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Energetic constraints on an early developmental stage: a comparative view

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Biologists have long sought a means by which to quantify similarities and differences in embryonic development across species. Here we present a quantitative approach for predicting the timing of developmental events based on principles of allometry and biochemical kinetics. Data from diverse oviparous species support model predictions that most variation in the time required to reach one early developmental stage—the time to first heartbeat—is explained by the body size and temperature dependence of metabolic rate. Furthermore, comparisons of this stage with later developmental stages suggest that, after correcting for size and temperature, the relationship of metabolic rate to the rate of embryogenesis is approximately invariant across taxonomic groups and stages of ontogeny.

Keywords: scaling; growth; embryology; metabolic theory; energetics

1. INTRODUCTION

Since the time of Haeckel and von Baer in the nineteenth century, biologists have sought to understand similarities and differences in rates of embryonic development across species. Interspecific comparisons are currently hindered by the lack of a general theoretical framework that yields quantitative predictions on rates of embryogenesis (Richardson & Keuck 2002). Such a framework is needed to provide a basis for comparing developmental times across species (Reiss 1989).

Historically, efforts to develop theoretical or conceptual models of embryonic development have highlighted potential linkages between development and allometric growth or bioenergetics (e.g. Dettlaff & Dettlaff 1961; Alberch *et al.* 1979). While such models have yielded important insights, they have not been shown to be capable of predicting variation in rates of embryogenesis among species—particularly for early developmental stages. This is partially due to the difficulties involved in estimating embryo size, and some would argue, due to the inherent complexity of the developmental process (Burggren & Crossley 2002).

Here we present and test an approach for predicting the timing of early developmental events. We begin by presenting a model that extends a recently proposed model of ontogenetic growth, which is based on principles of bioenergetics (West *et al.* 2001; Gillooly *et al.* 2002; Moses *et al.* in press). This growth model has been shown to predict the growth rates of diverse species, including growth rates to both birth and maturity (West *et al.* 2001; Gillooly *et al.* 2002). Next, we build on this work by testing the usefulness of this model for predicting early developmental events. To date, neither this model nor any other bioenergetics model of which we are aware has yet been shown to successfully predict differences in the timing of early developmental events across species. We perform this test by analysing newly compiled data on the timing of one developmental stage—the time to first heartbeat (TFH)—for species that include amphibians, birds, fishes and invertebrates. Finally, we build on previous work by comparing the TFH data with data compiled for two other developmental stages, the time to hatching (TH) and the minimum time to maturity (TM). This allows us to assess the relationship of metabolic energy flux to the rate of development across different stages of ontogeny. In particular, it allows us to estimate the rate of energy expenditure per unit biomass production through ontogeny.

2. MODEL DEVELOPMENT

The model presented here is based on the hypothesis that rates of growth are controlled by metabolic rate, and thus by the two primary factors that control metabolic rate, body size and temperature (Tyler 1939; Dettlaff & Dettlaff 1961; Reiss 1989; Gillooly *et al.* 2002). It builds on previous work that addresses the effects of size and temperature on metabolic rate (Robinson *et al.* 1983; see also Peters 1983), and more recent work showing that the combined effects of body size, m (g), and body temperature, T (K), on mass-specific metabolic rate, $\bar{B}$, can be characterized using the following equation (Gillooly *et al.* 2001):

$$\bar{B} = B/m = b_0 m^{-1/4} e^{-E/kT}, \tag{2.1}$$

where B is the individual metabolic rate and b_0 is a normalization constant independent of body size and temperature ($\mathrm{W\,g^{-3/4}}$). The one-quarter power scaling exponent for the body size term, $m^{-1/4}$, is well established empirically (Peters 1983; Savage *et al.* 2004*a*,*b*), and has been attributed to biophysical constraints on the delivery of energy and materials to cells (West *et al.* 1997). The Boltzmann–Arrhenius factor, $e^{-E/kT}$, characterizes the exponential effects of temperature on individual metabolic rate, where E is the activation energy of the respiratory complex (approx. 0.65 eV) and k is Boltzmann's constant ($8.62\times10^{-5}\,\mathrm{eV\,K^{-1}}$).

