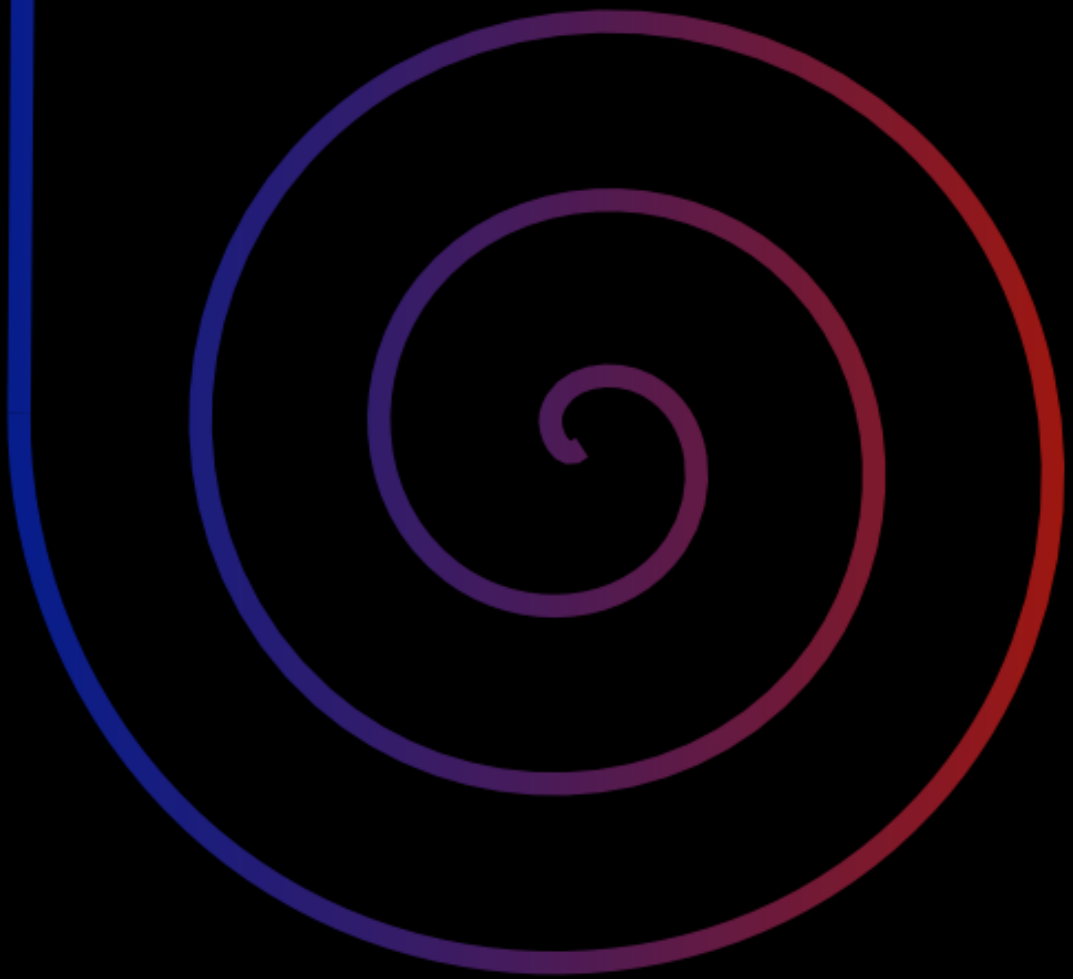
The pond snail under stress

Interactive effects of food limitation, toxicants and copulation explained by Dynamic Energy Budget theory



Elke I. Zimmer

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The pond snail under stress: Interactive effects of food limitation, toxicants and copulation explained by Dynamic Energy Budget theory

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door

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geboren te Norden, Duitsland

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1. General introduction

> ***Ecotoxicology*** *is the study of the effects of toxic chemicals on biological organisms, especially at the population, community, ecosystem level. Ecotoxicology is a multidisciplinary field, which integrates toxicology and ecology. The ultimate goal of this approach is to be able to predict the effects of pollution so that the most efficient and effective action to prevent or remediate any detrimental effect can be identified.*[1]

Ecotoxicology is commonly defined as a field which aims at predicting effects of compounds on the environment, and to prevent harmful effects. Nevertheless, current investigations mainly involve descriptive approaches, which can only be used to interpolate rather than extrapolate (Jager, 2011, 2012; Landis and Chapman, 2012). Traditionally, ecotoxicological tests are used to derive a single concentration to specify the toxicity of a compound. For example, from a range of concentrations, the highest concentration at which no statistically-significant effect is observed is reported (NOEC). Although the NOEC has been criticized in the scientific literature for some 20 years, it is still widely used in risk assessment, and the NOEC is still included in guidelines for standard ecotoxicity tests (as e.g. provided by the OECD[2] for Europe). Since the proposition to ban the NOEC (Kooijman, 1981; Laskowski, 1995), the so-called X% effect concentration (or rather EC_X) has been more widely used in research in ecotoxicology. The subscript X stands for the influence (in percent) of the toxicant on the endpoint[3] under consideration in comparison to the control[4] at a defined time point. Although using the EC_X has advantages compared to the NOEC, it has its drawbacks as well. For example, the EC_X depends substantially on the endpoint, the duration of the test, and on experimental conditions such as food availability and temperature (Jager, 2012).

Moreover, effects on multiple endpoints have to be treated separately when using the EC_X, although they are most likely connected. For example, let us consider a standard OECD reproduction test with the water flea *Daphnia magna* (OECD, 1998). Following the standard test protocol, young female daphnids should be exposed to at least 5 concentrations for 21 days. Only the cumulative number of offspring at the end of the test is required as an endpoint, although the daily

[1] Wikipedia, 25/02/2013

[2] The Organisation for Economic Co-operation and Development (OECD) provides guidelines for standard ecotoxicity testing. These include lists of test organisms and detailed laboratory test protocols.

[3] An endpoint in ecotoxicology is any measurable property of the organism, such as growth, reproduction rate or survival.

[4] The control is the situation without toxicants

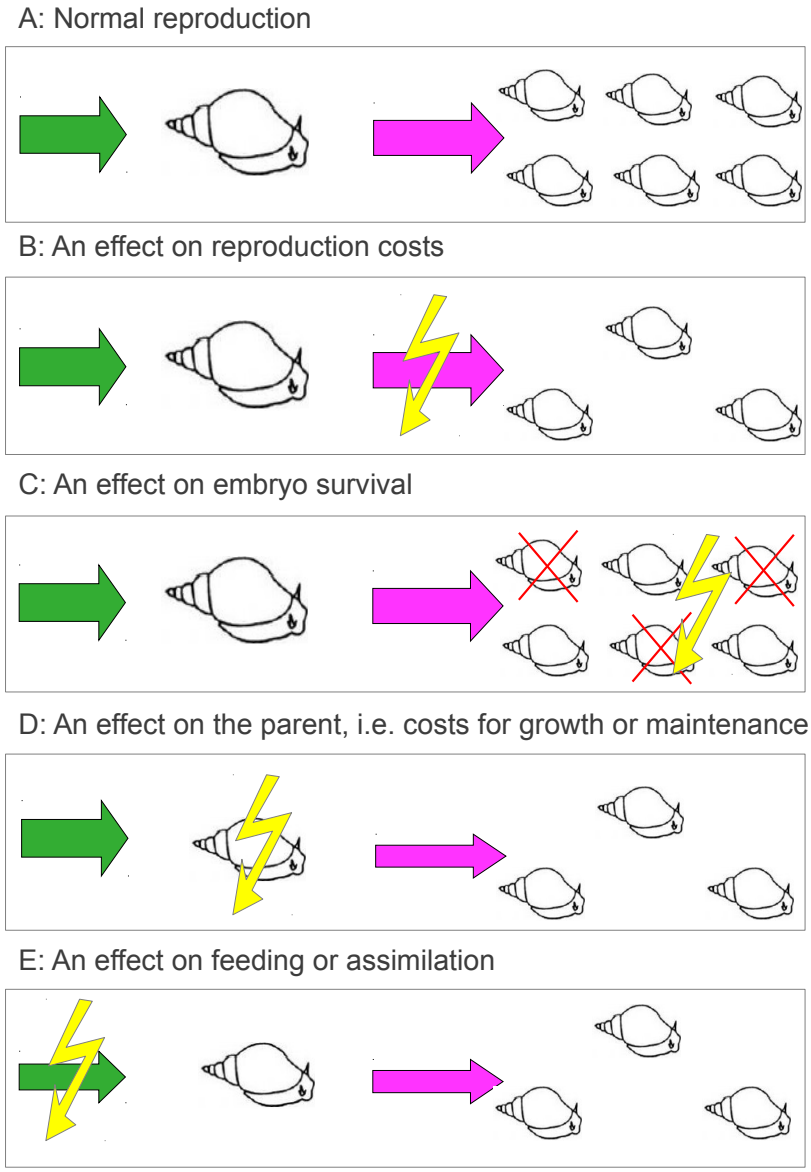

Figure 1.1.: Different options that may lead to a 50 % reduction in reproduction. Note that although I use a snail for demonstration, these patterns hold for any organism.

counting of living offspring produced is mentioned as possible additional endpoint. Besides reporting the NOEC, it is suggested to analyze the data with a regression model for the calculation of an EC_X, if possible. However, there are multiple mechanisms of effect on an individual which lead to a reduction in reproduction rate (see Figure 1.1). It is important to distinguish between the different mechanisms behind the reduction in reproduction, because they have a substantially different impact on population dynamics (Martin et al., 2013).

1.1. Mechanistic effect modeling

For informed risk assessment, we need coherent methods that can explain effects on multiple endpoints of individuals simultaneously over time, and means to extrapolate to higher levels of organization, e.g. the population or ecosystem level. Currently, mechanistic effect modeling is promoted as a means to make sense of ecotoxicity data and for ecological risk assessment (Grimm et al., 2009; Preuss et al., 2009; Hommen et al., 2010; Galic et al., 2010). Although the basic idea behind mechanistic effect modeling lies in the consideration of the underlying mechanisms to understand toxic effects, a common perception in the ecotoxicological community seems to be that it is equivalent to population modeling. However, mechanistic effect modeling starts at the individual level, as population effects are determined by effects on individual life histories.. One well-tested approach which is particularly suitable for this purpose is Dynamic Energy Budget (DEB) theory. It provides a set of rules that capture how much energy organisms assimilate from food, and how this energy is allocated to growth, development, reproduction and maintenance.

DEB theory was originally developed with the aim to understand how organisms change the allocation of energy in response to a toxicant (Kooijman and Metz, 1984): when a daphnid eats the same amount of algae, but produces less offspring, the energy has to be invested for something else. Following the idea that the energy metabolism is organized very similarly among organisms, DEB can in general be applied to all organisms (from bacteria to whales) and on all levels of organization (from the molecular to the ecosystem level, Nisbet et al. (2000)). The general DEB animal undergoes three life stages: embryo, juvenile and adult. During each stage, organisms basically follow the same rules for energy allocation, but the switch between life stages indicates a switch in metabolic behavior. The embryo is defined as an organism that does not feed and only lives from the reserves that were handed over from the mother. Once it starts feeding (first switch), it is considered a juvenile. When it reaches maturity and starts reproducing (second switch), it is considered an adult until it dies. However, for some organisms, additional life-stages have been included, and for others only two of the three stages are needed (Kooijman, 2010). Models for other organisms than animals, such as plants and bacteria, have been developed as well. In DEB, effects of toxicants are interpreted as changes in parameters that determine the energy allocation (e.g., Kooijman and Metz (1984); see Figure 1.2).

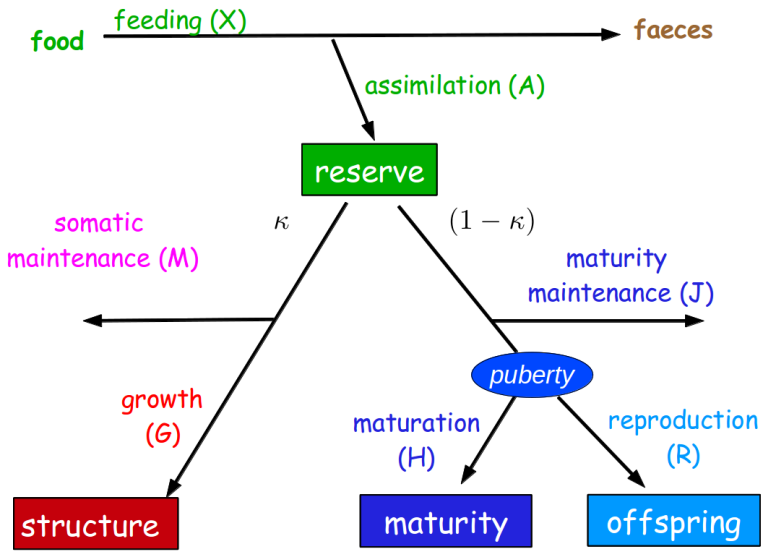

Figure 1.2.: Conceptual scheme of metabolic organization in the DEB framework. A perturbation on a single process such as somatic maintenance (M), costs for growth (G), assimilation efficiency (A) or reproduction efficiency (R) has effects on multiple measurable endpoints. Simulations with different metabolic modes of action (mMoA) are presented in Figure 1.3.

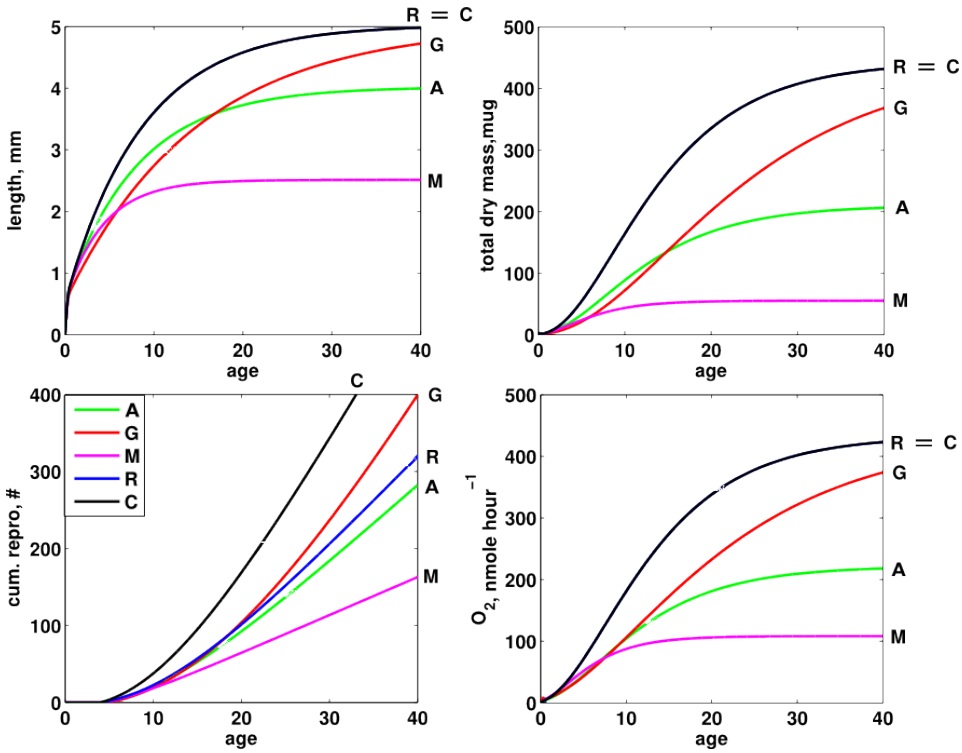

Figure 1.3.: Simulations for life-cycle consequences of the different modes of action presented in Figure 1.2. The colors of the lines correspond to the colors in the model scheme. Black line: control, green line: 80 % reduction of assimilation efficiency, pink: somatic maintenance costs are doubled, blue: 80 % reduction of reproduction efficiency, red: costs for growth are doubled. Simulations were run by Starrlight Augustine with the DEB parameter set for *Daphnia magna* from the add_my_pet - collection, but the general effect patterns are expected for all organisms.

Since DEB follows the development, growth and reproduction of organisms throughout the whole life-cycle, it can be used to investigate effects of stressors over time. This feature can be used to either study the propagation of effects from earlier to later life stages, or to the next generation (and thus the population level). Because of its generality and ability to consider the connections between endpoints, DEB theory holds great potential to become a generally applied method to make sense out of ecotoxicological experiments (Jager et al., 2006).

In more than 30 years of research in DEB theory, many of the most urgent questions have been resolved, but there are still some topics that need to be addressed which are of crucial importance for its use in ecotoxicology.

1.2. Deviations from von Bertalanffy growth

It was observed nearly a century ago by Ludwig von Bertalanffy (1934) that the change in length over time for most organisms follows a certain shape under constant conditions: when expressed as a length measure, growth is continuous, linear in the beginning, and approaches a maximum size asymptotically (see Figure 1.3). The von Bertalanffy pattern in general applies to most isomorphic[5] animals under constant conditions (Kooijman, 2010). A deviation from this pattern often points to a deviation from constant conditions. However, some organisms do not grow following this pattern, even under presumably constant and non-limiting conditions. In some cases, the deviation is caused by a change in shape (Pecquerie et al., 2009; Augustine et al., 2011). In other cases, the deviation can be caused by a food limitation in one part of the life cycle. This partial food limitation can be caused by differences in the ability of food handling depending on body size (Jager et al., 2005). Additionally, a deviation could be caused by changes in the nutritional needs of the organisms during the life cycle. In the laboratory, we mostly feed the test organisms with the same food source throughout the life cycle. In nature, many organisms change their diet during the early life stages. One well known example are mammals, who feed their young on milk in the first part of their life. Another example are ducks, who feed on insects shortly after birth, and change their diet to be mainly herbivorous as adults. One hypothesis is that juveniles in general have a higher need for proteins during their fast growth phase than adults have. If we do not account for a change in the nutritional needs of ecotoxicological test organisms, the juveniles might be more affected by toxic stress due to the interaction with stress by food limitation. Therefore, some standard test protocols already include the use of different food sources along the life-cycle of the test species (e.g., full life-cycle test with fish OECD, 2008). Because feeding is explicitly taken into account in DEB, deviations in feeding conditions or nutritional needs can be included in a DEB model to study the implications for ecotoxicology.

[5] An isomorphic organism does not change in shape during ontogeny.

1.3. Effect patterns: Hormesis

One very interesting phenomenon in ecotoxicology, hormesis, has led to much controversy. Hormesis is a phenomenon that describes a reversed dose-response relationship between low and high concentrations. It is commonly used to describe a dose-response relationship for a single endpoint showing a reversed response between low and high doses of a stressor (Kendig et al., 2010). Usually, a stimulated response to a compound at low concentrations, and an inhibition at higher concentrations is observed. Stressors tend to affect different traits in different degrees (Forbes, 2000), and the effect intensity depends on exposure duration (Jager, 2011). Therefore, it is important to study (hormetic) effects on multiple traits simultaneously and over a longer period of time. A stimulation at low doses is fascinating from an energy budget point of view: the energy that is used for the stimulation must be taken from another part of the energy budget. Since effects on all endpoints can be interpreted in DEB theory simultaneously, it holds great potential for understanding hormetic effect patterns. Kooijman (2009) suggested that an increase of the costs for growth ($[E_G]$ in DEB) could explain a hormetic effect pattern observed in reproduction. Jager et al. (2013) recently discussed many possible physiological mechanisms for hormesis in a DEB context, including an increase in acquisition of energy, a change in energy allocation, or a "medication" (the toxicant reliefs the organism from an unknown stressor in the control) at higher doses. The authors lay the foundation for more mechanism-based research on hormetic responses, and discuss case studies where the various options may have applied. However, hormesis has not been studied in detail in a DEB context yet.

1.4. Maternal effects

Before scaling up to the population level, it is important to understand how the condition of the parents affects the offspring. This applies to toxic stress, but also to the nutritional status. In the standard DEB model, it is assumed that parents produce offspring which have the same relative amount of reserve as themselves. Well-fed mothers produce large eggs, and the offspring have an optimal reserve density. Poorly-fed mothers produce smaller eggs, and the offspring have a less-than-optimal reserve density.

This rule seems to apply to many species (Kooijman, 2010), but the opposite pattern has been observed as well. For example, it has been reported that some species of water flea produce fewer but larger and heavier offspring when food is scarce (Guisande and Gliwicz, 1992; Gliwicz and Guisande, 1992). In general, offspring from smaller eggs are less fit than offspring from large eggs (e.g., Marshall et al. (2003)). Thus, the reserve density of offspring determines their ability to cope with stress, which in turn plays an important role in the population dynamics. Cases where the default DEB hypothesis does not apply still need further investigation.

1.5. Extrapolation to the population level

To extrapolate effects on individuals to the population level, modeling approaches are needed. In general, three fundamentally different approaches are used in ecotoxicology. i) the Euler Lotka equation, ii) Matrix modeling, and iii) individual based modeling.

In the Euler-Lotka approach, the exponential population growth rate in a constant environment is calculated. This approach allows for a quick assessment of a general possible impact on a population when e.g., comparing the effect of different toxicants. However, since only constant conditions can be considered, it cannot be a representation of actual population dynamics.

Matrix models divide individuals in age classes. One state variable for the organism has to suffice, and all individuals in one class have the same characteristics and experience the same conditions. Changing environmental conditions can be considered to some extend, which allows for a rather realistic assessment of population consequences when the differences between individuals in a size or age class can be neglected, and neither reserve nor toxicokinetics play a role.

Individual based models (IBM) are designed for following every single individual in a population. The main advantage is that individuals of the same age can have a different size when they have experienced different environmental conditions. Differences between individuals and multiple state variables per individual can be taken into account, which is closer to reality. One disadvantage of IBMs is the calculation time, which is substantially increased compared to the other two other approaches.

DEB has been applied in combination with all three approaches. While mainly matrix models (Klok and de Roos, 1996; Klanjscek et al., 2006; Billoir et al., 2007) and the Euler-Lotka equation (Kooijman and Metz, 1984; Jager et al., 2004) have been studied, only few attempts have been made to use DEB in an IBM context (Kooijman et al., 1989; Alver et al., 2006; Bacher and Gangnery, 2006). This is rather surprising, since the link between DEB and IBM seems natural: DEB is a model for individuals, in which individuals of the same age can have different sizes depending on their history, due to the separation of maturity and structure. One reason for the reluctance in combining the two approaches might be the complexity of implementation. Learning how to use DEB requires some investment, and implementing an IBM can be very challenging due to the fact that each individual has to be followed separately.

1.6. The pond snail *Lymnaea stagnalis*

In my thesis, I used the pond snail *Lymnaea stagnalis* (see Figure 1.4) to study the above mentioned questions. The pond snail has recently been proposed as a standard test organism for OECD guidelines, which makes it a very relevant model organism for ecotoxicology. The pond snail *L. stagnalis* is a holarctic species,

Figure 1.4.: The shell of the pond snail *L. stagnalis* (left), and two pond snails in the laboratory, preparing for copulation (right). Photographs by M. Dugué.

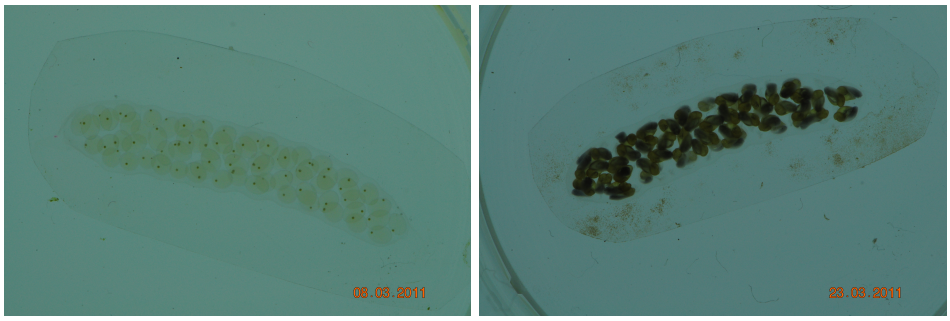

Figure 1.5.: An egg clutch of the pond snail *L. stagnalis* on the day of egg laying (left) and on the last day before hatching (right). These clutches were photographed during the experiment presented in Chapter 5

and occurs in freshwater ponds and slow-flowing streams all over Europe. It is a simultaneous hermaphrodite, that prefers to reproduce sexually (out-crossing) but is also able to reproduce by self-fertilization (selfing). It reaches a shell height of ≈ 4 cm when fed *ad libitum* with lettuce in the laboratory. It can produce an egg clutch with up to 200 eggs every 2-3 days (see Figure 1.5). In response to mating conditions, the pond snail changes its allocation into the male or female function, leading to high plasticity in the investment per egg (Hoffer et al., 2012). Its shell growth has been found to be isomorphic (Kooijman, 1993), so that it is expected to grow following the von Bertalanffy pattern. The pond snail has been studied in a DEB context before (Zonneveld and Kooijman, 1989). However, the authors used a different parameter set for the embryonic and adult period, and for starvation responses. This suggests a deviation from standard DEB, and points to metabolic acceleration (Kooijman et al., 2011).

1.7. The pond snail in my thesis

In my thesis, I studied the growth of the pond snail, and the potential implications of a deviation from the von Bertalanffy growth pattern for ecotoxicity testing, with a simple formulation of the von Bertalanffy growth model (Chapter 2). Using a simplified DEB model (Jager and Zimmer, 2012), I explored how a hormetic effect pattern could be explained in an experiment where the pond snail was exposed to the herbicide diquat (Chapter 3). I designed and conducted an experiment to investigate the interactions of copulation and food limitation in the pond snail, and used a full DEB model parameterized for the pond snail (Chapter 4) to explore potential deviations from the standard DEB formulations for reproduction patterns under various feeding regimes (Chapter 5). To enable the exploration of population-level consequences, we implemented DEB in an IBM software (Chapter 6), which can be used to study the population dynamics of any standard DEB animal. Finally, I come back to the major topics that I introduced here in a general context, and discuss the major results and conclusions of my thesis (Chapter 7).

2. Juvenile food limitation in standardized tests - a warning to ecotoxicologists

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abstract Standard ecotoxicological tests are as simple as possible and food sources are mainly chosen for practical reasons. Since some organisms change their food preferences during the life-cycle, they might be food limited at some stage if we do not account for such a switch. As organisms tend to respond more sensitively to toxicant exposure under food limitation, the interpretation of tests results may then be biased. Using a reformulation of the von Bertalanffy model to analyze growth data of the pond snail *Lymnaea stagnalis*, we detected food limitation in the early juvenile phase. The snails were held under conditions proposed for a standardized test protocol, which prescribes lettuce as food source. Additional experiments showed that juveniles grow considerably faster when fed with fish flakes. The model is based on Dynamic Energy Budget (DEB) theory, which allows for mechanistic interpretation of toxic effects in terms of changes in energy allocation. In a simulation study with the DEB model, we compared the effects of three hypothetical toxicants in different feeding situations. The initial food limitation when fed with lettuce always intensified the effect of the toxicants. When fed with fish flakes, the predicted effect of the toxicants was less pronounced. From this study, we conclude that (i) the proposed test conditions for *L. stagnalis* are not optimal, and require further investigation, (ii) fish flakes are a better food source for juvenile pond snails than lettuce, (iii) analyzing data with a mechanistic modeling approach such as DEB allows identifying deviations from constant conditions, (iv) being unaware of food limitation in the laboratory can lead to an overestimation of toxicity in ecotoxicological tests.

2.1. Introduction

Selecting a single food type for test organisms throughout the test duration can be a challenge, especially in full life-cycle tests. Clearly, we do not try to reproduce the natural environment in the laboratory. The test conditions for the species of interest (light regime, food availability, temperature, etc.) are standardized to conditions which are easy to replicate and have proven to maintain the species in good conditions. These conditions are kept as constant as possible to facilitate the interpretation of test results: only under constant conditions, we can distinguish the effects caused by the chemical of interest from any side effects resulting from the experimental conditions. Since we usually do not know exactly what the test organisms eat in nature, food is mainly chosen for practical reasons: e.g., the pond snail *Lymnaea stagnalis* is usually fed with lettuce (Ducrot et al., 2010b). However, we know that a range of species change their dietary preferences during the life cycle: organisms mainly require proteins to build up new structures, and during the rapid growth in the juvenile stage, the protein requirements are higher than in the adult stage. Well known examples are mammals, that feed their young on protein-rich milk after birth, and chicken and ducks, which feed on insects directly after hatching, but switch to a mainly vegetarian diet as adults. For some ecotoxicological standard test protocols, the switch in diet has been accounted for already, e.g. in the early life-stage toxicity test for fish (OECD, 1992). For each of the five recommended species, detailed information is provided regarding food item(s) for the newly hatched fish, the juveniles, and the adults.

If we aim to maintain the test organisms at constant *ad libitum* feeding conditions, we aim to do so during the whole experiment. A deviation from the *ad libitum* feeding situation results in stress by food limitation. Heugens et al. (2001) reviewed how additional stressors such as nutritional state influence the toxicity of test compounds to aquatic organisms. A decrease in food level usually increases toxicity: depending on the nutritional state of the organism, the toxicity can be 10-fold higher in comparison to well-fed organisms (Heugens et al., 2001).

If the nutritional status is so important for the sensitivity of organisms, how can we monitor it? One simple way to test for the constancy of environmental conditions for organisms, such as the availability of proper food, is to scrutinize the growth curves. It was observed nearly a century ago by Ludwig von Bertalanffy (1934) that the change in length over time for most organisms follows a certain shape under constant conditions: when expressed as a length measure, growth is continuous, linear in the beginning, and approaches a maximum size asymptotically. The von Bertalanffy pattern applies for most animals under constant conditions (Kooijman, 2010). Therefore, a deviation of the growth curve from this pattern points to a deviation from constant conditions for the organism in its environment. For example, for bacterivorous nematodes, it was found that the deviating growth curves could be explained by juvenile food limitation: the mouth parts of newly hatched worms do not allow efficient feeding on the bacterial food provided in experimental tests (see Jager et al., 2005).

Investigations on the toxicity of chemicals generally aim to assess the impact caused by the chemicals in the environment, where organisms hardly ever experience constant conditions. We therefore need mechanistic approaches that take into account interactions between toxicants and environmental factors such as food conditions and/or temperature. Mechanistic modeling approaches receive more and more attention in this context (Grimm et al., 2009; Preuss et al., 2009).

In this study, we combined experiments and modeling to investigate juvenile food limitation, and assess the potential for bias in the interpretation of ecotoxicological test results. We chose the great pond snail (*Lymnaea stagnalis*) as model organism, because it has been identified as a relevant candidate species for the development of toxicity test guidelines by the OECD (OECD, 2010). The proposed laboratory conditions are currently under investigation regarding their suitability for standardization. While investigating growth data from a full life-cycle experiment (Ducrot et al., 2010a), we found a deviation from the von Bertalanffy pattern. We hypothesize that this deviation is due to food limitation in the early juvenile phase. We use a reformulation of the von Bertalanffy growth model that is applied in Dynamic Energy Budget (DEB) theory (e.g., Kooijman et al., 2008), because it allows for mechanistic interpretation of toxic effects and their interaction with food availability. In DEB, the effect of a toxicant can be understood as a change in the acquisition or allocation of resources (Jager and Zimmer, 2012). We used existing partial and full life-cycle data on different food levels without toxicant exposure to parameterize an individual model for the pond snail. To test our hypothesis of juvenile food limitation, we conducted experiments with different types and amounts of food with freshly hatched snails. Using model simulations with hypothetical toxicants, we investigated the combined effect of juvenile food limitation and toxicity on the interpretation of ecotoxicological experiments.

2.2. Materials and Methods

2.2.1. Growth model

The von Bertalanffy growth model has been shown to apply to the pond snail *Lymnaea stagnalis* by Zonneveld and Kooijman (1989). The shell length L (in cm) is given as

$$\frac{dL}{dt} = \dot{r}_B(L_\infty - L), \tag{2.1}$$

where $\dot{r}_B$ is the von Bertalanffy growth rate constant (in d^{-1}), and L_∞ is the maximum shell length at abundant food. The growth rate constant $\dot{r}_B$ determines how fast the organism reaches its maximum size L_∞. The parameters of Eq. 2.1 are only constant for organisms that experience constant conditions. A change in food level will both affect $\dot{r}_B$ and L_∞. To explain these effects, we use the reformulation of the von Bertalanffy growth model that is used in DEB theory (Kooijman et al., 2008).

DEB theory provides a conceptual framework that explains how organisms allocate energy from food into growth, reproduction, development, and maintenance. The same framework can be applied to all organisms; inter-species differences are mainly expressed as differences in parameter values. The model parameters determine how much energy is invested in which process, i.e. how expensive one process is relative to the others. We will here only shortly mention the parameters that are important for the present work, i.e. the parameters that play a role in the growth model. A more detailed description of the model and the underlying assumptions can be found in Appendix A, Section A.1. For more introduction on DEB theory, we refer the reader to Van der Meer (2006), and for a deeper insight, to Kooijman (2010).

The two parameters of the von Bertalanffy equation can be expressed in terms of DEB parameters as

$$L_m = \frac{\dot{v}}{\dot{k}_M g}, \quad L_\infty = f L_m \quad \text{and} \quad \dot{r}_B = \frac{g\dot{k}_M}{3(f+g)}, \tag{2.2}$$

which reveals how L_∞ and $\dot{r}_B$ are linked to each other and to the food level. In Eq. 2.2, the following DEB parameters appear: g, the energy investment ratio $(-)$, $\dot{v}$, the energy conductance $(\mathrm{cm\,d^{-1}})$, $\dot{k}_M$, the somatic maintenance rate coefficient $(\mathrm{d^{-1}})$, and the scaled functional response f $(-)$. The scaled functional response f is the actual ingestion rate of an animal divided by the maximum ingestion rate for its size. For an individual under *ad libitum* feeding conditions, $f = 1$, whereas for a starving individual, $f = 0$, so that for limiting conditions $0 < f < 1$. Note that we assume fast reserve dynamics and thus use the scaled functional response f in $\dot{r}_B$ instead of the scaled reserve density e (see Appendix A, Section A.1.2, Fig. A.1. How these parameters are linked to metabolic processes is explained in detail in Jager and Zimmer (2012).

