



# Metabolic acceleration in the pond snail *Lymnaea stagnalis*?



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## ABSTRACT

Under constant environmental conditions, most animals tend to grow following the von Bertalanffy growth curve. 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. 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 as implemented in the DEB theory are an s-shaped growth curve (when body size is expressed as a length measure) and a prolongation of the hatching time. The great pond snail *Lymnaea stagnalis* was earlier found to be food limited during the juvenile phase in laboratory experiments conducted under classical ecotoxicity test protocols. The pond snail has isomorphic shell growth but yet does not exhibit the expected von Bertalanffy growth curve under food limitation. When applying the standard DEB model to data from such life-cycle experiments, we also found that the hatching time is consistently underestimated, which could be a sign of metabolic acceleration. We here present an application of the DEB model including metabolic acceleration to the great pond snail. We account for the simultaneous hermaphroditism of the snail by including a model extension that describes the relative investment into the male and female function. This model allowed us to adequately predict the life history of the snail 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. Since the change in shape is not the only explanation for metabolic acceleration in animals, we discuss the possible other explanations for this pattern in *L. stagnalis*.

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## 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 animals that do not (considerably) change their shape during ontogeny (isomorphy), and 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 the 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 environmental conditions. The von Bertalanffy growth rate, as well as the ultimate size, has a particular physiological interpretation in the context of the DEB theory. The DEB model 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/species.html](http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/species.html)).

In applications of the DEB theory, it is usually first assumed that the standard model can be used. If the standard model cannot be applied to fit the observed patterns in data, this often points to some interesting phenomenon in either the physiology of the organism or the conditions under which the data was obtained. For example, using deviations from the von Bertalanffy growth curve, the DEB framework can be used to reconstruct the feeding conditions that organisms experience. This can be done either 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

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the life cycle (Jager et al., 2005; Zimmer et al., 2012). However, the von Bertalanffy growth pattern only applies to isomorphically growing organisms. A deviation from the von Bertalanffy growth curve can thus also point to changes in the shape of the organism under investigation. Recently, a model extension dealing with morphological changes in fish during ontogeny has been developed (Pecquerie et al., 2009; Augustine et al., 2011). One of the major elements of the model extension is that the fish accelerate their metabolism during ontogeny due to the changes in surface-area to volume ratio. In isomorphically growing shapes, surface area is proportional to volume to the power 2/3. Since the formulation of the standard DEB model is based on the assumption of isomorphy, changes in shape modify some of the DEB parameters which have length in their dimension. The extension leads to an S-shaped deviation from von Bertalanffy growth, and to a prolongation of the embryonic development. A more detailed explanation of this extension will follow in the [Material and methods](#) section.

The bioenergetics of the pond snail *Lymnaea stagnalis* has first been studied many years ago by Zonneveld and Kooijman (1989), whose findings suggest that the pond snail does not comply with the standard DEB rules. 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 stages 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 over time predict an embryonic development that is much too short. This problem can be understood when considering that in the DEB theory, the whole life cycle of an organism is treated in an integrated manner. The rules for energy allocation only differ slightly between life stages. The model parameters that capture the growth of an adult snail should thus also be able to capture the growth and development of an embryo. However, if the embryo grows slower than expected from the adult parameters, modifications in the model parameters or model structure are needed. This in turn also influences the investment into egg production: an embryo, which stays longer in the egg, needs more energy for maintenance, which makes the egg more expensive.

The pond snail has now been proposed as standard test organism for ecotoxicity testing of chemicals (OECD, 2010). Using the DEB theory, it is possible to make better use of toxicity data in comparison to standard analyses (Jager et al., 2006). In a recent DEB application to the pond snail, it was revealed that when considering full life-cycle data, the growth of the great pond snail is S-shaped under standard laboratory conditions (Zimmer et al., 2012). That paper suggested that the juvenile pond snails are food limited up to a certain size, after which they are able to grow unhampered. The food limitation might lead to the observed deviations from the von Bertalanffy pattern. The authors argued in that article that not being aware of this limitation will lead to an overestimation of the toxicity of test compounds. However, that study only used the growth formulation of DEB, and did not consider predictions for reproduction.

