The trade-off between maturation and growth during accelerated development in frogs

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Abstract
Developmental energetics are crucial to a species’ life history and ecology but are poorly understood from a mechanistic perspective. Traditional energy and mass budgeting does not distinguish between costs of growth and maturation, making it difficult to account for accelerated development. We apply a metabolic theory that uniquely considers maturation costs (Dynamic Energy Budget theory, DEB) to interpret empirical data on the energetics of accelerated development in amphibians. We measured energy use until metamorphosis in two related frogs, *Crinia georgiana* and *Pseudophryne bibronii*. Mass and energy content of fresh ova were comparable between the species. However, development to metamorphosis was 1.7 times faster in *C. georgiana* while *P. bibronii* produced nine times the dry biomass at metamorphosis and had lower mass-specific oxygen requirements. DEB theory explained these patterns through differences in ontogenetic energy allocation to maturation. *P. bibronii* partitioned energy in the same (constant) way throughout development whereas *C. georgiana* increased the fraction of energy allocated to maturation over growth between hatching and the onset of feeding. DEB parameter estimation for additional, direct-developing taxa suggests that a change in energy allocation during development may result from a selective pressure to increase development rate, and not as a result of development mode.

1. Introduction

How energy and matter are mobilized and allocated during development is a basic problem in developmental biology. It also has critical ecological implications by affecting the duration of the embryo stage and the environmental requirements (oxygen, temperature, water) for development (Seymour et al., 1991; Rombough, 1994; Marsh et al., 1999; Gillooly et al., 2002; Kamler, 2008). Most species develop as eggs but there is enormous interspecific variation in factors such as initial egg size and energy density, the cost of development, developmental rate and the developmental stage at hatching. Classic energy budgeting approaches partition energy use into maintenance, growth, reproduction and storage (Vleck et al., 1980; Hoyt, 1987; Vleck and Vleck, 1987; Vleck and Hoyt, 1991; Charnov et al., 2001; Gillooly et al., 2002) but do not explicitly consider the costs of ‘maturation’ such as tissue differentiation, nor its maintenance. Under such energy budget frameworks, it is therefore difficult to account for changes in the relationship between energy use, growth and differentiation, as occurs in accelerated development.

The energetics of amphibian development is strongly tied to developmental mode, which ranges from the ancestral state of entirely aquatic eggs and larvae, to terrestrial eggs with aquatic larvae, to direct development where fully-formed metamorphs emerge directly from eggs (Duellman and Trueb, 1986). The generally high oxygen availability in air allows for an increase in egg size in terrestrial breeders (Packard and Seymour, 1997) and, as a consequence, terrestrial and direct developers have the largest eggs (Salthe and Duellman, 1973). Interspecific studies indicate that larger egg size slows the rate of embryonic development (Bradford, 1984, 1990; Pauly and Pullin, 1988). However, one Australian Myobatrachid frog appears to be an exception to this rule. The aquatic breeding *Crinia georgiana* produces relatively large eggs that are comparable in size to those of the closely related terrestrial eggs of *Pseudophryne bibronii*. The two species also have similar adult size, produce loose egg clutches of similar egg number and breed under comparable temperatures (Seymour and Roberts, 1995). Despite these parallels in egg characteristics and reproductive traits, studies have shown that *C. georgiana* embryos develop almost two times faster than *P. bibronii* (Seymour and Roberts, 1995; Seymour, 1999).
We therefore examine the development, growth and O2 consumption of *C. georgiana* and *P. bibronii* under an identical incubation temperature until metamorphosis and compare the partitioning of energy using dynamic energy budget (DEB) theory (Kooijman, 2010).

DEB theory is a metabolic theory that is based upon mechanistic assumptions that are grounded within a number of stylized biological facts (Sousa et al., 2008; Kooijman, 2010; Lika et al., 2011; Nisbet et al., 2012). An organism’s energy assimilation and utilization are described as functions of its state (age, size, etc.) and the state of the environment (temperature, food, etc.) (Nisbet et al., 2000). Developmental transitions, such as from embryo to juvenile (equivalent to amphibian larva), are linked to the level of maturity, a state variable of the model quantified as the cumulative energy used for development, $E_{i\text{p}}$. The other two state variables are structure, which can be quantified as volume, length or mass, and reserve, quantified as energy content or mass.

In DEB theory all energy acquired by an organism follows a unidirectional flow, going first into reserve before it is allocated to different processes. The flow of energy can split but does not merge (Fig. 1). The main split in energy flow from reserve, called the $\kappa$-rule for allocation, indicates that a fixed fraction, $\kappa$, of mobilized reserve is used for somatic growth and maintenance and the remaining, $1 - \kappa$, is used for maturity maintenance and maturation (in embryos and juveniles) or reproduction (in adults) (Kooijman, 2010). The concept of maturation as a destination of mobilized reserve energy makes very specific predictions for how variation in allocation of energy to maturity should affect respiration and growth, yet it is one of the least tested aspects of DEB theory. This distinction between growth and maturation allows us to not only examine energy used to reach a certain size, but also to reach a developmental stage (or level of maturity). We apply this theoretical framework to empirical data collected for *C. georgiana* and *P. bibronii* to understand the relationship between energy allocation to growth, maintenance and maturation and resultant developmental trajectories. Furthermore, DEB theory is applied to previously published data on two direct developers, *Crinia nimbus* and *Geocrinia vitellina*, to examine the influence of development mode on energy allocation.

