

## Energy budgets for tadpoles approaching metamorphosis

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### ABSTRACT

We describe a dynamic energy budget (DEB) model for tadpoles over the course of metamorphosis. The model accounts for details in the tadpoles feeding behavior, as feeding and indirectly respiration are reduced in the late developmental stages preceding metamorphosis to an immature froglet.

We propose two versions of our DEB model, one where the energy reserves of the organism are accounted for explicitly (a variant on Kooijman's "standard" DEB model), and one where reserves and structural biomass are lumped together so that only the size of the organism is tracked (a variant on DEBkiss). Both models are parameterized using a time series of measurements on a cohort of tadpoles of the Pacific tree frog, *Pseudacris regilla*. The models describe tadpoles from the middle of their development as tadpoles until they emerge as froglets. Visually, both models fit the growth and respiration empirical data reasonably well; statistically the fit to the full DEB model is slightly better.

The models highlight the metabolic changes during the life of a tadpole and demonstrate how morphological changes in developing organisms can be accommodated in the DEB framework.

### 1. Introduction

Dynamic Energy Budget (DEB) theory describes how energy is obtained and used for growth, reproduction, and development in an organism (Kooijman, 2010). A common and reasonable assumption in many DEB models for heterotrophs, is that the rate of energy uptake (such as through feeding) is proportional to the resource availability and the surface area of the organism. Kooijman (2010) proposed a "standard" DEB model for idealized "isomorphic" organisms (i.e., organisms that do not change shape over their lifetime), for which the rate of energy assimilation is proportional to the square of some measure of the length of the organism. However the feeding behavior of organisms can also depend on factors other than size and resource availability. One such factor can be the developmental state of an organism, which can affect what kind and how much food is consumed.

Previous DEB applications that accounted for changes in feeding behavior during development focused on discrete changes. For example, one such application was for holometabolous insects that experience strikingly different body shapes through ontogeny (Llandres et al., 2015; Maino and Kearney, 2014). In these works, the life cycle of the organism is divided into distinct stages with specific feeding properties. Here, we generalize this approach by not only allowing feeding to depend on discrete stages but also to change continuously over the

course of development within a stage. Our study system is a developing tadpole, which gradually ceases feeding towards the end of its metamorphosis - a process that accompanies a range of changes to the organisms body: absorption of the gills, change of body shape (i.e. loss of the tail), and adaptation of the mouth and digestion system to eventually switch from a omnivorous to a carnivorous diet.

We set out to describe two versions of the model (variants on standard DEB and DEBkiss), and parameterize them with empirical data on developing tadpoles of the pacific tree frog *Pseudacris regilla*. Fig. 1 shows a tadpole and a frog of this species. The data we use was collected in the lab tracking a cohort of tadpoles over a period of about one month. Measurements include daily feeding, body length, respiration and Gosner stage at different time points. The Gosner stage system quantifies the maturity state of a tadpole by morphological traits (Gosner, 1960). The data and models describe tadpoles from Gosner stage 28 (i.e., tadpole has hatched from egg and is independently feeding on external sources) until they reach the final stage 46, when they are classified as froglets.

Our model adds an additional layer of biological detail to the standard DEB approach by tracking how surface-area specific feeding changes during development. Whether additional complexity is needed depends generally on the organism of interest and on what one tries to understand. The advantage of simple models that unify different life

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**Fig. 1.** Tadpole (Gosner stage 40 or 41) and froglet (Gosner stage 46) of *Pseudacris regilla*. The scale bar is 10mm in length, and can be used in both images. Photo by Samuel S. Sweet.

stages is “coherence” in the model description. Coherence is not only intellectually appealing, but it also offers a route to model organisms experiencing stress across life stages. Nevertheless, specific application can demand a greater level of biological detail on developmental changes. An important example can be investigations on how stressors (e.g., diseases or toxicants) affect an organism during a particular time span of development. For the case of amphibians, the details of such stage-specific stressors could be important for instance with trematodes that attack tadpoles (Johnson et al., 1999) or endocrine disruptors that alter the further development of certain stages (Kloas et al., 1999). Similar examples can be presumably found for any class of developing organisms, potentially triggering analogous considerations.

This paper is structured as follows. In Section 2, we describe how the empirical data was collected in the lab. In Section 3, we present the DEB model variants. In Section 4, we explain how we estimated the parameters using empirical data. Finally, in Section 5, we discuss the results, consider possible shortcomings of our models, and give an outlook on future DEB developments for organisms that undergo major physiological changes through ontogeny.

## 2. Data collection

**Field collection**– We collected clutches of *Pseudacris regilla* eggs from Atascadero Creek in Santa Barbara, California in May of 2017 (DD coordinates: 34.433604, -119.780062). We housed egg clutches in separate 4 oz. Glad® containers with four holes poked through the sides. Thirteen to seventeen of these containers were placed in a large bin where water could move through the smaller containers. We placed air bubblers throughout each large bin and housed one to two bins in each of three Percival® incubators at 15 °C. After hatching and muscle development, tadpoles were moved to one of five large aquaria tanks.

**Animal husbandry**– We individually housed all tadpoles in plastic 9.7 x 6.6 x 4 inch Ziploc® containers with 1 L of water at 21–23 °C. Tadpole water consisted of 50:50 mixture of Sparkletts® bottled water and DI water treated with Kents® liquid R/O Right.

A total of 66 individuals was used for this study. The tadpoles were euthanized at different time points because they were principally used for another experiment. The experiment was ended after one month, around the time when the remaining tadpoles reached metamorphosis.

At the beginning of the experiment, on every feeding day, and on the days on which the tadpoles were euthanized, we determined tadpole snout-to-vent length by measuring the distance from the tip the snout to the vent using an ocular micrometer. The snout-to-vent length does not include the tail and therefore can be used as a proxy for the size of the tadpole even when the tail shrinks towards the end of the

metamorphosis. At the time points at which length was measured, we also assessed the developmental state through the Gosner stage system by examining morphological traits (Gosner, 1960).

We did not change water throughout the experiment or use air bubblers to oxygenate the water because of the large amount of water relative to tadpole size in each container.