Metabolic rates are known to depend on temperature, and usually, the Arrhenius relation provides a good description for the temperature dependence across species (Gillooly et al., 2006). In DEB, this is accounted for: all parameters that have the dimension per time $(\mathrm{d^{-1}})$ are multiplied by a temperature correction factor that can be derived from the Arrhenius relation (see Freitas et al. (2007) for the procedure). We need to account for the temperature dependence of the parameters because one of the experiments (see description below) has been conducted at a different temperature than the others. For the Arrhenius temperature, we use a typical value of 11900 K (see Kooijman, 2010, and Appendix A, Section A.1.4).

To describe the effect of juvenile food limitation on growth, we make the scaled functional response f a function of body size. For simplicity, we use a linear function for f related to length:

$$f(L) = a f_0 \frac{L}{L_m} \qquad \text{for} \qquad L < L_f, \tag{2.3}$$

where a is the *food quality factor*. This is a dimensionless constant, which relates

to the quality of the provided food source. Note that we use the normalized length (L/L_m) to keep a dimensionless. The parameter f_0 is the scaled functional response for $L > L_f$ in that treatment. It will be set to 1 for *ad libitum* feeding with lettuce, and for the highest amount of fish flakes given in the juvenile feeding experiment (see description below). The growth of the snails follows the von Bertalanffy pattern after they reach a certain shell length (see Fig. 2.1). We call this length the critical length L_f (cm), and assume that above that size, the snails are not limited by food quality anymore (i.e. $f = f_0$ in the model).

2.2.2. Simulation experiments with hypothetical toxicants

The DEB model allows for mechanistic interpretation of toxic effects, which enter the model as changes in parameter values (Jager et al., 2010). To study the interaction between toxicants and juvenile food limitation, we use model simulations where we include different metabolic mechanisms of action (mMoA) in terms of DEB. The inclusion of toxic effects in the DEB model is explained in detail in Jager and Zimmer (2012). Each mMoA leads to a specific combination of effect patterns (e.g. effects on growth, reproduction, development, feeding, respiration). All scenarios were run with the control value for all model parameters (i.e., the parameters that were estimated from experiments without toxicants), and with a range of values to simulate stress due to three hypothetical toxicants with different mMoAs. We use the three general mMoAs that affect growth in the context of DEB theory: (1) an increase of costs for somatic maintenance (i.e., $\uparrow \dot{k}_M$), (2) a decrease of assimilation efficiency (i.e., $\downarrow f$), and (3) an increase of costs for growth (i.e., $\downarrow \dot{k}_M$, $\uparrow g$) (see Jager and Zimmer (2012), and the Appendix A, Section A.1.3, and Table A.2). We assumed that a given toxicant would only impact one metabolic process. For the effect on assimilation and the effect on maintenance, the range of increase was simulated up to 15 %, while for the effect on costs for growth, it was simulated up to 600 %. These mMoAs have been found in several analyses of ecotoxicological data sets, for example in the nematodes *Acrobeloides nanus* and *Caenorhabditis elegans*, which were tested with carbendazim, cadmium, pentachlorobenzene, and aldicarb (Alda Álvarez et al., 2006; Wren et al., 2011). E.g., in the highest exposure concentration with Cadmium, the effect on the growth costs on *A. nanus* was predicted to increase from some 200% up to 600% over the life cycle. We additionally implemented three feeding scenarios: (A) individuals under true *ad libitum* feeding ($f(L) = 1$ throughout the life-cycle), (B) individuals with food limitation in the juvenile phase ($0 < f(L) < 1$), which were assumed to be fed with lettuce and (C) individuals with food limitation in the juvenile phase, which were assumed to be fed with fish flakes. In combination with the three different mMoAs, we therefore have nine different simulation studies. To facilitate the comparison with the life-cycle experiments, the duration of the simulations was 400 d.

2.2.3. Description of available data: the full life-cycle (FLE) and the partial life-cycle (PLE) experiments

To determine the DEB parameters that govern growth, it is essential to have data on body size over the life-cycle at different food levels (Kooijman et al., 2008). We used data from a full life-cycle experiment (336 d, from now on FLE) at *ad libitum* food, and data from a partial life-cycle experiment (184 d, from now on PLE) at three different food levels for the parameterization. Those experiments had been conducted earlier for other projects at the French National Institute for Agricultural Research, INRA, in Rennes. The experimental protocol, the setup, and part of the data of the FLE have been published by Ducrot et al. (2010a). The PLE has been conducted using a very similar protocol and setup, where only the initial conditions (i.e., initial age and size of snails) and the feeding conditions were slightly different. Therefore, we only give a short description of both experiments here. All snails mentioned in the present work originate from the culture at INRA in Rennes. A detailed description of the culture conditions and the experiments as well as the data used for the parameterization of the model can be found in the Appendix A, Section A.2.

The FLE was conducted to assess the effects of diquat on the life-cycle of *L. stagnalis* (Ducrot et al., 2010a). We used the growth data of the controls in the present work. The whole experiment was conducted under a photoperiod of 14/10 L/D at 21 ± 1°C. Freshly hatched snails were transferred to the test vessels in groups of five (24 replicates). They were fed abundantly with weighed slices of organic lettuce (*Lactuca sativa*), but only if no leftover remained, to avoid spoiling the water quality due to the degradation of leftovers. The freshly hatched snails were fed with one slice of 21 mm Ø. The number of slices of lettuce given was doubled when half of the replicates had fully consumed the previously provided lettuce on the next day. The test vessel volume was gradually increased along the snail life-cycle, to ensure relatively constant conditions concerning competition for space and food (see Appendix A, Fig. A.2).

The PLE was conducted to assess the impact of different food levels on the growth and reproduction of the pond snail. It was conducted under the same photoperiod and temperature as the FLE. In contrast to the FLE, this experiment started with juveniles (age 113 d and shell size 12.7 ± 1.3 mm) that had been reared under culture conditions. The size at the beginning of the PLE was chosen based on the outcome of the FLE: from a size of around 1 cm, the snails in the FLE grew following the von Bertalanffy growth pattern. The snails were kept at three different constant food levels. The first regime was supposed to be *ad libitum*, where the amount of food given the first day was determined from the amount of lettuce that was eaten in the FLE from snails of similar size. After that, the amount of lettuce given was doubled each time when half of the regimes had consumed the previously given amount of lettuce. To determine the amount of lettuce that was consumed, the lettuce was weighed before feeding, and the leftovers on the next day were weighed as well. The second and third regime received half of that weight and a quarter of

that weight.

2.2.4. The juvenile feeding experiment (JFE)

To test the hypothesis of juvenile food limitation in the FLE, we conducted a juvenile feeding experiment (from now on JFE) with different types and quantities of food. We took clutches from the culture and let them develop under the same light regime as for the longterm experiments, but at a temperature of 23.5 ± 0.75°C. We collected hatchlings of similar age (1 ± 1 d) and size (1.4 ± 0.1 mm) and transferred them into 100 ml test vessels in groups of five. The complete water volume was renewed weekly and they were fed three times a week with different amounts of lettuce or TetraPhyll® fish flakes (see Appendix A, Table A.4). We tested two regimes were the snails were fed *ad libitum* with lettuce. In one regime (Lettuce 1), sand was added, under the hypothesis that it would facilitate food digestion. The amount of lettuce given was determined in terms of numbers of slices of 21 mm Ø. The amount of fish flakes was chosen based on a recommendation for well-fed adults of *Potamopyrgus antipodarum*(OECD, 2010), which reaches the same size as juveniles of *L. stagnalis* (≈ 0.5 cm). We used a value that was slightly higher than the recommended value (0.3 mg/day/ind., recommended 0.25) for the maximum food level as a starting value, and increased the amount given if all had been eaten on the next feeding day. The two lower levels were calculated as half and a quarter of the maximum amount. Food was only provided if no leftover remained. Shell size was measured weekly using a binocular microscope fitted with a micrometer. The experiment lasted 28 days.

2.2.5. Obtaining model parameters and error structure from experimental data

All model parameters were estimated from the growth data of the FLE, PLE and JFE simultaneously, whereby the initial length L_0 was estimated for each experiment. The food quality factor a (Eq. 2.3) was estimated for the different food sources: one a was estimated for fish flakes from the JFE, whereby a different f_0 was estimated for each of the three food levels. In both the JFE and the FLE, lettuce was provided *ad libitum*, so that we set $f_0 = 1$ in both experiments. However, we needed to estimate one a for the juveniles in the FLE (i.e., $L < L_f$), and a different a for the lettuce-fed regime in the JFE to be able to capture the observed patterns. Note that we use the same L_f for lettuce and fish flakes since the JFE was too short to estimate a separate value the fish flakes experiment treatment.

The estimation routines were implemented in Matlab 2010a; scripts to perform these calculations can be found on http://www.debtox.info/debtoxm.php. We used maximum likelihood optimization, and derived the confidence intervals by profiling the likelihood (e.g., Meeker and Escobar, 1995). For the maximum likelihood estimation, we need to make an assumption for the error structure of the data. An analysis of the scatter structure of the data showed that the variance increased with

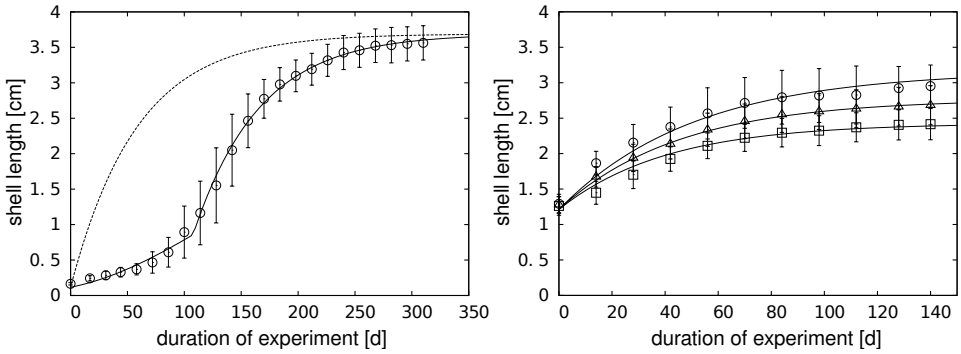

Figure 2.1.: The growth curves obtained in the full life-cycle experiment (FLE, left panel) and the partial life-cycle experiment (PLE, right panel). The symbols are the mean values of the measured shell length, the error bars the corresponding standard deviations. The lines correspond to the model predictions: the dashed line is the prediction without the juvenile food limitation function for the FLE. Right panel: ∘ *ad libitum* lettuce, △ 50 % of *ad libitum*, □ 25 % of *ad libitum*.

mean shell length for the PLE and the JFE (see Appendix A, Fig. A.3). However, the error structure of the FLE shows a different pattern: the growth data has a high variance in the fast growth phase, and a lower variance in the slower growth phases, i.e. at the beginning and at the end of the experiment (see Appendix A, Fig. A.3). We used an empirical spline function to describe the variance as a function of length, and used the actual error pattern in the likelihood function (see also Jager and Zimmer, 2012).

2.3. Results

2.3.1. Experiments and model fit

The growth curves from the FLE and the PLE and the corresponding model fits are shown in Fig. 2.1.

The growth pattern in the FLE cannot be reproduced with the standard DEB model in constant environmental conditions which follows the von Bertalanffy growth pattern (Fig. 2.1, left panel). With the juvenile food limitation (see Eq. 2.3), the pattern can be captured. The PLE was started with larger individuals and the growth pattern follows the von Bertalanffy pattern from the beginning (Fig. 2.1, right panel). The corresponding model parameters are shown in Table 2.1. We use shell length as our measure of body size, which implies that all parameters with length in their dimension (including $\dot{v}$) are based on this size measure. Note that the maximum shell length reached by the snails fed with the maximum food level in the PLE is lower than the one in the FLE, although both were supposed to be

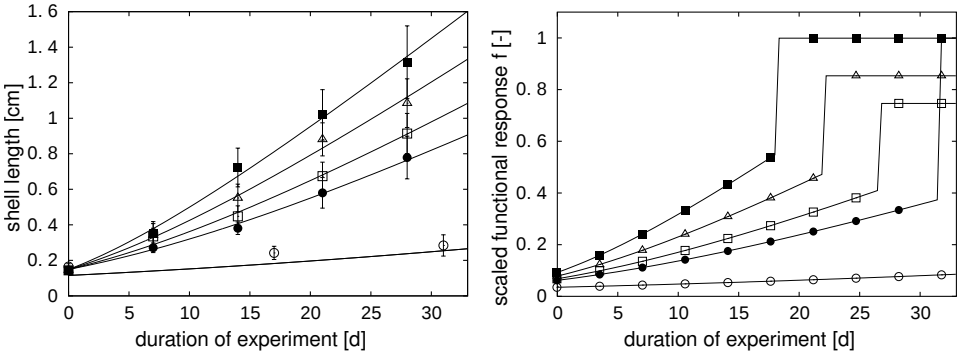

Figure 2.2.: Growth of the juvenile pond snails in the full life-cycle experiment (FLE) and juvenile feeding experiment (JFE), and the corresponding model predictions (left panel). JFE: ■ maximum level fish flakes, △ medium level fish flakes, □ minimum level fish flakes, • *ad libitum* lettuce; FLE: ○ *ad libitum* lettuce. The scaled functional response f (as a proxy for food availability), resulting from the linear food limitation function (Eq. 2.3 , Table 2.1) is presented as a function of time (right panel). The symbols on the model lines stand for the same regimes as in the left panel, but do not represent data points.

fed *ad libitum*. Thus, the scaled functional response that was estimated for the PLE is smaller than 1 (see Table 2.1).

The growth curves of the JFE are shown in Fig. 2.2 (left panel). The newborn pond snails grow much faster when fed with fish flakes, and reach double the size of the lettuce-fed snails after four weeks. Note that we used the mean growth of regime Lettuce 1 and 2, since they were not significantly different (see Appendix A, Table A.5). Additionally, there is a difference between the juveniles fed with lettuce in the JFE compared to the juveniles in the FLE: the juveniles in the FLE only reached half the size of the juveniles in the JFE after four weeks, which is reflected in the difference between a_{let1} and a_{let2}. The estimated food quality factors a as well as the constant scaled functional responses f_0 for each regime are listed in Table 2.1. The scaled functional responses resulting from Eq. 2.3 over time are presented in Fig. 2.2 (right panel). Note that *f(L)* is still between 0 and 1 by definition.

2.3.2. Model simulations

The simulated growth curves for the scenarios with the three hypothetical toxicants are shown in Fig. 2.3.

In each graph, the top line represents the simulation with the default parameter, and the lines from top to bottom represent simulations with the changed parameter value. Note that for effects on maintenance (1) and assimilation (2), the parame-

Table 2.1.: Model parameters and values as fitted simultaneously using maximum likelihood optimization from the FLE, PLE, and JFE. Confidence intervals were derived using profile likelihoods.

Parameter	Unit	Value	95 % conf. interval	Definition
$\dot{k}_M$	d^{-1}	0.4882	0.3246 – 0.8781	somatic maintenance rate coefficient (21°C)
$\dot{v}$	cm d^{-1}	0.2121	0.197 – 0.227	energy conductance (21°C)
g	–	0.1176	0.0627 – 0.1821	energy investment ratio
L_f	cm	0.8687	0.7718 – 0.9789	critical length
L_{01}	cm	0.1151	0.07589 – 0.165	initial length FLE
L_{02}	cm	1.212	1.154 – 1.271	initial length PLE
L_{03}	cm	0.1491	0.1385 – 0.1596	initial length JFE
food level FLE				
f	–	1	–	*ad libitum* feeding with lettuce (fixed to 1)
food level PLE				
f_1	–	0.8579	0.8236 – 0.9145	highest level
f_2	–	0.7505	0.7311 – 0.7736	medium level
f_3	–	0.6679	0.6431 – 0.6733	lowest level
food level JFE				
f_{01}	–	1	–	highest quantity fish flakes (fixed to 1)
f_{02}	–	0.8549	0.8247 – 0.8813	middle quantity fish flakes
f_{03}	–	0.7482	0.7166 – 0.7745	lowest quantity fish flakes
food quality factor				
a_{tet}	–	2.359	2.198 – 2.577	food quality factor fish flakes
a_{let1}	–	1.235	1.183 – 1.303	food quality factor lettuce, FLE
a_{let2}	–	1.61	1.548 – 1.688	food quality factor lettuce, JFE

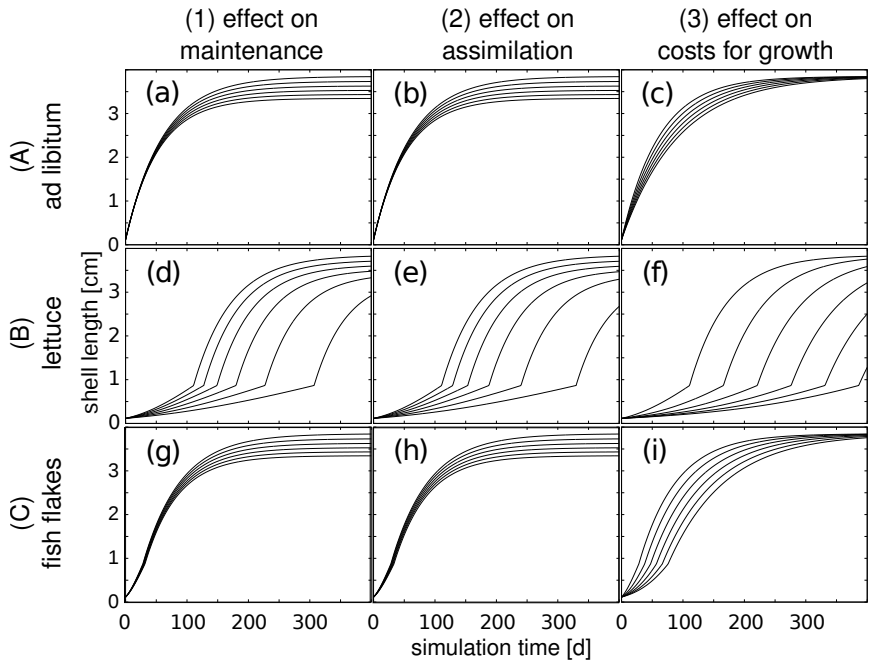

Figure 2.3.: The simulation of growth curves of snails exposed to the hypothetical toxicants. From left to right, the different mechanisms of effect are shown, while from top to bottom, the feeding scenarios are displayed. Scenarios: a - c without food limitation; d - f with the linear food limiting function, assumed to be fed on lettuce; g - i with the linear food limiting function, assumed to be fed with fish flakes. Top line: control conditions. Lines from top to bottom represent the scenarios were the respective parameters are changed.

ters were increased up to 15 %, so that each next line denotes an increase of the corresponding parameter value of 3 %. For the effect on costs for growth, the parameter was increased up to 600 %, so that each next line denotes an increase of 100 %. An effect on maintenance (left panel, (1)) decreases the maximum size (see Fig. 2.3a). While the initial growth is hardly influenced in the fish flakes scenario (see Fig. 2.3g), the initial growth of the lettuce fed juveniles is strongly impacted (see Fig. 2.3d), and the maximum size is only reached at the end of the simulation time for all levels of impact. The effect on assimilation (middle panel, (2)) strongly resembles the effect on maintenance: the maximum size is decreased under real *ad libitum* feeding conditions (see Fig. 2.3b). However, for the lettuce scenario (e), juvenile growth is stronger impacted than with an effect on maintenance with the same percentage of effect (d). The effect on costs for growth (right panel, (3)) shows a different pattern: the maximum size is not impacted, but the growth rate is decreased (see Fig. 2.3c). When fed with fish flakes, the effect is slightly stronger in the early juvenile phase (see Fig. 2.3i). However, when fed with lettuce (see Fig. 2.3f), the growth is strongly impacted in the juvenile phase, and the growth pattern resembles the two other toxicants, when fed with lettuce.

2.4. Discussion

2.4.1. Deviation of the von Bertalanffy growth pattern

The growth curve that was obtained in the FLE deviates from the von Bertalanffy pattern, and thus from the predictions of the standard DEB model in constant environmental conditions (Fig. 2.1). A deviation from the von Bertalanffy pattern could have two reasons: (1) the assumptions that underlie the model structure do not hold for the pond snail, or (2) the conditions, to which the model applies, are not met. The von Bertalanffy model applies to organisms that grow isomorphically (i.e., do not change in shape). Kooijman (1993) shows that the shell of the pond snail does grow without a change in shape. Thus, it should follow the von Bertalanffy growth pattern under constant conditions. The lack of this pattern in Fig. 2.1 (left panel) suggests that the snails do not experience the experimental conditions as constant. In nature, the pond snail can be considered a generalist, and its food consists of detritus and decomposed macrophytes (Kolodziejczyk and Martynuska, 1980). In the adult stage, the main part of its food is thought to consist of macrophytes (Elger and Lemoine, 2005), while juveniles and hatchlings probably mainly feed on periphyton. This switch in diet is not accounted for in the experimental setup. Therefore, we hypothesize that the snails do not experience a constant food level, although they are constantly fed with an *ad libitum* amount of lettuce. Instead, the juveniles are limited, either by the composition or accessibility of the provided food. The outcome of the JFE supports our hypothesis: the newborn pond snails grow much faster when fed with fish flakes, and reach double the size of the lettuce-fed snails after four weeks (Fig. 2.2).

2.4.2. Food limitation in the pond snail

The simplest relation that could provide a good description of the growth pattern was a linear function of body size (see Eq. 2.3). With this function, we were able to capture the observed pattern with the DEB model (see Fig. 2.1).

With the linear food quality factor, we can qualitatively compare the nutritional status of the juveniles in the JFE: it is higher for fish flakes than for lettuce (see Table 2.1). These differences might be due to the accessibility of the provided food. Fish flakes have a very low density: they first float on the water and sink to the bottom of the test vessels when they soak up enough water. In contrast, the lettuce only floats on the water surface. The pond snail is a grazer, and searches for food on all surfaces of the test vessel (i.e., water surface and vessel walls). The fact that the fish flakes are both on the water surface and on the bottom of the vessel increases the chance of the juvenile snails to find a food item. Moreover, the small size of fish flakes might facilitate the uptake by the small mouth openings of the juvenile snails.

Apart from accessibility, the food limitation might as well result from the composition of the provided food. Among the types of food that have been tested in the laboratory, dry matter content (DMC) and protein content are the two main determinants of food preference for the pond snail. In a study on the palatability of macrophytes, the two species with the highest protein content in combination with the lowest DMC were the most appealing to the snails (*Berula erceta*: DMC 11.3 %, protein 10.8 %, and *Sagittaria sagittifolia*: DMC 5.4 % and protein 12.8 %, see Elger and Lemoine, 2005). The composition of lettuce is in general similar to macrophytes, and seems to fulfill the requirements of the adult pond snails: the protein content of lettuce varies between 20 % (McKeehen et al., 1996) and 40 % (Selck et al., 2006), whereas the DMC varies between 5 % and 12 %. In the juvenile stage, pond snails might be mainly feeding on periphyton and biofilms. For both, the protein content and DMC highly depends on the species composition and substrate. The protein content of biofilm can vary between $5-20$ %, depending on the species compostion, (Fernandes Da Silva et al., 2008),while the protein content of periphyton can vary between $13-32$ %, depending on the substrate (Azim et al., 2002). The DMC of periphyton can be around 10% (Sladecek and Sladeckova, 1963). The protein content of TetraPhyll® fish flakes given by the manufacturer is 46%. The high protein content and softness of the fish flakes seems to make it a better food for the juvenile snails than lettuce. Yet, further experiments are needed to investigate the effects on the rest of the life-cycle.

2.4.3. Protein content of food in other aquatic invertebrates

Finding the right food type to culture aquatic organisms is a challenge. The influence of different food sources is mainly studied concerning the growth of animals, not only in eco(toxico)logical experiments (e.g., Ristola, 1995; Egeler et al., 2010), but also in bioproduction for human consumption (e.g., van Dam et al., 2002). In

aquaculture, juvenile growth under laboratory conditions has been studied intensively, and recently higher growth efficiency has been linked to protein content of the food (e.g. for queen conch *Strombus gigas*, Garr et al., 2011). Additionally, in the snail *Potamopyrgus jenkinsi*, lamb heart versus lettuce has been tested (Dorgelo et al., 1995), and in *Marisa cornuarietis*, growth in the juvenile phase has been investigated using Tetramin®, baby cereals and spinach (Selck et al., 2006). In both studies, the motivation was to test types of food with higher protein content than lettuce, and to compare the performance. However, only in *M. cornuarietis*, fast growth could be directly linked to protein content, while in *P. jenkinski*, protein content was not the only determinant for fast growth: the snails grew fastest on a mixture of lambs heart and lettuce (Dorgelo et al., 1995).

2.4.4. Differences in patterns following from experimental setup

Interestingly, the juveniles in the FLE grow much slower than the slowest ones in the JFE, although both are fed *ad libitum* with lettuce (see Fig. 2.2). The experiments have been conducted under different temperatures: the FLE at 21 °C, and the JFE at 23.5 °C. All rate constants in organisms tend to depend on temperature in a very similar way (Gillooly et al., 2006). By including the temperature dependence of the rate parameters $\dot{k}_M$ and $\dot{v}$, part of the difference in growth can be explained. However, even with the inclusion of the temperature dependence, we need a lower food quality factor in the FLE than in the JFE to be able to capture the growth pattern. One reason might be a difference in the quality of the provided lettuce: the nutrient content of lettuce is known to vary with growing season (Fallovo et al., 2009), and the two experiments were started at different times of the year (see Appendix A, Section A.2.3).

Surprisingly, the snails in the FLE and the PLE reach a different maximum length, although both are supposedly fed *ad libitum* with lettuce. The reason for the difference might be a difference in the determination of the *ad libitum* feeding regime in the two setups. The amount of lettuce was doubled whenever half of the replicates had eaten all that was provided the day before in both setups. However, while in the FLE the amount given was determined in terms of surface area (i.e. number of slices), in the PLE the amount was determined by weight (see Appendix A, Section A.2.3). Additionally, the maximum size of the snails in both experiments is much smaller than the maximum size of *Lymnaea stagnalis* as observed in nature ($\approx$ 6 cm, see Appendix A, Section A.2.3). Both the FLE and the PLE may thus not have represented *ad libitum* feeding conditions for adults.

2.4.5. Model simulations

The DEB formulation of the von Bertalanffy growth model allows us to study possible implications of the food limitation for ecotoxicological tests. The simulation studies in Fig. 2.3 show that food limitation has synergistic effects with all tested hypothetical toxicants. Under real *ad libitum* conditions, the effect of the toxi-

cant is not very pronounced: for effects on maintenance costs and assimilation, the maximum size is reduced slightly (Fig. 2.3a and b), while with an effect on costs for growth, the growth rate is decreased, but there is no effect on ultimate length (Fig. 2.3c). In the simulations where the juveniles are assumed to be fed with lettuce, and thus food limited, the same degree of effect on the target parameter leads to stronger effects on growth. In addition to the effects on growth observed in the simulations, the juvenile phase is prolonged by all hypothetical toxicants (Fig. 2.3d, e, f). In organisms that start reproducing at a given size (e.g., the pond snail), a decrease in juvenile growth would imply a delay in the start of reproduction, which can have a substantial impact on the population growth rate (Kammenga et al., 1996). Moreover, even for organisms that start reproducing at a given age, the population would be affected: smaller organisms eat less and have less energy available to invest in reproduction than larger organisms. In the simulations where the juveniles are assumed to be fed with fish flakes, there is hardly any visible additional effect (Fig. 2.3g, h, i). Only for the simulation with an increase in the costs for growth, the juvenile phase is slightly prolonged.

The simulation studies thus indicate that juvenile pond snails feeding on lettuce may show larger response to the same toxic stress than juvenile snails feeding on fish flakes. If we are not aware of the food limitation, this can lead to an overestimation of the toxicity of the tested compound. However, the interaction between the food limitation and the chemical depends on its mMoA.

2.4.6. Implications for ecotoxicology and risk assessment

Eventually, we want to assess the impact of a compound on the test organisms under natural conditions. Since organisms hardly ever experience unlimited food conditions in nature, the response under food limitation might be a more realistic representation of toxicity. However, in the test system we describe (the pond snail fed on lettuce), the degree of food limitation changes with the size of the snails. In mechanistic modelling of the toxic effect with DEB, we can account and compensate for that fact in the interpretation of the data (see Jager and Klok, 2010). However, when using a descriptive analysis of the data (such as the ECx from a dose-response curve), the size-dependent (and thus also time-dependent) feeding limitation in a toxicity test will lead to bias in the results. The simulation study demonstrates that an unrecognised food limitation can lead to serious overestimation of toxicity, compared to the effects under good nutritional status. Even though food limitation may be realistic, such a bias in our toxicity data hampers the comparison of toxicity between chemicals and between species. The interaction between food limitation and toxicants is strong and ecologically relevant, but we need to study it in a controlled way. We need to separate the effects of the toxicant from the effects of the food limitation to be able to understand the mechanisms behind their interaction. Only then we can develop reliable models that can predict effects under different feeding scenarios, and thus support a scientifically sound risk assessment.

2.5. Conclusions

In this study, we demonstrate the importance of food selection for ecotoxicological experiments. We showed that *L. stagnalis* is food limited in the juvenile phase, under conditions which are under consideration as standard conditions for OECD guidelines. Our simulation studies show that food limitation exaggerates the response to toxic stress. The interpretation of the results of life-cycle experiments (which include the early juvenile phase) may thus be biased when lettuce is used as the sole food source. Alternative food sources should be considered to avoid potential overestimation of toxicity. By using a mechanistic effect model (e.g. DEB), we can include the observed food limitation in the analysis and interpretation of test results. This modelling approach could thus potentially serve as a tool for extrapolating to other environmental situations for ecological risk assessment. Although food limitation is an ecologically relevant stress factor, we need to be able to separate these effects from the toxicity of the chemical stressor to make sense of the underlying mechanisms. Growth curves that deviate from the von Bertalanffy pattern are a good indication (but not a proof) of experimental problems with the food supply. Since a change in diet is a common strategy among organisms, this phenomenon does not only apply to the pond snail. So ecotoxicologists, be warned!

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3. Interaction between food and toxicant leads to hormesis in the pond snail *Lymnaea stagnalis*

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abstract The degree of toxicity of a chemical compound depends critically on the nutritional condition of the affected organism. However, in many cases, the actual nutritional status of the organisms in an ecotoxicological test is unclear. Here, we investigate a previously published study on the pond snail *Lymnaea stagnalis* exposed to the herbicide diquat under environmentally relevant concentrations. A hormetic effect pattern in juvenile feeding rate and time to first reproduction was observed. In the experiment, the pond snail was fed with lettuce. Diquat is a non-selective herbicide and acts on the lettuce, with a dose-dependent degradation of the food source as a consequence. To study the interaction between food and toxicant, we use a Dynamic Energy Budget (DEB) model. The observed effect patterns can be fully explained by effects on assimilation efficiency, with assimilation enhanced by low doses of diquat, and decreased by higher doses. With an effect on assimilation efficiency, the same amount of lettuce consumed leads to differences in the amount of energy that is obtained. Higher assimilation leads to faster growth, which in turn leads to differences in feeding rate and a shift in time to first reproduction.

3.1. Introduction

The quality and quantity of food have a major impact on the results of ecotoxicity testing. It is often observed that the toxic effects are more pronounced under limiting food conditions compared to unlimited conditions (Heugens et al., 2001).

Food limitation can occur via two different routes: either the amount of food is too low, or the quality of the provided food is insufficient to meet the nutritional demands of the organism. Both situations should be avoided in laboratory testing because they can lead to a misinterpretation of test results (see Chapter 2).

To interpret toxic effects in relation to food availability, it is advisable to distinguish between 'apparent' and 'intrinsic' sensitivity (Pieters et al., 2006). Apparent sensitivity is the relationship between the external concentration of a toxicant and the effect on a life-history trait (e.g., quantified by an ECX). In contrast, intrinsic sensitivity can be seen as the relationship between the internal concentration of the toxicant and the affected metabolic process (e.g., maintenance costs or assimilation). An increased apparent sensitivity to toxicants due to food limitation is not necessarily caused by an increase in intrinsic sensitivity; the same effect on a metabolic process may lead to an increased impact on a trait under food shortage (Kooijman, 1985; Pieters et al., 2006). Distinguishing between apparent and intrinsic sensitivity requires the use of mechanistic effect models that explicitly take feeding into account. Using a simple mechanistic effect model, we recently showed how unrecognized food limitation in juveniles of the pond snail *Lymnaea stagnalis* can dramatically increase effects on growth without affecting intrinsic sensitivity (see Chapter 2).

There are several ways in which toxicity and food availability can interact. The reasons for these interactions can be understood when considering that toxicokinetic (TK) and toxicodynamic (TD) processes are both linked to body size, but in a different way. Every biological and physico-chemical process involved in the uptake of a compound into an organism is part of TK. All processes that link the internal concentration to the measured endpoint are part of TD. Food availability affects growth, and thus the surface area to volume ratio, which in turn affects the TK rate constants. Furthermore, growing organisms constantly dilute their internal concentration of a compound. TD processes are also affected by changes in food availability. A change in food level alters energy uptake, and thus the energy available for the metabolic processes. Under limiting conditions, organisms are able to reduce the investment in growth and reproduction, while maintenance costs cannot be easily reduced, resulting in increased relative maintenance costs (Kooijman and Metz, 1984; Jager et al., 2006). Consequently, an effect of a fixed percentage of increase in maintenance costs has a larger impact on individuals under food limitation.

To examine the interactions between food, food limitation and toxic effects, we scrutinize a study with *L. stagnalis* exposed to the herbicide diquat, previously published by Ducrot et al. (2010a). This study is interesting for several reasons. We recently found that snails are food limited as juveniles when fed with lettuce, which is the typical food source in ecotoxicological experiments with the snail (see Chapter 2). In addition, diquat was observed to affect the quality of the lettuce (Ducrot, personal observation). Moreover, *L. stagnalis* is about to become a standard organism for aquatic freshwater testing in the OECD guidelines. The test had been conducted under the protocol that is currently under investigation for

standardization, and thus represents a good example to scrutinize the suitability of the test conditions.

Ducrot et al. (2010a) found a hormetic effect pattern in juvenile feeding and time to first reproduction. Recently, different mechanistic explanations for hormesis have been presented (Jager et al., 2013), namely the stressor (i) increases the energy uptake from food, (ii) influences the allocation of energy to the different metabolic processes, or (iii) acts as a cure for a disease or infection that is not recognized. We suspect that in the present case study, the hormetic effect pattern results from an increase of the energy taken up from food. Since diquat is a non-selective herbicide, it inevitably acts on the lettuce in the experiment. We hypothesize that the observed effects on maturation time result from effects on the growth rate due to effects on the assimilation process, in turn resulting from effects on the lettuce leaves that were degraded by the herbicide.

