The effect of egg size on hatch time and metabolic rate: theoretical and empirical insights on developing insect embryos

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Running title: Insect egg size and embryonic development

Summary:

1. Body size scaling relationships allow biologists to study ecological phenomena in terms of individual level metabolic processes. Recently dynamic energy budget theory has been shown to offer novel insights on the effect of body size on biological rates.
2. We test whether dynamic energy budget theory and its unique partitioning of biomass into reserve and structural components can explain the effect of egg size on hatch time and the time course of respiration in insect embryos.
3. We find that without any parameterisation (calibration), DEB theory is able to predict hatch time for eggs sizes spanning four orders of magnitude from fundamental biological processes.

4. We find, however, that the standard DEB model poorly predicts the timecourse of respiration, particularly in early embryonic development where a strong effect of egg size is observed. Further, we show that other theoretical models also poorly predict early embryonic respiration.

5. By modifying the assumption that a fresh egg is entirely reserve, we show that embryonic respiration and hatch time can be better predicted by the DEB model.

6. Useful theories in metabolic ecology, such as DEB theory, can help explain universal scaling patterns in development times. However, simple theoretical models must be expanded if they are to capture the scaling of metabolic rate in insect eggs.

Key-words:
Body size, Dynamic Energy Budget (DEB) theory, embryo, invertebrate, oxygen consumption, respiration

Introduction
Body size has long been known to correlate strongly with biological rates, whether they be rates of evolution or nutrient turnover in ecosystems (Kleiber 1932; Peters 1986; Brown et al. 2004). However, the biological basis of these relationships has been the subject of much intellectual effort and debate (Agutter & Wheatley 2004; Glazier 2005; Isaac & Carbone 2010; Maino et al. 2014). Since at least the 19th century, the scaling of biological rates has been predicted to scale more slowly than body size due to simple physical reasoning based on the mismatched scaling of surface area and volume mediated processes (Sarrus & Rameaux 1839; Hulbert 2014). More recently, the surface area scaling relationship has been replaced in favour of quarter power scaling relationships (Kleiber 1932; Hemmingsen 1960; Savage et al. 2004) with some groups proposing other physical constraints on nutrient supply systems that result in the observed quarter-power scaling relationships (Sernetz, Gelléri & Hofmann 1985; West, Brown & Enquist 1997; Maino et al. 2014). However, regardless of the precise scaling exponent, it is widely accepted that biological rates tend to decrease with size, while biological times (the reciprocal) increase (Calder 1984; Kooijman 1986; Gillooly et al. 2002).
An explanation of body-size scaling relationships that is receiving increasing attention is given by dynamic energy budget (DEB) theory (Kooijman & Metz 1984; Kooijman 2010; Maino et al. 2014; Maino & Kearney 2014b, 2015a, b). DEB theory considers the whole lifecycle of the organism, including embryonic development. The vast majority of multicellular species are oviparous, which has a simple interpretation within the context of a DEB theory. A freshly laid egg is viewed as consisting entirely of ‘reserve’, which is defined as a generalised chemical compound with a fixed stoichiometry and energy content. DEB theory explains egg development as the transformation of reserve to another generalised compound with different dynamics called ‘structure’ (see Figure S1 in Supporting Information). Structure may have a different composition to reserve but always differs from reserve in that it has a maintenance rate and fixed turnover. The standard DEB model’s assumption that a fresh egg consists entirely of reserve is made for pragmatic reasons as it removes a model parameter (initial structure), and has been justified on the basis that freshly laid eggs for diverse taxa have very low respiration. For example, plant seeds (analogous to eggs in DEB theory) may lay dormant for years before development begins. However, there also exists evidence which does not support this assumption, such as substantial oxygen consumption rates in unfertilized eggs (Whitaker 1931; Borei 1948; Horwitz 1965; Tyler, Tyler & Piatigorsky 1968; Davenport & Lonnig 1980). This evidence will be discussed later in more detail.