### 2. Materials and methods

#### 2.1. Egg collection and incubation

Embryos and juveniles were staged throughout experiments according to Gosner (1960). We refer to these developmental stages with respect to maturity level, $E_{i\text{p}}$, where $i$ represents Gosner stages 1 (oviposition)–46 (metamorphosis). Clutches of terrestrial *P. bibronii* eggs were collected from Watt’s Gully Native Forest Reserve, 50 km from Adelaide, South Australia. Three clutches ($E_{21\text{p}}$, approximately 22 days old) were collected in May 2008 and another clutch ($E_{0\text{p}}$, approximately 3 days old) in June 2008. The clutches were taken to the laboratory, cleaned and raised until metamorphosis at 12 °C as described by Mueller and Seymour (2011). Field site temperature in the three weeks prior to collection of the older clutches averaged 12 °C (Australian Bureau of Meteorology, http://www.bom.gov.au), and therefore development rate was assumed to be similar to the clutches incubated in the laboratory from 3 days old at 12 °C.

One clutch of aquatic *C. georgiana* eggs (laid the previous night) was collected from the field, near Brookton Highway, 35 km southeast of Perth, Western Australia, in August 2008. Another four clutches (laid the previous night) were collected from captive adults held at the University of Western Australia in August 2009. Clutches were held at the University of Western Australia, transported by air to the University of Adelaide and raised until metamorphosis at 12 °C as described by Mueller and Seymour (2011). The incubation method was highly successful for both species, with approximately 90% survival to metamorphosis. Data for each clutch, which were of similar size within and between species and showed no significant differences in development, mass or respiration, were pooled.

#### 2.2. Mass and energy density

Ova were dissected from fresh eggs, dried to constant mass over silica gel and weighed to 0.01 mg on an electronic balance (Mettler AE183, Greifensee, Switzerland). Embryos and juveniles were selected at random throughout development, killed by freezing and placed in Tyler’s preservative (Tyler, 1962). They were dissected into body and yolk, dried over silica gel and weighed. Dried samples of fresh ova, hatchling gut-free body and yolk and metamorph gut-free body were homogenized using a mortar and pestle to make a pellet of at least 25 mg. The energy density of the pellets was measured with an 1107 semi-micro bomb of a 1261 bomb calorimeter (Parr, Moline, USA) after calibration with dry benzoic acid.

#### 2.3. Oxygen consumption

O2 consumption rates ($M_{i\text{O}_2}$) of embryos and juveniles were determined at 12 °C as described by Mueller and Seymour (2011). Briefly, $M_{i\text{O}_2}$ until $E_{25\text{p}}$ (hindlimb toe maturation) were determined for individuals from the decrease in $P_{i\text{O}_2}$ within sealed water-filled respiratory chambers (0.67 mL, model 1271, Diamond General, Ann Arbor, MI).

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**Fig. 1.** Metabolism in a juvenile DEB organism. Rectangles represent state variables, ovals are processes, solid lines are flows from state variables to processes and vice versa and dashed lines are flows of minerals (O2, CO2, NH3, H2O) to and from processes. The processes of feeding and assimilation are absent in embryos.
USA) fitted with Clark-type O2 electrodes (model 730, Microelectrodes Inc., Bedford, NH, USA). After $E_H^{27}$, when juveniles outgrew the chambers, custom-made syringe respirometers, in which the electrodes were inserted, were used. Measurements were taken for a minimum of 3 h. Water-filled respirometers were used for both species as a previous study found no differences in $M_O2$ in *P. bibronii* eggs measured in air or water (Bradford and Seymour, 1985).

Total $O2$ consumed was calculated for the entire development period and also to $E_H^{22}$, between $E_H^{22}$ and $E_H^{27}$ and between $E_H^{27}$ and $E_H^{46}$. A polynomial function was fitted to the data for $M_O2$ against the age corresponding to each maturity level and the equation integrated to calculate the area under the curve. The $O2$ cost of development, which encompasses all developmental process (growth, maturation, maintenance), was calculated from the total $O2$ consumed divided by dry gut-free mass produced within each interval.