**Feeding rates**– We prepared food for the tadpoles from a mixture consisting of 100 mL of water, 5 g of spirulina, 5 g of TetraMin, and 2 g of agar. We dried this mixture and cut pellets from sheets of this gelatin mixture using a standardized pellet cutter. The wet weight of a pellet was  $550 \pm 10$  mg (*mean*  $\pm$  *SD*), and the dry weight was  $70 \pm 4$  mg. The tadpoles were fed either one or two pellets at intervals of three to eight days. When we added new pellets to tadpole tanks, we removed all previous solid pellet remains, and we did not change the water in the tanks. We observed that pellets left alone in tanks dissolved over time, but that the water did not become turbid when a tadpole was present - suggesting that the tadpoles consumed suspended particles in the water. To estimate the feeding rates, we dried the remaining pellets in a drying oven for at least 24 hrs on a low heat. When pellets no longer contained moisture, we weighted them on an analytical scale to the closest 1 mg. We calculated the feeding rates (mg/day) by subtracting the weight of the dried pellets from the product of the number of pellets fed and the mean dry weight of a new pellet, and dividing this quantity by the number of days between the feeding events. We then converted this to J/day by using the energy densities of the ingredients. TetraMin has an energy density of 14.0 J/mg (Prevedelli and Vandini, 1998). Dry agar has an energy density of about 1.3 J/mg and dry spirulina of about 1.2 J/mg (<https://fdc.nal.usda.gov>). Using the proportions from the recipe we find that the food pellets have an energy density of 6.5 J/mg.

**Respirometry**– We set up respirometry chambers by modifying 1-pint mason jars (473 mL). We cut out a circular hole on the lid and inserted the top of a 50 mL falcon tube sealed with aquaria sealant. This small opening created an air tight seal, while allowing easy access to the contents inside the chamber to insert the oxygen probe. We also divided the mason jar using a circular cut-out plastic mesh with two zip-ties to avoid harming the tadpole while measurements were recorded. We filled each mason jar with treated water that had been sitting with a bubbler for at least 30 minutes prior to the respirometry procedure to ensure the water was saturated with oxygen.

We filled the mason jars entirely with treated water and recorded an initial oxygen concentration using a YSI® MultiLab 4010 and YSI® ProBOD optical BOD probe. The tadpole and mesh were then quickly added to the mason jar, and the modified falcon tube cap on the jar lids was screwed on to create an air tight seal. After 90 minutes, the falcon tube cap was removed and the oxygen probe was inserted to record the final oxygen concentration. We sterilized the YSI® ProBOD optical BOD probe in between recordings of treatment groups by dipping in into a bleach solution, followed by a rinse with DI water and drying with a kim wipe. Four control jars containing only treated water were used to ensure that seals were airtight. The controls showed no decrease of oxygen in the absence of tadpoles. We excluded significant respiration by microorganisms because respiration of tadpoles was measured in fresh water. Respirometry recordings were performed one day prior to euthanasia; no individual was sampled twice. The change of the  $O_2$  concentration in the respirometry chamber was used to calculate the individuals oxygen consumption rate ( $O_2$  mg/day).

Laboratory temperature was maintained at 20–23 °C and water temperature was recorded before and after respirometry procedure. We also recorded temperature of the water in the jars, which remained constant between 20–22 °C. It is important that water temperature is constant throughout the trial because it would affect the dissolved oxygen concentration in the water.

## 3. Models

We describe two versions of the model: a “standard” DEB model

with reserves (Kooijman, 2010) and a DEBkiss model (Jager et al., 2013) which assumes that assimilates are used directly. Fig. 2 gives an overview of the general structure of the models. Table 1 lists the state variables of the models, and the derived auxiliary variables used to relate the models to the data.

The ingestion rate is  $\dot{p}_X$ . A fraction  $\kappa_X$  of this food is assimilated so that the assimilation rate is

$$\dot{p}_A = \kappa_X \dot{p}_X \quad (1)$$

We start with the standard DEB assumption for isomorphic organisms, that food uptake is proportional to the individuals surface

$$\dot{p}_X = \{\dot{p}_X\} L^2 \quad (2)$$

where  $L$  is the volumetric length  $L = V^{1/3}$ .

We generally adopt the formulation that square brackets  $[\ ]$  indicate quantities per volume  $V$  and curly brackets  $\{\ \}$  indicate quantities per volumetric surface area  $L^2$ . We also adopt the notation that a dot above a quantity indicates a rate measured per time.

To account for changing feeding rates through development, we depart from the standard assumption that  $\{\dot{p}_X\}$  depends only on the resource level, and let it instead depend on the tadpoles maturation state.

We assume  $\{\dot{p}_X\}$  is fixed (at a given developmental stage) because animals were fed ad libidum. In principle however resource dependence can be added to the model in the standard way by setting

$$\{\dot{p}_X\} = f\{\dot{p}_{Xm}\} \quad (3)$$

where  $f$  is the resource dependent functional response and with values between 0 and 1, and  $\{\dot{p}_{Xm}\}$  is the maximum area specific assimilation rate.

Maturation of the organism is tracked by an additional state variable  $G$ . We assume that maturation in terms of Gosner stages (Gosner, 1960) progresses at a constant rate  $\dot{s}$  until the final stage 46 is reached

$$\frac{d}{dt}G = \begin{cases} \dot{s} & G < 46 \\ 0 & \text{afterwards} \end{cases} \quad (4)$$

We do not explicitly model the energy used for maturation/reproduction. We instead assume that maturation costs are included in the somatic maintenance term. In standard DEB formulation, this would correspond to the case that all energy used goes to growth and somatic maintenance,  $\kappa = 1$ , with the addition of an independently progressing maturation variable. We decided to simplify the model in this fashion because with our data it seems impossible to distinguish between metabolic work for maturation and metabolic work for somatic maintenance.

As in the standard DEB model, we assume that somatic maintenance costs  $\dot{p}_S$  are proportional to the volume of the organism

$$\dot{p}_S = [\dot{p}_S]V \quad (5)$$

where  $[\dot{p}_S]$  is the volume specific maintenance rate. We assume there is no separate term for the maintenance of maturity (in the standard DEB model, this term would be proportional to the total energy spent for maturation). We also assume that the energy reserves in the full DEB model do not require metabolic work to be maintained.

The volume of the organism is assumed to grow according to

$$\frac{dV}{dt} = \dot{r}V \quad (6)$$

The growth rate  $\dot{r}$  will depend on the model we choose.

### 3.1. Model with reserves: DEB

This model version is close to the canonical DEB model described in Kooijman (2010). Assimilated energy is first saved in energy reserves  $E$ , from where it is then drawn to fuel the various processes in the

organism, such as growth and maintenance.