Building on the expression above for metabolic rate (equation (2.1)), Gillooly *et al.* (2002) characterized the combined effects of body size and temperature on the ontogenetic growth rate ($\mathrm{g\,s^{-1}}$) of an organism as

$$\frac{dm}{dt} = \frac{B}{E_m}\left(1-\left(\frac{m}{M}\right)^{1/4}\right) \approx \frac{b_0}{E_m} m^{3/4} e^{-E/kT}. \tag{2.2}$$

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Table 1. Slopes and intercepts of the fitted lines in figure 1, along with 95% confidence intervals in parentheses.

		slope		
data	figure	predicted	fitted	fitted intercept
body temperature models				
TFH	1*a*	0.65 eV	0.59 eV (0.37–0.81)	−16.17 (−24.83 to −7.52)
TM	1*c*	0.65 eV	0.69 eV (0.53–0.86)	−20.22 (−26.81 to −13.63)
TH	not plotted	0.65 eV	0.64 eV (0.36–0.92)	−18.11 (−28.98 to −7.24)
body size models				
TFH	1*b*	0.25	0.20 (0.09–0.31)	−19.06 (−19.93 to −18.19)
TM	1*d*	0.25	0.20 (0.17–0.22)	−18.46 (−18.72 to −18.20)
TH	not plotted	0.25	0.26 (0.17–0.35)	−18.47 (−18.98 to −17.96)

The approximation in equation (2.2) applies when m is small relatively to the asymptotic adult mass, M. This equation is theoretically derived, based on the conservation of energy, since the production of biomass requires energy, E_m (J g^{-1}), and represents a portion of the total energy expenditure by the organism, B (see West *et al.* 2001; Moses *et al.* in press). In principle, equation (2.2) should apply to any stage of development that exhibits the size and temperature dependence of metabolic rate described by equation (2.1). By integrating equation (2.2), the time required to reach some arbitrary developmental stage i, τ_i (s), can then be characterized as

$$\tau_i = \left(\frac{4E_m}{b_0}\right)\left(\frac{m_i}{\delta_i}\right)^{1/4} \ln\left[\frac{1-(m_c/M)^{1/4}}{1-\delta_i^{1/4}}\right] e^{-E/kT} \approx \left(\frac{4E_m}{b_0}\right) m_i^{1/4} e^{-E/kT}, \quad (2.3)$$

where m_c is the mass of the embryo at the time of fertilization; m_i is the mass of the embryo at stage i; and δ_i is the ratio of embryo mass to the asymptotic adult mass ($=m_i/M$) (Gillooly *et al.* 2002). The approximation in the final expression of equation (2.3) applies if the embryo mass is small relative to the asymptotic adult mass (i.e. $\delta_i \ll 1$), but still much larger than a single cell (i.e. $m_i \gg m_c$). Under these conditions, equation (2.3) directly links developmental times to mass-specific metabolic rate and to the factors that control metabolic rate, namely body size and temperature.

Equation (2.3) yields three quantitative predictions regarding TFH. Prediction 1 is that the logarithm of the body size-corrected time to this developmental stage, $\ln(\tau_i m_i^{-1/4})$, should be a linear function of inverse absolute temperature, $1/kT$, with a slope of $E \approx 0.65$ eV, reflecting the exponential effects of temperature on metabolic rate (equation (2.1)). Prediction 2 is that the logarithm of the temperature-corrected TFH, $\ln(\tau_i e^{-E/kT})$, should be a linear function of the logarithm of embryo size, $\ln(m_i)$, with a slope of 1/4, reflecting the size dependence of metabolic rate (equation (2.1)). Prediction 3 is that the size- and temperature-corrected times to *other* stages of embryonic and postembryonic development should adhere to the *same* predicted relationships as the TFH data, as expressed in predictions 1 and 2. This last prediction is expected to hold if the size- and temperature-corrected rate of metabolism, characterized by b_0, and the energy required to produce biomass, E_m, are similar for different species and stages of ontogeny.

3. MATERIAL AND METHODS

We evaluate predictions 1 and 2 using all known TFH data (see electronic supplementary material). For each species, we obtained estimates of TFH, yolk-free embryo size at TFH and developmental temperature. To the best of our knowledge, this is the only early developmental stage with sufficient data to perform comparative analyses with respect to size and temperature. These species, which vary considerably with respect to taxonomy, life history and habitat affinity, span six orders of magnitude in embryo size (approx. 10^{-5} g dry mass for *Daphnia magna* to approx. 4 g for *Tyto alba*) and a 30°C range in developmental temperature (approx. 7°C for *Salmo gairdneri* to approx. 38°C for *Gallus gallus*). We evaluate prediction 3 by comparing TFH with TH (table 1) and TM (data from Savage *et al.* 2004*a*) for a broader assortment of species.