### 2.4. DEB model

We applied the standard DEB model (Kooijman, 2010) to the data we collected to estimate energy parameters for *C. georgiana* and *P. bibronii*. We took initial energy content of an egg, $E_0$, to be equal to the observed mean energetic content of the egg for both species (Section 3.1, Table 1). We excluded water dynamics from the analysis by using dry biomass (body plus gut) and assumed, for the feeding stages, constant food availability (equivalent to constant food density in the environment). Each stage is quantified by the cumulated energy invested to reach that stage, known as maturity level, $E_M$. The cumulated energy invested to reach each stage is dissipated in the form of minerals ($O2$, $CO2$, $NH3$, $H2O$) and contributes to oxygen consumption. The transitions from embryo to juvenile, called birth, and from juvenile to metamorph are represented by $E_H^{22}$ and $E_H^{46}$, respectively. Birth corresponds with hatching in *P. bibronii* as the species fed immediately, but as *C. georgiana* did not feed immediately upon hatching, the maturity level at hatch ($E_H^{22}$) was included in the estimation routine to compare to empirical data. The effect of temperature on biological rates was described by the Arrhenius relationship. Rate data from this study and Seymour et al. (1991) was used for *P. bibronii*, with a resultant Arrhenius temperature of 12,000 K (online Supplementary material, Fig. S3(c)). Due to limited rate data for *C. georgiana* we used the same Arrhenius temperature. Further specifications of the model and computation of model $M_O2$ are detailed in the online Supplementary material. Likewise, parameter estimation and additional data that is too extensive to present in the main text of this study are summarized in the online Supplementary material. We use DEB theory terminology such that the term mineral fluxes refers to the fluxes of $O2$, $CO2$, $NH3$ and $H2O$ (Supplementary material, Eqs. (S12, S13)), hatch refers to leaving the egg capsule, birth represents to the onset of feeding and juveniles are equivalent to larvae (juveniles also refers to post-metamorphic stages prior to the onset of reproduction).

The standard DEB model was also applied to data for two additional Australian Myobatrachid amphibians, *C. nimbus* and *C. vitellina*. Both are terrestrial direct developers that do not exogenously feed until after metamorphosis. Data for *C. nimbus* were taken from Mitchell and Seymour (2000) and data for *C. vitellina* from Mitchell (2001).

### 3. Results

#### 3.1. Empirical results

Fresh ova of *C. georgiana* had a similar dry mass to *P. bibronii* (Mann–Whitney $U = 1434$, $P = 0.43$, Table 1). Likewise, energy density of fresh ova was comparable between the two species ($U = 19$, $P = 0.17$), and therefore energy content was similar.

Development was faster in *C. georgiana* than *P. bibronii* (Fig. 2). Metamorphosis was completed by 108 days in *C. georgiana* compared to 185 days for *P. bibronii*. Hatching also occurred earlier in *C. georgiana* (19 ± 2 days) compared to *P. bibronii* (39 ± 2 days). However, *C. georgiana* hatched at $E_H^{22}$ compared to $E_H^{27}$ in *P. bibronii*. Dry gut-free body mass at $E_H^{22}$ was similar between the species ($U = 68$, $P = 0.058$, Table 1), as was dry yolk mass ($U = 208$, $P = 0.08$). At $E_H^{27}$ dry gut-free

## Table 1

Summary empirical data for *Crinia georgiana* and *Pseudophryne bibronii* development at four stages: fresh ovum, $E_H^{22}$ (hatch in *C. georgiana*), $E_H^{27}$ (birth in *C. georgiana*), hatch and birth in *P. bibronii* and $E_H^{46}$ (completion of metamorphosis in both species). Data are presented as mean ± 95% CI (n). Stages: $E_H^i$ in which $i$ refers to Gosner (1960) stages 1–46.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C. georgiana</th>
<th>P. bibronii</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ovum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mass (mg)</td>
<td>2.74 ± 0.12 (91)</td>
<td>2.68 ± 0.11 (69)</td>
</tr>
<tr>
<td>Energy density (J mg⁻¹)</td>
<td>25.6 ± 1.2 (6) a</td>
<td>24.3 ± 0.9 (4) a</td>
</tr>
<tr>
<td>Energy content (J)</td>
<td>70.3</td>
<td>65.0</td>
</tr>
<tr>
<td>Dry gut-free body mass (mg)</td>
<td>0.69 ± 0.06 (56)</td>
<td>0.89 ± 0.15 (5)</td>
</tr>
<tr>
<td>Energy density of body (J mg⁻¹)</td>
<td>20.7</td>
<td>–</td>
</tr>
<tr>
<td>Energy content of body (J)</td>
<td>14.1</td>
<td>–</td>
</tr>
<tr>
<td>Dry yolk mass (mg)</td>
<td>1.98 ± 0.22 (56)</td>
<td>1.40 ± 0.27 (5)</td>
</tr>
<tr>
<td>Energy density of yolk (J mg⁻¹)</td>
<td>26.0 ± 2.5 (2) a</td>
<td>–</td>
</tr>
<tr>
<td>Energy content of yolk (J)</td>
<td>51.4</td>
<td>–</td>
</tr>
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<td>–</td>
</tr>
<tr>
<td>Total $O2$ consumed ($E_H^{22}$) (μmol)</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>$O2$ cost of development ($E_H^{22}$) (μmol mg⁻¹)</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td><strong>$E_H^{27}$</strong></td>
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<td></td>
</tr>
<tr>
<td>Dry gut-free body mass (mg)</td>
<td>1.49 ± 0.20 (5)</td>
<td>1.48 ± 0.06 (167)</td>
</tr>
<tr>
<td>Energy density of body (J mg⁻¹)</td>
<td>–</td>
<td>21.4 ± 0.7 (4) a</td>
</tr>
<tr>
<td>Energy content of body (J)</td>
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<td>31.7</td>
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<tr>
<td>Dry yolk mass (mg)</td>
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<td>0.92 ± 0.05 (167)</td>
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<tr>
<td>Energy density of yolk (J mg⁻¹)</td>
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<td>23.1 ± 0.5 (4) a</td>
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<tr>
<td>Energy content of yolk (J)</td>
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<td>21.2</td>
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<tr>
<td>Total energy content (J)</td>
<td>–</td>
<td>52.8</td>
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<tr>
<td>Total $O2$ consumed ($E_H^{22}$–$E_H^{27}$) (μmol)</td>
<td>22</td>
<td>22</td>
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<tr>
<td>$O2$ cost of development ($E_H^{22}$–$E_H^{27}$) (μmol mg⁻¹)</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td><strong>$E_H^{46}$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry gut-free body mass (mg)</td>
<td>2.52 ± 0.35 (8)</td>
<td>23.19 ± 2.07 (19)</td>
</tr>
<tr>
<td>Energy density of body (J mg⁻¹)</td>
<td>17.1</td>
<td>20.0 ± 0.4 (6) a</td>
</tr>
<tr>
<td>Energy content of body (J)</td>
<td>43.0</td>
<td>464.0</td>
</tr>
<tr>
<td>Dry gut mass (mg)</td>
<td>0.13 ± 0.03 (8)</td>
<td>1.17 ± 0.14 (19)</td>
</tr>
<tr>
<td>Total $O2$ consumed ($E_H^{27}$–$E_H^{46}$) (μmol)</td>
<td>364</td>
<td>2310</td>
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<tr>
<td>$O2$ cost of development ($E_H^{27}$–$E_H^{46}$) (μmol mg⁻¹)</td>
<td>353</td>
<td>106</td>
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<tr>
<td><strong>Overall</strong></td>
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</tr>
<tr>
<td>$O2$ cost of development (μmol mg⁻¹)</td>
<td>394</td>
<td>2388</td>
</tr>
<tr>
<td>$O2$ cost of development (μmol mg⁻¹)</td>
<td>156</td>
<td>101</td>
</tr>
</tbody>
</table>

