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

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Summary

1. Body size scaling relationships allow biologists to study ecological phenomena in terms of individual level metabolic processes. Recently, dynamic energy budget (DEB) theory has been shown to offer novel insights on the effect of body size on biological rates.
2. We test whether DEB 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 parameterization (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 time course 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 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 2014, 2015a,b). Dynamic energy budget theory considers the whole life cycle 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 generalized chemical compound with a fixed stoichiometry and energy

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content. DEB theory explains egg development as the transformation of reserve to another generalized compound with different dynamics called 'structure' (see Fig. S1, 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 conceptualizes reserve as a generalized 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 organization of diverse taxa. This, however, does not preclude rigorous testing of the theory.

Kearney & 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 interspecific 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 time course 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

Dynamic energy budget 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 is specified by the standard DEB model without maturity. Reserve is depleted at a rate equal to the reserve mobilization 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 mobilization 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 (cm³) through time are given by the differential equations:

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

Order	Species	Dry egg weight (mg) at		κ_H : ratio of egg mass at hatch: lay	Source
		Egg lay	Hatching		
Orthoptera	*Poecilocerus pictus*	4·1	3·33	0·812	Delvi & Pandian (1972)
Coleoptera	*Cryptolestes ferrugineus*	0·0030	0·0025	0·833	Campbell & Sinha (1974)
Coleoptera	*Rhyzopertha domini*	0·0047	0·0042	0·894	Campbell & Sinha (1974)
Coleoptera	*Triobolium castaneum*	0·0197	0·0172	0·873	Klekowski, Prus & Zyromska-Rudzka (1967)
Coleoptera	*Mellasoma collaris*	0·298	0·266	0·893	Hågvar (1975)
Coleoptera	*Sitophilus granarius*	0·0112	0·0098	0·875	Campbell, Singh & Sinha (1976)
Lepidoptera	*Cyclophragma leucosticte*	2·45	1·48	0·604	Mackey (1978)
	Mean value	–	–	0·826	

$$\frac{\mathrm{d}E}{\mathrm{d}t} = -\dot{p}_{\mathrm{C}} \qquad \text{eqn 1}$$

$$\frac{\mathrm{d}V}{\mathrm{d}t} = (\dot{p}_{\mathrm{C}} - \dot{p}_{\mathrm{M}})/[E_{\mathrm{G}}] \qquad \text{eqn 2}$$

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

$$\dot{p}_{\mathrm{C}} = E\frac{[E_{\mathrm{G}}]\dot{v}/V^{\frac{1}{3}} + [\dot{p}_{\mathrm{M}}]}{E/V + [E_{\mathrm{G}}]} \qquad \text{eqn 3}$$

The conductance parameter $\dot{v}$ (cm/d) relates to the turnover of reserve. Eqn 3 is fully derived in Appendix S1, but here we note that the 1 of 3 exponent in the numerator is a consequence of the central role that surface area and volume-based processes in DEB theory (1 of 3 emerges from the ratio of the two). The energy available for metabolism $\dot{p}_{\mathrm{C}}$ 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.

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 $[E_{\mathrm{G}}]$, multiplied by the proportion of energy that is not expended as overhead costs in forming structure κ_{G}. This gives us an expression for biomass energy M in terms of reserve and structure:

$$M = E + V[E_{\mathrm{G}}]\kappa_{\mathrm{G}} \qquad \text{eqn 4}$$

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 Eqns 1 and 2, where hatching is triggered when egg biomass (Eqn 4) drops to some proportion of the starting biomass or, equivalently, when $M = \kappa_{\mathrm{H}}M_0$. With the exception of the parameter κ_{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 generalized animal and are the usual uncalibrated starting point before the fine tuning required in fitting a full life cycle DEB model to a species (Lika,

Table 2. Uncalibrated parameter list for embryonic DEB model

Description	Parameter	Value	Units
Unfixed parameter			
Egg size	M_0	–	J
Fixed parameters*			
Specific maintenance	$[\dot{p}_{\mathrm{M}}]$	18	J cm^{-3} day^{-1}
Cost of structure	$[E_{\mathrm{G}}]$	8400	J cm^{-3}
Conductance	$\dot{v}$	0·02	cm day^{-1}
Growth efficiency	κ_{G}	0·80	Dimensionless
Hatch ratio	κ_{H}	0·83	Dimensionless

*Fixed parameters are set at the default values given in Lika *et al.* (2011) for a generalized animal with 70% water content, except for κ_{H} (see Table 1).

Kearney & Kooijman 2011). This integration is performed numerically in R using the DESOLVE library (Soetaert, Petzoldt & Setzer 2010).