To test our hypotheses, we use the simplified DEB model that has been proposed for use in ecotoxicology (Jager and Zimmer, 2012), and combine it with the juvenile food limitation function proposed for the pond snail (see Chapter 2). Furthermore, we discuss the implications for ecotoxicological investigations with the pond snail in the proposed test protocol.

3.2. Methods

3.2.1. Experimental work

The life-cycle experiment (336 days) was conducted to assess the effect of pulses of the herbicide diquat on growth, reproduction and survival of the pond snail *L. stagnalis* under a realistic exposure scenario. The experimental design and the main results have been published earlier (Ducrot et al., 2010a). In the first half of the experiment, the snails were exposed to four pulses of the herbicide diquat. After 168 days, they were transferred to clean water. Exposure treatments comprised seven diquat concentrations in the range of 5 to 320 μg/L (nominal peak concentrations). In this study, we only used data obtained for the two lowest concentrations (5 μg/L and 10 μg/L) as only in these exposures, the snails survived the juvenile phase and started reproducing. The snails were fed with organic lettuce, which was weighed to assess the feeding rate. They were only fed when no leftover remained from the previous feeding. Growth was assessed by measuring the shell length biweekly, and the reproductive output was measured as numbers of clutches per day per snail.

The main result of the original publication (Ducrot et al., 2010a) was that the authors observed a hormetic response in juvenile feeding and time to first reproduction. At 5 μg/L, the snails grew faster than in the control, and at 10 μg/L, the snails grew slower than in the control. The age at first reproduction in the control was 172 $\pm$ 2 days. The snails exposed to 5 μg/L started reproducing earlier (149 $\pm$ 2 days), and the snails exposed to 10 μg/L started reproducing later than the control (215 $\pm$ 2 days). The cumulative feeding rate was calculated after 84 days to compare the juvenile feeding rates in the different treatments. It was highest

Table 3.1.: Model parameters and values as fitted simultaneously using maximum likelihood optimization. Confidence intervals were derived using profile likelihoods.

Parameter	Unit	Value	95 % conf. interval	Definition
g	–	1	n.e.	energy investment ratio
L_0	mm	1.09	$0.978 - 1.22$	initial shell length
L_f	mm	7.26	$6.78 - 7.71$	critical shell length for food limitation
L_p	mm	14.3	$12.7 - 15.7$	shell length at puberty
L_m	mm	38.0	$37.1 - 38.9$	maximum shell length
$\dot{r}_B$	d^{-1}	0.0161	$0.0150 - 0.0175$	vB growth rate
R_m	# clutches / d	0.526	$0.491 - 0.564$	max. reproduction rate
food related parameters				scaled ingestion
f_0	–	0.939	$0.936 - 0.945$	in control
f_5	–	1	n.e.	at 5 μg/L
f_{10}	–	0.834	$0.822 - 0.849$	at 10 μg/L
a	–	1.62	$1.54 - 1.69$	food quality factor

at 5 μg/L (330 $\pm$ 70 mg), and lower for the control and for the snails exposed to 10 μg/L (control: 129 $\pm$ 32 mg; 10 μ/L: 137 $\pm$ 10 mg).

3.2.2. Model analysis

We analyze the effects on feeding, growth and reproduction over time using the simplified Dynamic Energy Budget (DEB) model that has been published previously (Jager and Zimmer, 2012). A detailed model derivation has been provided by the authors and will not be repeated here. In the DEB model, the feeding module consists of two steps: food intake and assimilation of energy from food. Food intake is taken proportional to the squared body length (i.e., related to a surface area of an animal), which was confirmed for the pond snail (Zonneveld and Kooijman, 1989). Assimilation efficiency is assumed to be constant. The food availability is included through the scaled functional response f, which is a hyperbolic function of the food density in the environment (zero when there is no food, and one under non-limiting conditions).

Because juvenile pond snails are food limited when fed with lettuce, the simplified DEB model was extended (see Chapter 2); the juvenile food limitation is taken as a function of shell length. When the juvenile snails are smaller than a critical shell length L_f, the food intake scales with cubed shell length, i.e., is proportional to body volume. Juvenile food limitation has severe consequences for growth, which is reflected in the shape of the growth curve. Under unlimited conditions, the model reduces to the von Bertalanffy growth curve, while it becomes s-shaped with the juvenile food limitation function. As a demonstration, we show simulations of the

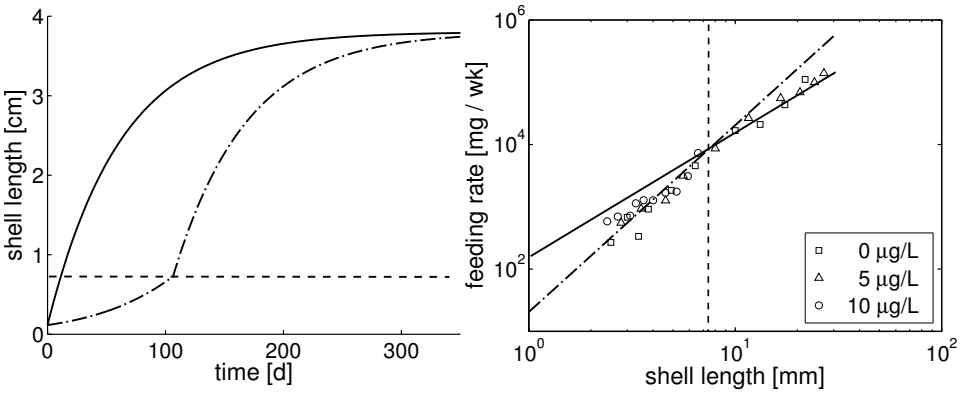

Figure 3.1.: The left panel shows a comparison between simulations with the DEB model with (dashed line) and without (solid line) the juvenile food limitation. The right panel shows the feeding rate as a function of shell length. Below L_f (vertical line), food intake scales with L^3 (dashed line), and above with L^2 (solid line). The symbols mark the different diquat treatments.

simplified DEB model with and without the juvenile food limitation (see Figure 3.1, left panel). When the snails are larger than L_f, food intake scales again with squared shell length.

The parameter estimation was done in Matlab 12. We used maximum likelihood optimization (Jager and Zimmer, 2012), and derived the confidence intervals by profiling the likelihood function (Meeker and Escobar, 1995). The model parameters with estimates and confidence intervals are given in Table 3.1. We assumed that all effects of diquat can be explained from changes in f. Further, we ignore TK, and take a constant value of f in each treatment. Since the snails exposed to 5 μg/L reached a higher maximum size than the control snails, we fixed the scaled functional response f_5 for this treatment to 1 (highest possible value, per definition), and fitted the scaled functional response for the other treatments. It should be noted that the parameter values that we obtained are somewhat different from the ones that have been published for the pond snail earlier (compare Chapter 2, and Zonneveld and Kooijman, 1989). From the experimental data in this study, it was not possible to determine a value for the energy investment ratio g; detailed data for growth and reproduction over time at different constant food levels would be needed to do so. We set $g = 1$ (as in earlier in DEBtox applications, see e.g., Jager and Klok, 2010), because the goodness of fit of the model to the data was insensitive to the choice of this value. However, the estimated model parameters (see Table 3.1) are slightly different when g is fixed to 10 or 0.1, which should be considered when using the model for extrapolations to other environmental conditions.

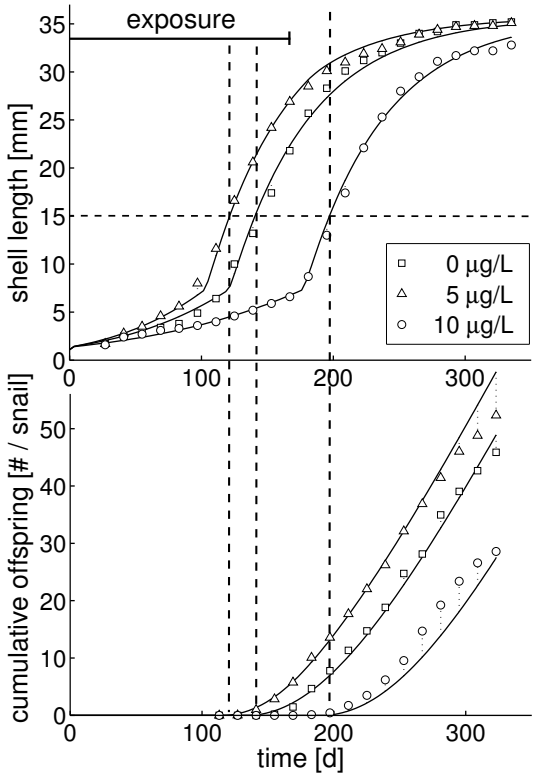

Figure 3.2.: Growth (top) and reproduction (bottom) over time. The lowest concentration (5 μ g/L, $\triangle$) shows stimulated growth compared to the control ($\square$), and the higher concentration (10 μ g/L, $\circ$) shows delayed growth. The black solid lines are the model predictions. The vertical dashed lines mark the age at puberty in the different regimes as predicted by the model. The horizontal dashed line marks the size at puberty as predicted by the model. It seems that the shift in age at first reproduction results from the differences in growth between the regimes, and is thus only an indirect consequence of the exposure to diquat.

3.3. Results & Discussion

The data and model fits for growth and reproduction over time are shown in Figure 3.2. From this figure, we can see that the snails start reproducing at the same shell length in all treatments, i.e. 15 mm, which is close to the value of 20 mm observed by Berrie (1966) in the field. The effect of diquat on the start of reproduction is thus likely a consequence of the stimulated or delayed growth, and not a specific effect on maturation.

Faster growing snails spend more energy on growth than slower growing snails. A difference in growth may therefore result from either a difference in the energy acquisition from food, or from a change in the energy allocation within the snail (Jager et al., 2013). Since both growth and reproduction are enhanced, a reallocation seems unlikely. Indeed, when assuming an effect on the feeding module, we can capture the observed pattern with the DEB model (see Figure 3.2). Even though the diquat concentration fluctuated over time, we could use a constant value for the scaled functional response f to explain both the hormetic response at 5 $\mu g/L$ and the negative effect at 10 $\mu g/L$ (Table 3.1). An effect on the feeding module in DEB means that the energy acquisition from food is changed, either due

to a change in feeding rate or due to a change in assimilation efficiency.

Ducrot et al. (2010a) suggest a difference in feeding rate caused by diquat. The cumulative amount of lettuce that the snails had eaten in the first 84 days of the experiment was indeed substantially different. While the juvenile snails exposed to 5 μg/L had eaten more than the control snails, the snails exposed to 10 μg/L had eaten less. However, when looking at the feeding rate as a function of shell length, we find that the treatment has no effect on the relative feeding rate (Figure 3.1). After 84 days, the juveniles snails had simply eaten different amounts of lettuce because of their size differences. An effect on food intake can therefore be ruled out; the difference in growth most likely results from a difference in assimilation efficiency. The size-dependent food limitation means that for juveniles ($L < L_f$) the feeding rate should scale with body length cubed (i.e., body volume). This pattern was confirmed by the observations on feeding rate (Figure 3.1). In a log-log plot, the switch from a cubed to a squared relationship is shown as a change in slope. This confirms our earlier hypothesis that juvenile snails eat less than expected based on their body size when fed with lettuce (see Chapter 2).

Different mechanistic explanations for a hormetic response from an energy budget point of view have been recently discussed by Jager et al. (2013). One of the possibilities is a hormetic response due to an interaction of the compound with the food. Diquat is a non-selective herbicide and degrades the lettuce leaves. In a dose-dependent manner, the lettuce leaves become more and more degraded, which led to the need to frequently replace the lettuce in the highest tested concentrations ($> 20\ \mu g/L$, Ducrot, personal observation). When given the choice, the pond snail prefers slightly degraded leaf material above fresh leaves (Kolodziejczyk and Martynuska, 1980). A mechanistic hypothesis to explain the stimulated growth at 5 $\mu g/L$ could be that diquat improves the snail's ability to assimilate energy from the lettuce by slightly degrading it. At 10 $\mu g/L$, the food might have become too degraded for optimal assimilation. An interaction with the food source was suggested earlier to explain hormesis in the toxicity of copper in the earthworm *Dendrobaena octaedra* (Jager and Klok, 2010) and of nonylphenol in the marine polychaete *Capitella teleta* (Jager and Selck, 2011). This indicates that hormesis due to interactions with food could be relatively common, and requires further attention.

The effect pattern of the reduced growth in Figure 3.2 strongly resembles the simulations for food-limited pond snails with hypothetical toxicants affecting assimilation, which have been discussed in Chapter 2. Effects of the same intensity (i.e., percentage of decrease of assimilation efficiency) have a much more pronounced impact on the snails that are food limited as juveniles compared to snails that are not. In the present study, the overall effect of diquat is intensified by the interaction with the juvenile food limitation.

Although we can interpret the observed effects as effects on assimilation efficiency, it is unlikely that these effects are solely caused by mechanical effects of diquat on the lettuce. The substantial mortality in the higher concentrations suggests that diquat is taken up and is causing toxicity. Muller et al. (2010) suggest

that in a DEB context, the observed patterns can often be better explained by assuming effects on assimilation together with effects on maintenance, rather than assuming only one of them. The effect patterns of these two mechanisms strongly resemble each other (see simulation studies in Chapter 2). Indeed, using an effect on maintenance costs alone produces a comparable goodness of fit, if we allow for a decrease in maintenance costs at 5 μg/L (model fit not shown). Diquat induces oxidative stress in the pond snail, which leads to damage of cell membranes (Lagadic, 2007). The repair involves an increased production of enzymes (Bouétard et al., 2013). Therefore, diquat might induce an increase in maintenance costs for repair or replacement of damaged cells. However, a decrease in maintenance costs due to a toxicant would be unlikely (Jager et al., 2013). We choose the simplest possible explanation, but cannot rule out that other hypotheses might explain the effect patterns as well.

3.4. Implications for ecotoxicology

When using lettuce as a food source for *L. stagnalis*, the test results should be treated with caution. The predicted interaction of toxic effects with the juvenile's food limitation (see Chapter 2) was confirmed in this study. As juveniles cannot feed effectively on lettuce, they experience a multiple-stress situation in a part of their life-cycle. This leads to a bias in the interpretation of test results, as juveniles will then be considered as being much more sensitive than they intrinsically are. When testing the effect of herbicides on animals fed with plants, it is difficult to separate the toxic effect on the animal from the effects through the degradation of the food source. Other food types than lettuce should therefore be considered for the pond snail (e.g., fish flakes, see Chapter 2). The hormetic response of low diquat doses can be explained by an effect on the assimilation efficiency of energy from lettuce, although further testing would be needed to corroborate this hypothesis (e.g., determining the caloric content of food and feces). Higher doses of diquat reveal negative effects on growth and reproduction, which are consistent with a decrease in the assimilation of energy from food. However, other explanations, (e.g., additional effects on the maintenance costs) cannot be ruled out using the present data set. Using mechanistic effect models helps to make sense of the observed effect patterns. To this end, DEB models are an excellent tool to investigate the dynamic interactions between toxic effects and food quality and quantity on multiple endpoints simultaneously.

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4. Modeling the full life cycle of *Lymnaea stagnalis*

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abstract Most animals tend to grow following the von Bertalanffy growth curve under constant conditions. Deviations from this curve can point to changes in the environment that the animals experience, such as food limitation when the available food is not sufficient or suitable for juveniles. However, such deviations can also point to a phenomenon called metabolic acceleration, which is receiving increasing attention in the field of dynamic energy-budget (DEB) modeling. Reasons for such an acceleration are usually changes in shape during ontogeny, which cause changes in the surface area to volume ratio of the organism. Those changes in turn lead to changes in some of the model parameters that have length in their dimension. The life-history consequences of metabolic acceleration are an S-shaped growth curve (when body size is expressed as a length measure) and a prolongation of the hatching time. The pond snail *Lymnaea stagnalis* was earlier found to be food limited in the juvenile phase when fed with lettuce. In applications of the standard DEB model, however, we also find that the hatching time is consistently underestimated, which indicates an acceleration. We here present an application of the DEB model including metabolic acceleration to the pond snail. Overall, the life history of the snail is well represented by this model, over the entire life cycle. However, the pond snail does not change in shape substantially after birth, so the original explanation for the metabolic acceleration does not hold. We discuss the possible explanations for this pattern.

4.1. Introduction

The von Bertalanffy growth model (von Bertalanffy, 1934) is the most commonly used model for indeterminate growth of animals (Charnov, 1993). A von Bertalanffy growth curve is linear in the beginning, and approaches a maximum size asymptotically, when body size is expressed as length. It can in general be applied to all isomorphically growing animals that experience a constant environment (Kooijman, 1988, 2010). For ecological applications, a growth curve under constant conditions does not suffice, and we need models that account for the interactions of animals with the environment through feeding, and that make predictions for reproduction. One well-tested theory, which accounts for feeding, growth, and reproduction in one framework, is Dynamic Energy Budget (DEB) theory (e.g., Kooijman et al. (2008); Van der Meer (2006)). The standard DEB model predicts von Bertalanffy growth under constant feeding conditions. It can, in general, be applied to any organism, and a growing community of DEB users has contributed to a library of parameters for species from most large animal phyla and all chordate classes (add_my_pet library, see Lika et al. (2011b), `http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/`).

Using deviations from the expected von Bertalanffy growth curve, the DEB framework can be used to reconstruct the feeding conditions that organisms experience. This can either be done to reconstruct the feeding history when analyzing observations on field-collected animals (e.g. Pecquerie et al. (2012), using otoliths), or to scrutinize the feeding conditions that organisms experience in the laboratory. Under controlled conditions, a deviation from the von Bertalanffy pattern can help to identify food limitation in a part of the life cycle (Jager et al., 2005; Zimmer et al., 2012). However, the von Bertalanffy growth pattern only applies to isomorphically growing organisms, and the standard DEB model assumes isomorphy: surface area is proportional to structural body volume to the power 2/3. Therefore, organisms that change in shape during ontogeny can not be modeled with the standard DEB model. Changes in shape modify some of the DEB parameters which have length in their dimension. Recently, a model extension dealing with morphological changes in fish during ontogeny has been developed (Augustine et al., 2011). One of the major implications of the model extension is that the fish accelerate their metabolism during ontogeny due to the changes in surface-area to volume ratio. Surface area is taken proportional to volume between birth and metamorphosis, a condition called V1-morphy. The metabolism thus accelerates between birth and a moment that is called (metabolic) metamorphosis. In the model extension, this is captured with one extra parameter that defines the magnitude of acceleration.

The V1-morph extension translates to differences in the model output: the embryonic development is prolonged, followed by a deviation in the shape of the growth curve. After birth, exponential growth is predicted, which turns into von Bertalanffy growth after (metabolic) metamorphosis. The duration of the exponential phase is thereby fixed by the length at metamorphosis. In some cases, the

acceleration leads to strongly S-shaped growth curves, whereas in others, the only visible implications concern the embryonic development. What can be considered as an acceleration of metabolism during ontogeny, can be regarded as a retardation of metabolism when considering ontogeny backwards. The duration of the embryonic development is prolonged, and the egg costs increase due to the additional maintenance costs. In general, if the duration of the embryonic development is strongly increased, the S-shape of the growth curve is more pronounced. It is of crucial importance to have a reliable estimate the egg costs, since it strongly interacts with predictions for reproduction rate. Therefore, it is important to be able to distinguish which model formulation is best suitable for the organism of choice.

Recent developments in DEB research suggest that many organisms undergo this so-called metabolic acceleration, even if they do not apparently change in shape (Kooijman et al., 2011). As potential alternative explanations for this deviation from the standard model, the authors discuss a diapause in the embryonic development, a difference in the local temperature (eggs develop under a different temperature than parents), or a change in food preference throughout the life-cycle.

The pond snail *Lymnaea stagnalis* is a holarctic species and widely spread in central Europe (Berrie, 1966). Its ecological relevance has recently been acknowledged by the fact that it has been proposed as future standard test organism of the OECD for toxicity testing of chemicals (OECD, 2010). *L. stagnalis* has previously been modeled with the standard DEB model by Zonneveld and Kooijman (1989). However, the authors presented four different parameter combinations for four different data sets on the pond snail, one of which concerned the embryonic development. One reason for treating the embryonic and adult stage separately is a substantial misfit between prediction and observation of the duration of the embryonic phase when using the standard model: the parameters that lead to good predictions of adult growth and reproduction over time predict an embryonic development that is much too short. The pond snail has been found to be food limited by lettuce (the standard food in the laboratory), and less or not limited by fish flakes (Zimmer et al., 2012). Since it was suggested that a metabolic acceleration could be resulting from changes in food preference (Kooijman et al., 2011), we now suggest that a standard DEB model with metabolic acceleration could explain the observed pattern as well. In the following, we present the application of the standard DEB model with the metabolic acceleration to the pond snail, and discuss potential reasons for its applicability.

4.2. Material and methods

We used a standard DEB animal model with the metabolic acceleration (Kooijman et al., 2011; Augustine et al., 2011), and a combination of earlier published data on *L. stagnalis* and data from our own experiments for parameterization. In the following, we briefly describe the earlier published methods and data.

4.2.1. The standard model

DEB theory provides a set of rules that determine how much energy organisms assimilate from food, and how this energy is allocated to growth, development, reproduction and maintenance. It was originally developed with the aim to understand how organisms change the allocation of energy in response to a toxicant (Kooijman and Metz, 1984). Following the idea that the energy metabolism is organized very similarly among organisms, DEB theory can in general be applied to all organisms (Kooijman, 2001; Nisbet et al., 2000).

The standard DEB model has three state variables: structural length L (cm), reserve E (J) and maturity E_H (J) (captured as energy invested into maturation). Structural length L is linked to physical length L_w via the shape coefficient δ_M, where $L = \delta_M L_w$. Additionally, we will consider the reproduction buffer E_R (J). A simplified model scheme with the description of the energy fluxes is presented in Figure 4.1, and the model parameters are presented in Table 4.1. Body mass is composed of structure and reserve, which allows linking model predictions for growth to different nutritional conditions of organisms. Maturity has no mass or energy, but keeps track of the developmental stage of the organism: the amount of energy invested into maturation determines the switch from one life stage to another. Thus, the same DEB model can be used to model the whole life cycle of an organism, whereby small differences between life stages exist. During the embryonic development, organisms do not feed ($\dot{p}_X = 0$) or reproduce (see Figure 4.1). When reaching the maturity threshold for birth (E_H^b), organisms start feeding and are considered as juveniles. After reaching the maturity threshold for reproduction (E_H^p), organisms start reproducing and are considered as adults. The flux that was used for maturation ($\dot{p}_H$) is then allocated to reproduction ($\dot{p}_R$).

The dynamics of the state variables are specified by

$$\begin{aligned}
\text{Reserve:} \quad \frac{d}{dt}E &= \dot{p}_A - \dot{p}_C \quad \text{if} \quad E_H > E_H^b \\
\text{Structural length:} \quad \frac{d}{dt}L &= \frac{\dot{r}}{3}L \\
\text{Maturity:} \quad \frac{d}{dt}E_H &= (1-\kappa)\dot{p}_C - \dot{k}_J E_H \quad \text{if} \quad E_H \leq E_H^p \\
\text{Reproduction buffer:} \quad \frac{d}{dt}E_R &= (1-\kappa)\dot{p}_C - \dot{k}_J E_H^p \quad \text{if} \quad E_H > E_H^p
\end{aligned}$$

The mobilization flux $\dot{p}_C$, the assimilation flux $\dot{p}_A$, and the specific volumetric growth rate $\dot{r}$ are given by

$$\begin{aligned}
\dot{p}_C &= E(\dot{v}/L - \dot{r}) \\
\dot{p}_A &= f\{\dot{p}_{Am}\}L^2, \\
\text{and} \quad \dot{r} &= \frac{E\dot{v}/L^4 - [\dot{p}_M]/\kappa}{E/L^3 + [E_G]/\kappa},
\end{aligned}$$

The parameters $\dot{v}$, $\{\dot{p}_{Am}\}$, $[\dot{p}_M]$, κ and $[E_G]$ are explained in Table 4.1.

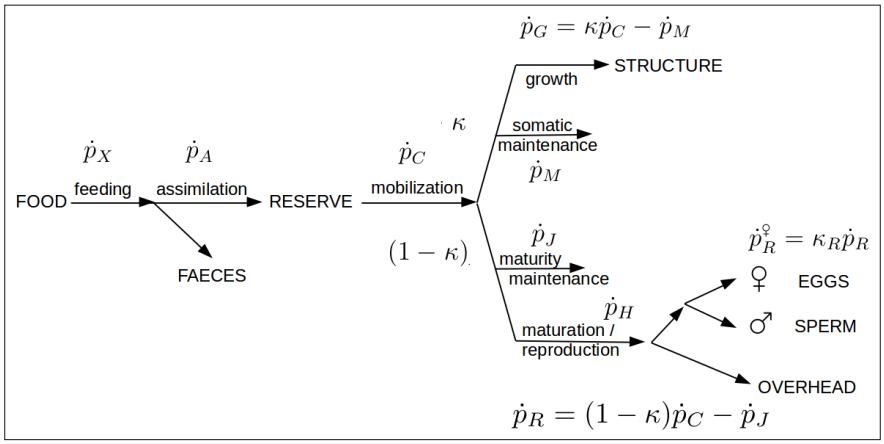

Figure 4.1.: A simplified scheme of the Dynamic Energy Budget (model). $\dot{p}$ denote the energy fluxes. Food is taken up ($\dot{p}_X$) and party assimilated ($\dot{p}_A$) into reserve. The reserves are mobilized ($\dot{p}_C$) and divided into the flux that goes to soma ($\kappa\dot{p}_C$) and the flux that goes into maturation and reproduction ($(1-\kappa)\dot{p}_C$). From the flux that goes into soma, first somatic maintenance is paid ($\dot{p}_M$), and the rest is used for growth ($\dot{p}_G$). From the other flux, first maturity maintenance is paid ($\dot{p}_J$), and the rest is used for maturation ($\dot{p}_H$) or reproduction ($\dot{p}_R$).

4.2.2. The metabolic acceleration

We use the standard DEB animal model with a V1-morphic extension (Kooijman et al., 2011; Augustine et al., 2011). The motivation to develop this model extension was based on the fact that some fish species change their shape in the early juvenile period. A change in shape alters the surface-area to volume ratio, and has an influence on all parameters that have length in their dimension: energy conductance $\dot{v}$, surface-area specific assimilation efficiency $\{\dot{p}_{Am}\}$, and the specific searching rate for food $\{\dot{F}_m\}$. Since we will only study conditions of constant food, the latter parameter is not considered here. The parameters increase proportional to length during the acceleration period, but stay constant before and after (see Fig. 4.2). As a result, growth at constant food is exponential after birth and changes into von Bertalanffy growth after (metabolic) metamorphosis. Consequently, the hatching time is altered with the extension: when using the extension, the energy conductance $\dot{v}$ is lower during the embryonic stage than the adult $\dot{v}$, which leads to a prolongation of the predicted hatching time. Since the growth of the pond snail *L. stagnalis* deviates from the von Bertalanffy pattern, and the prediction for hatching time was too short, we assumed that we can use this model extension to model the pond snail as well.

The mobilization flux $\dot{p}_C$, the assimilation flux $\dot{p}_A$, and the specific growth rate

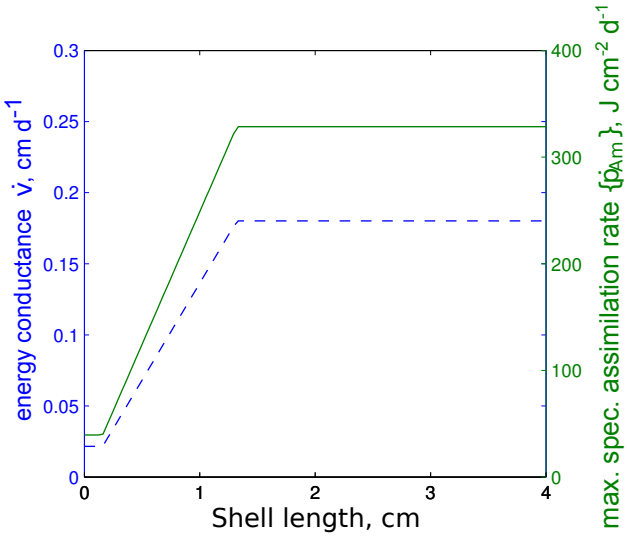

Figure 4.2.: The energy conductance $\dot{v}$ and the surface-area specific maximum assimilation rate $\{\dot{p}_{Am}\}$ have cm in their unit and change with length during the metabolic acceleration phase.

$\dot{r}$ are then modified to

$$\begin{aligned} \dot{p}_C &= E(\dot{v}\mathcal{M}(L)/L - \dot{r}) \\ \dot{p}_A &= f\{\dot{p}_{Am}\}\mathcal{M}(L)L^2, \\ \text{and} \quad \dot{r} &= \frac{E\dot{v}\mathcal{M}(L)/L^4 - [\dot{p}_M]/\kappa}{E/L^3 + [E_G]/\kappa} \end{aligned}$$

The shape correction function $\mathcal{M}(L)$ is given by:

$$\begin{aligned} \mathcal{M}(L) &= \frac{L_b}{L_b} \quad \text{if} \quad E_H < E_H^b && \text{(embryo)} \\ \mathcal{M}(L) &= \frac{L}{L_b} \quad \text{if} \quad E_H^b < E_H < E_H^j && \text{(early juvenile)} \\ \mathcal{M}(L) &= \frac{L_j}{L_b} \quad \text{if} \quad E_H > E_H^j && \text{(late juvenile)} \end{aligned}$$

Usually, metamorphosis is reached before puberty ($E_H^j < E_H^p$), but not in all cases.

4.2.3. Reproduction

When we assume that the reproduction buffer is emptied continuously, the reproduction rate is given by $\dot{R} = \kappa_R \dot{p}_R / E_0$ with $\dot{p}_R = \frac{d}{dt} E_R$, where E_0 is the amount of energy which is invested per egg.

DEB theory has been mainly applied to model female organisms. The main reason for this is that sperm production is rarely quantified, and in most experiments, only the female reproductive output is measured (e.g., numbers of eggs or

offspring). It is then usually assumed in the DEB model that 5 % of the available energy for reproduction is lost as overhead costs for producing offspring, which is captured by the reproduction efficiency $\kappa_R = 0.95$. The pond snail is a simultaneous hermaphrodite, and changes its allocation into male and female function in response to mating opportunities. We can capture this in the model by considering the reproduction flux as the sum of the female reproduction flux $\dot{p}_R^{\female} = \kappa_R \dot{p}_R$, the male reproduction flux and the overhead costs. For simplicity, we assume that all overhead costs for reproduction are included in the male reproduction flux, so that $\dot{p}_R^{\male} = (1-\kappa_R)\dot{p}_R$. The fraction κ_R is thus the fraction of energy invested into eggs.

Although the pond snail is able to reproduce via selfing, it prefers to reproduce via out-crossing (Hoffer et al., 2012, and references therein). Findings of De Visser et al. (1994) suggest that the snails invest roughly the same amount of energy into the male and female function when unlimited mating opportunities are provided. Therefore, in a situation with constant mating possibilities, as occurs when snails are kept in groups, we assume that the snails allocate approximately the same amount of energy into male and female function, so that $\kappa_R = 0.5$. The snails can store allosperm for a maximum of 116 days (Cain, 1956), so that after having mated once, they can reproduce via out-crossing without necessarily having to produce sperm.

4.2.4. The data used for parameterization

Following the definition of Lika et al. (2011a), we present the available data as uni- or zero-variate data. Uni-variate data is any data that contains information on how two state variable change in relation to each other. Usually, measurements as a function of time are used (e.g. length over time, reproduction over time). Zero-variate data are data points, such as length at puberty, size at birth, or maximum reproduction rate. A combination of uni-and zero-variate data is optimal to fully determine the DEB parameters (Lika et al., 2011a,b). In the following, we describe the data we used for parameterization and validation.

Uni-variate data

We use data on growth and reproduction from a partial life-cycle experiment (PLE) that has been published and described in detail in Chapter 2. We therefore only shortly repeat the experimental conditions here. The experiment was conducted under a photoperiod of 14 hours light, 10 hours dark (14/10 L/D) at 21 ± 1°C, and the snails were held in groups of five. Growth was evaluated biweekly by shell length measurements. Numbers of eggs produced per replicate were counted to measure reproduction rate during the whole experiment. We averaged the shell length per snail and numbers of eggs per snail for each replicate, and then calculated the average over all replicates per feeding regime. The experiment started with juveniles of homogeneous age (113 d) and similar size (12.7 ± 1.3 mm). They were fed at three different feeding regimes and monitored for 184 days. Each day, an *ad*

libitum quantity of lettuce was weighed and given to the snails in regime PLE_{100}, and leftovers were weighed on the next day. The food for regime PLE_{50} was determined as 50 % of the *ad libitum* value from the day before, and the in PLE_{25} as 25 %, respectively.

Zero-variate data

We used data from an experiment that has been conducted at INRA to determine the relation between length and weight of the snails in the culture (unpublished data, Marc Roucaute). Snails at different sizes were taken from the culture, and shell length, dry weight of the whole body, and dry weight of the soft body were determined. We used the size and weight of the largest snail that was found to define the maximum asymptotic size and weight for the model (see Table 4.2).

Additionally, we used data from an experiment where dry weight of eggs, size at birth, age at birth, and dry weight at birth (whole body) were determined (unpublished data, Alpar Barsi). The experiment was conducted to investigate the influence of endocrine disrupting components on the offspring of exposed adults. We used the data from the controls only (see Table 4.2).

4.2.5. The data used for validation

In Chapter 2, we fitted a food limitation function to a growth curve that was obtained in a full-life cycle experiment (FLE) with *L. stagnalis*. In the FLE, freshly hatched snails were fed *ad libitum* with lettuce during the whole life-cycle. The experimental conditions were very similar to the ones in the PLE. For more detailed information, we refer to Appendix A, Section A.2.3. In this chapter, we use the growth curve of the FLE to compare the predictions of the model with the metabolic acceleration used in this chapter with predictions of the model with the food limitation function.