Since the pond snail will soon be used for ecological risk assessment, correct predictions for reproduction rate are crucial when analyzing the test results with a DEB model. Both the S-shaped growth and the underestimation of the embryonic development point to the fact that the metabolic acceleration extension might be necessary to capture the whole life cycle of the pond snail with one parameter set in the DEB model.

In the following, we present the application of the standard DEB model with metabolic acceleration to the pond snail. We discuss potential explanations for its applicability, as well as alternative explanations for the deviations from the standard model. Lastly, we suggest additional experiments needed to confirm or disprove our findings.

## 2. Material and methods

We used a standard DEB animal model with metabolic acceleration (Kooijman et al., 2011; Augustine et al., 2011). For the model

parametrization, we used a combination of previously published data on *L. stagnalis*, and data from our own experiments. In the following, we briefly describe the previously published methods and data.

### 2.1. The standard model

The 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, the DEB theory can 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 cumulated energy invested into maturation). Structural length  $L$  is linked to physical length  $L_w$  via the shape coefficient  $\delta_M$ , where  $L = \delta_M L_w$ . We will consider an additional state variable, the reproduction buffer  $E_R$  (J), in which the energy that is available for investment into reproduction is collected. A simplified model scheme with the description of the energy fluxes is presented in Fig. 1, and the model parameters are presented in Table 1. Body mass is composed of structure and reserve, which allows the linkage of model predictions for growth to different nutritional conditions of organisms. Structure is thereby defined as any component of biomass requiring maintenance, and reserve is defined as any component that does not, but is able to fuel metabolism. Maturity has no mass or energy, and represents 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 Fig. 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 energy flux that was used for maturation ( $\dot{p}_H$ ) in embryos and juveniles is then allocated to reproduction ( $\dot{p}_R$ ) in adults.

The dynamics of the state variables are specified by

$$\begin{aligned} \text{Reserve : } \quad & \frac{d}{dt}E = \dot{p}_A - \dot{p}_C, \quad \text{with } \dot{p}_A = 0 \quad \text{if } E_H < E_H^b \\ \text{Structural length : } \quad & \frac{d}{dt}L = \frac{\dot{r}}{3}L \\ \text{Maturity : } \quad & \frac{d}{dt}E_H = (1-\kappa)\dot{p}_C - \dot{k}_J E_H \quad \text{if } E_H \leq E_H^p \\ \text{Reproduction buffer : } \quad & \frac{d}{dt}E_R = (1-\kappa)\dot{p}_C - \dot{k}_J E_H^p \quad \text{if } E_H > E_H^p. \end{aligned}$$

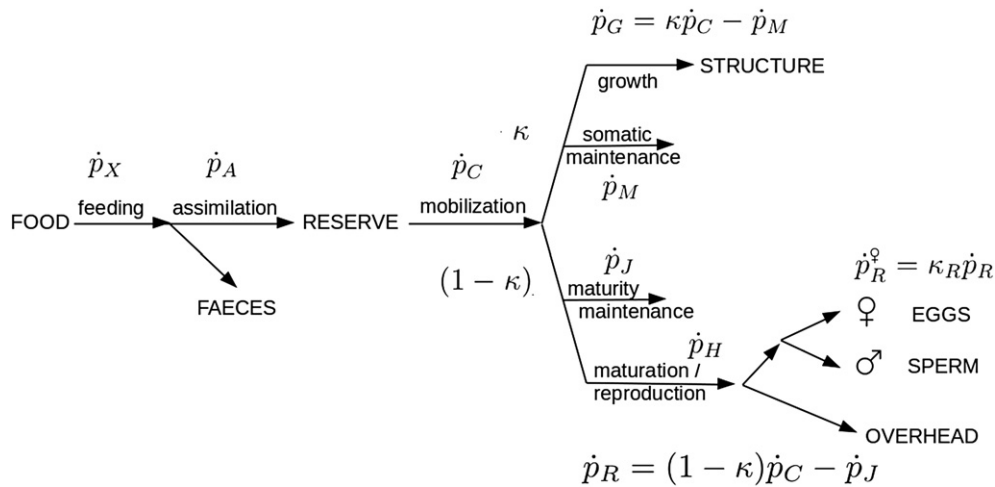
The mobilization flux  $\dot{p}_C$ , the assimilation flux  $\dot{p}_A$ , and the specific volumetric growth rate  $\dot{r}$  are given by

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

The parameters  $\dot{v}$ ,  $\{\dot{p}_{Am}\}$ ,  $[\dot{p}_M]$ ,  $\kappa$  and  $[E_G]$  are explained in Table 1. The assimilation of energy,  $\dot{p}_A$  is taken proportional to surface area in isomorphically growing organisms.