Reserve and structure are preferable to mass as state variables in DEB models because mass is an unreliable indicator of metabolic state (Maino et al. 2014). In eggs, for example, there is little change in mass, despite significant energetic and developmental transformations (Maino & Kearney 2014b). In the same way, the relative contribution of reserve and structure to biomass allows a starved animal and a well-fed animal of identical weights to be distinguished metabolically. After an egg hatches and the juvenile begins feeding, DEB theory assumes assimilated food is used to replenish reserve at a rate that depends on the amount of structure, while reserve continues to fuel the growth of structure. In this way, DEB theory conceptualises reserve as a generalised chemical intermediate between food and structure. An increased number of reserve and structural compartments can be added to capture more complex dynamics, such as in plants where mineral and carbohydrate availability are highly independent (Chapin, Schulze & Mooney 1990). However, for many heterotrophic organisms, a one reserve and one structure model is sufficient (Kooijman 2010). These core concepts of the DEB framework are somewhat abstract in order to capture the
metabolic organisation of diverse taxa. This, however, does not preclude rigorous testing of the theory. Kearney and White (2012) outline several testable hypotheses that emerge from DEB theory. Some of these hypotheses have been tested, such as predicted differences between intra- and inter-specific scaling, which have been found to support predictions deriving from DEB theory (White et al. 2011; Maino & Kearney 2015a).

Here we test whether we can use DEB theory to model the metabolism of insects during embryonic development. We show that an uncalibrated DEB model can predict egg hatch times over large mass ranges (five orders of magnitude), but highlight that the predicted timecourse of respiration until hatch (for 16 species) can be improved by incorporating a larger starting amount of structure. While insects are used as a case study, the presented model applies to all oviparous organisms.

Materials and methods

Model formulation

DEB theory traditionally assumes a freshly laid egg to be entirely reserve, which specifies the initial conditions of the integration with starting reserve equal to the mass of the egg and starting structure equal to zero. The point of hatching can be specified by assuming that hatching occurs after an egg loses a certain percentage of its starting dry mass. This departs from the standard DEB model, which uses the state variable of maturity as a trigger for stage transitions (Kooijman 2010). In the standard model, maturity represents energy that is expended in increasing the functional complexity of an organism, without increasing the energy content of the organism (e.g. the development and maintenance of immune systems). However, our simplifying assumption is approximated by the standard DEB model, which predicts neonate mass and egg mass to scale in proportion for very small and large masses (Maino et al. 2014). It can also be shown empirically, for at least several species for which data exist, that the ratio of hatching mass to freshly laid egg mass is approximately constant across species (see Table 1). During egg development, the depletion of reserve and growth of structure are specified by the standard DEB model without maturity. Reserve is depleted at a rate equal to the reserve mobilisation rate $\dot{p}_C$ (J/d), which is the total energy flux available for metabolism. The energy flux allocated to structural growth is equal to the mobilisation of reserve $\dot{p}_C$ after subtracting the maintenance of structure, $\dot{p}_M$ (J/d). Thus, the change in reserve $E$ (J), and structure $V$ (cm$^3$) through time are given by the differential equations:
\[
\frac{dE}{dt} = -\dot{p}_c
\]  

(1)

\[
\frac{dV}{dt} = (\dot{p}_c - \dot{p}_M)/[E_o]
\]  

(2)

where \(\dot{p}_M\) is equal to the volume specific maintenance rate \([\dot{p}_M]\) (J/d/cm\(^3\)) multiplied by structure or \(\dot{p}_M = [\dot{p}_M]V\); the cost per unit structure is \([E_c]\) (J/cm\(^3\)), which includes both the overhead costs of biosynthesis and the resulting energy stored as synthesised biomass; and \(\dot{p}_c\) is given by the formula:

\[
\dot{p}_c = E [E_c]\dot{\nu}/V^{\frac{1}{3}} + [\dot{p}_M]/E/V + [E_c]
\]  

(3)

The conductance parameter \(\dot{\nu}\) (cm/d) relates to the turnover of reserve. Equation 3 is fully derived in the online supplement (Appendix S1), but here we note that the 1/3 exponent in the numerator is a consequence of the central role that surface area and volume-based processes in DEB theory (1/3 emerges from the ratio of the two). The energy available for metabolism \(\dot{p}_c\) is a function of the state variables structure and reserve, and this function does not change throughout embryonic, immature and reproductive phases. Importantly, none of these variables depend on egg size, except the starting amount of reserve. Thus, the scaling of hatch time with egg size can be predicted by modifying the starting amount of reserve, which can be determined by simply measuring egg size.