Additionally, we used data on respiration at birth (Zotin, 2009) and of adult snails (Fernando Monroy, in prep.) to compare to the model predictions (see Table 4.2). The data from Zotin (2009) was obtained at another temperature than the data from our experiment. In DEB, all rates depend on temperature following the Arrhenius relation (e.g., Freitas et al., 2007). A further elaboration on how we performed the calculation can be found in Appendix A, Section A.1.4. Because we did not use respiration data in the parameterization process, we consider the prediction for this endpoint a solid validation. Respiration in DEB is the sum of different fluxes that result from the metabolic activity of the organisms, in contrast to other theories in ecology where metabolic rate is considered to be a driving force. In DEB, overhead costs for growth and reproduction as well as maintenance and maturation contribute to oxygen consumption. The calculation for the predictions is described in detail in Appendix B, Section B.2.1.

4.2.6. Parameterization

The parameterization procedure has been described in detail in Lika et al. (2011a) and Lika et al. (2011b). The estimation was done using the downloadable software DEBtool (Kooijman et al. (2008), `http://www.bio.vu.nl/thb/deb/deblab/debtool/`) run in Matlab (Mathworks, MA, USA). All parameters were estimated simultaneously using weighted sum of squares regression routines (nmregr.m) with a Nelder-Mead simplex method, generally followed by a Newton Raphson optimization.

4.2.7. Assumptions and simplifications

We assume that the snails with the maximum observed shell length in the culture were experiencing unlimited feeding conditions, so we set the scaled functional response $f = 1$ for these predictions. Because the snails in the PLE were held in groups of five, we assume that they invest equally in the male and female function, so we set $\kappa_R = 0.5$. We set $[E_G] = 2800$ J, which is a commonly used value in the add_my_pet collection (Lika et al., 2011b), to remove a parameter from the system. We correct the validation data of Zotin (2009), which was obtained at 18 °C using the Arrhenius temperature $T_A = 8000$ K.

4.3. Results and discussion

4.3.1. General patterns

The data we used to parameterize the model is generally well represented by the model predictions (see Figure 4.3, and Table 4.2). Even though the length at puberty is underestimated, the start of reproduction is well captured at the three food levels.

However, there is a slight discrepancy between the predictions for the zero-variate data points for length and weight: while maximum length (L_i) and dry weight (W_i) are underestimated, length (L_b) and weight at birth (W_b) are overestimated. In Zonneveld and Kooijman (1989), a time lag of 2 days at the beginning of the development was included to better fit the observed growth curves during the development in the egg. Indeed, in the first days of the development, the embryo mainly seems to increase in complexity, and not much in size (see Horstmann (1958)). In the current model, this time lag is not included, which might explain why the weight and length at birth are overestimated, while the duration of the development is not. This is also reflected in the predictions for respiration: while the adult respiration is predicted very well, the respiration at birth is overestimated by a factor of 10. Future investigations with the model could contain a time lag, in which mostly maturation is taking place, but hardly any growth. One could think of implementing this in a similar way as was done in Mueller et al. (2012). These authors implemented a switch in the fraction of energy allocated to soma κ

Table 4.1.: The parameters for the DEB model. Note that the specific surface-linked maintenance rate p_T is zero in ectotherms.

Symbol	unit	interpretation	value	stdev
Primary parameters				
T_A	K	Arrhenius temperature	8000	-
z	-	Zoom factor	0.1951	0.002334
δ_M	-	Shape correction coefficient	0.4272	0.005795
$\dot{v}$	cm/d	Energy conductance	0.02161	0.0006765
κ	-	Fraction of mobilized reserves allocated to the soma	0.7785	0.003241
$[\dot{p}_M]$	J/d.cm^3	Specific volume-linked maintenance rate	157.3	2.588
$\dot{k}_J$	1/d	Maturity maintenance rate coefficient	0.03804	0.001972
$[E_G]$	J/cm^3	Volume-specific costs for structure	2800	-
E_H^b	J	Maturity threshold at birth	0.3417	0.01154
E_H^p	J	Maturity threshold at puberty	721.7	16.74
E_H^j	J	Maturity threshold at metamorphosis	217.3	3.086
Scaled functional response				
f_{100}	-	in regime PLE 100	0.8818	0.02385
f_{50}	-	in regime PLE 50	0.7793	0.0241
f_{25}	-	in regime PLE 25	0.6945	0.02347
Initial size				
L_0^{100}	cm	in PLE 100	1.459	0.04388
L_0^{50}	cm	in PLE 50	1.446	0.04762
L_0^{25}	cm	in PLE 25	1.342	0.0413
κ_R	-	reproduction efficiency	0.5	-
$\{\dot{p}_{Am}\}$	J / d.cm^2	surface-area specific maximum assimilation rate, $\{\dot{p}_{Am}\} = z[\dot{p}_M]/\kappa$	39.42	-
Adult parameters (after acceleration)				
$\dot{v}_{adult}$	cm/d	$\dot{v}_{adult} = M(L)\dot{v}$	0.1802	-
$\{\dot{p}_{Am}\}_{adult}$	cm/d	$\{\dot{p}_{Am}\}_{adult} = M(L)z[\dot{p}_M]/\kappa$	328.7371	-

Table 4.2.: The zero-variate data that was used for parameterization and validation. Note that the data used for validation was not used to estimate the parameters.

Source	Symbol	unit	interpretation	data	prediction
Data for parametrization					
INRA	a_b	d	age at birth	13.5	12.6
(unpublished)	L_b	cm	size at birth	0.147	0.172
	W_b	mg	dry weight at birth	0.114	0.162
	d_w^{egg}	mg	dry weight per egg	0.140	0.122
	L_i	cm	ultimate physical length	4.02	3.81
	W_i	g	ultimate dry weight	1.92	1.78
PLE	L_p	cm	physical length at puberty	2.30	2.11
Data for validation					
Zotin (2009)	J_O^b	L/h	oxygen consumption at birth	$3.50\ 10^{-8}$	$2.77\ 10^{-7}$
	L_b^{18}	cm	size at birth at 18 °C	0.125	0.172
	a_b^{18}	d	age at birth at 18 °C	20	16.6
Monroy	J_O^{ad}	L/h	oxygen consumption of adults	$512\ 10^{-6}$	$467\ 10^{-6}$
(unpublished)			(L = 2.75 cm)		

to capture the deviating observations on maturation in two related frogs (*Crinia georgiana* and *Pseudophryne bibronii*).

The shape of the predicted cumulative reproduction curve does not fully match the observations: the DEB model predicts an increase in reproduction rate as long as the animals grow, which is reflected in the upcurving of the model curve, while the data show a constant reproduction rate (see Figure 4.3). It has been observed that the pond snail produced larger eggs with increasing body size (Hoffer et al., 2012). The DEB model predicts a constant egg size under constant feeding conditions such as in our experiments. Unfortunately, the weight of the egg clutches was not determined in the PLE, so we can not further investigate this hypothesis.

4.3.2. Food limitation vs. metabolic acceleration

Interestingly, we can almost predict the growth pattern observed in the FLE, to which we have earlier fitted the food limitation function in Chapter 2 (see Figure 4.4). This suggests that at least part of what we earlier interpreted as food limitation, can be captured by assuming that the snails accelerate their metabolism. Kooijman et al. (2011) proposed that organisms that change their diet during ontogeny could be modeled with the metabolic acceleration, despite the fact that it was developed for organisms that change in apparent shape. We suggest that in case of the pond snail, part of the observed pattern can be explained by changes in the microbial community in the gut. To be able to digest cellulose, animals need a microbial community in their guts. Directly after birth, the gut can not digest cellulose efficiently, because the community has not fully developed yet. We propose that the microbial community in the gut of the snail is slowly building up after hatching, which leads to an increase of the efficiency of the gut. This could be interpreted such that the efficient gut surface does not grow isomorphi-

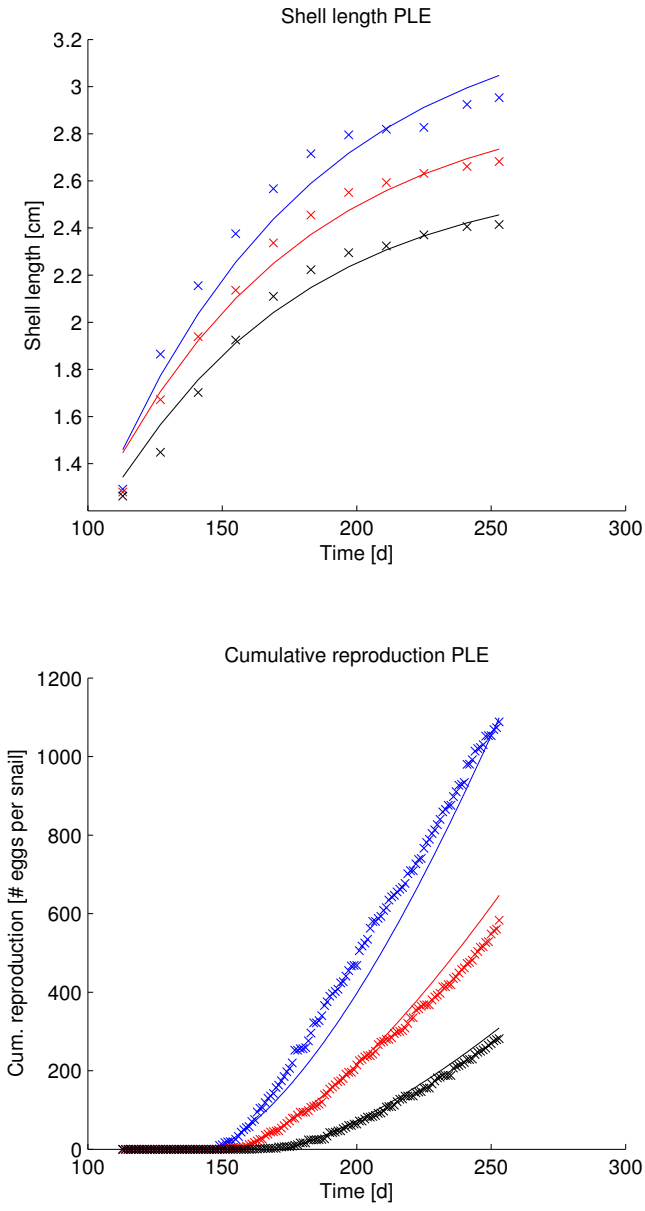

Figure 4.3.: Fits of the DEB model to the growth data (upper panel) and reproduction data (bottom panel) from the PLE tests. Blue: PLE_{100}, red: PLE_{50}, Black: PLE_{25}.

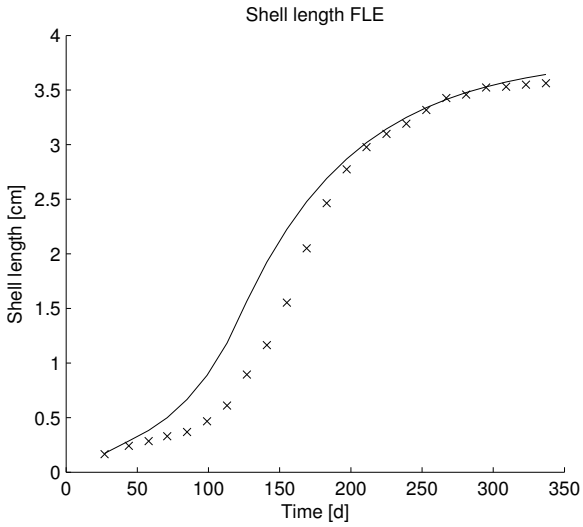

Figure 4.4.: The growth data from the FLE and the predictions of the DEB model with the metabolic acceleration. Note that the FLE data was not used for parameterization.

cally with the rest of the snail, which could lead to the slowly increasing values of $\{\dot{p}_{Am}\}$. In Chapter 2, we could show that the juvenile snails grow faster when fed with Tetraphyll fish flakes instead of lettuce. An explanation might be that the gut fauna needed for digestion of Tetraphyll can be built up faster than the one needed for lettuce. In the same chapter, we needed different food quality factors to be able to fit the growth in the different experiments where the juvenile snails were fed with lettuce (see Figure 2.2). This suggests that either the lettuce was different in terms of nutritional quality, or other environmental factors had an influence on the development of the gut fauna. The fact that juvenile growth is still slower than predicted from the DEB model with the metabolic acceleration (see Figure 4.4) suggests that the snails are still somewhat food limited, on top of the acceleration. However, although a slowly developing gut fauna could explain the changes in $\{\dot{p}_{Am}\}$, there is no reason to assume that $\dot{v}$ would need to change accordingly. Kooijman (1988) suggested that another type of acceleration exists, which includes only an increase in assimilation, without an increase of the energy conductance. However, with the lower energy conductance, the model predictions come close to the observations for hatching time and size at birth. Possible further model extensions could include a switch of κ after birth, which would also lead to longer developmental times (see Mueller et al., 2012). Moreover, we observed in experiments on the embryonic development (unpublished data) that the pond snails start feeding on the egg yolk before hatching. In the standard DEB model, the embryonic period ends with the start of feeding. In our application, we assume

that the snails only start feeding after hatching. We might be able to capture the longer hatching time by including the onset of feeding in the egg. Such a model extension would involve two extra model parameters, e.g. a maturity threshold for the onset of feeding in the egg, and the assimilation efficiency for the yolk. If the assimilation efficiency is very low when feeding on the yolk, the predictions for hatching time should be longer.

In nature, juvenile snails are thought to feed on periphyton and biofilms, whereas adult snails mainly feed on macrophytes (Kolodziejczyk and Martynuska, 1980). While the nutritional content of lettuce is relatively close to the one of macrophytes, periphyton is in general not used as food in the laboratory, and usually no noteworthy biofilm develops in laboratory experiments where the snails are fed with lettuce (V. Ducrot, personal observation). Recently, it has been suggested that juveniles of *L. stagnalis* should be fed with sweet potatoes, because they grow very well on the biofilm that develops on the potatoes (Munley et al., 2013). Interestingly, the authors present life-cycle experiments of a duration of 56 days, and they report that the snails start reproducing after 32 days. The pond snail does not start reproducing before it reaches approximately 2 cm shell length (Berrie, 1966). Unfortunately, Munley et al. (2013) do not report observations on growth. However, the wet weight that is reported is in the range of values that are usually found for sexually mature snails (E. Zimmer, unpublished data). This suggests that the snails in Munley et al. (2013) reach the size at first reproduction much faster than the ones in experiments where they are fed with lettuce. In the experiment that was discussed in Chapter 3, the size at first reproduction was reached after approximately 170 days, and the life-cycle experiment lasted 336 days. Further life-cycle experiments with sweet potato and lettuce as food source that include detailed measurements on growth and reproduction would shed light on the reproducibility of the short time to first reproduction as suggested by Munley et al. (2013), and on the life-cycle consequences of such a fast development. However, even though other food than lettuce might lead to Von Bertalanffy growth from the early juvenile stage on, the predicted hatching time would still be too short when using the standard DEB model.

4.3.3. Metabolic acceleration in other organisms

In the current add_my_pet-collection (14/03/2012), 62 out of 241 species have been modeled with the metabolic acceleration extension. Of those 62, only a small fraction actually change in apparent shape during ontogeny. The main reason for using the metabolic acceleration is that the developmental times of the embryo are not well captured with the standard model in these cases. Kooijman et al. (2011) discuss a diapause in the embryonic development, a difference in the local temperature between eggs and adults, or a change in food preference throughout the life-cycle as potential reasons for the acceleration. Apparently, the list of accelerating species mainly includes particular taxa, such as Cnidaria (e.g., sea anemones, corals, jellyfish), Echinodermata (e.g., starfish, sea urchins),

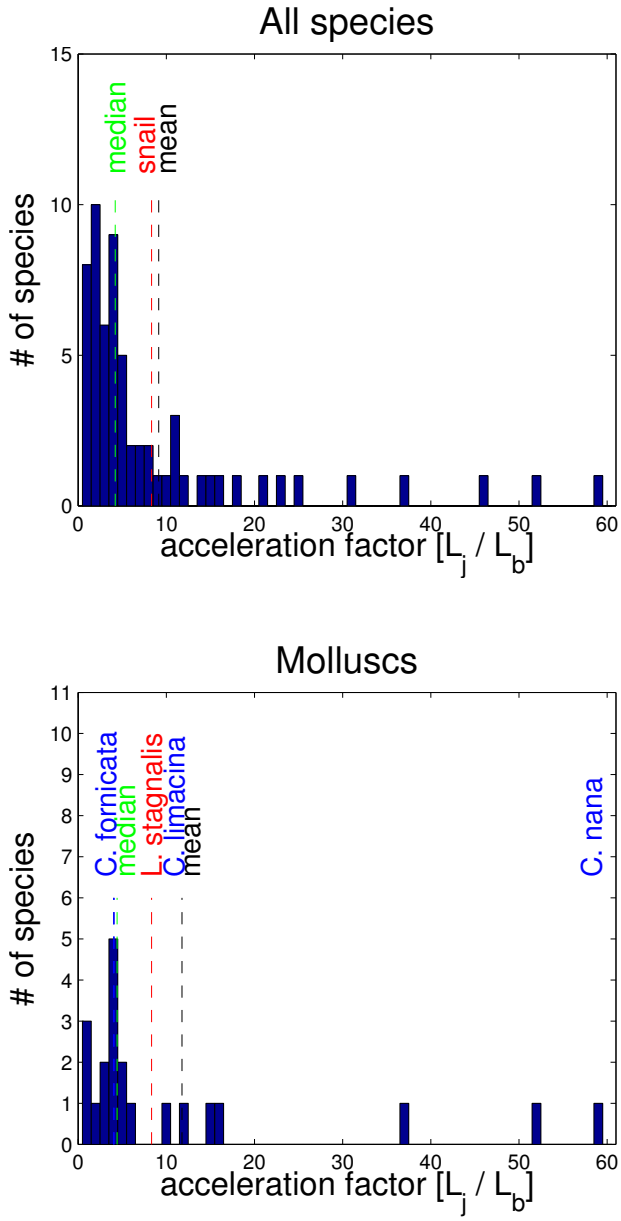

Figure 4.5.: Acceleration factor of the pond snail in comparison with all accelerating species in the add_my_pet-collection (top panel) and only all other molluscs (bottom panel). The acceleration factor is calculated as $\mathcal{M}(L) = \frac{L_j}{L_b}$, so for the pond snail $\mathcal{M}(L) = \frac{1.44}{0.172} = 8.37$.

Actinopterygii (ray-finned fish), Crustacea (e.g., crabs, crayfish, copepods), and Mollusca (e.g., bivalves, snails, slugs; Kooijman, personal communication). Remarkably, all species with larval development seem to accelerate their metabolism (all bivalves and Echinodermata, some of the fish), but also ones with less clear larval stages are included (Cephalopods). All but two of the molluscs seem to undergo the metabolic acceleration. The acceleration factor of *L. stagnalis* thereby lies in between median and mean of all species (see Figure 4.5, left panel). Most of the species with a very high acceleration factor are actually molluscs (see Figure 4.5).

4.4. Conclusions

We show in this work how the entire life-cycle of the pond snail can be modeled with the DEB model including the metabolic acceleration extension. The comparison to data from other experiments and laboratories shows that the model seems to be generally applicable to the pond snail when fed with lettuce in the laboratory. It is still an open question how the model could be used for extrapolation to real-life scenarios. From the different growth patterns that are observed when the snails are fed with different food sources (i.e. lettuce, fish flakes, sweet potatoes), it is questionable whether any of those situations reflect how the snails grow and reproduce in the field. The choice of food source might have an effect on the extent of the metabolic acceleration, which might hold an explanation for the fact that specimen of *L. stagnalis* that are found in the wild grow to larger sizes than the ones we find in the laboratory (Berrie, 1966). To gain a better understanding of the general applicability of the model to the snail, more experiments with both realistic and unrealistic food sources are required.

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5. The dynamics of male/female functions in a starving hermaphrodite

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abstract Understanding how external stressors on parents influence the fitness of their offspring is of crucial importance for investigations at the population level. Egg size generally provides a good indication of offspring fitness: juveniles that hatched from small eggs usually have more difficulties coping with stress than juveniles hatching from large eggs. When food is scarce, some species produce smaller eggs, others produce fewer but larger eggs, while still other species do not vary the size of their eggs at all. In simultaneous hermaphroditic species, the allocation into reproduction has to be divided into investment for the male and female function at the same time. Thereby, investment per egg tends to vary with mating opportunity. In general, the egg sizes increase with increasing mating frequency, whereas food limitation may lead to a relatively higher allocation to the male function, as compared to the female function. We investigated the interaction of food limitation and mating opportunity in the pond snail *Lymnaea stagnalis*, which we analyzed using a Dynamic Energy Budget (DEB) model. We performed experiments where the snails were held in four different feeding and two different mating regimes. The snails were fed *ad libitum* during an acclimation period, to ensure that they all had a full complement of reserves at the start of the experiment. Surprisingly, only the full starvation regime showed effects on egg size, which in turn led to decreased offspring fitness. Overall, the feeding regimes had a large effect on fecundity, whereas the mating regimes only showed a slight effect in the highest feeding regime after three weeks of the experiment. From the application of

the DEB model, it seems likely that the snails in the intermediate feeding regimes invest a smaller fraction of the available energy into the female function during their acclimation to the new food regime. The remaining energy might have been used to fuel a (relative) boost of the male function. We discuss the consequences of the observed patterns for the interpretation of experimental studies in stress ecology.

5.1. Introduction

Offspring fitness is an essential endpoint for assessing population fitness (Forbes et al., 2010) and thus of high ecological relevance. Usually, offspring fitness is related to egg size (e.g., Marshall et al., 2003), and for example measured as survival under starvation (e.g., Calado et al., 2007). Offspring from smaller eggs usually starve earlier than the ones from larger eggs (e.g., Gliwicz and Guisande, 1992; Ito, 1997); they have less reserves and are less fit to cope with starvation. Thus, it is important to study the rules for maternal effects: how do parents decide how much to invest in their offspring?

In many species, it has been found that well-fed mothers produce large eggs, and poorly-fed mother produce small eggs: e.g. the sea urchin *Pseudechinus huttoni* (Poorbagher et al., 2010), the wolf spider *Lycosa tarantula* (Moya-Laraño, 2002), the sea slug *Tenellia adspersa* (Chester, 1996), and the yellow dung fly *Scathophaga stercoraria* (Blanckenhorn and Heyland, 2004). But the opposite pattern has been observed as well: in some species, poorly-fed mothers produce larger eggs: e.g., the water fleas *Daphnia pulicaria* and *Daphnia hyalina* (Guisande and Gliwicz, 1992; Gliwicz and Guisande, 1992) and the cichlid fish *Simochromis pleurospilus* (Taborsky, 2006). A single, generally applicable maternal effect rule thus does not seem to exist.

In simultaneous hermaphrodites, the investment into reproduction is additionally influenced by the potential for conflict over sex allocation (Schaerer, 2009). Simultaneous hermaphrodites maintain both the male and the female sexual function at the same time. Although they are able to produce offspring by self-fertilization, they prefer to reproduce via out-crossing (fertilization with foreign sperm, see Schaerer, 2009). Thereby, mating with different partners (polyandry) may lead to larger offspring sizes (e.g., Sprenger et al., 2008a), whereas mating with too many different partners can also lead to opposite observations (Sprenger et al., 2008b). In contrast, self-fertilization can have a negative effect on offspring survival (e.g., Ramm et al., 2012; Johnson, 2010). In a review, Arnqvist and Nilsson (2000) suggested that maximum offspring sizes (and fitness) in general occur at intermediate mating regimes, which they consider to be most natural.

In addition to the effects on egg size, the fecundity of simultaneous hermaphrodites may be influenced by the mating regime as well. Frequent mating may lead to a reduction of fecundity and an increase of the investment into the male function (Trouve et al., 1999). As reviewed by Arnqvist and Rowe (2005), the reduction in

fecundity can be caused by substances in the seminal fluid.

Sex allocation theory predicts that the allocation to one sexual function will automatically reduce the allocation to the other sexual function (Charnov, 1982). Under food limitation, usually the investment into the female function is decreased to a higher extent than the investment into the male function (e.g., in the land snail *Arianta arbustorum* (Locher and Baur, 2002), and in the flatworm *Macrostomum lignano* (Vizoso and Schaerer, 2007)). This leads to a higher sex allocation (calculated as male over female investment) toward the male function. Thus, both mating frequency and food limitation affect the sex allocation in simultaneous hermaphrodites.

Above cited results show that many factors act simultaneously as determinants of the fecundity and egg quality in simultaneous hermaphrodites. In order to better understand which determinants are at play and how they interact, we investigate the combined effects of resource limitation and mating opportunity in the simultaneous hermaphrodite *Lymnaea stagnalis*. The pond snail has been commonly studied concerning sexual selection processes and the ensuing sexual conflict (Koene et al., 2006; Puurtinen et al., 2007; Koene et al., 2010) and inbreeding depression (i.e. the reduction of the fitness of a population due to low genetic variability, see Coutellec and Caquet, 2011). The effect of the sexual conflict on egg size was investigated in detail by Hoffer et al. (2012), who found a generally higher investment per egg in regimes with higher mating frequency. The effect of food availability on fecundity has been studied by Ter Maat et al. (2007), but egg size has not been investigated in that study. In the experiments that are presented in Chapter 4, the snails were fed at different food levels, however, only numbers of eggs of were determined.

Based upon these findings, we designed and conducted laboratory experiments to study the interaction between food limitation and mate availability on egg laying in *L. stagnalis*. Since we expect a trade-off between the male and female function, we use a modeling approach to account for the investment in both functions. For this purpose, we apply a Dynamic Energy Budget (DEB) model (Kooijman, 2010), that we adapted to capture both reproductive functions. This model was used in combination with an additional model extension that captures the starvation response of the snail.

5.2. Material and methods

5.2.1. Experimental approach

To study the combined effects of food level and mating opportunity, we conducted a partial life-cycle experiment with four feeding regimes and two mating regimes. The snails that we used in the experiment (i.e. RENILYS® strain) were taken from the culture of the INRA Experimental Unit of Aquatic Ecology and Ecotoxicology (Rennes, France). The laboratory conditions of the culture have been described in Coutellec and Lagadic (2006). We took snails from the culture at the age of

108 days since birth, with a similar size (2.7 ± 0.2 cm shell length). This size was chosen to ensure that all snails had mated and started reproducing before the start of the experiment (see Chapter 3). At that size, the snails already follow the von Bertalanffy growth and are not food limited anymore when eating lettuce (see Chapter 2).

Culture and test medium consisted of dechlorinated, charcoal-filtered tap water with the following physico-chemical characteristics: a pH of 7.7 ± 0.2, conductivity of 623 ± 60 μS/cm, dissolved oxygen of 7.3 ± 2 mg/L and water hardness of 254 ± 7 mg $CaCO_3$/L. During the experiment, the water was changed biweekly in order to maintain appropriate water quality. The entire experiment was conducted under a photoperiod of 14 hours light, 10 hours dark (14/10 L/D) at 21 ± 1°C. Snails were fed daily with weighed slices of organic lettuce (*Lactuca sativa*). All snails were held under *ad libitum* feeding conditions during an acclimation period of 2 weeks. The snails were held in two mating regimes, singles and pairs, which were divided into four feeding regimes after the acclimation period. The mating regimes were inspired by Hoffer et al. (2012), who identified significant differences in the reproductive output of snails that had mated once compared to snails that were continuously mating with the same partner. The singles snails were held in 250 ml jars, and the pairs in 500 ml jars. The experimental period lasted 4 weeks after acclimation.

The four different food levels were designed to be as different from each other as possible. Therefore, regime 'A' snails were fed *ad libitum*, regime 'B' snails were fed a very small amount of lettuce every day (1/4 of a slice of ø = 4.3 cm per snail, which is ≈ 3.6 cm^2), regime 'C' snails were fed an excessive amount of lettuce every third day, and left without food in between, and regime 'D' snails were not fed at all (see scheme C.1, Appendix C). Because all snails were fed *ad libitum* in the acclimation phase, the snails in all regimes but A had to adapt to the new feeding situation after the acclimation. The acclimation period was started with 170 snails, out of which 10 were killed after the acclimation period to determine a reference dry weight. We divided the remaining snails into 12 pairs (24 snails) and 16 single snails per feeding regime.

Measured endpoints

One adult snail died during the experiment (in regime D). The snail was from a pair, and the corresponding replicate was excluded from the results. We measured shell length weekly, and snail dry weight (with shell) at the end of the experiment. Once per day, we collected the egg clutches, and placed them into plastic six-well plates (volume of 10 ml). To assess the numbers of eggs, we took photos of the eggs through a microscope; the photos were then used to count the eggs using the software ImageJ (Schneider et al., 2012). For the pairs, individual measurements of reproductive output was not possible. We thus used the average of numbers of eggs and dry weight per clutch produced per day. For the determination of the dry weight of the clutches, all but a few clutches were used. One clutch per regime per

week was not dried, but kept in the plastic six-well plates, to assess the hatching rate, hatching time, and time to death of newborns by starvation (see Figure C.2, Appendix C). We counted the hatchlings every day and the 10 first and up to 10 late hatchlings were placed individually in test tubes where they were checked daily for survival. To identify differences between feeding and mating regimes, the data on cumulative egg numbers were analyzed using an ANOVA.

5.2.2. The modeling approach

We use the Dynamic Energy Budget (DEB) model (Kooijman et al., 2008) with the metabolic acceleration extension (Kooijman et al., 2011; Augustine et al., 2011). The parameterization for the pond snail is presented in Chapter 4; we use the same parameterization procedure here. In order to account for the specifics of the conditions in our experiment, we need to re-estimate κ_R, δ_M and the scaled functional responses f for the different feeding regimes. All other parameters are kept at values that were determined in Chapter 4 (see Table 4.1). The reasoning for the parameter modifications is detailed in the following.

Maternal effect (f): In the DEB model, the food level is included as the scaled functional response f, which is the actual ingestion rate of an animal divided by the maximum ingestion rate for its size. For an individual under *ad libitum* feeding conditions, $f = 1$, whereas for a starving individual, $f = 0$, so that for limiting conditions $0 < f < 1$. If the organism is in equilibrium with the environment, the reserve density $[E]$ (amount of reserve E divided by structural volume L^3) is constant. In DEB applications, often the the scaled reserve density e is used; e is the actual reserve density $[E]$ divided by the maximum reserve density $[E_m]$. The scaled reserve density e also takes values between 0 and 1. Under constant feeding conditions, when the organisms are in equilibrium with the food level, $e = f$. In DEB theory, the maternal effect rule is implemented such that offspring are born with the same reserve density as the parents had in the moment of egg production. This has an impact on egg size (dry weight of eggs): at low food levels, eggs are smaller than at high food levels. At a constant food level, egg sizes are expected to be constant.

The snails in our experiment were first all fed *ad libitum* (i.e., $f = 1$), before they were placed in the different feeding regimes. The feeding conditions in regime A were kept *ad libitum*, so we set the scaled functional response $f_A = 1$ for this regime. In regimes B and C, the food level was lower, so we estimate the values for f_B and f_C. For the full starvation regime D, we assume that f_D is slightly larger than zero. Because the snails first experience a high and than a low feeding regime, we expect to see the dynamics of adaptation to the new feeding condition.

Sex allocation (κ_R): In DEB theory, a fixed fraction of the mobilized energy is invested into reproduction in adult organisms (see Chapter 4, Figure 4.1). Thereby,

it is assumed that a fraction of that energy is used for the overhead costs for reproduction, which is captured by the reproduction efficiency κ_R. In general, only female reproductive output is measured (e.g. numbers of eggs), so that the reproduction efficiency κ_R is commonly used to quantify female reproductive investment. The overhead costs for reproduction are difficult to establish and therefore assumed to be 5 % from the total investment in reproduction, so that $\kappa_R = 0.95$.

In simultaneous hermaphrodites such as the pond snail, we have to account for the investment in both male and female function. The model in Chapter 4 was parameterized for snails that were held in groups of five, which means that they were able to mate continuously. Under the assumption that the snails invest roughly equally into male and female function in that particular mating regime, κ_R was set to 0.5. This means that half of the energy allocated to reproduction was assumed to be transformed into eggs, and the other half was assumed to be used for sperm production and overhead costs for both sexual functions. In this study, we investigate snails that are held single and in pairs. Since frequent mating decreases fecundity in *L. stagnalis* (Hoffer et al., 2012), we expect a higher fecundity in the singles in our experiment ($\kappa_R > 0.5$). Because the pairs can mate continuously, we expect to find a higher reproduction efficiency in the singles than in the pairs. However, surprisingly, we do not observe a strong effect of mating on fecundity in our experiment. We can use the same $\kappa_R = 0.95$ for both mating regimes, which is explained in detail later on.

Note that the reproduction efficiency κ_R only reduces fecundity, and has no impact on predictions of egg size within DEB theory.

Shell growth (δ_M): When we keep the remaining parameters to their values as derived in in Chapter 4, we still can not capture the patterns in growth (both dry weight and shell length) and reproduction in regime A. We assume that the differences are due to the fact that we do not explicitly model the shell formation of the pond snail. In DEB applications, the shape coefficient δ_M is used to go from physical to structural length, which is used as state variable in DEB. We use the measure of the shell length as a measure for physical length. The shape of the shell can vary a lot between individual snails. While some snails tend to have a long and slim shell, other have a shorter and broader shell. Therefore, we assume that the shape coefficient δ_M is different in this experiment compared to the one used for parameterization in Chapter 4. With a slightly different δ_M, we can capture the growth pattern in shell length sufficiently.

The dry weight data in our experiment includes the shell. The contribution of the shell to the full body mass can be very variable between individuals depending on factors such as feeding history and water composition. To keep it simple, we assume the shell to be a fixed fraction of the body, and include it by multiplying the dry weight of the soft body as predicted from the model with the factor s_{sh}.

5.2.3. Starvation response

The DEB model failed to predict the observed patterns in reproduction rate, which is why we tried a different model for the starvation response. In DEB applications, starvation responses are treated in a highly species specific manner. However, the initiation of the response is in general considered to be linked to the reserve density e, which is compared to the scaled length l (L divided by the maximum structural length L_m, so that $0 < l < 1$, see Kooijman, 2010). When well-fed organisms starve, e decreases immediately, while l does not (yet) change. When $e < l$, the starvation response is initiated. The organisms may then start to shrink, meaning that they can use part of their body tissue to fulfill their energy requirements. We assume that in the pond snail, this switch additionally activates higher investment in the male function, which results in a decrease of the female reproduction efficiency κ_R. We introduce a parameter κ_R^s for the reproduction efficiency under stress, to capture the reduced female investment during the stress response.