4. RESULTS

The data support all three model predictions. In support of prediction 1, a plot of the logarithm of mass-corrected TFH, $\ln(\tau_i m_i^{-1/4})$, versus inverse absolute temperature, $1/kT$, yields a significant linear relationship (figure 1*a*), with a fitted slope (0.59 eV) that has a 95% confidence interval (CI) that includes the predicted value of 0.65 eV (table 1). In support of prediction 2, a plot of the logarithm of temperature-corrected TFH, $\ln(\tau_i e^{-E/kT})$, versus the logarithm of embryo mass, $\ln(m_i)$, yields a significant linear relationship (figure 1*b*), with a fitted slope (0.20) that is statistically indistinguishable from the predicted value of 0.25 (table 1). Finally, in support of prediction 3, the TFH data depicted in figure 1*a*,*b* fall on virtually the same fitted lines as the TH and TM data depicted in figure 1*c*,*d*. The fitted slopes and intercepts for the TM and TH data depicted in figure 1*c*,*d* have 95% CI that overlap with those obtained for the TFH data depicted in figure 1*a*,*b* (table 1). ANCOVA analyses of the TFH and TH data indicate that the slopes and intercepts of the fitted lines are similar within and across taxonomic groups (see electronic supplementary material).

5. DISCUSSION

These results yield two insights regarding the relationship of embryonic development to individual energetics. First, the observed size and temperature

