). Limb 5 is used to close off the feeding chamber and prevent backflow of water as limbs 3 and 4 move inward (Ganf & Shiel, 1985b). In daphnids the relevant part of limbs 3 and 4, in combination with limb 5, comprise a pump that draws in water, and a fine-pored wall for abstracting particles from it (DeMott, 1985; Fryer, 1987). The whole process of filter feeding in cladocerans works as a pressure-pumping mechanism with an enclosed volume of water being actively pressed through the filter meshes (Brendelberger, 1985). The filtering process can be described by at least three steps (Geller & Müller, 1981):

- capture of particles by setae or setulae at first contact, or by electrostatic or ionic attraction (Rubenstein & Kohel, 1977; Gerritsen & Porter, 1982);

- removal of particles from the filter combs by means of long bristles; and
- transport of food along the food groove towards the mouth parts.

Particle chemistry determines the capture of particles smaller than the mesh size: positively charged or neutral particles are more readily captured than negatively charged particles; similarly, particles with reduced wettability are more readily captured, *e.g.* surfactants increase wettability of particles and animals, reducing capture (Gerritsen & Porter, 1982).

e) Maximum feeding rate

The area of the filtering appendages determines the maximum number of particles that can be removed from the water, whether they act as filters or paddles. The structures associated with the feeding process, *i.e.* area of filtering combs, scale approximately with the second power of the body length (Korínek & Machácek, 1980; Brendelberger & Geller, 1985; Ganf & Shiel, 1985b).

Thus the maximum feeding rate (3.4), F_{max}, is assumed to be size dependent

$$F_{max} = \eta L^{\delta} \tag{3.4}$$

where: L - body length

η - coefficient of the length-feeding relationship

δ - index of the length-feeding relationship

f) Gut structure and digestion of food

Daphnia has a tubular gut divided into three main parts: the foregut or exophagus, the midgut, and the hindgut. The foregut and the hindgut are short tubular connections between the midgut and the mouth or anus, respectively. Food boli are driven down the foregut by peristaltic contractions of the gut wall and by hydraulic pressure. The digestive and absorptive processes take place mainly in the anterior portion of the midgut (Peters, 1987b). It has been hypothesised that, depending on the gut clearance time, it is possible for particles which pass through the gut unprocessed, to be recaptured and reingested (Sibly & Calow, 1986). Thus, it is necessary to introduce a correction parameter to the maximum feeding rate to ensure a more or less accurate number of feeding particles (3.5). Since the gut does not grow isometrically, *i.e.* gut volume increases faster than surface area, the correction parameter was scaled to the surface area, *i.e.* through the second power of length.

$$F_{max} = \eta L^{\delta} \left(\frac{L_d^2}{L_d^2 + L^2} \right) \qquad (3.5)$$

where:

η	-	coefficient of the length-feeding relationship
δ	-	index of the length-feeding relationship
L	-	body length
L_d	-	length-dependent feeding depression factor

g) Feeding as a function of environmental conditions

Feeding rates change with environmental factors such as food concentration, toxicant concentration, temperature, and light. Michaelis-Menten kinetics (type II functional response) were used to relate feeding rate to food concentration (see section on **Functional feeding responses** for explanation). Feeding rate increases with food concentration at a decreasing rate until a maximum, F_{max}, is reached for very high food concentrations (Figure 3.4). Thus, the dependency of feeding on food concentration is defined as F_d:

$$F_d = \frac{F_{avail}}{F_h + F_{avail}} \tag{3.6}$$

where: F_{avail} - available food

F_h - half saturation factor of food.

Toxicants are known to depress feeding, *e.g.* pesticides (Kersting & van der Honing, 1981; Fernandez-Casalderrey *et al.*, 1994) and metals (Gulati *et al.*, 1988; Allen *et al.*, 1995). Metals and other positively charged particles appear to have more pronounced effects on feeding than other toxicants at non-lethal concentrations (Allen *et al.*, 1995). Neutral substances or negatively charged have the same effect only at concentrations approaching or exceeding lethal concentrations (Allen *et al.*, 1995).

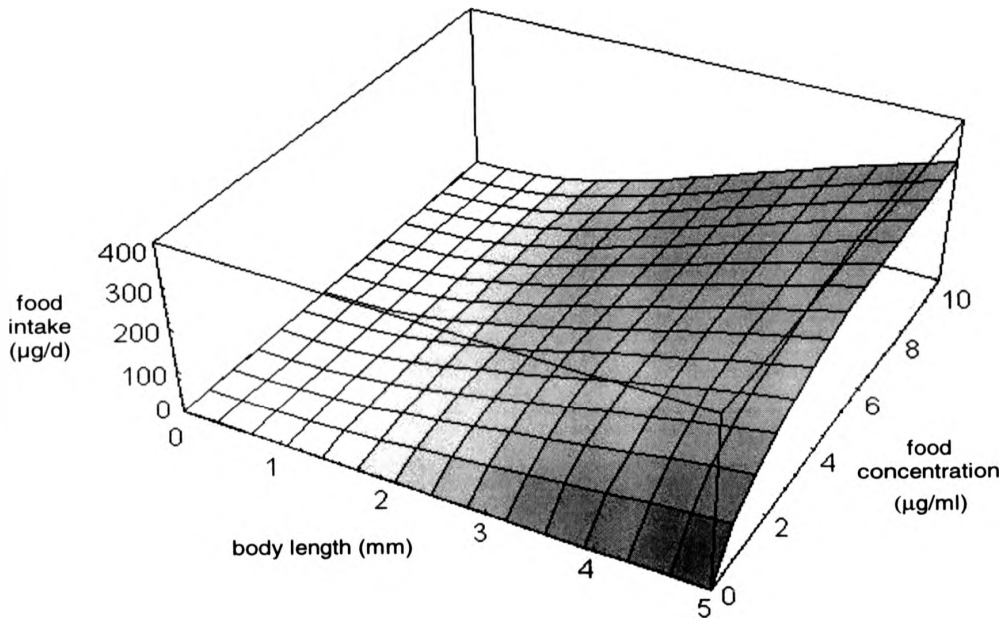


Figure 3.4 - Daily food intake as a function of body length and food concentration

The toxicant depression effect, T_d, follows an allosteric decay function that can be represented by (3.7).

$$T_d = \frac{T_h^{\kappa}}{T_h^{\kappa} + Tox^{\kappa}} \tag{3.7}$$

where T_h - toxicant concentration inducing 50% depression in feeding rate

Tox - toxicant concentration

κ - allosteric index

Total food intake (3.8) can be computed as the product of these individual effects, *i.e.* maximum feeding rate (3.5), food scaling function (3.6), and toxicant depression function (3.7).

$$Intake = F_{max}\, T_d\, F_d \tag{3.8}$$

in which food intake varies inversely with toxicant concentration, and directly with food concentration, and increases with a power of the body length (Figure 3.5).

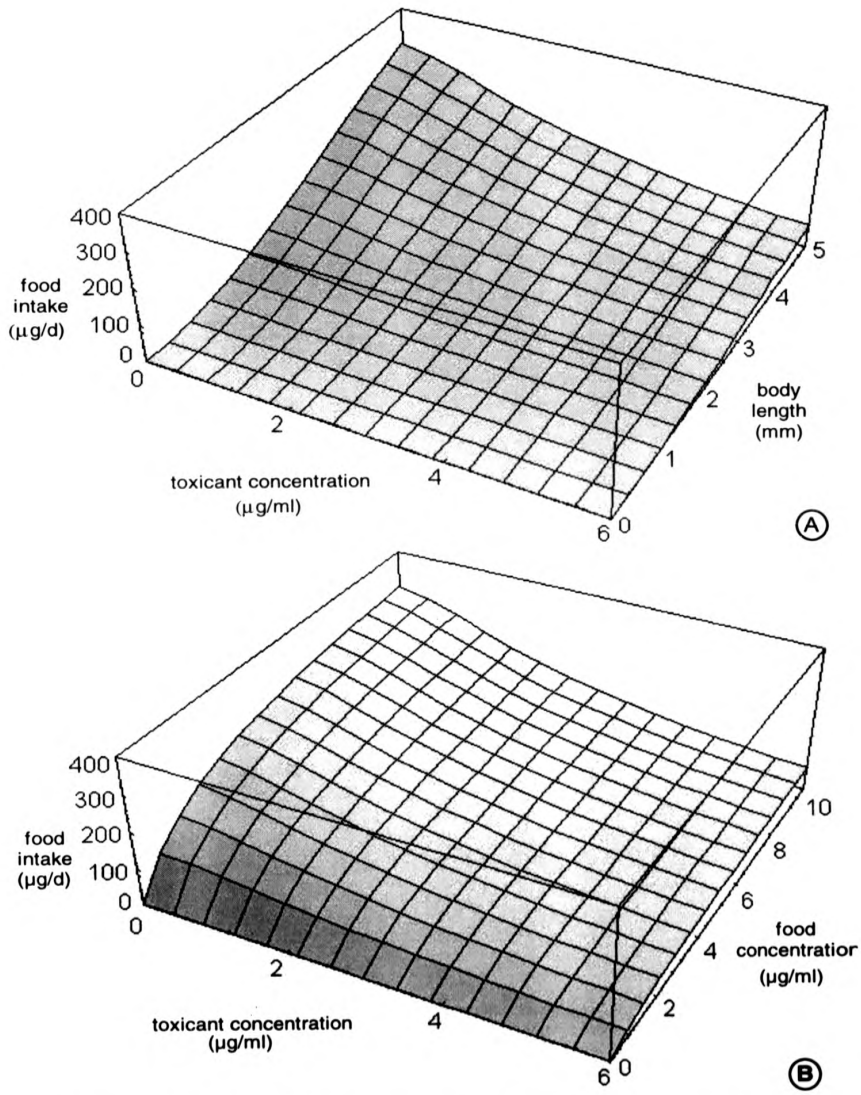


Figure 3.5 - Daily food intake as a function of (A) food concentration and body length, and (B) food and toxicant concentrations in a *Daphnia* with 5 mm of body length.

3.2.5. Assimilation

The gut residence time for food particles is inversely related to the feeding rate, *i.e.* food particles remain longer in the gut at low food levels since feeding rate increases with food concentration. Moreover, assimilation efficiency increases at a decreasing rate with gut residence time. The information in the literature about the relationship between assimilation efficiency and food concentration is scarce. Nevertheless, experimental observations with *Daphnia pulex* (Geller, 1975), *D. galeata* (Urabe & Wanatabe, 1991), *D. longispina* (Christoffersen, 1988) and *D. pulicaria* (Murtaugh, 1985; Christoffersen, 1988), the mysid *Neomysis mercedis* (Murtaugh, 1984), and the cephalopod *Octopus cyanaea* (Sibly, 1981; Sibly & Calow, 1986) suggest that a first order decay curve is appropriate to relate assimilation efficiency with food concentration using equation (3.6). Based on these observations, assimilation efficiency (A_ε) is described as

$$A_\varepsilon = A_{min} + (A_{max} + A_{min})\ (1 - F_d) \tag{3.9}$$

where A_{min} and A_{max} represent the assimilation efficiency of an individual feeding at its maximum and minimum feeding rate, respectively.

The product of the amount of ingested material (3.8), and the assimilation efficiency (3.9) represents the assimilated resources (A), *i.e.* the amount of resources which are absorbed across the gut wall

$$A = Intake\ A_\varepsilon \tag{3.10}$$

Assimilated materials include all ingested resources minus faecal waste. Higher assimilation efficiencies are achieved at low food levels by maximising gut retention time. Moreover, although assimilation efficiency is reduced in the presence of large amounts of food (Porter, 1975), the net intake of resources is higher.

3.2.6. Allocation rules

The allocation of resources between the main physiological process (Figure 3.6) shares some features of previous allocation models (Bradley *et al.*, 1991b; Hallam *et al.*, 1990a; see Chapter 2). However, the allocation between structural mass (growth) and reserves is dynamic (Figure 3.7), *i.e.* the fraction invested into reserves is determined by a feedback mechanism dependent on the balance between reserves and structure. Moreover, this model can be used to explain experimental results (Glazier & Calow, 1992), where only particular models seem to apply.

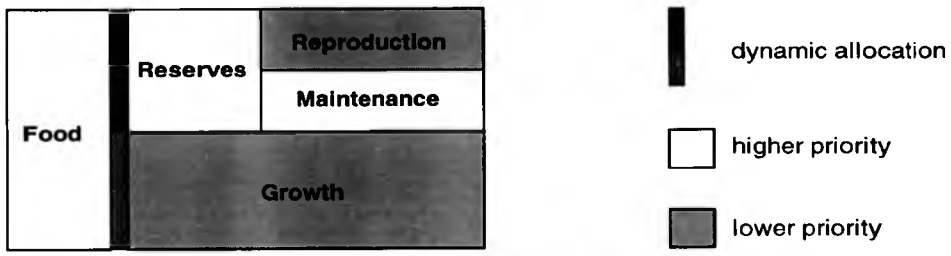


Figure 3.6 - Proposed allocation model for *Daphnia*.

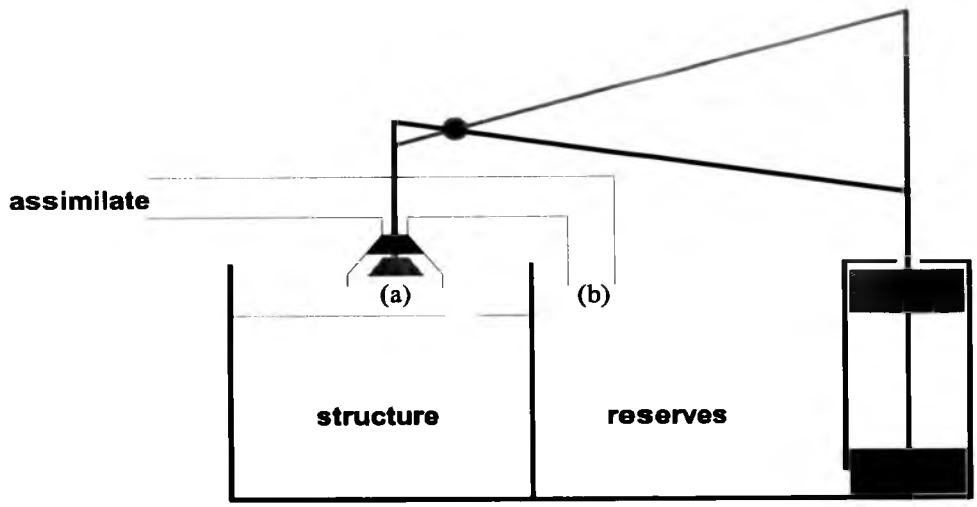


Figure 3.7 - Hydraulic representation of the dynamic allocation of assimilated materials into structural mass and reserves. (a) Partition to structure, (b) partition to reserves, (grey) the situation where reserves and structure are balanced, (black) the situation where reserves are nil.

In the model, an optimal reserves level (*reserves**) implies a balanced increment between the structural and reserves components. The ideal allocation to reserves, *QrL*, is established after moulting occurs remaining constant during the following intermoult period. The value of this function is minimal during the juvenile period (non-mature individuals) but increases rapidly before the individual achieves maturation size (L_m), reaching a maximum very quickly (3.11).

$$QrL = Q_{min} + (Q_{max} - Q_{min}) \left(\frac{L^{\beta}}{L^{\beta} + L_m^{\beta}} \right) \tag{3.11}$$

where:

- Q_{min} - minimum fraction of resources allocated to reserves
- Q_{max} - maximum fraction of resource allocated into reserves
- L_m - length threshold for maturation

However, when reserves and structure are not balanced the investment into reserves should be increased proportionally. This goal is achieved through an instantaneous evaluation of the relative level of reserves, Q_i, reflecting the prevailing balance between reserves and structural mass (3.12). This function corrects the amount of assimilated materials that will be effectively allocated to structure.

$$Qi = \frac{Storage\ (1 - QrL)}{Structure\ QrL} \tag{3.12}$$

Thus the actual partitioning of assimilated resources between structure and reserves (Figure 3.6) is based on the following assumptions:

i) the ideal amount of reserves is a function of existing structure,

$$reserves^* = f(structure)$$

ii) the ideal allocation to reserves is a function of body length,

$$QrL = f(length)$$

iii) a variable fraction of assimilated resources is allocated to structure,

$$(1\text{-}QrL)\ Q_i$$

while the remaining fraction allocated to reserves

$$1\text{-}(1\text{-}QrL)\ Q_i.$$

iv) when reserves are nil all resources are allocated to reserves

$$Q_i = 0 \text{ when } reserves = 0$$

iv) partitioning between structure and reserves approaches the optimal when reserves approach the optimal level for existing structure

$$Q_i \rightarrow 1 \text{ when } reserves \rightarrow reserves^*$$

3.2.7. Metabolism

Living animals require resources to generate the energy necessary to maintain all their normal functions, *e.g.* locomotion, feeding, escaping predators, reproduction. The energy an animal needs for all of these processes comes primarily from the chemical energy contained in available resources. The rate of energy usage is the metabolic rate.

The metabolic rate is frequently defined as the overall use, or turnover, of chemical energy. Oxygen consumption is a convenient way to measure oxidative metabolism. Furthermore, the determination of oxygen consumption is technically simple, and it is so commonly used for estimation of metabolic rate that the two terms are often used interchangeably (Schmidt-Nielsen, 1984).

The term metabolic rate usually refers to the respiration rate of an entire animal (Schmidt-Nielsen, 1984). The respiratory rate of an animal will vary depending on the amount of metabolically active tissue. Metabolic rates can be expressed in a variety of ways. Typical units for small organisms are µl O_2/hr, ml O_2/d, cal/hr. Similarly, since the specific metabolic rates are a customary way to express metabolism, usual units are: µl O_2/mg/hr, ml O_2/mg/d, cal/mg/hr. It appears more meaningful to relate metabolism with the dry mass of an organism since the body water does not contribute directly to the metabolism. Proteins, lipids and carbohydrates contribute differentially to metabolic rate; therefore, it is necessary to relate their use with the different ways, mentioned above, of expressing metabolism (Table 3.1). Thus, for modelling purposes, it is more convenient to express metabolic rates per unit of mass. To avoid confusion, "specific metabolic rate" will be used here to designate "metabolic rate per unit of mass". This is in accord with the definition "specific" when used before the name of a physical quantity, which means "divided by mass" (Royal Society, Symbols Committee, 1975 *in* Schmidt-Nielsen, 1984).

Table 3.1 - Calorimetric factors for the basic substrates used in metabolism (adapted from Wilson, 1972)

Factors and Units	Protein	Lipids	Carbohydrate
Gas exchange (*)			
Oxygen (µl/mg)	1037	2167	890
Carbon dioxide (µl/mg)	839	1531	890
Respiratory quotient (RQ)	0.81	0.71	1.00
Energy			
Total (J/mg)	18.4	39.8	4.2
Metabolisable (J/mg)	17.2	38.9	4.1
Caloric equivalents of metabolisable energy			
oxygen (J/ml)	16.6	18.0	19.3
carbon dioxide (J/ml)	20.4	25.4	19.3

(*) The use of oxygen consumption as a metabolic unit must be treated carefully since the volume occupied by a gas is temperature dependent, according to the equation of state of ideal gases (Bent, 1965), i.e. $PV = nRT$, where P is the pressure (atm), V is volume (ml), n is the number of moles of gas, R is the gas constant of Boltzmann (0.0820597 $atm.dm^3.mol^{-1}.K^{-1}$), and T is the temperature (K). Therefore, the molar volume of any gas at 20°C is 24.05662 l/mol while at 0°C is only 22.41543 l/mol, leading to a correction factor of 1.073217 was applied to the values tabulated by Wilson (1972, p. 634).

a) Standard respiration rate

The concept of basal metabolic rate is commonly defined as the minimal value of metabolic expenditure (Peters, 1983). The wide use of this concept has been criticised since it incorrectly implies that the metabolic rate will not fall below this level (Schmidt-Nielsen, 1984). However, the metabolic rate of ectotherms varies with temperature (Peters, 1983) and many other external factors, making the use of the term basal rather dubious.

As an alternative, resting metabolic rate or maintenance rate is preferred to designate the metabolism or oxygen consumption in the absence of external activity in non-reproductive, fasting animals within their thermal neutral zone (Peters, 1983; Schmidt-Nielsen, 1984).

The concept of basal metabolic rate is used only for endotherms. The metabolic rate is lower and constant within the thermal neutral zone if all other factors are kept constant The thermal neutral zone is bounded by two critical temperatures: lower critical minimum value, below which the metabolism increases to warm up the body, and upper critical temperature above which the metabolism increases since the animal expends energy in cooling processes (Schmidt-Nielsen, 1984).

The conditions for estimation of resting metabolic rate in endotherms are easily achieved. On the other hand, ectotherms do not have a thermal neutral zone since body temperature depends on external temperature. Thus, among ectotherms, the conditions for measuring metabolic rates can not be so closely defined and the term standard

metabolic rate is used. The standard metabolic rate must be measured under standardised conditions to yield low values for the metabolic rate, but not necessarily the lowest possible value (Peters, 1983).

The specific metabolic rate of *D. magna* has been independently measured by several authors, yielding average values of 0.1 µl O_2 / µg DW / d for an individual (Glazier, 1991; Perrin *et al.*, 1992; Barber *et al.*, 1994). Thus, oxygen consumption in *Daphnia* was converted into units of mass. Oxygen consumption was divided by a conversion factor of 1.34 µg O_2 / µg DW to express metabolic rate as mass depletion. This value was derived from the values presented in Table 3.1 assuming carbohydrates, proteins and lipids as anabolic sources, and carbohydrates and lipids as the major catabolic sources.

Respiration rate depends to a large extent on food level (Lampert, 1986; Perrin *et al.*, 1992). The enhancement of respiration due to feeding is usually referred as "Specific Dynamic Action", SDA, or the "calorigenic effect of food" (Klekowski & Duncan, 1975). Nonetheless, SDA can be used in a much broader sense to describe increases beyond standard respiration rate, due to environmental factors (*e.g.* food, toxicants). Modelling the respiratory costs of a daphnid under stress involves accounting for three basic components of respiration:

- specific standard metabolic rate (M_r), including costs with basic activity (swimming) and tissue turnover;
- specific dynamic action of food (SDA_f), including extra swimming costs with collection, processing and digestion of food particles, and supply-side effects

on feeding of toxicant exposure (*e.g.* reduction in palatability or wettability of food particles); and,

- specific dynamic action of toxicant (SDA_t), including all demand-side effects caused by toxicant exposure (*e.g.* increase in the synthesis of stress proteins) and direct poisoning (*e.g.* induction of oxidative processes). Metals are known to enhance the oxidative reactions responsible for cell protein damage and lipid peroxidation (Stohs & Bagchi, 1995; Broderius *et al.*, 1995).

b) Specific dynamic action of food

Respiration rate increases with feeding in a large variety of animals, including *Daphnia* (*e.g.* Porter *et al.*, 1982; Kiorboe *et al.*, 1985; Lampert, 1986; Philipova & Postonov, 1988a, b; Barber *et al.*, 1994; Boher & Lampert, 1988). However, a few studies by Kersting and co-workers report contradictory results; respiration rates decreased for very high food concentrations at which maximum feeding rates were observed (Kersting & van der Leeuw-Leegwater, 1976; Kersting, 1978). Thus, following the general trend, observed experimentally, respiration includes a component (SDA_f) which scales with feeding rate, *i.e.*

$$SDA_f = M_f \, F_d \, T_d \tag{3.13}$$

where:

SDA_f	-	specific dynamic action of food
M_f	-	maximum SDA of food
F_d	-	feeding saturation function
T_d	-	toxicant depression function on feeding.

Since feeding is depressed in the presence of toxicants both factors, F_d and T_d, are used to scale the specific dynamic action of food.

c) Specific dynamic action of toxicant

Toxicant concentration affects respiration rate through different modes of action:

- metals are known to increase the 'metabolic load' by inducing the synthesis of mixed-function oxidase enzymes (Sibly & Calow, 1986) or other detoxifying mechanisms associated the elimination of highly reactive free radicals (see Stohs & Bagchi, 1995);
- highly dissociated phenols are known to uncouple oxidative phosphorylation (see Broderius *et al.*, 1995), *i.e.* the normal flow of electrons from $NADH_2$ to O_2 is not interrupted during the process but the rate of electron transfer is increased;
- highly lipophilic molecules, *e.g.* organic pollutants, cause reversible polar and non-polar narcosis (see Broderius *et al.*, 1995).

Using the analogy of the SDA of food an SDA of toxicant was defined as

$$SDA_t = M_t\,(1 - T_d) \tag{3.14}$$

where:
- SDA_t - specific dynamic action of food
- M_t - maximum SDA of toxicant
- T_d - toxicant depression function on feeding

All body tissue (structure and reserves) is metabolically active involving reactions of synthesis (*e.g.* building structure or storing resources) and degradation (*e.g.* reserves usage) which use matter to generate energy. Thus, the specific metabolic rate (SMR), *i.e.* the overall sum of Mr, SDAf, and SDA_t, must be multiplied by the body mass (*Reserves+Structure*) to obtain the metabolic costs (R) of any individual *Daphnia* (Glazier, 1991), as respresented in equation (3.15).

$$R = \left[M_r + M_f F_d T_d + M_t (1 - T_d) \right] (Reserves + Structure) \qquad (3.15)$$

Barber *et al.* (1994) designed an elegant experiment involving different combinations of non-lethal cadmium concentrations and food concentrations, from which the M_r, M_f, and M_t, were derived for two clones of *D. magna* (Table 3.2). Examining their data it becomes clear that the SDAs (M_f, M_t) simply scales with M_r, and are independent of the genotype, *i.e.* the observed ratios were equal for both clones. Applying a linear regression between observed and expected metabolic rates gives a line with slope close to 1 passing through the origin (Figure 3.8).

Table 3.2 - Parameters of equation (3.15) for two clones of *Daphnia magna* (S-1, F), calculated from data of Barber *et al.* (1994) using multiple linear regression.

	S-1	F	Pooled
M_r (µl O_2 / d / µg DW) (% DW / d)	**0.112** (8.3)	**0.144** (10.7)	**0.128** (9.5)
M_f (µl O_2 / d / µg DW) (% DW / d)	**0.097** (7.2)	**0.154** (11.5)	**0.126** (9.0)
M_t (µl O_2 / d / µg DW) (% DW / d)	**0.014** (1.1)	**0.018** (1.3)	**0.016** (1.2)
M_f / M_r	**0.864**	**1.069**	**0.97**
M_t / M_r	**0.129**	**0.123**	**0.13**

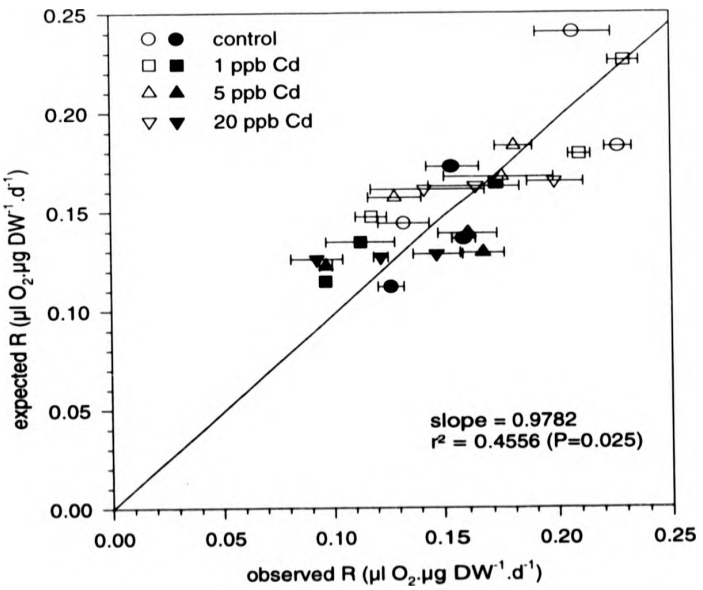


Figure 3.8 - Expected versus observed specific metabolic rate (SMR) for two clones (F - open symbols and S-1 - solid symbols) of *Daphnia magna* using parameters from Table 3.2 (data from Barber *et al.*, 1994).

The graphical plot for the overall specific metabolic rate (Figure 3.9) shows that respiration does not vary monotonically and independently with food and toxicant concentration (cadmium). Experimentally it is possible to register similar respiration values for a multitude of combinations between toxicant and food concentrations. Thus, experiments of this kind are useful in helping to understand the toxicity mechanisms and modes of actions of different compounds.

3.2.8. Recent feeding history

The nutritional status of an individual *Daphnia*, since the last moult, is used in the model to predict the values of two key functions: instar duration and average egg mass of a clutch. The recent feeding history of an individual (*RFH*) is modelled as the proportion of food effectively ingested relative to the amount of food that would be ingested during the same period if the individual was feeding at its maximum feeding rate, *i.e.*

$$RFH = \frac{F_{\max} - \left(\frac{F_{cum}}{Inst}\right)}{F_{\max}} \tag{3.16}$$

where: $Inst$ - time since last moult

F_{max} - maximum feeding rate

F_{cum} - cumulative feeding (instar feeding history).

Incorporation of the effects of past feeding conditions on egg size has previously been suggested by Threlkeld (1987a) and McCauley *et al.* (1990).

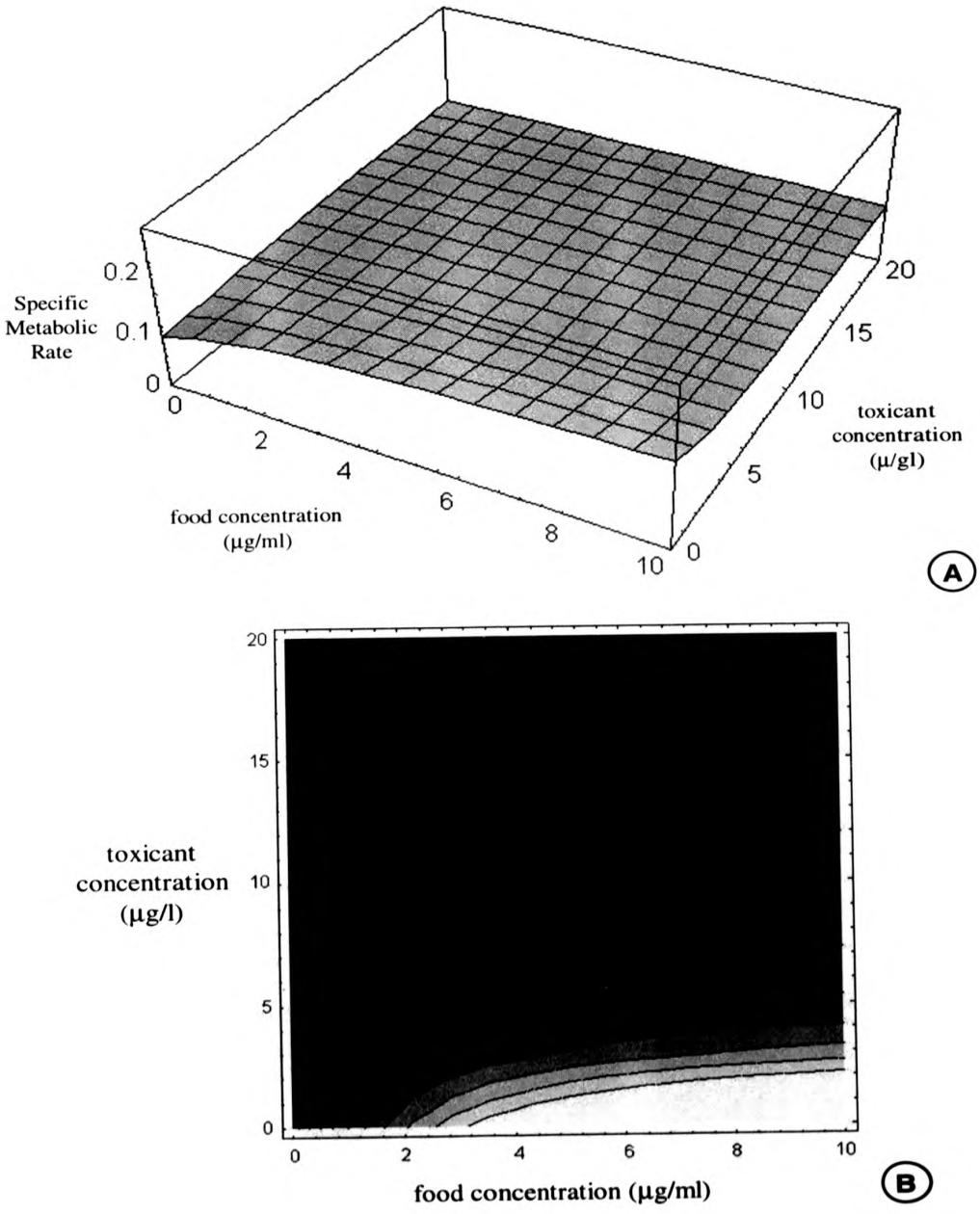


Figure 3.9 - Specific metabolic rate as a function of food and toxicant concentrations: (A) surface plot, (B) metabolic isolines (dark areas correspond to lower values of specific metabolic rate).

3.2.9. Moulting

Moulting is a discrete process, which can be considered as crucial component of the crustacean life-history. It is not possible for the animal to grow, reproduce or ultimately to survive without moulting. Every time an individual moults it loses an appreciable amount of mass. Thus, proper regulation of the timing for moult is vital for an individual, since if it moults too soon it is wasting mass while if it takes too long to moult it can compromise its survival and reproduction. In *Daphnia* the moulting cycle can be divided into four periods (Green, 1963; Wilson, 1972) during which several metabolic processes occur, co-ordinated and regulated by hormones:

- premoult or proecdysis, characterised by active preparation for moult during which materials, *e.g.* calcium, are partially removed from the old carapace and used in the formation of the new one. Many biochemical reactions occur to prepare materials for the formation of a new carapace, *e.g.* glycogen accumulates in the cells of the epidermal tissue and protein synthesis is accelerated;
- moult or ecdysis, in which the splitting and shedding of the old partially reabsorbed carapace occurs. At this point, uptake of water from the environment increases and the individual immediately increases in size;
- postmoult or postecdysis, characterised by rapid deposition of chitin, synthesised at the expense of the glycogen and stored beneath the newly forming carapace before the moult. The subsequent phenolisation of proteins combined with the deposition of calcium gives the carapace its rigidity; and

- the intermoult or interecdysis, during which the processes normally associated with moulting are absent. At this time the animal feeds, and replaces the water taken up in the postmoult with tissue deposition.

a) Carapace

The carapace of crustaceans is a rigid, semi-permeable structure that strains the body shape of an individual whilst giving some physical protection. Every time an individual moults it loses the carapace or *exuviae*, representing a significant non-recoverable fraction of it body mass. For *D. magna* the carapace was estimated to represent 10% of total dry weight (Waddell, 1993), whilst for *D. pulex* values between 4% and 15% of total dry weight were reported (Lynch *et al.*, 1986; McCauley *et al.*, 1990).

The size of the carapace, and consequently its mass, is associated with the amount of cellular tissue of the organism (*i.e.* structure). Thus, the mass of the carapace (C) can be calculated using a geometric relationship similar to the relationship between structure and length, *i.e.*

$$C = \sigma L^{\beta} \tag{3.17}$$

where: σ - coefficient of the carapace-length relationship

β -index of structure-length relationship,

and must be deduced from reserves at moult time

$$\frac{dReserves}{dt} = -C \tag{3.18}$$

b) Instar duration

Instar duration is a complex dynamic process which has not been properly addressed by other models. Some do not consider it, *i.e.* change in body length is forced to follow a von Bertallanffy growth curve (Gabriel, 1982; Kooijman & Metz, 1984; Kooijman, 1986a, b, 1993). Others consider it to be static throughout life, *e.g.* for *D. pulex* both 2 days (Gurney, 1990; McCauley *et al.*, 1990) and 3 days (Paloheimo *et al.*, 1982) have been used for the instar duration. Finally, Hallam *et al.* (1990a) consider it to be discrete, but do not describe its constraints, nor they do detail the function used to define it.

The instar duration varies with the life-stage of the individual (Anderson, 1932; Porcella *et al.*, 1969), and as function of environmental conditions such as light cycle (Buikema, 1973) and temperature (Porcella *et al.*, 1969; Bottrell, 1975; Goss & Bunting, 1983). Since the model assumes constant photoperiod and temperature, the instar duration is defined here as strictly associated with the recent feeding history (environmental conditions) and the body length (life-stage) of the individual. Instar duration is assumed to increase (Figure 3.10):

- geometrically with body length;

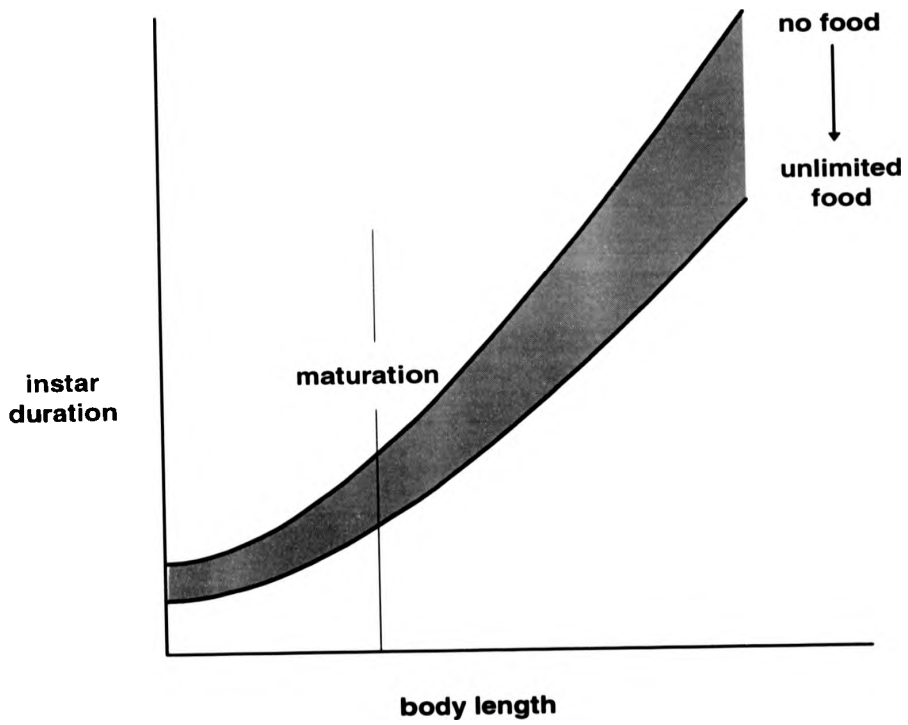


Figure 3.10 - Variation of instar duration with individual size and variable food conditions.

- linearly with the reduction in food availability. The "recent feeding history" of an individual is considered as the factor that determines the boundaries of the moulting cycle. The lower limit corresponds to the situation where food resources are unlimited and feeding is continuing at its maximum rate. The upper limit is the situation of complete deprivation of food and defines the point where survival is compromised if moulting does not occur.

Instar duration (*Tm*) is defined mathematically as

$$Tm = T_{min} + T_{coef}\, L^{\beta} + T_{delay}\, Inst\, RFH \qquad (3.19)$$

where:

T_{min}	-	minimum instar duration (length dependent)
T_{coef}	-	coefficient of the length-instar duration relationship
T_{delay}	-	proportion of delay caused by feeding history
β	-	index of the length-structure relationship
RFH	-	recent feeding history
Inst	-	elapsed time since last moult.