When organisms are placed into a new feeding regime, their reserve density changes until a steady state with the new food level is reached (see Figure 5.5). We assume that the snails keep producing eggs with the low reproduction efficiency κ_R^s until they are close to steady state with the new food level. After that, they return to their previous reproduction efficiency κ_R. We introduce another parameter s_f, which captures how close to the new scaled food density f the scaled reserve e has to be for the snails to readjust their investment (see Figure 5.5). We assume that a higher food level leads to earlier re-adaptation, so that we multiply the factor s_f with the scaled food density f to determine the stopping criteria for the starvation response. Factor s_f has the following interpretation: if s_f was 1, the snails would readjust their investment when their reserve density e exactly equals the scaled food density f. If factor s_f was 1.2, the snails would readjust their investment when their e is 20 % higher than f.

To summarize, this means that at the beginning of the experiments, the snails have a reproduction efficiency of $\kappa_R = 0.95$. When the reserve density e falls below l, the starvation response is initiated, and $\kappa_R = \kappa_R^s$ (see Fig. 5.5). When $e < f \times s_f$, the stress response stops, and the snails invest the old fraction $\kappa_R = 0.95$ into female reproductive output again.

Note that, other than this, we do not introduce any starvation rules. This means that when the snails start to shrink, we assume that they obtain the same amount of energy from the reduction in body tissue as they used to put in, which is an admittedly unrealistic simplification.

5.3. Results and discussion

With a few additional assumptions concerning the shell growth, we can use the same parameter estimates as we used in Chapter 4 to capture the general pattern in growth and reproduction at the highest food level (see Fig. 5.2). Surprisingly, we need to assume that the shell is only 10 % of the total body mass (in dry weight)

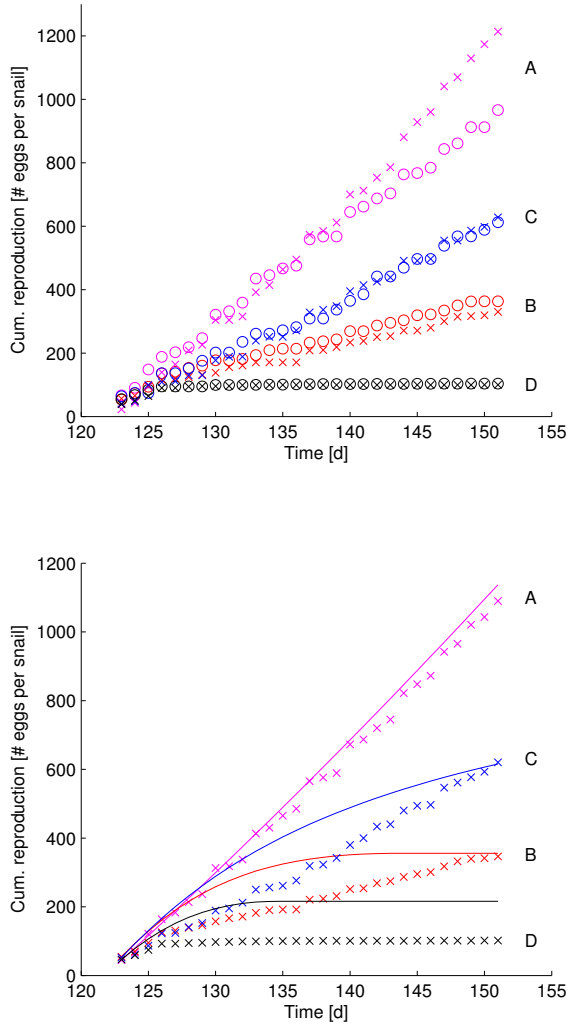

Figure 5.1.: **Upper panel:** Only in the highest feeding regime (A), an effect of mating regime is visible after 3 weeks in the cumulative number of eggs per regime. The circles represent the data from couples, and the crosses represent the data form the single snails. **Lower panel:** With the standard DEB model, the predictions of mean cumulative numbers of eggs do not capture the patterns observed in all feeding regimes but A. In this figure, $f_B = 0.18$ and $f_C = 0.4$. Note that we show the mean values of the mating regimes here, because mating had no significant effect during most of the experiment (see Section 5.3.3).

Table 5.1.: The parameter values for the model that was presented in Chapter 4. The parameters presented here are specific to the experiment presented in this study, and for the model extension. Note that all parameters are dimensionless.

Symbol	interpretation	value
Parameters		
κ_R	reproduction efficiency	0.95
δ_M	shape coefficient	0.464
s_{sh}	shell factor	1.1
Scaled functional response		
f_A	in regime A	1
f_B	in regime B	0.39
f_C	in regime C	0.56
f_D	in regime D	0.5 10^-3
Stress parameters		
κ_R^s	Reduced reproduction efficiency during stress	0.15
s_f	Factor for stopping the stress response	1.23

of the snail (see Tab. 5.1), which seems a bit low compared to values of around 40 - 70 % which have been found in experiments at INRA (personal communication). However, the measurements indicate that the contribution of the shell to the total body mass is variable, and not a constant fraction during the life cycle (unpublished data, Alpar Barsi, INRA). To critically investigate this, detailed measurements of dry weight with and without shell under controlled conditions in a (partial) life-cycle experiment are needed. A model formulation that explicitly considers shell formation could be necessary to fully understand the observed patterns. Since the shell does not require maintenance, it would be seen as a product in DEB. Product formation can be seen as a by-product from the fluxes that go into growth and maintenance, as was done for otolith (i.e. fish inner ear bone) formation (Fablet et al., 2011). The primary DEB parameters correspond to the metabolic processes such as growth, reproduction and maintenance, so that they should be linked to the soft body tissue in the snail when the shell is modeled separately from the body.

5.3.1. Maternal effects

In contrast to what we expected, there were no big differences in individual egg weight between the feeding regimes. Despite the low food levels, as evidenced by severe reduction in reproduction, the investment per egg did not decrease much during the experiment (see Figure 5.3). Only in the full starvation regime, the investment per egg decreased substantially. However, the observed egg sizes in all treatments were small compared to observations of Hoffer et al. (2012). These authors report eggs that were more than twice as big as the ones we find. The maternal effect that the DEB model predicts is very small as well (see Figure 5.3),

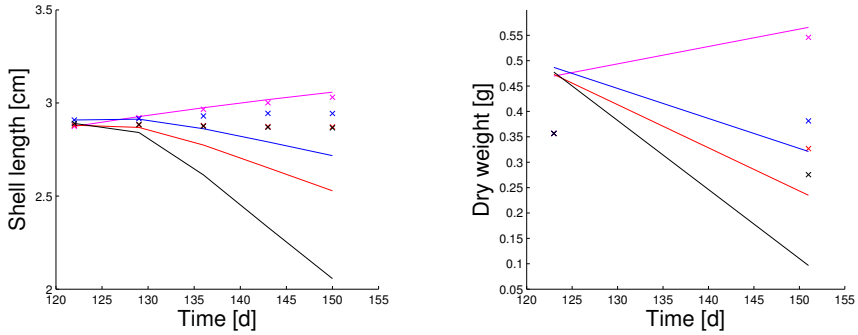

Figure 5.2.: Predictions for length (left panel) and full body dry weight (right panel). The model does not separate soft body tissue from the shell. Therefore, the starvation response in the model overestimates the reduction in weight due to starvation.

despite the huge changes in reserve density (see Figure 5.5, upper panel). It might be that the maternal effect of this magnitude was not that visible here because of the small size of the eggs and the large variation between egg sizes. Note that the model predictions are lower than the mean egg size, but lie within the standard deviation for all regimes but C (see Figure 5.3). The results are thus consistent with predictions of the standard DEB model.

Hoffer et al. (2012) kept the snails under a photoperiod of 12h light, 12h dark (12/12 L/D), whereas our experiment was conducted under a photoperiod of 14h light, 10h dark (14/10 L/D). This might be an explanation for the differences in egg sizes between the experiments. A difference in the investment in reproduction relative to growth caused by light regime has been discussed earlier for the pond snail in Zonneveld and Kooijman (1989). The authors stated that snails with a longer day (LD) spend a larger fraction on reproduction than snails with a shorter day (MD), and therefore also grow less. This phenomenon has been discussed as the "κ-effect"(Kooijman, 2010): in DEB theory, the value of κ determines the fraction of energy that is invested into somatic maintenance and growth, relative to what is available for maturation and reproduction (1 - κ). However, in Zonneveld and Kooijman (1989), only the reproduction rate of the LD and MD snails was discussed, not the investment per egg. Interestingly, when comparing the total investment into reproduction of the snails in Hoffer et al. (2012) to our experiment, it turns out that our snails invest much more energy in reproduction (see Table 5.2). The snails in Hoffer et al. (2012) have a shorter day than ours, which confirms the observations of Zonneveld and Kooijman (1989): the snails with the shorter day invest less energy in reproduction than the snails with the longer day. Indeed, using our DEB model, we can calculate egg sizes in the range of the ones Hoffer et al.

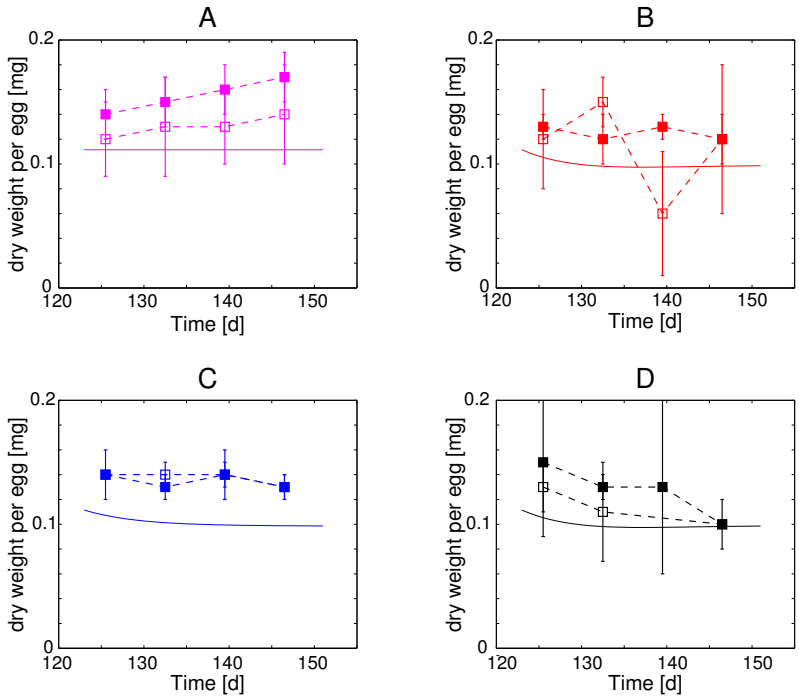

Figure 5.3.: Dry weight of eggs with standard deviation over time as mean values per week per regime. The data from pairs are represented by the filled boxes (■), and the data from single snails are represented by the empty boxes (□). The lines represent the model predictions.

(2012) report when changing κ to higher values (see Figure C.3, Appendix C). It would be interesting to see whether the growth and reproduction pattern over time as observed by Hoffer et al. (2012) can be matched with the same parameter values, or whether we would need to adapt more parameters.

5.3.2. Offspring fitness

Only in the full starvation regime, there was a decrease in egg weight (see Figure 5.4). It must be noted that not many clutches were laid in regime D after the first 3 days (see Figure 5.5, lower panel). From the clutches that were laid in regime D, we measured the dry weight of a few, and followed the development of some of the others (see Figure C.2, Appendix C). The clutches that were laid last in regime D did not hatch. Even though there was only a slight decrease in egg weight, it had a substantial impact on time to death by starvation (see Figure 5.4). This means that in the pond snail, too, the investment per egg relates to the fitness

Table 5.2.: The details on reproduction data in our experiment compared to Hoffer et al. (2012). The regime 'Cont. same' refers to snails that have the same partner during the experiment, and the regime 'Once' refers to a single snail which was allowed to mate once.

L / D	Hoffer et al. (2012) 12 / 12		Our experiment 14 / 10	
	Once	Cont. same	Singles	Pairs
reproduction rate [# eggs / d]	10	4.3	45	33
dry weight per egg [mg]	0.22	0.3	0.15	0.15
total dry weight of eggs [mg / d]	**2.2**	**1.3**	**6.8**	**5**

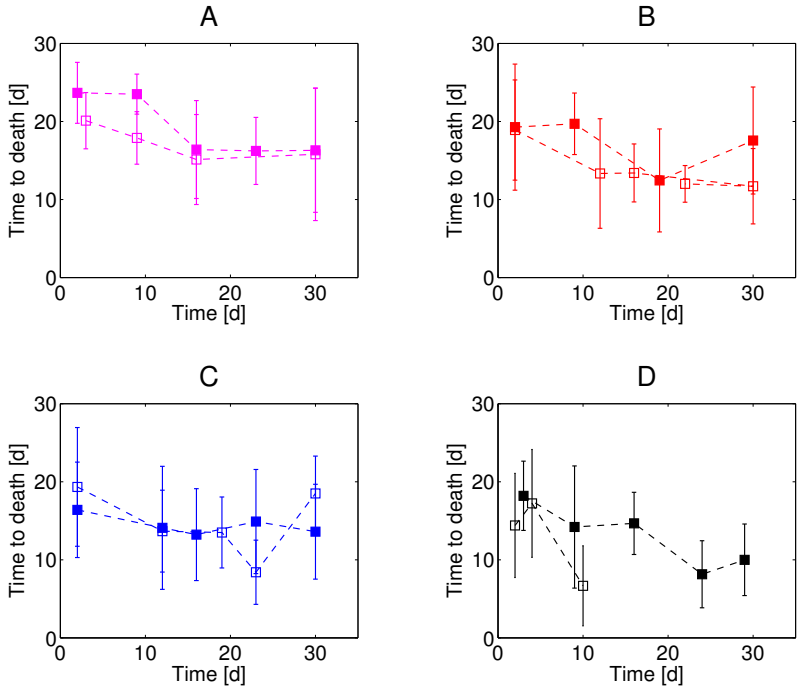

Figure 5.4.: Time to death by starvation as means per clutch with standard deviation. The data from pairs are represented by the filled boxes (■), and the data from single snails are represented by the empty boxes (□).

of the offspring. This was expected from observations on egg size and starvation handling in other species: offspring that hatch from smaller eggs can not cope as well with starvation as offspring from larger eggs (Gliwicz and Guisande, 1992; Ito, 1997).

5.3.3. Effects of mating opportunity

During most of the experiment, we do not see a strong effect of the mating regime. Up to the end of week three, the differences between the groups could be attributed solely to the feeding regime (ANOVA: $F=72.95$; $df=7, 101$; $P<0.0001$). Hence, each feeding regime differed significantly from the other regimes, irrespective of mating treatment (post hoc Student's t: $P<0.05$). After the fourth week there was still an overall statistical difference between all the treatments (ANOVA: $F=84.51$; $df=7, 100$; $P<0.0001$). But now, in addition to the differences between feeding regimes, the post-hoc tests also reveal a difference in egg numbers between the single and paired individuals in regime A (post hoc Student's t: $P<0.05$). Thus, we only found an effect of mating opportunity on egg laying in the highest feeding treatment, with the paired individuals laying less eggs than the single ones.

The reason why we expected a difference in the mating regimes lies in the effect of the seminal fluid. In a situation with a high mating rate, a suppression of egg laying is usually observed in the pond snail, which results from components of the seminal fluid that increase the interval between egg laying (Koene et al., 2009, 2010). Van Duivenboden et al. (1985) showed that the snails can recover from this suppression within 6 days. Therefore, we can expect that the single snails had recovered from any suppression caused by mating in the culture during the acclimation period. The snails in the paired regimes continuously had the opportunity to mate, and therefore continuously received the seminal fluid that has been shown to reduce egg laying. However, in regime B and C, we observe no suppression of egg laying, and it only starts to show in regime A after three weeks. This suggests that all snails do not invest much energy in the male function in this experiment, at least during the time when they are not in the starvation response mode. Because there was no difference between mating regimes during the most part of the experiment, we use the mean values per feeding regimes, and set $\kappa_R = 0.95$ like is usually done in DEB applications where only the female reproductive output is followed.

5.3.4. Effect of food limitation on fecundity: starvation response

With the suggested model extension for the starvation response, we get an excellent fit for the observed patterns in fecundity (see Figure 5.5). This means that during the acclimation phase from the high food level to the lower food levels, the snails in the intermediate feeding regimes invest less of the available energy in producing eggs: with a reproduction efficiency $\kappa_R = 0.15$, we can capture the observed fecundity in the acclimation interval (see Figure 5.5, lower panel). The reproduction

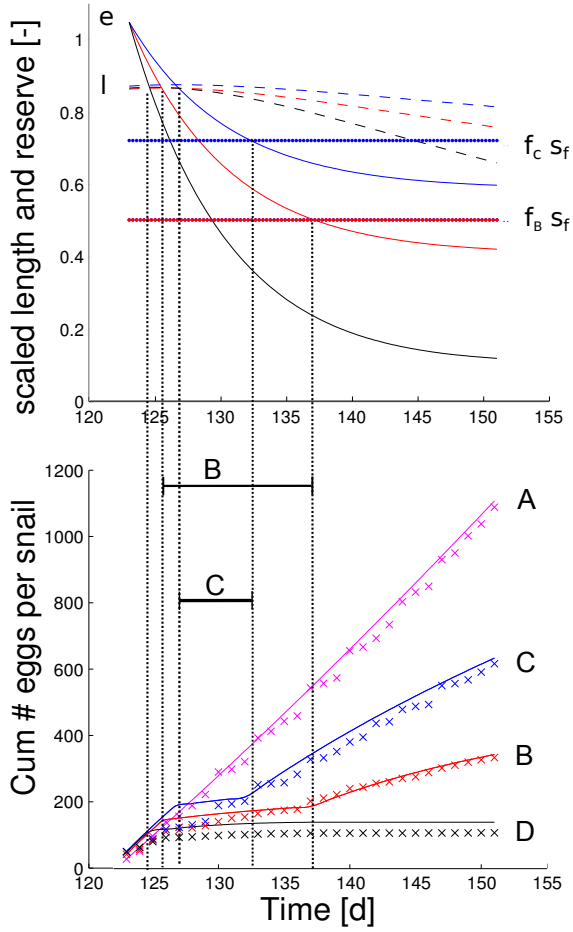

Figure 5.5.: The fecundity of the snails as mean values per feeding regime. Time corresponds to the age since birth of the snails. The starvation response can be captured as a response to the reserve dynamics (upper panel). When the scaled reserve density e (solid line) falls below the scaled length l (striped line), the starvation response is initiated in regime B (red lines) and C (blue lines). When e reaches a level which is s_f times higher than the new scaled food level f (dotted line), the snails go back to investing mainly in the female function.

efficiency κ_R denotes the fraction of energy of the investment into reproduction that is actually fixed into egg biomass. The largest fraction of the energy allocated to reproduction was thus available for other uses. It might be that the snails used that energy to produce sperm. Another possible explanation might be that the snails used this energy to cover their maintenance costs when they could not pay them from the mobilized flux allocated to the soma instead of shrinking. Indeed, the snails shrink less than the model predicts (see Figure 5.2). However, it is very unlikely that such a major change in energy allocation is first performed, only to be changed back after the acclimation to the new feeding regime. We thus have reason to assume that the pond snails either invest the remaining energy in sperm, or in processes that are associated with overhead costs for reproduction. This might also be the reason why no significant effect of mating is observed during the course of the experiment: the reproductive system is disturbed by the changes in food level, and that effect is stronger than potential suppression of egg laying. Relative higher sex allocation towards the male function under poor nutritional conditions was observed by Locher and Baur (2002) in a land snail, and by Vizoso and Schaerer (2007) in a flatworm. However, our hypothesis remains speculative, and to confirm this for the pond snail, we would need to measure the investment in sperm.

5.4. Conclusions

In the present study, the food level hardly affects the observed egg sizes. These observations are consistent with the predictions of the DEB model, which also does not show a strong effect of food level in this particular case. Apart from mating possibilities, many other factors such as food availability and light regime have an influence on investment per egg in *L. stagnalis*. We suggest that the pond snail responds to starvation with a temporarily enhanced investment into the male function relative to the investment into the female function. However, detailed experiments including the measurement of reproductive organs and fluids are needed to support this hypothesis. Future experiments investigating reproductive investment should consider the interaction between food level and mating in the experimental design and interpretation of the results. Especially in ecotoxicological investigations, the total investment in eggs should be monitored, and not only reproduction rate in terms of numbers of eggs (see e.g. Hammers-Wirtz and Ratte, 2000). Using a mechanistic effect model can improve the understanding of patterns in data. The deviations allow for identification of the underlying mechanisms that have not been considered in the model, but might be important to understand the pattern.

The trade-off between male and female function is commonly observed in simultaneous hermaphrodites, and an increase of investment into the male function during starvation has been found in other species. Thus, the model extension we suggest might be more generally applicable to simultaneous hermaphrodites.

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6. Dynamic Energy Budget theory meets individual-based modelling: a generic and accessible implementation

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Summary

1. Dynamic Energy Budget (DEB) theory was designed to understand the dynamics of biological systems from cells to populations and ecosystems via a mass balance approach of individuals. However most work so far has focused on the level of the individual. To encourage further use of DEB theory in a population context we developed DEB-IBM, a generic individual-based model (IBM) that is based on DEB theory.
2. The generic IBM is implemented as a computer program using NetLogo, a free software platform that is accessible to biologists with little programming background. The IBM uses DEB to represent assimilation, maintenance, growth, and reproduction of individuals. The model description follows the ODD (overview, design, details) protocol, a generic format for describing IBMs, and thereby provides a novel and accessible introduction to DEB theory and how it works in a population context.
3. DEB-IBM can be used to explore properties of both individual life history traits and population dynamics which emerge from the set of DEB parameters of a species, and their interaction with environmental variables such as food density. Furthermore, DEB-IBM can be adapted to address specific research questions, for example by including spatial effects. A User Manual explains how this can be done.
4. DEB-IBM is designed to both facilitate use and testing DEB theory in a population context and to advance individual-based modelling by basing the represen-

tation of individuals on well-tested physiological principles.
Key words: Dynamic Energy Budget, Individual-based model, Population dynamics

6.1. Introduction

Understanding how population dynamics emerge is one of the fundamental challenges in ecology. As the influence of individual variation, local interactions, and adaptive behaviour on population dynamics have become more appreciated, individual-based models (IBMs) are playing an increasing role in both basic and applied disciplines (DeAngelis and Mooij, 2005; Grimm and Railsback, 2005; Stillman and Goss-Custard, 2010). IBMs represent individual organisms as unique entities which differ from each other and change over their life cycle. Individuals are characterized by a set of state variables and attributes which are chosen according to the problem addressed with the model (Grimm et al., 2010). Individuals behave as autonomous entities according to behavioural rules. They interact with each other and their abiotic environment, including habitat structure and environmental drivers such as temperature, humidity, or disturbances. Population dynamics emerge from these interactions.

IBMs have been shown to be powerful and flexible tools. However, they have also been criticized for often being based on ad hoc assumptions and representations of individual dynamics and behaviour (Grimm, 1999). This makes the development of IBMs inefficient and the field of individual-based modelling incoherent (Grimm and Railsback, 2005). To facilitate re-usability of IBMs and their elements, and to facilitate distilling general insights from specific IBMs, it is desirable to base IBMs more on standardized and well-tested approaches for individual behaviour (Berger et al., 2002).

Dynamic Energy Budget (DEB) theory (Kooijman, 2010) is such an approach. It has been developed with the goal of understanding the dynamics of biological systems, from cells to ecosystems, via a balance approach for mass and energy. As in IBMs, in DEB theory individuals are considered the key unit of interest for understanding dynamic systems at higher levels of organisation. Focusing on the individual is motivated by the fact that mass and energy balances are easier to calculate for individuals than for higher or lower levels of biological complexity. Additionally, natural selection occurs at the level of the individual, which shapes the life-history traits of a species, and ultimately drives dynamics at higher levels of biological organisation. DEB theory provides a quantitative framework for modelling the acquisition and use of resources for organisms over the entire life cycle. It thereby generates a quantitative explanation for the time patterns of life-history traits such as growth, maturity, and reproduction in dynamic environments.

An overview of DEB theory and its applications can be found in Nisbet et al. (2000), Kooijman (2001), Van der Meer (2006) and Sousa et al. (2008). A key assumption in the theory is that the mechanisms governing metabolic organiza-

tion are similar among species. Therefore, the same basic model structure can be used for, in principle, all animal species; species differ in life-history primarily as a result of differences in their set of DEB parameters, not because of differences in model structure. The generality of DEB facilitates a growing understanding of how life history traits co-vary among and within taxa. In spite of DEBâĂŹs generality, it is an empirically grounded and well tested theory, and has been applied in a range of disciplines including ecotoxicology (Jager et al., 2006) and aquaculture (Alunno-Bruscia et al., 2009), and to species from a wide range of taxonomic groups including bacteria, yeast, arthropods, fish, and mammals. Yet to understand behaviour at higher levels of biological organisation, tools are needed to scale from the individual model to populations. We believe both DEB and IBMs can benefit each other, however to date these approaches have rarely been used in combination. Below we discuss how each of these approaches can benefit each other, and then describe the DEB-IBM framework which we have developed to facilitate the use of DEB in an individual-based context.

6.2. How can DEB benefit IBMs?

A common problem with the application of IBMs is their complexity. IBMs are often developed for very specific research questions, and the structure and parameterization of models defining the life-history of organisms differ widely. This creates a problem not only for model developers who often start from scratch when modeling a new species, but also for the scientific community which must try to reconcile different models, or try to understand how conclusions for one species relate to another. DEB is appropriate as a building block for IBMs because it is aÂĂrelativelyÂĂsimple model which translates environmental conditions to individualÂăperformanceÂă(growth, survival and reproduction) and isÂăconsistentÂăwith first principles such as conservation of energy. This is important because the trade-offs in life history traits that DEB specifies (growth vs reproduction, time and size to maturation) turn out toÂăstronglyÂăinfluence population dynamics (Denney and Reynolds, 2002; Sæther and Bakke, 2000). Moreover, because DEB is a generic theory, it can be applied to virtually all species which would facilitate broader insight from specific studies and comparisons between species.

6.3. How can IBMs benefit DEB?

Because DEB models specify behaviour of an individual, tools are needed to extrapolate to the population level. So far, most of such population predictions based on DEB theory were made using matrix models (Klanjscek et al., 2006; Klok and de Roos, 1996; Billoir et al., 2007) or the Euler-Lotka equation (Kooijman and Metz, 1984; Jager et al., 2004). The disadvantage of these approaches is that only one state variable can be easily considered (age, stage or size), whereas the consistent application of DEB often requires considering more state variables, especially

in time-varying environments. Another method for simulating population dynamics based on a model of individual performance is provided by physiologically structured population models (PSPM) (e.g. the escalator boxcar train from De Roos et al. (1992)). PSPMs can be used to model population dynamics in dynamic environments. However for all of these approaches (Matrix, Euler-Lotka equation, PSPM) as opposed to IBM's, variation among individuals, local interactions, or adaptation cannot be easily considered in a rigorous manner. IBMs are the natural link to the population for DEB because both approaches focus on the behaviour of individuals, as a key aspect in understanding higher levels of biological complexity. Additionally, use of DEB in a population context has generally used a deterministic approach. DEB-IBM allows for the inclusion of stochasticity, and thus provides a framework to investigate its effect at the population level.

6.4. DEB-IBM links DEB theory with IBMs

Despite this potential, so far DEB theory has not been widely used in IBMs. We only know of three published examples (Kooijman et al., 1989; Alver et al., 2006; Bacher and Gangnery, 2006). A reason for this might be that to implement DEB theory in IBMs, skills in both mathematics and computer programming are required, which many ecologist lack. Therefore, to encourage further development and use of DEB theory we have developed a generic framework for DEB-based IBMs using a software platform that is accessible to biologists with little programming background: NetLogo (Wilensky, 1999). DEB-IBM is a generic IBM, which can be linked to specific species by using species-specific parameters. It is thus rather a framework than a specific model. We here focus on the general framework which is designed to facilitate using DEB and IBM in combination for tackling all kinds of generic and specific questions for a wide range of species. We present a transparent and complete yet concise implementation of the DEB model for a generic isomorphic (i.e., organisms retain the same shape during growth) and ectothermic animal (Kooijman et al., 2008) within an IBM. In the following, we first briefly describe the DEB-IBM framework and then present the IBM and its scope.

6.5. The DEB-IBM Framework

We implemented a scaled version of the standard DEB model as described in Kooijman et al. (2008). A full description of the model, following the ODD protocol for describing IBMs (Grimm et al., 2006, 2010), a User Manual, and the NetLogo file of DEB-IBM are all included in the supplementary material (http://cream-itn.eu/projects/wp-1/daphnia-2/deb-ibm). In the following we provide a brief overview of DEB-IBM and describe how it can be used. Each model individual is characterized by four primary state variables (called "DEB state variables" hereafter) that describe the energy content of four different compartments: "structure", which determines actual size, feeding rates, and maintenance costs; "reserves",

which serve as a buffer between feeding and metabolic processes that require energy; "maturity", a continuous state variable which regulates transitions between the three development stages (embryo, juvenile, adult) at fixed maturity levels, and a "reproduction buffer", into which mature individuals direct energy for reproduction and which is converted into embryos during reproductive events.
In DEB theory, metabolic processes are mechanistically driven by surface/volume ratios. Individuals update their primary DEB state variables based on a set of differential equations. Individuals assimilate food from the environment which enters the reserve. Energy is mobilized from the reserve and is distributed to two distinct pathways: somatic growth and maintenance on one side, and maturity maintenance, development (for immature individuals) or reproduction (for mature individuals) on the other (maintenance costs need to be satisfied first). Here, κ is the proportion of the mobilized energy allocated to the soma, and (1 - κ) the proportion allocated to maturity maintenance, development, or reproduction. Based on the updated DEB state variables, a set of discrete events may occur. An individual dies when it cannot mobilize enough energy to pay somatic maintenance. At each time step, for each mature individual, it is calculated whether the individual has enough energy for an offspring, if it does, it produces one offspring. In the next time step of the numerical simulation, this individual is added to the population; it will start to feed exogenously when the maturity level reaches the threshold for birth, however this default reproduction process can be easily adapted to replicate other types of reproduction behaviour. In addition to this standard model, we have included optional submodels for the ageing process, intra-specific variation, and simple predator-prey dynamics.
Species in the model are specified by the 8 âĂIJscaledâĂİ DEB parameters (see User Manual), with two additional parameters for the ageing submodel (optional), and two parameters needed for the foraging submodel (you also need the two parameters [r and K] of the logistic growth formula of the prey to run the population dynamics under logistic prey dynamic conditions).
Our implementation is compatible with a database of DEB parameters for a rapidly growing number of species: `"Add_my_pet"` (`http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.php`). In the user manual we provide a detailed explanation of how to obtain parameters from this data base and input them into DEB-IBM. The `"Add_my_pet"` database is relatively new, with parameters values for approximatly 60 species, with varying degree of support. However, users can assess the degree of support for a species in the database, because the data used to derive the parameter set for each species is given in a corresponding data file, and within the file the references from which the data were taken are listed.
Many users still will have to obtain DEB parameters themselves. There are currently two thorough reviews and guides for parameterizing a DEB model for a species (Van der Meer, 2006; Kooijman et al., 2008). If data are very limiting, a general set of parameters can be estimated from maximum body size of an individual (Kooijman et al., 2008). While users can cope with less, generally data for growth and reproduction at multiple food densities provides enough

information to get a good set of parameters for use in DEB-IBM. Parameterization tools, DEBtool (available in both Matlab and the free software Octave) and DEBtoxM (specific for toxic stress, Matlab only) can be obtained from (`http://www.bio.vu.nl/thb/deb/deblab/`), which perform the required optimization techniques for varying levels of data availability. This level of use requires deeper investment into DEB theory.

There are two levels of application for our generic framework. First, it can be used to explore properties of both individual life history traits and population dynamics which emerge from the set of DEB parameters of a species, and their interaction with environmental variables such as food density. For this, no programming or technical understanding of DEB theory are required. Users need only input DEB parameters and environmental conditions in the graphical user interface, from which they can monitor and record various individual and population level output such as fecundity, population density, and size structure. The second level of use, involves adapting DEB-IBM to address a specific research question. For this, users must learn how to change the code of the generic model. For example the research question might be: how are the population dynamics of a species influenced by changes in land use? In this case, the user would adapt the generic DEB-IBM to include space and movement behaviour of individuals, with DEB theory acting as the energetic model for the individual. We provide detailed examples of how the model can be adapted to include both spatial and behavioural aspects. The NetLogo implementation is flexible enough to add all kinds of modules or alter existing ones, including ones that are in DEBtool or DEBtoxM.

This more advanced use of the model requires users to learn programming in NetLogo. However, NetLogo is an exceptionally well documented software platform that was specifically designed for implementing IBMs; moreover, a recent textbook on individual-based modelling which is based on NetLogo is available (Railsback and Grimm, 2012). NetLogo comes with powerful built-in procedures, leading to a shallow learning curve. This makes both IBMs and DEB more accessible to ecologists without formal training in computer programming. NetLogo has some limitations, in particular regarding computation time, number of agents and spatial units it can deal with, and the lack of a tool for debugging the software. However, these limitations turn out to not be constricting for many models and problems in population ecology. Moreover, since NetLogo slowly but surely is turning into a standard platform for implementing IBMs, we expect that these limitations will be overcome in the near future (see, for example, the recent link between NetLogo and the computationally more powerful platform RePast: `http://repast.sourceforge.net/repast_simphony.html`).

For DEB-IBM we did not chose a general programming language such as C++ or Java because learning these languages to the point were users can implement or modify individual-based models would usually be too time-consuming for most ecologists. Likewise, we considered none of the alternative software platforms, e.g. Repast (repast.sourceforge.net) or MASON (Luke et al., 2005) suitable because they are much harder to learn and not as thoroughly documented (for compara-

tive reviews of software platform for individual-based or agent-based models, see Railsback et al. (2006); Nikolai and Madey (2009)).

