

Energetics of Lizard Embryos at Fluctuating Temperatures

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ABSTRACT

Most animals experience fluctuations in temperature during development, but studies of energetics have ignored the potential influence of these thermal fluctuations. We measured the energetics of *Sceloporus undulatus* lizard embryos under two conditions that differ realistically in the mean and variance of temperature (diel cycles of 20°–30° and 20°–34°C). Our goal was to determine whether embryos in warm nests would expend more energy to develop than embryos in cool nests. We quantified metabolic rates during development, durations of incubation, and sizes at hatching. To describe changes in metabolic rate during incubation, we used the Akaike Information Criterion to determine the best statistical model among a set of six candidates. Once the best model was determined, the energetic cost of development was estimated by integrating metabolic rates over the period of incubation. We found that some form of sigmoidal model provided the best fit to the data for the majority of embryos (75%). Although embryos in the warmer treatment hatched earlier, the cost of development (≈ 1.6 kJ) did not differ significantly between embryos in the two treatments. This estimate of energy expenditure at fluctuating temperatures accords with previous estimates of energy expenditure at constant temperatures, suggesting that embryonic metabolism under realistic thermal conditions does not differ substantially from that under constant conditions.

Introduction

As with other ontogenetic stages, an organism's performance during the embryonic stage depends greatly on body temperature (Deeming and Ferguson 1991; Booth 2006). Over a broad range of temperatures, the warming of an embryo speeds its

metabolism, growth, and development. These thermal effects ultimately determine the energetic cost of development (i.e., the energy lost to catabolism during the embryonic stage). Specifically, higher temperatures affect the energetic cost of development by raising the rate of metabolism (Vleck and Hoyt 1991) and shortening the duration of development (Deeming and Ferguson 1991). These two effects combine to generate a counterintuitive pattern: embryos incubated at a high temperature require the same or less energy to develop than do embryos incubated at a low temperature (Booth and Thompson 1991). For example, an increase in the temperature of embryonic lizards by 10°C caused a doubling of metabolic rate (Angilletta et al. 2006). Nevertheless, rapid growth and development at high temperatures seems to compensate for a high metabolic rate because the cost of development appears relatively insensitive to temperature (Angilletta et al. 2000). Acclimation of metabolic rate can further reduce the cost of development at high temperatures (O'Steen and Janzen 1999). The balance between metabolic and developmental rates might canalize the energetics of embryos over a wide range of thermal environments.

Despite the intriguing patterns of thermal physiology generated in laboratories, the extent to which these patterns have ecological relevance remains unknown. Our state of ignorance stems from a serious disconnect between the thermal treatments used in laboratory experiments and those of natural nests. Specifically, most laboratory experiments have been conducted at two or more constant temperatures, but natural nests can fluctuate greatly on a daily basis (Cagle et al. 1993; Shine and Harlow 1996; Andrews 2000). For example, nests of eastern fence lizards (*Sceloporus undulatus*) varied by as much as 20°C on sunny days (Angilletta et al. 2009). These thermal fluctuations have important implications for the phenotype. First, embryos grow and develop rapidly during acute exposures to temperatures that would prove lethal during prolonged exposures (Christian et al. 1986; Oufiero and Angilletta 2006; Storm and Angilletta 2007). Second, rates of physiological performance usually change nonlinearly in response to changes in body temperature, approaching a maximum at some intermediate temperature (Huey and Stevenson 1979; Angilletta et al. 2002). Consequently, the mean and variance should interact to determine an individual's performance in a fluctuating environment. In other words, a high variance of temperature could speed development at low mean temperatures but would slow development and threaten survival at high mean temperatures. Finally, theoretical models suggest that the patterns of physiological acclimation should differ between constant and fluctuating environments (Gabriel 2005). Taken together, these factors might explain why rates of growth and survival in fluctuating environments differ from those observed in constant environments (Shine and Harlow 1996; Shine et al. 1997; Shine

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and Elphick 2001; Du and Ji 2006). Thus, studies of metabolic rate in fluctuating environments should enhance our understanding of embryonic energetics.