This function is constantly updated in the model and the individual moults when *Tm* equals *Inst*. Due to the importance of moulting as a key process and to the lack of literature information some experiments were conducted within the framework of this thesis to parameterise the above function (see Chapter 4).

3.2.10. Growth

Growth, in the sense of this work, is defined as increase in the quantity of organised cellular building blocks (Structure), as opposed to Reserves that is the fraction of materials which are used to meet the metabolic demands of the organism. Body length and structure are interdependent, *i.e.*

$$Structure = \alpha L^{\beta} \tag{3.20}$$

where: a - coefficient of length-structure relationship

b - index in length-structure relationship

Thus reserves can be obtained by subtracting structure from body mass, *i.e.*

$$Reserves = mass - structure \tag{3.21}$$

Structure and reserves are dynamically modelled through differential equations defining the partitioning of assimilated materials between these two compartments (Figure 3.7, page 68).

$$\frac{dStructure}{dt} = A\left(1 - QrL\right) Qi^{\varepsilon} \tag{3.22}$$

where: A - assimilated materials

QrL - optimal partition into reserves

Qi - instantaneous evaluation of the relative level of reserves

ε - partition index

The remaining assimilate is partitioned into reserves (Figure 3.7, page 68) after deducting metabolic costs (M), *i.e.*

$$\frac{dReserves}{dt} = A - M - \frac{dStructure}{dt} \tag{3.23}$$

The optimal partitioning function (QrL) has a sigmoid shape. Thus, the proportion of reserves increases rapidly with body size until maturation size is reached (Figure 3.11), increasing at a decreasing rate after that. Growth investment is higher during the juvenile phase while reserves allocation increases after maturity. During the juvenile stages reserves are used only for metabolism and moulting while, after maturity, they also have to provide the necessary resources for egg provisioning.

3.2.11. Reproduction

During reproduction, *Daphnia* can provision a variable number of eggs under variable periods of food deprivation, during an instar (Tessier *et al.*, 1983; Kooijman, 1986a; Enserink *et al.*, 1990; McCauley *et al.*, 1990; Tessier & Consolatti, 1991). The strategy of resource allocation into eggs has originated some controversy in the literature; while some authors treat allocation into eggs as a continuous process (Gabriel, 1982; Kooijman & Metz, 1984; Kooijman, 1990; McCauley *et al.*, 1990; Gurney *et al.*, 1990) while others consider egg investment a discontinuous event, occurring immediately prior to moulting (Hallam *et al.*, 1990a, b; Lassiter & Hallam, 1990).

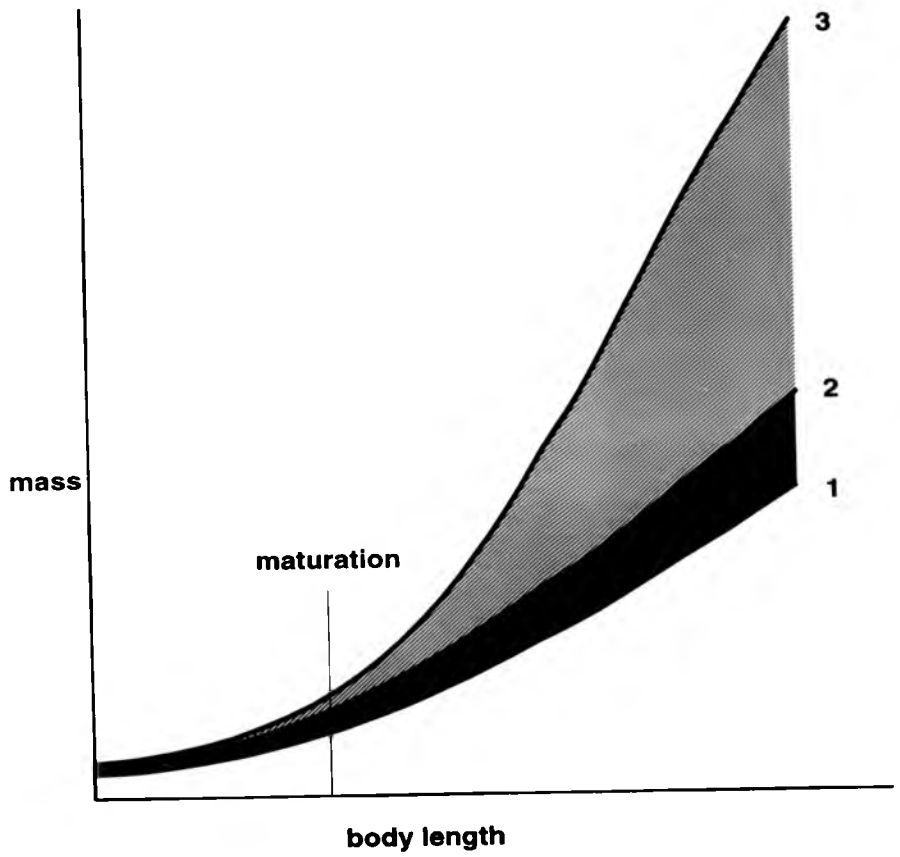


Figure 3.11 - Theoretical relationships between body length and mass of an individual. (1) structural mass, (2) structural mass + mass of residual reserves, (3) whole body mass during the instar.

During an instar, *Daphnia* continuously accumulates lipids as lipid droplets that tend to be more concentrated near the ovaries (Tessier & Goulden, 1982) until a point close to the end of the instar. This observation provides a reasonable support for the idea that most of the egg mass is mobilised from available maternal reserves (mostly lipids) before ecdysis. In addition, it is worth noting that strong evidence exists that an individual does not always produce eggs with the same size (Smith, 1963; Tessier *et al.*, 1983; Enserink, 1990; Glazier, 1992; Glazier & Calow, 1992). However, different *Daphnia* species exhibit the same type of variation in egg size. It was observed that there is a three fold difference between maximum egg size and minimum egg size in *D. pulex* (Bell, 1983; Tessier and Consolatti, 1991) and *D. pulicaria* (Tessier & Consolatti, 1991).

Life-history theory states that, under conditions of high food availability, organisms should invest resources in reproduction in the form of large numbers of small eggs (Sibly & Calow, 1986). In *Daphnia* data are generally supportive of this prediction (Smith, 1963; Enserink, 1990; Glazier, 1992; Glazier & Calow, 1992) although there are some exceptions (Tessier *et al.*, 1983; Glazier, 1992; Glazier & Calow, 1992). This prediction was therefore incorporated as a model assumption, using the recent feeding history function to calculate average egg mass:

$$E_m = \mathrm{E}_{\min} + 2\, E_{\min}\, RFH \tag{3.24}$$

where: $\mathrm{E}_{\min}$ - minimum mass of an egg

RFH - recent feeding history.

The average egg mass, E_m, is used to compute an integer number of eggs for the clutch from available reserves. Physiologically, ecdysis and clutch release into the brood pouch are synchronised; however, ecdysis must occur first followed by egg release into the brood pouch before the new carapace becomes hard. Thus, since the new carapace and the eggs are assumed to be provisioned from reserves, the matter required to build a carapace must first be discounted. Similarly, the individual should avoid exhausting its reserves completely, since it must retain reserves, Q_{res} *Structure*, to ensure a short period of survival if conditions become suddenly unfavourable. After deducting these components, clutch size can be finally computed, as:

$$Brood = \text{Integer}\left\{\frac{Reserves - C - Q_{res}\ Structure}{E_m}\right\} \tag{3.25}$$

where:

- C - carapace mass (see page 82)
- Q_{res} - proportion of residual reserves retained to maintain structure under adverse conditions
- E_m - average mass of an egg

and total clutch mass must be taken from reserves

$$\frac{dReserves}{dt} = -E_m\ Brood \tag{3.26}.$$

In the first IM-2 instar (*i.e.* the instar where maturity is achieved), individuals provision eggs from available reserves. The maturation threshold-size is set constant (following Ebert, 1994a) and body length in the IM-2 instar varies with environmental conditions (Ebert, 1991, 1992, 1994a). Therefore, the number of eggs in the first clutch tends to vary considerably between individuals under relatively constant food conditions (Barata & Baird, personal communication).

3.2.12. Embryonic development and juvenile size

The duration of embryonic development in cladocerans is negatively temperature-dependent (Kankaala & Wulff, 1981; Goss & Bunting, 1983). In *Daphnia magna*, it takes about 2.5 days, at 20 °C, for a new-born to develop to the point of hatching from the egg (Goulden *et al.*, 1987). Egg mass and juvenile size (in terms of both mass and length) are positively correlated, with small-diameter lighter eggs producing shorter length, light neonates and large-diameter heavier eggs producing longer length, heavy neonates (Green, 1956; Kerfoot, 1974; Glazier, 1992).

A theoretical relationship between egg mass and neonate length was calculated, based on the observed range for egg mass in *Daphnia magna* (3 to 9 µg DW) and the observed range of neonate length (0.75 to 1.1 mm). Numerical comparisons between the range of egg mass variation and the range of newborn length showed that newborn length can be derived from egg mass (Figure 3.12) using parameters already defined in the model:

$$L = \beta \sqrt{Mass \frac{(1 - Q_{min})}{\alpha \ (1 - Q_{min} Q_{res})}} \tag{3.27}$$

where:

β	-	index of the length-structure relationship
α	-	coefficient of the length-structure relationship
Mass	-	mass of an egg
Q_{min}	-	minimum fraction of resources allocated into reserves

During embryonic development the physiological dynamics of an individual are described by equations (3.27), (3.20), (3.21) and the modified forms of equations (3.15), (3.22) and (3.23) as follows:

$$Structure = \alpha \, L^{\beta} \tag{3.20}$$

$$Reserves = Mass - Structure \tag{3.21}$$

$$R = \left[M_r + M_t \ (1 - T_d) \right] (Structure + Reserves) \tag{3.15}$$

$$\frac{dStructure}{dt} = 0 \tag{3.22}$$

$$\frac{dReserves}{dt} = -R \tag{3.23}$$

Neonates hatch from the eggs after a fixed time, t_e, and start feeding independently and behaving according to the physiological dynamics previously presented.

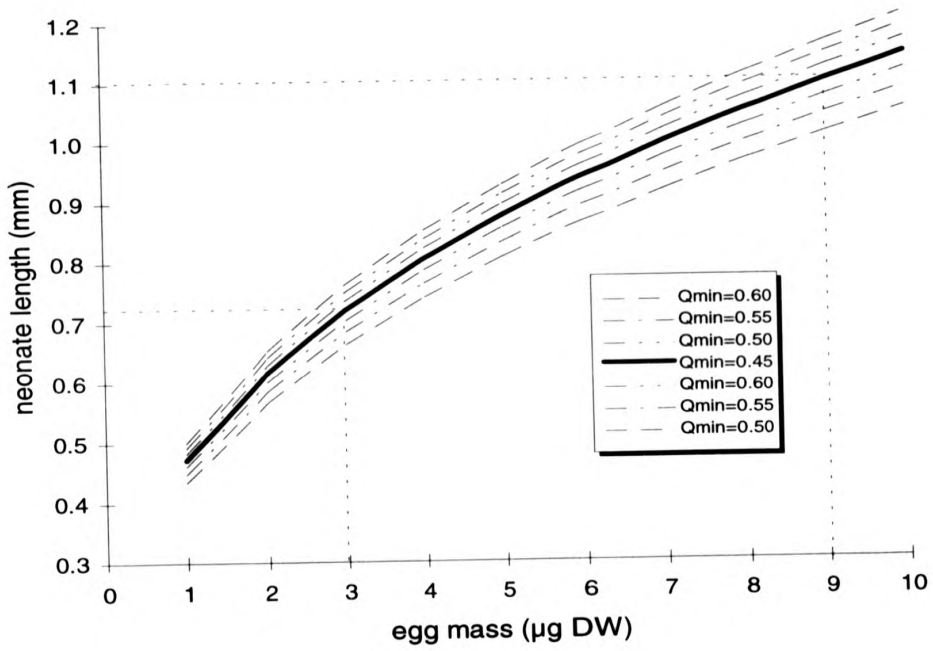


Figure 3.12 - Theoretical derivation of neonate length from egg mass for different values of Q_{min}. The solid line correspond to the value of Q_{min} used in the model.

3.3. MODEL FORMULATION

3.3.1. Structural formulation

The physiological model of an individual *Daphnia*, presented here, uses two primary compartments: Structure (cellular building blocks) and Reserves (resources not included as building blocks, but used as metabolic fuel). Functional responses outlined in previous sections are assembled under a dynamic structure (Figure 3.13) where mass flows are subject to feedback control. The parameterisation of the model (Table 3.3) was based partly on published data and partly on experimental work described in this thesis (Chapter 4).

Some processes not occur continuously (medium renewal, food addition to the medium, moulting). Therefore, it is necessary to detect changes in state of these phenomena using switching functions to determine precisely when the shift in state occurred. Key switching states (moulting and consequently reproduction) are a dynamic function of the nutritional state of the individual while the others (medium renewal and addition of food to the medium) occur at fixed time intervals.

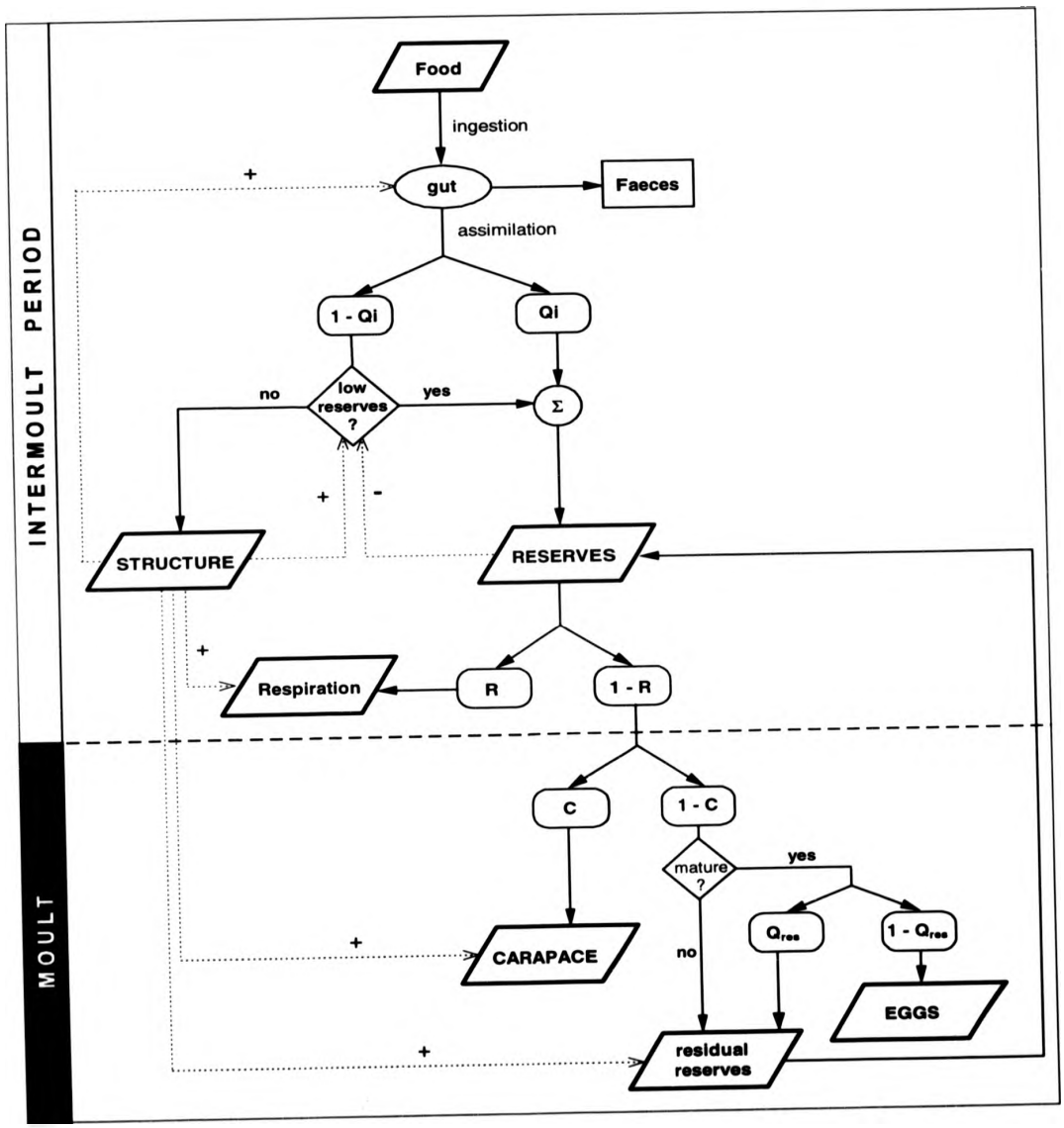


Figure 3.13 - Mass flow in *Daphnia*. Slanted rectangles - static and dynamic sinks, rectangles with rounded corners - partitioning factors, solid arrows - flow paths, dotted arrows - feedback effects.

Table 3.3 - *Daphnia magna* parameters for the model described in the text. Mass is always expressed as dry weight.

Parameter	Value	Units	Description	Page	Sources
Volume	-	ml	Volume of culture medium		experiments
Ration	-	$\mu g\ ml^{-1}$	Ration level		experiments
Feed	-	d^{-1}	Frequency of food replenishment		experiments
Transfer	-	d^{-1}	Frequency of medium renewal		experiments
Length	-	mm	Initial body length (*)		experiments
Mass	-	μg	Initial body mass (*i.e.* mass of an egg)		experiments
Tox	-	$\mu g\ l^{-1}$	Toxicant concentration		experiments
α	5.00	$\mu g\ mm^{-\beta}$	Coefficient of the length-structure relationship	86	(a)
β	2.60	none	Index of the length-structure relationship	82	(b)
δ	2.50	none	Index of the length-feeding rate relationship	60	(a), (c)
ε	1.50	none	Index of the mass-partition function	86	estimated
η	33.00	$\mu g\ mm^{-\beta}\ d^{-1}$	Coefficient of the length-feeding relationship	60	(d)
κ	1.55	none	Allosteric index of the toxicant depression function	64	(d)
σ	1.50	$\mu g\ mm^{-\beta}$	Coefficient of the length-carapace mass relationship	82	(a)
L_d	3.40	mm	Length-dependent feeding depression factor	61	(a)
L_m	2.30	mm	Length threshold for maturation	69	(a), (e)
F_h	3.00	$mg\ ml^{-1}$	Half saturation factor of food	62	(a)
T_h	2.63	$mg\ l^{-1}$	Toxicant concentration inducing 50% depression in feeding	64	(d)
M_r	0.08	none	Specific standard respiration rate	77	(f), (g)
M_f	0.14	none	Maximum SDA of food	75	(f), (h)
M_t	0.02	none	Maximum SDA of toxicant	76	(i)
A_{min}	0.45	none	Minimum food assimilation efficiency	66	(j)
A_{max}	0.95	none	Maximum food assimilation efficiency	66	(j)
Q_{min}	0.45	none	Minimum fraction of resources allocated into reserves	69	(k)
Q_{max}	0.85	none	Maximum fraction of resources allocated into reserves	69	(k)
Q_{res}	0.50	none	Proportion of residual reserves retained to maintain structure under stress conditions	90	estimated
t_s	7.00	d	Settling time for half of the food particles	58	(l)
E_{min}	3.00	μg	Average mass of an egg (minimum value)	90	(l)
T_{min}	1.00	d	Minimum instar duration	85	(l)
T_{coef}	0.06	$d\ mm^{-\beta}$	Coefficient of instar-length relationship	85	(l)
T_{delay}	0.25	d	Delay factor of feeding in instar duration	85	(l)
t_e	2.50	d	Average embryonic developmental time	92	(m)

(*) calculated from egg mass; (a) Waddell (1993); (b) Burns (1969); (c) Lynch *et al.* (1986); (d) Allen *et al.*, (1995); (e) Kooijman (1986a); (f) Perrin *et al.* (1992); (g) Glazier (1991); (h) Lampert (1986); (i) Barber *et al.* (1994); (j) Lampert (1987); (k) Yampolsky & Ebert (1994); Lynch, 1980; (l) This work; (m) Goulden *et al.* (1987)

3.3.2. Mathematical formulation

The model was implemented on an IBM-PC compatible using a modified version SOLVER 4.1 (Bence *et al.*, 1986) running under Turbo-Pascal 7.0 (O'Brien & Nameroff, 1992). SOLVER is a package implementing the fourth-order Runge-Kutta method for solving sets of first order non-linear differential equations (Press *et al.*, 1986). This "self-starting" method uses estimates of first derivatives for times greater than those at which the state variables were last estimated, making it easier to manipulate situations where some of the state variables must be reset. *Daphnia* cultures are systems where occurrence processes, like medium renewal, feeding and moulting affect the values of some state variables.

SOLVER uses a non-linear interpolation algorithm to detect at which time the switching of state in a process occurred. However, the method originally implemented was sometimes unable to find the switching point, causing the program to halt. On further investigation, the time step used in the integration and the scale of the values, were found to be critical to the process. For this reason, I implemented a new algorithm in SOLVER, based on the cubic interpolation procedure (Press *et al.*, 1986), making the detection of switching points more robust and stable. The new algorithm was tested using systems of equations similar to the ones used in the model for which exact analytical solutions were known. Observed solutions were found to yield values correct to better than 10 significant figures.

Turbo-Pascal does not have a large library of mathematical functions. Therefore I developed and included in SOLVER a library with the most common mathematical

functions commonly used in modelling (*e.g.* power functions, trigonometric functions). SOLVER was also subjected to some minor customisations of the user interface (assigning parameter descriptors) and the structure of the program (addition of the ability to internally stop the program when some predefined condition is met).

SOLVER and the *Daphnia* model itself uses DOUBLE precision values for calculations, which increase the accuracy of the system, especially with regard to calculations involving very low values. Modelling under SOLVER involves the creation of three basic types of files related to: global constants and variables, definitions of differential equations and related functions, and definition of switching functions and reset procedures.

The model (summarised in Table 3.4) is a mass model incorporating length-dependent relationships. The individual is regarded as consisting of two compartments (structure and reserves) whose dynamics are defined coupled differential equations. Two of the most important processes (moulting and clutch provisioning) are a dynamic result of the integration of previous feeding conditions.

Table 3.4 - Summary specification of the model (functions and variables are shown in italic, while parameters appear in plain text).

	Referred in page
Start-up conditions	
Volume	96
Ration	96
Transfer	96
Feed	96
Length	96
Mass	96
Tox	96
Initial state	
$Food = \text{Ration}$	58
$F_{cum} = 0$	79
$Inst = 0$	79
$t_f = 0$	58
$L = \beta \sqrt{Mass \frac{(1 - Q_{\min})}{\alpha (1 - Q_{\min} Q_{\text{res}})}}$	92
$Structure = \alpha L^{\beta}$	86
$Reserves = Mass - Structure$	86
During embryonic development (time<=t_e)	
$T_d = \frac{T_h^{\kappa}}{T_h^{\kappa} + Tox^{\kappa}}$	64
$R = \left[M_r + M_t (1 - T_d) \right] (Structure + Reserves)$	77
$\frac{dStructure}{dt} = 0$	92
$\frac{dReserves}{dt} = -R$	92

(continued on next page)

Table 3.4 (continued)

After embryonic development (time>t_e) — **Referred in page**

At feeding (controlled through a fixed-step switch set-up at start-up)

$$\frac{dFood}{dt} = \text{Ration} \qquad 96$$

$$t_f = time \qquad 96$$

At transfer (controlled through a fixed-step switch set-up at start-up)

$$Food = \text{Ration} \qquad 96$$

$$t_f = time \qquad 96$$

At Moult time (controlled through a continuously updated dynamic switch)

$$C = \sigma L^{\beta} \qquad 82$$

$$\frac{dReserves}{dt} = -C \qquad 83$$

$$L = \sqrt[\beta]{\frac{Structure}{\alpha}} \qquad 92$$

$$QrL = Q_{min} + (Q_{max} - Q_{min}) \left(\frac{L^{\beta}}{L^{\beta} + L_m^{\beta}} \right) \qquad 69$$

$$F_{max} = \eta L^{\delta} \left(\frac{L_d^2}{L_d^2 + L^2} \right) \qquad 61$$

$$T_d = \frac{T_h^{\kappa}}{T_h^{\kappa} + Tox^{\kappa}} \qquad 64$$

When ($\mathbf{L > L_m}$) (mature individuals)

$$E_m = E_{min} + 2\, E_{min}\, RFH \qquad 89$$

$$Brood = \text{Integer} \left\{ \frac{Reserves - C - Q_{res}\, Structure}{E_m} \right\} \qquad 90$$

$$\frac{dReserves}{dt} = -E_m\, Brood \qquad 90$$

$$F_{cum} = 0 \qquad 79$$

$$Inst = 0 \qquad 79$$

(continued on next page)

Table 3.4 (continued)

Between moults	**Referred in page**
$RFH = \left(\frac{F_{\max} - \left(\frac{F_{cum}}{Inst} \right)}{F_{\max}} \right)$	79
$Tm = \mathrm{T_{min}} + \mathrm{T_{coef}}\ L^{\beta} + \mathrm{T_{delay}}\ Inst\ RFH$	85
$F_{avail} = Food \frac{\mathrm{t_s}}{\mathrm{t_s} + (time - \mathrm{t_f})}$	58
$F_d = \frac{F_{avail}}{\mathrm{F_h} + F_{avail}}$	58
$Intake = F_{\max}\ T_d\ F_d$	64
$A = Intake \left[\mathrm{A_{min}} + (\mathrm{A_{max}} + \mathrm{A_{min}})\ (1 - F_d) \right]$	66
$R = \left[\mathrm{M_r} + \mathrm{M_f}\ F_d\ T_d + \mathrm{M_t}\ (1 - T_d) \right] (Structure + Reserves)$	77
$Qi = \frac{Reserves\ (1 - QrL)}{Structure\ QrL}$	69
$\frac{dFood}{dt} = -\frac{Intake}{\mathrm{Volume}}$	-
$\frac{dF_{cum}}{dt} = Intake$	-
$\frac{dStructure}{dt} = A\ (1 - QrL)\ Qi^{\varepsilon}$	86
$\frac{dReserves}{dt} = A - R - \frac{dStructure}{dt}$	87
$\frac{dInst}{dt} = 1$	-

3.4. CONCLUSIONS

The model outlined here is a dynamic physiological model of an individual *Daphnia* that could be easily adapted to other organisms with minor adjustments. My goal was to establish a dynamic model of an individual consistent with the physiological essence of the processes involved, yet powerful enough to gain insight on physiological interactions. Key processes for which there was not enough information available in the literature (*e.g.* time for moult, allocation rules, effects of toxicants), had to be modelled, and thus experimental protocols were established to parameterise and validate them (see next chapters). The model, like other existing models, deals with an average egg size, without considering the egg size distribution of each clutch. For the individual level this approach is close enough to be appropriate, although a future development could give valuable information for analysing life-history strategies. The embryonic development approach needs to be tested and improved to find a valid general rule.

4. FOOD DEPRIVATION EXPERIMENTS

"An experiment is a device to make nature speak intelligibly"

George Wald (1967), Nobel Lecture

4.1. INTRODUCTION

Information available in the literature involving precise measurements of instar duration under variable environmental conditions is scarce. Moreover, resource allocation priorities (Bradley *et al.*, 1991b; Glazier, 1992; Glazier & Calow, 1992) and reproduction strategies (Bradley *et al.*, 1991a; Perrin *et al.*, 1990) derived from experiments using pulsed food regimes are not biologically consistent.

Moulting is perhaps the most important process for crustaceans. Moulting allows body length to increase whilst allowing the release of eggs into the brood pouch. Furthermore, if an individual cannot moult, it will potentially die, compromising its fitness. The resources to build a carapace are obtained from the reserves compartment since an individual can moult even in the absence of food. It is also well documented that the provisioning of the eggs occurs a few hours before moulting, when lipid reserves accumulate inside the ovaries (McCauley *et al.*, 1990; Tessier and Goulden, 1982). It has also been shown that individuals deprived of food for increasing periods of time produce a decreasing number of eggs. Moreover, an individual only grows if it is fed (Bradley *et al.*, 1991b; this work).

The aims of this chapter are to present and discuss results from experimental work carried out to:

- generate precise data on moulting intervals to use for model parameterisation;
- validate the general allocation model presented in the previous chapter, and its influence in determining growth, fecundity, maturation, and survival.

These objectives will be achieved using pulsed food deprivation experiments at different points of the life cycle of individual *Daphnia* using an approach similar to that of other authors (*e.g.* Perrin *et al.*, 1990; Bradley *et al.*, 1991a, b). These experiments allow us to test hypotheses concerning allocation priorities between growth, reproduction and survival. It will also test whether growth and reproduction recover simultaneously from food deprivation. Enhanced feeding is known to occur when individuals are moved from starvation into food conditions probably biasing the results. However, the feeding rate gradually resumes standard levels within a few hours (Ringelberg, & Royackers, 1985; Kremer & Kremer, 1988).

The experimental design presented in page 122 will be used to test the following hypothesis:

I) instar duration is expected to vary geometrically with the body length of the individual since both feeding and body mass scale geometrically with body length and the instar duration is associated with increase in body mass;

II) instar duration is expected to increases proportionally with the duration of the food deprivation period, since it will take longer to increase in mass;

III) growth in length is expected to be reduced proportionally with the duration of the food deprivation period since it depends upon increase in structure (directly associated with body mass);

IV) the biochemical composition of an individual is a good indicator of recent feeding conditions since individuals use biochemical components at different rates.

In addition to these hypotheses, for each experiment some specific hypotheses concerning age at maturity and size-fecundity relationships will be tested.

4.2. MATERIALS AND METHODS

4.2.1. *Daphnia* culture

Daphnia stock cultures and experiments were maintained in an artificial medium, ASTM hard water (ASTM, 1980). The culture medium was prepared with nanopure distilled water with conductivity values < 0.055 µS/cm of conductivity and Analar grade chemicals. Nanopure distilled water was produced coupling a water distiller (GFL-2002, GFL, Germany) with a batch of ionic resin filters (Seralpur Pro 90 CN, Seral, Germany). After dilution of the chemicals (Table 4.1), and before use, the medium was left standing overnight.

The culture medium was enriched with a seaweed-based organic additive (Table 4.2; Baird *et al.*, 1989b), supplied by Glenside Organics Ltd. Seaweed extract, or Marinure, is a water soluble concentrate of the seaweed *Ascophyllum nodosum*. This extract can provide a source of trace elements for *Daphnia*, such elements being essential for the long-term maintenance of *Daphnia* in the laboratory (Baird *et al.*, 1989b).

Table 4.1 - Chemical composition of artificial freshwater used in Daphnia culture (ASTM hard water) and physical properties

Chemical	Amount required (mg/l)
$NaHCO_3$	192
$CaSO_4.2H_2O$	120
$MgSO_4$	120
KCl	8
pH	7.6-8.0
hardness (as mg/l of $CaCO_3$)	160-180

Table 4.2 - Typical composition of seaweed-extract in dry powder form, and in the solutions used for *Daphnia* culture: (a) organic and inorganic fractions, and (b) chemical composition.

(a)

Fraction	Minimum (%) (*)	Maximum (%) (*)	Average (%)	Dry Weight (%)	Stock (µg/ml) (**)	Added to culture (µg/ml)
Dry matter	92	95	93.5	-	6524.5	65.25
Organic matter	50	55	52.5	56.2	3663.5	36.63
Inorganic matter	40	45	42.5	45.5	2965.7	29.66

(b)

Compound	Average composition (% DW) (*)	Stock composition (µg/ml) (**)	Added to culture (µg/l) (**)
Chlorine	3.2086%	209.343	2093.43
Sulphur	2.8877%	188.409	1884.09
Potassium	2.6738%	174.452	1744.52
Nitrogen	1.4973%	97.693	976.93
Calcium	1.2834%	83.737	837.37
Magnesium	0.8556%	55.825	558.25
Phosphorus	0.0535%	3.489	34.89
Iodine	0.1925%	12.561	125.61
Iron	0.1604%	10.467	104.67
Boron	0.0118%	0.768	7.68
Zinc	0.0107%	0.698	6.98
Manganese	0.0014%	0.091	0.91
Aluminium	0.0005%	0.035	0.35
Nickel	0.0005%	0.035	0.35
Copper	0.0003%	0.021	0.21
Cobalt	0.0002%	0.011	0.11
Vanadium	0.0001%	0.005	0.05
Growth stimulants	0.0209%	1.361	13.61

(*) values supplied by Glenside Organics Ltd, UK.
(**) concentration of solutions based on the relative contribution of each element, as supplied by Glenside Organics Ltd, and the total amount of dry of matter used in the preparation of the stock solution.

The absorbance spectrum of the seaweed extract stock solution, originally used at Sheffield University, was determined to select the best wavelength to quantify the relative concentration of stock solutions (Figure 4.1). The absorbance value of 0.62 at 400 nm of the stock solution at one tenth dilution, measured with a visible spectrophotometer (Jenway 6100, Jenway Ltd, UK), was used as a benchmark to ensure good reproducibility in the preparation of new seaweed extract stock solutions. The final solution, after mixture of the seaweed extract with the ASTM hard water, will be hereinafter referred as "enriched ASTM hard water", and was used for all *Daphnia* cultures. Three types of cultures were used (Figure 4.2):

- long-term bulk cultures at 10 °C
- long-term bulk cultures at 20 °C
- individual cultures at 20 °C.

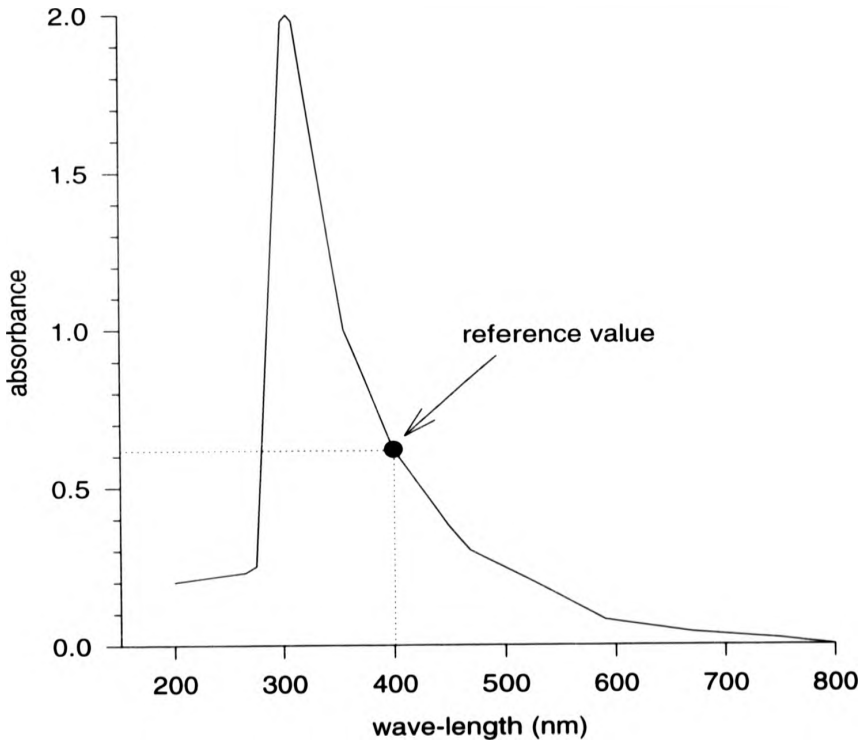


Figure 4.1 - Absorbance spectrum of the seaweed-extract stock solution at one tenth dilution. The benchmark value is the absorbance at 400 nm (*i.e.* approximately 0.62).

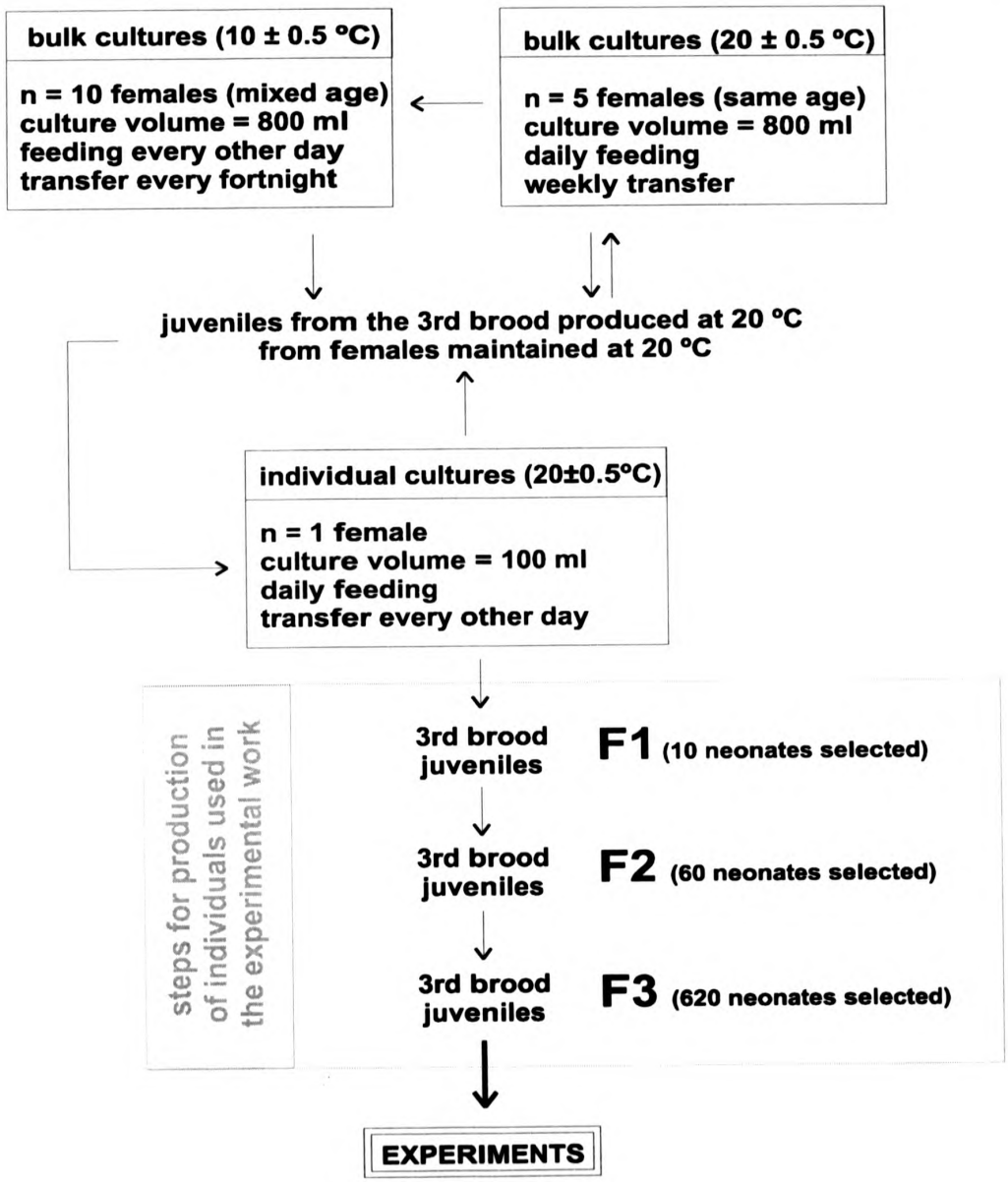


Figure 4.2 - Long term cultures of *Daphnia* for experimental use. F1, F2, and F3 refer to the 1st, 2nd and 3rd generation, respectively, used to select sufficient neonates with synchronous birth as experimental individuals.

a) Long-term bulk cultures at 10 °C

The clone used in this study was maintained at 10 °C in bulk cultures. These cultures were initiated with 10 individual females, placed in 800 ml of enriched ASTM hardwater in 1000 ml glass covered beakers: three adult females with eggs present in the brood pouch, four adolescents, and four neonates. This mixed age group was fed every other day with a ration of 6.5 x 10^5 cells/ml of the green alga *Chlorella vulgaris* Beijerinck, and kept under a photoperiod of 14:10 (light:dark). These conditions are known to prevent the occurrence of males and ephippia, thus ensuring continuous supply of parthenogeneticaly produced offspring (Banta & Brown, 1929; Banta, 1939; Slobodkin, 1954; Stross & Hill, 1965; Berge, 1978; Carvalho & Hughes, 1983; Doma, 1979). The *Daphnia* medium was completely replaced every 15 days and the culture restarted with the same age structure.

b) Long-term bulk cultures at 20 °C

Since all experiments were carried out at 20 °C, individuals were also kept in bulk cultures at 20 °C, with a photoperiod of 14:10 (light:dark). These cultures were routinely established using the third or later clutch of offspring from females reared at 20 °C. However, if the individual cultures deteriorated, *e.g.* due to fungal infections, bulk cultures at 20 °C were restarted by transferring an egg-bearing female from 10 °C into a 175 ml screw top jar with 100 ml of culture enriched ASTM hardwater at 20 °C, receiving the usual treatment for individual cultures. The first two clutches released by the female after transfer were discarded to allow acclimation to the new temperature before neonates were used in experiments since a temperature shock is known often to

cause production of males. The offspring from the third clutch after transfer were then used to restart the bulk culture at 20 °C.