**Table 1.** The ratio of egg mass at hatching to egg mass at lay \((\kappa_H)\) is calculated from published values

<table>
<thead>
<tr>
<th>Order</th>
<th>Species</th>
<th>Dry egg weight (mg) at egg lay</th>
<th>Dry egg weight (mg) at hatching</th>
<th>(\kappa_H): ratio of egg mass at hatch:lay</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthoptera</td>
<td><em>Poecilocerus pictus</em></td>
<td>4.1</td>
<td>3.33</td>
<td>0.812</td>
<td>(Delvi &amp; Pandian 1972)</td>
</tr>
<tr>
<td>Coleoptera</td>
<td><em>Cryptolestes ferrugineus</em></td>
<td>0.0030</td>
<td>0.0025</td>
<td>0.833</td>
<td>(Campbell &amp; Sinha 1978)</td>
</tr>
<tr>
<td>Coleoptera</td>
<td><em>Rhycopertha dominica</em></td>
<td>0.0047</td>
<td>0.0042</td>
<td>0.894</td>
<td>(Campbell &amp; Sinha 1978)</td>
</tr>
<tr>
<td>Coleoptera</td>
<td><em>Triobolium castaneum</em></td>
<td>0.0197</td>
<td>0.0172</td>
<td>0.873</td>
<td>(Klekowski, Prus &amp; Zyromska-Rudzka 1967)</td>
</tr>
<tr>
<td>Coleoptera</td>
<td><em>Mellasoma collaris</em></td>
<td>0.298</td>
<td>0.266</td>
<td>0.893</td>
<td>(Hågvar 1975)</td>
</tr>
<tr>
<td>Coleoptera</td>
<td><em>Sitophilus granarius</em></td>
<td>0.0112</td>
<td>0.0098</td>
<td>0.875</td>
<td>(Campbell, Singh &amp; Sinha 1976)</td>
</tr>
</tbody>
</table>
We measure total biomass in units of energy (J) so structure, which is given as volume, must be converted to joules and added to the amount of reserve (already in joules). To convert volumetric structure to units of energy it is multiplied by the energy invested in building a unit volume of structure $[E_g]$, multiplied by the proportion of energy that is not expended as overhead costs in forming structure $\kappa_G$. This gives us an expression for biomass energy $M$ in terms of reserve and structure:

$$
M = E + V [E_g] \kappa_G
$$

Using the initial conditions of $E_0 = M_0$ and $V_0 = 0$, the evolution of structure and reserve through time can be calculated using Equations 1 and 2, where hatching is triggered when egg biomass (Equation 4) drops to some proportion of the starting biomass or, equivalently, when $M = \kappa_H M_0$. With the exception of the parameter $\kappa_H$, which is taken as 0.83 from published values (Table 1), all parameters are the default parameters of the standard DEB model and are given in Table 2. These default parameters are considered typical for a generalised animal and are the usual uncalibrated starting point before the fine tuning required in fitting a full lifecycle DEB model to a species (Lika, Kearney & Kooijman 2011).

This integration is performed numerically in R using the deSolve library (Soetaert, Petzoldt & Setzer 2010).