As explained in Kooijman (2010)<sup>1</sup> reserve energy density  $[E]$  (per structural volume of the organism) changes according to

$$\frac{d}{dt}[E] = \frac{\{\dot{p}_A\} - [E]\dot{v}}{L} \quad (7)$$

where  $\{\dot{p}_A\}$  is the energy assimilation per square volumetric length  $L^2$ , and  $\dot{v}$  is the energy conductance determining how fast reserves are mobilized.

Referring to Kooijman (2010)<sup>2</sup> the growth rate of the organism is

$$\dot{r} = \frac{[E]\dot{v}/L - [\dot{p}_S]}{[E] + [E_G]} \quad (8)$$

This equation holds for non-starving organisms which mobilize more energy than needed for maintenance,  $[E]\dot{v}/L \geq [\dot{p}_S]$ .

DEB theory leaves the rules for starving organisms open; several possibilities are described in Kooijman (2010). For our application, we assume that structural biomass is formed with an efficiency of  $y$  between 0 and 1, and that in times of starvation structure can be again metabolized to cover maintenance costs. Assuming that all the energy of metabolized structure can be used for maintenance, we find that for starving organisms ( $[E]\dot{v}/L < [\dot{p}_S]$ ) the (negative) growth rate is

$$\dot{r} = \frac{1}{y} \frac{[E]\dot{v}/L - [\dot{p}_S]}{[E] + [E_G]} \quad (9)$$

Note that  $y$  does not appear in the growth equation for non-starving organisms because the parameter  $[E_G]$  captures all volume-specific costs for building new structure (including the overhead on growth, which adds to respiration).

### 3.2. Model without reserves: DEBkiss

The DEB model can be simplified to a DEBkiss model by assuming that energy turnover is fast  $\dot{v} \rightarrow \infty$ , Jager et al. (2013). With this simplification, reserves vanish,  $[E] \rightarrow 0$  and assimilated energy is used directly to fuel growth and maintenance because  $\dot{v}[E] \rightarrow \{\dot{p}_A\}$ .

Plugging this into the growth equation, growth is then simply given by the energy left after paying maintenance costs so that - given the per volume costs of structure  $[E_G]$  - the growth rate of the organism is

$$\dot{r} = \frac{\{\dot{p}_A\}/L - [\dot{p}_S]}{[E_G]} \quad (10)$$

where  $\{\dot{p}_A\}$  is the energy assimilation per square volumetric length  $L^2$  and  $[\dot{p}_S]$  is the volume specific maintenance rate.

This equation describes growth as long as the organism takes up enough energy to cover the maintenance costs,  $\{\dot{p}_A\}/L \geq [\dot{p}_S]$ . If assimilation is lower, the organism starves. Analogously to Eq. (9) in the DEB model, it reverts structure to cover maintenance costs and the (negative) growth rate is

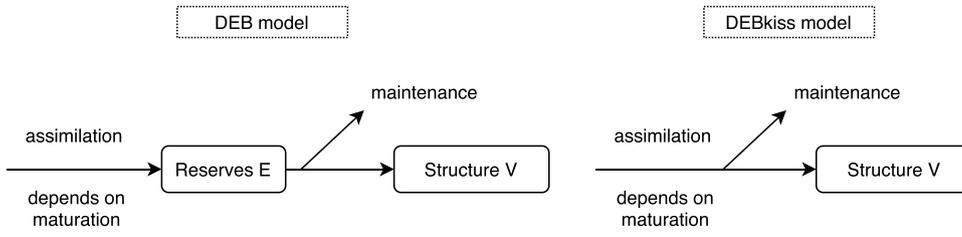
$$\dot{r} = \frac{1}{y} \frac{\{\dot{p}_A\}/L - [\dot{p}_S]}{[E_G]} \quad (11)$$

### 3.3. Respiration

To relate the models to the respiration measures, we formulate respiration  $R$ . We assume that all assimilated carbon used for maintenance and for overhead costs on growth is respired. In this process  $O_2$  is transformed to  $CO_2$ . With  $\eta_R$  being mg oxygen used per  $J$  energy, this leads for non-starving organisms with  $\frac{dV}{dt} > 0$  to the rate of  $O_2$  consumption

<sup>1</sup> equation (2.10), with  $\kappa = 1$

<sup>2</sup> equation (2.13)



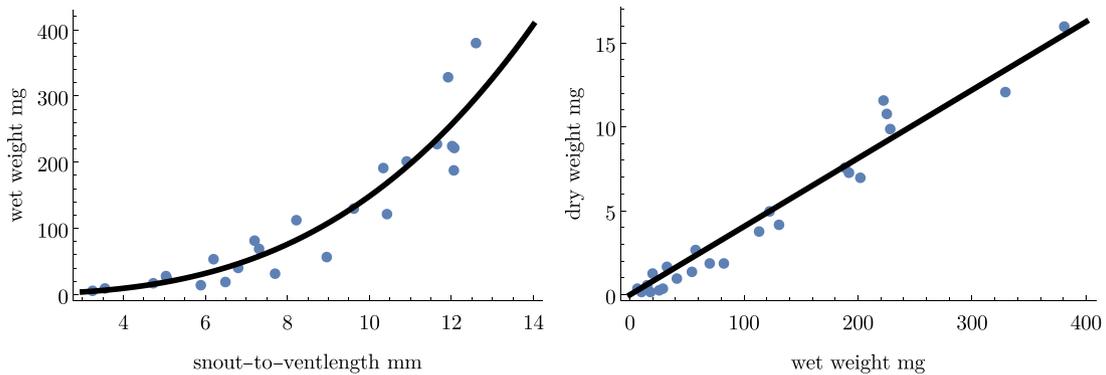
**Fig. 2.** The two versions of the model. In both models, assimilated energy is used to fuel maintenance and growth. The feeding rate depends on the developmental state (Gosner stage). In the "full" DEB model (left), assimilates are first stored in reserves from where energy is drawn to fuel growth and maintenance. In the DEBkiss model (right), reserve dynamics are assumed to be fast so that reserves can be ignored and assimilates are used directly.

**Table 1**  
State variables and auxiliary variables of the models.

Symbol	Definition	Unit
State variables		
$V$	Volume of organism	$mm^3$
$L$	Volumetric length	$mm$
$G$	Maturation, Gosner stage	-
$E$	Energy reserves (only full DEB model)	$J$
Auxiliary variables		
$L_w$	Snout-to-vent length	$mm$
$R$	Respiration flux	$mgO_2/day$

**Table 2**  
The parameter values used. The parameters which are fitted with the likelihood function are found matching the trajectories for snout-to-vent length and respiration as described in Section 4.1. The quantities in parentheses show 95% confidence intervals.