6.6. Discussion

DEB-IBM can be used without modification to make general estimations of population characteristics, such as population growth rate, in simple environments, and as a learning tool for understanding how the physiological properties of individuals can influence population dynamics. While other tools, such as matrix models or the Euler-Lotka equation can be used to estimate population growth rates in constant environments, they cannot as easily be extended to dynamic environments. A further advantage of DEB-IBM is that we can consider the interactions between a predator population and its prey. In the default version of DEB-IBM we have allowed the option to model dynamic predator-prey population dynamics assuming the prey follows a logistic growth pattern and is depleted via predation, however this can be adapted when needed to model prey dynamics of a specific system. Thus, DEB-IBM can be used to estimate carrying capacity of the predator population as a function of its environment. Because DEB-IBM predicts dynamics in time, it lends itself to more rigorous testing with population-level data which often consist of time series observations of population density and/or size structure. This is important because validation of models against population data is necessary to build confidence in the model for applied uses.

It should be noted that although DEB-IBM facilitates applying DEB theory in individual-based population models, using it still requires commitment. For specific research questions, DEB-IBM merely serves as a starting point. Researchers will have to consider species-specific processes such as the rules for converting the reproduction buffer into offspring. In the generic model, individuals reproduce when they have enough energy to produce one offspring. However, many animals produce clutches of offspring, either at fixed time intervals or when triggered by an environmental cue. These differences in life-history strategy can easily be incorporated into the generic model, and in the user manual we give examples of how to do so. Additionally, relevant behaviours such as dispersal or habitat selection may have to be considered. Users of DEB-IBM should thus be prepared to learn basic skills in NetLogo, but this requires, due to the design and excellent documentation of NetLogo, usually not more than a few days.

Like any useful theory, DEB theory is not static, and there are still plenty of open questions within DEB that require dedicated research. A growing international community is currently working with this theory, so we can expect new developments in the near future. One benefit of using DEB in a population context is that it highlights aspects of the individual dynamics which are especially relevant for population dynamics. Often these are areas which have been overlooked by those focusing solely on individuals. Our own initial use of DEB-IBM has highlighted important questions where further research is needed. For example, within DEB

a general pattern of intraspecific variation in parameter values has been suggested (Kooijman et al., 1989), however little research to date has investigated how DEB parameters (co)vary among individuals within a population. Additionally, little research has so far been carried out on the process of starvation. Kooijman (2010) offers some possibilities to handle starvation within a DEB context, but these rules are probably highly species-specific and require further evaluation.
Nevertheless, the advantage of using a mechanistic framework like DEB is that once these questions are addressed, and the major processes understood, they are more likely to apply in untested conditions, whereas phenomenological approaches can only be applied within the range of tested conditions. Additionally, research on starvation within a DEB context may help shed light on how similar the mechanisms of the starvation process are among a wide range of taxa. DEB has a lot to offer for solving specific problems, but to exploit its benefits as a general theory it needs to be used and tested more widely at the population level. This would increase confidence in the model, clarify its limitations, and possibly lead to further improvement.

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7. General discussion

7.1. Choosing the right model

In my thesis I present three different approaches that we used to investigate the life history of the pond snail *L. stagnalis*. The application of each of the approaches helped understand the physiology of the snail in more detail. In Chapter 2, we started with the DEB formulation of the von Bertalanffy growth model as the simplest possible option to investigate the growth at different types and amounts of food. From this, we learned that lettuce is not an optimal food source for the juvenile pond snails. However, we only used this formulation because it is the easiest way to demonstrate the juvenile food limitation and its potential implications for ecotoxicity testing. In the following, I will discuss i) the implications of metabolic acceleration (as discussed in Chapter 4) in comparison to the food limitation (see Chapter 2) and ii) the usefulness of the full DEB model (Chapters 4 and 5) in comparison to the simplified DEBtox model (Chapter 3). Further, I will come back to the discussion points I mentioned in the introduction and finally discuss my ideas on the "perfect" ecotoxicological experiment.

7.1.1. Acceleration vs. food limitation

Both the metabolic acceleration and the juvenile food limitation function capture the growth and reproduction of the pond snail (see Chapter 3 and 4). However, neither one of the two models is true: both are simplifications of reality. The life history of the pond snail can not be modeled with the standard DEB model with one consistent parameter set: when fitting the parameters to data on juvenile/adult growth and reproduction, the predicted values for hatching time were too short. Additionally, the shape of the growth curve deviates from prediction of the standard DEB model: after birth, juvenile pond snails grow slower than expected from the von Bertalanffy growth pattern (see Figure 2.1, Chapter 2). When using the food limitation function, we can capture the deviation of the growth curve, however, the predicted hatching time is still too short. We could repair that by assuming a switch in the energy conductance $\dot{v}$ after birth, which would result in a slower use of reserve by the embryo, and thus a slower development. However, this would be a very 'ad hoc' solution, and without any other support than goodness of fit. The metabolic acceleration extension (Kooijman et al., 2011) that we used in Chapter 4 has as a consequence that the embryonic development is slower. However, it was developed for organisms that change in shape during ontogeny, until they reach their final shape after metamorphosis. As a consequence from the changes in shape, the

energy conductance $\dot{v}$ and the surface area specific maximum assimilation efficiency $\{\dot{p}_{Am}\}$ are low at birth, and increase until metamorphosis. The snail does not apparently change in shape after birth. A change in $\{\dot{p}_{Am}\}$ seems reasonable: the food limitation might be a result from an insufficiently developed gut fauna. All animals have to build up a functioning gut fauna before they are able to digest cellulose. If this holds true for the pond snail, this might be the reason for the slow growth of the early juveniles when fed with lettuce (see Chapter 2). The fact that the juveniles can grow faster when fed with Tetraphyll could then be explained by assuming that the gut fauna needed for digesting Tetraphyll can be built up faster. However, this hypothesis is essentially similar to the assumption of juvenile food limitation. When the gut fauna for eating Tetraphyll can be built up faster than the one for lettuce, the juvenile snails fed with lettuce are food limited relative to the ones fed with Tetraphyll. However, a slowly building up gut fauna would not likely result in a change in $\dot{v}$. The pond snail might not change in shape after birth, but during the embryonic development. Approximately five days after egg laying, they reach metamorphosis and their adult-like shape. If a change in $\dot{v}$ would coincide with the point of physical metamorphosis, the acceleration period would only be five days. The difference between the value for $\dot{v}$ before and after acceleration is determined by the acceleration factor $\mathcal{M}(L)$, which is the ratio of length at birth and length at metamorphosis (see Section 4.2.2). It is thus indirectly determined by the duration of the acceleration period. For the pond snail, $\mathcal{M}(L) = 8.37$, which could not likely be achieved within five days.

7.1.2. Simplified model vs. full DEB model

The simplified DEBtox model (Jager and Zimmer, 2012) is a simplification from the full DEB model. Two major simplifications have been applied: first, the ratio between structure and maturity is set constant. As a consequence, the maturity maintenance rate coefficient $\dot{k}_J$ and the somatic maintenance rate coefficient $\dot{k}_M$ have the same value. This means that the size at first reproduction does not vary with food availability, but is a constant, and maturity is not followed as a state variable anymore. This is a very handy simplification, because we lose one of the three state variables. However, it restricts the applicability of the model to organisms that do not vary in their size at first reproduction. The second simplification concerns egg costs. In the standard DEB model, the maternal effect rule applies, meaning that well-fed mothers produce well-fed offspring, and poorly-fed mothers produce poorly-fed offspring. In the simplified DEBtox model, the maternal effect is removed, and egg costs are taken constant. The embryonic development is not explicitly modeled.

The full DEB model requires more data input for parameterization, and it is more difficult to understand, but also more flexible in its application. In case of the pond snail, it seems that under certain light conditions (14 hours light, 10 hours dark, see Chapter 5), the egg size does not change much with food availability. Additionally, the pond snail tends to start producing offspring at a similar size (see Figure 3.2).

In such a situation it might be more handy to use the simplified DEBtox model to analyze an ecotoxicological experiment, because it is easier to use. However, in other light regimes, the pond snail produces larger eggs, and the size of eggs is more variable (e.g., Hoffer et al., 2012, 12 hours light, 12 hours dark). Therefore, we need more investigations to assess whether the simplified model could be applied for other light regimes as well.

Because the egg sizes can vary so much, it is in general recommendable to measure egg sizes in experiments where the reproductive output is assessed. This does not only hold for the pond snail, but also for other species. In cases where the maternal effect rule applies, or when the size at puberty is not constant, the full DEB model is much more suitable than the simplified model.

7.1.3. Deviations from von Bertalanffy growth

The pond snail deviates from the von Bertalanffy growth pattern although the shell grows isomorphically (Kooijman, 1993). When fed with lettuce, the snails grow slower compared to when they are fed with Tetraphyll (see Chapter 2, Figure 2.2). Even in the highest Tetraphyll level, they still do not reach the von Bertalanffy growth rate we would expect from the growth in the late juvenile and early adult stage. When using the food limitation function, we can capture the observed growth pattern and compare the relative food quality of lettuce and Tetraphyll. However, we ignored the embryonic development in those investigations.

When we use the metabolic acceleration extension (see Chapter 4), the deviating pattern in growth can nearly be matched without assuming an additional food limitation factor (see Figure 4.4). In that case, we can also get a good prediction for the duration of the embryonic development without further modification of the parameters (see Table 4.2). But the snails are still growing a bit too slow so that we cannot fully capture the observed pattern. Both the food limitation and the acceleration influence the assimilation flux. It might be that both scenarios contribute, and that we need to apply them together to fully capture the observed pattern. Considering the changes in model parameters, the main difference between the two approaches is that the energy conductance is changed as well in the acceleration scenario, but not in the food limitation scenario. The main effect of a change in $\dot{v}$ is a difference in the egg costs and the duration of the embryonic development.

7.1.4. Effect patterns: Hormesis

Our analysis of a data set where hormesis[1] was observed (see Chapter 3) confirms the benefit of using DEB models for revealing the mechanism underlying the biological response in the analysis of ecotoxicity experiments. Although we did not uniquely identify the chemical's mechanism of action behind the observed pattern,

[1] Hormesis is a phenomenon that describes a reversed dose-response relationship between low and high concentrations. Usually, a stimulated response to a compound at low concentrations, and an inhibition at higher concentrations is observed.

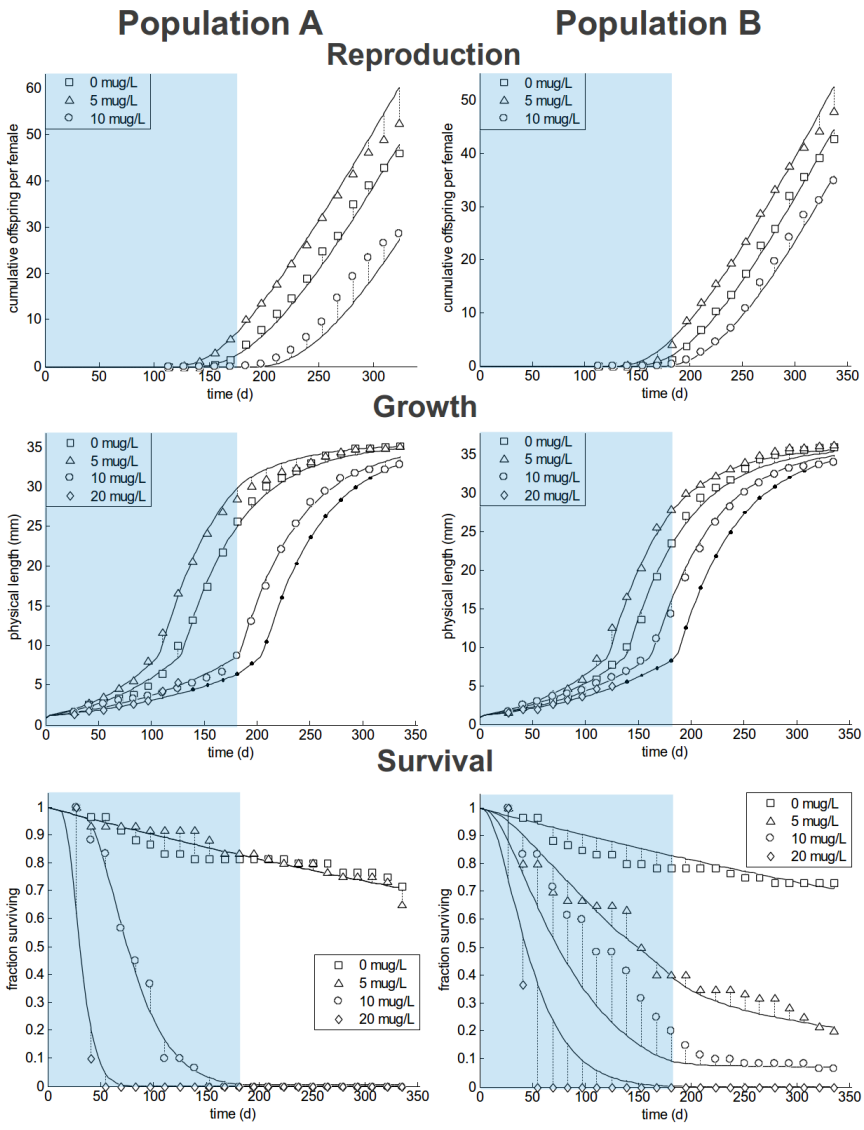

Figure 7.1.: The data of the life cycle experiment with population A (left panels) and B (right panels). Both populations were exposed to diquat until day 180 and then transferred to clean water (blue region corresponds to time of exposure). Upper panel: cumulative reproduction [number of clutches], middle panel: shell length [cm], bottom panel: survival [fraction surviving].

we indicated the most likely scenario and determined the factors that need to be investigated in more detail. In the original publication (Ducrot et al., 2010a), it was suggested that the feeding rate was influenced by diquat. We now found that there was no effect on feeding (corrected for the individuals' size), but on assimilation efficiency, which induced the effect cascade (see Figure 3.1). Although the same energy flux is affected, the implications for the interpretation of the effects is very different, when it comes to extrapolating the effects to the population level. If assimilation efficiency is affected, the snails eat normally, but cannot extract as much energy as usual from the food. If feeding is affected, the snails eat less, which leaves more food for other competitors for food.

Ducrot et al. (2010a) investigated two genetically different populations. During five years, populations with a different size at foundation were kept in outdoor mesocosms. Population A was founded from 8 snails from two different lineages. Population B was founded from only unrelated snails (80 snails from 80 different lineages). Their general effect pattern in response to diquat was very similar, so we decided to only include one of the populations in the article (see Chapter 3). In Figure 7.1 both populations are shown, as well as the effect on survival (parameters are shown in Table 7.1). The effect on assimilation that we estimated for population B was lower than the one on population A. Interestingly, the reproduction rate in population B was also lower. In this experiment, only clutches were counted, and no information on clutch weight or number of eggs per clutch over time was collected. We therefore can not further determine whether there is an extra effect on reproduction or not. In addition to the effect on assimilation, very high mortality was observed in both experimental populations (see Figure 7.1). The mortality in population B was higher than in population A in the lowest concentration of diquat. The original assumption was that the genetically more diverse population B should be less sensitive to diquat than population A. However, we decided not to focus on the mortality pattern because of the way the data were generated: dead snails were replaced by siblings of similar size, which had been treated in the same way, to ensure constant snail density. Because snail density is known to affect feeding behavior, this is the right choice when the main concern is to keep feeding rate constant. The snails were not marked, so when the survival data was collected, there was no way to tell whether a newly introduced snails had died or an old one. Therefore, we decided against putting too much emphasis on this data, and excluded it from the article (see Chapter 3). However, it is likely that there is a connection between the effects on assimilation and the high mortality.

Applying a DEB model can help identify the underlying effect cascade. Instead of multiple endpoints being influenced individually, we could show that all of the separately observed effects are interconnected. We could now use this outcome for informed risk assessment, e.g. by including the model in DEB-IBM (see Chapter 6), and investigate possible population-level effects of the hormetic response.

Table 7.1.: The parameters of the DEBtox model as estimated for the two genetically different populations from Ducrot et al. (2010a). The parameters that were estimated independently in both populations are emphasized in **bold**.

parameter	A	B	unit
elimination rate	0.0004273	0.0004273	1/d
blank hazard rate	0.001022	0.001022	1/d
scaled no-effect concentration for survival	**0.1139**	**0.02605**	mg / d
scaled killing rate	**0.6442**	**0.1095**	L / mg d
energy investment ratio	1	1	-
initial body length	0.9288	0.9288	mm
food limitation length	8.7	8.7	mm
length at puberty	15.12	15.12	mm
maximum length	38.12	38.12	mm
von Bertalanffy growth rate	0.01737	0.01737	1/d
maximum reproduction rate (clutches)	**0.5257**	**0.4449**	#/d
scaled ingestion rate control	**0.9312**	**0.9519**	-
scaled ingestion rate 5 $\mu g/L$	1	1	-
scaled ingestion rate 10 $\mu g/L$	**0.8271**	**0.9023**	-
scaled ingestion rate 20 $\mu g/L$	**0.7875**	**0.8641**	-
food quality factor	**1.664**	**1.583**	-

7.1.5. Maternal effects

The maternal effect as it is implemented in DEB theory is an elegant concept, which requires few parameters. However, it is computationally intensive because the costs for an egg have to be calculated for each clutch under changing feeding conditions: the costs for an egg are calculated from the maturity at birth and the reserve density the mother has at the moment of egg production (Kooijman, 1986, 2009). Interestingly, in the experiment we designed to investigate the maternal effect in the pond snail (see Chapter 5) we did not find such strong maternal effects as we expected. Throughout all feeding and mating regimes, the egg weight was rather constant. Only in the complete starvation regime, the egg weight decreased noticeably with decreasing reserve density. This finding suggest that we could use constant egg costs for the largest part of this experimental data set.
In contrast, it has been observed (Hoffer et al., 2012) that the snails change their investment per egg when they grow older/larger, and keep the number of eggs rather constant. Other organisms have been observed to produce larger eggs in scarce feeding conditions (e.g., Taborsky, 2006), which would not be captured with the standard DEB model either. In those cases, we might need a more descriptive formulation for the maternal effect, because it might be challenging to find a mechanistic formulation for such patterns. Possible ways of implementing could be descriptive formulations for the costs per egg, or for the maturity at birth E_H^b, or both. Similarly, in experiments with compounds that influence the size of the

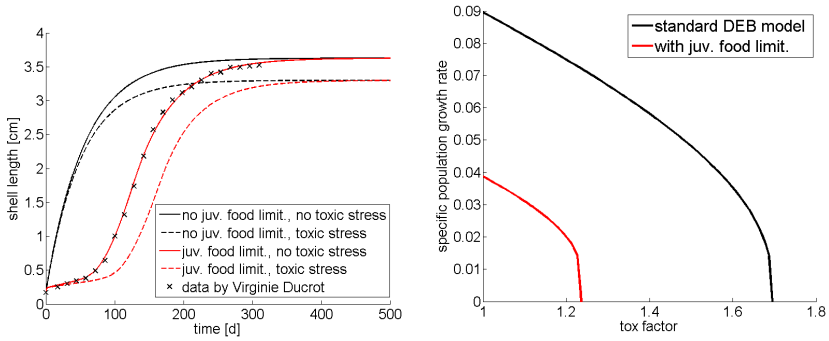

Figure 7.2.: Simulations with the juvenile food limitation and the effects on extrapolation to the population level using the Euler-Lotka equation. The tox factor is applied to the somatic maintenance rate coefficient $\dot{k}_M$, i.e., at tox factor 1.4, $\dot{k}_M$ is 40 % increased.

produced offspring (e.g., Hammers-Wirtz and Ratte, 2000), the maternal effect rule does not seem to apply in strict form any more. In any case, when using the standard DEB model with the maternal effect rule, one should pay special attention to test the predictions of egg size, especially because the investment per egg determines the predictions for the reproduction rate.

However, the maternal effect rule of DEB can easily be replaced with a variety of other rules without affecting the model structure. All those other rules will have at least one or more parameters, so one should consider in an extension whether the number of parameters is matched by a sufficient increase in realism.

7.1.6. Extrapolation to the population level

When studying the interaction of the juvenile food limitation with toxic effects (see Chapter 2), we also investigated the impact on predictions for the population growth rate with the Euler-Lotka equation. We found a relatively stronger effect on population growth rate, and extinction at lower toxic exposure, when the juveniles are food limited compared to the control (see Figure 7.2). This means that if we are unaware of the juvenile food limitation, we will overestimate the effect on the population level. One might argue that it is better to be overprotective than underprotective. However, in my opinion, it is better to be consciously overprotective than accidentally. Moreover, unnoticed juvenile food limitation introduces a bias when comparing toxicity between species and between compounds.

DEB-IBM now offers the possibility to study the population dynamics under dynamic food conditions. However, I am not aware of longterm population data on the pond snail in a form that allows for comparison with the output of an

IBM. Therefore, this study would remain a theoretical exercise like the population growth rate, with only slightly increased ecological realism.

7.2. Factors influencing the outcome of ecotoxicological experiments

Environmental conditions can have a complex influence on the endpoints that are usually studied in ecotoxicological experiments. External factors such as photo period, temperature, water quality and food source influence the condition of the test organisms, which in turn affect the outcome of ecotoxicity tests. We thus need to be aware how the experimental conditions influence our test organisms, to be able to make sense out of our test results.

The pond snail *L. stagnalis* is about to become a standard test organism for OECD guidelines for aquatic invertebrates. My experiment, and most of the experiments I analyzed, have been conducted under conditions that have been similar or identical to a protocol that is currently under investigation for standardization (OECD, 2010). While analyzing these data, I realized that there is room for improvement of the proposed protocol. Although I have focused on investigating the pond snail, I think that my findings are more generally applicable for ecotoxicological experiments.

7.2.1. Feeding

To be able to extrapolate our findings to the environment, it is of major importance to understand how the test organisms experience the laboratory conditions. For many organisms, we are not sure what exactly they feed on in nature, and the established food sources in the laboratory are partly chosen because they can easily be obtained, handled and standardized. Many organisms change their diet during the life-cycle, which might lead to a misinterpretation of the toxicity of the tested compounds if we do not account for this in the analysis (see Chapter 2). Additionally, there might be an interaction between the food source and the toxicant(s), which can lead to complex interactions between direct toxicity and indirect effects due to food limitation, or enhanced digestibility caused by the interaction (see Chapter 3). When designing an ecotoxicological experiment, we should be aware of these factors. Different food sources have different nutritional value for the organisms, so we should be careful when designating a feeding condition as *ad libitum*. This can even hold for the same food source, produced under different circumstances: e.g., the pond snail was fed *ad libitum* with lettuce both in the full life-cycle experiment (FLE) and in the partial life-cycle experiment (PLE) we discussed in Chapter 2. Yet, the final size they reach at the end of the experiment is different (see Figure 2.1). One reason for this could be a different nutritional value of the lettuce in both experiment. This finding questions the use of lettuce for

ecotoxicological experiments even more, in addition to the juvenile food limitation and the interaction potential with herbicides as demonstrated in Chapters 2 and 3.

For the experiments with the pond snail, I think it would be a good idea to test the different food sources over the full life cycle of the snail to assess the impact on growth and reproduction. Subsequently, one could choose one food source or a mixture that provides optimal growth and reproduction during the whole life cycle, and test the effects of compounds at different food levels, to be able to make more realistic extrapolations to effects on populations in the field. If the proposed protocol will be used, where feeding with lettuce is foreseen, I suggest to use a mechanistic effect model such as the DEB models I used in my thesis to be able to account for the food limitation when analyzing the test results. Otherwise, it would perhaps be better not to test juveniles as they will be far more sensitive than adults due to the multiple stress that they experience.

7.2.2. Other environmental factors

Apart from food source and food availability, other environmental factors have an influence on the physiological condition of the test organisms. For the pond snail, light regime has a major impact on its energy allocation to the different major functions such as growth and reproduction (Zonneveld and Kooijman, 1989; Ter Maat et al., 2007). Additionally, the snail is stimulated to lay eggs when the water is changed (clean water stimulus (CWS), see Ter Maat et al., 1989). In other organisms, similar responses might occur. In any case, we should be aware of those influences, to allow realistic extrapolations to the environment. The question is: what are the most informative experimental conditions for ecotoxicity tests?

The pond snail seems to lay smaller eggs in the light regime that we used in our experiments compared to other light regimes (see Chapter 5). We did not observe effects of food level or mating, although under other light conditions, effects of feeding and mating on egg size have been detected (Ter Maat et al., 2007; Hoffer et al., 2012). We assume that we did not see effects on egg size because the snails were already producing the smallest possible eggs under the experimental conditions that we used. Variations in very small eggs are very difficult to detect and might even be absent. In scenarios with shorter days, the observed eggs are much bigger, and effects on egg size are easier to detect. For ecological realism, it would be best to study the effect of a compound at different light regimes. Because this is not practical, I think it would be a good idea to choose conditions where the eggs that are laid are big enough to detect changes in the investment, which might be caused by food limitation or toxicants.

The CWS has also been shown to influence the reproductive output. Although it is more expensive to maintain, I believe that using flow-through systems to do experiments with the pond snail might be more suitable to remove as many environmental disturbances as possible.

7.2.3. The sublethal endpoints growth and reproduction

Multiple effect mechanisms can yield effects on reproduction (see Figure 1.1). Therefore, we need to measure at least growth and reproduction over time to be able to identify the cause for a shift in the energy budget (the metabolic mechanism of action, (mMoA), see Chapter 1, Figure 1.2). For example, an effect on costs for growth affects the shape of the growth curve, but not the final size, whereas an effect on maintenance costs mainly appears late in the life cycle, when the maintenance costs make a larger part of the energy budget (compare Figure 1.3). If we only assess the growth at the end of the experiment, the duration of the experiment determines whether we can identify the mMoA or not. In combination with other endpoints such as reproduction or respiration, the mMoA can be detected with much more certainty. The choice of the mMoA has a crucial impact on population structure and dynamics (Martin et al., 2013). Therefore, measuring growth and reproduction on several time points during an experiment strongly increases the informative value of an experiment.

Growth can be assessed as body length (shell length for the snail) or weight. When measuring weight, we can decide to measure wet or dry weight. Although dry weight gives more information, wet weight is the only option if we follow individuals over time. Thereby, we need to pay attention to factors that influence the weight. For example, if the reproduction buffer makes a large fraction of the body weight, we should consider the buffer in the model, or e.g. the shell for the pond snail (see Section 5.3).

Reproduction is usually assessed as number of offspring produced per female. However, the size of an egg can be very variable in some organisms. If we do not measure the dry weight per egg, which likely corresponds to the energy investment of the parents, we miss effects on reproduction that cause the production of smaller or larger offspring. For example, in Chapter 3, we did not observe an effect on reproduction. However, only the number of clutches were determined, so we do not know whether there might have been an effect on numbers or size of eggs, which is of substantial importance for the fitness of the offspring, and for the quantification of the investment in reproduction. To detect effects on maturation we need to start the experiment before the organisms reach sexual maturity, and then follow reproduction for some time.

If the offspring are to be followed, or other reasons make it impossible to determine dry weight of offspring, the size at birth and incubation time already provide enough information to calculate egg weight in a DEB context.

7.3. The perfect ecotoxicological experiment with the pond snail

If I were to decide on the perfect test protocol for the pond snail, without the chance to do further experiments before, I would propose the following:

Initial conditions: The snails should be chosen at a size at which they are not food limited by lettuce any more, to avoid interaction with the juvenile food limitation ($L > 0.9$ cm, see Chapter 2). They should not have reached sexual maturity yet, to be able to detect effects on maturation ($L < 1.4$ cm, see Chapter 3). Before starting the experiment, they should be fed *ad libitum* for a week, to be sure of the current nutritional status of the snail (see Chapter 5, Figure 5.5 for reserve dynamics).

Feeding conditions: The snails should be fed *ad libitum* with lettuce, meaning that lettuce should be present in substantial amounts at all times. A possible interaction of the toxicant with lettuce should be tested before the experiment (see Chapter 3). The feeding rate should be measured, so that potential effects on feeding rate can be detected.

Mating conditions: The snails should be tested individually to exclude potential interaction of the toxic effect with the sexual conflict (see Chapter 5), and to allow modeling individuals (needed when there is a lot of variation between individuals). To avoid the potential negative effects of selfing, one might consider to let them mate once after they reach the size at sexual maturation.

Light conditions and temperature: The water temperature should be constant, and in an environmentally relevant range. To be able to detect effects on egg size, if present, tests should be conducted in a light regime of 12 hours day light, since the snails seem to lay the largest eggs when kept in that photoperiod (see Chapter 5). For comparable results, the light regime should be the same in all tests.

Endpoints: Growth should both be measured as shell length and wet weight during the experiment. Preferably, the dry weight should be measured after the experiment, and a reference dry weight should be measured before the experiment (see Chapter 5). Reproductive output should be measured as total investment, i.e. dry weight of clutches, and the numbers of eggs should be counted. This way, potential effects on egg size can be detected. Additionally, hatching time should be monitored, to be able to detect effects on development.

Test duration: The test should be conducted for several weeks after the snails start reproducing. In my experiments, the effects of the mating regime only started showing after more than four weeks (see Chapter 5). Therefore, I suggest to test at least 4 weeks after the start of reproduction.

Ecological relevance: For ecological relevant testing, all factors that have an influence on the physiological performance of the snail should be tested in interaction. Optimally, experiments should be conducted at different light and temperature conditions, different mating regimes as well as different levels of food availability. Since

this is not feasible, mechanistic effect models should be used to study the possible interactions of effects with these environmentally relevant factors. All these interactions (hopefully) only need to be determined once. If it can be shown that we can capture these interactions for one (or a few compounds) with a mechanistic effect model such as DEB, we can have more confidence to make predictions for interactions with an untested toxicant.

7.4. Outlook / Perspective

There are several questions that remain unsolved after finishing my thesis. In the following, I will list the ones that I consider to be the most burning issues.

7.4.1. How does metabolic acceleration influence the rest of the life cycle?

From the theory behind the concept of metabolic acceleration, the food history before metamorphosis determines the adult parameters. Since the factor L_j/L_b determines the adult parameters $\{\dot{p}_{Am}\}$ and $\dot{v}$, changing feeding conditions can create large differences between adult parameters in organisms that do not have a constant size at puberty ($\dot{k}_J \neq \dot{k}_M$). If this is true, the acceleration can cause large differences between individuals of the same species. Moreover, it should cause complex interactions at the population level: when the mother experiences good feeding conditions, she lays eggs with plenty of reserves, which lead to a high length at birth L_b. When the offspring then encounters worse feeding conditions, it reaches maturity at metamorphosis at a smaller size L_j than the mother did. The factor L_j/L_b thus should vary a lot in a dynamic food environment. To demonstrate this, I calculated L_b and L_j for situations where the mother had different (constant) food conditions than the offspring (see Figure 7.3). This could be a potential explanation for the variability between individuals of the same species in nature. To study this in the pond snail, a first step would be to make life-cycle experiments where the parents are kept at a high food level, and their offspring are kept at low food level and vice versa, and compare the maximum sizes. Additionally, we could investigate the gut fauna, to further test the hypothesis of acceleration due to the development of the gut fauna.

7.4.2. How important are the proposed changes of κ with light regime for realistic extrapolation of effects?

From the comparison of my own data on egg weight in the pond snail with data that has been obtained by Hoffer et al. (2012), the suggestion of Zonneveld and Kooijman (1989) of plasticity in κ with light regime was confirmed. Indeed, using our DEB model, we can reach predictions for egg sizes in the range of the ones

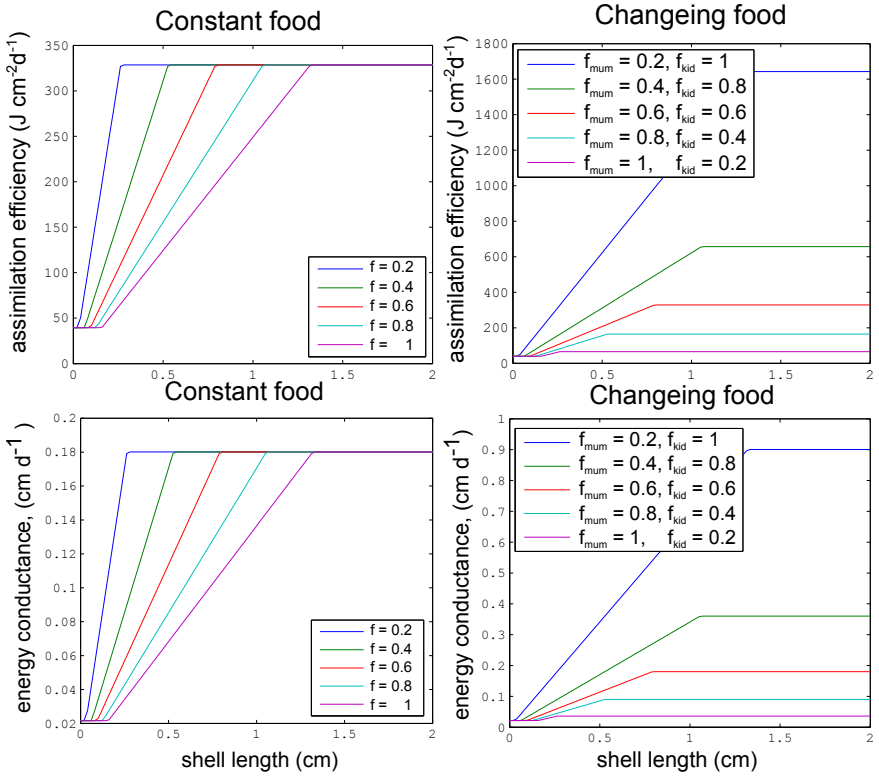

Figure 7.3.: The fraction $\mathcal{M}(L) = \frac{L_j}{L_b}$ determines the adult parameter values for the surface-area specific maximum assimilation rate $\{\dot{p}_{Am}\}$ and the energy conductance $\dot{v}$ in the metabolic acceleration scenario. $\mathcal{M}(L)$ does not differ between two generations that experience the same constant food level (left panels). However, it can be very different when parent and offspring do not experience the same food level (right panels). In the legend, the parameter values chosen for the parent (f_{mum}, determines L_b) and for the offspring (f_{kid}, determines L_j) are shown. In some of these scenarios, the differences between food level of mother and offspring are extreme. Note the different scaling of the y-axes.