Neonates from the subsequent generation were then used to restart the bulk culture at 20 °C by transferring them into 1000 ml glass covered beakers with 800 ml of medium. The culture was reduced to 5-6 daphnids with synchronous reproduction when the first eggs were observed in the brood pouch of the females. Neonates were removed from the culture within one day after release from the brood pouch. Cultures were re-established after the release of 5-6 broods. Cultures were fed daily 6.5 x 10^5 cells/ml of *C. vulgaris*, and the medium renewed weekly. This ration level is equivalent to 7 µg DW ml^{-1} d^{-1} (see Physical characterisation of *C. vulgaris*, page 121).

c) Individual cultures at 20 °C

The use of bulk cultures of *Daphnia* for experimental purposes has been popular in the past (*e.g.* Tunstall & Solinas, 1977; Berge, 1978; APHA, 1980; Dave *et al.*, 1981; Barera & Adams, 1983; Frieser *et al.*, 1986). However, this methodology has some disadvantages (see Baird *et al.*, 1989b) if one needs to produce individuals of consistent quality and performance. So, to minimise possible sources of variation due to culturing methods, culturing *Daphnia* individually has become more common in both physiological (e.g. Ebert, 1992, 1993, 1994a) and ecotoxicological experimental work (*e.g.* Enserink *et al.*, 1995).

4.2.2. Algal culture

Cultures of *D. magna* have been successfully maintained by feeding a wide range of algal species (*e.g. Chlamydomonas reinhardtii* Dang., *Ankistrodesmus falcatus* Corda emend. Ralfs, *C. vulgaris*). The green alga *C. vulgaris* is one of the recommended diets for maintaining *Daphnia* cultures (AFNOR, 1974; EC, 1986; ISO, 1982; OECD, 1981). Moreover, despite some arguments against the use of this algae, *e.g.* production of chlorellin (McMahon & Rigler, 1965; Pratt & Fong, 1940; Pratt *et al.*, 1945; Ryther, 1954; Taub & Dollar, 1968), *Chlorella* species have long been widely used as the sole (*e.g.* Ringelberg & Royackers, 1985; Baird *et al.*, 1989a, b, 1991a, b; Bradley *et al.*, 1991a, b; Barber *et al.*, 1994; Allen *et al.*, 1995; Bodar *et al.*, 1988; Cox *et al.*, 1992; Naylor *et al.*, 1992; Schlinder, 1968; Soares *et al.*, 1992; Perrin *et al.*, 1990; Stuhlbacher *et al.*, 1993) or the primary food source (*e.g.* Stephenson & Watts, 1984) in *Daphnia* studies.

C. vulgaris has been proved to be a suitable diet to satisfactorily sustain (*i.e.* maximise fecundity without compromising long-term viability) stock cultures of *D. magna* (see Soares, 1989). All our cultures were fed with *C. vulgaris* grown axenically by semi-continous batch cultures. The culture medium chosen to grow *C. vulgaris* was Woods Hole MBL (pH=7.2) (Stein, 1973) (Table 4.3). All glassware and media were autoclaved at 120 °C and 124.1 kPa for one hour using an AJC autoclave (AJC, Portugal), and all algal culture procedures were carried out under sterile conditions.

Table 4.3 - Woods Hole MBL (pH=7.2) culture medium for *C. vulgaris*. the macro- and micro-nutrients are prepared as 1000-fold stock solutions, which are stored at 4ºC. Vitamin stocks are membrane filtered and stored frozen. The medium is prepared by consecutive addition of a, b, c and d to nanopure distilled water.

a. Macronutrients (use 1 ml per each litre of medium)	
Chemical	g/l
$CaCl_2.2H_2O$	36.76
$MgSO_4.7H_2O$	36.97
$NaHCO_3$	12.60
K2HPO4	8.71
$NaNO_3$	85.01
$NaSiO_3.9H_2O$	28.42
b. Micronutrients (use 1 ml per each litre of medium)	
Chemical	g/l
$Na_2.EDTA$	4.360
$FeCl_3.6H_2O$	3.150
$CuSO_4.5H_2O$	0.010
$ZnSO_4.7H_2O$	0.022
$CoCl_2.6H_2O$	0.010
$MnCl_2.4H_2O$	0.180
$NaMo_4.2H_2O$	0.006
c. Vitamins (concentration in the culture medium)	
Thiamine.HCl	0.1 mg/l
Biotin	0.5 µg/l
Cyanocobalamine	0.5 µg/l
d. Tris (use 2 ml per each litre of medium)	
Tris (hydroximethyl)-aminomethane	250 g/l
Adjust pH to 7.2 with HCl	

The concentration of the algal cells used to feed *Daphnia* was calculated from the nomograph on Figure 4.3 where the absorbance at 440 nm (which is the absorbance wave length of chlorophyll-a, hereinafter referred to as A_{440}) of the concentrated stock of suspended algal cells, diluted to one tenth, was plotted versus the concentration of cells in the stock. The calibration was performed using independent measurements, carried out by two persons, and using two methods of cell counting: the haemocytometer and the Coulter Counter (Coulter Counter TAII, Coulter Electronics, UK).

a) Maintenance and inoculation of slopes

Algae were maintained on slopes of proteose agar (2%) (Table 4.4) at room temperature. New slopes were prepared every six months. To obtain single colonies of algae, a number of agar (proteose-peptone agar 2%) plates were inoculated from the slope. These plates were incubated under continuous light (2000 lux) at 25 °C. New plates were routinely cultured every three to four weeks.

b) Production of the inoculum

Once a week, five 250 ml Erlenmeyer flasks, containing 100 ml of Woods Hole MBL, were inoculated with algae taken from the plates with a flamed platinum loop. The flasks, stoppered with non-absorbent cotton wool, were placed into an orbital incubator and shaken at 120 rpm at a temperature of 25 °C and under continuous light (small batch cultures).

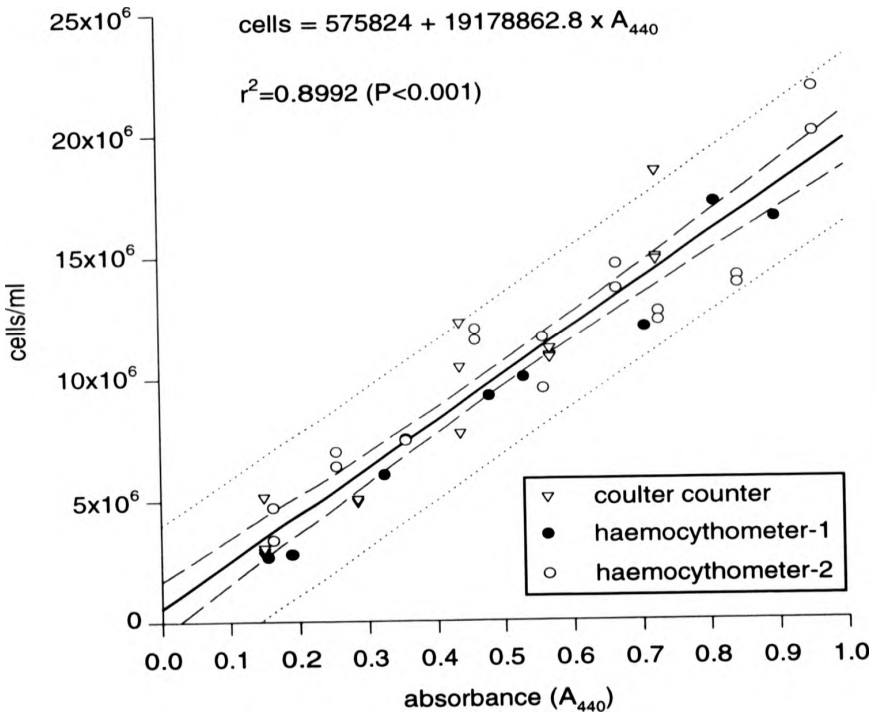


Figure 4.3 - Relationship between the concentration of *C. vulgaris* cells and the absorbance at 440 nm of a one-tenth diluted sample (A_{440}) (solid line is the regression line while dashed and dotted lines are the 95% confidence and prediction levels respectively). Points correspond to independent measurements involving two independent operators and two independent methods of cell counting.

Table 4.4 - Proteose-peptone agar medium for algal culture (solutions prepared with nanopure distilled water)

Chemical	g / l of water
Proteose peptone	1.00
KNO_3	0.20
K_2HPO_4	0.02
$MgSO_4.7H_2O$	0.02
Agar	10.00

After three to five days the A_{440} of the inoculum was measured by withdrawing 1 ml of algal suspension. Simultaneously, the purity of the inoculum was checked by viewing the algal suspension under a high power microscope.

c) Inoculation of the fermenter and culturing conditions

The large scale semi-continous culture of *C. vulgaris* was carried out in 10 litre fermenters, containing 6 litres of Woods Hole MBL (Figure 4.4). To start a culture, the fermenter was inoculated with approximately 100 ml of inoculum (see above). The cultures were maintained at 20 ± 2 °C, under continuous light conditions (2000 lux). The media was aerated with clean air using an air pump (Maxima 4W, Weltweit, Germany) at a rate of 2 - 4 litres of air per minute.

Approximately 6 days after inoculation, the culture was fully established (*i.e.* the medium was dark green), and a sample of approximately 100 ml was removed and the absorbance at 440 nm checked to be between 0.6 to 0.8. Cultures were then harvested every other day in order to maintain them below the maximum standing crop and thus in an exponential growth phase to ensure a healthy, constant, and consistent food supply. Usually 3 l of culture were removed, replaced with equal volume of sterile culture medium, and used to prepare food.

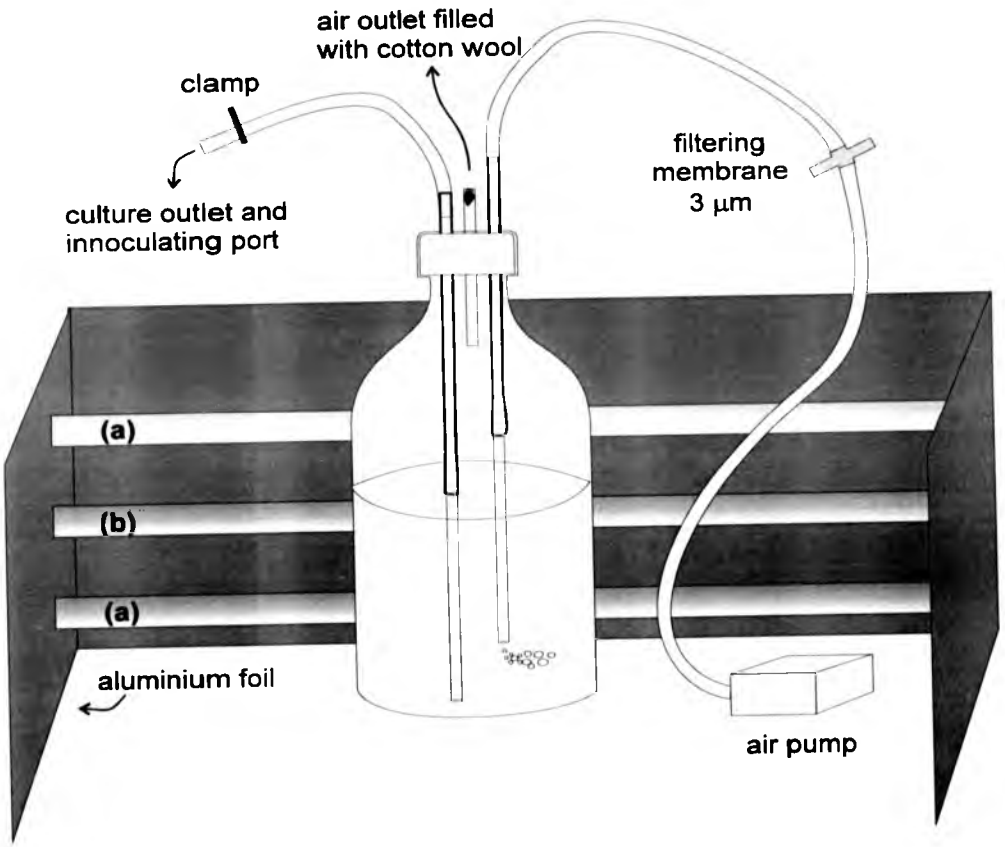


Figure 4.4 -Semi-continous culture system of *C. vulgaris*. (a) white and (b) red fluorescent light tubes: Osram L36W/10 and Osram TLD 36W/15, respectively.

The cultures were harvested a maximum of five times to avoid cell ageing effects, changes in the quality of algal cells (Baird *et al.*, 1989a; Pratt *et al.*, 1945; Stein, 1973) and possible infections. Occasionally it was noticed that cultures were contaminated with bacteria (this was easily detected when cultures appeared cloudy, foamy or yellowish coloured): these were discarded. A minimum of four cultures of different ages was maintained at any time in order to prevent food shortage.

d) Preparation of daphnid food

The culture medium and algae removed from the fermenter were spun down in a bench centrifuge (Sigma 3K10, Sigma, Germany) at 4000 rpm for 5 minutes. The supernatant was poured off and the pellets of algal cells resuspended in ASTM hard water until the A_{440} (*i.e.* absorbance of the stock diluted to one tenth) was between 0.6 and 0.8. The exact concentration of the food stock was determined using the nomograph from Figure 4.3. The algal stock was maintained at 10 °C and used to feed the daphnids. Stocks of *C. vulgaris* were discarded after 3 days. Thus, only stocks less than 3 days old were used for maintaining stocks and experimental individuals (*i.e.* high quality cells with consistent composition).

e) Physical characterisation of *C. vulgaris*

C. vulgaris is a non-motile algal species, existing as spherical cells with an average diameter of 4.29 ± 0.09 µm and weight of 11.47 ± 0.16 µg DW (mean ± 95% confidence limits). The relative chemical composition of *C. vulgaris* (*i.e.* carbon, hydrogen and nitrogen content) relative to dry weight was determined using a CHN

analyser (Perkin Elmer Series II CHN S/O Analyzer 2400, Perkin Elmer, USA) (Figure 4.5).

Similarly, the biochemical composition of algal cells of *C. vulgaris* (*i.e.* proteins, lipids, and carbohydrates content) relative to dry weight, and corrected to 100%, was determined using the biochemical methods described elsewhere in following sections (Figure 4.6). The relative carbon content of *C. vulgaris* of 40.2% was very similar to the value of 44.1% measured by others (*e.g.* Ahlgren *et al.*, 1992), although I observed a higher protein and lipid content than quoted by these authors. The carbon content of 0.127 pg C/μm^3 observed is also in close agreement with values of 0.1 pg C/μm^3 already reported (Ketchum & Redfield, 1949 *in* Rocha & Duncan, 1985) for cells of comparable size. Nonetheless, it is lower than the 0.27 pg C/μm^3 reported for small cells (Chalk, 1981 *in* Rocha & Duncan, 1985).

4.2.3. Experimental design

Two experimental approaches were used to test the effects of variable periods of food deprivation, at different point of the life cycle. The 3rd instar was chosen as representative of the adolescent instars whilst the 1st adult instar was used as representative of the adult instars.

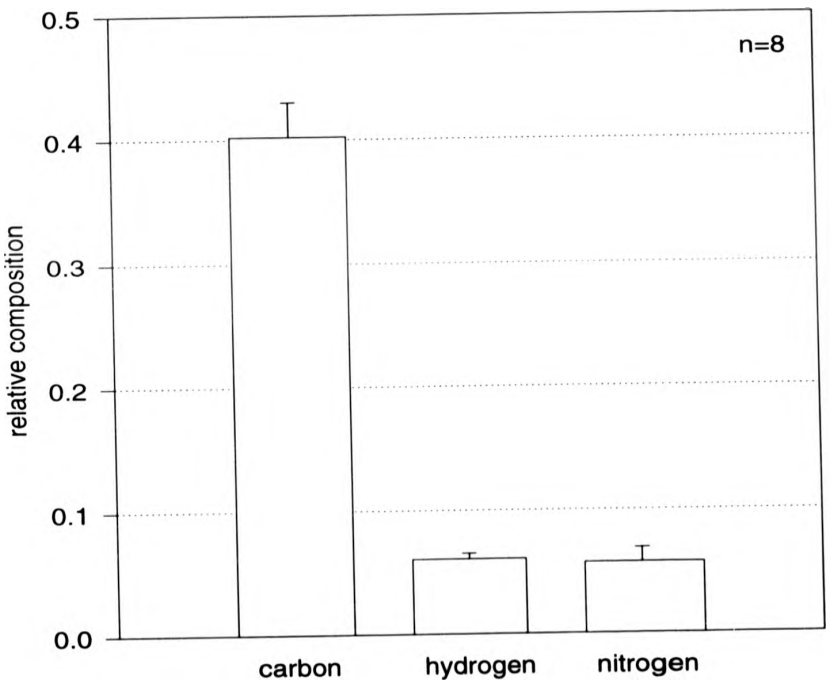


Figure 4.5 - Relative chemical composition of *C. vulgaris* cells: carbon, hydrogen, and nitrogen (error bars are 95% confidence limits of the means of eight values).

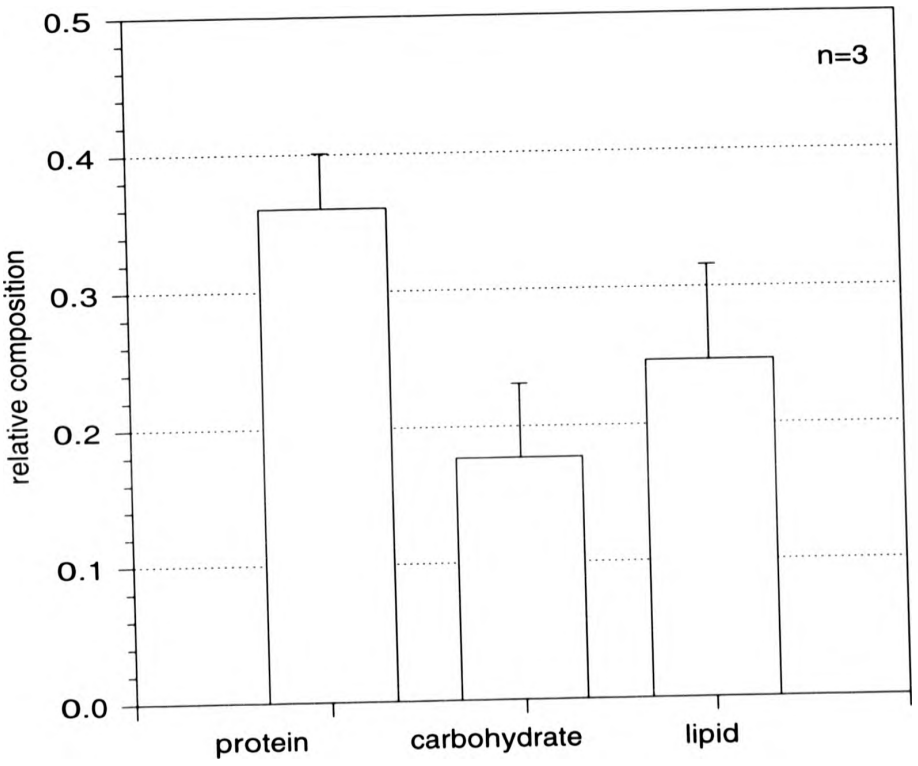


Figure 4.6 - Biochemical composition of algal cells: proteins, carbohydrates and lipids relative to dry weight (error bars are 95% confidence limits of the means of three samples).

a) Response to food deprivation in an adolescent instar (third instar)

In this experiment, along with the common hypotheses, it was intended also to check if increasing periods of food deprivation during adolescent instars resulted in increased age at maturity. In this experiment, females released from the maternal brood pouch within 3 h of each other were cultured using the method outlined in Figure 4.2. These neonates were randomly assigned to one of three treatments: control (no food deprivation), 18 h of food deprivation, and one instar (approximately 36 h or more) of food deprivation according to a predefined experimental design (Figure 4.7). Samples of individuals selected randomly from each treatment were taken for biochemical analysis (Table 4.5).

Body length was determined for each instar in all individuals. Body length in *Daphnia* is usually measured as the distance between the anteriormost point of the head to the base of the spine. Since this procedure implies manipulation of animals and is potentially harmful, an indirect method of body length determination was used (see Soares, 1989). This method used the relationship between the length of the first exopodite of the second antennae and the body length of an individual calculated by Soares (1989) as:

$$body\ length = 11.04 \times exopodite\ length - 0.51 \quad (4.1)$$
$$(r^2 = 0.99, P < 0.001).$$

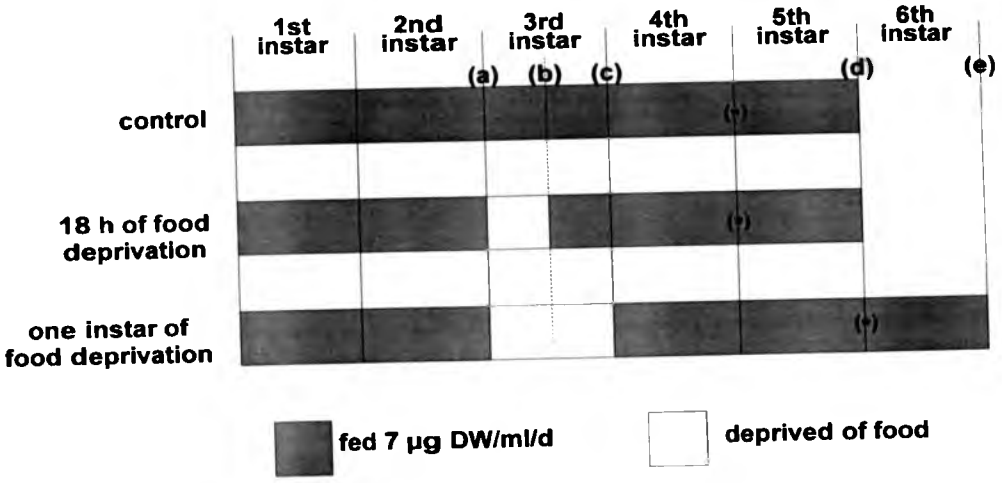


Figure 4.7 - Experimental design of food deprivation on the 3rd adolescent instar. The vertical solid lines represent the moulting process and (*) mark the release of the first clutch of eggs into the brood pouch.

Table 4.5 - Assignment of 610 neonates between treatments, and the samples collected, following the experimental design of Figure 4.7.

	number of individuals	Sampling periods (sample size)				
		(a)	(b)	(c)	(d)	(e)
Control	270	80	75	80	35	
18h of food deprivation	210		80	80	50	
one instar of food deprivation	130			80		50

Once it has hardened, the *Daphnia* carapace does not increase in length until next moult (Green, 1956). Thus, the length of the exopodite on the moult was measured under a binocular microscope with a precision of 0.0125 mm. The exopodite length was used to predict the body length in the instar that precedes the moult. Body length was thus estimated with a precision of 0.14 mm.

Individuals from all samples were measured and debrooded, and eggs counted whenever present. Egg-bearing females were debrooded by flushing water, through a micropipette, into the brood pouch, a method which does not injure the eggs or the mother (Glazier, 1991).

b) Response to food deprivation in the first adult instar

In this experiment I used individuals releasing their first clutch in the 5^{th}, 6^{th} and 7^{th} instar respectively. With this experiment, along with the common hypothesis, I intended to assess the effects of variable periods of food deprivation on the fecundity of reproducing egg-bearing adults. In this experiment I used 620 females released from the maternal brood pouch within 3 h of each other using the method outlined in Figure 4.2 (page 111).

Individuals were separated into three groups according to the instar where the first release of eggs into the brood pouch occurred (*i.e.* beginning of 1st adult instar). Within each group, individuals were randomly assigned to each of the treatments used, and fed accordingly (Figure 4.8, Figure 4.9, and Figure 4.10), while samples for biochemical analysis were taken from each treatment as outlined in Figure 4.8, Figure 4.9, and Figure 4.10. All individuals were measured (body length) and debrooded (except when mentioned otherwise) and the eggs counted whenever present. Egg-bearing females were debrooded using the method already referred in the previous section.

In this experiment I wanted to test the validity of the allocation model proposed in Chapter 3. According to the allocation model, fecundity should be reduced and ultimately cease with increasing food deprivation. However on return to normal feeding conditions it should resume the length-dependent relationship observed for the controls.

Clutches of eggs provisioned in the 2nd adult instar were checked for egg-size distribution differences between treatments and related to eventual body length differences between the mothers. For each treatment, samples of 10-12 females were debrooded, egg diameters measured, and the body length of the mothers recorded.

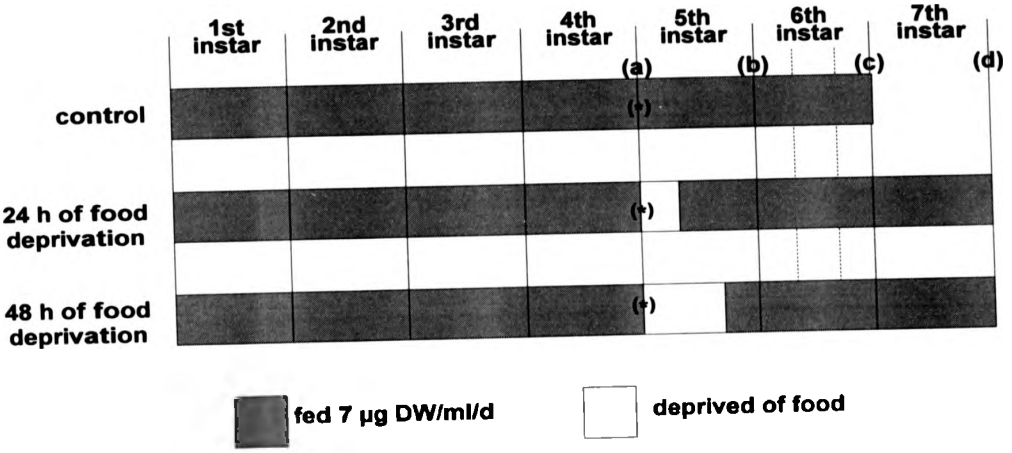


Figure 4.8 - Experimental design for individuals maturing in the fourth instar. The vertical solid lines represent the moulting process and (*) mark the release of the first clutch of eggs into the brood pouch.

Table 4.6 - Assignment of 114 neonates between the different treatments, and the samples collected, following the experimental design of Figure 4.8.

	number of individuals	Sampling periods (sample size)			
		(a)	(b)	(c)	(d)
Control	34	14	10	10	
24h of food deprivation	40		20	10	10
48h of food deprivation	40		20	10	10

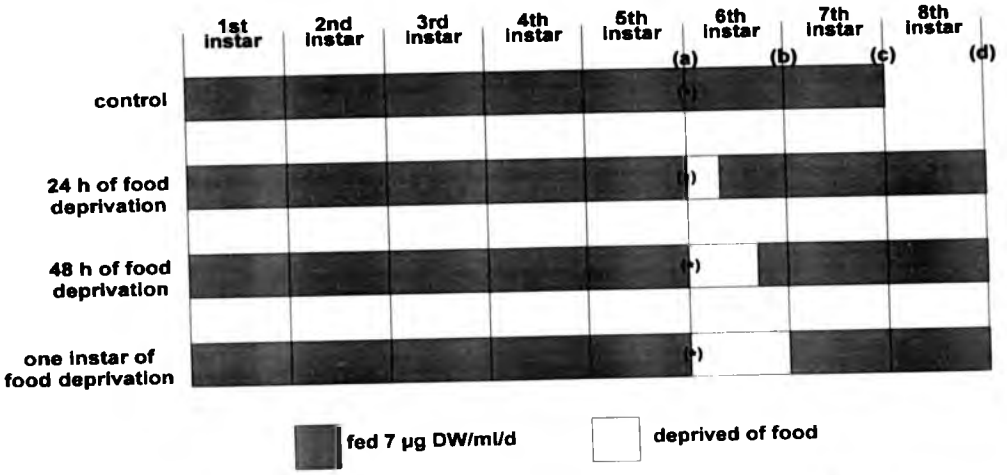


Figure 4.9 - Experimental design for individuals maturing in the fifth instar. The vertical solid lines of the graph represent the moulting process and (*) mark the release of the first clutch of eggs into the brood pouch.

Table 4.7 - Assignment of 377 neonates between the different treatments, and the samples collected, following the experimental design of Figure 4.9.

	number of individuals	Sampling periods (sample size)			
		(a)	(b)	(c)	(d)
Control	101	40 -	30 - 20 +	11 -	
24h of food deprivation	83		30 - 20 +	18 -	15 -
48h of food deprivation	90		40 -	30 -	20 -
one instar of food deprivation	103		45 -	38 -	20 -

- debrooded individuals + individuals carrying eggs

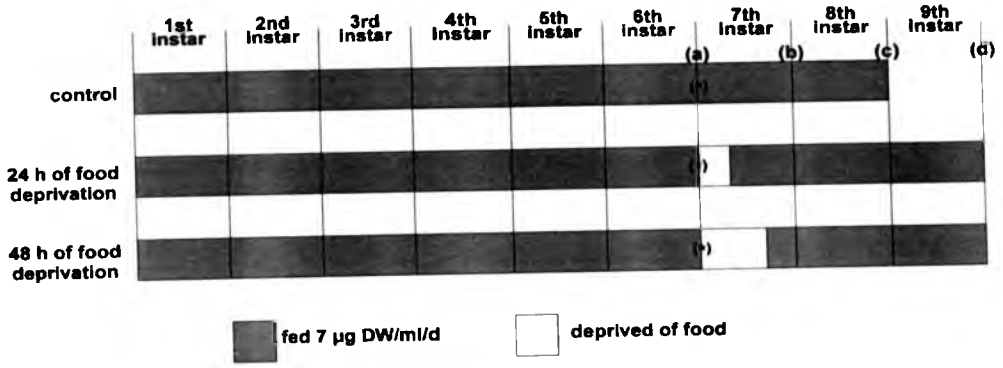


Figure 4.10 - Experimental design for individuals maturing in the sixth instar. The vertical solid lines represent the moulting process and (*) mark the release of the first clutch of eggs into the brood pouch.

Table 4.8 - Assignment of 114 neonates between the different treatments, and the samples collected, following the experimental design of Figure 4.10.

	number of individuals	Sampling periods (sample size)			
		(a)	(b)	(c)	(d)
Control	35	15	10	10	
24h of food deprivation	45		20	15	10
48h of food deprivation	45		20	15	10

4.2.4. Preparation of *Daphnia* samples

Samples of individuals collected during the experiments (Table 4.5, Table 4.6, Table 4.7, Table 4.8) were immediately frozen with liquid nitrogen inside a drop of distilled water in previously weighed aluminium boats kept inside capped Eppendorf tubes. Samples were stored individually in a deep freezer at -80°C until further processing.

All samples were freeze-dried later for 24 h at less than -50 °C and 40 mT of vacuum pressure on a freeze-dryer (FD-3-55D-MP, FTS Systems Inc, USA) coupled with a vacuum pump (TRIVAC D4A, Leybold Vacuum Products Inc, USA). Aluminium boats used to freeze the samples were weighed, prior to use, to the nearest 1 µg to measure the bulk dry weight of each sample on a microbalance (Mettler UMT2, Metler-Toledo AG, Switzerland).

4.2.5. Biochemical analyses

Several biochemical analyses were performed on each sample for protein, lipids and carbohydrate composition. All reagents used in the analysis were Analar grade chemicals. Micro-methods for each analysis were adapted from published methods and their sensitivity was evaluated before sample analysis.

Individual samples were homogenised in 1 ml of sodium chloride 0.15 M, at approximately 5000 rpm, using a previously washed electrical micro-homogeniser (X 1020, Ystral GmbH, Germany) equipped with a speed regulator. During

homogenisation the sample was kept on ice whilst the speed of the homogeniser was carefully controlled to avoid protein denaturation.

a) Protein

The method of Lowry *et al.* (1951) for protein analysis has been adapted for micro-analysis. An aliquot from each sample homogenate (0.1 ml) was mixed with 20 µl of 20% sodium dodecyl sulfate. An alkaline copper reagent was prepared by mixing the alkaline reagent (2% Na_2CO_3 in 0.1N NaOH) with a copper reagent (5% $CuSO_4$ in 1% sodium citrate), in a proportion of 50 to 1 respectively. The mixture was reacted with homogenate samples for about 20 minutes. Then, the Folin & Ciocalteu's phenol reagent (0.25 ml) was added and vigorously mixed. Color was allowed to develop for 30 minutes. All tubes were gently warmed in water, to avoid crystallisation of potassium dodecyl sulfate, and the absorbance was measured at 750 nm with a JENWAY spectrophotometer (6100, Jenway, Germany). Standards ranging from 0 to 200 µg of protein were prepared from a stock solution of 0.2% bovine serum albumin. Standards received the same treatments as samples.

b) Lipids

The lipid fraction of each sample was extracted using a modification of the Bligh & Dyer (1959) method. An aliquot of sample homogenate (0.5 ml) was mixed with 2.5 ml methanol in a centrifuge tube and extraction proceeded for 5 minutes. Following this, 2.5 ml of chloroform were added and the mixture shaken on a vortex mixer (ZX,

Uniequip, Germany) for 3 minutes, with the tube covered by Teflon tape. The mixture was then centrifuged for 5 minutes at 3000 rpm in a bench centrifuge to speed up the sedimentation of solid residues such as carapace fragments. The sediment was discarded and 1.25 ml of chloroform and 2.5 ml of water were mixed with the chloroform:methanol fraction. The mixture was vigorously shaken on the vortex mixer while covered with Teflon tape. The mixture was centrifuged at 3000 rpm for 5 min to speed up the phase separation process. Then the lower phase was carefully collected with a pipette equipped with a rubber pump. The sample was dried in a nitrogen stream and resuspended with 3 ml of chloroform and stored in the freezer in appropriate vials for further analysis.

Total lipids

Total lipids were determined using a modification of the gravimetric method of Barnes & Blackstock (1973). Aliquots of 1 ml of the lipid fraction were evaporated to dryness in previously weighed aluminum boats. The aluminum boats were weighed with a resolution of 0.1 µg in microbalance and the lipid residue calculated.

Phospholipids

The determination of phospholipids was carried out using the Bartlett method (Bartlett, 1959). Aliquots of 1 ml of the lipid fraction were evaporated to dryness in a boiling water bath. The phosphate standards, ranging from 0 to 300 nmol, were prepared from a stock solution of 1 mM potassium dihydrogeno phosphate and the water carefully evaporated in a Bunsen burner. The residues remaining in the tubes of samples and standards were treated with 0.2 ml of 70% perchloric acid, at 180 °C for 2 h. Following

this, 3 ml of 0.22% ammonium molybdate solution in 2% sulfuric acid were added to each tube. The mixture was supplemented with 0.12 ml of recently filtered Fiske-Subbarow reagent (0.25% amino-naphthol-sulphonic acid, 15% sodium disulphide, 0.5% sodium sulphite). The tubes were boiled in a water bath for 15 minutes and the absorbances read at 830 nm in a spectrophotometer. Phospholipid phosphate contents were determined against the orthophosphate standards. The phosphate content of phospholipid was estimated by using an average molecular weight of 775 daltons, *i.e* the molar amounts of phospholipid phospate (expressed in µmol) were multiplied by 775 to express the phospholipid amounts in units of mass (µg).

c) Carbohydrates

Carbohydrate analysis is generally based on a colour development when a phenolic glucose solution is heated with concentrated sulphuric acid (Mendel & Bauch, 1926; Mendel *et al.*, 1954). Phenol in the presence of hot sulphuric acid can be used for the quantitative colorimetric microdetermination of sugars and their methyl derivatives, oligosaccharides, and polysaccharides (Dubois *et al.*, 1956). The method used here is a modification of the method described by Marshall & Orr (1962) based on that of Dubois *et al.* (1956).

Glycogen is the main form of carbohydrate present as animal reserves. Thus, in quantitative estimation of carbohydrates it is useful to separate insoluble carbohydrates, such as glycogen, from soluble carbohydrates, otherwise all tissue sugars will be quantified (Kemp *et al.*, 1954). The glycogen was separated from soluble sugars by

precipitation in the presence of methanol. Methanol (10 ml) was added to 0.2 ml aliquots of sample homogenates in conic centrifuge tubes, shaking vigorously on the vortex mixer. The mixtures were then centrifuged for 15 minutes at 3000 rpm. Supernatants were collected into separate tubes, and the precipitates resuspended in 10 ml methanol and shaken vigorously on the vortex mixer. The suspensions were then centrifuged as before, the supernatants were collected and pooled with those from the previous extraction. The supernatants were used for quantification of soluble sugars whilst the precipitates were used for quantification of glycogen.

*Soluble carbohydrates (*e.g. *glucose)*

Soluble fractions were evaporated to total dryness in a water bath at 100 °C. The residue was resuspended with 3 ml of 1.67% phenol, shaking vigorously on the vortex mixer. Then, concentrated sulphuric acid (5 ml) was rapidly mixed and heated at 100 °C in a water bath for 10 minutes. Standards ranging from 0 to 100 µg of glucose were prepared from a stock solution of 0.1% glucose. The standards received the same treatment as samples.

Insoluble carbohydrates (glycogen)

The sulphuric acid method can be used to quantify glycogen in the samples since the glycogen is hydrolysed into glucose in the presence of hot sulphuric acid (Kemp & Heijningen, 1941). Thus, insoluble carbohydrates were also analysed using the method presented in previous section.