* n refers to number of bomb calorimetry pellets.
body mass was similar \((U=446, P=0.80)\), but dry yolk mass was greater in \textit{C. georgiana} \((U=55, P<0.001)\). Energy content was measured at hatch in each species (Table 1), so a direct comparison cannot be made due to the different hatching stage. Mass increased quite rapidly during \textit{P. bibronii} juvenile development (Fig. 3b), but this did not occur in \textit{C. georgiana} (Fig. 3a). Upon completion of metamorphosis \((E_{M}^{26})\) the dry gut-free body mass of \textit{P. bibronii} was much larger than \textit{C. georgiana} \((U=152, P<0.001)\). Energy density of the gut-free body was similar, but due to the much larger mass, \textit{P. bibronii} metamorphs had higher energy content (Table 1).

During the majority of embryonic development, \(M_{O_2}\) was higher in \textit{C. georgiana}, especially after \(E_{H}^{22}\) at 19 days when \textit{C. georgiana} hatched. The higher \(M_{O_2}\) was attributed to release from the egg capsule, as the larvae were quiescent and \textit{P. bibronii} was still confined in the egg (Fig. 3c, d). However, juvenile \textit{P. bibronii} increased \(M_{O_2}\) quite rapidly beyond birth to reach a much greater maximum of approximately 1350 nmol h\(^{-1}\) at the onset of metamorphosis compared to 230 nmol h\(^{-1}\) in \textit{C. georgiana} (Fig. 3e, f).

Integration of \(M_{O_2}\) data indicated that embryos of \textit{C. georgiana} consumed more oxygen in total, but produced a similar dry gut-free body mass at \(E_{H}^{22}\), which resulted in a slightly higher mass-specific oxygen cost of development compared to \textit{P. bibronii} (Table 1). Between \(E_{H}^{22}\) and \(E_{H}^{27}\) the oxygen cost of development switched to being higher in \textit{P. bibronii}, due to a lower mass produced in relation to oxygen consumed. However, juvenile oxygen cost of development, from \(E_{H}^{27}\) to \(E_{M}^{46}\), was over three and a half times higher in \textit{C. georgiana}. Overall mass-specific oxygen cost of development was 1.5 times higher in \textit{C. georgiana} compared to \textit{P. bibronii} (Table 1).

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**Fig. 2.** Stage of development as a function of age in \textit{Crinia georgiana} and \textit{Pseudophryne bibronii}. Each stage is represented by \(E_{H}^{i}\) where \(i\) refers to stages 1–46 (Gosner, 1960). The dashed lines indicate first hatch then birth in \textit{C. georgiana} and the solid line represents both hatch and birth in \textit{P. bibronii}, which occur at the same age and stage.