### 2.2. 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



**Fig. 1.** A simplified scheme of the Dynamic Energy Budget (model).  $\dot{p}$  denotes the energy fluxes. Food is taken up ( $\dot{p}_X$ ) and partly assimilated ( $\dot{p}_A$ ) into reserve. The reserve is mobilized ( $\dot{p}_C$ ) and divided into the flux that goes into 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 ( $\dot{p}_R$ ).

species change their shape in the early juvenile period, leading up to a metamorphosis after which they reach the adult shape. A change in shape alters the surface-area to volume ratio, and has an influence on the parameters that have length in their dimension. Most importantly, the surface-area specific assimilation efficiency  $\{\dot{p}_{Am}\}$  is influenced. The reason is as follows: for any isomorphic shape, surface area is proportional to volume to the power 2/3 at all times. The assimilation flux  $\dot{p}_A$  is defined via the structural surface. For an isomorph, the surface-area specific assimilation  $\{\dot{p}_{Am}\} = \dot{p}_A/L^2$  is constant, while the volume-specific assimilation  $[\dot{p}_{Am}] = \dot{p}_A/L^3$  changes with length. For a V1-morph, where surface area is proportional to volume, the surface-area specific assimilation  $\{\dot{p}_{Am}\} = \dot{p}_A/L^2$  is variable, while the volume-specific assimilation  $[\dot{p}_{Am}] = \dot{p}_A/L^3$  is constant. During a V1-morphic acceleration phase,  $\{\dot{p}_{Am}\}$  thus has to be variable, and

increase with increasing length. The energy conductance  $\dot{v}$ , which determines reserve mobilization, is defined as  $\{\dot{p}_{Am}\}/[E_m]$ , where  $[E_m]$  is the maximum reserve density. During the acceleration phase, also  $\dot{v}$  has to increase with length. This is solved by defining the shape correction function  $\mathcal{M}(L)$ , with which  $\{\dot{p}_{Am}\}$  and  $\dot{v}$  are multiplied. The acceleration ends with the metamorphosis.

The mobilization flux  $\dot{p}_C$ , the assimilation flux  $\dot{p}_A$ , and the specific growth rate  $\dot{r}$  are then modified to

$$\dot{p}_C = E(\dot{v} \mathcal{M}(L)/L - \dot{r})$$

$$\dot{p}_A = f\{\dot{p}_{Am}\} \mathcal{M}(L) L^2,$$

$$\text{and } \dot{r} = \frac{E \dot{v} \mathcal{M}(L)/L^4 - [\dot{p}_M]/\kappa}{E/L^3 + [E_G]/\kappa}.$$

**Table 1**

The parameters for the DEB model. Note that the specific surface-linked maintenance rate  $\dot{p}_T$  is zero in ectotherms.