Hoffer et al. (2012) report, when changing κ to higher values (see Figure C.3, Appendix C). A change in κ would change the fraction of energy invested in maturity, and thus the time the offspring needs to reach maturity at birth. It would be interesting to investigate this further, e.g. study growth and reproduction (total investment) at different light regimes for the full life cycle, and also include studies on toxic effects. Because κ changes the energy allocation, effects of toxicants should be influenced as well. In a DEB model, effects of toxicants are interpreted as a change of the parameters that determine the energy fluxes for the different processes. For example, a toxicant that has an effect on somatic maintenance should be less toxic if κ is higher, because with a higher κ, more energy is available for somatic maintenance. Further, it would be interesting to see how population-level predictions with DEB-IBM differ in terms of e.g. population structure when the changes in κ are accounted for or not. However, with a varying κ, the maturity density (maturity per structure) cannot be constant any longer, which is one of the simplifying assumptions in the simplified DEB model. In such applications, one would have to use the full DEB model.

7.4.3. What is the best food source for the pond snail in a standard test?

Although we have shown that lettuce is not the most appropriate food source for ecotoxicity testing with *L. stagnalis*, the question remains which food source is better suitable. Different potential food sources would be Tetraphyl fish flakes (see Chapter 2), algae (e.g., *Spirulina sp.,* Lalah et al., 2007), macrophytes (e.g., Elger and Lemoine, 2005), or even sweet potatoes (e.g., Munley et al., 2013). These food sources should be tested alone or in combination in respect to life-cycle consequences, and their interaction potential with toxicants should be determined.

7.5. Final remarks

I will try to summarize the essence of what I learned during my PhD in a few final sentences.

While working with the different models, I learned that although deviations of the model predictions from the data can be frustrating, those deviations usually lead to the most interesting questions and hypotheses.

While working with DEB, I realized how important it is to look at things in a holistic manner, to be able to understand some of the complexity. I think that a more holistic approach to research in ecotoxicology can improve our understanding of test results, even without using a model such as DEB.

Often, the reanalysis of data with a different question in mind can lead to new

insights.

No model is better than another in every context. It always depends on the research question which model is more useful.

A. Supplement for Chapter 2

This Appendix has been published as Online Resource to Chapter 2.

A.1. Dynamic Energy Budget (DEB) theory

A.1.1. Concepts and ideas

Dynamic Energy Budget (DEB) theory provides a set of rules that capture how much energy organisms assimilate from food, and how this energy is allocated to growth, development, reproduction and maintenance. It was originally developed with the aim to understand how organisms change the allocation of energy in response to a toxicant (Kooijman and Metz, 1984): when a daphnid eats the same amount of algae, but produces less offspring, the energy has to be invested for something else. Following the idea that the energy metabolism is organized very similarly among organisms, DEB can in general be applied to all organisms (Kooijman, 2001; Nisbet et al., 2000). The general DEB animal undergoes three life stages: embryo, juvenile and adult. During each stage, organisms basically follow the same rules for energy allocation, but the switch between life stages indicates a switch in metabolic behavior. The embryo is defined as an organism that does not feed and only lives from the reserves that were handed over from the mother. Once it starts feeding (first switch), it is considered a juvenile. When it reaches maturity and starts reproducing (second switch), it is considered an adult until it dies. However, for some organisms, additional life-stages have been included, and for others only two of the three stages are needed (Kooijman, 2010). Models for other organisms than animals, such as plants and bacteria, have been developed as well. Here, we only consider the standard animal model because it has been applied in the present work.

A.1.2. The model

In DEB, the biomass of an organism is composed of structure and reserve, which are two of the three state variables in the standard DEB model. Structure consists of all parts of the organism that require maintenance (e.g. cell membranes). Reserve captures all parts of the organism that fuel other metabolic processes, but do not require maintenance. The third state variable is the most abstract one: maturity. Maturation relates to an increase in complexity of the organism, and determines the switches between life stages in DEB. A certain amount of energy needs to be invested into maturation before the embryo can become a juvenile, i.e., start

feeding, and before the juvenile can become an adult, i.e., start reproducing. All energy fluxes are fully determined by 12 primary parameters, which translate to measurable quantities via compound parameters (see e.g., Kooijman et al., 2008). Here, we are focusing on the energy fluxes from food into reserves and from reserves into growth and maintenance in juveniles. Therefore, maturity and reproduction do not play a role in the present study.

Structure is described in terms of structural body volume V (cm^3). The cubic root of the structural volume is the structural length L (cm). In the paper, we assume that shell length is proportional to structural length. Reserves are followed in terms of energy E (J). Before we dive into the details of the energy fluxes, we should specify a few more DEB parameters that we did not mention in the article, where we only mention g, the energy investment ratio $(-)$, $\dot{v}$, the energy conductance ($\mathrm{cm\,d}^{-1}$), $\dot{k}_M$, the somatic maintenance rate coefficient (d^{-1}), and the scaled functional response f $(-)$. The scaled functional response f is the actual ingestion rate of an animal divided by the the maximum ingestion rate for its size. For an individual under *ad libitum* feeding conditions, $f = 1$, whereas for a starving individual, $f = 0$, so that for limiting conditions $0 < f < 1$.

The parameters $\dot{k}_M$ and g are compound parameters in DEB, i.e. parameters that can be expressed in terms of primary parameters:

$$\dot{k}_M = \frac{[\dot{p}_M]}{[E_G]} \quad \text{and} \quad g = \frac{[E_G]\dot{v}}{\kappa\,\{\dot{p}_{Am}\}} \tag{A.1}$$

In DEB, the notation already indicates in what unit parameters are defined. For example, parameters that are defined per unit of structure are always written between square brackets (see Table A.1). Here, some primary parameters appear, such as $[E_G]$, the costs for one unit of structure, $[\dot{p}_M]$, the maintenance that needs to be paid for one unit of structure, and $\{\dot{p}_{Am}\}$, the maximum surface area specific assimilation rate. The parameter $\{\dot{p}_{Am}\}$ is a species specific parameter that determines the maximum rate at which the energy that is extracted from food can be assimilated into reserves (more information about general DEB notation can be found under
http://www.bio.vu.nl/thb/research/bib/Kooy2010_n.pdf). The parameter κ, $(-)$, determines the fraction of the mobilized energy that is allocated to the soma (i.e. somatic maintenance and new structure). The energy fluxes in DEB are always noted as $\dot{p}_i$, where the i stands for the different fluxes.

Let us follow the energy that is taken up from food, and see how much of it is converted into new structure. The energy that is assimilated from food ($\dot{p}_A$) is defined as follows:

$$\dot{p}_A = fL^2\{\dot{p}_{Am}\}. \tag{A.2}$$

Assimilation is assumed to be proportional to a square of the structural length L, based on a simple physical principle: mass transport from one environment to the another, as for example to an organism, must be across a surface (for illustration see Kooijman, 2010). The assimilated energy is first fixed into reserves before it is

Table A.1.: DEB model parameters used in this article with their symbols, interpretation and units.

Symbol	interpretation	units
state variables		
E	reserve	J
L	(stuctural) body length	cm
primary parameters		
$[E_G]$	volume-specific costs for structure	$\mathrm{J\,cm^{-3}}$
$[\dot{p}_M]$	volume-specific somatic maintenance	$\mathrm{J\,cm^{-3}\,d^{-1}}$
$\{\dot{p}_{Am}\}$	surface-specific maximum assimilation	$\mathrm{J\,cm^{-2}\,d^{-1}}$
$\dot{v}$	energy conductance	$\mathrm{cm\,d^{-1}}$
κ	fraction of mobilized reserves allocated to the soma	–
s	stress factor (0 in control)	–
compound parameters		
f	scaled functional response ($0 < f < 1$)	–
g	energy investment ratio	–
k_M	somatic maintenance rate coefficient	$\mathrm{d^{-1}}$
powers / energy fluxes		
$\dot{p}_A$	assimilation power	$\mathrm{J\,d^{-1}}$
$\dot{p}_C$	mobilization power	$\mathrm{J\,d^{-1}}$
$\dot{p}_M$	somatic maintenance power	$\mathrm{J\,d^{-1}}$

mobilized. The main reason for this assumption lies in the observation that organisms can survive in times of starvation, and even continue growing and reproducing for a while. The mobilization flux $\dot{p}_C$ is defined as:

$$\dot{p}_C = E\left(\frac{\dot{v}}{L} - \dot{r}\right) \quad \text{with} \quad \dot{r} = \frac{1}{L^3}\frac{d}{dt}L^3. \tag{A.3}$$

Mobilization is proportional to the amount of available reserve E. The possibilities for the reserve mobilization are severely restricted by the weak homeostasis assumption, which implies that the reserve density (i.e. reserve E per unit of structural volume) at constant food does not change, and thus cannot depend on body size. The relative growth rate $\dot{r}$ is included in the expression for reserve mobilization to counteract dilution by growth to allow for weak homeostasis. Kooijman (2010) argues that Eq. A.3 is the only formulation that satisfies all requirements.
The dynamics of the reserves are given as:

$$\frac{d}{dt}E = \begin{cases} -\dot{p}_C & \text{for embryos} \\ \dot{p}_A - \dot{p}_C & \text{otherwise} \end{cases} \quad \text{with } E(0) = E_0 \tag{A.4}$$

A useful quantifier for reserve is the scaled reserve density e, which is the relative amount of reserve an organism has compared to its highest possible reserve density. For a well fed organism, $e = 1$.

$$\frac{de}{dt} = (f - e)\frac{\dot{v}}{L} \tag{A.5}$$

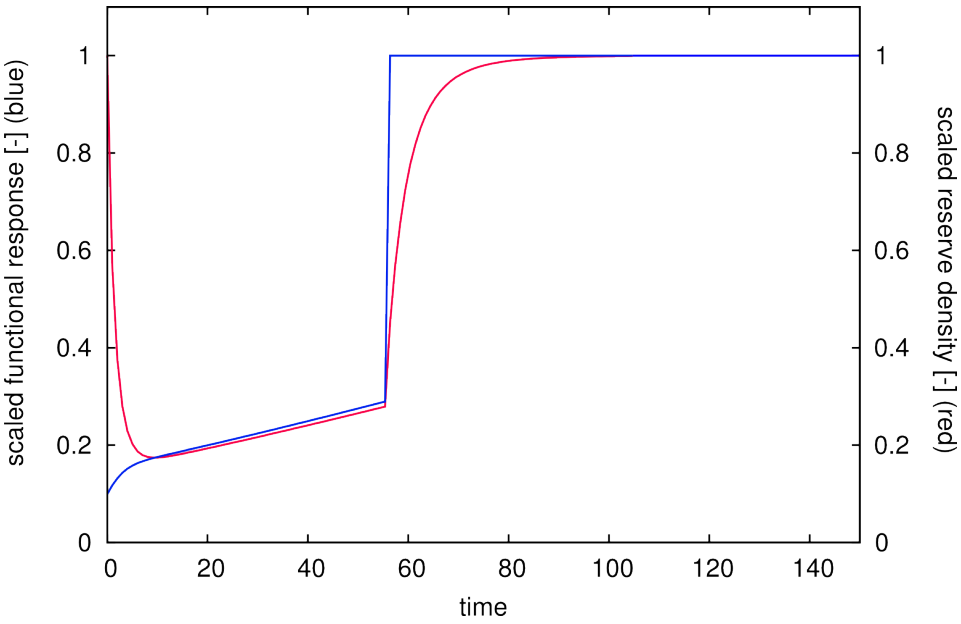

Figure A.1.: When reserves are included dynamically, the scaled reserve density e (red) follows the scaled functional response f (blue) very fast.

Under constant food conditions, $f = e$, and the Von Bertalanffy growth pattern is followed. In that case, we do not need to consider reserve dynamics explicitly. Since the energy conductance is rather high compared to the standard value ($\dot{v}_{std} = 0.02\,\mathrm{cm\,d^{-1}}$), we can consider the reserve dynamics to be fast. Indeed, when considering reserve dynamics with this particular parameter combination, e follows f rapidly (see Fig. A.1).
A fixed fraction κ is of the mobilized energy is invested into the soma, and the rest $(1 - \kappa)$ is invested into maturation or reproduction. The energy available for the soma is used for somatic maintenance $\dot{p}_M$, and the rest is used to build up new structure (growth). Somatic maintenance is proportional to the amount of structural volume:

$$\dot{p}_M = [\dot{p}_M] L^3 \tag{A.6}$$

The energy available for growth is thus the energy available for the soma $\kappa \dot{p_C}$ minus the energy needed for somatic maintenance $\dot{p}_M$:

$$\dot{p}_G = \kappa \dot{p}_C - \dot{p}_M \tag{A.7}$$

The new structure that is build is the available energy divided by the costs for one unit of structure $[E_G]$:

$$\frac{d}{dt} L^3 = \frac{1}{[E_G]} \left(\kappa \dot{p}_C - \dot{p}_M\right) \quad \text{with } L(0) \approx 0 \tag{A.8}$$

Table A.2.: The three metabolic Mechanisms of Action (mMoAs) and their translation from effects on primary parameters into effects on compound parameters.

mMoA	primary parameter	compound parameter
(1) increase of maintenance costs	$\uparrow [\dot{p}_M]$	$\uparrow \dot{k}_M$
(2) decrease of assimilation	$\downarrow \{\dot{p}_{Am}\}$	$\downarrow f$
(3) increase of costs for growth	$\uparrow [E_G]$	$\downarrow \dot{k}_M, \uparrow g$

To explain how we get from this equation to the Von Bertalanffy growth is beyond the scope of this paper. It can be found in Jager and Zimmer (2012).
The energy that is not invested into the soma is used for maturation and reproduction (for more detail, see Kooijman et al., 2008).

A.1.3. Inclusion of toxic effects

In the article, we analyze three different metabolic mechanisms of action (mMoA). We are using compound parameters in the article, but the toxic effects are actually effects on primary parameters. Thus, we explain in the following how the effects of primary parameters translate to the compound parameters.

An effect on a parameter p can be expressed using the stress factor s . If the stress decreases the parameter, $p = p_0(1 - s)$, and if the stress increases the parameter, $p = p_0(1 + s)$, where p_0 is the parameter without stress (i.e., in the organisms in the control).
Using Eq. A.1, we can now see how the above mentioned mMoAs are placed in the DEB model (see Table A.2): effect (1) is actually an increase of the somatic maintenance costs per unit of structure $[\dot{p}_M]$, effect (2) is a decrease of the maximum surface area specific assimilation efficiency $\{\dot{p}_{Am}\}$, and effect (3) is an increase of the volume specific costs for structure $[E_G]$. From Eq. A.2 we can see that f enters the assimilation flux in the exact same manner as $\{\dot{p}_{Am}\}$, so that an effect on both would result in the same effect pattern. Thus, it does not make a difference for the individual whether the effect is on $\{\dot{p}_{Am}\}$ or f. From ecotoxicological data, it is generally not possible to distinguish them.

A.1.4. Temperature dependence

In DEB, the temperature dependence of rate constants can be accounted for by multiplying them with a temperature correction factor c_T that can be derived from the Arrhenius relation (see Freitas et al., 2007). The temperature correction is given by

$$c_T = \exp\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right). \tag{A.9}$$

All temperatures are given in Kelvin. In Eq. A.9, T_{ref} is the temperature for which we know the parameter value, T is the temperature for the new situation, and T_A is the Arrhenius temperature, which can be estimated if sufficient data is available. Since both the FLE and the PLE have been conducted at $21 \pm 1°C$, we set $T_{ref} = 273 + 21$. The JFE has been conducted at $23.5 \pm 1°C$, so we set $T = 273 + 23.5$. For the Arrhenius temperature T_A, we use a typical value of $T_A = 11900$ (Kooijman, 2010). Typical data that would be suitable to estimate T_A would be respiration rates, ingestion rates or development times at different temperatures.

A.2. Experimental part

A.2.1. Life-cycle of *Lymnaea stagnalis*

Lymnaea stagnalis is a large freshwater snail with a maximum observed shell length of up to 6 cm (OECD, 2010) and a maximum observed age in the laboratory of 700 days (Slob and Janse, 1988). The hatching time highly depends on temperature, and ranges from 10 days (Horstmann, 1958) up to 27 days (pers. observation, FLE). It reaches maturity around 2-2.2 cm, with age at maturity depending on food conditions (Janse et al. (1994), pers. observation).

A.2.2. Rearing conditions at the snail culture

The culture of *Lymnaea stagnalis* is kept at a constant water temperature of 20±1 °C and a constant light/dark period of 14/10 hours with a light intensity of 250-500 lux (natural daylight spectrum). We use dechlorinated charcoal filtered tap water, with the following properties: pH comprised between 7 and 8, oxygen concentration: > 6 mg/l at 20 °C, conductivity between 500 and 700 μS/cm. One third of the water volume is renewed weekly. Snails are fed three times a week with lettuce (organic quality, as certified by the european label ECOCERT, and washed in culture water). The amount of food delivered depends on the development stage, e.g. 160 g of fresh food/ 100 ind./week in adults. The maximum number of snails is 120 adults per 35 l.

A.2.3. Experiments

The full life-cycle experiment (FLE)

We use the growth data of a life-cycle experiment that has been conducted to investigate effects of diquat on the pond snail *Lymnaea stagnalis*. A detailed description of the experimental setup and part of the data has been published in Ducrot et al. (2010a).

The whole experiment was conducted under a photoperiod of 14/10 L/D using charcoal filtered tap water at $21 \pm 1°C$. Freshly laid clutches (less than 24 h, laid

on the 01/08/2007) were individually placed in plastic six-well plates (V = 10 ml). Hatching times were variable, but only newborns that hatched after 28 days were used for the life-cycle experiment. They were transferred to 100 ml Petri dishes (five snails per dish), where they were fed immediately. The snails were fed daily with weighted slices of organic lettuce, but only when no leftover from the day before remained, so that the risk of an effect on water quality due to disintegration of the lettuce was minimized. The amount of lettuce that was provided was gradually increased using the following rule: when the quantity given the day before was consumed in half of the replicates, the amount of lettuce given was doubled in terms of surface area. We started with one slice of 21 mm Ø in small juveniles. Then we doubled the Ø of the slices (from 21 to 43 mm). This resulted in the doubling of the leaf surface, and a corresponding increase by a factor of ca. 2.3 in weight. When lettuce was consumed in half of the replicates, we doubled the number of 43 mm slices that were provided and increased it to 2, then 4, and then 8 slices of 43 mm on day 168, which was the end of the contamination period in the regimes that were exposed to diquat. From that day on, the amount of lettuce given was not assessed anymore, but lettuce was provided *ad libitum*.

Water was regularly analyzed to determine physico-chemical characteristics regarding O_2, NH_4^+, NO_2^- and HPO_4^{2-} contents. Oxygen supply was not mandatory because the snail is a pulmonate. O_2 content in the water varied between 37.2% and 48.7% of the saturation value. In these conditions of water aeration, without any food leftover accumulating in the test vessels, weekly water renewals and the regular removal of feces with a pipette sufficed to maintain nitrogen products at values that are sufficiently low not to hurt the snails: e.g. NO_3^- varied in the range 7-15 mg/l and NH_4^+ varied in the range 0.02-0.5 mg/l.

The water volume was doubled each time when the amount of food was doubled. Water was renewed weekly during the whole experimental period. The volume of water that was renewed increased from 10% per week to 100% per week, depending on the amount of food that was consumed by the snails (the more they eat, the more feces they produce that needs to be removed). The actual volumes that were renewed as a function of age are indicated in Fig. A.2.

To keep the number of snails per replicate constant, dead snails were removed daily from the vessels and replaced by siblings of similar age (±3 days) and size (±0.5 mm), which had been collected from the same clutches, and subsequently batch-reared under the same feeding conditions as the snails used in the life-cycle experiment. We kept batches of replacement snails for every tested concentration and controls. We stopped replacing snails when there were no replacement snails left that had the same size as the dead snail we wanted to replace (day 239). Shell length was measured every other week using a digital caliper (see Table A.3). The experiment was stopped after 336 days, when survival fell below 75% (i.e., upper limit of the survival threshold for validity of toxicity test data, see American Society for Testing of Materials, 2000).

Figure A.2.: A schematic representation of the experimental setup of the FLE.

Table A.3.: Mean shell size (mm), standard deviation (st. dev.) and numbers of individuals (# of ind.) of the FLE and the PLE. Note that day zero is defined as the day of egg-laying, and not as hatching.

	FLE			PLE *ad libitum*			PLE 50%			PLE 25%		
age	mean size [mm]	st. dev. [mm]	# of ind.	mean size [mm]	st. dev. [mm]	# of ind.	mean size [mm]	st. dev. [mm]	# of ind.	mean size [mm]	st. dev. [mm]	# of ind.
27	1.65	0.16	72	-	-	-	-	-	-	-	-	-
44	2.42	0.24	120	-	-	-	-	-	-	-	-	-
58	2.84	0.42	120	-	-	-	-	-	-	-	-	-
71	3.29	0.47	120	-	-	-	-	-	-	-	-	-
85	3.69	0.52	120	-	-	-	-	-	-	-	-	-
99	4.66	0.90	120	-	-	-	-	-	-	-	-	-
113	6.10	1.25	120	12.92	1.35	30	12.80	1.13	30	12.62	1.31	30
127	8.94	2.48	120	18.65	1.68	30	16.72	1.45	30	14.48	1.63	29
141	11.64	3.23	120	21.55	2.56	29	19.39	1.93	30	17.02	1.96	29
155	15.53	4.16	120	23.76	2.80	28	21.36	2.12	29	19.25	1.74	28
169	20.50	4.21	120	25.67	3.61	28	23.37	2.34	29	21.10	1.81	28
183	24.64	3.08	120	27.15	3.58	26	24.55	2.44	29	22.23	1.91	28
197	27.74	2.34	120	27.95	3.79	26	25.51	2.51	28	22.95	2.01	28
211	29.78	1.65	120	28.19	3.80	26	25.93	2.52	28	23.24	2.11	28
225	30.98	1.56	120	28.27	4.09	25	26.32	2.68	28	23.71	2.05	26
239	31.92	1.60	117	-	-	-	-	-	-	-	-	-
241	-	-	-	29.24	3.05	23	26.61	2.73	28	24.06	2.14	26
253	33.17	1.74	117	29.53	2.97	21	26.82	2.77	28	24.15	2.20	26
267	34.27	1.92	116	-	-	-	-	-	-	-	-	-
281	34.59	2.07	114	-	-	-	-	-	-	-	-	-
295	35.23	2.16	114	-	-	-	-	-	-	-	-	-
309	35.31	2.22	114	-	-	-	-	-	-	-	-	-
323	35.49	2.22	113	-	-	-	-	-	-	-	-	-
337	35.63	2.15	110	-	-	-	-	-	-	-	-	-

The partial life-cycle experiment (PLE)

The partial life-cycle experiment has been conducted to assess the effect of different food levels on growth and reproduction of the pond snail *L. stagnalis*. They were conducted under a similar protocol as was used for the FLE. The differences lie in the initial conditions, the duration of the experiment, the strategy of providing food, and the snail density per volume. The PLE was started with juveniles of homogeneous age (113 d) and similar size (12.7 ± 1.3 mm) that had been reared under culture conditions. The clutches were laid on the 15/09/2008, and the experiment started on the 06/01/2009. The juveniles were placed in 500 ml test vessels per groups of 5 (6 replicates per feeding regime). We tested four different food levels. The highest level was fed *ad libitum*, the second 50% of this quantity, the third 25 %, and the fourth was not fed. The initial value for the regimes with the highest food level was based on the results of the FLE: the snails received the same amount of lettuce as was given to the snails of a similar size in the FLE (80 g/ind. lettuce fresh weight). The amount of lettuce in this experiment was determined on a weight basis in contrast to slices (i.e. surface area) in the FLE. Each day, an *ad libitum* quantity of lettuce was weighted and given to the snails, and leftovers were weighted on the next day. The food for the regime with the second food level was determined as 50 % of the *ad libitum* value from the day before, and the third food level as 25 % respectively. In this experiment, dead snails were not replaced, so that the snail density per water volume slightly decreased over time. Shell length and wet weight were measured every other week, over 184 days. The complete water volume was renewed once a week.

The juvenile feeding experiment (JFE)

A detailed description of the juvenile feeding experiment can be found in the main text. The clutches were laid on the 22/06/2010. Details concerning the amount of food given in each feeding regime can be found in Table A.4. The size at corresponding age is listed in Table A.5.

A.3. Analysis of the error structure

The error structure was different in the three experiments (see Fig. A.3), so the error structure of the model used to describe the data was adapted accordingly. The variance increased with shell length in both the PLE and the JFE (i.e. large variance for large shell lengths). Interestingly, the variance of the FLE does not show this pattern: it is small for small shell length and small for large shell length, and has its peak around a shell length of $1.5 - 2$ cm (see Fig. A.3). One possible reason for this variation might be a difference in the way individual snails are limited by food: since the individuals are competing for food, a small difference in food generates a subsequent large difference in growth during the fast growth phase.

Table A.4.: The number of slices per replicate (i.e. 5 snails) and mg fish flakes per snail for the five experimental groups. Lettuce 1: without sand, Lettuce 2: with sand, Tetra 1: highest level of fish flakes, Tetra 2: mean level, Tetra 3: lowest level of fish flakes.

day	Lettuce 1 [# slices/rep.]	Lettuce 2 [# slices/rep.]	Tetra 1 [mg/ind.]	Tetra 2 [mg/ind.]	Tetra 3 [mg/ind.]
0	1	1	1.8	0.9	0.45
3	0	0	0	0	0.3
5	0	0	0	0.6	0.3
7	1	1	1.8	0.9	0.45
10	1	1	1.2	0.6	0.3
12	0	0	2.4	1.2	0.6
13	1	1	0	0	0
14	1	1	3.6	1.8	0.9
17	1	1	4.8	2.4	1.2
19	1	1	4.8	2.4	1.2
20	2	2	7.2	3.6	1.8
23	2	2	9.6	4.8	2.4
24	0	0	9.6	4.8	2.4
25	2	2	19.2	9.6	4.8

Table A.5.: Growth data and standard deviation of the juvenile feeding experiment (JFE). Lettuce 1: fed with lettuce without sand, Lettuce 2: fed with lettuce with sand, Tetra max: highest level of fish flakes, Tetra mid: middle level of fish flakes, Tetra min: lowest level of fish flakes.

	Lettuce 1		Lettuce 2		Tetra max		Tetra mid		Tetra min	
age	shell length	st. dev.	shell length	st. dev.	shell length	st. dev.	shell length	st. dev.	shell length	st. dev.
17	1.42	0.09	1.42	0.10	1.43	0.09	1.46	0.10	1.44	0.10
24	2.71	0.38	2.70	0.27	3.52	0.66	3.43	0.64	3.35	0.46
31	3.81	0.61	3.81	0.35	7.23	1.09	5.51	0.78	4.48	0.59
38	5.80	0.68	5.77	0.86	10.19	1.42	8.81	0.93	6.74	0.79
45	7.79	0.88	7.62	1.20	13.16	2.04	10.85	1.37	9.14	1.13

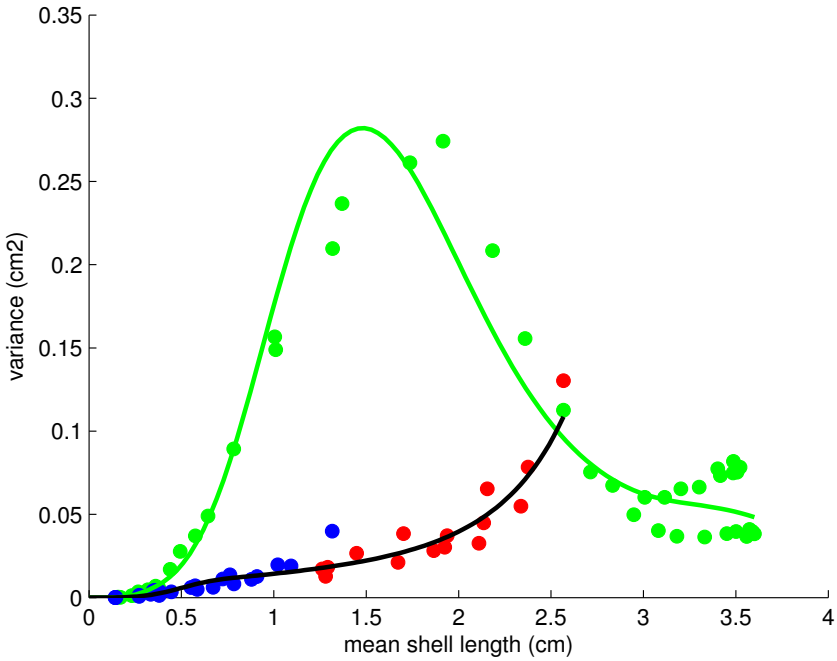

Figure A.3.: The mean variance over shell length of all experiments. Green: FLE, red: the PLE, blue: the JFE.

B. Supplement for Chapter 4

B.1. Assumptions that specify the standard DEB model

Below, I summarize the assumptions that specify the standard DEB model (adapted from Kooijman, 2010, Table 2.4)

- **State variables**: the state varaibles of the individual are the structural volume, V, reserve energy, E and maturity, expressed in terms of cumulative energy investment into maturation, E_H; reserve and structure have a constant composition (strong homeostasis) and maturity represents information
- **Maturity:** Two switches in the energy allocation occur that are related to maturity: if the maturity threshold for birth is reached, substrate (food) uptake is initiated; if the maturity threshold for puberty is reached, the allocation to maturation is redirected to reproduction
- **Feeding rate:** the feeding rate is proportional to the surface area of the individual, and the food handling time is independent of food density
- **Assimilation:** the energy assimilated from food is first converted to reserve, before it can be used; the mobilisation rate depends on the state variables
- **Embryonic development:** initially an embryo has a substantial amount of reserve, and negligible amounts of structure and maturity
- **Reserve density:** the reserve density (i.e. current amount of reserve per structure divided by maximum possible amount of reserve per structure) at constant food density does not depend on the amount of structure (weak homeostasis)
- **Maternal effect:** the reserve density at birth equals the reserve density of the mother at the moment of egg formation
- **Somatic maintenance:** the somatic maintenance is proportional to body structure (i.e. reserve does not require maintenance), but some components (e.g. osmosis in aquatic organisms, heating in endotherms) are proportional to surface area
- **Maturity maintenance:** maturity maintenance is proportional to the level of maturity

- **The κ - rule:** a fixed fraction of the mobilised reserve is allocated to the soma (i.e. somatic maintenance + growth), and the rest is allocated to maturity maintenance and maturation / reproduction
- **Maintenance:** maintenance always has to be paid first
- **Isomorphism:** the individual does not change in shape during growth (standard DEB model only!)

Table B.1.: The 12 individual specific parameters of the standard DEB model.

Parameter	dimension	description
$\dot{F}_m$	$l^3L^{-2}t^{-1}$	specific searching rate
κ_X	-	assimilation efficiency
$\dot{p}_{Am}$	$Jt^{-1}m^{-2}$	maximum specific assimilation rate
$\dot{v}$	$Lt-1$	energy conductance
κ	-	allocation fraction to soma
κ_R	-	reproduction efficiency
$[\dot{p}_M]$	$Jd^{-1}m^{-3}$	volume-specific somatic maintenance costs
$\dot{p}_T$	$Jt^{-1}m^{-2}$	surface-area specific somatic maintenance costs
$\dot{k}_J$	d^{-1}	maturity maintenance rate coefficient
$[E_G]$	Jm^{-3}	specific costs for structure
E_H^b	J	maturity at birth
E_H^b	J	maturity at puberty

B.2. The basic fluxes

In DEB theory, all energy and mass fluxes are a weighted sums of the three basic basic powers assimilation, $\dot{p}_A$, growth $\dot{p}_G$ and dissipation $\dot{p}_D$, which are specified as follows:

$$\dot{p}_A = f\{\dot{p}_{Am}\}L^2 \tag{B.1}$$

$$\dot{p}_G = \kappa\dot{p}_C - [\dot{p}_M]L^3 \tag{B.2}$$

$$\dot{p}_D = \dot{p}_M + \dot{p}_J + \dot{p}_H \tag{B.3}$$

The dissipation power has contributions from somatic maintenance $\dot{p}_M$, maturity maintenance $\dot{p}_J$, and maturation $\dot{p}_H$. After puberty, $\dot{p}_H$ is replaced by overhead costs for reproduction $(1-\kappa_R)\dot{p}_R$.
The mineral fluxes follow from the organic fluxes, and the organic fluxes follow from the assumptions underlying DEB theory. Because the elements C, H, O and N comprise more than 95% of the total dry weight of most organisms, DEB theory focuses on those four elements only. However, the inclusion of more elements is straightforward and might be necessary for some organisms.

In DEB theory, reserve and structure are seen as generalized compounds: they consist of different mixtures of C, H, O and N that do not change in composition (strong homeostasis). A 'molecule' of structural biomass can be described by $CH_{n_{HV}}O_{n_{OV}}N_{n_{NV}}$ and a 'molecule' of energy reserve by $CH_{n_{HE}}O_{n_{OE}}N_{n_{NE}}$. The indices n_{*V} and n_{*E} denote the relative abundance of an element in dry biomass relative to carbon. For example, n_{OV} is the relative abundance of oxygen in structure relative to carbon. For any randomly chosen microorganism, there are usually 1.8 H-atoms, 0.5 O-atoms and and 0.2 N-atoms for each C-atom (see Kooijman, 2010, p.83), so that the coefficient matrix for the organics is given by:

$$\boldsymbol{n}_{\mathcal{O}} = \begin{pmatrix} 1 & 1 & 1 & 1 \\ 1.8 & 1.8 & 1.8 & 1.8 \\ 0.5 & 0.5 & 0.5 & 0.5 \\ 0.2 & 0.2 & 0.2 & 0.2 \end{pmatrix}. \tag{B.4}$$

The fluxes of the chemical elements between the four organic compartments food (X), structure (V), reserve (E) and faeces (P) are expressed as $\dot{J}_*$, as the flux of compound * in c-mol / day. The, $\dot{\boldsymbol{J}}_{\mathcal{O}}$ denotes the matrix of organic fluxes, with the $\dot{J}_X$ flux of food, $\dot{J}_V$ the flux of structure, $\dot{J}_E$ the flux of reserve and $\dot{J}_P$ the flux of faeces.