**Table 2. Uncalibrated parameter list for embryonic DEB model**

<table>
<thead>
<tr>
<th>Description</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfixed parameter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg size</td>
<td>$M_0$</td>
<td>-</td>
<td>J</td>
</tr>
<tr>
<td>Fixed parameters*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific maintenance</td>
<td>$\bar{\rho}_M$</td>
<td>18</td>
<td>J cm$^{-3}$ d$^{-1}$</td>
</tr>
<tr>
<td>Cost of structure</td>
<td>$[E_g]$</td>
<td>8400</td>
<td>J cm$^{-3}$</td>
</tr>
<tr>
<td>Conductance</td>
<td>$\bar{V}$</td>
<td>0.02</td>
<td>cm d$^{-1}$</td>
</tr>
<tr>
<td>Growth efficiency</td>
<td>$\kappa_G$</td>
<td>0.80</td>
<td>dimensionless</td>
</tr>
<tr>
<td>Hatch ratio</td>
<td>$\kappa_H$</td>
<td>0.83</td>
<td>dimensionless</td>
</tr>
</tbody>
</table>

*Fixed parameters are set at the default values given in Lika et al. (2011) for a generalised animal with 70% water content, except for $\kappa_H$ (see Table 1)
In the same way, respiration $MR$ (J/d) is predicted as the sum of growth overheads and structural maintenance or:

$$MR = [\dot{p}_H]V + \frac{dV}{dt}[E_G](1 - \kappa_G)$$

The assumption that a fresh egg is 100% reserve can be relaxed by allowing a fresh egg to consist of some starting proportion of structure, $\kappa_V$. This change only affects the initial conditions of the integration and has the advantage that the introduced parameter $\kappa_V$, like other parameters in the model, does not depend on egg size. In addition, the standard DEB assumption can be viewed as a special case of this modified model where $\kappa_V = 0$.

Past analyses have suggested that predicted respiration is sensitive to starting amounts of structure (Maino & Kearney 2014a), so in addition to the commonly assumed starting structure of 0% (standard DEB model) we also chose a starting value of 5% to represent some small amount of starting structure (with this value later tested in a sensitivity analysis) to explore the consequences of this assumption.

We compare the explanatory power of this model with other theoretical models in the literature (Smith 1957; Gillooly et al. 2002; Kooijman 2010; Maino & Kearney 2014; see discussion) on the basis of how much variance in the data was explained and Akaike Information Criteria values for each competing model (Burnham & Anderson 2002). These alternate models predict embryonic respiration to vary with time as some polynomial function (with a highest order of three) and can be thought of as special cases of a third-degree polynomial function, which we represented by a best-fit third-degree polynomial function.

**Data set**

Data used in the analyses of hatch times and egg size were taken from the literature (68 studies) and compiled in a new dataset deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.pn1j6 (Maino, Pirtle & Kearney 2016). An attempt to represent a large taxonomic diversity was made resulting in data on 98 species from 12 insect orders (Coleoptera, Hemiptera, Lepidoptera, Orthoptera, Megaloptera, Hymenoptera, Diptera, Ephemeroptera, Heteroptera, Odonata, Plecoptera, and Trichoptera). The range of egg masses in the data set spans five orders of magnitude. Often egg masses were calculated from size...
measurements and the appropriate volume formula (sphere, ellipsoid, etc.) assuming a wet weight density of 1 g/ml. Egg size was standardised to units of energy (J) and, when necessary, converted assuming a water content of 70% and energy density of 25 J/mg dry mass. Where development rates at multiple temperatures are given, only one temperature was taken closest to the midpoint of the reported range of experimental temperatures that permit normal development.

Egg respiration was taken from a data set recently published by authors of the present study (Maino & Kearney 2014b). Respiration is given in units joules per day. All times and rates are standardised to a constant temperature of 20°C ($T_{\text{ref}} = 293K$) using the Boltzmann-Arrhenius equation with the Arrhenius temperature parameter ($T_A$) set to 8000K (Gillooly et al. 2001). This equation does not have a strong mechanistic underpinning, but adequately describes the empirical relationship of the temperature dependence of biological rates. The temperature standardisation factor ($\dot{k}$) can be calculated as $\dot{k} = \exp \left( \frac{T_A}{T_{\text{ref}}} - \frac{T_A}{T} \right)$ where $T$ is the measurement temperature in Kelvin.