Symbol	Unit	Interpretation	Value	Stdev
<i>Primary parameters</i>				
$T_A$	K	Arrhenius temperature	8000	–
$z$	–	Zoom factor	0.1951	0.002334
$\delta_M$	–	Shape correction coefficient	0.4272	0.005795
$\dot{v}$	cm/d	Energy conductance	0.02161	0.0006765
$\kappa$	–	Fraction of mobilized reserves allocated to the soma	0.7785	0.003241
$[\dot{p}_M]$	J/d · cm <sup>3</sup>	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 <sup>3</sup>	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
$E_H^m$	J	Maturity threshold at metamorphosis	217.3	3.086
<i>Scaled functional response</i>				
$f_{100}$	–	In regime PLE <sub>100</sub>	0.8818	0.02385
$f_{50}$	–	In regime PLE <sub>50</sub>	0.7793	0.0241
$f_{25}$	–	In regime PLE <sub>25</sub>	0.6945	0.02347
<i>Initial size</i>				
$L_0^{100}$	cm	In PLE <sub>100</sub>	1.459	0.04388
$L_0^{50}$	cm	In PLE <sub>50</sub>	1.446	0.04762
$L_0^{25}$	cm	In PLE <sub>25</sub>	1.342	0.0413
$\kappa_R$	–	Reproduction efficiency	0.5	–
$\{\dot{p}_{Am}\}$	J/d · cm <sup>2</sup>	Surface-area specific maximum assimilation rate, $\{\dot{p}_{Am}\} = z[\dot{p}_M]/\kappa$	39.42	–
<i>Adult parameters (after acceleration)</i>				
$\dot{v}_{adult}$	cm/d	$\dot{v}_{adult} = M(L) \dot{v}$	0.1802	–
$\{\dot{p}_{Am}\}_{adult}$	J/d · cm <sup>2</sup>	$\{\dot{p}_{Am}\}_{adult} = M(L)z[\dot{p}_M]/\kappa$	328.7371	–

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

$$\begin{aligned}\mathcal{M}(L) &= \frac{L_b}{L} = 1 \quad \text{if } E_H < E_H^b \quad (\text{embryo}) \\ \mathcal{M}(L) &= \frac{L}{L_b} \quad \text{if } E_H^b < E_H < E_H^j \quad (\text{early juvenile}) \\ \mathcal{M}(L) &= \frac{L_j}{L_b} \quad \text{if } E_H > E_H^j \quad (\text{late juvenile and adult}).\end{aligned}$$

Usually, metamorphosis is reached before puberty ( $E_H^j < E_H^p$ ).

The parameters  $\{\dot{p}_{Am}\}$  and  $\dot{v}$  increase proportionally to length during the acceleration period, but stay constant before and after (see Fig. 2). This explains why the acceleration has an influence on the duration of the embryonic development. Indeed,  $\dot{v}$  of the embryo is smaller than the adult  $\dot{v}_{adult}$ . The surface-area specific assimilation  $\{\dot{p}_{Am}\}$  with which the juveniles start, is smaller than the  $\{\dot{p}_{Am}\}_{adult}$ . Since  $\dot{v}$  determines the mobilization of energy, the embryonic development is slower compared to predictions of the standard model. As a result, growth under constant food conditions is exponential after birth and changes into von Bertalanffy growth after metamorphosis.

### 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  $\kappa_R$  is the reproduction efficiency, and  $E_0$  the amount of energy which is invested per egg. Since the pond snails produce an egg clutch every 2–3 d, and we look at the average reproduction of 5 snails, we simplify and assume that the reproduction buffer  $E_R$  is emptied continuously. To go more into details of the reproductive behavior of individual organisms, one could adapt so-called buffer handling rules, as has been suggested by e.g. Pecquerie et al. (2009) or Augustine et al. (2011). The value of  $E_0$ , the costs for producing one egg, follows from the maternal effect rule of DEB: reserve density at birth equals that of the mother at egg formation (Kooijman, 1986).

The 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  $\kappa_R = 0.95$ . However, the pond snail is a simultaneous hermaphrodite, and changes its allocation into male and female function in response to mating opportunities (Hoffer et al., 2012). We can capture this in the model by considering the reproduction flux as the sum of the female reproduction flux  $\dot{p}_R^\phi = \kappa_R \dot{p}_R$ , 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  $\dot{p}_R^\phi = (1 - \kappa_R) \dot{p}_R$ . The fraction  $\kappa_R$  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. These findings were corroborated in Hoffer et al. (2010), who specifically looked at the investment into male or female function only by experimentally limiting some of the snails to one or the other function. Therefore, in a situation with constant mating possibilities, as occurs when snails are kept in groups during the toxicity test, 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 several months (Nakadera et al., 2014).

### 2.4. The data used for parametrization

Following the definitions 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 one state variable changes against time, or against another state variable (e.g. length over time, reproduction over time, weight over length). 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 parametrization and validation.