4.2.6. Statistical analysis

Statistical analysis was performed using the STATGRAPHICS package (SGC, 1989) using standard methodology (Zar, 1984). Nonlinear regression analysis was used to establish the descriptive or predictive relationships between body length and other biological parameters (*i.e.* instar duration, fecundity), and between age and body length. The parameters from these models were determined using a routine based on the Marquardt algorithm (Marquardt, 1963).

Descriptive models are defined herein as reference models describing the causal relationship between two variables (Zar, 1984), and are used only for comparison between stressed and unstressed individuals. Predictive models, as used herein, are descriptive models whose functional relationships are used in the physiological *Daphnia* models with predictive purposes.

Linear models, based on least-squares regression, were established for each experiment (*i.e.* food deprivation in non-mature instars and food deprivation in mature instars) to describe the effects of the duration of the period of food deprivation on instar duration and individual growth (*i.e.* the increase in body length). The degree of association between the regressed variables was judged using the regression coefficient (r^2) and the respective probability level. One way analysis of variance was also used, if the criteria of normality and homoscedasticity of the data were met, complemented by the Tukey test for pairwise multiple comparison should the ANOVA show statistically significant differences between treatments (Zar, 1984). When the normality and homoscedasticity criteria were not met a Kruskal-Wallis one way analysis of variance on ranks was used,

complemented with pairwise multiple comparisons using the Dunn's method when statistically significant differences between treatments were obtained from the ANOVA (Zar, 1984).

4.3. RESULTS

4.3.1. Instar duration

In *Daphnia magna*, instar duration, as predicted by hypothesis I (page 105), is geometrically correlated with body length (Figure 4.11) and is almost proportional to the surface area (*i.e.* exponent = 2). The data do not exhibit any trend associated with the instar where maturation occurs (*i.e.* residuals from individuals maturing at different instars are randomly distributed). Although there are insufficient data available to be sure that instar duration continues to increase geometrically after maturation this assumption was made and used in the model.

Prevailing food conditions instantaneously affect instar duration. Thus, while instar duration is delayed if the food supply is reduced (Figure 4.12, Figure 4.13, Figure 4.14, Figure 4.15, under stress) it returns to control values after control conditions have been resumed (Figure 4.12, Figure 4.13, Figure 4.14, Figure 4.15, after stress), *i.e.* instar duration continues to vary randomly around the regression line. However the recovery is not as efficient when food deprivation occurs for the whole instar (Figure 4.14, after stress).

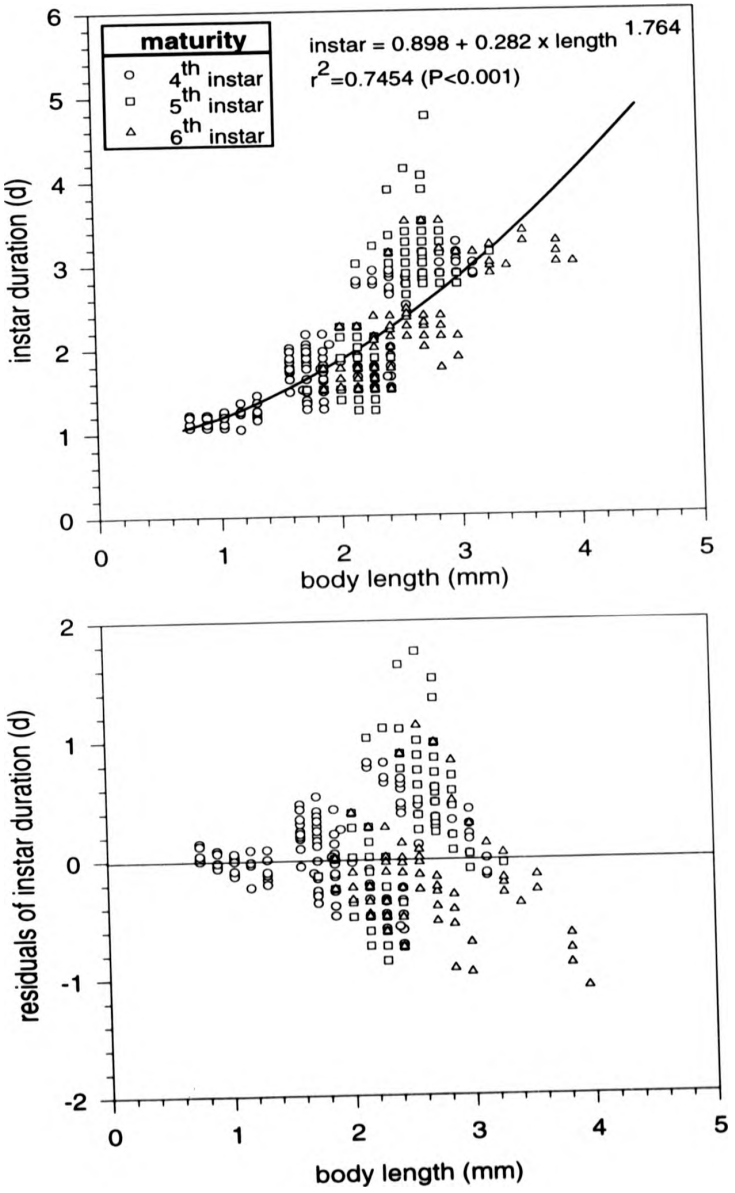


Figure 4.11 - Predictive model (top) and residual (bottom) of the relationship between size at the beginning of the instar and the instar duration of control individuals maturing in the 4th, 5th, or 6th instar.

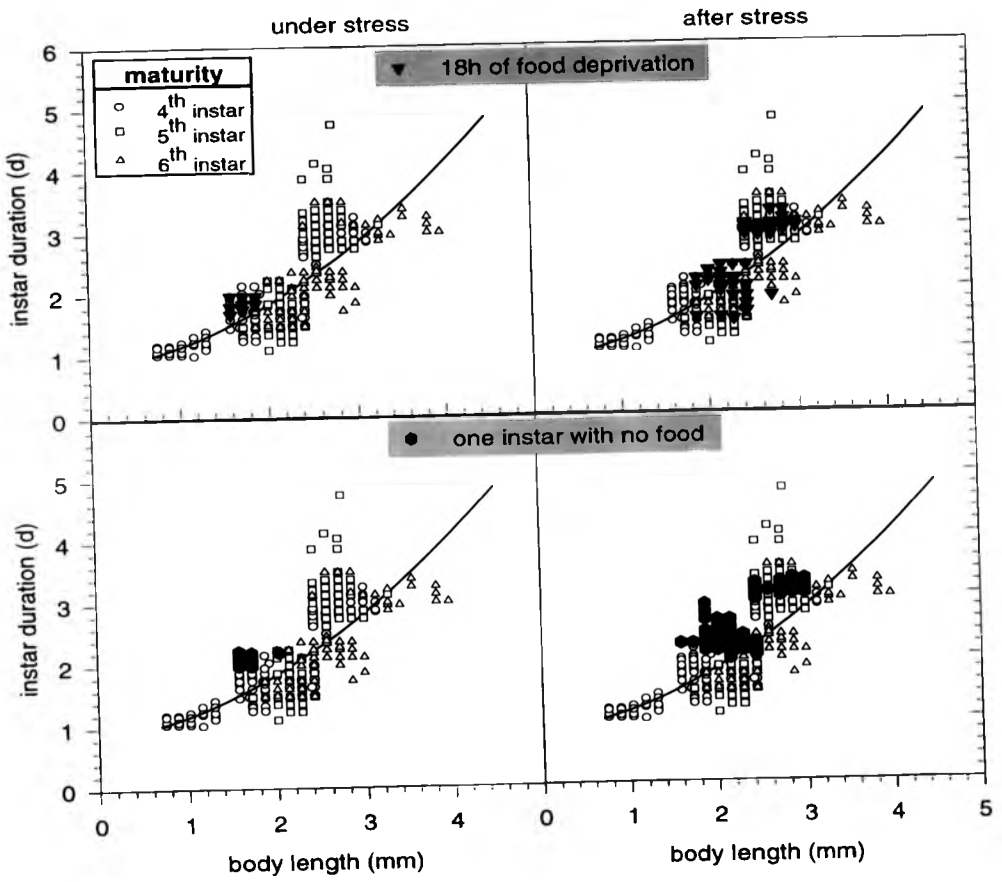


Figure 4.12 - Relationship between instar duration on individuals exposed to, and recovering from food deprivation on the 3rd adolescent instar. Open symbols are control individuals.

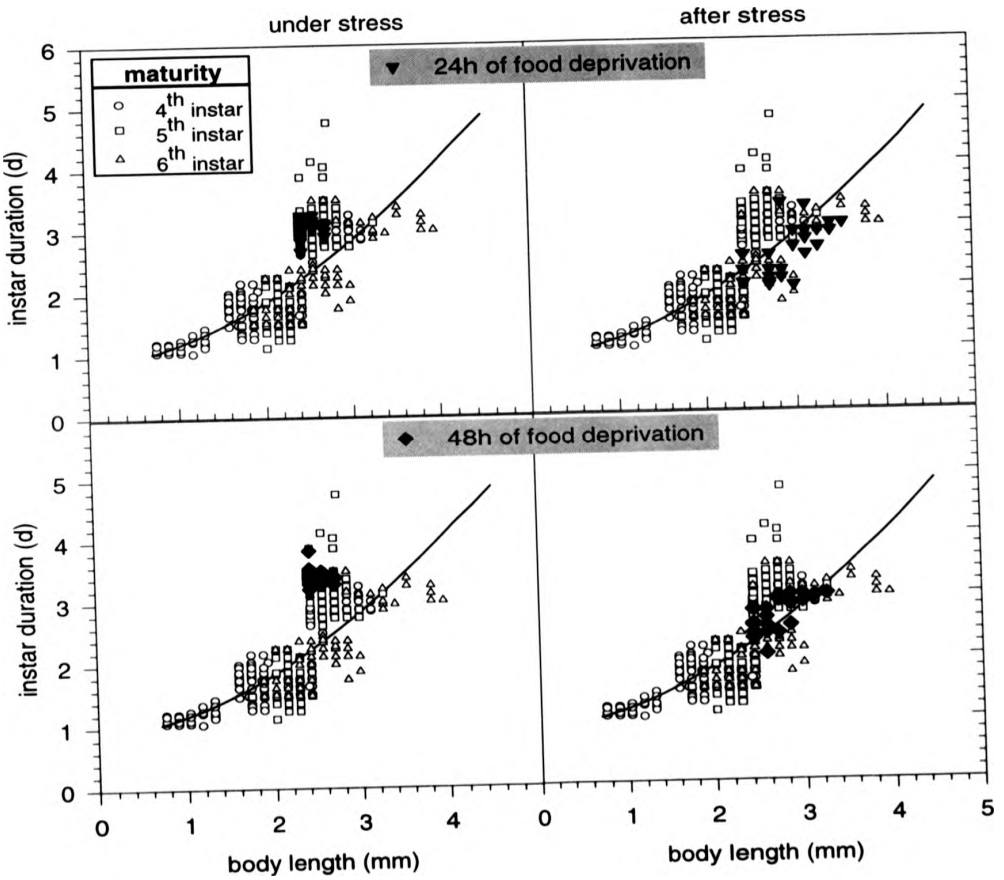


Figure 4.13 - Relationship between instar duration on individuals exposed to, and recovering from food deprivation on the 5th instar (first adult instar). Open symbols are control individuals.

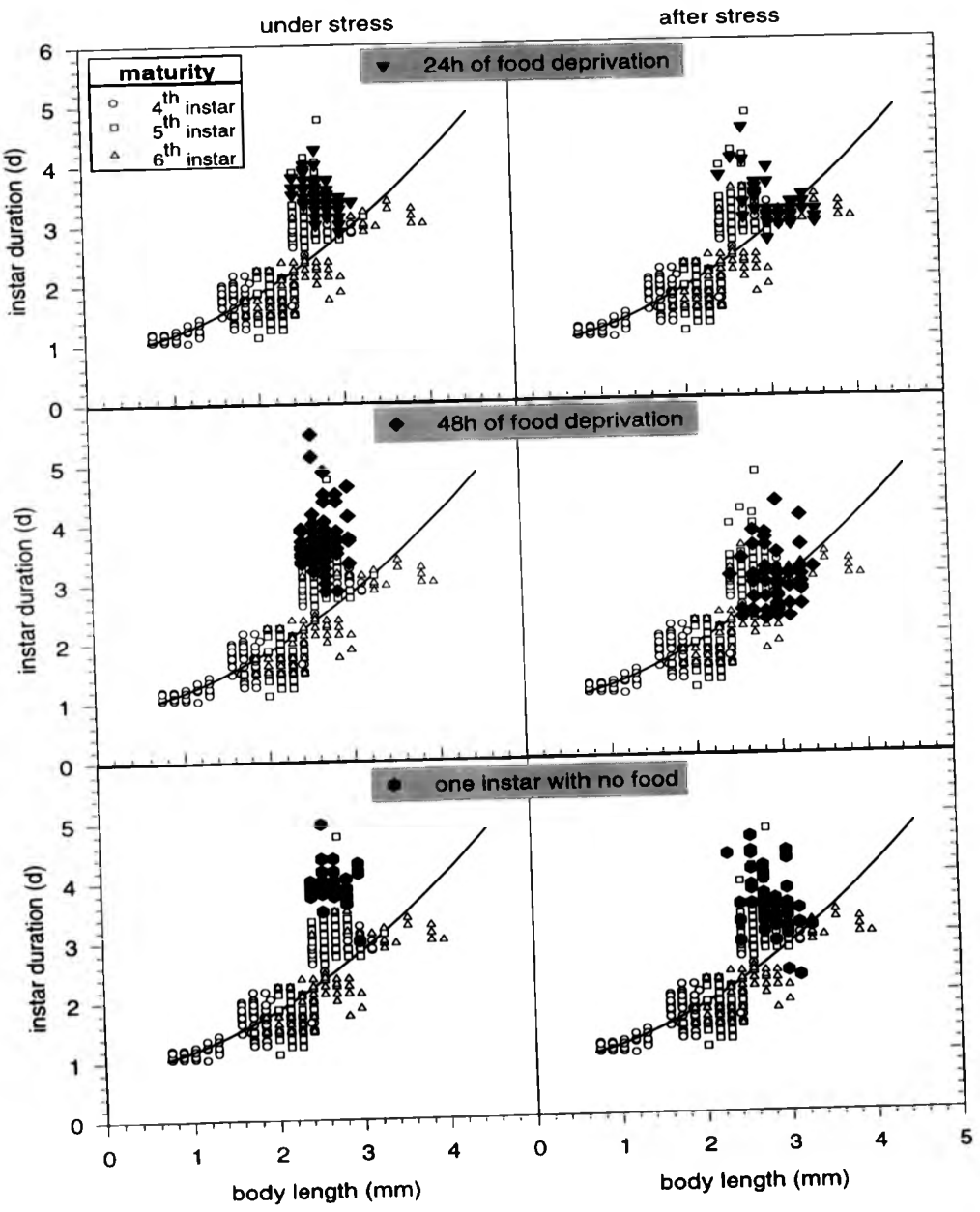


Figure 4.14 - Relationship between instar duration on individuals exposed to, and recovering from food deprivation on the 6th instar (first adult instar). Open symbols are control individuals.

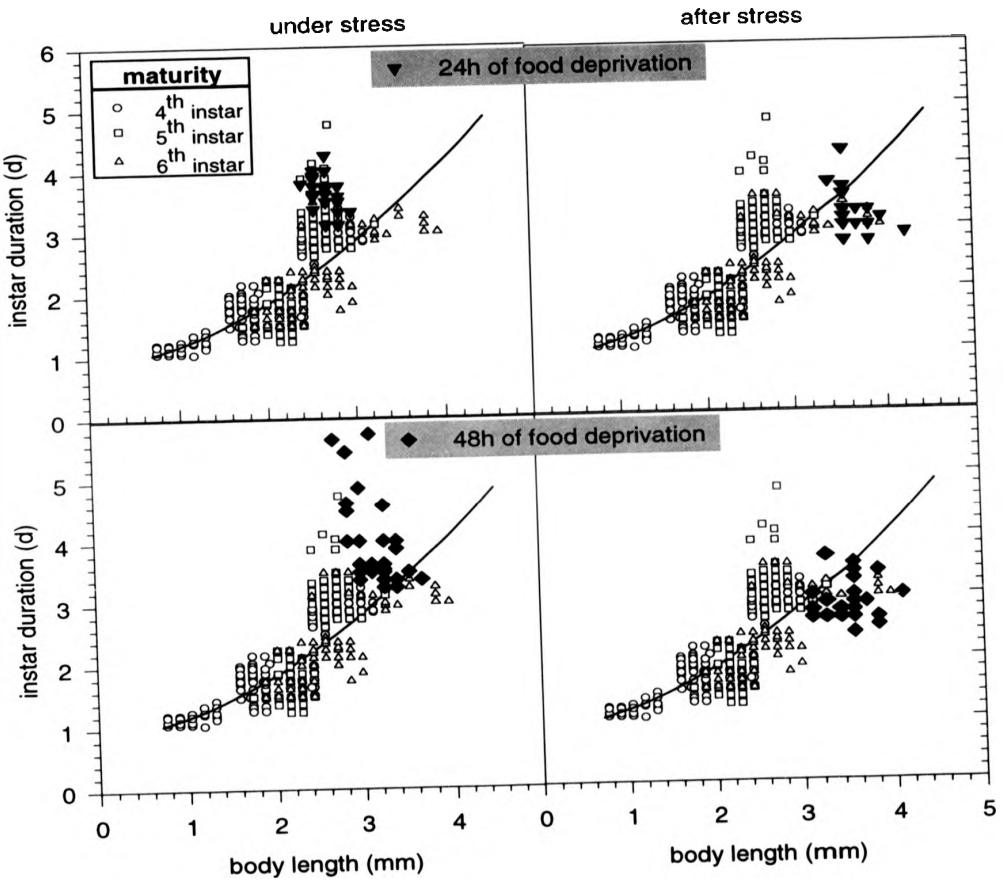


Figure 4.15 - Relationship between instar duration on individuals exposed to, and recovering from food deprivation on the 7th instar (first adult instar). Open symbols are control individuals.

As predicted by hypothesis II (page 105), instar duration increases proportionally to the duration of the food deprivation period (Figure 4.16). Food deprivation in the 3rd adolescent instar and food deprivation in the 1st adult instar exhibited similar dependence upon food conditions during the instar duration. The comparison of the two slopes using the standard t-test procedure (Zar, 1984) is not statistically significant ($t=-0.604$, $df=655$, $p=0.546$). Since the effect of the food deprivation period on the delay of instar duration is proven statistically to be instar-independent, a common slope of 0.24 can be computed.

Thus, it can be concluded that under constant temperature two factors determine the duration of an instar: body length at the beginning of the instar and food availability during the instar. Under *ad libitum* food conditions an individual will take a minimal time (size-dependent) to moult (*i.e.* to build a new carapace and replace the old one). Similarly when food conditions deteriorate the individual takes longer to moult, with the maximum duration of the instar occurring in the complete absence of food. The existence of a limit for instar duration is probably explained by a reduction of the quality of the carapace with time (micro-organisms can be seen growing on it whilst detritus present in the environment become attached to the carapace over time). Thus, it is assumed that the permeability (porosity) of the carapace must be affected, resulting in death for the individual if it is not capable of replacing the carapace.

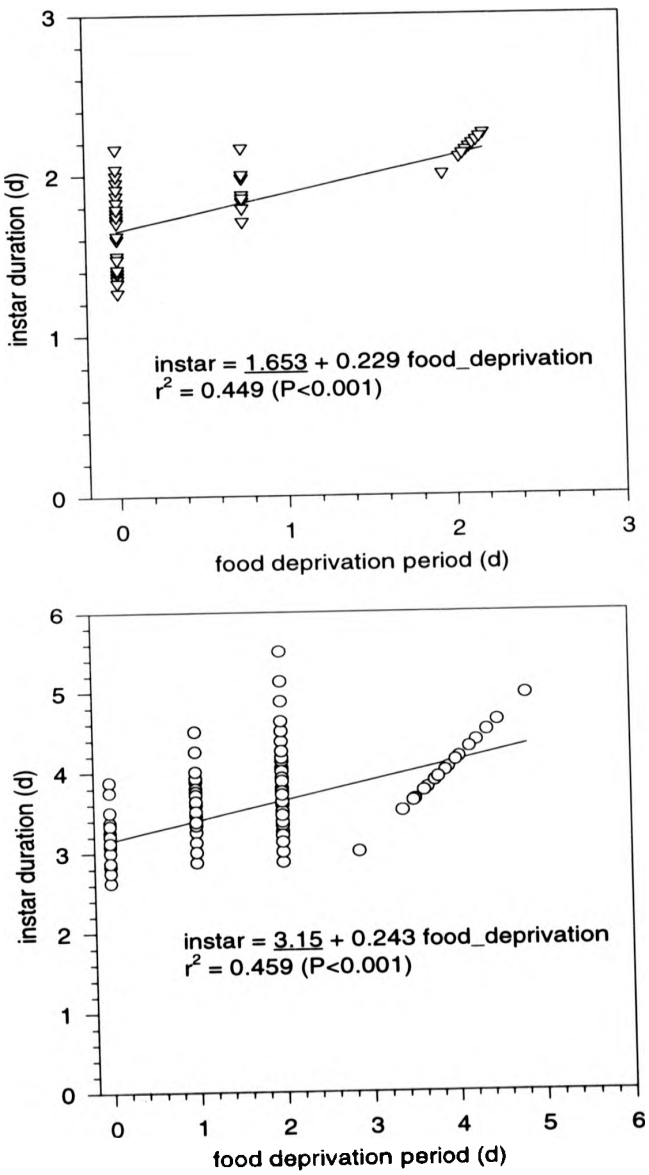


Figure 4.16 - Predictive model of the relationship between instar duration and the duration of the food deprivation period on the third adolescent instar (top), and on individuals on first adult instar (bottom).

4.3.2. Growth

A descriptive non-linear regression model, assuming asymptotic growth, was calculated using the variation of individual body length with age of control individuals maturing at different instars (Figure 4.17). The regression line was then used to graphically evaluate the effects of periods of food deprivation at two points of the life cycle, *i.e.* third instar (Figure 4.18) and 2nd reproductive instar (Figure 4.19, Figure 4.20, Figure 4.21). Growth is not much affected, relative to the controls, if reproduction is not taking place when the individuals are deprived of food for short periods (Figure 4.18, under stress) since growth is compensated by an increase in instar duration (Figure 4.16, page145). Although growth stops completely in the absence of food (Figure 4.18, Figure 4.20, under stress) it resumes normal rates when food levels are restored (Figure 4.18, Figure 4.20, after stress).

Growth is more strongly affected when reproduction is also taking place (*i.e.* when individuals are deprived of food during adult instars, Figure 4.19, Figure 4.20, Figure 4.21). Growth rates return to normal values (relative to the body sizes at the beginning of the instar) when control conditions are resumed, independent of the duration of the food deprivation period (Figure 4.19, Figure 4.20, Figure 4.21, after stress).

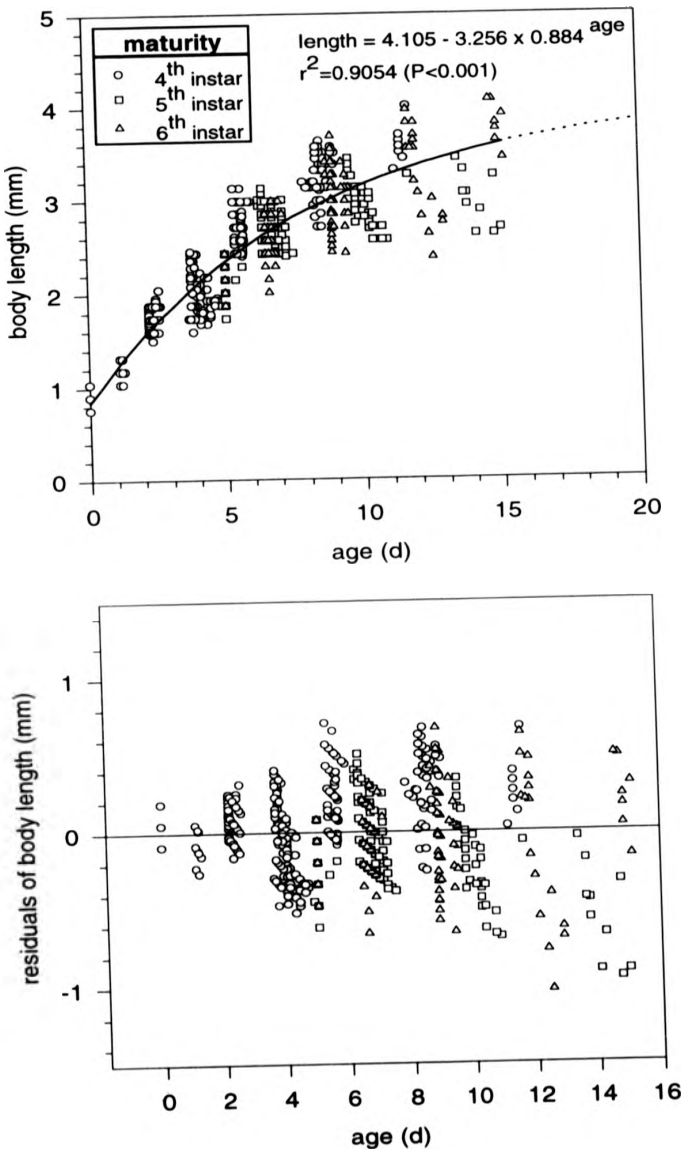


Figure 4.17 - Descriptive model (top) and residuals (bottom) of the relationship between age and body size of control individuals releasing maturing at different instars.

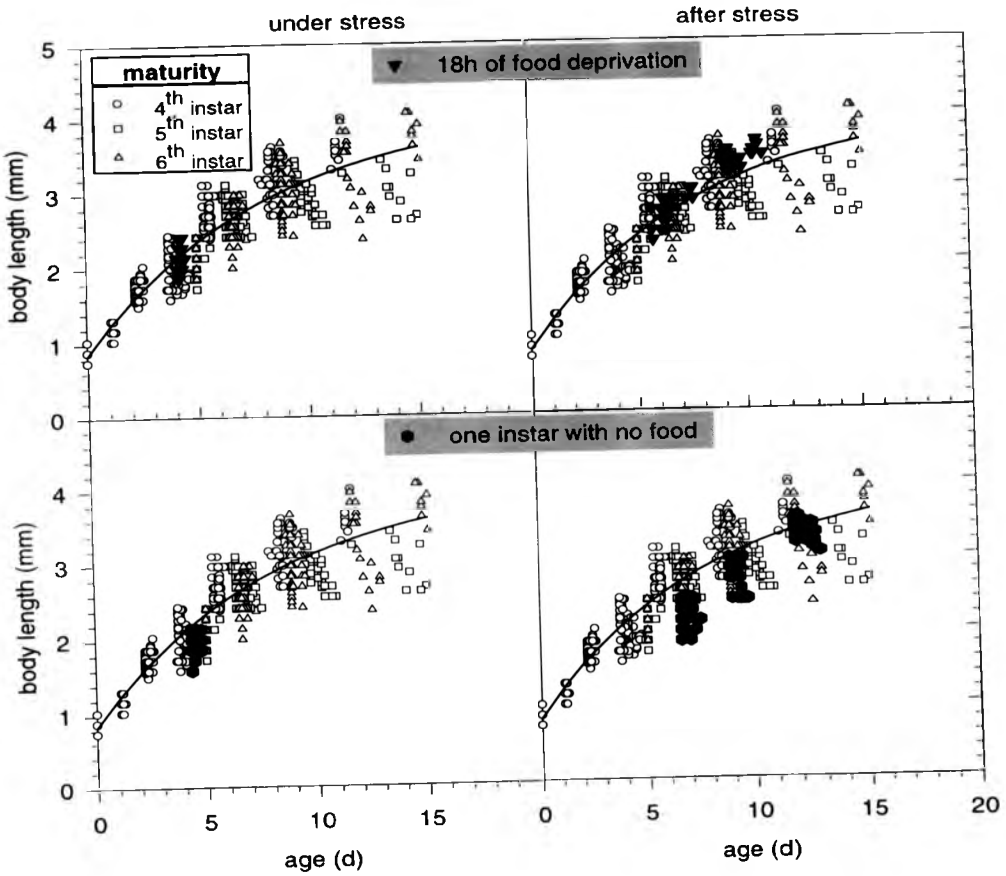


Figure 4.18 - Effects of food deprivation on growth on individuals exposed to, and recovering from, variable food deprivation periods on the 3rd adolescent instar. Open symbols are control individuals.

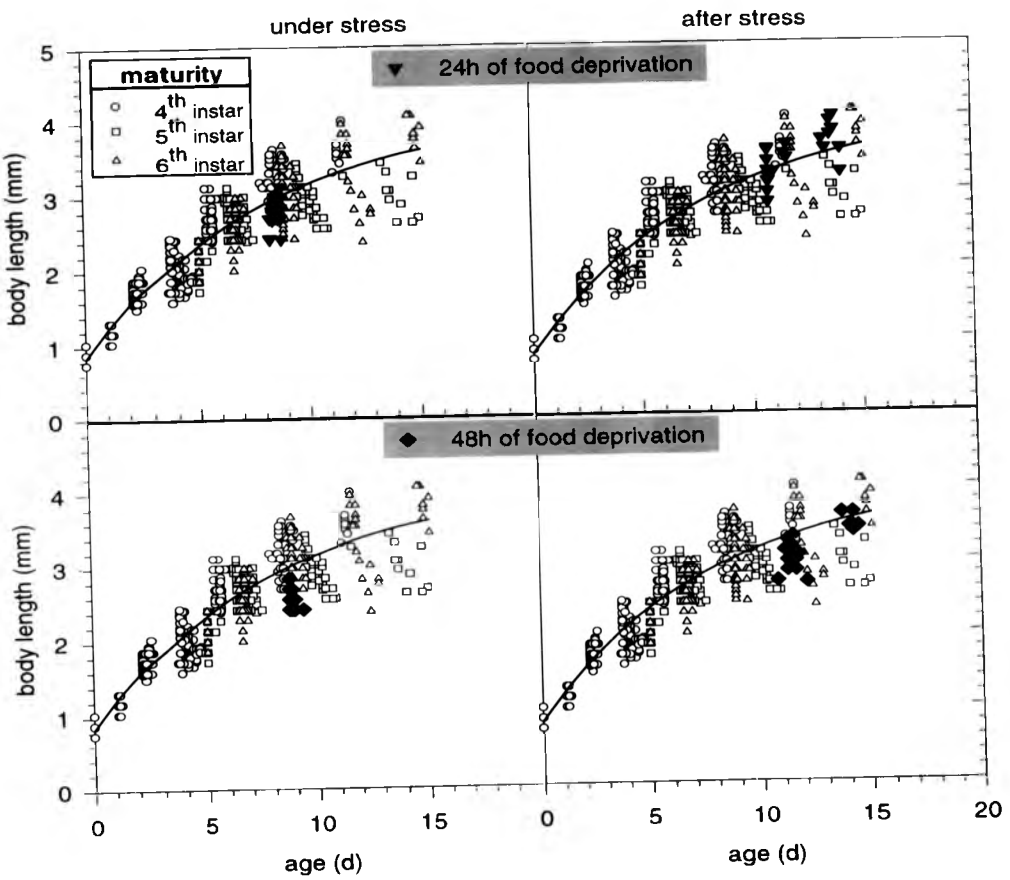


Figure 4.19 - Effects of food deprivation on growth on individuals exposed to, and recovering from variable food deprivation periods on the 5th instar (1st adult instar). Open symbols are control individuals.

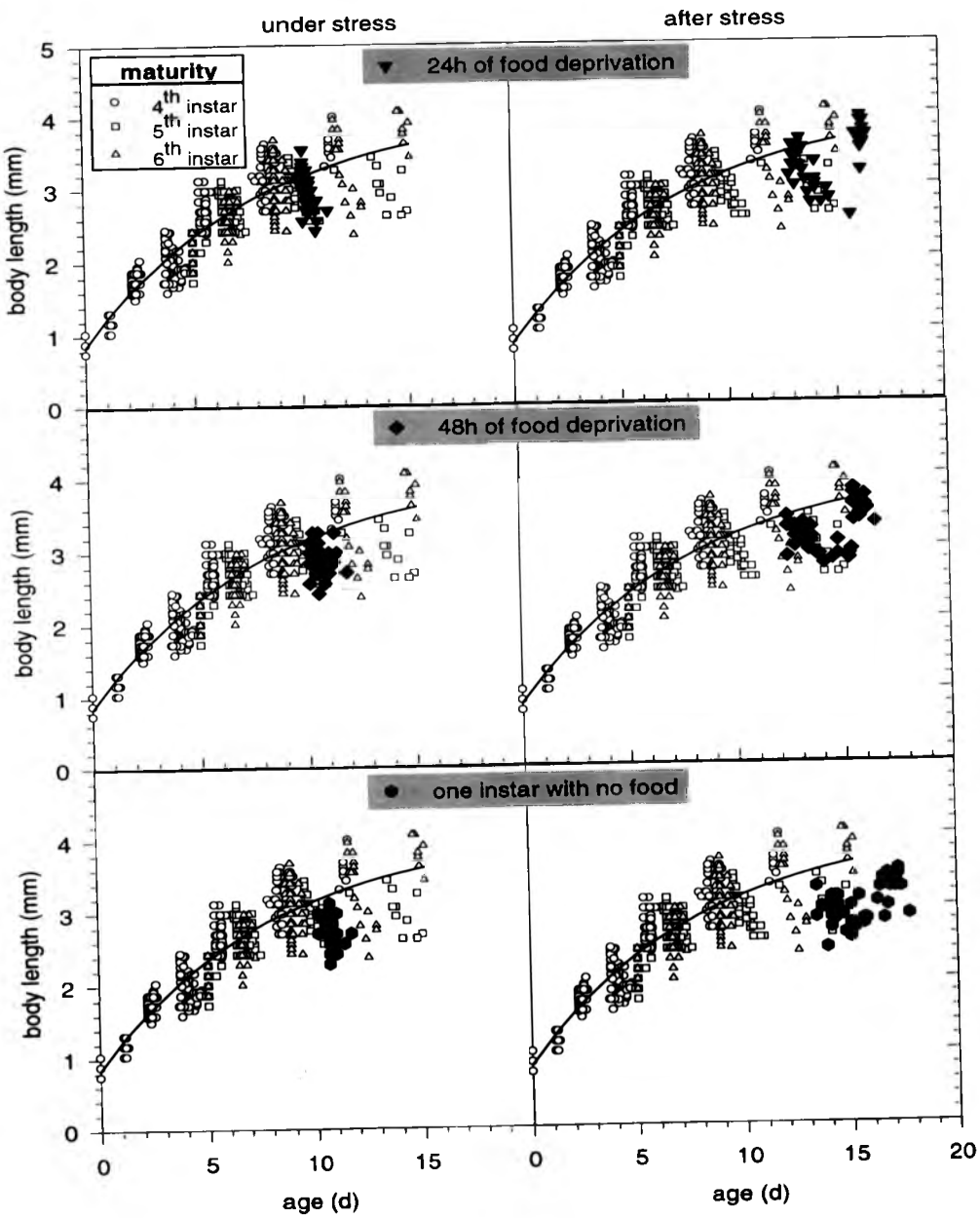


Figure 4.20 - Effects of food deprivation on growth of individuals exposed to, and recovering from variable food deprivation periods on the 6th instar (1st adult instar). Open symbols are control individuals.

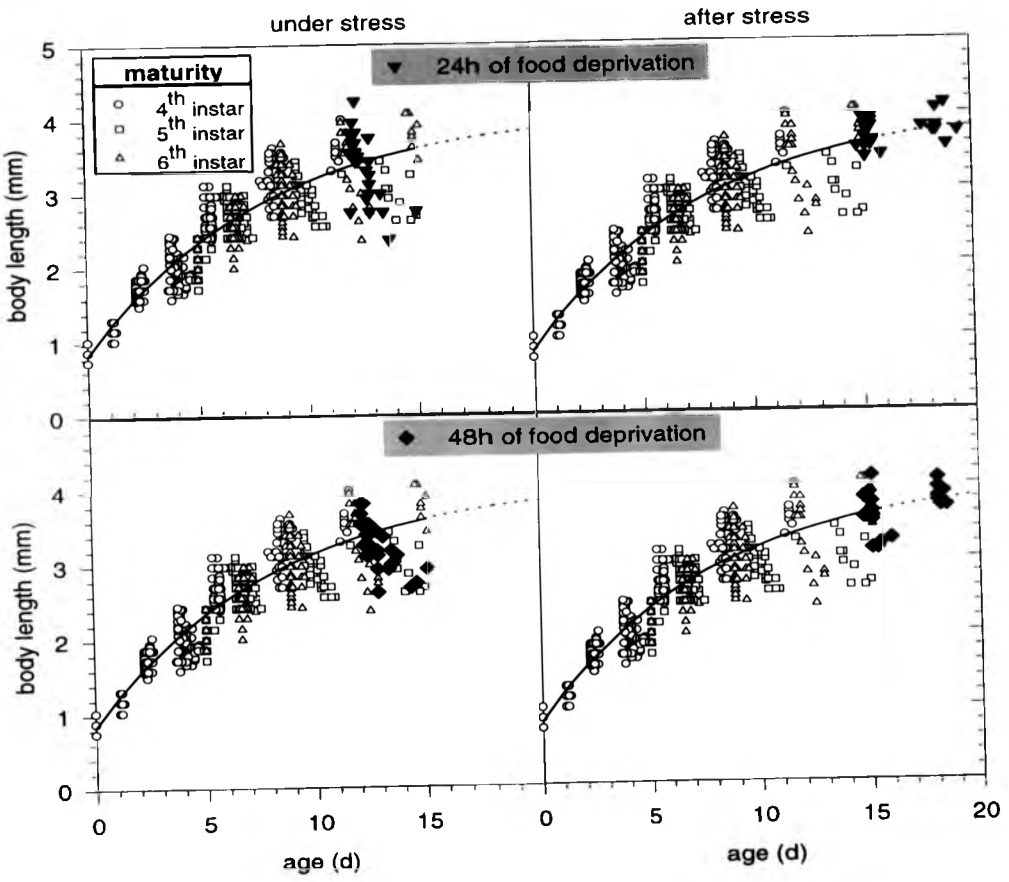


Figure 4.21 - Effects of food deprivation on growth of individuals exposed to, and recovering from variable food deprivation periods on the 7th instar (1st adult instar). Open symbols are control individuals.

As predicted by hypothesis III (page 105), increase in length (growth) varies inversely with the duration of the food deprivation period (Figure 4.22). A standard t-test comparison of the slopes (Zar, 1984) does not show any statistically significant difference between them ($t=0.12$, $df=490$, $p=0.904$). Thus, a common slope (-0.065) could be computed as the proportionality constant between growth and the duration of the food deprivation period. Growth (considered as increase in length) is reduced with increasing food deprivation periods stopping when food is absent for the whole instar. Thus, these results demonstrate clearly that investment in growth comes directly from food resources. Moreover, growth is depressed when adulthood is reached as a result of simultaneous growth and reproduction (Figure 4.17).