Parameters					
Symbol	Definition	Unit	Estimates		Details
			DEB	DEBkiss	
$\delta_M$	Shape coefficient	$mm/mm$		0.53	Section 4.2
$\eta_R$	Respiration of oxygen per combusted assimilate	$mg/J$		0.07	Section 4.4
$\dot{s}$	Maturation speed	$1/day$		0.63	Section 4.6
$\kappa_X$	Food assimilation efficiency	$J/J$	0.54 (0.46-0.64)	0.54 (0.46-0.62)	Fitted with likelihood function
$\dot{v}$	Energy conductance	$mm/day$	2.8 (1.5-7.5)	-	Fitted with likelihood function
$[\dot{p}_S]$	Volume-specific maintenance	$J/(mm^3 \text{ day})$	0.10 (0.04-0.14)	0.12 (0.09-0.15)	Fitted with likelihood function
$y$	Growth efficiency	$J/J$	0.20 (0.12-0.41)	0.40 (0.27-0.61)	Fitted with likelihood function
$[E_G]$	Volume-specific costs of structure	$J/mm^3$	2.3 (1.1-3.7)	2.3 (1.5-3.2)	From $y$ and Section 4.3



**Fig. 3.** Left: relation between wet weight to snout-to-vent length  $L_w$ . Right: relation between dry weight and wet weight. Data for tadpoles of *Pseudacris regilla* between Gosner stage 23 and 33. Courtesy of Pieter Johnson.

$$R = \eta_R \left( [\dot{p}_S]V + (1 - y)[E_G] \frac{dV}{dt} \right) \quad (12)$$

and for starving organisms to

$$R = \eta_R [\dot{p}_S]V \quad (13)$$

#### 4. Parameter estimations and data fitting

Here, we estimate the various parameters for the two model variants. Some of the parameters are estimated from different sources, and some are fitted to the data of our experiment. Table 2 summarizes the estimates. We used *Wolfram Mathematica* version 12.0 to estimate the model parameters.

##### 4.1. Maximum likelihood function

The parameters  $[\dot{p}_S]$ ,  $\kappa_X$ , and  $y$  both models, and additionally  $\dot{v}$  for

the full DEB model, are fitted using a maximum likelihood approach. The procedure is described in (Jager and Ashauer, 2018).

We denote the times when length was measured with  $t_{L_i}$  and the times when respiration was measured with  $t_{R_j}$ . To speed up the fitting procedure, we use the mean values over individuals at each time point. We denote the (mean) length data points with  $(t_{L_i}, L_i)$  and the respiration data points with  $(t_{R_j}, R_j)$ . The corresponding model predictions are  $\hat{L}(t_{L_i})$  and  $\hat{R}(t_{R_j})$ . Now the likelihood function  $\mathcal{L}$  is

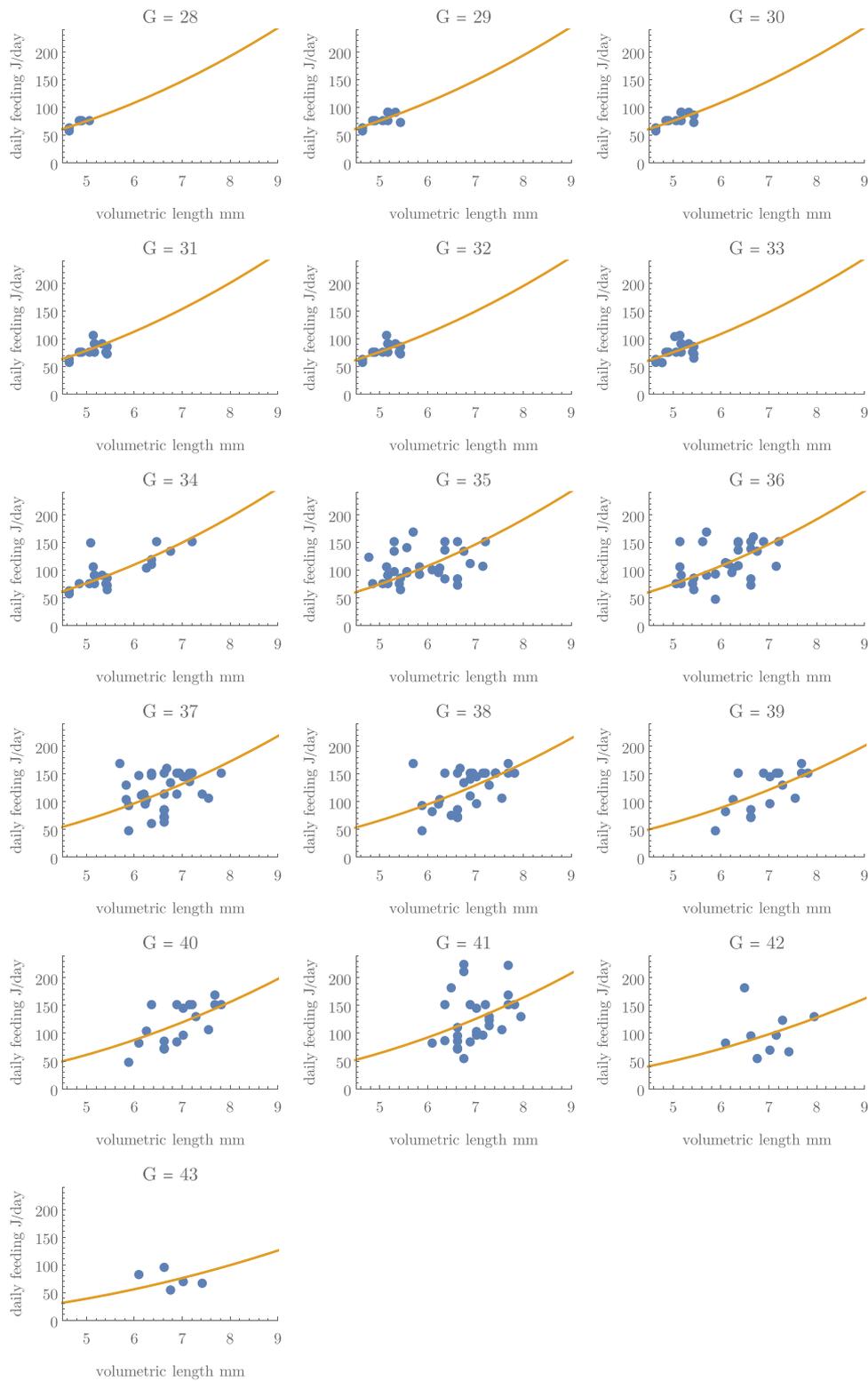


Fig. 4. Daily feeding (in J/day, vertical axis) at different Gosner stages  $G$  in dependence of the volumetric length  $L$  (in mm, horizontal axis). For each stage a separate curve  $\{\hat{p}_x\}L^2$  is fitted.