**Results**

*Timecourse of Respiration*

Observations of the timecourse of respiration for 16 insects is shown in Fig. 1A. Both forms of the DEB model considered (0% and 5% starting structure) predict a positive relationship between time and respiration rate. Moreover, both correctly predict that larger eggs will exhibit a higher maximum respiration rate. However, the standard DEB model, assuming 0% starting structure, tends to severely under-predict early respiration, with all data points higher than predicted until roughly day three (Fig 1E). Regardless of egg size, the model predicts respiration rates will follow the same trajectory until insect species begin to diverge around day five (Fig. 1C). Interestingly, while the standard model predicts no size effect in early embryonic development, Fig. 1B shows that there is a significant positive relationship between day one respiration rate and egg size in our data set ($p < 0.001$). Day one respiration scales positively with egg mass raised to an exponent of $1.21 \pm 0.45$ (95% confidence interval). When the DEB model is modified to include 5% structure in a fresh egg, the model predicts this positive effect of egg size on early respiration (Fig. 1B). Moreover, the modified DEB model better fits the observed timecourse of respiration (Fig. 1D and 1F). When
compared against a third-degree polynomial function (Table 3), the modified DEB model explained higher levels of variance in the timecourse of respiration, and was far more likely to be the ‘best’ model as determined by AIC scores. In contrast, the standard DEB model performs no better than a third-degree polynomial function.

Table 3. Statistical comparison of model fits

<table>
<thead>
<tr>
<th></th>
<th>Time course of respiration (Figure 1C and 1D)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>variance explained</td>
<td>AIC weight</td>
<td></td>
</tr>
<tr>
<td>Third-order polynomial</td>
<td>0.102</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>DEB (0% structure)</td>
<td>0.139</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>DEB (5% structure)</td>
<td>0.346</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Statistical comparison of model fits

<table>
<thead>
<tr>
<th></th>
<th>Hatch time (excluding univoltine aquatic) (Figure 2)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>variance explained</td>
<td>AIC weight</td>
<td></td>
</tr>
<tr>
<td>Power function</td>
<td>0.374</td>
<td>0.927</td>
<td></td>
</tr>
<tr>
<td>DEB (0% structure)</td>
<td>0.282</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>DEB (5% structure)</td>
<td>0.309</td>
<td>0.059</td>
<td></td>
</tr>
</tbody>
</table>

**Hatch Time**

As found by Gillooly and Dodson (2000b), univoltine aquatic insects (consisting of Plecoptera, Odonata, Tricoptera and Ephemeroptera) exhibit much longer hatch times than all other insects. Despite our addition of 64 insects to Gillooly and Dodson’s data set of 34 insects, there was still no overlap between data from univoltine aquatic insects and all other insects, which is confirmed statistically by the lack of overlap in confidence intervals of the
fitted least squares regression. The regression lines estimate that hatch time scales with an exponent of 0.29±0.19 (95% confidence interval) for univoltine aquatic insects and an exponent of 0.21±0.06 for all other insects. While not a pure power function, the DEB prediction (0% structure) approximates one with a logarithmic slope of 0.26 at the lower range of insect masses and 0.23 at the higher range. When compared to the fitted power function (Table 4), the standard DEB model (0% structure) can be seen to explain less variance in hatch time and has a lower AIC weight (likelihood of being the ‘best’ model). However, given that the DEB model (0% structure) has not been fit to the data, it still explains a comparable levels of variance and lies close to the 95% confidence interval of the estimated scaling relationship. For smaller masses, the standard DEB model prediction lies close to the 95% confidence interval of the best fit to insects (excluding univoltine aquatic) but tends to over-predict development times for larger insects. When we modify our assumptions to allow 5% structure within a fresh egg, the prediction is slightly improved, but not to a significant degree as can be seen in the similar AIC weights of the two models (Table 4).