In the same way, respiration MR (J/d) is predicted as the sum of growth overheads and structural maintenance or:

$$\mathrm{MR} = [\dot{p}_{\mathrm{M}}]V + \frac{\mathrm{d}V}{\mathrm{d}t}[E_{\mathrm{G}}](1 - \kappa_{\mathrm{G}}) \qquad \text{eqn 5}$$

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, κ_{V}. This change only affects the initial conditions of the integration and has the advantage that the introduced parameter κ_{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 $\kappa_{\mathrm{V}} = 0$.

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 and Kearney 2014, 2015a, 2015b; 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 data set 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 standardized 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 mid-point 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 standardized to a constant temperature of 20 °C ($T_{\mathrm{ref}} = 293\mathrm{K}$) 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 standardization factor ($\dot{k}$) can be calculated as $\dot{k} = \exp\left(\frac{T_{\mathrm{A}}}{T_{\mathrm{ref}}} - \frac{T_{\mathrm{A}}}{T}\right)$ where T is the measurement temperature in Kelvin.

Results

TIME COURSE OF RESPIRATION

Observations of the time course of respiration for 16 insects are 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 ± 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 time course of respiration (Fig. 1d,f). When compared against a third-degree polynomial function (Table 3), the modified DEB model explained higher levels of variance in the time course 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.

HATCH TIME

As found by Gillooly & Dodson (2000b), univoltine aquatic insects (consisting of Plecoptera, Odonata, Trichoptera and Ephemeroptera) exhibit much longer hatch times than
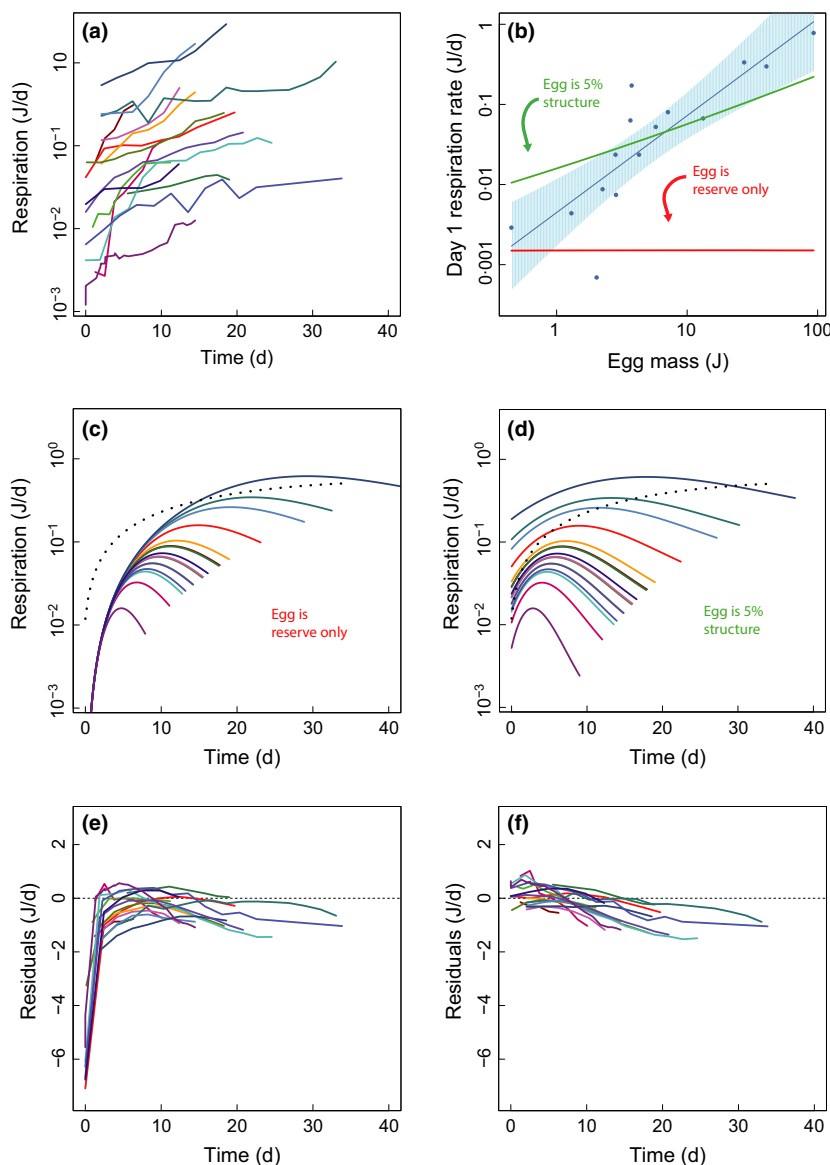
Fig. 1. (a) The time course of respiration from oviposition to hatching for 16 insects. (b) Day one respiration rate for 15 insects was plotted against egg size with a quadratic function used for interpolation (data for *Rhodnius prolixus* was omitted because its earliest measurement was 3·5 days since oviposition, whereas for all other insects day 1 respiration was reported). The prediction for the DEB model that assumes a fresh egg is all reserve is denoted by the lower bold line. Assuming an egg is 5% structure results in the prediction denoted by the upper bold line. The least-squares regression line, and 95% confidence interval is also given and has a slope of 1.21 ± 0.45. (c) The solid lines show the standard DEB model's prediction of time course of respiration from oviposition to hatching, assuming 0% starting structure, for each of the 16 insects in panel A. The dotted line shows the least-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.