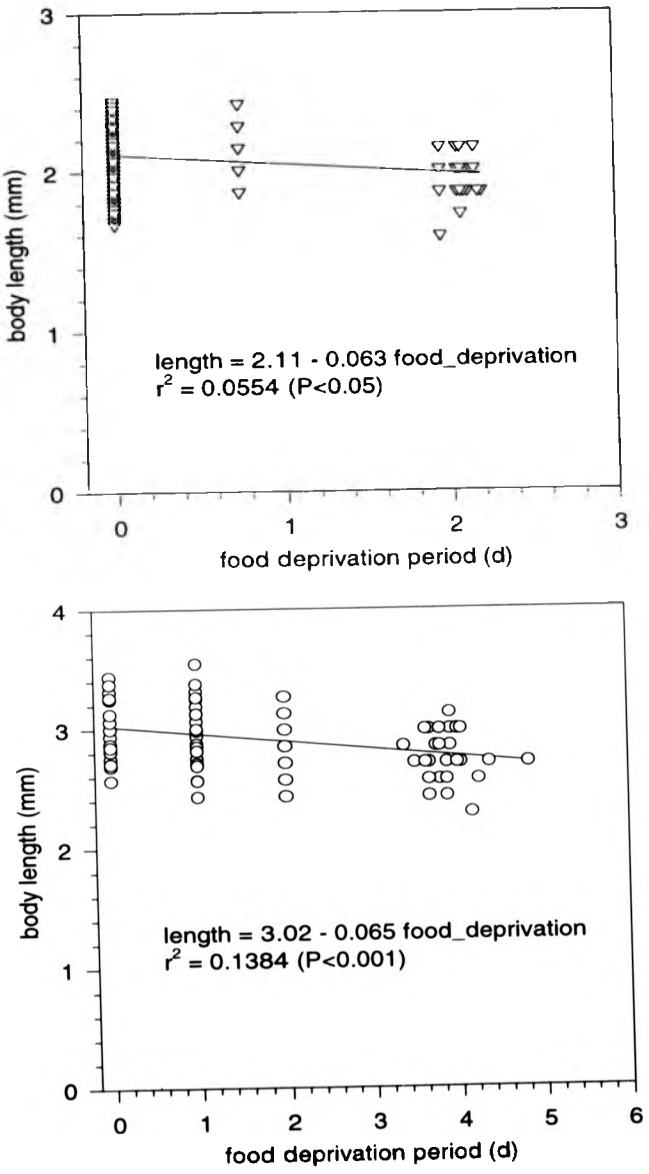


Figure 4.22 - Descriptive model of the relationship between growth in length and the duration of the food deprivation period on non-reproducing individuals (3rd instar), and on individuals on their first adult instar (6th instar).

4.3.3. Fecundity

A geometrical model was calculated to relate body length when eggs are released into the brood pouch with the number of eggs released by control individuals releasing their first clutch into the brood pouch in different instars (Figure 4.23). This model is used in later figures to graphically compare individuals exposed to different periods of food deprivation with control individuals (Figure 4.24, Figure 4.25, Figure 4.26, Figure 4.27).

Food deprivation in adolescents (3rd instar) does not affect the relationship between body length and fecundity since all points are uniformly distributed around the regression line (Figure 4.24). However, as predicted fecundity is greatly depressed or ceases altogether with increasing food deprivation periods during reproductive instars (Figure 4.25, Figure 4.26, Figure 4.27, under stress). Individuals under the same feeding conditions but maturing in later instars are less affected than individuals maturing earlier. Individuals which matured in the 4th instar did not produce eggs after 48 h of food deprivation (Figure 4.25), while some individuals which matured in the 6th instar produced clutches with fewer eggs than the controls (Figure 4.27).

In all treatments, among those instars where feeding conditions have been restored back to control values, the relationship between body length and fecundity resumes control values, *i.e.* values are plotted randomly around the regression line (Figure 4.25, Figure 4.26, Figure 4.27, after stress).

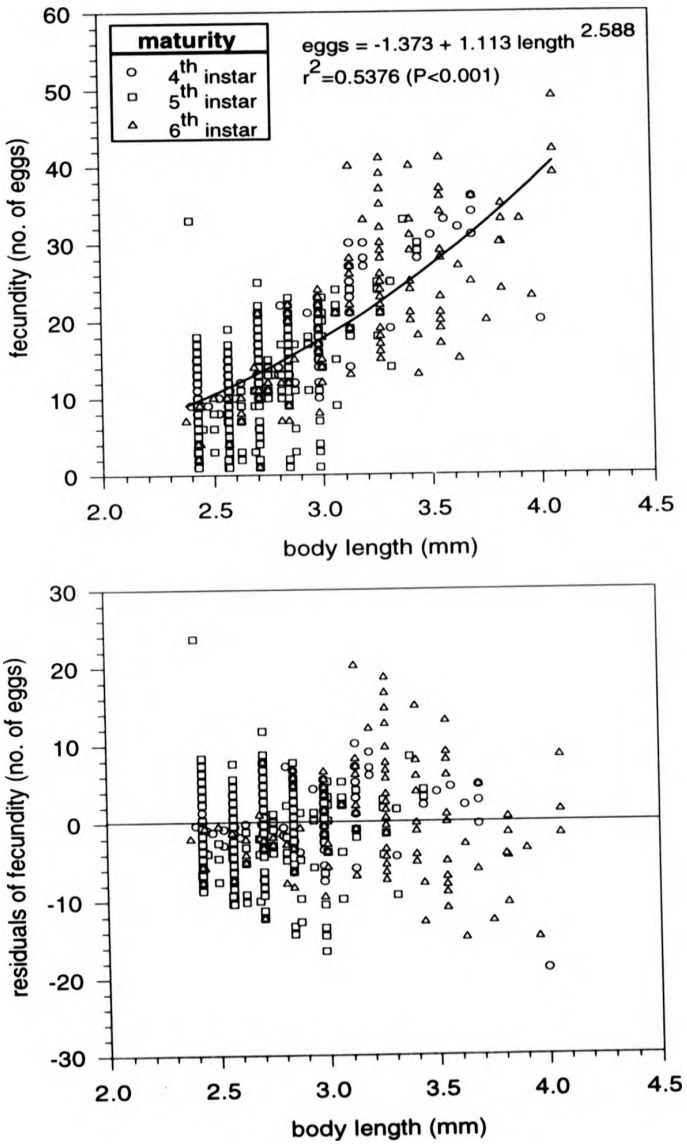


Figure 4.23 - Descriptive model (top) and residuals (bottom) of the relationship between body length and fecundity of control individuals releasing their first clutch on different instars.

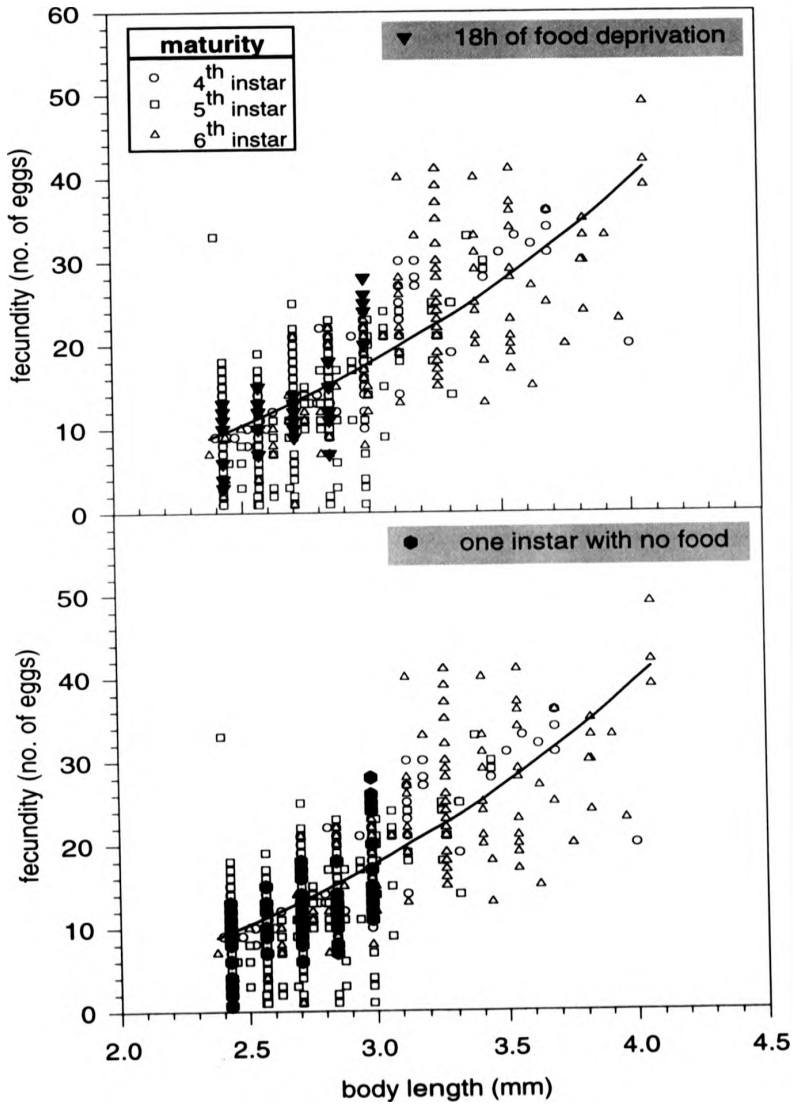


Figure 4.24 - Effects of food deprivation on the relationship between body length and fecundity relationship of individuals previously exposed (3rd adolescent instar) to different food deprivation periods. Open symbols are control individuals.

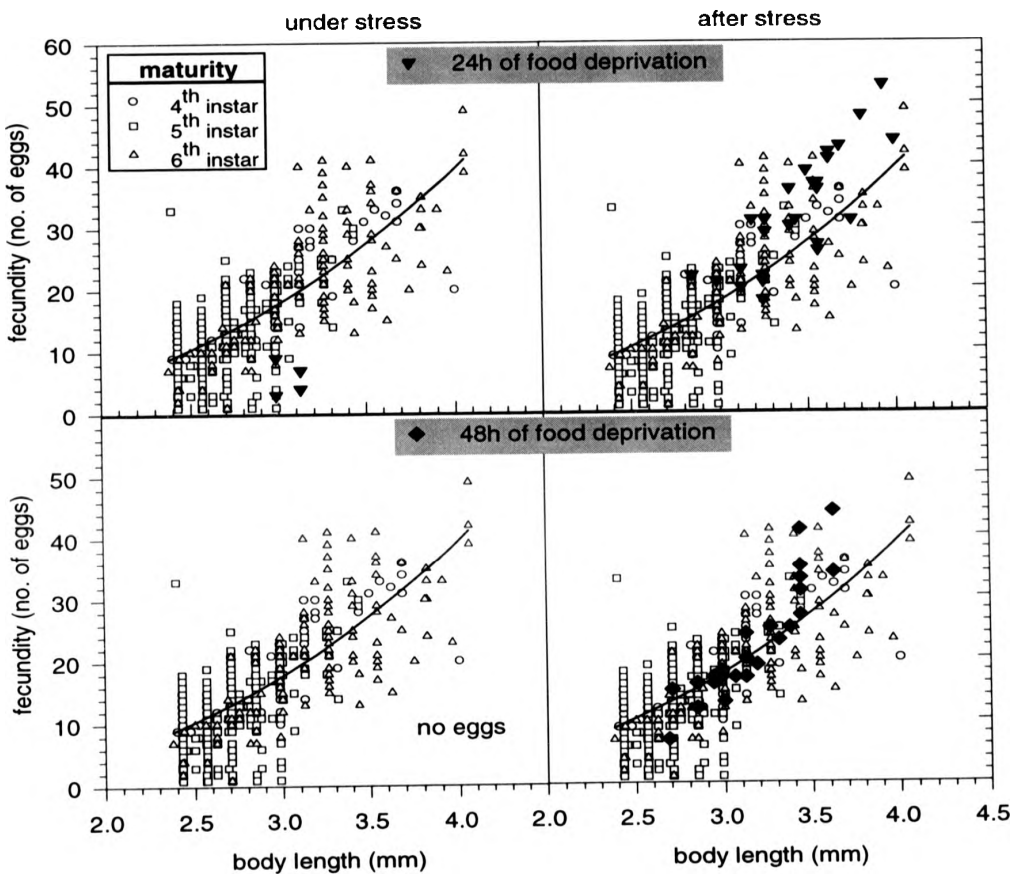


Figure 4.25 - Effects of food deprivation on the relationship between body length and fecundity of individuals exposed to, and recovering from different periods of food deprivation in their first adult instar (5^{th} instar). Open symbols are control individuals.

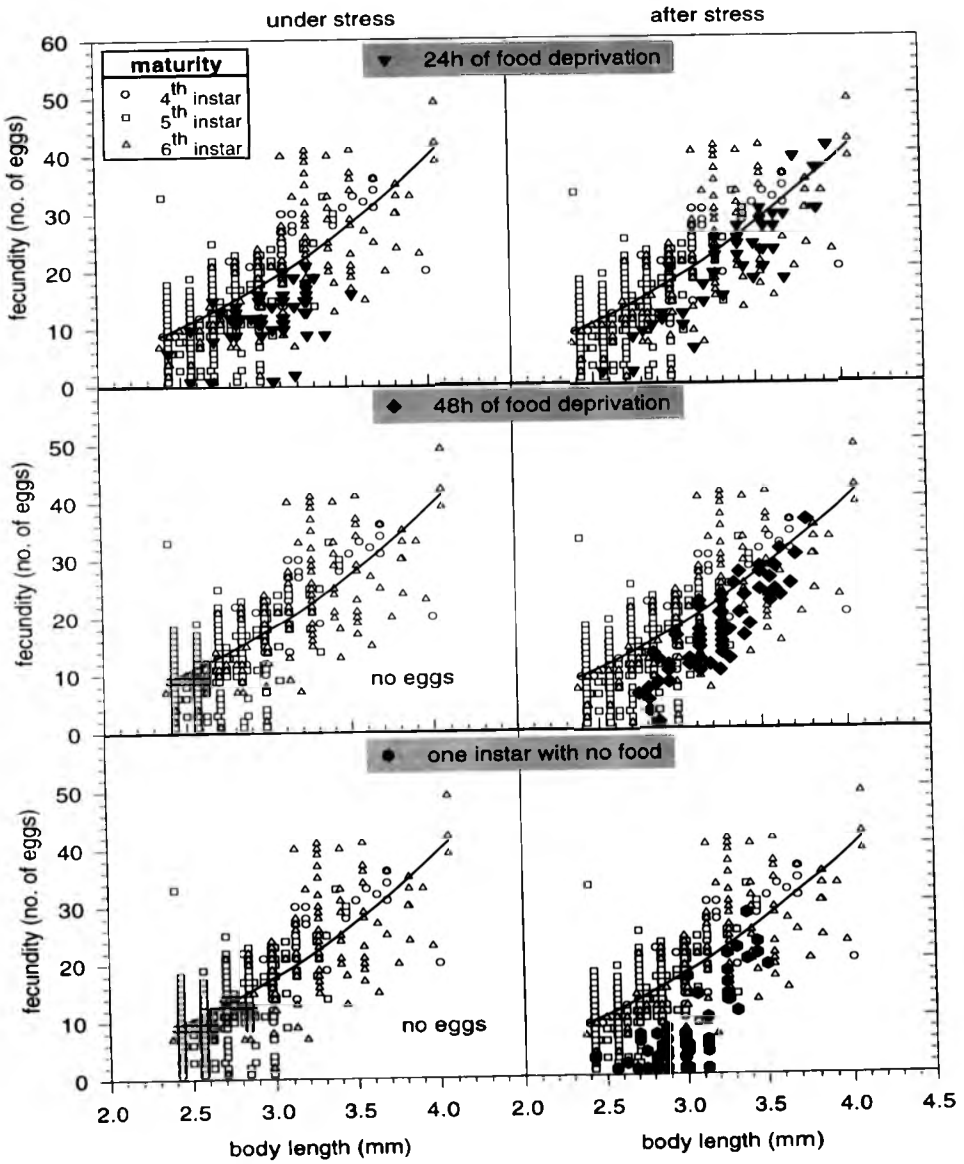


Figure 4.26 - Effects of food deprivation on the relationship between body length and fecundity of individuals exposed to, and recovering from different periods of food deprivation in their first adult instar (6th instar). Open symbols are control individuals.

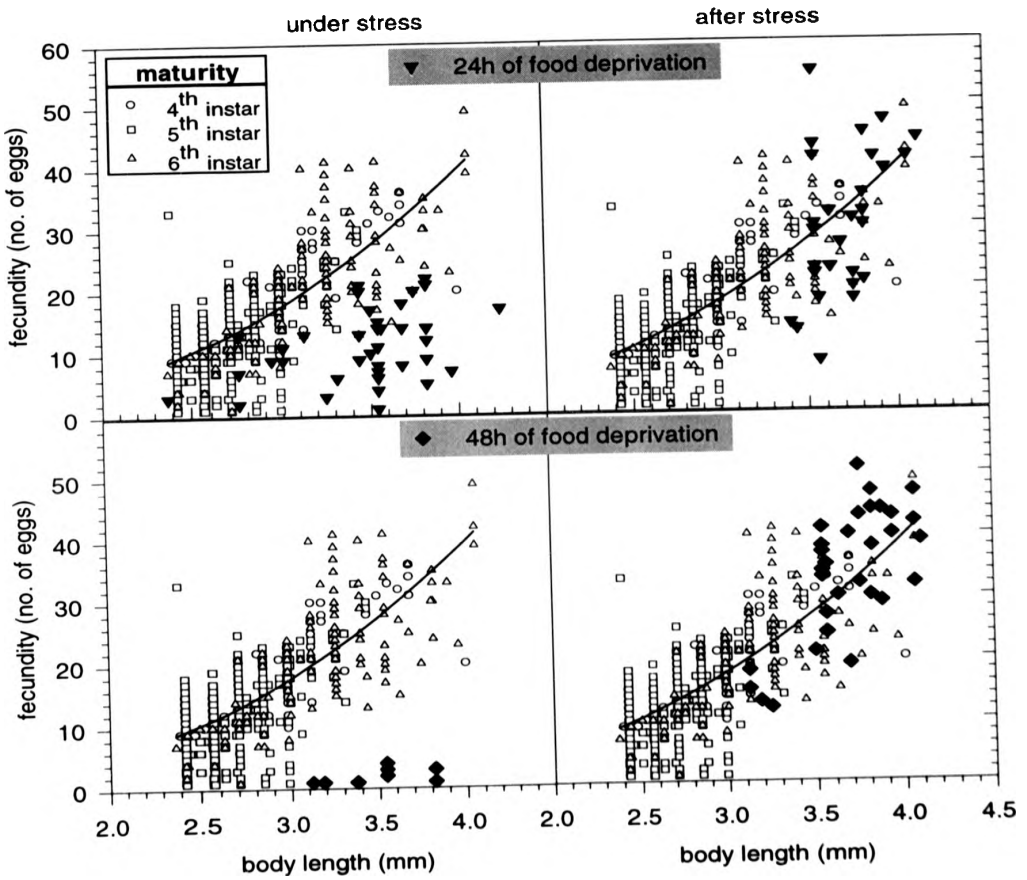


Figure 4.27 - Effects of food deprivation on the relationship between body length and fecundity of individuals exposed to, and recovering from different periods of food deprivation in their first adult instar (7th instar). Open symbols are control individuals.

4.3.4. Maturity

Maturity is a crucial point in an animal life cycle. In *Daphnia magna*, when maturity is reached, resources acquired from the environment must be shared between maintenance (including moulting, hence survival), growth and reproduction. Previous studies have noted that the first clutch of *Daphnia* has frequently been referred to as highly variable in egg number (*e.g.* Ebert, 1991). The first clutch is also the one to which the intrinsic rate of increase, r, is most sensitive (Cole, 1954; Lewontin, 1965).

Reaching maturity has been considered as equivalent to the release of the first clutch of eggs (*e.g.* Ebert, 1992, 1994a). However, I think this is not appropriate from an energetics standpoint since maturity is reached when the ovaries become fully functional and eggs can be provisioned. Moreover, maturation must be reached at some point before the first release of the eggs into the brood pouch since they must be provisioned before being released. Thus, since size or age at maturity are vague and difficult to determine it is preferable to speak about the size and age at first clutch (*e.g.* Bell, 1983; Ebert, 1991).

Food conditions determine both the size at first clutch and the age at the release of the first clutch (Figure 4.28). Thus, if two mothers in the same food conditions mature in different instars, the mothers which mature later will be larger, and hence produce a larger first clutch, in terms of egg number (Figure 4.29). Moreover, in both cases fecundity (clutch size) will be strongly correlated with body length at clutch release (Figure 4.28, Figure 4.29) following the model previously established (Figure 4.23).

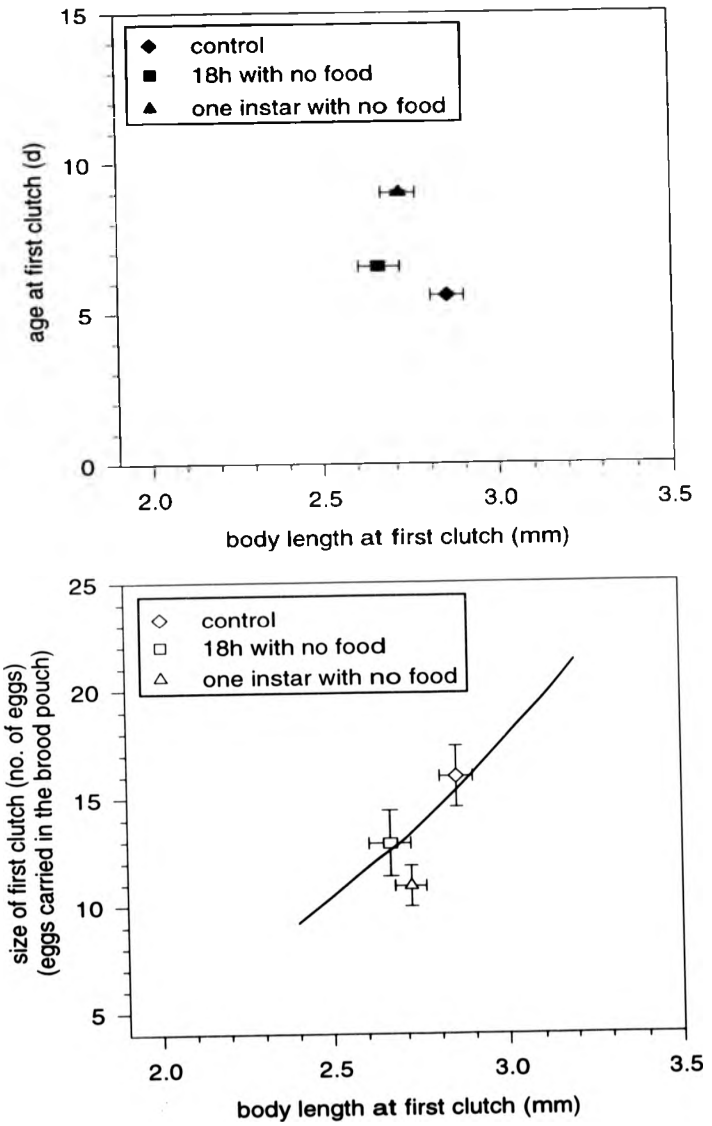


Figure 4.28 - Relationship between body length, and age at first clutch (top), and size of first clutch (bottom) of individuals exposed to different periods of food deprivation in the 3rd instar (regression line as in Figure 4.23). Error bars are 95% confidence limits for both variables.

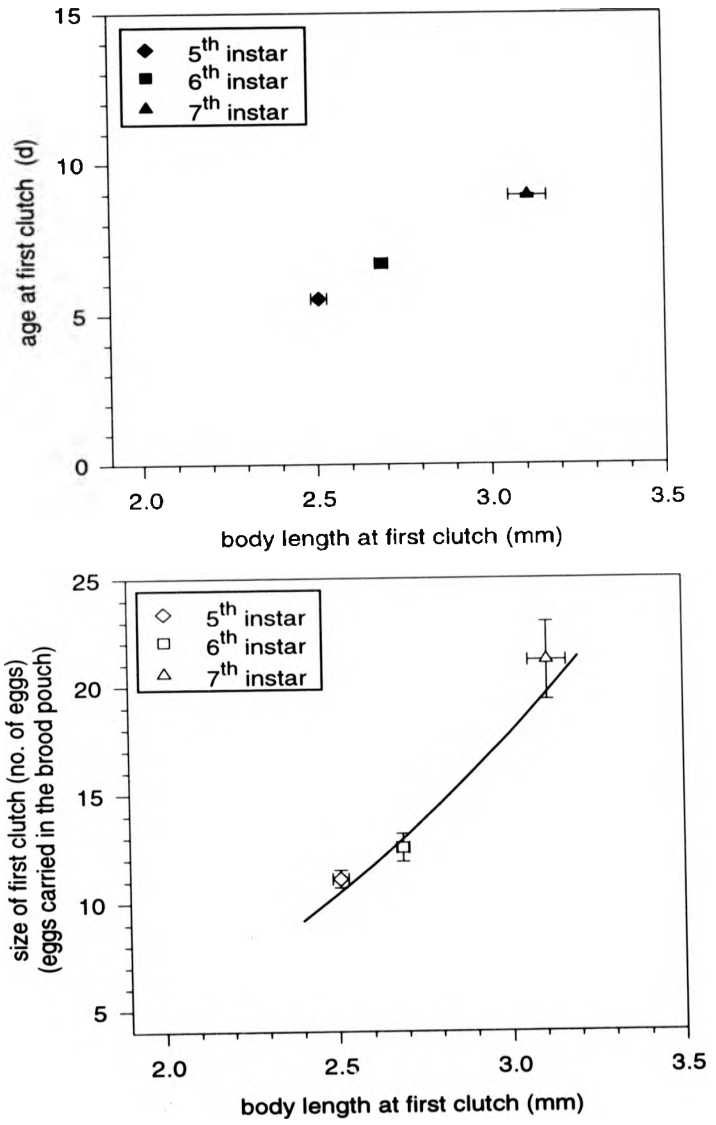


Figure 4.29 - Relationship between body length and age at first clutch (top), and size of first clutch (bottom) of control individuals carrying their first clutch on different instars (regression line as in Figure 4.23). Error bars are 95% confidence limits for both variables.

As predicted it takes longer for an individual to provision its first clutch when feeding conditions deteriorate, even for short periods of time, before maturity. Moreover, body length at first clutch is clearly the result of a trade-off between the size at maturity and the instar where maturity occurred (Figure 4.28, Figure 4.29).

The egg size distribution of clutches from the 2nd adult instar were studied together with maternal body length for three treatments (24 h, 48 h, and one full instar of food deprivation) plus a control. Statistically significant differences in mean body length of the females were found between treatments using one way ANOVA ($F=24.6$, $df=2,30$, $P<0.0001$). The Tukey test showed statistically significant differences between the mean length of individuals deprived of food for one instar relative to the other two treatments which were statistically identical.

All egg-size distributions have the same mode (Figure 4.30). However, the frequency distribution of egg-size from smaller individuals (deprived of food for one instar) is skewed to the left (*i.e.* eggs are smaller), whilst the egg-size distribution from larger individuals (deprived of food for 24 and 48 hours) is skewed to the right (*i.e.* eggs are larger).

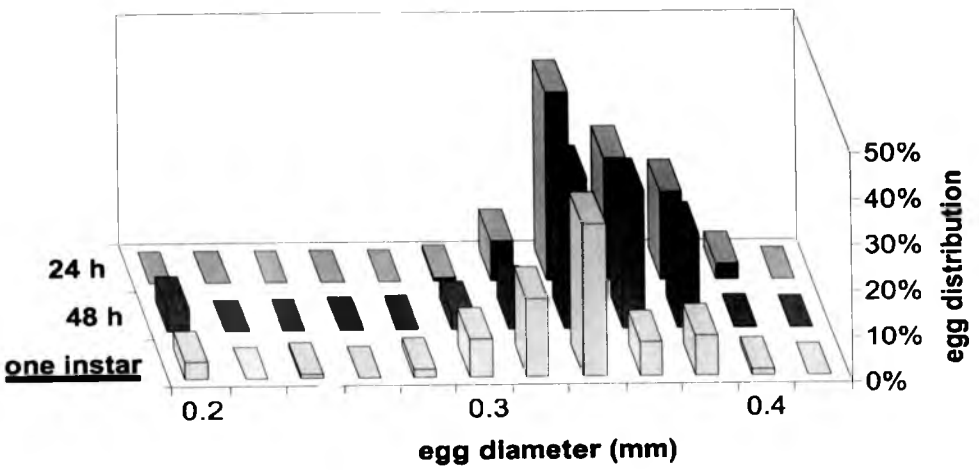


Figure 4.30 - Egg size distribution of the 3rd clutch (mothers exposed to 24 h of food deprivation in the second reproductive instar) and 2nd clutch (mothers exposed to 48 h and one instar of food deprivation in the 2nd reproductive instar). The treatment with mean body length of the mothers and median egg-size significantly different from the other two treatments is underlined.

Statistically significant differences were also observed between the medians of the egg-size distributions of the different treatments, using the Kruskal-Wallis one way ANOVA on ranks ($H=102.3$, $df=2$, $P<0.0001$). Dunn's method, used for pairwise multiple comparisons, showed that only individuals which were deprived of food for one full instar were significantly different from individuals of the other two treatments ($P<0.001$).

4.3.5. Biochemical analysis

The data obtained from the biochemical analysis of the samples collected during the experiment do not exhibit a consistent pattern between different experiments. Statistical analysis between treatments could not be carried out since individuals were pooled together within each sample without replication. Hypothesis IV (page 105) was not supported by the results from the biochemical analysis, probably because the amount of material used in each analysis was near the lower limits of detection and no sample replication was used. However, some trends emerge from the results of the graphical analysis of the data (Figure 4.31, Figure 4.32, Figure 4.33, Figure 4.34) and their relationship with the duration of the food deprivation periods:

- the relative amount of protein (µg protein/µg DW) increases with increasing food deprivation;
- the relative amount of lipid (µg lipid/µg DW) either increases (Figure 4.31) or remains approximately the same with increasing food deprivation;

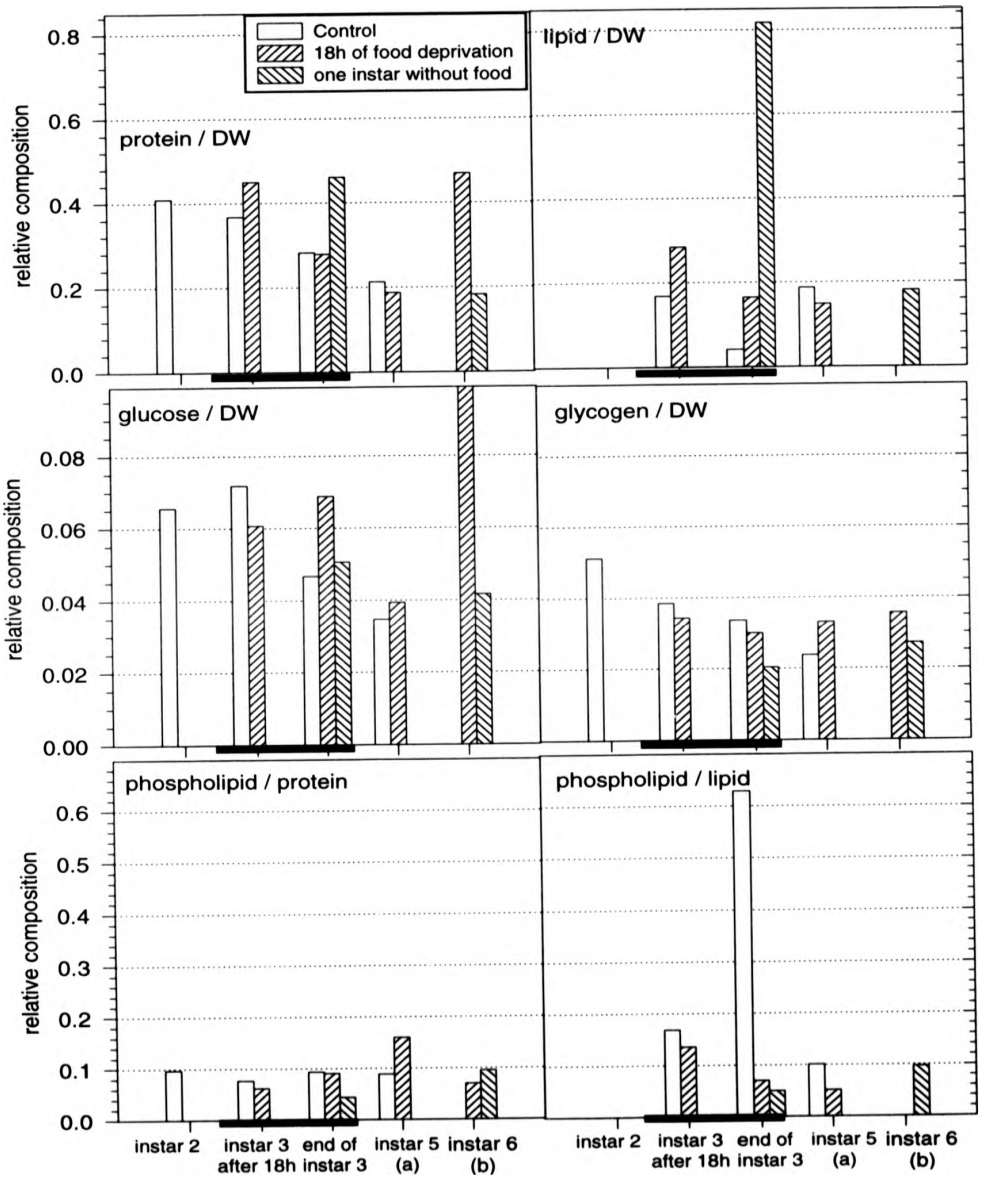


Figure 4.31 - Biochemical composition of individuals exposed to different periods of food deprivation during the 3rd instar. Samples were collected at the end of each instar unless stated otherwise. The black bar indicates the exposure period.

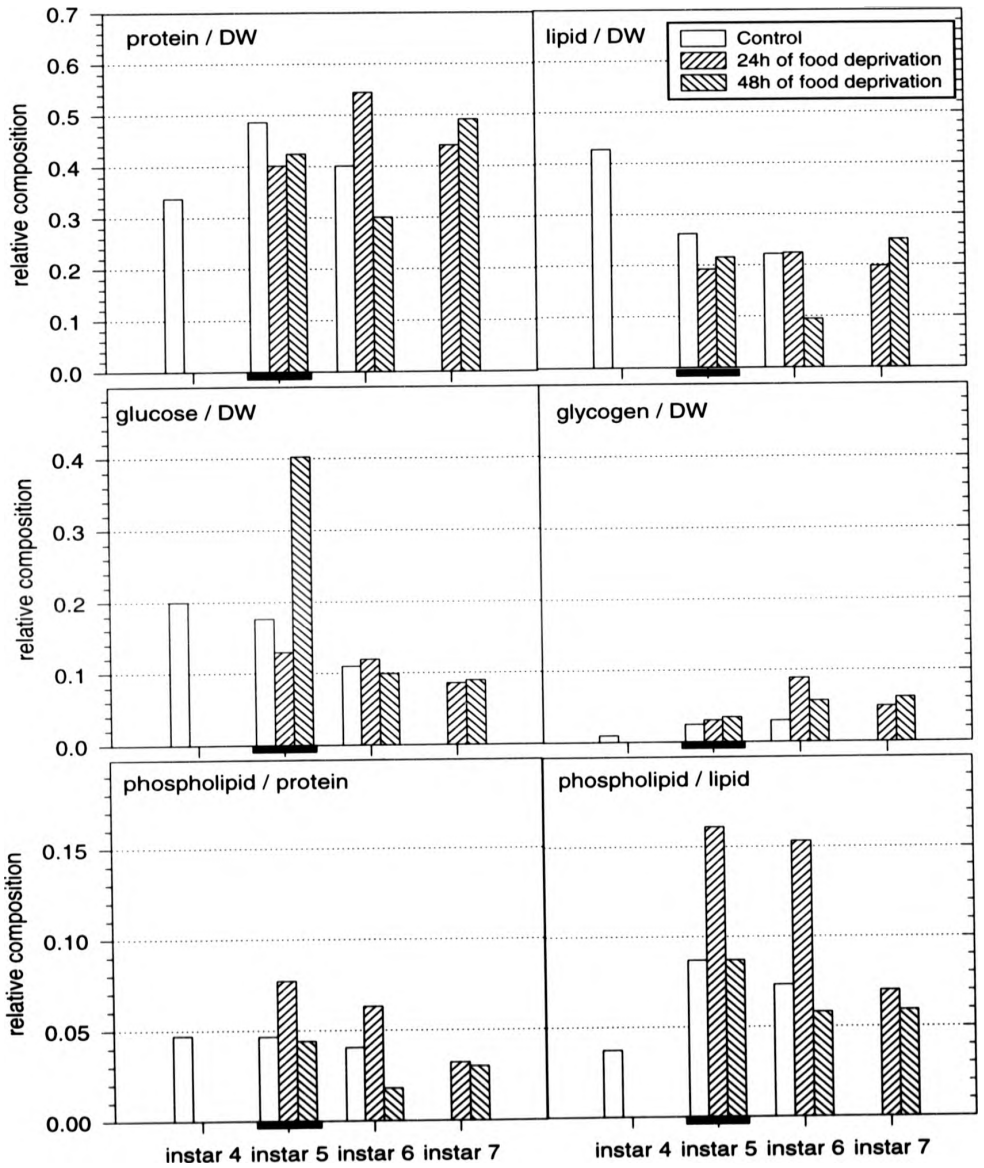


Figure 4.32 - Biochemical composition of individuals exposed to different periods of food deprivation during the first adult instar (5th instar). Samples were collected at the end of each instar. Black bars indicate the exposure period. Newly released eggs were included, if present in the brood pouch.