$$\begin{aligned}
 \mathcal{L} &= \mathcal{L}_L \mathcal{L}_R \\
 &\text{with} \\
 \mathcal{L}_L &= \prod_i f(\hat{L}(t_{L_i}), \sigma_L; L_i) \\
 \mathcal{L}_R &= \prod_j f(\hat{R}(t_{R_j}), \sigma_R; R_j)
 \end{aligned}
 \tag{14}$$

where  $f(\mu, \sigma; x)$  is the probability density function of the normal

distribution with mean  $\mu$  and standard deviation  $\sigma$  at the value  $x$

$$f(\mu, \sigma; x) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left(-\frac{1}{2}\left(\frac{\mu - x}{\sigma}\right)^2\right)
 \tag{15}$$

The likelihood is maximized with respect to the parameters ( $[\hat{p}_S]$ ,  $\kappa_x$ ,  $y$ , and for the full DEB model additionally  $v$ ) and the standard deviations  $\sigma_L$  and  $\sigma_R$ .

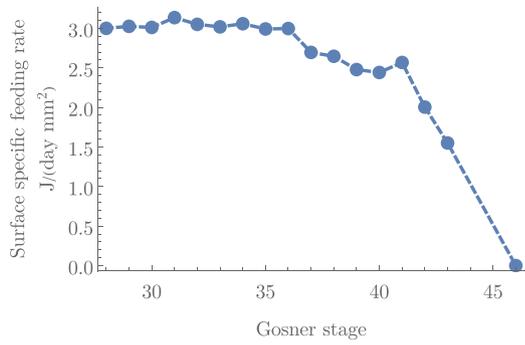


Fig. 5. Surface specific feeding rate  $\{\dot{p}_X\}$  as a function of maturation (Gosner stage).

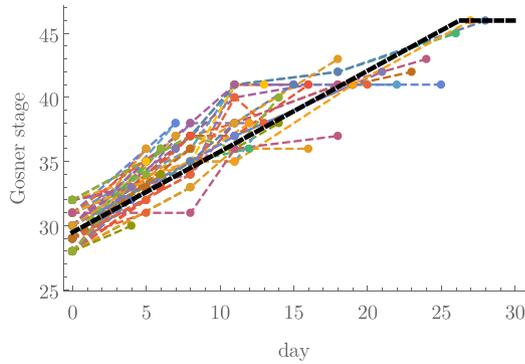


Fig. 6. Maturation. Individual trajectories for tadpole Gosner stage shown by colored lines, and linear fit displayed with a thick black line. Gosner stage 46 is the final stage, a froglet.

To obtain likelihood profiles for the parameters, we find the maximum likelihood  $\mathcal{L}_0$  with all parameters fitted, and compare it to the maximum likelihood  $\mathcal{L}_1$  with a focal parameter fixed to a range of values while the other parameters are fitted again. We then find the 95% confidence intervals for the parameter by using Wilks' theorem (Jager and Ashauer, 2018). According to this theorem, the test statistic

$$D = -2 \log \left( \frac{\mathcal{L}_1}{\mathcal{L}_0} \right) \quad (16)$$

is approximately  $\chi^2$  distributed with one degree of freedom. The 95% confidence interval is thus given by all parameter values for which  $D$  is lower than the  $p = 95\%$  quantile of the  $\chi^2$  distribution.

#### 4.2. Volumetric length to snout-to-vent length $\delta_M$

We assume that the volumetric length  $L = V^{1/3}$  is proportional to the snout-to-vent length  $L_w$ , such that

$$L = \delta_M L_w \quad (17)$$

To estimate the parameter  $\delta_M$ , we use measures on snout-to-vent length and wet weight of tadpoles shown in Fig. 3. We assume that 1 mg wet weight corresponds to 1 mm<sup>3</sup> volume (because their volume is mostly filled with water and they are neutrally buoyant). Finally, we fit the parameter minimizing the square error of the equation

$$V = L^3 = (\delta_M L_w)^3 \quad (18)$$

and find  $\delta_M = 0.53 \text{ mm/mm}$ .

#### 4.3. Cost per structure $[E_G]$

Dried tadpoles of a similar species reportedly have an energy content of about 23 J/mg (Turnipseed and Altig, 1975). To find the energy content per volume, we combine this with data on dry weight per

volume from Pieter Johnson shown in Fig. 3. Using linear regression we find the density of dry weight to volume of 0.04 mg/mm<sup>3</sup> (assuming again that wet tadpoles have a density similar to water). This leads to an energy density of  $\delta_E = 0.9 \text{ J/mm}^3$ . We use this energy density and the growth efficiency  $y$  (which is fitted to the growth trajectories together with the other free parameters after fixing all other parameters) to estimate the energetic costs for producing structure.

##### 4.3.1. DEBKiss

For the DEBKiss model, all mass is assumed to be structural mass i.e., reserves are lumped together with structure). Using the growth efficiency  $y$ , we calculate the energetic cost per structure

$$[E_G] = \frac{\delta_E}{y} \quad (19)$$

##### 4.3.2. DEB

For this model, we need to distinguish between structural weight and reserve weight. Absent information we could use to estimate the ratio of structure to reserves, we assume that  $\rho = 0.5$  of the dry weight in the data is structural weight and the rest is reserves. Thus, only a fraction  $\rho$  of the weighted body mass counts towards structural cost and we find

$$[E_G] = \rho \frac{\delta_E}{y} \quad (20)$$

This estimation is close to that of Grably and Piery (1981), who measured the effect of starvation on adult frogs of another species. Taking into account the differences they found for wet weight and water content, their experiment suggests that starved frogs have a 60% lower dry weight than non-starved frogs of the same length.