Table 3. Statistical comparison of model fits

	Time course of respiration (Fig. 1c,d)	
	Variance explained	AIC weight
Third-order polynomial	0·102	0·000
DEB (0% structure)	0·139	0·000
DEB (5% structure)	0·346	1·000

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.

Table 4. Statistical comparison of model fits

	Hatch time (excluding univoltine aquatic) (Fig. 2)	
	Variance explained	AIC weight
Power function	0·374	0·927
DEB (0% structure)	0·282	0·014
DEB (5% structure)	0·309	0·059

Upon varying each parameter by ±20%, we found our results were sensitive only to the growth efficiency parameter κ_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 κ_G could be estimated to a high degree of accuracy with a mean of 0·80 and a 95% 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 *a priori* 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 to 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 & Dodson (2000b), univoltine aquatic insects exhibit a scaling pattern of
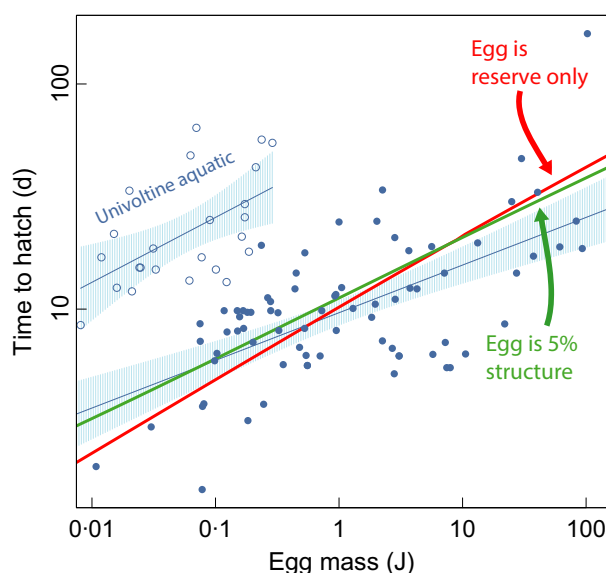
Fig. 2. The effect of egg size on time to hatch in 98 insect species. Univoltine aquatic insects are separated from other insects following Gillooly & Dodson (2000b). The upper bold line denotes the prediction of the DEB model that assumes a fresh egg is entirely reserve. The lower bold line denotes 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.

development time that is distinct from other insects. Indeed, the exceptionality of univoltine insects appears to extend beyond the insects. Gillooly & 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 generalized 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 synchronized development. Gillooly & Dodson (2000a) hypothesized 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 synchronized 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 Fig. S2). 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 time course 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. Figure 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 eqn 3 when taken

at the limit of large reserve, which occurs during early embryonic development when structure is small; the term for reserve E 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 were 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 parameterization, 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 mobilization of reserve is simply $\dot{p}_C = aV^{\frac{2}{3}}$ where a is some constant. This is simpler than eqn 2. Another model proposed by Gillooly et al. (2002) assumes metabolic rate (MR) scales with the mass of the embryo (m) as $r = am^{\frac{3}{4}}$, where a is the normalization constant. Mass is predicted to increase with time as $m = (at/4)^4$ and so respiration as $MR = a(at/4)^3$. In both of these alternative models, the time course 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 time course 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 hypothesized (Maino & Kearney 2014b) that the nonzero 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 nonzero 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 fertilized 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 fertilized 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, Tyler and Piatigorsky 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, Tyler and Piatigorsky 1968). A measureable 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 formalizing further constraints on the metabolic organization of organisms, such as constraints on uptake, or mobilization 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.

Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.pn1j6 (Maino *et al.* 2016).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Schematic of embryonic development in the DEB model.
Fig. S2. Sensitivity analysis of model parameters.
Appendix S1. Derivation of the eqn 3.