**Fig. 3.** Experimental data (circles) compared to dynamic energy budget model results (lines) for \textit{Crinia georgiana} and \textit{Pseudophryne bibronii}. Experimental data presented as means with 95% CI error bars. Vertical dotted lines indicate first hatching and then birth for \textit{C. georgiana} and birth for \textit{P. bibronii}. (a) and (b) show model output for total dry biomass against age. (c) and (d) show model embryonic \(O_2\) consumption partitioned into different energetic processes against age. (e) and (f) show model \(O_2\) consumption until metamorphosis partitioned into different energetic processes against age. Note the different scales on the axes.
3.2. DEB model

We successfully determined a set of DEB parameters that quantified the development times, dry biomass and $M_{O_2}$ patterns measured throughout $C.\text{georgiana}$ and $P.\text{bibronii}$ development (Table 2). The standard DEB model did not fit the data for $C.\text{georgiana}$ so we extended the model by allowing the allocation fraction $\kappa$ to decrease at a constant rate after hatching until birth. The decrease in $\kappa$ occurred as a function of maturity (online Supplementary material) rather than structure so that it could be linked to developmental stages, returning to the original value only after metamorphosis. This has the effect of accelerating maturation at the expense of growth, while enhancing respiration considerably. The model results are consistent with the entire life history of the species (Tables S2, S3). The chemical potential and molar weights of reserve and structure were taken to be identical for both species (Table S1), which together with the similar initial dry mass of the ova, indicate that $C.\text{georgiana}$ and $P.\text{bibronii}$ had near-identical initial conditions in the model.

Model outputs for dry biomass and $M_{O_2}$ against age are well matched to empirical results (Fig. 3). The model captured the abrupt increase in $M_{O_2}$ at birth in both species as the onset of assimilation involves a conversion of food into reserve, which comes with overhead costs. This is likely the case for $C.\text{georgiana}$, but $P.\text{bibronii}$ was not fed prior to the first $M_{O_2}$ measurement after birth (which corresponds to hatching), and so the observed increase in $M_{O_2}$ is attributed to the removal of the diffracive barrier of the egg capsule (Mueller and Seymour, 2011).

Model $M_{O_2}$ was partitioned into contributions from somatic maintenance, growth, maturation (and maturity maintenance) and assimilation (for juveniles) (Fig. 3c, d, e, f). Supplementary material Eqs. (S14–171). Throughout embryonic development $C.\text{georgiana}$ allocated more oxygen to growth than did $P.\text{bibronii}$ (Fig. 3c, d). Between $C.\text{georgiana}$ hatching and birth the fraction $\kappa$ of mobilized reserve allocated to growth and somatic maintenance decreased from 0.86 at $E_{H}^{22}$ to 0.61 at $E_{H}^{27}$ (Table 2); the complementary fraction (1−$\kappa$) to maturation and maturity maintenance thus increased, meaning an acceleration of maturation. This resulted in $\kappa$ being initially higher in $C.\text{georgiana}$ during embryogenesis, before falling below the constant $\kappa$ of $P.\text{bibronii}$ by birth (Table 2). This switch in $\kappa$ matched well with the decrease in biomass and rapid increase in $M_{O_2}$ that occurred between hatch and birth in $C.\text{georgiana}$ (Fig. 3a, e). The contribution to maturation increased between $E_{H}^{22}$ and $E_{H}^{27}$ while the contribution to growth declined and continued to do so throughout juvenile development (Fig. 3e) due to limited feeding, quantified by a low scaled functional response $f$ (Table S2).

In comparison, $P.\text{bibronii}$ continued to partition energy by the same proportion after birth so that there was no rapid increase in contribution to maturation (Fig. 3f).

The model determined that 14 $\mu$mol and 9 $\mu$mol of oxygen were consumed to reach $E_{H}^{22}$ in $C.\text{georgiana}$ and $P.\text{bibronii}$, respectively. Between $E_{H}^{22}$ and $E_{H}^{27}$ the predicted total oxygen consumed was 37 $\mu$mol in $C.\text{georgiana}$ and 34 $\mu$mol in $P.\text{bibronii}$. The values for both intervals were higher than empirical data (Table 1). Predicted total oxygen values for juvenile development, between $E_{H}^{27}$ and $E_{H}^{46}$, were 307 and 2095 $\mu$mol in $C.\text{georgiana}$ and $P.\text{bibronii}$, respectively, which were lower than empirical data. The overall predicted total oxygen consumed was 358 and 2138 $\mu$mol in $C.\text{georgiana}$ and $P.\text{bibronii}$, respectively. Both values are within 90% of empirical calculations (Table 1).

Until $E_{H}^{22}$, $C.\text{georgiana}$ partitioned a greater percentage of total oxygen to growth and less to maturation than $P.\text{bibronii}$ (Fig. S6). The decrease in $\kappa$ between $E_{H}^{22}$ to $E_{H}^{27}$ in $C.\text{georgiana}$ resulted in an increase in the percentage of oxygen allocated to maturation, compared to the slight decrease in $P.\text{bibronii}$. From $E_{H}^{27}$ to $E_{H}^{46}$ the difference in partitioning between the two species was most pronounced, with $C.\text{georgiana}$ partitioning 37% of its total oxygen to maturation while $P.\text{bibronii}$ partitioned only 28%. The decrease in allocation to growth, and increase in somatic maintenance, as development progresses are also clear in both species. For the entire development period until metamorphosis, $C.\text{georgiana}$ allocated 7% more of its total oxygen to maturation, 9% more oxygen to growth and 8% less to somatic maintenance than $P.\text{bibronii}$.