#### 4.4. Oxygen used per assimilate $\eta_R$

To find the constant  $\eta_R$  for how much oxygen (in mg) is used to metabolize assimilates (in J), we assume that assimilates are comparable to glucose. Using the caloric value of glucose (Linstrom and Mallard, 2014), we find that one J of assimilates corresponds to 0.06 mg glucose. From the chemical equation for the oxidation of glucose and the molar weights of the reactants, we find that 1 mg glucose is combusted with 1.1 mg oxygen. This leads to

$$\eta_R = 0.06 \frac{\text{mgGlucose}}{\text{J}} \cdot 1.1 \frac{\text{mgO}_2}{\text{mgGlucose}} = 0.07 \frac{\text{mgO}_2}{\text{J}} \quad (21)$$

To check the sensitivity of the assumption on the composition of assimilates, we imagined an extreme scenario where the assimilate was entirely composed of lipids, re-estimated the parameter  $\eta_R$  and repeated the fitting. The fitted curves were close to indistinguishable but the estimated values of some parameters (notably  $\kappa_X$  and  $y$ ) were different (not shown).

#### 4.5. The stage dependent feeding rate $\{\dot{p}_X\}$

We estimated the stage-dependent ingestion rates (J/day) from the amount of food consumed between feeding events. For each Gosner stage between 28 and 46 we used the data from individual feeding periods where the individual was in this Gosner stage at the beginning, the end or between the feeding events (including the last event where only the remaining food was weighted but no food was added). We then calculated the mean snout-to-vent-length  $L_w$  during the feeding periods from the measurements at the beginning and the end of the period, converted this to volumetric length  $L$  and fitted a curve for the feeding rate

$$\dot{p}_X = \{\dot{p}_X\} L^2 \quad (22)$$

for each Gosner stage as shown in Fig. 4. The average maturation

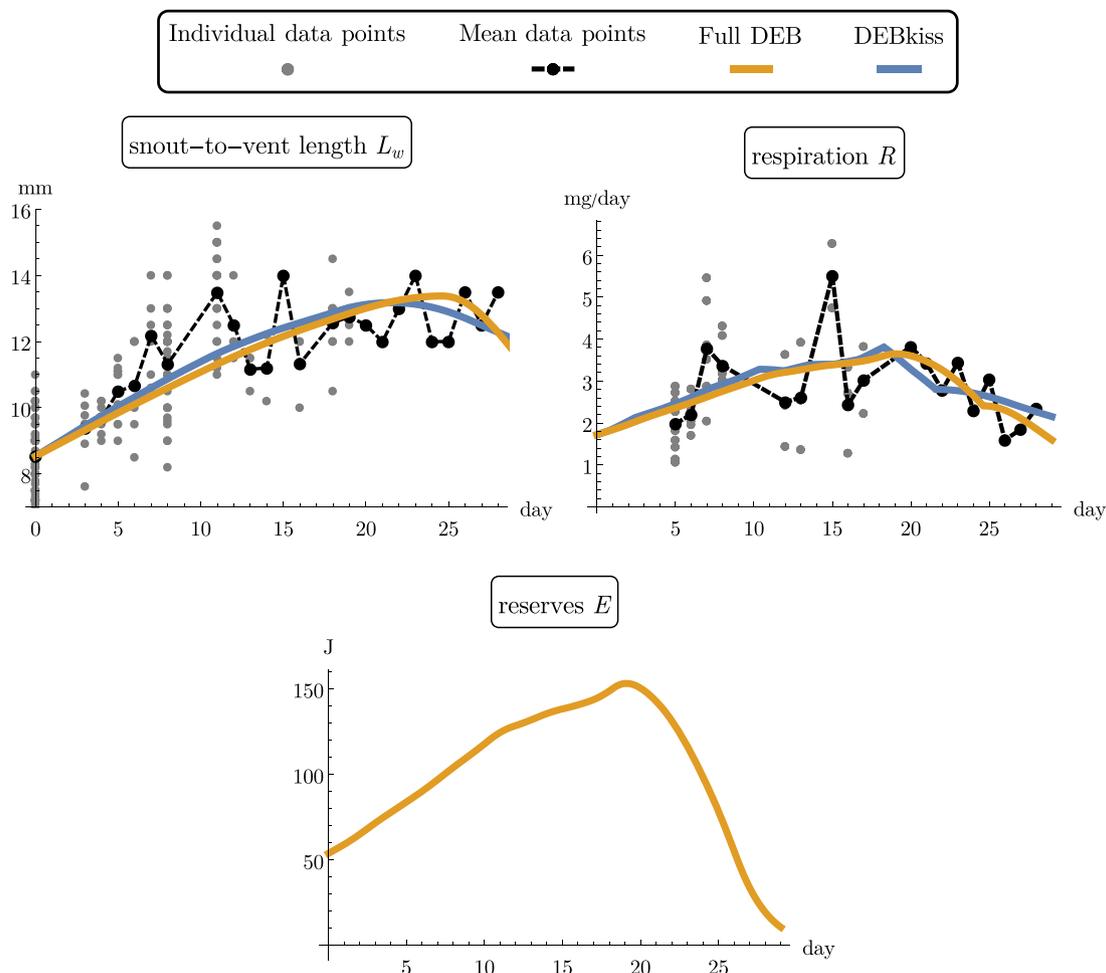


Fig. 7. The model fits with the best fitting parameter values.

between feeding event is 3.0 Gosner stages, so the estimated feeding rates are smoothed across this range. Because we have only a few data points on the final stages (and smoothed over stage 43–46), we assume that the animals entirely stop feeding when turning into a froglet at stage 46 and interpolate the feeding rates between stage 43 to 46. Afterwards the froglets would start eating again i.e., their diet consisting predominantly of insects), but our experiment did not continue into this life stage. The surface area specific feeding rates  $\{p_x\}$  at the different Gosner stages are shown in Fig. 5.

#### 4.6. The parameter $\dot{s}$

The maturation speed  $\dot{s}$  is fitted to the experimental data shown in Fig. 6. We choose all individuals for whom the final measure was at least one week after the start of the experiment thereby focusing on individuals that arrive to higher stages, when feeding is reduced). For each of these individuals, we calculate how many stages were passed per day on average. We then calculate the mean value of all individuals, finding the maturation speed  $\dot{s} = 0.64/\text{day}$ . The maturation state progresses until the final Gosner stage 46 is reached. Note that we make maturation a continuous variable, connecting the original discrete stages defined by Gosner (1960).