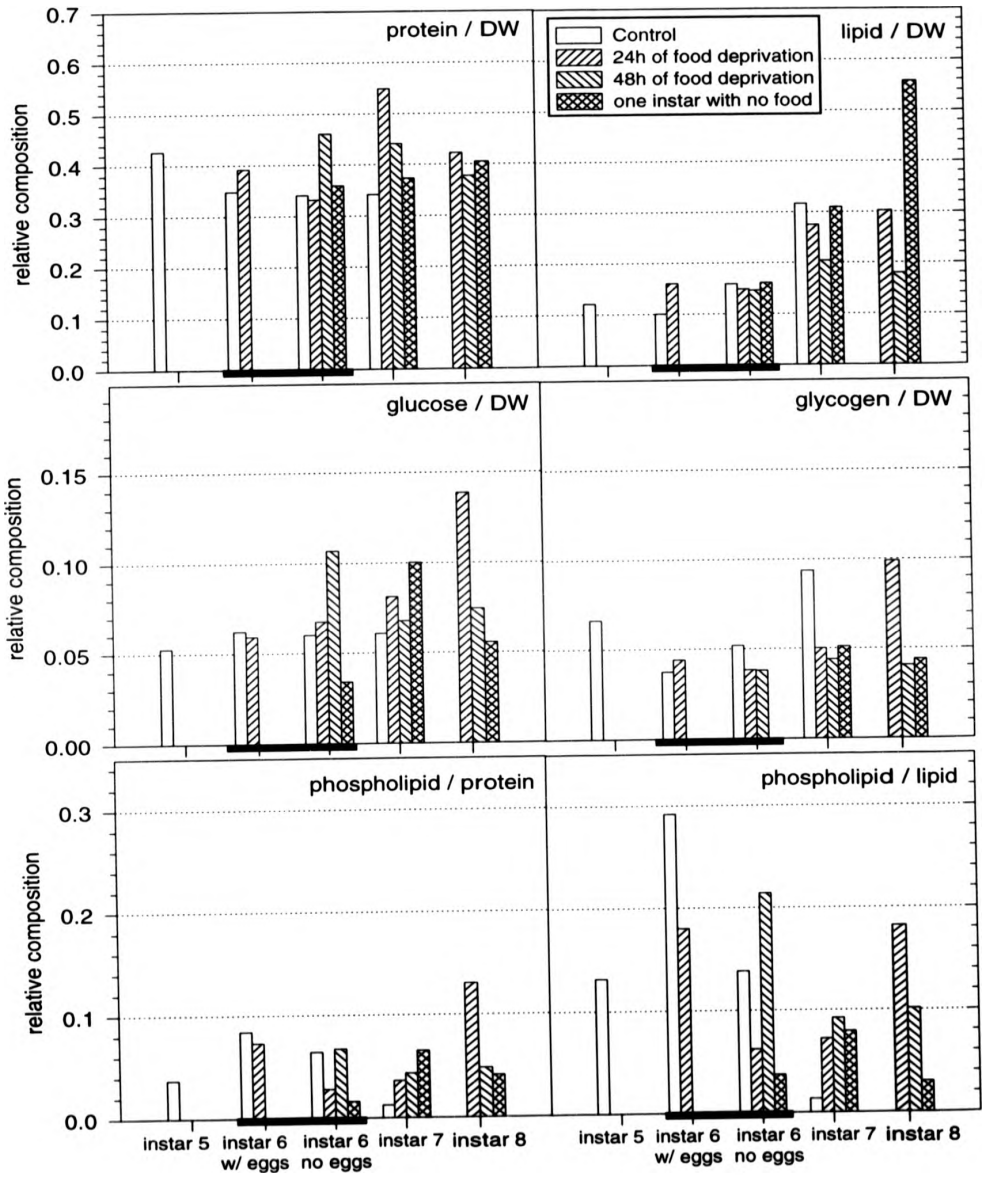


Figure 4.33 - Biochemical composition of individuals exposed to different periods of food deprivation during the first adult instar (6th instar). Samples were collected at the end of each instar. Black bars indicate the exposure period. Newly released eggs were included, if present in the brood pouch, unless stated otherwise.

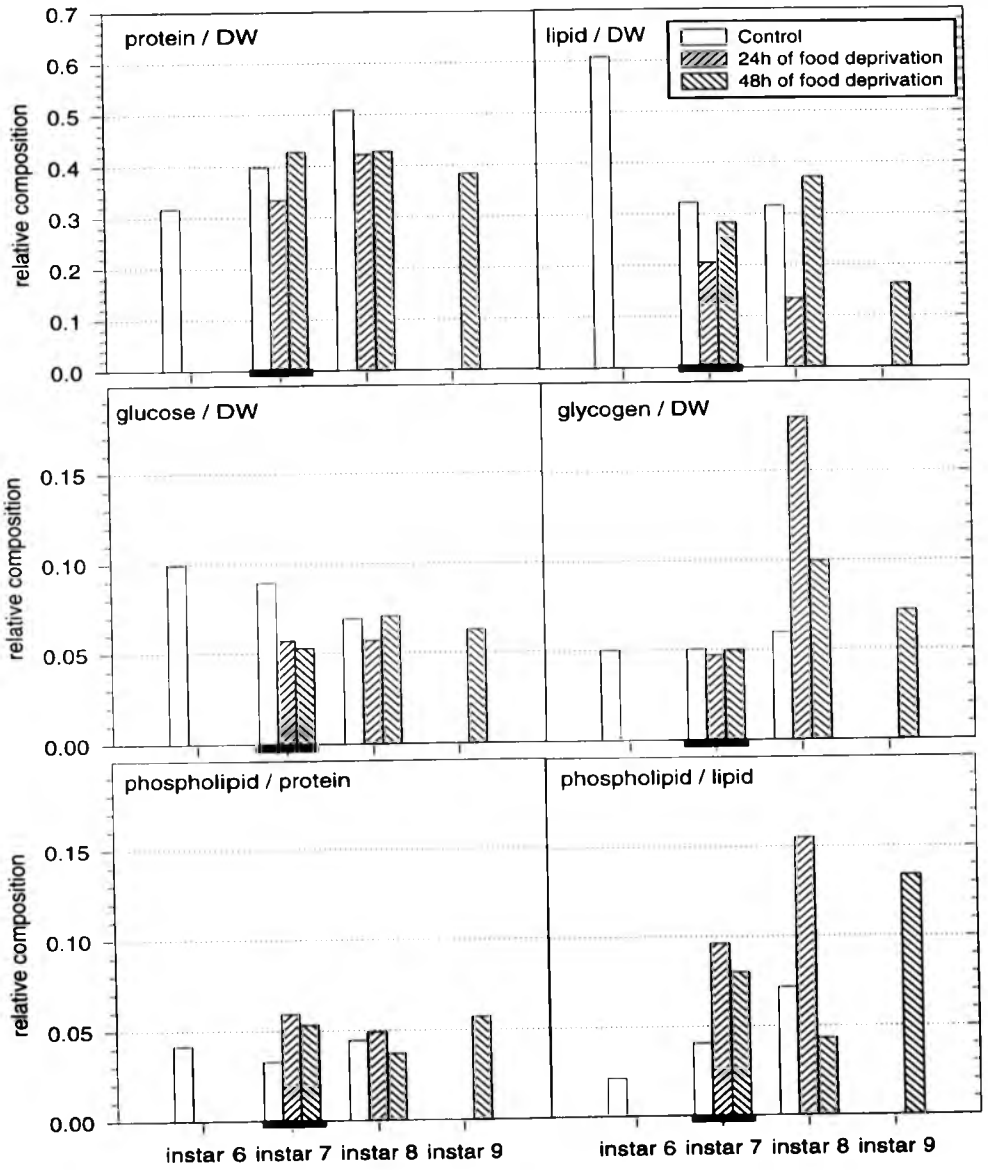


Figure 4.34 - Biochemical composition of individuals exposed to different periods of food deprivation during the first adult instar (7th instar). Samples were collected at the end of each instar. Black bars indicate the exposure period. Newly released eggs were included, if present in the brood pouch.

- the relative amount of sugars (µg sugar/µg DW), in particular glycogen which is more affected than previous groups of compounds, decrease with increasing food deprivation;
- phospholipids were used here as reference compounds since they can not be utilised as fuel for metabolic process (they are the main building blocks of biological membranes). Thus, phospholipid relative to protein ratio did not exhibit a consistent trend associated with the duration of the food deprivation period. Similarly, the phospholipid to lipid ratios do not exhibit a consistent trend although the main trend favours a decrease of the phospholipid ratio with increasing food deprivation in particular when the food deprivation period is more extended (compare Figure 4.31 - one instar of food deprivation, Figure 4.32 - 48 h of food deprivation, and Figure 4.33, one full instar of food deprivation).

4.4. DISCUSSION

The results presented here support the allocation model presented in section 3.2.6, since:

1. individuals grow faster during the adolescent stages while growth is reduced in the adult stages (Figure 4.17, page 147) implying a dynamic allocation between growth and the other physiological compartments;
2. growth stops in the absence of food (Figure 4.18 - page 148, Figure 4.20 - page 150, Figure 4.22 - page 153);

3. reproduction can occur under variable periods of food deprivation in the adult instars (Figure 4.19 - 24 h of food deprivation, page 149, Figure 4.20 - 24 h of food deprivation, page 150, Figure 4.22 - 24 h and 48 h of food deprivation, page 153);
4. under stress reproduction stops before survival is compromised (Figure 4.19 - 48 h of food deprivation, page 149, Figure 4.20 - 48 h and one instar of food deprivation, page 150); and
5. combining evidences 4 and 5 suggests the existence of a common pool (reserves) from which resources used for reproduction and survival are derived. Moreover, the existence of a residual fraction of reserves restricted tor maintenance is supported by evidence 4.

As predicted, the allocation of resources during the adolescent instars favours growth whilst in adults favours reserves. Moreover, reproduction stops far before survival is compromised, supporting the idea of a residual component for reserves. This approach is not difficult to accept if we consider the costs involved at these two stages: adolescents are only concerned with growing, moulting, and surviving whilst adults must also provide resources for reproduction. Moreover, adolescents have to survive and reach maturation as soon as possible to maximise their fitness whilst adults must also maximise their fecundity to achieve the same goal.

Assuming that the metabolic needs of an individual *Daphnia* must be provided first, it is possible to confirm the predicted allocation rules governing the different processes. The allocation priorities involve two separate approaches:

(1) metabolism > reserves storage >= growth

(2) metabolism (survival) > moulting (survival) > reproduction

The first approach considers the situation where the individual is feeding and the partitioning of resources involves only metabolism, reserves and growth. The second approach deals with the usage of reserves for metabolism, moulting, near future survival and egg provisioning when moulting is due to occur.

Survival is described in the model as the inability to cope with metabolic needs and/or moulting costs from existing reserves (including residual reserves). Only one of the existing models addressed the idea of residual reserves, applying the concept of weight-for-length (McCauley *et al.*, 1990; Gurney *et al.*, 1990). This concept involved the idea that for each length there was a weight below which the individual would invest all resources in regaining weight without investing in reproduction. Moreover, a continuous and irreversible commitment into egg provisioning during the whole instar has frequently been assumed (*e.g.* Kooijman & Metz, 1984; Kooijman, 1986; McCauley *et al.*, 1990; Gurney *et al.*, 1990; Bradley *et al.*, 1991a, b). This irreversible investment into eggs, without any knowledge of the chances of surviving to release the eggs, could seriously compromise individual fitness. This approach is difficult to accept, since death could result if environmental conditions did deteriorate ultimately to interfere with moulting.

The maturation threshold is probably one of the most important traits in *Daphnia* life-history. Adding one more instar to reach maturity will result in increased age at maturity and a larger first clutch. It has been assumed several times that female *Daphnia* must reach a certain size (Anderson, 1932; Taylor, 1985; Urabe, 1988; Lynch, 1989), rather than a certain age (Sinko & Streifer, 1969) before maturation is reached. However, Ebert was the first to determine this threshold size (Ebert, 1991, 1992, 1994a).

Two basic assumptions underline the theory concerning the evolution of offspring size: limited resources dictate a trade-off between offspring size and number, and gains in offspring fitness are a diminishing function of offspring size. Nonetheless, it has been found that there is a substantial within-clutch and among-clutch variation in size of eggs of single *Daphnia* clones (Green, 1954, 1956; Lynch, 1985).

The variability in size of the first clutch has been explained considering that females maturing later could compensate for their delayed maturity by investing in a larger number of eggs (Ebert, 1991). However, as observed, a more simple explanation is the relationship between female body length and the number of eggs it produces.

Egg size and the body length of the female are positively correlated (Glazier, 1992; Boersma, 1995). It was also been observed that neonate size increases with the age at maturity of the mother (Bell, 1983). Under the same food conditions females maturing later have a larger size at first clutch (Figure 4.29). These results can be easily explained

by a faster food depletion in vessels with larger females resulting in higher values for the RFH and consequently in larger egg size.

If it often assumed that offspring produced under poor food conditions should be larger than those produced under good conditions (Green, 1966; McCauley *et al.*, 1990; Ebert, 1994a, b). This pattern is supported by experimental evidence when the number of eggs per clutch is greater than three (Ebert, 1993; Urabe, 1988; Tessier & Consolatti, 1991; Glazier, 1992). However, the alternative situation (*i.e.* clutches with less than three eggs) should only occur under very poor food conditions. Under very low food conditions the trade-off between egg size and clutch size is favourable with regard to the former, probably maximising the fitness of the mother (Glazier, 1992; Ebert, 1993). The simplest explanation for this strategy is that a female provisioning a very small number of eggs will decrease the average egg mass, maximising egg number to the nearest whole integer number of eggs.

The size of a neonate is probably one of the most important life-history traits in *Daphnia,* since both age and size at first clutch are strongly correlated with it (Ebert, 1991). The hatchability of the eggs increases with egg mass (Bell, 1983) and larger neonates take fewer instars to reach maturity (Green, 1954, 1956; Ebert, 1991) whilst having better chances of survival (Gliwicz & Guisande, 1992; Tessier & Consolatti, 1989). Thus, it has been generally assumed that offspring fitness is an asymptotic function of size (Bell, 1983; Tessier & Consolatti, 1989) and thus a trade-off between clutch size and egg mass must exist (Glazier, 1992; S ibly & Antonovics, 1992).

The relationship between neonate mass and starvation time follows a power function with an exponent of 0.25 (Threlkeld, 1976; Tessier *et al.*, 1983). This exponent, found for several organisms, is expected on the basis of decreased specific metabolic rate with increased body size (Peters, 1983). This agrees with measurement of triacylglycerol utilisation which followed a power function with exponent of 0.73, which is close to the predicted value of 0.75 (Goulden *et al.*, 1987).

5. MODEL VALIDATION

"...the physicist is not concerned with what *is* true, but only with what he can measure"

Papoulis (1965), Probability, Random Variables and Stochastic processes

5.1. INTRODUCTION

A model must be tested to ensure that it is reliable, error-free, and has credibility with those who are to use it. This process involves three major steps (Davies and O'Keefe, 1989):

- **experimentation** - once the model structure and assumptions are fully tested, the parameters are varied to optimise their estimates and to determine the behaviour of the model (see Chapter 3);
- **validation** - the process of checking whether the model looks and behaves like a real system rather than the checking the programming logic, and
- **sensitivity analysis** - assesses the responsiveness of the model, particularly the most important endpoints (*i.e.* growth in length, age at release of first clutch, and cumulative fecundity), to different assumptions and changes in factor levels

5.2. MODEL VALIDATION

The *Daphnia* model presented here is a physiological model incorporating the description of non-lethal effects of toxicants on individuals. Thus, these two aspects of the model were validated independently using: different food conditions without toxic stress, and similar food conditions with differing toxic stress. The evaluation of the model predictions was performed both graphically and statistically.

5.2.1. Statistical analysis

Statistically the model predictions were evaluated in two steps:

- calculation of the regression line between observed and predicted values for the different endpoints, together with a correlation coefficient for the association between the two sets of values, and
- testing the hypothesis of an exact prediction, *i.e.* an exact prediction would be equivalent to a line with intercept equal to zero and slope of one. Thus the standard t test procedure (Zar, 1984) was used to compare the intercepts with zero and the slopes with one.

5.2.2. Validation for different food regimes

Chronic toxicity tests with *Daphnia* are usually run for 21 days starting with individuals less than 24 hours-old. A control without toxicant must be used every time a chronic toxicity test is run. The feeding regime used is usually approximately 5 µg $DW.ml^{-1}.d^{-1}$ that is equivalent to 5×10^5 cells ml^{-1} d^{-1} of *Chlorella vulgaris* (*e.g.* Barber *et al.*, 1994; Allen *et al.*, 1995). Nonetheless, it was considered also important to evaluate model performance under lower food conditions to check robustness of the model.

Two data sets corresponding to two food regimes (0.5 µg DW ml^{-1} d^{-1} and 5 µg DW ml^{-1} d^{-1}) from Waddell (1993) were used (Figure 5.1, Figure 5.2). The simulation and experimental conditions were the same:

- culture volume: 120 ml,
- feeding regime: daily,
- medium renewal: every other day,
- simulation period: 25 days.

Data from controls used in the experimental work (Chapter 4) were also used (Figure 5.3). The data were generated using a slightly different experimental scenario:

- food regime: 7 µg DW ml^{-1} d^{-1}
- culture volume: 100 ml,
- feeding regime: daily,
- medium renewal: every other day,
- simulation period: 25 days.

The model endpoints used in the validation were: change in body length and clutch size. Since the model uses egg size as a starting point, an average egg size was arbitrarily chosen. Model simulations for the previously outlined conditions (Figure 5.1, Figure 5.2, Figure 5.3) were overlaid with the 95% and 99% confidence limits of the means of experimental data. Mass predictions from the model were also plotted versus the average mass of pooled samples of individuals (Chapter 4) of known age (Figure 5.4).

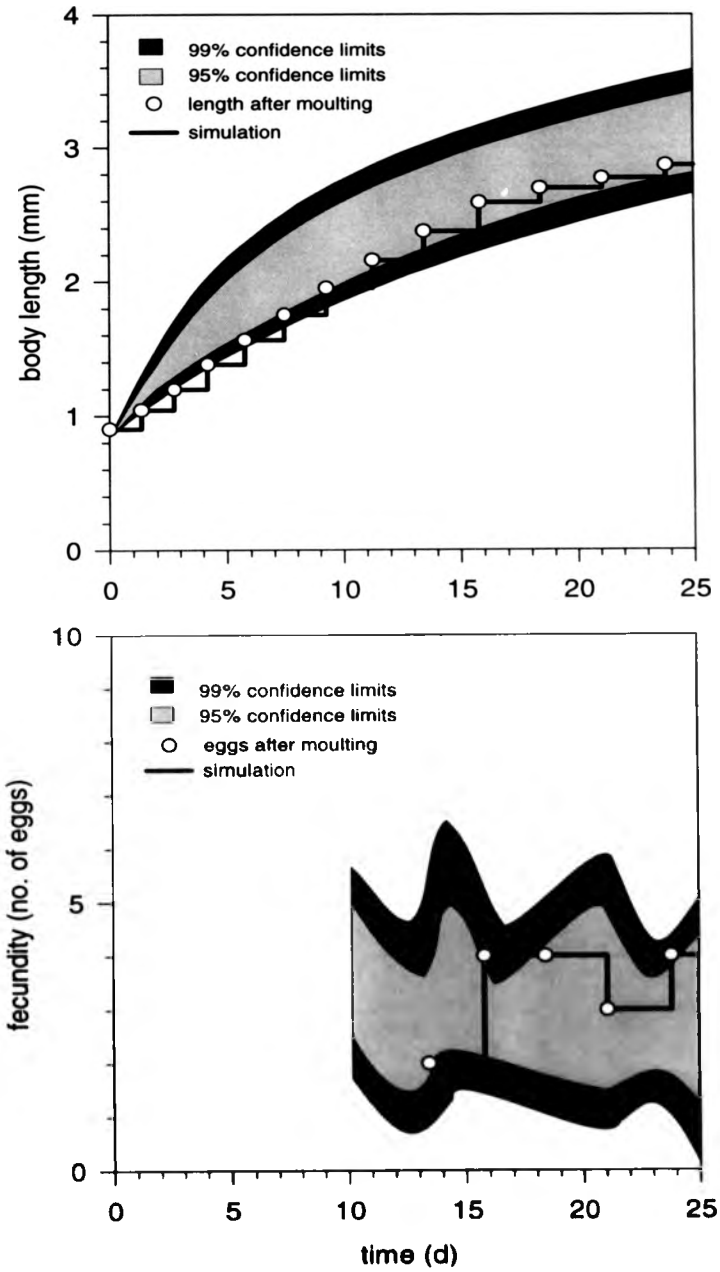


Figure 5.1 - Model validation at a low ration level (0.5 µg DW ml^{-1} d^{-1}). Shaded areas are confidence limits of experimental data from Waddell (1993).

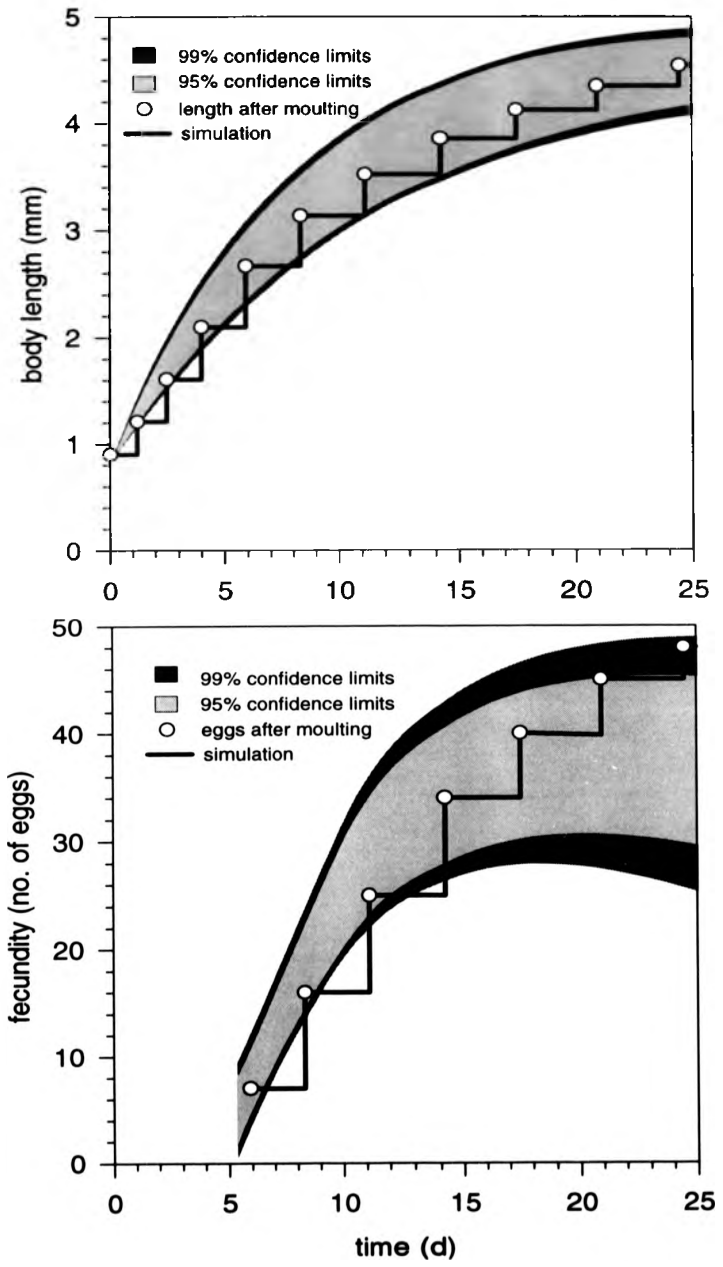


Figure 5.2 - Model validation at a high ration level (5 µg DW ml^{-1} d^{-1}). Shaded areas are confidence limits of experimental data from Waddell (1993).

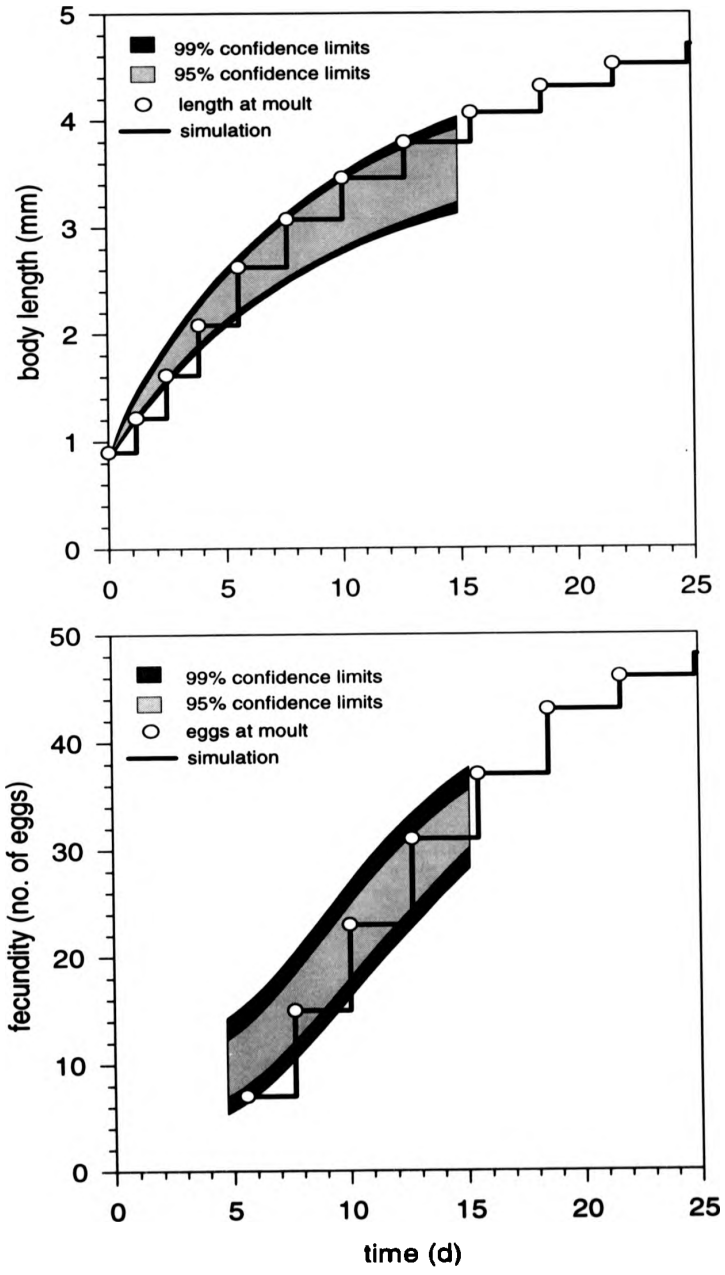


Figure 5.3 - Model validation at a non limiting ration level (7 µg DW ml^{-1} d^{-1}). Shaded areas are confidence limits of experimental data (controls) from Chapter 4.

a) Body length and fecundity

Graphically, the simulated values constantly fall always within the 99% confidence limits of the experimental means, although most values fall also within the narrower band of the 95% confidence limits, particularly after maturation occurred. Daily experimental averages, for both body length and fecundity, are strongly correlated with the model predictions (P is always less than 0.001). Model predictions were regressed against daily averages of experimental values of body length and fecundity, whilst regression parameters were tested as mentioned before (Table 5.1).

All regression models, except one, exhibited a slope that did not differ significantly from one. Model intercepts were more variable with two not differing significantly from zero, two differing significantly from zero and only two that exhibited a highly significant difference from zero. Statistically it can be stated that a good agreement was found between model predictions and experimental data (significant correlation coefficients and slopes statistically not different from one) although sometimes predictions slightly underestimated some of the endpoints (intercepts significantly greater than zero).

Table 5.1 - Statistical testing of the hypotheses of null intercept (α=0) and slope equal to one (β=1) for the regressions between experimental and simulated values of body length and fecundity for three food regimes: (NS) not significant, (*) significant, and (**) highly significant.

Food regime	Endpoint	α	α=0		β	β=1	
			t	p		t	p
0.5 µg DW.ml^{-1}.d^{-1}	body length	0.39	2.29	0.035 (*)	0.98	-0.23	0.820 (NS)
	fecundity	3.31	10.69	0.0001 (**)	-0.12	-10.54	0.0001 (**)
5 µg DW.ml^{-1}.d^{-1}	body length	0.23	1.21	0.241 (NS)	0.98	-0.27	0.790 (NS)
	fecundity	8.16	2.45	0.028 (*)	0.76	-1.98	0.067 (NS)
7 µg DW.ml^{-1}.d^{-1}	body length	0.24	1.36	0.206 (NS)	0.90	-1.32	0.22 (NS)
	fecundity	6.18	8.23	0.004 (**)	0.86	-2.70	0.074 (NS)

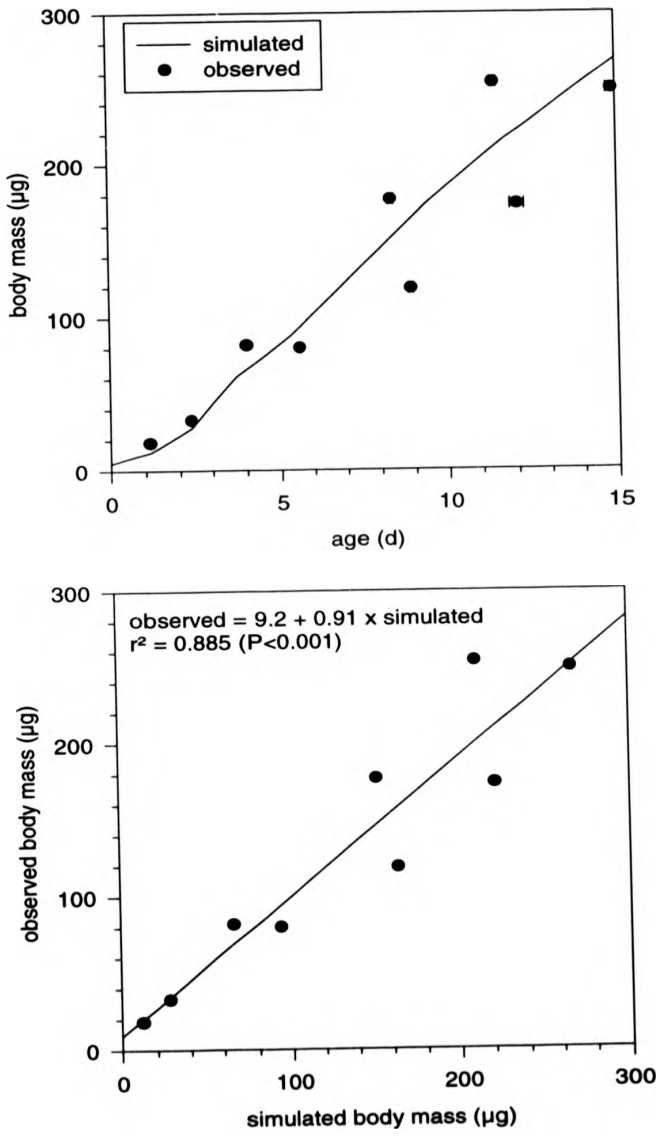


Figure 5.4 - Validation of mass predictions of the model: mass at different ages (top) and predicted mass versus observed mass (bottom). Error bars are 95% confidence limits of the mean age.

The prediction of the fecundity at a food regime of 0.5 µg $DW.ml^{-1}.d^{-1}$ was an exception (intercept significantly different from zero and slope significantly different from one) although predicted results fell within the 95% confidence limits. These apparently contradictory results can be easily explained. At low food regimes, the regularity of food addition to the culture medium becomes critical. A slight change in the feeding schedule can result in drastic changes in food concentration and consequently in the feeding rate of the individuals during that period. Thus, body length and the amount of available reserves, which determines the number of eggs provisioned, can be highly variable within an instar.

b) Mass prediction

Although the model is accurate in predicting growth and fecundity under different food conditions the mass measured experimentally at different instars was compared with the model predictions (Figure 5.4). Average weights of individuals killed after moulting and without eggs (if they were present) were plotted against the simulated values for residual mass (structure + residual reserves). Predictions and experimental values were strongly correlated ($r = 0.941$, $P < 0.001$) while the intercept and the slope were not statistically different from zero and one, respectively (intercept: 9.2 $t = 0.47$, $P = 0.655$; slope: 0.91, $t = 0.74$, $P = 0.482$).

5.2.3. Simulation of different exposures to cadmium

Cadmium is an abundant, non-essential metal that accumulates in the environment as a result of industrial practices such as electroplating and galvanising, zinc and lead mining, smelting, and battery production (Friberg *et al.*, 1974; Nriagu, 1980). Cadmium toxicity and modes of action (Stohs & Bagchi, 1995), carcenogenicity (Manca *et al.*, 1991; Koizumi & Li, 1992), and bioaccumulation (see Stoeppler & Piscator, 1988) are well documented among vertebrates. Cadmium does not generate free radicals (Ochi *et al.*, 1987) but induces lipid peroxidation in tissues (Muller, 1986), and it is known to be an inducer of metallothioneins and other stress proteins (Bauman *et al.*, 1993).

Cadmium was chosen as the reference toxicant for model calibration since effects of cadmium on feeding (Allen *et al.*, 1995) and respiration (Barber *et al.*, 1994) are well documented. Thus, toxicant-dependent parameters could be determined with reliability (see Chapter 3). Moreover, I had access to raw data on growth of *Daphnia* individuals under several non-lethal cadmium concentrations (0.2 µg/ml of Cd, 0.4 µg/ml of Cd, and 1 µg/ml of Cd) (Waddell, 1993).

All predicted and experimental values were strongly correlated ($P < 0.001$) and only the control exhibited a slope that was statistically different from one (Table 5.2). Thus, it can be stated that individual-level effects of non-lethal exposure to cadmium in *Daphnia* are properly described by the model (Chapter 3) for all experimental concentrations. Moreover, the model is able to accurately predict non-lethal toxic effects on growth and reproduction using the effect of toxicants on feeding (see Allen *et al.*, 1995).

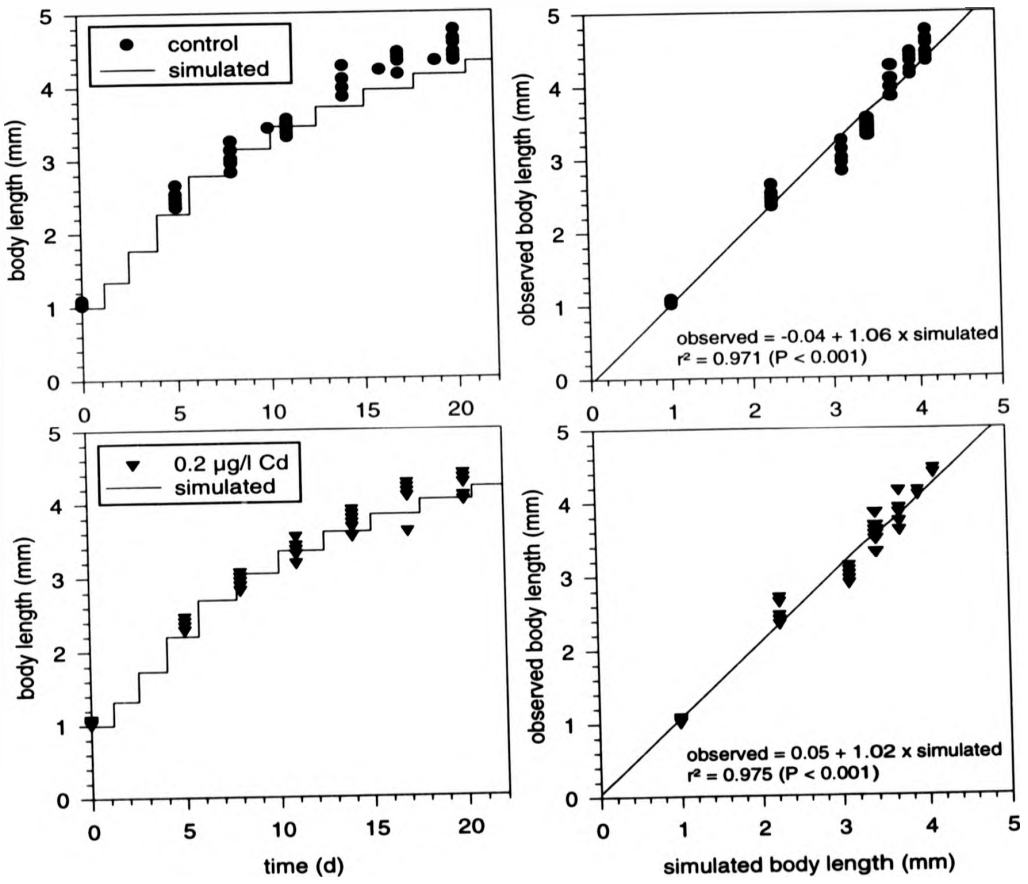


Figure 5.5 - Simulation of chronic stress for different cadmium exposures using experimental data from Waddell (1993): control and 0.2 µg/l of Cd.

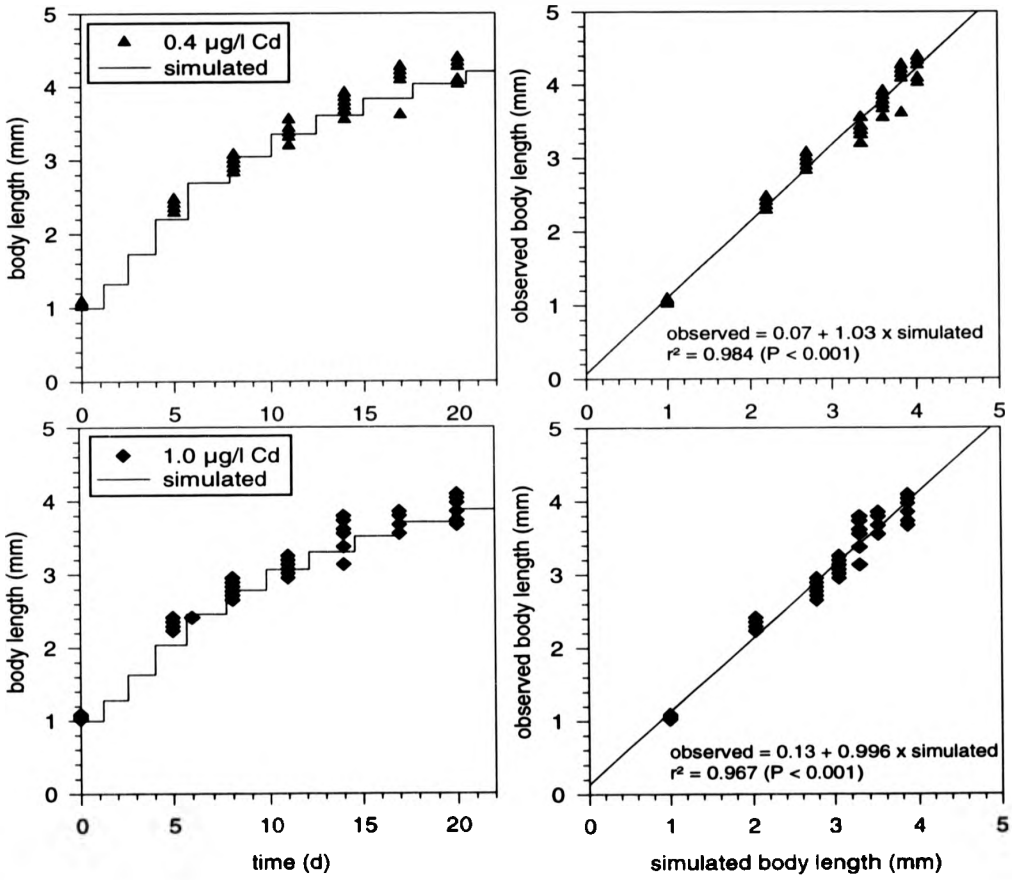


Figure 5.6 - Simulation of chronic stress for different cadmium exposures using experimental data from Waddell (1993): 0.4 µg/l of Cd and 1 µg/l of Cd.

Table 5.2 - Statistical testing of the hypotheses of null intercept (α=0) and slope equal to one (β=1) for the regressions between experimental and simulated values of body length for three cadmium concentrations plus a control: (NS) not significant, (*) significant, and (**) highly significant.

Toxicant	α	α=0		β	β=1	
		t	p		t	p
0.0 µg/l Cd	-0.04	-0.57	0.573 (NS)	1.06	2.66	0.010 (*)
0.2 µg/l Cd	0.05	0.72	0.472 (NS)	1.02	0.97	0.339 (NS)
0.4 µg/l Cd	0.07	1.30	0.200 (NS)	1.03	2.05	0.044 (*)
1.0 µg./l Cd	0.13	1.97	0.053 (NS)	0.99	-0.17	0.866 (NS)

5.3. SENSITIVITY ANALYSIS

The sensitivity analysis studies the responsiveness of a model to different levels of changes in the parameters. The sensitivity analysis is an important step in the overall validation for several reasons:

- to ascertain that the model still produces sensible and credible results when factors are varied;
- to look for ways of simplifying the model structure. For instance, if a certain level of change in a parameter does not affect the model outputs, then it may be possible to simplify the model by removing it; and
- to check the effects of using parameters which are based on poor quality data.