DEB parameters were also successfully estimated for two direct developers, $C.\text{nimbus}$ and $G.\text{vitellina}$ on the basis of published literature (Table 2). DEB model predictions for life history traits (age at birth, puberty, etc.) are summed up in Tables S4 and S5. Both $C.\text{nimbus}$ and $G.\text{vitellina}$ had lower embryo energy conductance ($V$) values than the other two species, but the value was particularly low for $C.\text{nimbus}$. A decrease in $\kappa$ described the increase in $M_{O_2}$ after hatch in $C.\text{nimbus}$, similar to $C.\text{georgiana}$, however $\kappa$ was much lower in $C.\text{nimbus}$. Further details of parameter estimation and results can be found in the online Supplementary material and the parameter estimation routines are freely downloadable from the Add_My_Pet collection (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/).

4. Discussion

At fertilization the dry biomass and energy density of the eggs of $C.\text{georgiana}$ and $P.\text{bibronii}$ are equivalent (Table 1). Therefore, the

Table 2

<table>
<thead>
<tr>
<th>Symbol</th>
<th>C. georgiana</th>
<th>P. bibronii</th>
<th>C. nimbus</th>
<th>G. vitellina</th>
<th>Unit</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$</td>
<td>547</td>
<td>565</td>
<td>263</td>
<td>565</td>
<td>J $d^{-1}cm^{-2}$</td>
<td>Maximum surface area specific assimilation rate</td>
</tr>
<tr>
<td>$V$</td>
<td>0.056</td>
<td>0.040</td>
<td>0.009</td>
<td>0.017</td>
<td>cm $d^{-1}$</td>
<td>Embryo energy conductance</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>0.86</td>
<td>0.69</td>
<td>0.50</td>
<td>0.43</td>
<td>$cm$</td>
<td>Fraction of energy allocated to soma at hatch/birth</td>
</tr>
<tr>
<td>$E_{H}$</td>
<td>0.61</td>
<td>0.69</td>
<td>0.30</td>
<td>0.43</td>
<td>$cm$</td>
<td>Fraction of energy allocated to soma at hatch/birth</td>
</tr>
<tr>
<td>$\mu$</td>
<td>368</td>
<td>491</td>
<td>205</td>
<td>170</td>
<td>J $d^{-1}cm^{-3}$</td>
<td>Volume specific somatic maintenance</td>
</tr>
<tr>
<td>$k_{s}$</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>J $d^{-1}$</td>
<td>Maturity maintenance rate</td>
</tr>
<tr>
<td>$E_{c}$</td>
<td>6502</td>
<td>5397</td>
<td>5185</td>
<td>5185</td>
<td>J $cm^{-3}$</td>
<td>Cost of synthesis of structure</td>
</tr>
<tr>
<td>$E_{22}$</td>
<td>1.5</td>
<td>0.8</td>
<td>9.0</td>
<td>0.0</td>
<td>J</td>
<td>Cumulated energy invested in maturity at hatching</td>
</tr>
<tr>
<td>$E_{27}$</td>
<td>7.7</td>
<td>9.2</td>
<td>60.1</td>
<td>54.5</td>
<td>J</td>
<td>Cumulated energy invested in maturity at birth</td>
</tr>
<tr>
<td>$E_{46}$</td>
<td>70.8</td>
<td>313.5</td>
<td>60.1</td>
<td>54.5</td>
<td>J</td>
<td>Cumulated energy invested in maturity at metamorphosis</td>
</tr>
</tbody>
</table>
species have the same amount of initial energy at their disposal for embryonic development. Despite these similarities, the time to reach hatching is much shorter in *C. georgiana* (Fig. 2). However, in contrast to previous studies that reported hatching at $E_P^{27}$ for both species (*Seymour and Roberts, 1995; Seymour, 1999*), this study found that *C. georgiana* hatches at $E_P^{21}$. The discrepancy in reported hatching stage in *C. georgiana* may be due to the rapid development of stages $E_P^{21}$–$E_P^{23}$ (Fig. 2), as these stages are difficult to recognize at 15 °C (*Seymour and Roberts, 1995*). At hatching *C. georgiana* has yet to develop eyes, mouthparts and intestinal loops at 12 °C, all of which usually occur at $E_P^{21}$–$E_P^{27}$, but they do have hindlimb buds, which normally form at $E_P^{26}$ (*Gosner, 1960*). However, the development of mouthparts and intestinal loops was used to assign stages, so hatching is considered to occur at $E_P^{22}$ in *C. georgiana*. Therefore, a comparison of time to hatching is less informative than time to metamorphosis ($E_R^{20}$) (Fig. 2).

Empirical data and the DEB model indicate that the two species invest vastly different amounts of energy to reach metamorphosis (Table 2). Embryonic $M_O$ does differ somewhat between the species (Fig. 3c, d), but it is during juvenile development that $M_O$ greatly diverges, with a much higher maximum $M_O$ in *P. bibronii* compared to *C. georgiana* (Fig. 3e, f). The substantial difference in $M_O$ between the two species is directly related to their different development rates, energy partitioning and feeding.