Table 4 shows that although the DEB model that assumed 5% structure did not perform as well as the fitted power function in capturing the mass scaling of hatch times, it explained comparable levels of variance, lied within the confidence bounds of the estimated scaling relationship, and was able to capture the time course of respiration with greater accuracy.

To ensure that the results obtained were robust, we conducted a sensitivity analysis on all model parameters. Upon varying each parameter by ±20% we found our results were sensitive only to the growth efficiency parameter \( \kappa_g \) which was taken as the default value of 0.80. To increase our confidence in this ‘default’ value we analysed 389 species for which this parameter had been estimated. Parameter estimates and supporting data for the 389 are available online in the ‘add_my_pet’ library (www.bio.vu.nl/thb/deb/deblab/add_my_pet/). We found that \( \kappa_g \) could be estimated to a high degree of accuracy with a mean of 0.80 and a 95 percent confidence interval of \{0.796, 0.810\}. This value is also very close to the value independently estimated by Ricklefs (1974).

**Discussion**

Dynamic Energy Budget theory’s unique interpretation of embryonic development predicted the broad effect of body size on development time without any parameter fitting. This article is protected by copyright. All rights reserved
Prediction is facilitated by the mechanistic basis of the model; the model parameters have a physical interpretation so that a reasonable estimate can be made before even seeing the observational data to which the prediction is compared (Maino et al. 2014). This approach contrasts with most scaling studies where power functions are fitted to log transformed data using a least squares or similar approach with analysis carried out on the estimated exponents. While not a pure power law, the DEB function for hatch time approximates one and appears linear on a double logarithmic plot (Fig. 2).

As first demonstrated by Gillooly and Dodson (2000b), univoltine aquatic insects exhibit a scaling pattern of development time that is distinct from other insects. Indeed, the exceptionality of univoltine insects appears to extend beyond the insects. Gillooly and Dodson (2000b) found that univoltine aquatic insects were outliers in the general relationship between egg size and hatch time in a broad analysis that included zooplankton, fish, amphibians reptiles (Gillooly & Dodson 2000a). However, the relationship for multivoltine insects was nearly indistinguishable from most other animals. After contributing 66 new insect species to Gillooly and Dodson’s data set, this separation is confirmed with univoltine aquatic insects exhibiting a delayed embryonic development that does not overlap with data from all other insects. Our prediction based on typical parameters for a generalised animal did not capture univoltine aquatic insects, which appear to be outliers even when wide taxonomical groups are included in the analysis.

This non-conformity to the general trend may be related to the degree of diapause frequently exhibited in these species and requirements for synchronised development. Gillooly and Dodson (2000a) hypothesised that the degree of diapause in a species may be related to the level of voltinism. For aquatic insects in particularly, it may be important that hatching is synchronised to avoid cannibalism when many size classes are present at one time (Hopper, Crowley & Kielman 1996). Delayed development time through diapause may be one strategy to ensure synchronicity of development. In our analysis DEB parameters were held constant at the values expected for a general organism. However, selective pressures will modify parameters for particular taxa from the general trend and we can use DEB theory to build hypotheses for such parameter shifts. For example, the delayed development of univoltine aquatic insects (Fig. 2), could be explained by a decrease in structural maintenance, an increase in growth efficiency, or a decrease in conductance (see Figure S2 in Supporting Information). As changes to these parameters will also modify other aspects of an organism’s life history, these alternate hypotheses can be tested by collecting more data on these insects.
The presented DEB model also predicted the effect of egg size on the timecourse of embryonic respiration. The standard DEB model assumes all embryos begin development with zero structure, predicting that increased amounts of reserve will not affect development (the growth rate of structure) until reserve becomes sufficiently depleted such that the amount of reserve is limiting. In other words, while reserve is not limiting, eggs of different size will follow the same trajectory of respiration rate. This is analogous to increasing the raw inputs into a factory that is already operating at capacity, and observing no effect on the rate of production. Fig. 1C shows this prediction, where respiration rates at early embryonic development follow identical trajectories. However, as reserve is depleted and begins to limit the energy available for metabolism, the trajectories diverge, with smaller eggs diverging first as their reserves are the first to deplete. This can also be seen analytically from inspection of equation 3 when taken at the limit of large reserve, which occurs during early embryonic development when structure is small; the term for reserve cancels out, showing energy for metabolism is not dependent on reserve. However, this prediction does not align well with observations of early development respiration where it was found that data was under-predicted by the model. Moreover, respiration rate during early development appears to vary widely across species, suggesting early egg metabolism may not be independent of egg size (starting structure). This positive effect of body size on the respiration of day old eggs is shown in Fig. 1B.