5.3.1. Methodology

The sensitivity of the model to the parameters used to describe the physiology of an individual, under non toxic conditions, was determined using three food regimes: 0.5 μg DW ml^{-1} d^{-1}, 5 μg DW ml^{-1} d^{-1}, and 50 μg DW ml^{-1} d^{-1}. The endpoints used were age at first clutch (defined as release of the first clutch into the brood pouch), and body length and cumulative fecundity after 30 d. Variations of ±10% and ±50% were imposed, individually, to each parameter (although the degree of uncertainty about each parameter was always within ±10% of the values used in the model). The sensitivity of the parameters, S_x, was determined for each endpoint using the formula proposed by Jorgensen (1988):

$$S_x = \frac{\frac{\partial x}{x}}{\frac{\partial Param}{Param}} \qquad (5.1)$$

where: x - selected state variable

Param - examined parameter.

5.3.2. Results

The results of the sensitivity analysis, which are given in Appendix A, were summarised as average sensitivity scores, representing the average sensitivity of each parameter when all food regimes and parameter variations were considered (Figure 5.7). I chose to classify the parameters under three categories of sensitivity using the following criteria:

- low sensitivity when a relative change in the parameter caused a relatively reduced change in the endpoints (0 - 0.5),
- medium sensitivity when a relative change in the parameter caused a similar level of relative change in the endpoints (0.5 - 1.0), and
- high sensitivity when the relative change in the endpoints was relatively greater than the change in the parameter (> 1.0).

The parameters for which I did not have any experimental support (*i.e.* ε, Q_{res}) were classified as low sensitivity parameters. Moreover, highly sensitive parameters were associated, as could be expected, with structure to length relationships (*i.e.* α, β), length to feeding relationships (*i.e.* δ, η, L_d), and maximum partition into reserves (Q_{max}) (see

Figure 5.8). The model is more sensitive to the values of the parameter when either a very high or a very low food regime is used. However, the robustness of the model increases when an intermediate food regime, similar those usually used in experimental work, is chosen. Thus, simulations of experimental conditions are expected to be more accurate when feeding regimes close to 5 μg DW ml^{-1} d^{-1} are used. The deposition speed of the food particles (t_s) is the only parameter, included in the medium sensitivity group, which is highly sensitive only under low food regimes. Thus, it must be carefully defined for the type of food used if it is intended to run simulations under low food regimes.

Clutch size was more affected by parameters associated with length relationships (α, β, δ, η) than body length. The maximum partitioning into reserves (Q_{max}), whilst being critical to increase in length, has only a small effect on clutch size. The length depression factor on feeding (L_d) has a negligible effect at ration level of 5 μg DW ml^{-1} d^{-1} (Figure 5.8) and above, considering a variation of ±10% (Appendix A, Table A-3), but plays an important role at low ration levels (*i.e.* 0.5 μg DW ml^{-1} d^{-1}) (Appendix A, Table A-1).

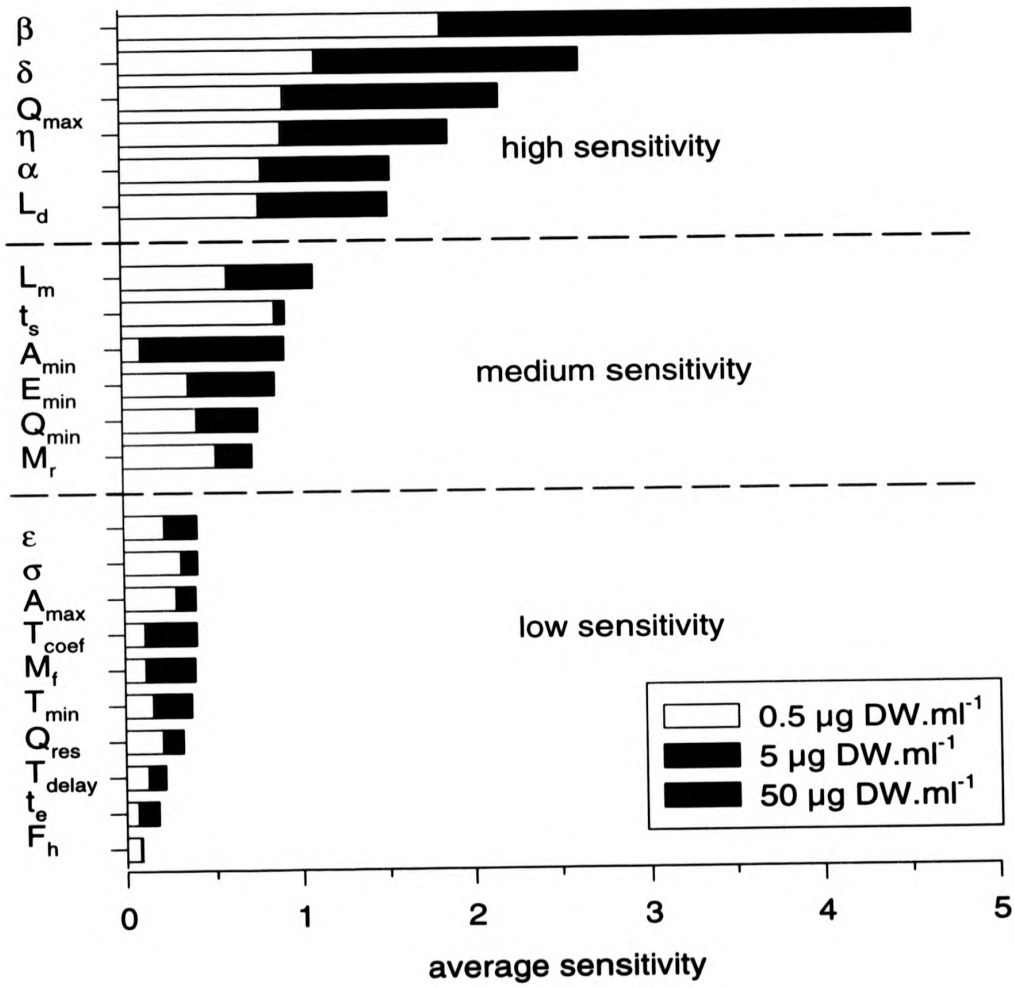


Figure 5.7 - Average sensitivity scores of model parameters for different food regimes (see text for explanation)

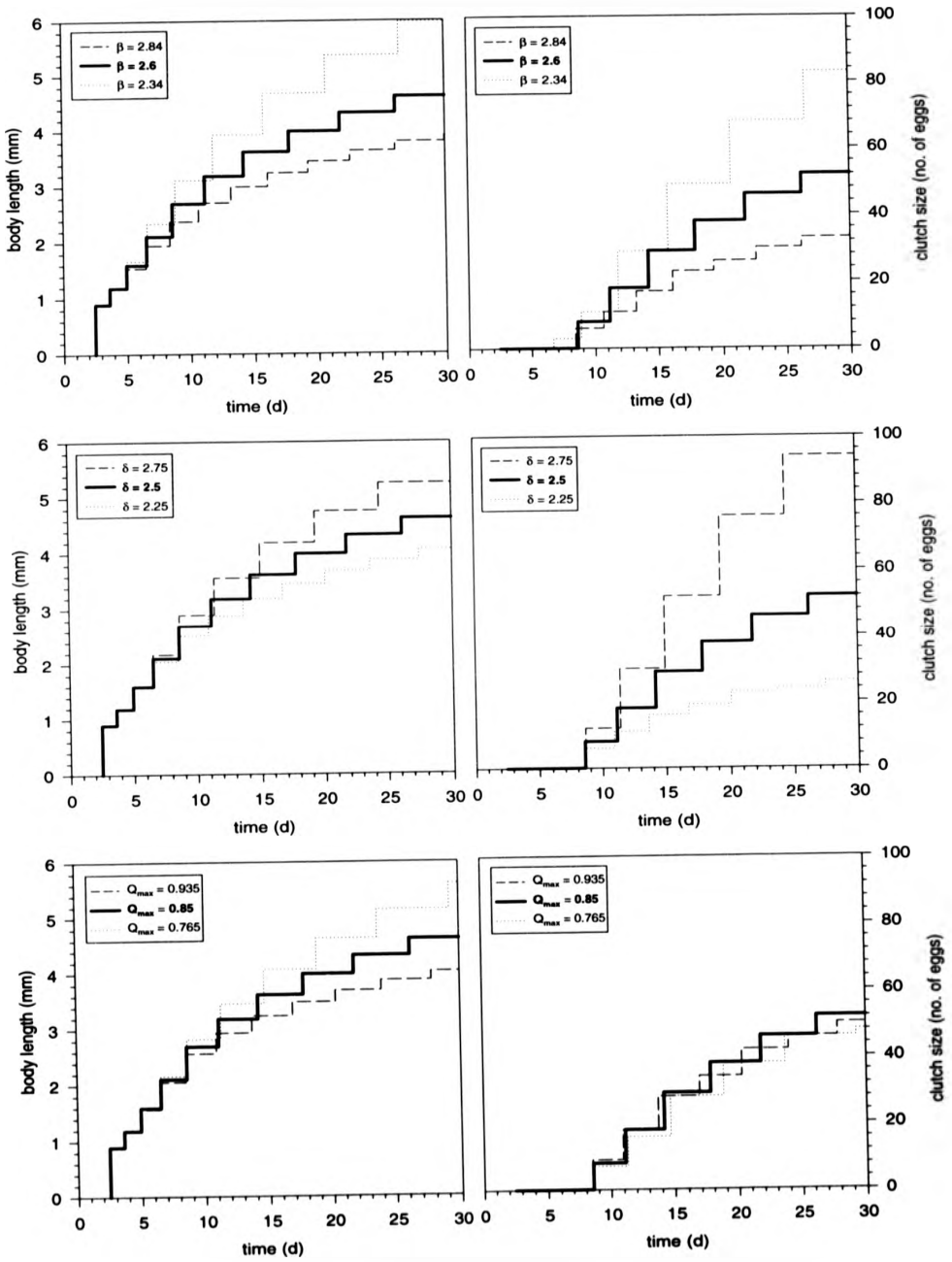


Figure 5.8 - Effects on growth and clutch size of changing the most sensitive parameters of the model (±10%).

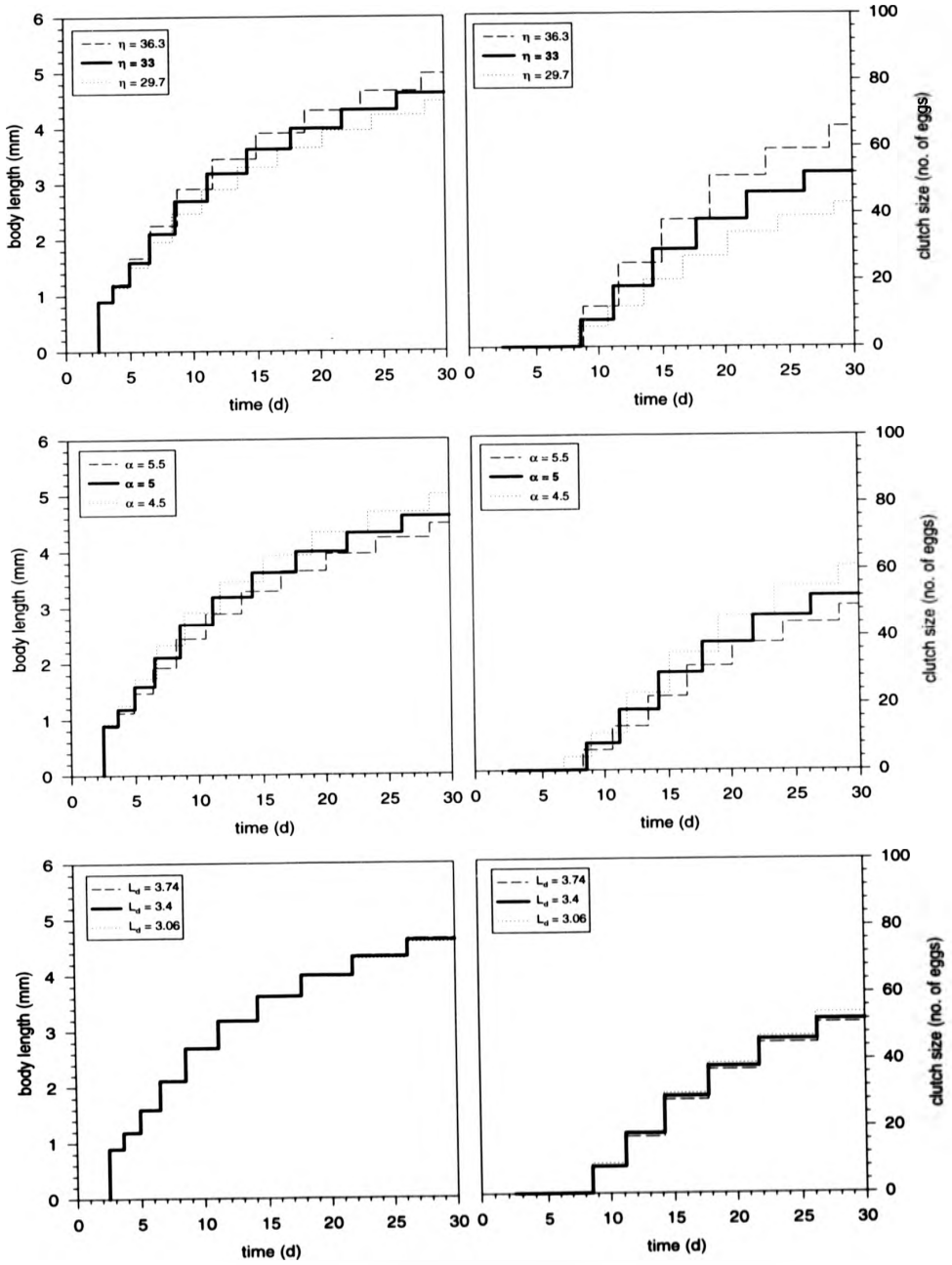


Figure 5.8 (continued) - Effects on growth and clutch size of changing the most sensitive parameters of the model (±10%).

5.4. CONCLUSIONS

The results from the model validation can be summarised as:

1. the model is well-posed and robust; *i.e.* the model managed to capture essential and basic properties of the living organism such as
   - the allocation priorities of an individual *Daphnia*,
   - variable instar duration,
   - flexibility of adjustment to variable food conditions,
   - environmental determination of egg-size, and
   - life-cycle development from egg to adult.
2. the model behaviour is consistent at both low and high food regimes since it is able to reasonably predict model endpoints for differing food regimes;
3. all highly sensitive parameters were calculated from published data, thus they were determined with reasonable accuracy;
4. the model is relatively insensitive to all estimated parameters, *i.e.* parameters for which data was not available.

6. DISCUSSION

"Truth is rarely pure, and never simple"

Oscar Wilde (1854-1900)

Daphnia has several characteristics that make it an ideal species for use in ecological and ecotoxicological studies. The increased interest in investigating details of *Daphnia* life-history traits and their sensitivity to toxic stress has resulted in the development of several models describing the physiology of parthenogenetic individuals (Gabriel, 1982; Paloheimo *et al.*, 1982; Kooijman & Metz, 1984; Kooijman, 1986a, 1993; Gurney *et al.*, 1990; Hallam *et al.*, 1990a, b; Lassiter & Hallam, 1990; McCauley *et al.*, 1990). Some models have been concerned with capturing basic properties of individuals to be used in individual-based populations models (Kooijman & Metz, 1984; Nisbet *et al.*, 1989). Others have also tried to describe the interactions between the physiology of individuals and their response to toxic stress at population level (Lassiter & Hallam, 1990; Hallam *et al.*, 1990b). However, these interactions were described only for lipophilic substances for which Quantitative Structure Activity Relationships (QSAR) could be derived. Although these interactions are important for the bioaccumulation of organic pollutants they do not address the problem of other classes of pollutants (*e.g.* metals).

The main purpose of this thesis was to produce and validate an individual model of a *Daphnia* using a general framework of basic rules derived from life-history biology. The model was designed to have a simple structure with as few parameters as possible whilst linking the physiology of an individual with its response to toxic stress. The main weaknesses of existing models was their inability to predict the physiological state (growth, survival and reproduction) of an individual, over a wide range of food and toxicant concentrations.

Moulting is a crucial process in a crustacean life-cycle. However, none of the existing models above describe it as a dynamic process, probably since existing information is poor and contradictory. Thus, the experiments which were carried out concentrated on the interactions between moulting, other physiological process (growth, survival, and reproduction) and environmental conditions (food levels).

Several conclusions could be drawn from this thesis regarding the experimental results:

1. Individuals retain a certain amount of resources to support their metabolic needs for some time whilst still allowing them to moult. However, these resources are not available to allow increase in body length (growth).
2. Increases in reserves and increases in length (growth) under feeding, occur in parallel, with growth stopping when feeding stops.
3. Egg are provisioned from available reserves.
4. Egg size and instar duration are correlated with the integration of most recent feeding conditions (recent feeding history)
5. The biochemical composition of individuals exposed to different food conditions can be a good indicator of physiological conditions only if larger replicated samples are used.
6. Pulsed periods of food deprivation are a useful disturbance factor which can give further insights concerning the physiological adaptations of individuals.

The model proposed in this thesis is very simple and makes very few assumptions since it is based on the understanding of the biological processes underlying resource

allocation in individual animals. Only egg mass and culture conditions must be specified as initial conditions for the model to run, since the individual development is simulated from the egg stage.

Although the model has good predictive abilities there are a few points which deserve further investigation to generate accurate information about different processes:

1. The relationship between instar duration and body length must be validated for larger individuals to ascertain if the relationship follows a power function or a sigmoid curve (*i.e.* if instar duration increases at an increasing rate with the body length of the individual or if it increases at an increasing rate until maturation, and after that at a decreasing rate, until gradually levelling off).
2. Feeding and metabolic rates need to be determined for different clones since they will determine the responses to stress and the survival of an individual hence its fitness. Special attention needs to be given to obtain accurate measurements of specific metabolic rates under different food levels and toxicant concentrations (it must be checked if the specific metabolic rate is a linear or decreasing function of body mass).
3. Egg mass variation within and among clutches under different environmental conditions (food levels, toxic stress) needs more study since it can have drastic consequences for the adaptative abilities of a species or a clone to a specific environment. Moreover, this knowledge will be critical if in the future we wish to convert the model into a stochastic model.

The model is adaptive, and its ability to predict dynamic changes in allocation of energy to different processes can help track environmental fluctuations and their effects in accelerated real time. Since the model is built upon the understanding of some basic 'rules of life' it is possible to carry out simulations under conditions different from those under which it was parameterised. Thus, the model can be used:

a) to extrapolate from short-term responses (*e.g.* feeding under toxicant exposure) to long-term responses;

b) to study the effects of pulsed phenomena (*e.g.* cyclic and non-cyclic food fluctuations, episodic pollution).

c) as a tool to help design experiments;

d) to extrapolate from unitary environmental conditions to a range of novel environments, offering a potential breakthrough in the use of toxicity data in risk assessment;

e) as a teaching aid, as it allows students to run simulations of individual development.

I foresee some future development to the model. Using the basic rules of the model it can be gradually extended to wider range of organisms (*e.g.* cnidarians, planarians, isopods, vertebrates) with different feeding strategies (*e.g.* selective feeding) or occupying other ecological niches (*e.g.* terrestrial environment). The model can also evolve to incorporate genetic variation into model parameters (stochasticity) and higher-order processes like group effects or predator-prey interactions.

References

Adema, D.M.M. & G.J. Vink (1981). A comparative study of the toxicity of 1,1,2-trichloroethane, dieldrin, pentachlorophenol and 3,4-dichloroaniline for marine and freshwater organisms. *Chemosphere* **10**(6): 533-554.

AFNOR (1974). *Determination de l'inhibition de la mobilite de Daphnia magna* Straus (crustace cladocere). Norme experimentale T 90-301..

Ahlgren, G., I.-B. Gustafsson & M. Boberg (1992). Fatty acid content and chemical composition of freshwater micro-algae. *J. Phycol.* **28**: 37-50.

Allen, Y., P. Calow & D.J. Baird (1995). A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. *Environ. Toxicol. Chem.* **14**(9): 1625-1630.

Anderson, B.G. (1932). The number of pre-adult instars, growth, relative growth, and variation in *Daphnia magna*. *Biol. Bull.* **63**: 81-98.

Anderson, B.G. & J.C. Jenkins (1942). A time study of events in the life span of *Daphnia magna*. *Biol. Bull.* **83**: 260-272.

Anderson, B.G., A.L. Buikema, Jr. & J. Cairns, Jr. (1980). Aquatic invertebrates in tolerance investigations from Aristotle to Naumann. *In*: *Aquatic invertebrates bioassays*, ASTM/STP-715, pp. 3-35.

APHA (1980). *Standard methods for the examination of water and wastewater*. 15th edn. American Public Health Association. Water Pollution Control Federation. New York, NY.

Argentesi, F., R. De Bernardi, G. Di Cola & M. Manca (1987). Mathematical modelling of *Daphnia* populations. *Mem. Ist. Ital. Idrobiol.* **45**: 389-412.

Arnold, D.E. (1971). Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue-green algae. *Limnol. Oceanogr.* **16**(6): 906-920.

Arruda, J.A. (1983). Daphnid filtering comb area and the control of filtering rate. *J. Freshwat. Ecol.* **2**(3): 219-224.

ASTM (1980). *Standard practice for conducting acute toxicity tests with fishes, macroinvertebtrates, and amphibians*. E-729-80. American Standards for Testing and Materials. Philadelphia, PA.

Baird, D.J., I. Barber, M. Bradley, P. Calow & A.M.V.M. Soares (1989a). The *Daphnia* bioassay: a critique. *Hydrobiologia* **188/189**: 403-406.

Baird, D.J., A.M.V.M. Soares, A. Girling, I. Barber, M.C. Bradley & P. Calow (1989b). The long-term maintenance of *Daphnia magna* Straus for use in ecotoxicity tests: problems and propects. *In*: Lokke, H., H. Tyle & F. Bro-Rasmussen, (ed.) *Proceedings of the 1st European Conference ion Ecotoxicology*, Lyngby, Denmark, pp. 144-148.

Baird, D.J., I. Barber, M. Bradley, A.M.V.M. Soares & P. Calow (1991a). A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. *Ecotoxicol. Environ. Saf.* **21**: 257-265.

Baird, D.J., I. Barber, A.M.V.M. Soares & P. Calow (1991b). An early life-stage test with *Daphnia magna* Straus: an alternative to the 21-day chronic Test?. *Ecotoxicol. Environ. Saf.* **22**: 1-7.

Banta, A.M. & L.A. Brown (1929). Control of sex in Cladocera. I. Crowding the mothers as a means of controlling male production. *Physiol. Zool.* **2**: 80-92.

Banta, A.M. (1939). *Studies on the physiology, genetics and evolution of some Cladocera.* Carnwegie Institution. Washington, D.C..

Barber, I., D.J. Baird & P. Calow (1994). The effect of cadmium and ration level on oxygen consumption, RNA concentration and RNA-DNA ratio in two clones of *Daphnia magna* Straus. *Aquat. Toxicol.* **30**(3): 249-258.

Barera, Y. & W.J. Adams (1983). Resolving some practical questions about *daphnia* acute toxicity tests. *In*: Bishop, W.E., R.D. Cardwell & B.B. Heidolph, (ed.) *Aquatic Toxicology and Hazard Assessment: Sixth Symposium. ASTM 802*, American Society for Testing and Materials. Philadelphia, PA., pp. 509-518.

Barnes, H. & J. Blackstock (1973). Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilin method for "total lipids". *J. exp. mar. Biol. Ecol.* **12**: 103-118.

Barnes, R.S.K., P. Calow, P.J.W. Olive & D.W. Golding (1990). *The invertebrates: a new synthesis*. Chapter 4. Blackwell Scientific Publications, Oxford.

Barreto, J., J. Lefèvre & W.C. Lima (1984). Physiological systems modelling with bond graphs. *System Sci.* **10**: 93-111.

Bartlett, G.R. (1959). Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466-468.

Baudo, R. (1987). Ecotoxicological testing with *Daphnia*. *Mem. Ist. Ital. Idrobiol.* **45**: 461-482.

Bauman, J.W., J. Liu & C.D. Klaassen (1993). Production of metallothionein and heat-shock proteins in response to metals. *Fund. Appl. Toxicol.* **21**: 15-22.

Bell, G. (1983). Measuring the cost of reproduction: III. The correlation structure of the early life history of *Daphnia pulex*. *Oecologia (Berl.)* **60**: 378-383.

Bent, H.A. (1965). *The second law: an introduction to classical and statistical thermodynamics*. O.U.P., New York.

Bence, J., S. Blythe, W.S.C. Gurney, P. Maas & R.M. Nisbet (1986). *SOLVER - a delay-differential equation solver. Applied Physics Industrial Consultants, University of Strathclyde, Glasgow, Scotland.* Applied Physics Industrial Consultants, University of Strathclyde, Glasgow, Scotland.

Berge, W.F. (1978). Breeding *Daphnia magna*. *Hydrobiologia* **59**(2): 121-123.

Bern, L. (1990). Size-related discrimination of nutritive and inert particles by freshwater zooplankton. *J. Plankton Res.* **12**(5): 1059-1067.

Bligh, E.G. & W.J. Dyer (1959). A rapid method of lipid extraction for use in determining vitamin E-lipid ratios. *Can. J. Biochem. Physiol.* **37**: 911-917.

Bodar, C.W.M., I. Van der Sluis, P.A. Voogt & D.I. Zandee (1988). Effects of cadmium on consumption, assimilation and biochemical parameters of *Daphnia magna*: Possible implications for reproduction. *Comp. Biochem. Physiol.* **90C**(2): 341-346.

Boersma, M. (1995). The allocation of resources to reproduction in *Daphnia galeata*: against the odds?. *Ecology* **76**(4): 1251-1261.

Bohrer, R.N. & W. Lampert (1988). Simultaneous measurement of the effect of food concentration on assimilation and respiration in *Daphnia magna*. *Funct. Ecol.* **2**: 463-471.

Bottrell, H.H. (1975). Generation time, length of life, instar duration and frequency of moulting, and their relationship to temperature in eight species of Cladocera the river Thames, Reading. *Oecologia (Berl.)* **19**: 129-140.

Bradley, M.C., N. Perrin & P. Calow (1991a). Energy allocation in the cladoceran *Daphnia magna* Straus, under starvation and refeeding. *Oecologia* **86**: 414-418.

Bradley, M.C., D.J. Baird & P. Calow (1991b). Mechanisms of energy allocation to reproduction in the cladoceran *Daphnia magna* Straus. *Biol. J. Linn. Soc.* **44**: 325-333.

Brendelberger, H. (1985). Filter mesh-size and retention efficiency for small particles: Comparative studies with Cladocera. *Arch. Hydrobiol. Beih.* **21**: 135-146.

Brendelberger, H. & W. Geller (1985). Variability of filter structures in eight *Daphnia* species: mesh sizes and filtering areas. *J. Plankton Res.* **7**(4): 473-486.

Broderius, S.J., M.D. Kahl & M.D. Hoglund (1995). Use of joint toxic response to define the primary mode of toxic action for diverse industrial organic chemicals. *Environ. Toxicol. Chem.* **14**(9): 1591-1605.

Brooks, J.L. (1959). Cladocera. *In*: Edmondson, W.T., (ed.) *Freshwater biology*, 2nd edn. Wiley, New York, pp. 587-656.

Buikema, A.L. (1972). Oxygen consumption of the cladoceran, *Daphnia pulex*, as a function of body size, light and light acclimation. *Comp. Biochem. Physiol.* **42A**: 877-888.

Buikema, A.J., Jr. (1973). Some effects of light on the growth, molting reproduction and survival of the cladoceran, *Daphnia pulex*. *Hydrobiologia* **41**(3): 391-418.

Buikema, A.L., Jr., J.G. Geiger & D.R. Lee (1980). *Daphnia* toxicity tests. *In*: Buikema, A.L. & Cairns, J, (ed.) *Aquatic Invertebrate Bioassays*, ASTM STP 715, pp. 48-69.

Burns, C.W. (1968). The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. *Limnol. Oceanogr.* **13**: 675-678.

Burns, C.W. (1969). Particle size and sedimentation in the feeding behavior of two species of *Daphnia*. *Limnol. Oceanogr.* **14**: 392-402.

Butler, G.C. (Ed.) (1978). *Principles of ecotoxicology*. SCOPE Report 12, Wiley, New York.

Calow, P. & C.R. Townsend (1981). Energetics, ecology and evolution. *In*: Townsend, C.R. & P. Calow, (ed.) *Physyiological ecology. An evolutionary approach to resource use*, Blackwell Scientific Publications, Oxford, UK, pp. 3-19.

Carvalho, G.R. & D.J. Crisp (1987). The clonal ecology of *Daphnia magna* (Crustacea: Cladocera). 1. Temporal changes in the clonal structure of a natural population. *J. Anim. Ecol.* **56**(2): 453-468.

Carvalho, G.R. & R.N. Hughes (1983). The effect of food availability, female culture density and photoperiod on ephippia production in *daphnia magna* Straus (Crustacea: Cladocera). *Freshwat. Biol.* **13**: 37-46.

Caswell, H. & A.M. John (1992). From the individual to the population in demographic models. *In*: DeAngelis, D.L. & L.J. Gross, (ed.) *Individual-based models and approaches in ecology: populations, communities and ecosystems*, Chapman & Hall, London, UK, pp. 36-66.

Christoffersen, K. (1988). Effect of food concentration on gut evacuation of *Daphnia pulicaria* and *Daphnia longispina* measured by the fluorescence technique. *Verh. Internat. Verein. Limnol.* **23**: 2050-2055.

Cole, L.C. (1954). The population consequences of life-history phenomena. *Quart. Rev. Biol.* **29**: 103-137.

Cox, E.J., C Naylor, M.C. Bradley & P. Calow (1992). Effect of differing maternal ration on adult fecundity and offspring size in laboratory cultures of *Daphnia magna* Straus for ecotoxicology testing. *Aquat. Toxicol.* **24**: 63-74.

Dave, G., K. Anderson, R. Berglind & B. Hasselrot (1981). Toxicity of eight solvent extraction chemicals and of cadmium to water fleas, *Daphnia magna*, rainbow trout, *Salmo gairdneri*, and zebrafish, *Brachydanio rerio*. *Comp. Biochem. Physiol.* **69C**: 83-98.

Davies, R.M. & R.M. O'Keefe (1989). *Simulation modelling in Pascal*. Prentice Hall, New York.

De Bernardi, R. & R.H. Peters (1987). Why *Daphnia*?. *Mem. Ist. Ital. Idrobiol.* **45**: 1-9.

DeAngelis, D.L. & L.J. Gross (eds) (1992). *Individual-based models and approaches in ecology: populations, communities and ecosystems*. Chapman & Hall, London, UK.

DeMott, W.R. (1982). Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*. *Limnol. Oceanogr.* **27**(3): 518-527.

DeMott, W.R. (1985). Relations between filter mesh-size, feeding mode, and capture efficienty for cladocerans feeding on ultrafine particles. *Arch. Hydrobiol. Beih.* **21**: 125-134.

DeMott, W.R. (1986). The role of taste in food selection by freshwater zooplankton. *Oecologia (Berlin)* **69**: 334-340.

DeMott, W.R. (1988). Discrimination between algae and detritus by freshwater and marine zooplankton. *Bull. Mar. Sci.* **43**(3): 486-499.

Doma, S. (1979). Ephippia of *Daphnia magna* Straus - a technique for their mass production and quick revival. *Hydrobiologia* **67**(2): 183-188.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers & F. Smith (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350-356.

Ebert, D. (1991). The effect of size at birth, maturation threshold and genetic differences on the life-history of *Daphnia magna*. *Oecologia* **86**: 243-250.

Ebert, D. (1992). A food-independent maturation threshold and size at maturity in *Daphnia magna*. *Limnol. Oceanogr.* **37**(4): 878-881.

Ebert, D. (1993). The trade-off between offspring size and number in *Daphnia magna*: the influence of genetic, environmental and maternal effects. *Arch. Hydrobiol.* **Suppl. 90**(4): 453-473.

Ebert, D. (1994a). A maturation size threshold and phenotypic plasticity of age and size at maturity in *Daphnia magna*. *Oikos* **69**: 309-317.

Ebert, D. (1994b). Fractional resource allocation into a few eggs: *Daphnia* as an example. *Ecology* **75**(2): 568-571.

EC (1979). Council directive 79/831/EEC amending for the sixth time directive 67/548/EEC on the classification, packaging and labelling of dangerous substances. *OJ L259/10 15 October 1979.*

EC (1986). *Current test guideline: prolonged toxicity study with Daphnia magna* effects on reproduction. Draft 4. XI/681/86.

Edmondson, W.T. (1987). *Daphnia* in experimental ecology: notes on historical perspectives. *Mem. Ist. Ital. Idrobiol.* **45**: 11-30.

Enserink, L., W. Luttmer & H. Maas-Diepveen (1990). Reproductive strategy of *Daphnia magna* affects the sensitivity of his progeny in acute toxicity tests. *Aquat. Toxicol.* **17**: 15-26.

Fernandez-Casalderrey, A., M.D. Ferrando & E. Andreu-Moliner (1994). Effect of sublethal concentrations of pesticides on the feeding behavior of *Daphnia magna*. *Ecotox. Environmental Saf.* **27**: 82-89.

Fernando, C.H., J.C. Paggi & R. Rajapaksa (1987). *Daphnia* in tropical lowlands. *Mem. Ist. Ital. Idrobiol.* **45**: 107-141.

Frank, P.W. (1952). A laboratory study of intraspecific and interspecific competition in *Daphnia pullicaria* (Forbes) and *Simocephalus vetulus* O.F. Müller. *Physiol. Zool.* **25**: 178-204.

Frank, P.W. (1957). Coactions in laboratory populations of two species of *Daphnia*. *Ecology* **38**: 510-579.

Friberg, L., M. Piscator, G.E. Nordberg & T. Kjellstrom (1974). *Cadmium in the environment.* 2nd edn. CRC Press, Cleveland, USA.

Frieser, A.H., J.L. Sykora, M.S. Kostalos, Y.C. Wu & D.W. Weyel (1986). Effects of fluorides on survival and reproduction of *Daphnia magna*. *J. Water Pollut. Control. Fed.* **58**(1): 82-86.

Fryer, G. (1987). The feeding mechanisms of the Daphnidae (Crustacea: Cladocera): Recent suggestions and neglected considerations. *J. Plankton Res.* **9**(3): 419-432.

Gabriel, W. (1982). Modelling reproductive strategies of *Daphnia*. *Arch. Hydrobiol.* **95**(1/4): 69-80.

Ganf, G.G. & R.J. Shiel (1985a). Particle capture by *Daphnia carinata*. *Aust. J. Mar. Freshwat. Res.* **36**(3): 371-381.

Ganf, G.G. & R.J. Shiel (1985b). Feeding behaviour and limb morphology of two Cladocerans with small intersetular distances. *Aust. J. Mar. Freshw. Res.* **36**: 69-86.

Geller, W. & C. Knisely (1988). Drag forces and energetic costs in *Daphnia* filter-feeding. *Verh. Internat. Verein. Limnol.* **23**: 2061.

Geller, W. (1975). Die nahrungsaufnahme von *Daphnia pulex* in Abhangigkeit von der futterkonzentration, der temperatur, der korpergrosse und dem hungerzustand der tiere. *Arch. Hydrobiol. Suppl.* **48**: 47-107.

Geller, W. & H. Müller (1981). The filtration apparatus of Cladocera: filter mesh-sizes and their implications on food selectivity. *Oecologia (Berl.)* **49**(3): 316-321.

Geller, W. & H. Müller (1985). Seasonal variability in the relationship between body length and individual dry weight as related to food abundance and clutch size in two coexisting *Daphnia* species. *J. Plankton Res.* **7**(1): 1-18.

Gerritsen, J. (1984). Size efficiency reconsidered: a general foraging model for free-swimming aquatic animals. *Am. Nat.* **123**(4): 450-467.

Gerritsen, J & K.G. Porter (1982). The role of surface chemistry in filter feeding by zooplankton. *Science* **211**: 1225-1227.

Glazier, D.S. (1991). Separating the respiration rates of embryos and brooding females of *Daphnia magna*: Implications for the cost of brooding and the alometry of metabolic rate. *Limnol. Oceanogr.* **36**(2): 354-362.

Glazier, D.S. (1992). Effects of food, genotype, and maternal size and age on offspring investment in *Daphnia magna*. *Ecology* **73**(3): 910-926.

Glazier, D.S. & P. Calow (1992). Energy allocation rules in *Daphnia magna*: clonal and age differences in the effects of food limitation. *Oecologia* **90**: 540-549.

Gliwicz, Z.M. & C. Guisande (1992). Family planning in *Daphnia*: resistance to starvation in offspring born mothers grown at different food levels. *Oecologia* **91**(4): 463-467.

Gliwicz, Z.M. & E. Siedlar (1980). Food size limitation and algae interfering with food collection in *Daphnia*. *Arch. Hydrobiol.* **88**(2): 155-177.

Gophen, M. & W. Geller (1985). Filter mesh size and food particle uptake by *Daphnia*. *Oecologia*. **64**(3): 408-412.

Goss, L.B. & D.L. Bunting (1983). *Daphnia* development and reproduction: Responses to temperature. *J. Therm. Biol.* **8**(4): 375-380.

Goulden, C.E., L. Henry & D. Berrigan (1987). Egg size, post-embryonic yolk, and survival ability. *ecologia (Berlin)* **72**: 20-31.

Green, J. (1954). Size and reproduction in *Daphnia magna* (Crustacea: Cladocera). *Proc. Soc. Zool. London* **124**: 535-545.

Green, J. (1956). Growth, size and reproduction in *Daphnia magna* (Crustacea: Cladocera). *Proc. Soc. Zool. London* **126**: 173-204.

Green, J. (1963). *A biology of Crustacea*. H.F. & G. Whitherby Ltd, London, UK.

Green, J. (1966). Season variation in egg production by Cladocera. *J. Anim. Ecol.* **35**: 77-104.

Guisande, C. & Z.M. Gliwicz (1992). Egg size and clutch size in two *Daphnia* species grown at different food levels. *J. plankton res.* **14**(7): 997-1007.

Gulati, R.D., C.W.M. Bodar, A.L.G. Schuurmans, J.A.J. Faber & D.I. Zandee (1988). Effects of cadmium exposure on feeding of freshwater planktonic crustaceans. *Comp. Biochem. Physiol., C* **90**(2): 335-340.

Gulati, R.D. (1978). The ecology of common planktonic Crustacea of the fresh waters in the Netherlands. *Hydrobiologia* **59**: 101-112.

Gurney, W.S.C., E. McCauley, R.M. Nisbet & W.W. Murdoch (1990). The physiological ecology of *Daphnia*: a dynamic model of growth and reproduction. *Ecology* **71**(2): 716-732.

Hallam, T.G., R.R. Lassiter, J. Li & L.A. Suarez (1990a). Modelling individuals employing an integrated energy response: application to *Daphnia*. *Ecology* **71**(3): 938-954.