With the capacity to explicitly include maturation as an energetic process, DEB theory improves our ability to model energy partitioning. The relationship between maturity level, a concept of DEB theory, and morphologically defined stage of development, allows us to think about development as an energy allocation strategy. The developmental stages of *C. georgiana* and *P. bibronii* are linked to maturity levels (Fig. S4), as specified by DEB theory, in a similar way to the zebrafish (*Danio rerio*) (*Augustine et al., 2011*).

The juvenile stages of *C. georgiana* and *P. bibronii* provide the greatest insight into the effects of energy partitioning on development. After hatching, *C. georgiana* begins to partition more energy into maturation (Figs. 3, S6), so that the species completes metamorphosis 1.7 times faster than *P. bibronii* (Fig. 2). In comparison, *P. bibronii* juveniles continue the same energy use strategy as the embryos (Fig. 3d, f) and, as a consequence, the percentage of oxygen used for maturation decreases (Fig. S6) and development is slower. The increase in energy allocation to maturation (indicated by a decrease in model $k$ in *C. georgiana* is matched by a rapid increase in $M_O$ (Fig. 3a, e), an unusual biomass pattern as growth is reduced (Fig. 3a), and much smaller metamorphs than *P. bibronii* (Table 1). This large difference in juvenile tissue contributes to the lower $M_O$ of *C. georgiana* (Figs. 3, S5). Both species were fed ad libitum during juvenile stages but *P. bibronii* consumed noticeably more exogenous food than *C. georgiana*, allowing for a higher growth rate. This is reflected in the higher scaled functional response, $f$, which relates ingestion as a function of food availability in the model (*Kooijman, 2010*) (Tables S2, S3). Food availability was the same for both species, therefore the difference in $f$ reflects differences in feeding. As adult characteristics of *C. georgiana* and *P. bibronii*, such as ultimate length, mass and reproduction rate, are similar between the two species (*Barker et al., 1995; Main, 1957; Smith and Roberts, 2003; Woodruff, 1976*), $k$ is modeled as switching back to the pre-hatch level at metamorphosis, allowing for increased allocation to growth in *C. georgiana*. Further research is required to confirm that this occurs.

The difference in energy partitioning between *C. georgiana* and *P. bibronii* influences the total oxygen required to complete metamorphosis. Total oxygen required during embryogenesis does not vary much between the species but the total oxygen required by *P. bibronii* juveniles is significantly higher than *C. georgiana* due to their greater mass (Table 1, Fig. 3a, b). The mass-specific oxygen cost of development is determined using a method of previous studies, in which the total oxygen consumed, or energy used, is divided by gut-free dry mass produced (*Whitehead and Seymour, 1990; Vleck and Hoyt, 1991; Thompson and Russell, 1999; Booth and Astill, 2001; Mueller et al., 2011*). The oxygen cost of development to reach $E_R^{22}$ and between $E_R^{22}$ and $E_R^{27}$ is not overly different between the species, but the oxygen cost of building 1 mg of juvenile gut-free dry mass is much higher in *C. georgiana*, which increases overall mass-specific oxygen cost (Table 1). The higher mass-specific oxygen cost indicates that the low $M_O$ of *C. georgiana* juveniles is coupled by a disproportionately lower mass production. This indicates that not only do differences in growth, and therefore mass, influence total $O_2$ used, but also the species must have different energy requirements for other processes, such as maturation. The benefits of faster development must outweigh the higher mass-specific $O_2$ cost of development of *C. georgiana*.

The trade-off between *C. georgiana* juvenile maturation and growth can be related to the species' reproductive mode. The earlier hatching stage in *C. georgiana*, together with its faster development to metamorphosis, is in accordance with the trend seen in other aquatic breeding species (*Bradford, 1990*), despite its similar egg size with *P. bibronii*. The species lays its eggs in ephemeral pools so fast development is advantageous in escaping the pools before they dry. Aquatic predation may also promote fast development, however no aquatic predators have been observed in the pools in which *C. georgiana* develops (*Doughty and Roberts, 2003*). A deteriorating incubation environment may contribute to the ability to decrease energy allocated to growth after hatch. While hatching and birth coincide in *P. bibronii*, hatching is well before birth in *C. georgiana*. Upon hatching, *C. georgiana* apparently detects water level which acts as an environmental trigger for faster development. In shallow water the species metamorphoses faster than in deeper water, and only when resource availability is high under such conditions is the species able to increase growth and maturation concurrently (*Doughty, 2002; Doughty and Roberts, 2003*).

The environmental cue of water level takes effect upon hatching, which matches the timing of the change in $\kappa$. Prior to hatching $\kappa$ is high so that *C. georgiana* can produce a certain amount of tissue before it switches to the energy use strategy which disfavors growth. Juveniles of *C. georgiana* did feed during this study but they are able to complete metamorphosis without feeding and therefore without large increases in mass (*Doughty, 2002; Doughty and Roberts, 2003*) and this strategy would be difficult without an initial mass buffer.