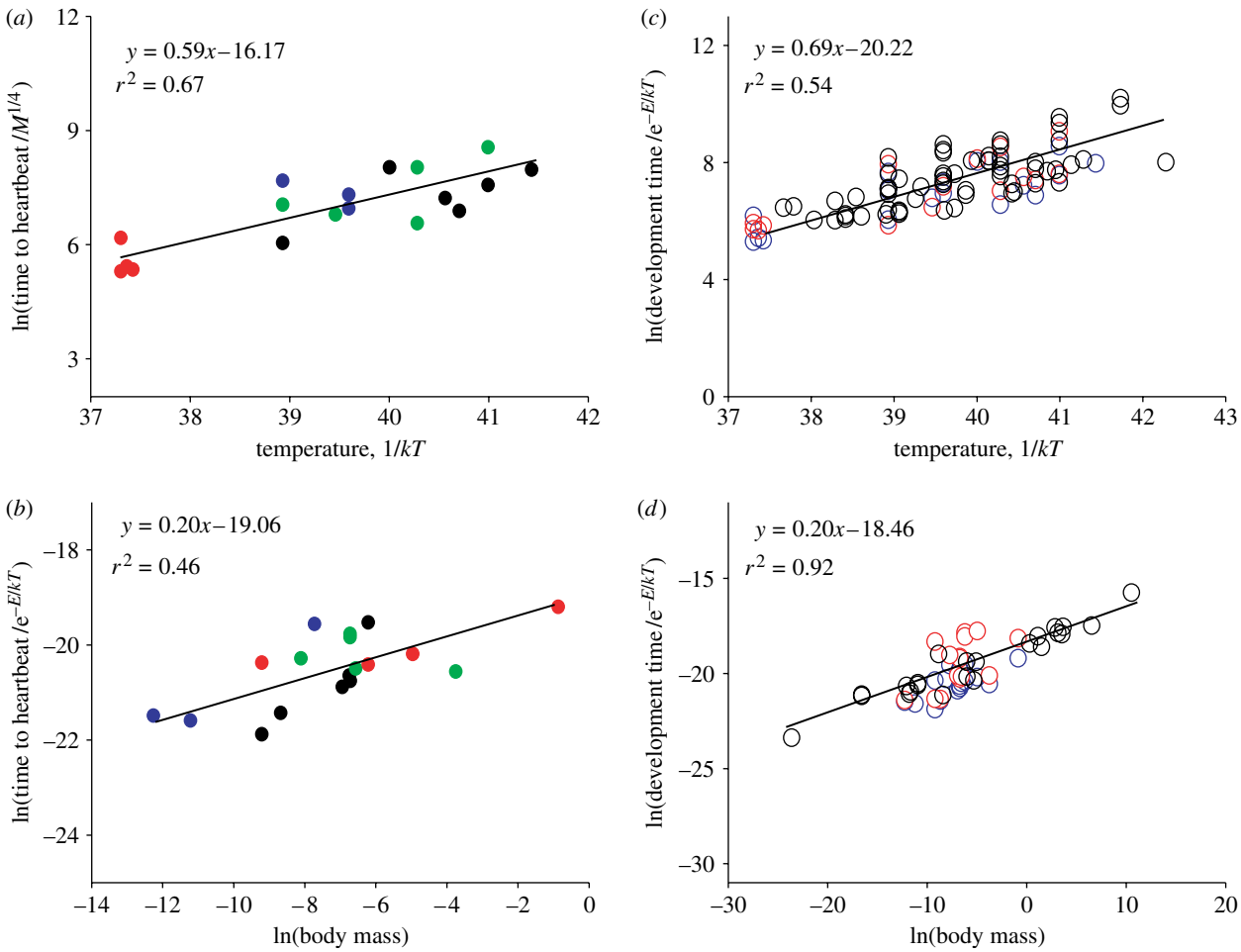

Figure 1. (*a*) Logarithm of body size-corrected TFH, $\ln(\tau_i m_i^{-1/4})$ (h $g^{-1/4}$), versus inverse absolute temperature, $1/kT$ (eV^{-1}) (black filled circle, fish; green filled circle, amphibian; blue filled circle, invertebrate; red filled circle, bird). (*b*) Logarithm of temperature-corrected TFH, $\ln(\tau_i e^{-E/kT})$ (hours), versus embryo mass, $\ln(m_i)$ (g dry mass), for the same data plotted in (*a*) (black filled circle, fish; green filled circle, amphibian; blue filled circle, invertebrate; red filled circle, bird). (*c*,*d*) Temperature- and body size-corrected TH and TM are plotted along with the TFH data, respectively. The regression lines depicted in (*c*,*d*) are fitted only to the TM data. (Open blue circle, time to heartbeat; open red circle, time to hatch; open black circle, time to maturity.)

dependence of TFH suggests that early embryonic development is fundamentally controlled by metabolic rate, which in turn is controlled by embryo size and temperature. Equation (2.3) predicts up to 67% of the variation in TFH, which is a relatively early developmental stage since the heart is among the first organs to develop. Furthermore, the similarity in intercepts between figure 1*a*,*b* and *c*,*d* suggests that the relationship of metabolic rate to developmental rate is approximately fixed over ontogeny. Specifically, these results indicate that the size- and temperature-corrected metabolic rate (characterized by b_0 in equation (2.3)) and the rate of energy expenditure per unit biomass production (characterized by E_m) remain essentially unchanged from the time of first heartbeat to the time of maturity. Given that the fitted intercepts in table 1 represent estimates of $\ln(4E_m/b_0)$, we can estimate the quantity E_m using the TFH data. Taking $\ln(4E_m/b_0) = -19.06$ h $g^{-1/4}$ (table 1) and $b_0 = 2.4 \times 10^8$ W $g^{-3/4}$ (average of b_0 values reported by Gillooly *et al.* (2005) for multicellular taxa), we estimate that $E_m = 1140$ J g^{-1}. This estimate is similar in magnitude to Vleck *et al.* (1980) estimate of $E_m \approx 1230$ J g^{-1} from bird embryos, which was obtained using entirely different methods (see also Moses *et al.* in press). The substantial unexplained variation about the fitted lines in figure 1, in addition to measurement error, probably reflects the importance of other factors such as food quantity and/or quality, or oxygen availability, all of which may alter rates of development through their effects on b_0 and E_m.

Second, these results point to a fundamental relationship between the processes of transcription, translation and protein synthesis at the cellular level and the process of metabolic energy flux at the level of the embryo. With respect to early heart development, it is well established that the sequence of developmental events is highly conserved across both invertebrates and vertebrates (Cripps & Olson 2002). Figure 1*a*,*b* further indicates that any changes in the timing of this developmental sequence must have evolved concurrently with changes in embryo mass. Yet, the linkages between the cellular-level processes leading to differentiation and controls on individual-level metabolic rate remain largely unexplored in developmental biology.