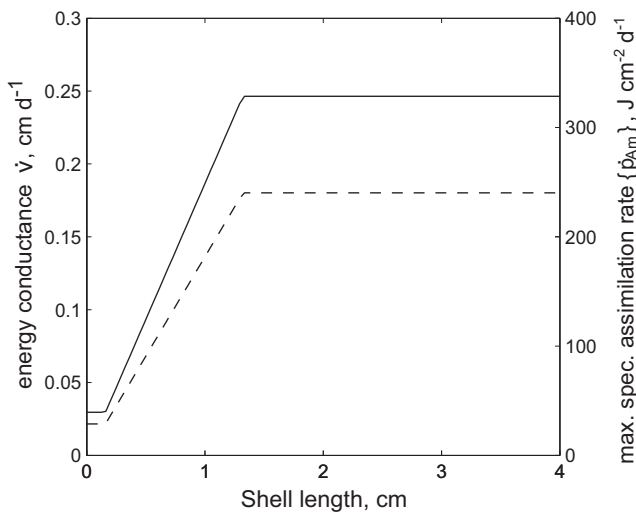
#### 2.4.1. Uni-variate data

We use data on growth and reproduction from a partial life-cycle experiment (PLE) that has been published and described in detail in Zimmer et al. (2012). We therefore only briefly describe the experimental conditions here. The experiment was conducted at INRA, the french National Institute of Agronomic Research, Rennes, under a photoperiod of 14 h light, 10 h dark at  $21 \pm 1^\circ\text{C}$ , and the snails were held in groups of five. The experiment started with juveniles of homogeneous age (113 d) and similar size ( $12.7 \pm 1.3$  mm). They were fed at three different feeding regimes and monitored for 184 d. Each day, an ad libitum quantity of lettuce was weighed and given to the snails in regime PLE<sub>100</sub>, and leftovers were weighed on the next day. The food for regime PLE<sub>50</sub> was determined as 50% of the ad libitum value from the day before, and in PLE<sub>25</sub> as 25%. 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.

#### 2.4.2. Zero-variate data

We used data from an unpublished experiment (INRA, Marc Roucaute) to determine the relation between length and weight of the snails in the culture. 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 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, INRA, Alpar Barsi).



**Fig. 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. The dashed line corresponds to  $\dot{v}$ , the solid line to  $\{\dot{p}_{Am}\}$ .



**Table 2**

The zero-variate data that was used for parametrization and validation. Note that the data used for validation was not used to estimate the parameters.

Source	Symbol	Unit	Interpretation	Data	Prediction
<i>Data for parametrization</i>					
INRA (unpublished)	$a_b$	d	Age at birth	13.5	12.6
	$L_b$	cm	Size at birth	0.147	0.172
	$W_b$	mg	Dry weight at birth	0.114	0.071
	$d_w^{egg}$	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
<i>Data for validation</i>					
Zotin (2009)	$J_O^b$	L/h	Oxygen consumption at birth	$3.50 \times 10^{-8}$	$2.77 \times 10^{-7}$
	$a_b^{18}$	d	Age at birth at 18 °C	20	16.6
Monroy (unpublished)	$J_O^{ad}$	L/h	Oxygen consumption of adults ( $L = 2.75$ cm)	$512 \times 10^{-6}$	$467 \times 10^{-6}$

### 2.5. The data used for validation

In Zimmer et al. (2012), the von Bertalanffy equation, modified by a food limitation function, was fitted to data 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. In the present paper, we use the growth curve of the FLE to compare the predictions of the model with the metabolic acceleration used in this paper with predictions of the model with the food limitation function, as presented in Zimmer et al. (2012).

Additionally, we used data on respiration at birth (Zotin, 2009) and of adult snails (unpublished data, VU Amsterdam, F. Monroy and J. Koene) to compare to the model predictions (see Table 2). The data from Zotin (2009) was obtained at another temperature than the data from our experiment. In the DEB theory, all rates depend on temperature following the Arrhenius relation (e.g., Freitas et al., 2007). In this article, the temperature is assumed to be constant. Because we did not use respiration data in the parametrization process, we consider the prediction for this endpoint relevant data for model validation. Respiration in the DEB theory 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 (Brown et al., 2004). In the DEB theory, overhead costs for assimilation, growth and reproduction as well as maintenance and maturation contribute to oxygen consumption. Respiration, i.e.  $O_2$  consumption, follows from the conservation law for chemical elements, as is evaluated simultaneously with  $CO_2$ ,  $NH_3$  and  $H_2O$  production (e.g., see Mueller et al., 2012).