#### 4.7. Initial values

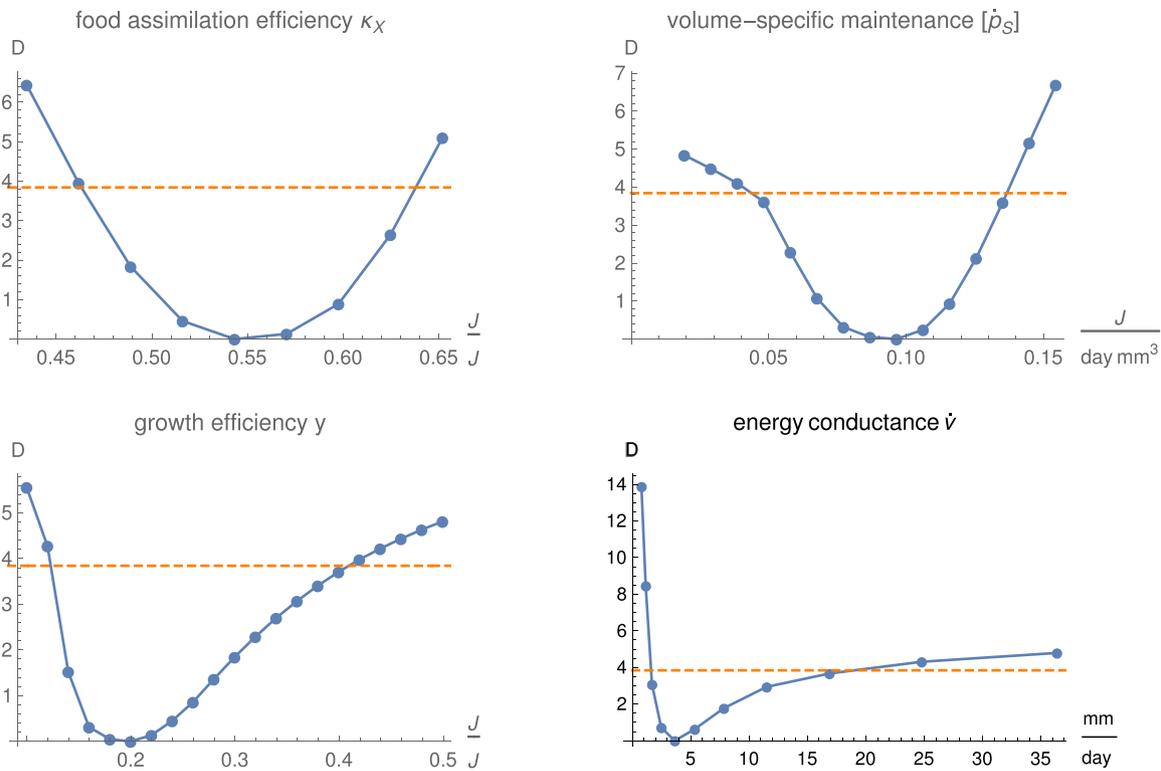
We initialize the model using the mean length and mean Gosner stage at the beginning of the experiment. The reserve energy density at the beginning is set to its (feeding and thus maturity dependent)

equilibrium given by Eq. (7).

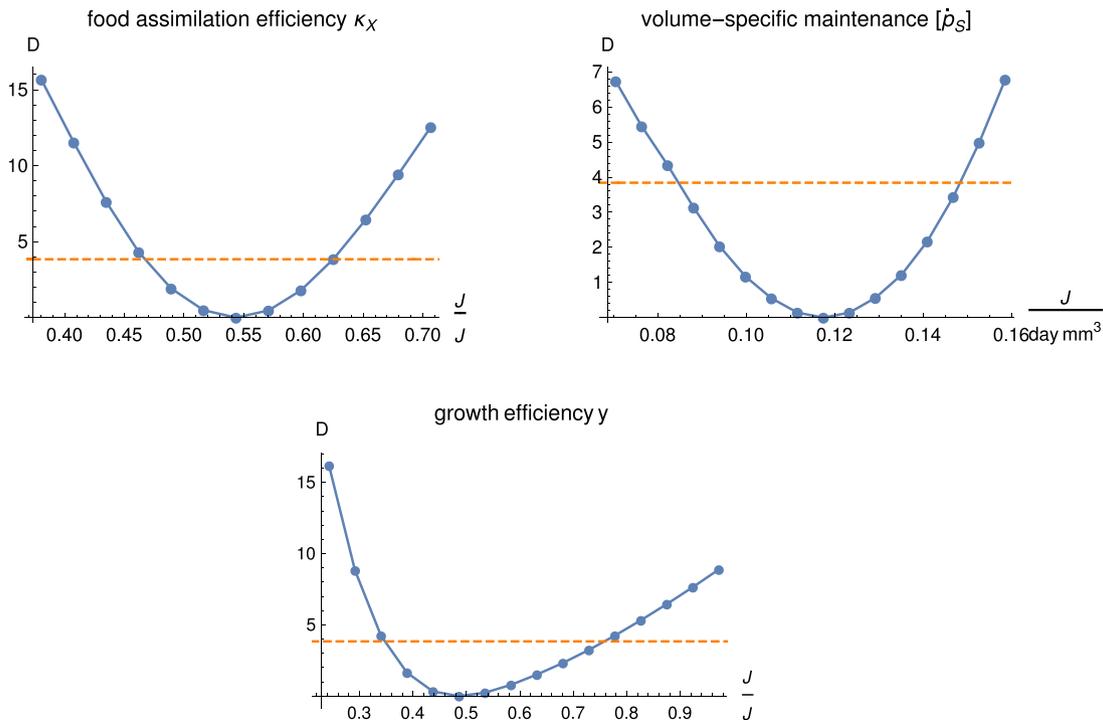
### 5. Results and discussion

In this work, we described two variants of a DEB model for a developing tadpole which changes its feeding behavior during metamorphosis. The first variant is a full DEB model, where assimilates are first stored as reserves and from there are used to fuel the various processes. The second variant is a DEBkiss model, where reserve turnover is assumed to be fast, so that reserves can be neglected and assimilated energy is used directly for growth and maintenance. The models do not distinguish between energy spent for maintenance or maturity work and are intended only to describe the tadpole stage until metamorphosis is reached.