Ultimately, this approach may provide a point of departure for quantifying differences in the timing

of early developmental stages (i.e. heterochrony) among taxa that have evolved through natural selection. However, this will require more and better data on the body sizes and temperatures of early embryos at specific developmental events (e.g. time to gastrulation). In these data, there is no indication that TFH represents some fixed fraction of the total energy expended by an organism to reach maturity, or some fixed fraction of the total TM. For example, given that the dry mass of *D. magna* at maturity is approximately 10^{-5} g and that of *S. gairdneri* is approximately 600 g, the TFH occurs at approximately 10% of adult mass in *Daphnia* and at approximately 0.0002% of adult mass in *Salmo*. Future research that applies this allometric framework to other developmental events, at both the cellular and whole-embryo levels of biological organization, may shed light on the extent to which the tempo of embryogenesis is conserved across species.

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Alberch, P., Gould, S. J., Oster, G. F. & Wake, D. B. 1979 Size and shape in ontogeny and phylogeny. *Paleobiology* **5**, 296–317.

Burggren, W. & Crossley, D. A. 2002 Comparative cardiovascular development: improving the conceptual framework. *Comp. Biochem. Physiol. A* **132**, 661–674.

Cripps, R. N. & Olson, E. N. 2002 Control of cardiac development by an evolutionarily conserved transcriptional network. *Dev. Biol.* **246**, 14–28. (doi:10.1006/dbio.2002.0666)

Dettlaff, T. A. & Dettlaff, A. A. 1961 On the relative dimensionless characteristics of the development duration in embryology. *Arch Biol.* **72**, 1–16.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. & Charnov, E. L. 2001 Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251. (doi:10.1126/science.1061967)

Gillooly, J. F., Charnov, E. L., West, G. B., Savage, V. M. & Brown, J. H. 2002 Effects of size and temperature on developmental time. *Nature* **417**, 70–73. (doi:10.1038/417070a)

Gillooly, J. F., Allen, A. P., Brown, J. H., Elser, J. J., Martinez del Rio, C., Savage, V. M., West, G. B., Woodruff, W. H. & Woods, H. A. 2005 The metabolic basis of whole-organism RNA and phosphorus content. *Proc. Natl Acad. Sci. USA* **102**, 11 923–11 927. (doi:10.1073/pnas.0504756102)

Moses, M. E., Hou, C., Woodruff, W. H., West, G. B., Nekola, J. C. & Brown, J. H. In press. Revisiting a model of ontogenetic growth: effects of size and temperature on developmental time. *Am. Nat.*

Peters, R. H. 1983 *The ecological implications of body size*. New York, NY: Cambridge University Press.

Reiss, J. O. 1989 The meaning of developmental time: a metric for comparative embryology. *Am. Nat.* **134**, 170–185. (doi:10.1086/284974)

Richardson, M. K. & Keuck, G. 2002 Haeckel's ABC's of evolution and development. *Biol. Rev.* **77**, 459–528. (doi:10.1017/S1464793102005948)

Robinson, W. R., Peters, R. H. & Zimmerman, J. 1983 The effects of body size and temperature on metabolic rate of organisms. *Can. J. Zool.* **61**, 281–288.

Savage, V. M., Gillooly, J. F., Brown, J. H., West, G. B. & Charnov, E. L. 2004*a* Effects of body size and temperature on population growth. *Am. Nat.* **163**, 429–441. (doi:10.1086/381872)

Savage, V. M., Gillooly, J. F., Woodruff, W. H., West, G. B., Allen, A. P., Enquist, B. J. & Brown, J. H. 2004*b* The predominance of quarter power scaling in biology. *Funct. Ecol.* **18**, 257–282. (doi:10.1111/j.0269-8463.2004.00856.x)

Tyler, A. 1939 *The energetics of embryonic differentiation*. Paris, France: Hermann.

Vleck, C. M., Vleck, D. & Hoyt, D. F. 1980 Patterns of metabolism and growth in avian embryos. *Am. Zool.* **20**, 405–416. (doi:10.1093/icb/20.2.405)

West, G. B., Brown, J. H. & Enquist, B. J. 1997 A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122–126. (doi:10.1126/science.276.5309.122)

West, G. B., Brown, J. H. & Enquist, B. J. 2001 A general model for ontogenetic growth. *Nature* **413**, 628–631. (doi:10.1038/35098076)