### 2.6. Parametrization

The parametrization procedure has been described in detail in Lika et al. (2011a,b). 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 (nmreg.m) with a Nelder–Mead simplex method, generally followed by a Newton Raphson optimization.

### 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  $\kappa_R = 0.5$ . To remove a parameter from the system, we set  $[E_C] = 2800$  J, which is a commonly used value in the add\_my\_pet collection (Lika et al., 2011b). We correct

the validation data of Zotin (2009), which was obtained at 18 °C using the Arrhenius temperature  $T_A = 8000$  K.

## 3. Results and discussion

### 3.1. General patterns

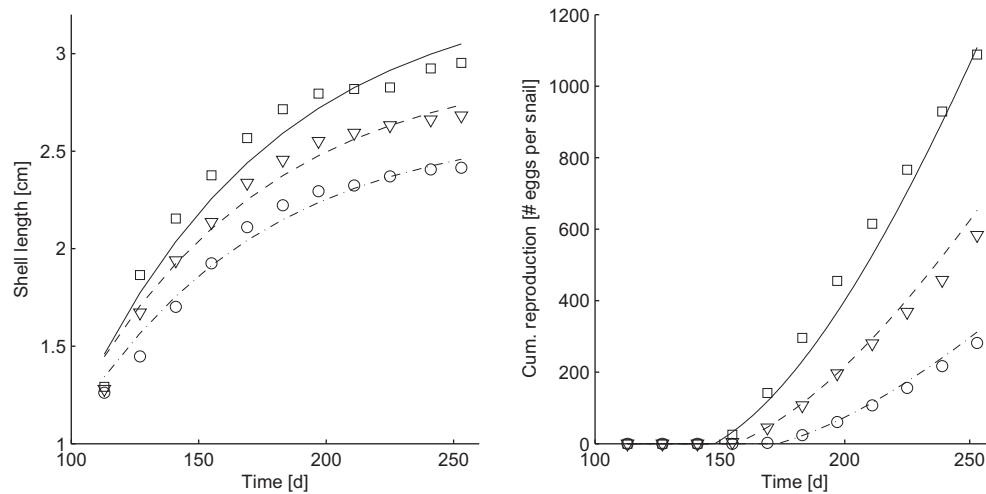
The data we used to parametrize the model is generally well represented by the model predictions (see Fig. 3 and Table 2). Even though the length at puberty is underestimated, the start of reproduction is well captured at the three food levels.

There is a slight discrepancy between the predictions for the zero-variate data points for length and weight: while maximum length ( $L_i$ ), dry weight ( $W_i$ ) and weight at birth ( $W_b$ ) are underestimated, length at birth ( $L_b$ ) is overestimated. Within the DEB theory, such a pattern is explained by the value of the shape coefficient which translates the structural length to physical length of the freshly hatched snail. Thus, this result points to the fact that the shell might not be growing isomorphically, which would be the most straightforward explanation for metabolic acceleration.

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 Fig. 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 cannot further investigate this hypothesis using present data.

### 3.2. Alternatives to metabolic acceleration

We use the V1-morphic extension of the standard DEB model, leading to metabolic acceleration in the juvenile phase, to explain the observed patterns (Kooijman et al., 2011). However, alternative approaches have now been suggested to offer explanations for metabolic acceleration (Kooijman, 2014, this special issue). In that article, the V1-morphic extension is newly defined as type-M acceleration, and discussed in relation to other explanations such as for example changes in temperature. In earlier applications, other adaptations or modifications to the standard DEB model had been made in order to fit the growth and/or reproductive output of the pond snail. As a reminder, the standard DEB model was not able to capture the slow embryonic development and the deviation from von Bertalanffy growth, despite the fact that the shell growth of the snail seems to be isomorphic (Zonneveld and Kooijman, 1989; Kooijman, 2010). The first application of the DEB theory to *L. stagnalis* was published by Zonneveld and Kooijman (1989). The authors fitted the embryonic development



**Fig. 3.** Fits of the DEB model to the mean growth data (left panel) and reproduction data (right panel) from the PLE tests. Solid lines and squares: PLE<sub>100</sub>, dashed line and triangle: PLE<sub>50</sub>, dash-dot line and circles: PLE<sub>25</sub>.

separately from the adult growth data. The embryo energy conductance  $\dot{v}$  was smaller than the adult  $\dot{v}$ . Data on reproduction was not included.