Interestingly, other alternative models also suffer from the problem of under-predicting the respiration during early embryonic development and predict no systematic pattern with egg size. Recently, a simplified DEB model known as DEBkiss has been developed for basic DEB theory applications and to simplify the process of parameterisation, which is quite involved for a full life-cycle standard DEB model (Jager, Martin & Zimmer 2013). Like the standard model presented here, a DEBkiss model also assumes that a fresh egg is all reserve from which structure grows. The difference is that the DEBkiss mobilisation of reserve is simply \( \dot{p}_c = a V^{3/2} \) where \( a \) is some constant. This is simpler than equation 2. Another model proposed by Gillooly et al. (2002) assumes metabolic rate (MR) scales with the mass of the embryo \( m \) as \( \dot{r} = a m^{3/2} \), where \( a \) is the normalisation constant. Mass is predicted to increase with time as \( m = \left( \frac{at}{4} \right)^4 \) and so respiration as \( MR = a \left( \frac{at}{4} \right)^3 \). In both of these alternative models, the timecourse of respiration will follow the same trajectory regardless of the size of the egg (but smaller eggs will hatch earlier). In other words, these models also do
not predict the apparent effect of egg size on embryonic respiration shown in Fig 1B. These
alternatives (as well as other less mechanistic models) frequently model the timecourse of
respiration as some polynomial function of time (Smith 1957; Gillooly et al. 2002; Kooijman
2010; Maino & Kearney 2014b) (represented by the third-order polynomial model in Table 3).

To bring theory in better accordance with observation it is necessary to modify the
assumption that a freshly laid egg is entirely reserve. In his formulation of the standard DEB
model, Kooijman highlights that the assumption of zero starting structure is made for
convenience, claiming “for all practical purposes the initial structural volume of the embryo
can realistically be assumed to be negligibly small” (Kooijman, 2010, pg. 4). We have shown
that this assumption is not practical in the case of insect embryonic respiration. It was
previously hypothesised (Maino & Kearney 2014b) that the non-zero respiration of fresh eggs
may have been due either to some development having already taken place inside the adult
prior to egg lay (Sander 1990), or the rapid speed at which many insect eggs develop, which
poses practical problems for attaining respiration measurements of completely undeveloped
eggs (Bownes 1975). Indeed, many insect eggs are already undergoing the first mitotic
division by the time of oviposition (Dunkel, Wensman & Lovrien 1979). However, the
positive relationship of egg size to early embryonic respiration cannot be explained by simply
adding a small lag time. This is because different theoretical models predict that the time
course of embryonic respiration rate will be the same for eggs of different size, at least during
early embryonic development. The modified assumption of non-zero starting structure is
consistent with the observation of non-zero respiration rate of unfertilized eggs and with
physiological observations of fertilized and unfertilized eggs. It is able to consistently explain
both the mass scaling and time course of respiration within a single framework.