Similarly, $\dot{\boldsymbol{J}}_{\mathcal{M}}$ denotes the mineral fluxes, with $\dot{J}_O$ the flux of dioxygen, $\dot{J}_H$ the flux of water, $\dot{J}_C$ the flux of carbon dioxide, and the $\dot{J}_N$ flux of nitrogen waste. Because the nitrogenous waste of the pond snail mainly consists of urea ($CO(NH_2)_2$, Dogterom, 1980), the coefficient matrix for the mineral compounds is given by

$$\boldsymbol{n}_{\mathcal{M}} = \begin{pmatrix} 1 & 0 & 0 & 1 \\ 0 & 2 & 0 & 4 \\ 2 & 1 & 2 & 1 \\ 0 & 0 & 0 & 2 \end{pmatrix}. \tag{B.5}$$

The matrix of coefficient that couples mass to energy fluxes is given by (Kooijman, 2010, p. 141):

$$\boldsymbol{\eta}_{\mathcal{O}} = \begin{pmatrix} \eta_{XA} & \eta_{XD} & \eta_{XG} \\ \eta_{VA} & \eta_{VD} & \eta_{VG} \\ \eta_{EA} & \eta_{ED} & \eta_{EG} \\ \eta_{PA} & \eta_{PD} & \eta_{PG} \end{pmatrix} = \begin{pmatrix} -1.5 & 0 & 0 \\ 0 & 0 & 0.5 \\ 1 & -1 & -1 \\ 0.5 & 0 & 0 \end{pmatrix} \tag{B.6}$$

B.2.1. Respiration

In DEB theory, respiration is calculated from the fluxes of elements between the four organic compartments food (X), structure (V), reserve (E) and faeces (P).

Table B.2.: We use typical values for the conversion parameters (Kooijman, 2010, Table 4.2).

Symbol	unit	value	Name
$\overline{\mu}_E$	kJ mol $^-1$	500	chemical potential of reserves
$\overline{\mu}_V$	kJ mol $^-1$	550	chemical potential of structure

The organic fluxes $\dot{\boldsymbol{J}}_{\mathcal{O}} = \boldsymbol{\eta}_{\mathcal{O}}\dot{\boldsymbol{p}}$ are given by

$$\begin{pmatrix} \dot{J}_X \\ \dot{J}_V \\ \dot{J}_E + \dot{J}_{E_R} \\ \dot{J}_P \end{pmatrix} = \begin{pmatrix} -\eta_{XA} & 0 & 0 \\ 0 & 0 & \eta_{VG} \\ \overline{\mu}_E^{-1} & -\overline{\mu}_E^{-1} & -\overline{\mu}_E^{-1} \\ \eta_{PA} & \eta_{PD} & \eta_{PG} \end{pmatrix} \begin{pmatrix} \dot{p}_A \\ \dot{p}_D \\ \dot{p}_G \end{pmatrix} \tag{B.7}$$

Considering the conservation of mass and energy, all organic and mineral fluxes must be described by $\mathbf{0} = \boldsymbol{n}_{\mathcal{M}}\dot{\boldsymbol{J}}_{\mathcal{M}} + \boldsymbol{n}_{\mathcal{O}}\dot{\boldsymbol{J}}_{\mathcal{O}}$. The fluxes of the mineral compounds can then also be expressed as a weighted sum of the organic compounds $\dot{\boldsymbol{J}}_{\mathcal{M}} = -\boldsymbol{n}_{\mathcal{M}}^{-1}\boldsymbol{n}_{\mathcal{O}}\dot{\boldsymbol{J}}_{\mathcal{O}}$, so that we can write

$$\boldsymbol{y}_{\mathcal{M}\mathcal{O}} = -\boldsymbol{n}_{\mathcal{M}}^{-1}\boldsymbol{n}_{\mathcal{O}} = \begin{pmatrix} y_{CX} & y_{CV} & y_{CE} & y_{CP} \\ y_{HX} & y_{HV} & y_{HE} & y_{HP} \\ y_{OX} & y_{OV} & y_{OE} & y_{OP} \\ y_{NX} & y_{NV} & y_{NE} & y_{NP} \end{pmatrix} \tag{B.8}$$

The total dioxygen consumption is than given as:

$$\dot{J}_O = y_{OX}\dot{J}_X + y_{OV}\dot{J}_V + y_{OE}\dot{J}_E + y_{OP}\dot{J}_P \tag{B.9}$$

C. Supplement for Chapter 5

C.1. The experimental setup

The snails were held in four feeding and two mating regimes. The feeding regimes were defined with letters: regime 'A' snails were fed *ad libitum*, regime 'B' snails were fed a very small amount of lettuce every day (1/4 of a slice of ø = 4.3 cm, which is ≈ 3.6 cm^2), regime 'C' snails were fed an excessive amount of lettuce every third day, and left without food in between, and regime 'D' snails were not fed at all (see scheme C.1. Singles were numbered with even numbers, and pairs with uneven numbers.

C.2. Development of clutches

One clutch per regime was monitored for hatching time (see Figure C.2), and the hatchlings were monitored to assess time to death by starvation. To reduce variability, we aimed at always following clutches from the same snail or couple. Usually, the clutch was collected at the beginning of the week. However, when the replicate that we originally followed did not produce a clutch until the middle of the week, we collected another clutch from the same regime.

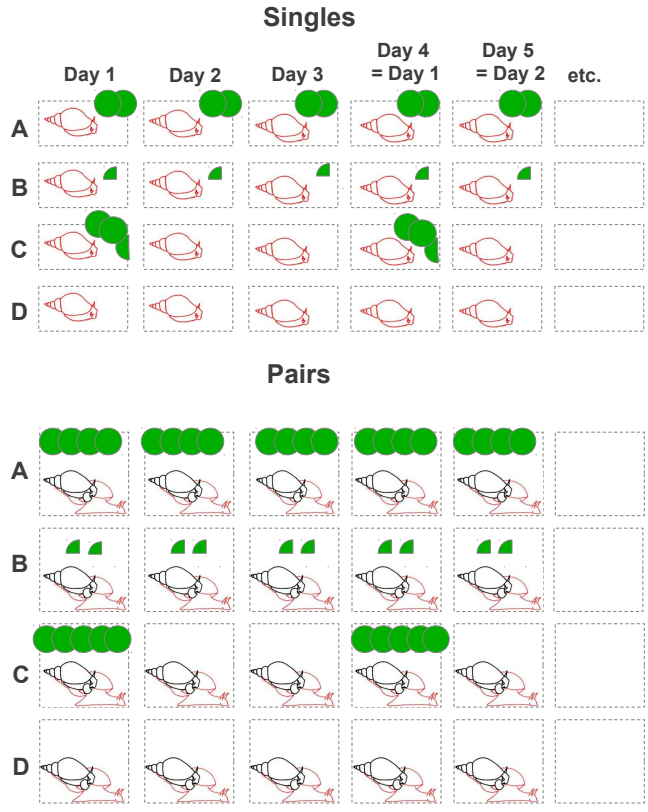

Figure C.1.: The experimental setup. The green circles symbolize a lettuce slice of $\varnothing = 4.3$ cm. The amount of slices fed in regime A and C approximately reflects the amount we fed: to ensure *ad libitum* feeding, we added more lettuce when snails were close to finishing the lettuce in the beaker. The snails in regime B received a quarter of a slice each per day, which was a constant food level because they did not grow. Scheme modified from Hoffer et al. (2012)

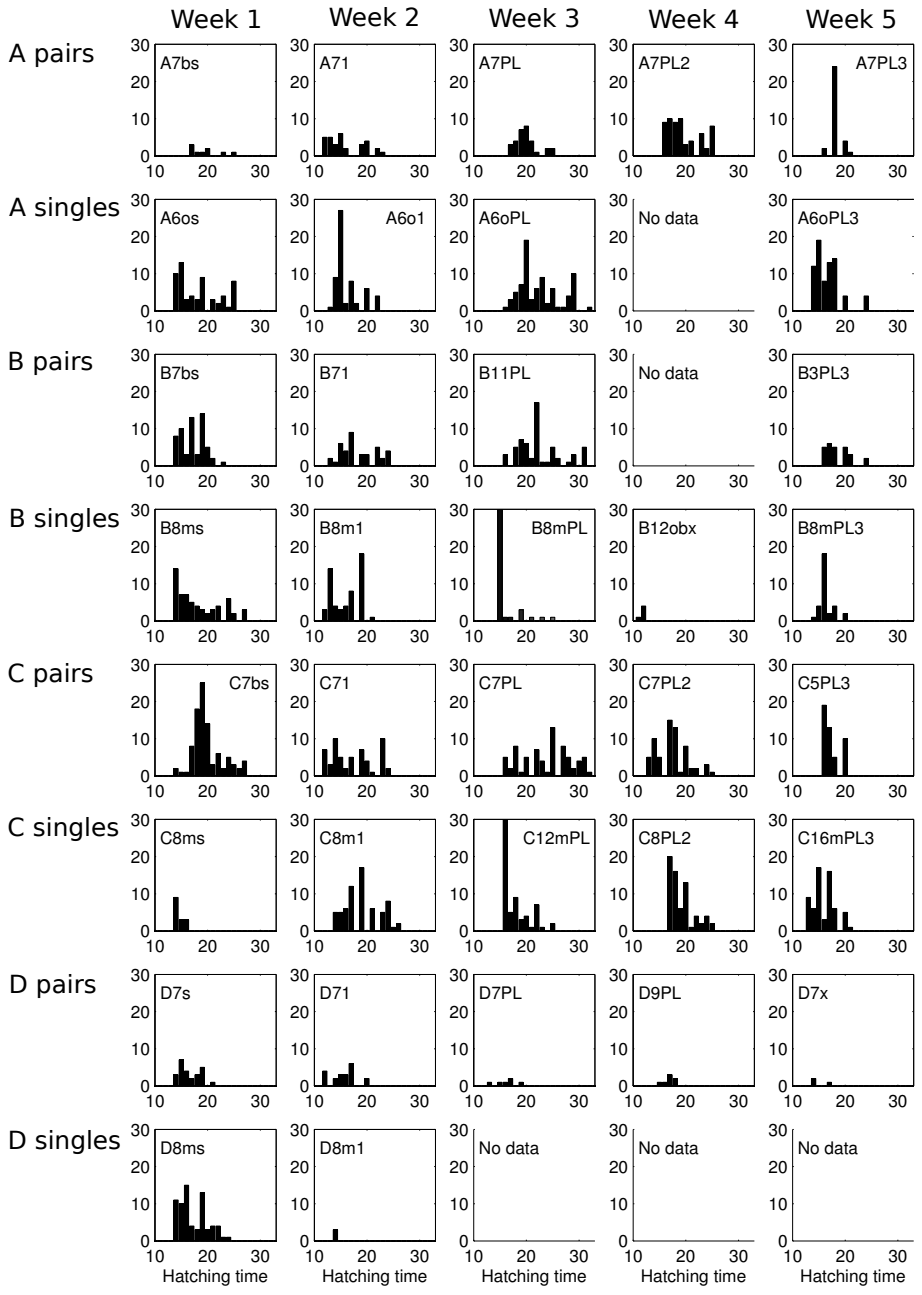

Figure C.2.: The hatching pattern of the followed clutches. The y-axis presents the number of hatchlings that hatched per day after egg laying.

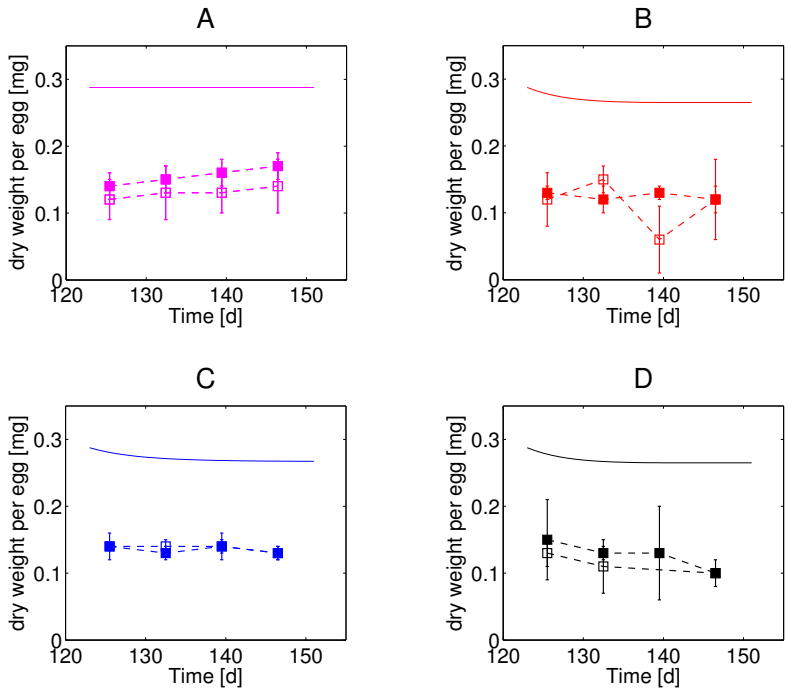

Figure C.3.: Dry weight of eggs with standard deviation over time as mean values per week from our experiment. The data of pairs are represented by the filled boxes (■), and the data of singles are represented by the empty boxes (□). The lines represent the model predictions when $\kappa = 0.91$, to simulate the experimental conditions in Hoffer et al. (2012).

Summary

Understanding the effects of toxicants for informed risk assessment requires a detailed understanding of the physiology of test organisms. The ultimate goal of risk assessment is to protect populations in nature, which requires means to extrapolate from investigations in the laboratory to effect patterns in the field. To assess effects at the population level, it is crucial to understand how external stress on the parents influences offspring fitness. Dynamic Energy Budget (DEB) theory simultaneously specifies ingestion, assimilation, growth, reproduction and maintenance over the whole life cycle of an individual organism. Effects of toxicants can be explained as changes in the model parameters that determine the allocation of energy to the various processes in an organism.

The pond snail *Lymnaea stagnalis* has been proposed as a standard test organism for aquatic invertebrates for future OECD guidelines. The pond snail has a rather complex reproductive system: as a simultaneous hermaphrodite, it maintains both male and female function at the same time. The decision on how much to invest into the male or female function is generally designated as the 'sexual conflict' of the snail. Mating opportunity, amongst other factors, influences both fecundity and egg size. The aim of this dissertation was to i) scrutinize the proposed experimental conditions for their suitability for standardization, ii) explore possibilities of using DEB theory to understand the effects of different stressors and their interactions on the reproductive system of the pond snail, and iii) investigate the potential population-level effects of the observed interactions.

We found that the juvenile pond snails are food limited by lettuce, which is the recommended food source in ecotoxicological investigations with this species. The initial food limitation can lead to an overestimation of the toxicity of compounds if it is not accounted for in the analysis of test results. Since food availability also determines the age at first reproduction, the initial food limitation also has serious impacts for the predictions of population-level effects. Moreover, using lettuce as a food source holds potential for interacting with compounds used in toxicity tests. In a case study with the herbicide diquat, we could demonstrate that the hormetic effect pattern in growth and reproduction was caused by the interaction of the herbicide with the lettuce. Both results are in general applicable for the suitability of food sources in ecotoxicity tests: juveniles commonly have different nutritional needs than adults, and might thus be unintentionally limited. Many food sources hold potential for interaction with toxicity, and we need to be aware of that as this interaction can strongly influence the interpretation of toxicity tests.

When applying the DEB model, we found that the pond snail seems to undergo a so-called metabolic acceleration, which has been commonly found in molluscs.

The acceleration plays a major role in the determination of egg costs in the model, and thus in population-level predictions. The investment per egg is generally also influenced by food availability that the mother experienced, a phenomenon commonly known as a maternal effect. In an experiment designed for investigating the interaction of the sexual conflict and the maternal effect, we discovered that the pond snail invests relatively more energy into the male function when food is scarce. A similar phenomenon has been observed in many simultaneous hermaphrodites, which suggest a broader applicability of our model. However, there was no clear influence of food availability on the investment per egg, but the rather small effect as predicted by the DEB model for the pond snail could easily have been missed. The population-level effects of the observed interactions can be further explored by using DEB-IBM, an individual based modeling framework, which was designed to investigate individuals that are modeled with DEB at the population level.

In conclusions, we can say that lettuce is not an optimal food source for ecotoxicity experiments with the pond snail. The juveniles are food limited when fed with lettuce, which inters with toxicant effects. If a better food source can not be found, a DEB model should be used for the interpretation of toxicity tests. Since the investment per offspring is potentially influenced by many maternal factors (including toxicants), it needs to be determined in toxicity tests. When under stress, pond snails can change the balance between investing in the male and female function. Such behavior can bias the interpretation of toxic effects on reproduction. Considering the energy budgets at the individual level is important to extrapolate to effects at the population level.

Samenvatting

Ecotoxicologie heeft als doel de effecten van stoffen op het milieu te begrijpen. Denk bijvoorbeeld aan bestrijdingsmiddelen, die gebruikt worden voor de landbouw. Voordat een nieuw product op de markt toegelaten kan worden, moet er eerst onderzocht worden of (en in hoeverre) het milieu geschaad wordt als men het gebruikt. Hiertoe worden allereerst experimenten in het laboratorium gedaan, waar onderzocht wordt welke effecten er op kunnen treden bij welke blootstellingsconcentraties. Vervolgens worden de resultaten gebruikt om voorspellingen te doen van wat er in het milieu zou kunnen gebeuren (of welke niveaus veilig zijn). Omdat er niet met elke diersoort een experiment kan worden gedaan, zijn er een paar "standaardsoorten" voor experimenten gekozen. Vanuit de effecten op deze soorten probeert men af te leiden wat de effecten op het gehele ecosysteem zijn.

In mijn proefschrift 'De poelslak onder stress: interactieve effecten van voedsellimitatie, toxicanten en copulatie, verklaard door Dynamische Energy Budget theorie' heb ik onderzoek met de gewone poelslak (*Lymnaea stagnalis*) gedaan. Deze slak leeft in zoet water, en is overal in stilstaande wateren zoals sloten en vijvers in Midden Europa te vinden. De poelslak is heel bijzonder voor wat haar voortplanting betreft: ze is tegelijkertijd zowel mannelijk als vrouwelijk (een zogenaamde "simultane hermafrodiet"). Als ze geen partner kan vinden, kan ze ook haar eigen eieren bevruchten. Men verwacht dat de poelslak om deze reden heel gevoelig op toxische stoffen reageert, vooral voor hormoon-verstorende stoffen. Het idee is dus dat als we de poelslak als voorbeeldsoort onderzoeken, het milieu waarschijnlijk goed beschermd wordt.

Om voorspellingen voor effecten in het milieu te kunnen doen moeten we eerst de resultaten uit het laboratorium goed begrijpen. Voor dit doel worden sinds geruime tijd mathematische modellen ontwikkeld. Als je met behulp van deze modellen de resultaten uit het laboratorium goed snapt, kan je het model vervolgens gebruiken om de effecten op het milieu te voorspellen.

Tijdens mijn onderzoek heb ik een mathematisch model gebruikt om resultaten van experimenten met de poelslak te analyseren. Behalve het analyseren van reeds bestaande experimentele resultaten heb ik ook zelf experimenten ontwikkeld en uitgevoerd. Het model bouwt voort op de zogenaamde Dynamisch Energie Budget (DEB) theorie. In het model wordt de energie gevolgd die de slak door voedsel opneemt. Vervolgens wordt beschreven hoe veel van die energie gebruikt wordt om te groeien, te ontwikkelen, om eieren te leggen, en hoeveel er nodig is om gewoon te overleven (onderhoud). Als je naar de effecten van stoffen kijkt, worden deze beschouwd als een afwijking van het gewone energiegebruik. Bijvoorbeeld zou en bepaalde stof induceren, dat het duurder wordt voor de slak om te groeien, waar-

door er minder energie blijvt, om te reproduceeren. Het DEB model is niet specifiek voor een bepaalde soort of stressor, dus zijn de resultaten van mijn onderzoek ook nuttig om andere soorten en stressors beter te kunnen begrijpen.

Voordat je de effecten van stoffen op een dier kunt begrijpen, moet je eerst de stofwisseling van het dier in een niet-gestressde situatie begrijpen. In mijn onderzoek heb ik dus eerst nauwkeurig naar de stofwisseling van de poelslak in een niet-gestressde situatie gekeken.

In experimenten wordt de poelslak meestal met sla gevoerd, hoewel ze in de natuur vooral waterplanten eet. Ze gebruiken in het laboratorium sla omdat het makkelijker beschikbaar is dan waterplanten. Door het model toe te passen hebben we gevonden dat de sla geen optimaal voedsel voor de jongen van de slak is. Nadat ze uit het ei komen groeien de jongen heel langzaam, waarschijnlijk omdat ze maar weinig energie uit te sla kunnen krijgen. Ik heb in een experiment laten zien dat ze veel sneller groeien als ze met visvoer of zoete aardappelen gevoerd worden. Een mogelijke verklaring voor het langzame groeien is dat ze eerst een goede darmflora op moeten bouwen, voordat ze de sla goed kunnen verteren. Direct nadat de slakjes uit het ei komen is deze nog niet goed ontwikkeld. Omdat ze niet genoeg energie krijgen zijn de jonge slakjes hongerig en in een minder goede staat. Als men ze nu aan stoffen blootstelt kunnen ze daar naar verwachting minder goed tegen dan als ze optimaal gevoerd worden. Als hier geen rekening mee wordt gehouden tijdens de analyse, krijg je een foute interpretatie van de toxiciteit. Met een DEB model kan dit voorkomen worden.

Als we het standaard DEB model gebruiken, voorspellen we een te snelle ontwikkeling in het ei. Er bestaat een modeluitbreiding waarmee dit probleem kan worden voorkomen. De uitbreiding houdt in dat de slak na geboorte de stofwisseling versnelt, en pas later de stofwiiselingssnelheid van de adulten bereikt. Dit fenomeen kun je ook met de ontwikkeling van het darmmilieu verklaren. Over de tijd wordt de vertering efficiÃńnter, totdat de darmflora volledig ontwikkeld is.

Dieren zijn gevoeliger voor toxische stoffen als ze geen goed of te weinig voedsel hebben. Dit is ook vaak het geval in het milieu. Daarom is het belangrijk te begrijpen hoe de stofwisseling verandert onder slechte voedselcondities. Om deze reden heb ik experimenten gedaan waar ik de poelslak bij verschillende voedseldichtheden bestudeerd heb. Het bleek dat de slakken veel minder eieren leggen dan verwacht als ze plotseling weinig voedsel krijgen. Als ze aan de nieuwe situatie gewend raken, leggen ze weer meer eieren. Een goede verklaring voor dit patroon zou zijn dat de slakken meer energie in de mannelijke functie stoppen (ten koste van de vrouwelijke) wanneer voedsel plotseling schaars wordt. Dit is een belangrijk punt als je effecten in het milieu wil voorspellen, omdat voedsel daar periodiek schaars of afwezig is.

Er bestaat in de ecotoxicologie een fenomeen dat op dit moment slecht begrepen wordt. Sommige stoffen hebben in een heel lage concentratie een stimulerend effect, bijvoorbeeld, de dieren groeien sneller. In een hogere concentratie zie je dan vaak een negatief effect, en de beestjes groeien langzamer dan zonder de stof. Dit patroon wordt hormesis genoemd. Ik heb heel nauwkeurig naar een experiment met een

herbicide (diquat) gekeken waar dit fenomeen ook gevonden was. Het eerste idee was dat de slakken meer sla eten bij kleine concentratie van de stof, en minder bij een hogere concentratie, waardoor de hormesis verklaard kan worden. Door het model toe te passen, hebben we de echte oorzaak kunnen vinden. De herbicide heeft de sla aangetast die als voedsel gebruikt wordt. Bij een lage concentratie kan de slak de sla beter verteren, sneller groeien, en vroeger eieren leggen. Bij een hogere concentratie kan de slak de sla slechter verteren, waardoor ze langzamer groeit, en later eieren legt. Met dit onderzoek konden we laten zien dat je ons model goed kan gebruiken om de oorzaken van hormesis beter te begrijpen. Dit geldt niet alleen maar voor de slak, maar ook voor andere organismen.

Zusammenfassung

Ich habe meine Doktorarbeit im Fachbereich der Ökotoxologie angefertigt. Die Ökotoxologie befasst sich mit Untersuchungen von Effekten von Giftstoffen auf die Umwelt. Giftstoffe bezieht beispielsweise auch Pflanzenschutzmittel mit ein, die zur Unterstützung des Getreideanbaus verwendet werden. Bevor ein neues Produkt (oder Pflanzenschutzmittel) auf dem Markt erhältlich ist und tatsächlich eingesetzt werden darf, untersucht man zuerst, wie schädlich es für die Umwelt sein könnte. Dafür werden Laborexperimente durchgeführt. Hierzu verwendet man ausgewählte Lebewesen, die repräsentativ für viele andere Lebewesen sein sollen. Anhand der im Labor festgestellten Reaktionen der Lebewesen auf die Giftstoffe werden Vorhersagen dafür getroffen, welche Folgen beim Einsatz des Mittels dann in der Umwelt auftreten.

In meiner Doktorarbeit habe ich Untersuchungen mit der sogenannten Spitzschlammschnecke (*Lymnaea stagnalis*) durchgeführt. Diese Schnecke ist eine Lungenwasserschnecke, die in Teichen und Schloten in Mitteleuropa beheimatet ist. Die Spitzschlammschnecke hat ein besonderes Fortpflanzungssytem: sie ist ein Zwitter. Das bedeutet, dass sie gleichzeitig männlich und weiblich ist. Sie kann somit ihre eigenen Eier befruchten, wenn sie keinen Partner zur Fortplanzung findet. Man glaubt, dass dies ein Grund sein könnte, weswegen sie besonders sensibel auf Giftstoffe reagiert, die den Hormonhaushalt stören. Die Schnecke soll in Zukunft standardmässig als Versuchstier eingesetzt werden. Zur Zeit werden die Versuchsprotokolle untersucht, die für Standardtests benutzt werden sollen.

Wenn man die Ergebnisse aus dem Labor verwenden möchte, um Vorhersagen für die Folgen in der Umwelt zu treffen, muss man zunächst genau verstehen, welche Prozesse im Labor ablaufen. Dazu werden seit einiger Zeit mathematische Modelle entwickelt. Wenn man mit diesen Modellen verstanden hat, wie ein Stoff auf das Versuchstier Einfluss nimmt, können weitere mathematische Modelle entwickelt werden, um Vorhersagen für die Folgen in der Umwelt zu treffen. Diese Modelle kann man sich ungefähr wie Klimamodelle vorstellen - nur etwas weniger komplex.

Das mathematische Modell, dass ich während meiner Doktorarbeit verwendet habe, basiert auf der sogenannten Dynamischen Energie Budget (DEB) Theorie. Es diente dazu, die Ergebnisse aus Experimenten mit der Spitzschlammschnecke zu untersuchen und zu analysieren. Ich habe hauptsächlich Daten analysiert, die bereits früher erhoben wurden, aber auch selbst Experimente durchgeführt. Mit dem von mir verwendeten Modell kann man erfassen, wie viel Energie die Schnecke bei der Nahrungsaufnahme aufnimmt. Das Modell gibt dann Aufschluss darüber, wie viel von der aufgenommenen Energie für Wachstum, Entwicklung und Fortpflanzung benutzt wird, und wie viel davon für den Unterhalt und das Überleben benötigt

wird. Wenn man nun die Auswirkungen von Giftstoffen auf Lebewesen untersucht, kann man mithilfe dieses Modells verstehen, welche der genannten Prozesse gestört werden. Das Modell kann für den gesamten Lebenszyklus der Schnecke verwendet werden: von der Entwicklung im Ei bis hin zum Erwachsenen Stadium, wenn die Schnecke beginnt, Eier zu legen. Das Modell ist allgemein gültig und kann für jedes Lebewesen verwendet werden. Die Modellstruktur bleibt dabei weitgehend dieselbe; nur die Parameter, die die Energieflüsse bestimmen, sind unterschiedlich von Art zu Art. Die Ergebnisse meiner Doktorarbeit werden daher dazu beitragen, dass auch andere Lebewesen besser verstanden werden können.

Um die Ergebnisse von Experimenten mit Giftstoffen zu verstehen, muss man zuerst die Versuchstiere ohne Giftstoffe verstehen. In meiner Doktorarbeit habe ich daher zuerst untersucht, wie die Spitzschlammschnecke ihren Energiehaushalt im Labor ohne Giftstoffe organisiert.

Zur einfacheren Handhabung wird die Schnecke im Labor mit Kopfsalat gefüttert, obwohl sie sich in der Natur hauptsächlich von Wasserpflanzen ernährt. Auch in dem Standardtest, der gerade entwickelt wird, ist bisher vorgesehen, dass die Schnecke mit Salat gefüttert wird. Mithilfe des Modells haben wir festgestellt, dass Salat als Futter für die Jungtiere nicht gut geeignet ist. Nach dem Schlüpfen wachsen sie sehr langsam, selbst wenn Salat im Überschuss vorhanden ist. Wir vermuten, dass die Jungtiere nicht genug Energie vom Salat aufnehmen können. Füttert man sie hingegen mit Fischfutter (oder Süsskartoffeln), wachsen sie schneller. Das liegt vermutlich daran, dass die Schnecke zuerst eine funktionierende Darmfauna entwickeln muss, die den Salat verdauen kann. Direkt nach dem Schlüpfen hat sich diese noch nicht entwickelt. Durch die unzureichende Energieversorung sind die Jungtiere nach dem Schlüpfen hungrig und in keiner guten Verfassung. Wenn man sie in diesem Zustand Giftstoffen aussetzt, können sie damit schlechter umgehen und sind empfindlicher als sie es normalerweise wären. Wenn man dies nicht in die Analyse der Ergebnisse mit einbezieht, besteht das Risiko, dass man die Daten falsch interpretiert. Mit unserem Modell kann man dies mit einbeziehen, und eine korrekte Datenanalyse vornehmen.

Verwendet man das Standard DEB Modell, um die Schnecke zu untersuchen, wird die Entwicklungszeit, die die Schnecke braucht, bis sie aus dem Ei schlüpft, unterschätzt. Gleichzeitig wird das Gewicht der Eier unterschätzt, was sich wiederum auswirkt auf die Vorhersagen für die Fortpflanzungsrate. Eine Erweiterung des Modells kann diese Abweichung beheben. In der Erweiterung nehmen wir an, dass die Schnecke ihren Stoffwechsel nach der Geburt nach und nach beschleunigt, bis er die Geschwindigkeit des Stoffwechsels einer ausgewachsenen Spitzschlammschnecke erreicht. Auch das Phänomen der Beschleunigung der Stoffwechsels können wir (zumindest teilweise) mit der Hypothese erklären, dass die Darmfauna sich entwickeln muss: nach und nach wird die Verdauung effizienter, bis die Darmfauna vollständig ausgebildet ist.

Wie bereits schon erwähnt, sind Lebewesen sensitiver, wenn sie schlechten Futterbedingungen ausgesetzt sind. Daher ist es wichtig, den Energiehaushalt der Schnecke auch unter schlechten Futterbedingungen zu verstehen. Während meiner

Experimente habe ich die Schnecke unterschiedlich guten Futterbedingungen ausgesetzt. Unsere Experimente zeigten, dass die Schnecken deutlich weniger Eier legen, wenn ihnen plötzlich wesentlich weniger Futter zur Verfügung steht. Sobald sie sich an die neue Situation gewöhnt haben, legen sie jedoch wieder mehr Eier. Eine Erklärung für dieses Phänomen könnte sein, dass die zwittrige Spitzschlammschnecke bei Futtermangel kurzfristig mehr Energie in die männliche Rolle investiert, um für schlechte Zeiten vorbereitet zu sein. Diese Feststellung ist besonders wichtig, wenn man Vorhersagen für die Umwelt machen möchte: in der Natur ändern sich die Futterbedingungen täglich. Desweiteren scheint es, dass die Schnecken die Eigröße in Abhängigkeit von der Tageslänge (Anzahl Stunden mit Licht im Labor) verändern: an kürzeren Tagen legen sie weniger aber größere Eier, und an längeren Tagen legen sie viele kleine Eier. Die Eigröße ist ausschlaggebend für die Überlebenschancen der Jungtiere. Schnecken, die aus kleinen Eier schlüpfen, können schlechter mit Stress umgehen als Schnecken, die aus größeren Eier schlüpfen.

Es gibt in der Ökotoxikologie ein weiteres Phänomen, das noch nicht besonders gut verstanden ist: manche Giftstoffe haben bei sehr geringen Konzentrationen einen stimulierenden Effekt auf die Versuchstiere, die dadurch beispielsweise schneller wachsen als normalerweise. In höheren Konzentrationen haben diese Stoffe dann einen negativen Effekt und die Tiere wachsen langsamer. Man nennt dieses Phänomen Hormesis. In einem Versuch, bei dem die Schnecke einem Pflanzenvernichtungsmittel ausgesetzt wurde, haben wir diesen Effekt auch beobachtet. Unsere erste Vermutung, dass die Schnecke bei geringen Konzentrationen mehr und bei weniger hohen Konzentrationen weniger frisst, hat sich nicht bestätigt. Mithilfe des Modells haben wir die wirkliche Ursache für das schnellere Wachstum herausgefunden. Das Pflanzenvernichtungsmittel hat den an die Schnecke verfütterten Salat angegriffen. Bei geringen Konzentrationen des Pflanzenschutzmittels kann die Schnecke den Salat besser verwerten, schneller wachsen und früher Eier legen. Bei hohen Konzentrationen kann die Schnecke den Salat schlechter verwerten, wächst langsamer, und legt die Eier später. Damit konnten wir zeigen, dass man mithilfe unseres Modells die Ursachen für Hormesis besser verstehen kann. Dies gilt nicht nur für die Schnecke, sondern auch für andere Versuchstiere.

Zusammenfassend kann man sagen, dass Salat kein optimales Futter ist für Experimente mit der Spitzschlammschnecke. Die Jungtiere sind Futter limitiert, was zu einer Wechselwirkung mit den Giftstoffen führt. Falls kein besseres Futter gefunden werden kann, sollte ein Modell so wie unser DEB Modell benutzt werden, um die Ergebnisse von Tests besser interpretieren zu können. Da viele verschiedene Faktoren die Eigröße beeinflussen, ist es wichtig, diese bei Experimenten mit der Schnecke zu messen. Wenn die Schnecken gestresst sind, verändern sie das Verhältnis zwischen Energie-Investierung in die männlichen oder weibliche Funktion. Dies kann Auswirkungen auf die Interpretation von Testergebnissen haben.

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