Zimmer et al. (2012) applied the von Bertalanffy growth model, expressed in terms of DEB parameters, to investigate the growth of the pond snail when fed with different food sources. The aim of this application was to investigate how suitable the test protocol (Ducrot et al., 2010) is, which has been proposed for standardization for ecotoxicity tests. When fed with lettuce, which is the proposed food source, the juvenile snails grow very slowly, and the shape of the growth curve is S-shaped. In additional tests, where freshly hatched snails were fed with fish flakes, the juvenile snails reached double the size of snails fed on lettuce within 4 weeks of experiment (Zimmer et al., 2012). We hypothesized that this is due to food composition: in nature, juvenile snails feed on periphyton, while adult snails feed mainly on macrophytes. While the composition of lettuce is close enough to macrophytes and seems to meet the needs of adult snails, the juvenile snails may need more protein in the rapid growth phase. The authors concluded that this will lead to an overestimation of toxicity, when the stress of a compound is added to the stress of food limitation, if this interaction is an unrecognized consequence of the experimental setup. However, this conclusion was based on the growth data only, and no predictions for reproduction were made.

Despite the V1-morphic metabolic acceleration, two other options to fit a slow embryonic development to the rest of the life cycle have been proposed in Kooijman et al. (2011). Either the local temperature might be lower, or the Arrhenius temperature (i.e., temperature dependence of parameters) could be different during the embryonic development. All metabolic rates depend on temperature, so that at a lower temperature, the developmental time of eggs should be longer than at a higher temperature. Because all experiments on which we base the parameter estimation have been conducted at the same temperature, a temperature difference cannot be the reason for a slow development. A difference in Arrhenius temperature might occur when the eggs usually develop under a different temperature (in nature). However, in the pond snail, the eggs are mostly laid on macrophytes or other surfaces close to the water surface (Nakadera and Koene, 2013), so there is no evidence that this explanation is more realistic than any other.

Yet another possibility has not been suggested earlier. We observed in experiments on the embryonic development (Elke Zimmer, personal observation) that the pond snails seem to 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. Moreover, the calculation for egg costs would be more complex, since the feeding in the egg would have to be taken into account. If the assimilation efficiency is low when feeding on the yolk, the predictions for hatching time will be longer. With the present data, this extension, which comes with additional parameters, will probably not balance the cost for an increase in goodness of fit.

### 3.3. Justification of the application of metabolic acceleration

We now suggest that the snails might be accelerating their metabolism, just like many species with larval development do. The model extension we are using was first published as an explanation for fish growth and development (Kooijman et al., 2011). This extension has now been applied to all organisms in the `add_my_pet` library which have a larval development. Possibly, the function of metabolic acceleration lies not in the acceleration, but in a retardation (Kooijman, 2014, this special issue). When considered from the 'adult point of view', a slower embryonic and juvenile development makes sense when considering that a lower metabolic rate means less energy expenditure, which in turns means higher survival probability under low food availability. This interpretation corresponds to the idea of Garstang (1951), who suggested that marine larvae sport a slower metabolism to increase dispersal (Kooijman, 2014, this special issue).

The pond snail still has a trochophora larval stage (i.e. the first free-swimming stage in many marine molluscs), but it passes through this stage during the development in the egg (e.g., Byrne et al., 2009), so it does not need to optimize dispersal. However, *L. stagnalis* is an air-breathing pond snail. Egg clutches are laid under water, and the freshly hatched snails need to find their way to the surface in order to start breathing. Following the argument for dispersal, a slow metabolism increases the chance for the freshly hatched snails to survive until they reach the surface to breathe.