In the context of DEB theory, the respiring unfertilized egg would represent an egg with
some structure but no allocation of resources to growth, while the fertilised egg is allocating
resources to the growth of structure. These theoretical representations of unfertilized and
fertilized eggs gain support from physiological observations. It has long been known that
fresh eggs consume oxygen, albeit small amounts, including even those that have not been
fertilised and are not undergoing cellular divisions (Boyd 1928; Borei 1948). As oocytes
develop into eggs inside the ovaries (or ovarioles in insects), reserve is synthesized and the
respiration rate is high (Borei 1948). Once the oocyte has become a ‘ripe’ unfertilized egg,
respiration rate falls (Borei 1948) and meiotic growth is halted (Went 1982). Depending on
the species, ripe eggs may remain viable in this state for several days (Whitaker 1931;
Holcomb et al. (2004) and, although meiotically halted, may be far from inactive. Measurable oxygen consumption rates have been observed in unfertilized eggs for a variety of species (Whitaker 1931; Borei 1948; Horwitz 1965; Tyler, Tyler & Piatigorsky 1968; Davenport & Lonnig 1980) and may indicate the generation of energy through the Krebs Cycle.

Unfertilized eggs contain all the necessary components of protein synthesis, including mRNAs (Tyler 1963; Tyler et al. 1968) and evidence of protein synthesis has been demonstrated in ripe, unfertilized eggs of sea urchins (Tyler et al. 1968), clams (Bell & Reeder 1967), polychaete worms (Gould 1969) and frogs (Smith, Ecker & Subtelny 1966). Protein synthesis in ripe, unfertilized eggs likely represents the turnover of degraded proteins and other cell maintenance processes, as ripe eggs may remain unfertilized for extended period without a change in the capacity for normal development (Tyler et al. 1968). A measurable respiration rate and evidence of active protein synthesis without growth supports the notion that an unfertilized egg should be comprised of some amount of structure.

There is also physiological evidence that the growth of structure from reserve may initiate quite soon after fertilization, further supporting the assumption that a freshly fertilized egg should be modelled with some amount of structure. Within the first few hours after fertilization, respiration may increase exponentially until respiration rate has nearly doubled (Boyd 1928; Whitaker 1931; Borei 1948). Respiration rate may also increase dramatically within the first few minutes after fertilization, up to three times the rate of respiration in the unfertilized egg, but falling again within minutes (Horwitz 1965). Protein synthesis in clam eggs has also been shown to increase rapidly immediately after fertilization, and well before the first meiotic division (Bell & Reeder 1967). In the context of DEB theory, this sharp change in respiration is captured when the growth of structure is initiated after egg fertilization, with the overhead costs of structural growth making an observable contribution to respiration. This advance in our knowledge of egg development brought by the application of DEB theory highlights the usefulness of process-based models from which higher levels patterns can be derived.

Metabolic theory is increasingly being applied in biology to understand a range of biological phenomena by currencies of mass and energy, which are relevant at all scales of biology (Brown et al. 2004; Kearney & White 2012; Maino et al. 2014). DEB theory builds on this notion by formalising further constraints on the metabolic organisation of organisms, such as constraints on uptake, or mobilisation of assimilated resources for metabolism. The broad reaching field of DEB theory, and metabolic theory in general, is in an active state of
development and will be further refined by conducting broad scaled tests of the model assumptions as we have shown here.

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Data accessibility


Supplementary information

Additional supporting information may be found in the online version of this article.

Appendix S1 Derivation of the Equation 3

Figure S1 Schematic of embryonic development in the DEB model

Figure S2 Sensitivity analysis of model parameters

References


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squares fit of a third-order polynomial. (D) The model shown in panel C is modified so that a fresh egg contains 5% structure. The solid lines show the revised model prediction. The dotted line shows the least-squares fit of a third-order polynomial. (E) Residual deviations of observations from predictions of the 0% structure model. (F) Residual deviations of observations from predictions of the 5% structure model.

**Figure 2.** The effect of egg size on time to hatch in 98 insect species. Univoltine aquatic insects are separated from other insects following Gillooly and Dodson (2000b). The upper bold line denotes the prediction of the DEB model that assumes a fresh egg is entirely reserve. The lower bold line denotes the prediction of the DEB model that assumes a fresh egg is 5% structure. Least square regression lines with 95% confidence intervals are also given.
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