Water depth (– 3 cm) during *C. georgiana* juvenile development in this study was higher than the depths (0.5–2 cm) used by *Doughty* (2002) and *Doughty and Roberts* (2003). It is unknown if the change in $\kappa$ is a direct response to this water level or if the change in energy allocation is an adaptation to development in ephemeral pools. Further investigations of *C. georgiana* developmental energy budgets under varying water depths and food availability are required to examine the relationship between $\kappa$ and environmental cues.

Facultative feeding is possible due to the relatively large eggs of *C. georgiana*, which provide more reserves for development (*Doughty, 2002*). The large eggs and facultative feeding of *C. georgiana* may be hypothesized as representing a development mode that lies somewhere in between the ancestral amphibian development of aquatic obligate feeding juveniles and direct development, in which all nutrition is provided by the yolk and small metamorphs emerge directly from large eggs. A reduction in feeding and growth, as described by a changing $\kappa$ value, may reflect a transition in developmental strategy.

To explore if the change in $\kappa$ in *C. georgiana* but constant $\kappa$ in *P. bibronii* is related to their different development modes, DEB theory was applied to two terrestrial-breeding direct developing Myobatrachid frogs, *C. nimbus* and *C. vitellina*. Both *C. nimbus* and *C. vitellina* have lower embryo energy conductance ($\nu$) values than the other two species, but the value is particularly low for *C. nimbus* (Table 2). A decrease in $\nu$ describes the increase in *C. nimbus* $M_O$, similar to *C. georgiana*, however $\kappa$ is much lower in *C. nimbus*. In comparison, $\kappa$ does not change in *C. vitellina*. Such a difference between two species
using a very similar mode of direct development suggests there may be no relationship between a direct development strategy and a change in $\kappa$. Furthermore, C. nimbus and C. vitellina have appreciably different incubation environments and egg sizes.

The slow incubation of large C. nimbus eggs in subalpine conditions at 5 to 15 °C is described by the DEB model via a low value for embryo energy conductance, even when temperature is corrected. Low energy conductance means slow mobilization of reserve, a high reserve capacity and the ability to survive starvation. This low value is likely a result of oxygen limitation of a large egg capsule, which confines C. nimbus to a cool climate and slows development (Seymour and Bradford, 1995; Mitchell and Seymour, 2003). Metamorphs can emerge before winter or juveniles overwinter and emerge in spring, but the cost of overwintering is greater (Mitchell and Seymour, 2000).

In comparison, G. vitellina develops in a temperate environment over spring and summer when nest temperatures can reach 20 °C (Mitchell, 2001). The increase in $\kappa$ in C. nimbus may reflect the greater instability in its environment and an increase in allocation to maturation increases the likelihood that development will be complete before winter. The smaller eggs in G. vitellina, in combination with a larger $\nu$, result in an already inherently faster development rate and so they, as in P. bibronii, are not under a selective pressure to increase energy allocation to maturation.

The model results for this study provide strong empirical support for the maturation concept of DEB theory (Kooijman, 2010), in which mobilized reserve not used for growth (and somatic maintenance) is used for maturation (and maturity maintenance) (Fig. 1). Juvenile C. georgiana convincingly demonstrate how energy used for maturation is dissipated as minerals (CO$_2$, NH$_3$, H$_2$O) without contributing to biomass. The standard DEB model describes the empirical data for P. bibronii and G. vitellina rather well. This standard model includes a constant $\kappa$ value, which was found to be the best model construct for describing energy use (Lika and Kooijman, 2011). However, C. georgiana and C. nimbus depart from the expected pattern with an increase in metabolism. This divergence is best explained in the model by a changing $\kappa$ value, supporting the notion that dissipation for maturity increases.

A changing $\kappa$, while a slight deviation from the standard DEB model, is not unique to C. georgiana or C. nimbus. $\kappa$ also changes in response to photoperiod in the pond snail, Lymnaea stagnalis (Zonneweld and Kooijman, 1989) and $\kappa$ may be influenced by other environmental and physiological factors (Kooijman, 2010). While the two Crinia species show a change in allocation to accelerate maturation before the onset of assimilation, quite a few other species, such as some fish and bivalves, show an acceleration of metabolism as a whole after the onset of assimilation (Pecquerie, 2007; Augustine et al., 2011; Jusup et al., 2011; Kooijman et al., 2011). In these examples, the surface area-specific assimilation and searching rates, as well as $\nu$, increase during a certain confined period, while $\kappa$ and other parameters remain constant. This not only leads to acceleration of development, but also of growth, unlike what we see in Crinia.

In conclusion, we have shown how explicit consideration of the trade-offs between maturation and growth via DEB theory helps to interpret the energetic consequences of accelerated development. In the context of DEB theory, $O_2$ consumption, a common measure of metabolic rate, can be broken down into different energetic processes and thus better understood. We have shown that an increase in respiration is not always associated with an increase in growth. In the case of C. georgiana and C. nimbus, time constraints placed upon their development by their incubation environments may act as triggers for an increase in energy allocated to maturation, with a resultant increase in metabolism. Empirical data and corresponding DEB parameter estimations for additional species, as well as within species under different environmental conditions, are now required for future investigation of the selective pressures that result in acceleration of metabolism during development.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpa.2012.05.190.

References