Hallam, T.G., R.R. Lassiter, J. Li & W. McKinney (1990b). Toxicant-induced mortality in models of *Daphnia* populations. *Environ. Toxicol. Chem.* **9**: 597-621.

Haney, J.F. & C. Buchanan (1987). Distribution and biogeography of *Daphnia* in the Arctic. *Mem. Ist. Ital. Idrobiol.* **45**: 77-105.

Hebert, P.D.N. (1978). The population biology of *Daphnia* (Crustacea, Daphnidae). *Biol. Rev. Camb. Philos. Soc.* **53**(3): 387-426.

Hebert, P.D.N. & R.D. Ward (1972). Inheritance during parthenogenesis in *Daphnia magna*. *Genetics* **71**: 639-642.

Holling, C.S. (1959). The components of predation as revealed by a study of small-mammal predation of the european pine sawfly. *Can. Entomol.* **91**: 293-320.

Holling, C.S. (1965). The functional response of predators to prey density and its role in mimicry and population regulation. *Mem. Entomol. Soc. Can.* **45**: 5-60.

Holling, C.S. (1966). The functional response of invertebrate predators to prey density. *Mem. Entomol. Soc. Can.* **48**: 1-86.

Horton, P.A., M. Rowan, K.E. Webster & R.H. Peters (1979). Browsing and grazing by cladoceran filter-feeders. *Can. J. Zool.* **57**(1): 206-212.

Hrbácek, J. (1987). Systematics and biogeography of *Daphnia* species in the northern temperate region. *Mem. Ist. Ital. Idrobiol.* **45**: 37-76.

ISO (1982). *Water quality - determination of the inhibition of the mobility of daphnia magna* Straus (Crustacea, Cladocera). International Standard 6341 E. ISO 6341-1982 (E).

Jeffers, J.N.R. (1978). *An introduction to systems analysis: with ecological implications.* Edward Arnold Ltd, London, UK.

Jorgensen, S.E. (1988). *Fundamentals of ecological modelling*. Elsevier, Oxford.

Jorgensen, C.B. (1990). *Bivalve feeding: hydrodynamics, bioenergetics, physiology and ecology*. Olsen & Olsen, Fredensborg, Denmark.

Kankaala, P. & F. Wulff (1981). Experimental studies on temperature-dependent embryonic and postembryonic developmental rates of *Bosmina maritima* (Cladocera) in the Baltic. *Oikos* **36**: 137-146.

Karnopp, D. & S. Azarbaijani (1981). Pseudo bond graphs for generalized compartmental models in engineering and physiology. *J. Franklin Inst.* **312**(2): 915-918.

Kemp, A. & A.J.M.K.V. Heijningen (1954). A colorimetric method for the determination of glycogen in tissues. *Biochem. J.* **56**: 646-648.

Kerfoot, W.C. (1974). Egg size cycle of a cladoceran. *Ecology* **55**: 1259-1270.

Kerfoot, W.C. & K.L. Kirk (1991). Degree of taste discrimination among suspension-feeding cladocerans and copepods: implications for detritivory and herbivory. *Limnol. Oceanogr.* **36**(6): 1107-1123.

Kersting, K. (1978). Some features of feeding, respiration and energy conversion of *Daphnia magna*. *Hydrobiologia* **59**(2): 113-120.

Kersting, K. & V. Van der Leeuw-Leegwater (1976). Effect of food concentration on the respiration of *Daphnia magna*. *Hydrobiologia* **49**(2): 137-142.

Kersting, K. & H. van der Honing (1981). Effect of the herbicide dichlobenil on the feeding and filtering rate of *Daphnia magna*. *In*: Sladecek, V., (ed.) *Verh. Int. Ver. Theor. Angew. Limnol./Proc. Int. Assoc. Theor. Appl. Limnol./Trav. Assoc. Int. Limno*, Vol. 21, Stuttgart (Frg), Schweizerbartsche Verlagsbuchhandlung, pp. 1135-1140.

Kiorboe, T., F. Mohlenberg & K. Hamburger (1985). Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Prog. Ser.* **26**: 85-97.

Kirk, K.L. (1991a). Suspended clay reduces *Daphnia* feeding rate: Behavioural mechanisms. *Freshwat. Biol.* **25**(2): 357-365.

Kirk, K.L. (1991b). Inorganic particles alter competition in grazing plankton: The role of selective feeding. *Ecology.* **72**(3): 915-923.

Kirk, K.L. & J.J. Gilbert (1990). Suspended clay and the population dynamics of planktonic rotifers and cladocerans. *Ecology* **71**(5): 1741-1755.

Klekowski, R.Z. & A. Duncan (eds) (1975). Physiological approach to ecological energetics. *In*: Grodzinki, W., R.Z. Klekowski & A. Duncan, (ed.) *IBP Handbook No 24. Methods for ecological bioenergetics*, Blackwell Scientific Publications, Oxford, UK, pp. 15-64.

Koizumi, T. & Z.G. Li (1992). Role of oxidative stress in single-dose cadmium-induced testicular cancer. *J. Toxicol. Environ. Health* **37**: 25-36.

Kooijman, S.A.L.M. (1986a). Populations dynamics on the basis of budgets. *In*: Metz, J.A.J. & O. Diekmann, (ed.) *The dynamics of physiologically structured populations*, Springer Lecture Notes in Biomathematics. Springer Verlag, Berlin, pp. 266-297.

Kooijman, S.A.L.M. (1986b). Energy budgets can explain body size relations. *J. theor. Biol.* **121**: 269-282.

Kooijman, S.A.L.M. (1993). *Dynamic energy budgets in biological systems. Theory and applications in ecotoxicology.* Cambridge University Press, Cambridge, UK.

Kooijman, S.A.L.M. & J.A.J. Metz (1984). On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. *Ecotoxicol. Environ. Saf.* **8**: 254-274.

Korinek, V. & J. Machacek (1980). Filtering structures of Cladocera and their ecological significance 1. *Daphnia pulicaria. Vestn. Cesk. Spol. Zool.* **44**(3): 213-218.

Koza, V. & V. Korinek (1985). Adaptability of the filtration screen in *Daphnia*: Another answer to the selective pressure of the environment. *In*: Lampert, W., (ed.) *Food Limitation And The Structure Of Zooplankton Communities.*, Ergeb. Limnol./Adv. Limnol., pp. 193-198.

Lam, R.K. & B.W. Frost (1976). Model of copepod filtering response to changes in size and food concentration of food. *Limnol. Oceanogr.* **21**(4): 490-500.

Lampert, W. (1977). Studies on the carbon balance of *Daphnia pulex* De Geer as related to environmental conditions. II. The dependence of carbon assimilation on animal size, temperature, food concentration and diet species. *Arch. Hydrobiol.* **Suppl. 48**(3/4): 310-335.

Lampert, W. (1986). Response of the respiratory rate of *Daphnia magna* to changing food conditions. *Oecologia (Berlin)* **70**: 495-501.

Lampert, W. (1987). Feeding and nutrition in *Daphnia*. *Mem. Ist. Ital. Idrobiol.*, **45**: 143-192.

Lassiter, R.R. & T.G. Hallam (1990). Survival of the fattest: implications for acute effects of lipophilic chemicals on aquatic populations. *Environ. Toxicol. Chem.* **9**: 585-595.

Lehman, J.T. (1976). The filter-feeder as an optimal forager, and the predicted shapes of feeding curves. *Limnol. Oceanogr.* **21**(4): 501-516.

Lehninger, A.L. (1973). *Bioenergetics: the molecular basis of biological energy transformations*. 2nd edn. W.A. Benjamin, Inc., New York.

Lei, C.H. & K.B. Armitage (1980). Energy budget of *Daphnia ambigua* Scourfield. *J. Plankton Res.* **2**: 261-281.

Lemcke, V.H.W. & W. Lampert (1975). Veranderungen im gewicht und der cemischen zusammensetzung von *Daphnia pulex* im hunger. *Arch. Hydrobiol.* **Suppl. 48**(1): 108-137.

Leonhard, S.L. & S.G. Lawrence (1981). *Daphnia magna* Straus, *Daphnia pulex* (Leydig) Richard. *In*: *Manual for the culture of selected freshwater invertebrates*, Vol. 54, Can. Spec. Publ. Fish. Aquat. Sci., pp. 31-50.

Lewontin, R.C. (1965). Selection for colonizing ability. *In*: Baker, H.G. & G.L. Stebbins, (ed.) *The genetics of colonizing species*, Academic Press, New York, pp. 79-94.

Lomnicki, A. (1988). *Population ecology of individuals*. Princeton University Press, Princeton, USA.

Lomnicki, A. (1992). Population ecology from the individual perspective. *In*: DeAngelis, D.L. & L.J. Gross, (ed.) *Individual-based models and approaches in ecology: populations, communities and ecosystems*, Chapman & Hall, London, UK, pp. 3-17.

Lowry, O.H., N.J Rosebrough, N.L. Farr & R.J. Randall (1951). Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.

Lycnh, M. (1985). Spontaneous mutations for life-history characters in an obligate parthenogen. *Evolution* **39**: 804-818.

Lynch, M. (1989). The life history consequences of resource depression in *Daphnia pulex*. *Ecology* **70**(1): 246-256.

Lynch, M., L.J. Weider & W. Lampert (1986). Measurement of the carbon content balance in *Daphnia*. *Limnol. Oceanogr.* **31**(1): 17-33.

Maltby, L., C. Naylor & P. Calow (1990). Effects of stress on a freshwater benthic detritivore: Scope for growth in *Gammarus pulex*. *Ecotoxicol. Environ. Saf.* **19**: 285-291.

Maltby, L. (1992). The use of physiological energetics of *Gammarus pulex* to assess toxicity - a study using artificial streams. *Environ. Toxicol. Chem.* **11**(1): 79-85.

Manca, D., A.C. Richard, B. Trotier & G. Chevalier (1991). Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. *Toxicology* **67**: 303-323.

Marquardt, D.W. (1963). An algorithm for least-squares estimation of nonlinear parameters. *J. Soc. Industrial Appl. Mathematics* **11**(2): 431-415.

Marshall, S.M. & A.P. Orr (1962). Carbohydrate as a measure of phytoplankton. *J. Mar. Biol. Ass. U.K.* **42**: 511-519.

May, R.M. (1973). *Stability and complexity in model ecosystems*. Princeton University Press, Princeton, USA.

McCabe, G.D. & W.J. O'Brien (1983). The effects of suspended silt on feeding and reproduction of *Daphnia pulex*. *Am. Midl. Nat.* **110**(2): 324-337.

McCauley, E. & W.W. Murdoch (1987). Cyclic and stable populations: plankton as paradigm. *The American Naturalist* **129**(1): 97-121.

McCauley, E. & W.W. Murdoch (1990). Predator-prey dynamics in environments rich and poor in nutrients. *Nature* **343**(6257): 455-457.

McCauley, E., W.W. Murdoch, R.M. Nisbet & W.S.C. Gurney (1990). The physiological ecology of *Daphnia*: development of a model of growth and reproduction. *Ecology* **71**(2): 703-715.

McMahon, J.W. & F.H. Rigler (1963). Mechanisms regulating the feeding behavior of *daphnia magna* Straus. *Cand. J. Zool.* **41**: 321-332.

McMahon, J.W. & F.H. Rigler (1965). Feeding rate of *Daphnia magna* Straus in different foods labelled with radioactive phosphorus. *Limnol. Oceanogr.* **10**: 105-113.

Mendel, B. & M. Bauch (1926). Eine colormetrische mikromethode zur quantitativen bestimmung des blutzuckers in 8 minuten. *Klin. Wschr.* **5**: 1329-1330.

Mendel, B., A. Kemp & D.K. Myers (1954). A colorimetric micro-method for the determination of glucose. *Biochem. J.* **56**: 639-646.

Metz, J.A.J. & A.M. de Roos (1992). The role of physiologically structured population models within a general individual-based modelling perspective. *In*: DeAngelis, D.L. & L.J. Gross, (ed.) *Individual-based models and approaches in ecology: populations, communities and ecosystems*, Chapman & Hall, London, UK, pp. 88-111.

Muck, P. & W. Lampert (1980). Feeding of freshwater filter-feeders at very low concentrations: poor evidence for 'threshold feeding' and 'optimal foraging' in *Daphnia longispina* and *Eudiaptomus gracilis*. *J. Plankton Res.* **2**(4): 367-379.

Muller, L. (1986). Consequences of cadmium toxicity in rat hepatocytes: mitochondrial dysfunction and lipid peroxidation. *Toxicology* **40**(3): 285-295.

Murdoch, W.W., E. McCauley, R.M. Nisbet, W.S.C. Gurney & A.M. de Roos (1992). Individual-based models: combining testability and generality. *In*: DeAngelis, D.L. & L.J. Gross, (ed.) *Individual-based models and approaches in ecology: populations, communities and ecosystems*, Chapman & Hall, London, UK, pp. 18-35.

Murtaugh, P.A. (1984). Variable gut residence time: Problems in inferring feeding rate from stomach fullness of a mysid crustacean. *Can. J. Fish. Aquat. Sci.* **41**(9): 1287-1293.

Murtaugh, P.A. (1985). The influence of food concentration and feeding rate on the gut residence time of *Daphnia*. *J. Plankton res.* **7**(3): 415-420.

Naylor, C., L. Maltby & P. Calow (1989). Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. *Hydrobiologia* **188/189**: 517-523.

Naylor, C., E.J. Cox, M.C. Bradley & P. Calow (1992). Effect of differing maternal food ration on susceptibility of *Daphnia magna* Straus neonates to toxic substances. *Aquat. Toxicol.* **24**: 75-82.

Needham, A.E. (1964). *The growth process in animals*. Pitman Publishing Company, New York.

Nisbet, R.M., W.S.C. Gurney, W.W. Murdoch & E. McCauley (1989). Structured population models: a tool for linking effects at individual and population levels. *Biol. J. Lin. Soc.* **37**: 79-99.

Nriagu, J.O. (1980). *Cadmium in the environment. Part II. Health Effects*. John Wiley & Sons, New York.

O'Brien, S.K.,; Nameroff, S. (1992). Turbo Pascal 7 complete reference. Borland, Osborne/McGraw-Hill, Berkley, US.

Ochi, T., K. Takahashi & M. Ohsawa (1987). Indirect evidence for the induction of a prooxidant state by cadmium chloride in cultured mammalian cells and a possible mechanism for the induction. *Mutat. Res.* **180**: 257-266.

OECD (1981). *Guidelines for the testing of chemicals*. Paris.

Oster, G.F., A.S. Perelson & A. Katchalsky (1973). Network thermodynamics: dynamic modelling of biophysical systems. *Quaterly Reviews of Biophysics* **6**(1): 1-134.

Paloheimo, J.E., S.J. Crabtree & W.D. Taylor (1982). Growth model of *Daphnia*. *Can. J. Fish. Aquat. Sci.* **39**: 598-606.

Penry, D.L. & P.A. Jumars (1987). Modelling animal guts as chemical reactors. *Am. Nat.* **129**(1): 69-96.

Perrin, N., M.C. Bradley & P. Calow (1990). Plasticity of storage allocation in *Daphnia magna* Straus. *Oikos* **59**: 70-74.

Perrin, N., D.J. Baird & P. Calow (1992). Resource allocation, population dynamics and fitness: some experiments with *Daphnia magna* Straus. *Arch. Hydrobiol.* **123**(4): 431-449.

Peschel, M. & W. Mende (1986). *The predator-prey model*. Springer-Verlag, New York.

Peters, R.H. (1983). *The ecological implications of body size*. Cambridge University Press, Cambridge, UK.

Peters, R.H. (1987a). Daphnia culture. *Mem. Ist. Ital. Idrobiol.* **45**: 483-495.

Peters, R.H. (1987b). Metabolism in *Daphnia*. *Mem. Ist. Ital. Idrobiol.* **45**: 193-243.

Peters, R.H. & R. De Bernardi (eds) (1987). *Daphnia*. *Mem. Ist. Ital. Idrobiol.*, Vol. 45, Consiglio Nazionale delle Richerche, Istituto Italiano di Idrobiologia, Verbania Palanza, Italy.

Peters, R.H. & J.A. Downing (1984). Empirical analysis of zooplankton filtering and feeding rates. *Limnol. Oceanogr.* **23**(5): 1039-1044.

Philippova, T.G. & A.L. Postnov (1988). Relationship between metabolism and feeding in Cladocera (Crustacea). *Zhurnal Obshchei Bilogii* **49**(4): 540-551.

Philippova, T.G. & A.L. Postnov (1988). The effect of food quantity on feeding and metabolic expenditure in Cladocera. *Int. Rev. Gesamt. Hydrobiol.* **73**(6): 601-615.

Phillipson, J. (1975). Introduction to ecological energetics. *In*: Grodzinki, W., R.Z. Klekowski & A. Duncan, (ed.) *IBP Handbook No 24. Methods for ecological bioenergetics*, Blackwell Scientific Publications, Oxford, UK, pp. 3-14.

Porcella, D.B., C.E. Rixford & J.V. Slater (1969). Molting and calcification in *Daphnia magna*. *Physiol. Zool.* **42**: 148-159.

Porter, K.G. (1975). Viable passage of gelatinous green algae ingested by *Daphnia*. *Verh. internat. Verein. Limnol.* **19**: 2840-2850.

Porter, K.G. (1977). The plant-animal interface in freshwater ecosystems. *Am. Sci.* **65**: 159-170.

Porter, K.G., J. Gerritsen & J.D. Orcutt, Jr. (1982). The effect of food concentration on swimming patterns, feeding behavior, ingestion, assimilation, and respiration in *Daphnia magna*. *Limnol. Oceanogr.* **27**(5): 935-949.

Porter, K.G., J.D. Orcutt, Jr. & J. Gerritsen (1983). Functional response and fitness in a generalist filter feeder, Daphnia magna (Cladocera: Crustacea). *Ecology* **64**(4): 735-742.

Pratt, R. & J. Fong (1940). Studies on *Chlorella vulgaris*. II. Further evidence that *Chlorella* cells for a growth inhibiting substance. *Amer. J. Bot.* **27**: 431-436.

Pratt, R., J.F. Oneto & J. Pratt (1945). Studies on *Chlorella vulgaris* X. Influence of the age of the culture on the accumulation of chlorellin. *Amer. J. Bot.* **32**: 405-408.

Press, , W.H., B.P. Flannery, S.A. Teukolosky & W.T. Vetterling (1986). *Numerical recipes*. Cambridge University Press, Cambridge, England.

Real, L.A. (1977). The kinetics of functional response. *Am. Nat.* **111**(978): 289-300.

Richman, S. (1958). The transformation of energy by *Daphnia pulex*. *Ecol. Monographs* **28**(3): 273-291.

Rigler, F.H. (1961). The relation between concentration of food and feeding rate of *Daphnia magna* Straus. *Can. J. Zool.* **39**: 857-868.

Rocha, O. & A. Duncan (1985). The relationship between cell carbon and cell volume in freshwater algal species used in zooplanktonic studies. *J. Plankton Res.* **7**(2): 279-294.

Rubenstein, D.I. & M.A.R. Koehl (1977). The mechanisms of filter feeding: some theoretical considerations. *Am. Nat.* **11**(981): 981-994.

Ryther, J.H. (1954). Inhibitory effects phytoplankton upon the feeding of *Daphnia magna* with reference to growth, reproduction, and survival. *Ecology* **35**(4): 522-533.

Schindler, D.W. (1968). Feeding, assimilation and respiration rates of *Daphnia magna* under various environmental conditions and their relation to production estimates. *J. Anim. Ecol.* **37**: 369-385.

Schmidt-Nielsen, K. (1984). *Scaling: Why is animal size so important?*. Cambridge University Press, Cambridge, UK.

Schrodinger, E. (1977). *What is Life? & Mind and Matter*. Cambridge University Press, Cambridge.

Sibly, R.M. (1981). Strategies of digestion and defecation. *In*: Townsend, C.R. & P. Calow, (ed.) *Phsyiological ecology. An evolutionary approach to resource use*, Blackwell Scientific Publications, Oxford, UK, pp. 109-139.

Sibly, R.M. (1989). What evolution maximises. *Funct. Ecol.* **3**: 129-135.

Sibly, R.M. & J. Antonovics (1992). Life-history evolution. *In:* Berry, R.J., T.J. Crawford & G.M. Hewitt, (eds) *Genes in ecology*, Blackwell Scientific Publications, Oxford, UK, pp. 87-122.

Sibly, R.M. & P. Calow (1986). *Physiological ecology of animals*. Blackwell Scientific Publications, Oxford, UK.

Sinko, J.W. & W. Streifer (1969). Applying models incorporating age-size structure of a population to *Daphnia*. *Ecology* **6**: 489-496.

Slobodkin, L.B. (1954). Population dynamics in *Daphnia obtusa* Kurz. *Ecol. Monogr.* **24**: 69-88.

Smith, F.E. (1963). Population dynamics in *Daphnia magna* and a new model for population growth. *Ecology* **44**(4): 651-663.

Soares, A.M.V.M., D.J. Baird & P. Calow (1992). Interclonal variation in the performance of *daphnia magna* Straus in chronic bioassays. *Environ. Toxicol. Chem.* **11**(10): 1477-1483.

Soares, A.M.V.M. (1989). *Clonal variation in life-history traits in Daphnia magna Straus (Crustacea, Cladocera). Implications for ecotoxicology*. PhD. thesis submitted at the Department of Animal and Plant Sciences at the University of Sheffield.

Starkweather, P.L. & K.G. Bogdan (1980). Detrital feeding in natural zooplankton communities: discrimination between live and dead algal foods. *Hydrobiologia* **73**(1/3): 83-85.

Starkweather, P.L. (1978). Diel variation in feeding behavior of *Daphnia pulex*. Influences of food density and nutritional history on mandibular activity. *Limnol. Oceanogr.* **23**(2): 307-317.

SGC (1989). *STATGRAPHICS 4.0 Statistical Graphics System*. Statistical Graphics Corporation, US.

Stein, J.R. (1973). *Handbook of phycological methods*. Cambridge University Press, London.

Stephenson, R.R. & S.A. Watts (1984). Chronic toxicity tests with *Daphnia magna*: the effects of different food and temperature regimes on survival reproduction and growth. *Env. pollution Ser. A* **36**: 95-107.

Stoepler, M. & M. Piscator (Eds.) (1988). *Cadmium*. Environmental Toxin Series 2. 3rd IUPAC Cadmium Workshop. Springler-Verlag, Berlin.

Stohs, S.J. & D. Bagchi (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biol. Med.* **18**(2): 321-336.

Stross, R.G. & J.C. Hill (1965). Diapause induction in *Daphnia* requires two stimuli. *Science* **150**: 1462-1464.

Stuhlbacher, A., M.C. Bradley, C. Naylor & P. Calow (1993). Variation in the development of cadmium resistance in *Daphnia magna* Straus; effects of temperature, nutrition, age and genotype. *Environmental Pollution* **80**: 153-158.

Taub, F.B. & A.M. Dollar (1968). The nutritional inadequacy of *Chlorella* and *Chlamydomonas* as food for *Daphnia pulex*. *Limnol. Oceanogr.* **13**: 607-617.

Taylor, B.E. (1985). Effects of food limitation on growth and reproduction of *Daphnia*. *In*: Lampert, W., (ed.) *Food limitation and the structure of zooplankton communities*, Ergeb. Limnol./Adv. Limnol., pp. 285-296.

Tessier, A.J. & N.L. Consolatti (1989). Variation in offspring size in *Daphnia* and consequences for individual fitness. *Oikos* **56**: 269-276.

Tessier, A.J. & N.L. Consolatti (1991). Resource allocation and offspring quality in *Daphnia*. *Ecology* **72**(2): 468-478.

Tessier, A.J. & C.E. Goulden (1982). Estimating food limitation in cladoceran populations. *Limnol. Oceanogr.* **27**(4): 707-717.

Tessier, A.J., L.L. Henry & C.E. Goulden (1983). Starvation in *Daphnia*: energy reserves and reproductive allocation. *Limnol. Oceanogr.* **28**(4): 667-676.

Thieme, H.R. (1988). Well-posedness of physiologically structured population models for *Daphnia magna*. How biological concepts can benefit by abstract mathematical analysis. *J. Math. Biol.* **26**: 299-317.

Threlkeld, S.T. (1976). Starvation and size structure of zooplankton communities. *Freshwat. Biol.* **6**: 489-496.

Threlkeld, S.T. (1987a). *Daphnia* life history strategies and resource allocation patterns. *Mem. Ist. Ital. Idrobiol.* **45**: 353-366.

Threlkeld, S.T. (1987b). *Daphnia* population fluctuations: patterns and mechanisms. *Mem. Ist. Ital. Idrobiol.* **45**: 367-388.

Townsend, C.R. & R.N. Hughes (1981). Maximizing net energy returns from foraging. *In*: Townsend, C.R. & P. Calow, (ed.) *Physiological ecology. An evolutionary approach to resource use*, Blackwell Scientific Publications, Oxford, UK, pp. 86-108.

Tunstall, E.W. & M. Solinas (1977). *Daphnia pulex* pulls its weight in pulp mill toxicity tests. *Pulp & Paper Can.* **78**(4): 84-90.

Urabe, J. & Y. Watanabe (1991). Effect of food concentration on the assimilation and production efficiencies of *Daphnia galeata* G.P. Sars (Crustacea: Cladocera). *Funct. Ecol.* **5**(5): 635-641.

Urabe, J. (1988). Effect of food conditions on the net production of *Daphnia galeata*: separate assessment of growth and reproduction. *Bull. Plankton Soc. Japan* **35**(2): 159-174.

Von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquiries on growth. II). *Hum. Biol.* **10**(2): 181-213.

Waddell, Y.T. (1993). *Feeding responses of Daphnia magna under exposure to toxic chemicals*. PhD. thesis submitted at the Department of Animal and Plant Sciences at the University of Sheffield.

Warren, C.E. & G.E. Davis (1967). Laboratory studies on the feeding bioenergetics and growth of fish. *In*: Gerking, S.D., (ed.) *The biological basis of freshwater fish production*, Blackwell Scientific Publications, Oxford, UK, pp. 175-214.

Williams, G.C. (1966). *Adaptation and natural selection*. Princeton University Press, Princeton, New Jersey.

Wilson, J.A. (1972). *Principles of animal physiology*. The Macmillan Company, New York, USA.

Wong, P.T.S. (1987). Toxicity of cadmium to freshwater micro-organisms, phytoplankton and invertebrates. *In*: Nriagu, J.O. & J.B. Sprague, (ed.) *Cadmium in the aquatic environment*, John Wiley and Sons, New York, pp. 117-138.

Yampolsky, L.Y. & D. Ebert (1994). Variation and plasticity of biomass allocation in *Daphnia*. *Funct. Ecol.* **8**: 435-440.

Yong, J.Z. (1950). *The life of vertebrates*. Clarendon Press, Oxford, UK.

Zaffagnini, F. (1987). Reproduction in *Daphnia*. *Mem. Ist. Ital. Idrobiol.* **45**: 245-284.

Zar, J.H. (1984). *Biostatistical analysis*. 2nd edn. Prentice Hall Inc., Englewood Cliffs, New Jersey.

APPENDIX A

Table A.1 - Sensitivity analysis of the parameters used in the model at a very low ration level (0.5 µg DW/ml). Tabulated values were obtained for each endpoint: final body length and cumulative fecundity after 30d, and age at first clutch. The formula presented in Chapter 5 was used while the value of each individual parameter was forced to ±10% and ±50% of the value used to set-up the model, except when change was out of range (*).

		10%			50%		
Parameter		body length	cumulative fecundity	age at maturity	body length	cumulative fecundity	age at first clutch
α	−	-0.39	0.00	0.86	0.07	0.25	-0.17
	+	3.72	13.75	-4.60	-0.38	-2.00	-2.00
A_{max}	−	0.67	5.00	-0.93	-0.11	-1.25	0.21
	+	-3.56	-10.00	-10.00	*	*	*
A_{min}	−	0.09	0.00	0.04	-0.02	0.00	-0.01
	+	-0.30	-2.50	-0.20	0.03	0.50	-0.23
β	−	-1.13	-5.00	2.35	0.17	1.25	-0.45
	+	24.66	22.50	-4.35	-0.68	-2.00	-2.00
δ	−	0.63	6.25	-1.13	-0.13	-1.50	0.24
	+	-2.78	-10.00	-10.00	0.45	6.25	-0.70
ε	−	-0.18	1.25	-0.06	0.03	0.00	0.01
	+	0.88	-5.00	-1.02	-0.13	0.00	-0.04
E_{min}	−	0.05	-1.25	0.00	0.00	0.25	0.00
	+	-0.05	11.25	0.00	0.02	-1.00	0.00
F_h	−	0.04	0.00	0.02	-0.01	0.00	0.00
	+	-0.21	-2.50	-0.13	0.02	0.00	0.01
η	−	0.27	2.50	-0.90	-0.09	-1.00	0.20
	+	-4.07	-10.00	-10.00	0.21	2.75	-0.58
L_{tdelay}	−	0.27	2.50	0.11	-0.01	-0.50	0.25
	+	-2.09	-10.00	-10.00	0.15	1.50	-0.22
L_m	−	0.62	-1.25	1.43	-0.09	0.50	-0.03
	+	-2.97	5.00	-4.93	0.30	-2.00	-2.00
M_f	−	-0.06	0.00	-0.02	0.01	0.00	0.00
	+	0.02	2.50	-1.25	-0.03	-0.50	-0.02
M_r	−	0.03	-2.50	1.26	0.03	0.25	0.02
	+	0.59	11.25	-1.01	-0.13	-1.50	0.19
Q_{max}	−	-0.76	2.50	1.21	0.15	-0.25	0.03
	+	3.85	-10.00	-10.00	-0.39	-2.00	-2.00
Q_{min}	−	-0.39	-1.25	1.04	0.09	0.25	-0.19
	+	1.40	-6.25	0.15	-0.39	-2.00	-2.00
Q_{res}	−	0.11	-1.25	0.00	-0.02	0.25	0.00
	+	-0.27	5.00	0.00	0.05	-1.00	0.00
σ	−	-0.03	-1.25	-0.05	0.01	0.25	0.01
	+	0.19	7.50	-1.15	-0.07	-1.25	0.22
T_{coef}	−	-0.03	0.00	0.52	0.03	0.25	0.18
	+	-0.18	-2.50	0.24	-0.02	-0.25	-0.13
T_{delay}	−	0.22	1.25	0.37	-0.02	-0.25	0.20
	+	-0.24	-1.25	-0.58	0.02	0.25	-0.17
te	−	0.03	0.00	0.13	0.00	0.00	-0.03
	+	-0.03	-1.25	-0.72	-0.04	-0.25	0.09
T_{min}	−	0.00	-1.25	0.65	0.03	0.25	0.15
	+	-0.28	-2.50	0.50	0.05	0.25	-0.04
t_s	−	-0.31	-2.50	1.15	0.08	0.50	0.04
	+	1.37	18.75	-3.19	-0.26	-1.75	0.84

Table A.2 - Sensitivity analysis of the parameters used in the model at a medium ration level (5 µg DW/ml). Tabulated values were obtained for each endpoint: final body length and cumulative fecundity after 30d, and age at first clutch. The formula presented in Chapter 5 was used while the value of each individual parameter was forced to ±10% and ±50% of the value used to set-up the model, except when change was out of range (*).

		10%			50%		
Parameter		body length	cumulative fecundity	age at maturity	body length	cumulative fecundity	age at first clutch
α	–	-0.86	-2.30	2.15	-0.84	-0.44	0.72
	+	-0.29	0.63	-0.34	-0.35	-0.37	0.64
A_{max}	–	-0.09	-0.94	0.12	0.26	0.81	-0.36
	+	0.44	1.78	0.12	*	*	*
A_{min}	–	-0.17	-1.26	0.12	0.19	0.60	-0.35
	+	0.36	1.47	0.13	0.19	0.68	-0.42
β	–	-2.96	-2.72	2.29	-10.20	0.70	0.35
	+	-1.45	-0.58	-0.22	-0.88	-1.87	2.16
δ	–	1.21	3.46	0.06	0.77	1.97	-0.90
	+	1.38	3.82	0.07	0.81	1.48	-0.47
ε	–	-0.26	-0.05	-0.02	-0.39	0.04	-0.03
	+	-0.24	0.00	-0.02	-0.10	0.51	-0.01
E_{min}	–	0.01	-1.15	0.00	0.00	-2.03	0.00
	+	-0.02	-0.89	0.00	0.00	-0.66	0.00
F_h	–	0.01	0.00	0.00	0.01	0.05	0.00
	+	0.01	0.05	0.00	0.00	0.03	0.00
η	–	0.30	0.52	0.25	0.59	1.60	-1.51
	+	0.77	3.14	0.25	0.51	2.06	-0.37
L_{cdelay}	–	0.17	0.00	0.06	0.59	1.62	-0.36
	+	0.61	2.36	0.05	0.33	0.94	0.03
L_m	–	0.10	-2.20	0.08	0.56	0.10	0.85
	+	0.57	0.42	0.07	0.38	-0.49	0.72
M_f	–	-0.08	-0.58	-0.02	-0.09	-0.64	-0.02
	+	-0.11	-0.52	-0.02	0.02	-0.04	-0.02
M_r	–	-0.08	-0.58	-0.03	-0.09	-0.63	-0.03
	+	-0.11	-0.47	-0.02	0.02	-0.03	-0.02
Q_{max}	–	-2.15	0.42	-0.08	-1.44	2.00	2.00
	+	-1.27	1.88	-0.08	-0.87	0.17	0.41
Q_{min}	–	-0.49	-0.94	-0.29	-0.27	-0.04	0.33
	+	0.02	1.78	-0.29	-0.35	0.02	0.56
Q_{res}	–	0.07	-0.31	0.00	0.07	-0.26	0.00
	+	0.06	-0.26	0.00	0.07	-0.30	0.00
σ	–	0.01	-0.31	-0.01	0.00	-0.27	-0.01
	+	-0.02	-0.21	-0.01	-0.01	-0.24	-0.01
T_{coef}	–	-0.12	-1.47	0.40	-0.08	-0.24	-0.08
	+	-0.37	2.57	-0.06	0.04	-0.36	-0.24
T_{delay}	–	0.10	0.21	0.10	-0.01	-0.05	-0.02
	+	0.17	1.57	-0.47	0.08	0.26	0.11
te	–	0.00	0.00	0.28	-0.11	-0.61	0.28
	+	0.00	0.00	0.29	-0.01	-0.03	0.28
T_{min}	–	-0.34	-1.99	0.52	-0.04	-0.16	-0.11
	+	-0.18	0.42	-0.76	0.05	0.05	-0.13
t_s	–	-0.04	-0.47	0.04	-0.04	-0.46	0.05
	+	-0.07	-0.37	0.03	-0.07	-0.37	0.03

Table A.3 - Sensitivity analysis of the parameters used in the model at a very high ration level (50 µg DW/ml). Tabulated values were obtained for each endpoint: final body length and cumulative fecundity after 30d, and age at first clutch. The formula presented in Chapter 5 was used while the value of each individual parameter was forced to ±10% and ±50% of the value used to set-up the model, except when change was out of range (*).

		10%			50%		
Parameter		body length	cumulative fecundity	age at maturity	body length	cumulative fecundity	age at first clutch
α	−	-0.33	0.45	2.08	0.07	-0.04	0.06
	+	5.01	4.28	-3.48	-0.39	-0.54	0.62
A_{max}	−	0.07	0.29	0.02	-0.02	-0.06	0.00
	+	-0.39	-1.44	-0.10	*	*	*
A_{min}	−	0.77	2.67	0.21	-0.15	-0.62	-0.04
	+	-2.97	-8.02	5.30	0.40	1.47	-0.37
β	−	-3.51	-4.14	2.21	0.38	0.61	0.04
	+	48.82	-4.60	-1.70	-0.93	-1.96	2.08
δ	−	1.40	4.33	0.06	-0.41	-1.48	-0.01
	+	-4.19	-9.92	4.37	3.02	9.16	-0.46
ε	−	-0.32	-0.03	-0.02	0.05	0.01	0.00
	+	1.43	-2.62	0.14	-0.20	-0.06	-0.01
E_{min}	−	0.00	-1.12	0.00	0.00	0.18	0.00
	+	0.00	10.05	0.00	0.00	-0.67	0.00
F_h	−	0.00	0.00	0.00	0.00	0.00	0.00
	+	0.02	-0.03	0.01	0.00	0.00	0.00
η	−	0.38	1.10	0.23	-0.05	-0.09	-0.05
	+	-3.21	-8.48	7.23	0.47	1.79	-0.36
L_{delay}	−	0.73	2.51	0.05	-0.15	-0.58	-0.01
	+	-3.23	-8.66	1.75	0.51	1.95	0.03
L_m	−	0.64	0.59	0.08	-0.12	-0.08	-0.01
	+	-2.92	-1.44	-4.14	0.40	-0.44	0.69
M_f	−	-0.14	-0.86	-0.02	0.02	0.16	0.00
	+	0.71	5.11	0.11	-0.12	-0.66	-0.02
M_r	−	-0.10	-0.51	-0.02	0.02	0.10	0.00
	+	0.44	2.94	0.11	-0.08	-0.44	-0.02
Q_{max}	−	-1.50	2.73	-0.07	0.29	-0.27	0.01
	+	6.15	-10.00	-10.00	-0.92	-0.14	0.40
Q_{min}	−	-0.47	-0.83	-0.27	0.11	0.20	0.05
	+	1.37	0.29	-1.62	-0.37	-0.11	0.54
Q_{res}	−	0.07	-0.32	0.00	-0.02	0.07	0.00
	+	-0.36	1.44	0.00	0.07	-0.35	0.00
σ	−	-0.02	-0.27	-0.01	0.00	0.05	0.00
	+	0.04	1.39	0.03	-0.01	-0.27	-0.01
T_{coef}	−	-0.06	-1.10	0.38	0.03	0.28	-0.08
	+	-0.59	1.42	-0.06	0.10	-0.21	-0.23
T_{delay}	−	0.00	0.03	0.01	0.00	-0.01	0.00
	+	-0.05	-0.13	-0.06	0.01	0.03	0.01
te	−	-0.01	-0.03	0.30	0.00	0.01	-0.06
	+	0.03	0.13	-1.49	0.00	-0.01	0.31
T_{min}	−	0.21	0.72	0.50	-0.04	-0.14	-0.10
	+	-0.21	0.19	-0.73	0.06	0.07	-0.12
t_s	−	0.01	-0.03	0.01	0.00	0.01	0.00
	+	-0.08	0.11	-0.07	0.02	-0.03	0.01

GLOSSARY

age at first clutch	age of the individual after releasing the first clutch of eggs into the brood pouch (equivalent to age at maturity as used by other authors)
functional feeding response	the ingestion rate of an organism as a function of food density
growth	increase in structural mass which is measured as equivalent to increase in length
maturity	developmental stage at which the body is functionally able to provision eggs
power function = allometric function	function describing the dependency on length, *e.g.* mass as a function of length
ration	amount of food used to feed a culture
recent feeding history	integration of the feeding conditions since the beginning of the instar relative to what the individual would feed if feeding at its maximum
reserves = storage mass	fraction of the body mass which can be used to cover any costs associated with metabolism, moulting and reproduction
size at first clutch	size of the individual after releasing the first clutch of eggs into the brood pouch (equivalent to size at maturity as used by other authors)
structure = structural mass	fraction of the body mass which is irreversibly committed to cellular materials