Moreover, like every animal, the pond snail needs to develop a microbial community in their guts before it is able to digest cellulose. Directly after birth, it is likely that the gut cannot digest cellulose efficiently, because the microbial community has not fully developed yet. This could be interpreted such that the efficient gut surface does initially not grow isomorphically with the rest of the snail, which could lead to the slowly increasing values of  $\{\dot{p}_{Am}\}$ .

Zonneveld and Kooijman (1989) had to fit the embryonic development separately from the adult stage. Indeed, the  $\dot{v}$  of the embryo was smaller than the adult  $\dot{v}$  in this article. However, the concept of metabolic acceleration had not been discussed by then, which is why the authors did not consider this possibility.

### 3.4. Implications for ecotoxicity tests with the pond snail

In Zimmer et al. (2012), we used the von Bertalanffy growth equation and only included data on growth at different food types and levels. Interestingly, part of the S-shaped growth pattern observed in the FLE, to which we have earlier fitted the food limitation function in Zimmer et al. (2012), can be captured with the metabolic acceleration (see Fig. 4). This suggests that at least part of what we interpreted as food limitation earlier, can be explained by assuming that the snails accelerate their metabolism. However, the snails in the FLE still grow slower than predicted by the model. Moreover, Zimmer et al. (2012) showed that the juvenile snails grow faster when fed with Tetraphyll fish flakes instead of lettuce. This means that the snails are still food limited in the FLE, on top of the acceleration. The conclusions of Zimmer et al. (2012), that the toxicity of a compound might be overestimated when juvenile snails are fed with lettuce in an ecotoxicity test, without accounting for food limitation, still hold.

An explanation for the differences in growth between juvenile snails fed with lettuce or Tetraphyll might be that the microbial community in the gut needed for digestion of Tetraphyll can be built up faster than the one needed for lettuce. In Zimmer et al. (2012), the authors used different food quality factors to be able to fit the growth in the different experiments where the juvenile snails were fed with lettuce. 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 microbial community in the gut. 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 that of macrophytes, periphyton is in general not used as food in the laboratory, and usually no noteworthy biofilm develops in laboratory experiments at INRA 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). However, experimental evidence is needed to determine whether the microbial community does actually change or not to test this hypothesis.

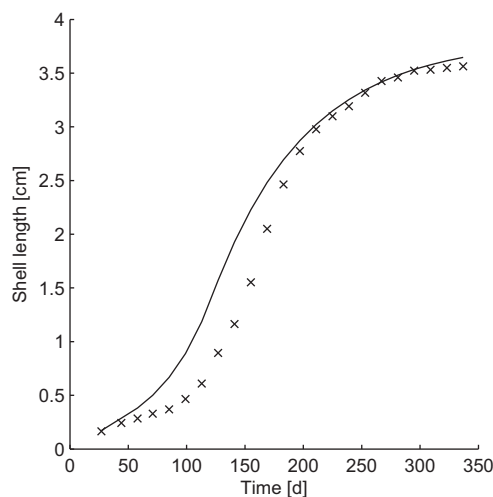


Fig. 4. The growth data from the FLE and the predictions of the DEB model with the metabolic acceleration. Note that the FLE data was not used for parameterization.

### 3.5. Experiments needed for verification

There are several possibilities to shed more light on our title question. When comparing the impact of different food sources on life-history traits, experiments based on the geometric framework (GF) of nutrients would be insightful (Simpson and Raubenheimer, 2012). The GF defines food sources in a state space of nutritional components that are functionally relevant to the animal. When given the choice, animals chose those foods that are closest to their momentary dietary need, which might change with ontogeny (Simpson and Raubenheimer, 2012). Experiments in which snails are offered food sources with different protein and carbohydrate contents (e.g., fish flakes vs lettuce vs sweet potato), preferably with snails in various life stages and with various feeding history, would help settle the above discussed questions. Additionally, caloric measurements of food and feces would be needed to confirm if there are indeed differences in digestion efficiency. Moreover, dry weight measurements during the lifetime of the soft body and shell separately would verify whether the pond snail does actually grow isomorphically or not.

## 4. Conclusions

We show in this work how the entire life-cycle of the pond snail can be modeled with a 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. However, we cannot settle the question whether the pond snail does actually accelerate its metabolism or not. 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 specimens 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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