We fitted both model variants to growth trajectories and respiration data of a cohort of tadpoles. The growth trajectories are shown in Fig. 7, and the likelihood profiles of the parameters are shown in Figs. 8 and 9. Visually, both models fit the data reasonably well, with the exception that the models predict a decrease of snout-to-vent length towards the end of the experiment. This trend is not clearly evident in the data. The models predict this decrease because they assume that when feeding is reduced, structure is reverted to cover maintenance. The full DEB model includes a reserve buffer through which growth and maintenance can continue for some time after feeding is reduced. This makes it fit arguably a bit better to the growth and respiration data. The limiting case in which the DEB model becomes the DEBkiss model (large energy conductance  $\dot{v} \rightarrow \infty$ ) does not lie within the 95% confidence interval. This means that the model fits are significantly better



**Fig. 8.** Likelihood profiles of the parameters in the DEB model ( $D$  is proportional to the negative logarithm of the likelihood ratio relative to the optimal value, thus lower values indicate a higher likelihood). The dashed horizontal line shows the 95% confidence intervals.



**Fig. 9.** Likelihood profiles of the parameters in the DEBkiss model ( $D$  is proportional to the negative logarithm of the likelihood ratio relative to the optimal value, thus lower values indicate a higher likelihood). The dashed horizontal line shows the 95% confidence intervals.

for the full DEB model, reflecting that reserves are an essential feature for the energy budget of tadpoles undergoing metamorphosis. Still, reserve dynamics and physiological changes can be implemented in different ways in a DEB framework. It is therefore important to scrutinize the model assumptions and discuss possible modifications that could improve the models.

A critical simplification of our models is the assumption that the shape coefficient and the volume specific costs of structure are constant over the course of development. However, it appears that when comparing a froglet to a tadpole, then (1) the volume to snout-to-vent length is reduced and (2) the dry weight per body volume is increased (unpublished data from Pieter Johnson and data for a related frog

species from (Takahara et al., 2008)). To part from the shape assumptions, one could either account explicitly for a changing shape coefficient or formally set out the model in terms of body weight instead of volume to make use of the preservation of body mass (as the reduced volume and increased dry weight per volume counterbalance each other to some extent). Another option could be to relax the assumptions on how reserves are handled in the model. In the standard DEB model, energy is mobilized for growth and maintenance at a rate proportional to the reserve density (reserves per structural volume), so that when surface specific feeding is constant, reserve density converges to an equilibrium (i.e. in our application, the reserve density stays virtually constant over the first proportion of the tadpole development and then decreases when feeding is reduced). This assumption might be not generally met by tadpoles, for instance they could steadily increase reserve density until they approach metamorphosis and reduce feeding. This kind of reserve dynamics have been observed in fish; a modification of the standard DEB model, DEBlipid, has been developed to capture such dynamics (Martin et al., 2017). Despite these possibilities we stick with body volume and with the DEB/DEBkiss reserve dynamics to stay close to the canonical DEB model and focus on the changing feeding pattern.

In this work, we have shown how DEB theory can be used readily to describe an organism which changes its surface-area specific energy assimilation over time. For this application, we used an interpolation of feeding rates at different developmental tadpole stages. This captures the patterns from the empirical data, but it could be also interesting to find more causative and mechanistic principles describing the change in feeding rates. For example, one could consider how the functional surface used for assimilation changes during development to infer the assimilation rates. We hope that theory from bioenergetic models will be further developed in this direction and help understand the energy balance of organisms that change feeding and other traits during their life.

#### CRedit authorship contribution statement

**Ferdinand Pfab:** Writing - original draft, Formal analysis. **Graziella V. DiRenzo:** Investigation, Conceptualization, Writing - original draft, Formal analysis. **Ariel Gershman:** Investigation, Writing - review & editing. **Cheryl J. Briggs:** Investigation, Conceptualization, Writing - original draft, Formal analysis. **Roger M. Nisbet:** Investigation, Conceptualization, Writing - original draft, Formal analysis.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### References

- Gosner, K., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183–190.
- Grably, S., Piery, Y., 1981. Weight and tissue changes in long term starved frogs *Rana esculenta*. *Compar. Biochem. Physiol. Part A* 69 (4), 683–688.
- Jager, T., Ashauer, R., 2018. Modelling Survival under Chemical Stress. A Comprehensive Guide to the GUTS Framework. Version 2.0. Toxicodynamics Ltd., York, UK.
- Jager, T., Martin, B.T., Zimmer, E.L., 2013. Debkiss or the quest for the simplest generic model of animal life history. *J. Theor. Biol.* 328, 9–18.
- Johnson, P.T., Lunde, K.B., Ritchie, E.G., Launer, A.E., 1999. The effect of trematode infection on amphibian limb development and survivorship. *Science* 284 (5415), 802–804.
- Kloas, W., Lutz, I., Einspanier, R., 1999. Amphibians as a model to study endocrine disruptors: ii. estrogenic activity of environmental chemicals in vitro and in vivo. *Sci. Total Environ.* 225 (1–2), 59–68.
- Kooijman, S., 2010. Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press.
- Shen, V.K., Siderius, D.W., Krekelberg, W.P., and Hatch, H.W., Eds., NIST Standard Reference Simulation Website, NIST Standard Reference Database Number 173, National Institute of Standards and Technology, Gaithersburg MD, 20899, <http://doi.org/10.18434/T4M88Q>, retrieved July 21 2020.
- Llandres, A.L., Marques, G.M., Maino, J.L., Kooijman, S., Kearney, M.R., Casas, J., 2015. A dynamic energy budget for the whole life-cycle of holometabolous insects. *Ecol. Monogr.* 85 (3), 353–371.
- Maino, J.L., Kearney, M.R., 2014. Ontogenetic and interspecific metabolic scaling in insects. *Am. Nat.* 184 (6), 695–701.
- Martin, B.T., Heintz, R., Danner, E.M., Nisbet, R.M., 2017. Integrating lipid storage into general representations of fish energetics. *J. Anim. Ecol.* 86 (4), 812–825.
- Prevedelli, D., Vandini, R.Z., 1998. Effect of diet on reproductive characteristics of ophryotrocha labronica (polychaeta: dorvilleidae). *Mar. Biol.* 132 (1), 163–170.
- Takahara, T., Miyasaka, H., Genkai-Kato, M., Kohmatsu, Y., 2008. Length-weight relationships in six amphibian species of Japan. *Curr. Herpetol.* 27 (1), 43–45.
- Turnipseed, G., Altig, R., 1975. Population density and age structure of three species of hylid tadpoles. *J. Herpetol.* 9 (3), 287–291.