In this article we present the first estimates of energetic costs of development under ecologically relevant thermal conditions. Our thermal treatments were designed to mimic the diel cycles of temperature experienced by embryos of *S. undulatus*. Therefore, we positively covaried the mean and variance of temperature as observed among nests in natural soils (Angilletta et al. 2009). We compared metabolic rates, incubation periods, and hatchling sizes of individuals incubated in the two thermal treatments. For each embryo, we used an information-theoretic approach to identify the best statistical model to describe the temporal changes in the metabolic rate. Using the best models, we integrated metabolic rates over time to estimate energetic costs of development under warm and cool conditions. We show that realistic fluctuations in temperature have little influence on the energetic cost of development in lizard embryos.

Material and Methods

Collection and Husbandry

Gravid females of *Sceloporus undulatus* ($n = 8$) were collected from Burlington County, New Jersey, in June 2003 and were transported to Indiana State University. These females were housed in 38-L glass terraria, which were kept in a controlled environment with a 12L : 12D light cycle and an ambient temperature of 23°C. Females were fed crickets ad lib. and were misted with water daily. An incandescent bulb was placed on one side of the cage to enable behavioral thermoregulation; operative temperatures within each cage ranged from 25° to 38°C.

Acquisition and Care of Eggs

We acquired eggs by hormonally inducing females to oviposit. Controlling the timing of oviposition served two purposes. First, we could weigh eggs immediately after laying to avoid changes in mass caused by water flux. Second, we could coordinate dates of oviposition among females to synchronize measures of embryonic respiration during development. Inducing females to oviposit might have caused variation in the embryonic stage at oviposition; nevertheless, this variation would have had a trivial effect on the energetic cost of development (Oufiero and Angilletta 2006) because of the very slow acceleration of metabolic rate at early stages (see "Results" and Angilletta et al. 2000). Oviposition was induced with an intra-coelomic injection of ≈ 0.5 mL of oxytocin (20 USP units mL⁻¹; Butler, Columbus, OH). After induction, each lizard was placed in a plastic container (4 L) with a substrate of moist sand. These containers were kept in a dark incubator set at 30°C. We monitored females closely for up to 9 h or until palpation revealed that all eggs had been deposited. Freshly laid eggs were

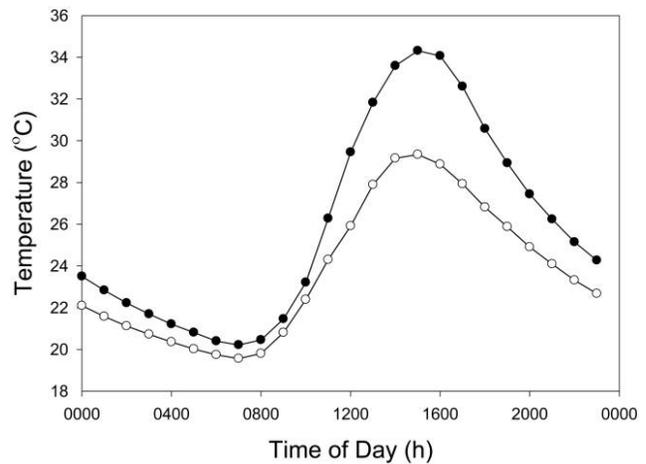


Figure 1. Diel cycles of temperature in our experiment were based on temperatures recorded within natural nests of *Sceloporus undulatus* (Warner and Andrews 2002; Angilletta et al. 2009). Mean temperatures of these treatments were 27°C (filled circles) and 24°C (unfilled circles).

assigned unique marks for identification and were weighed to the nearest 0.01 mg.

Eggs from each clutch were randomly assigned to each of two thermal treatments (Fig. 1): a warm cycle that ranged from 20° to 34°C with a mean of 27°C ($n = 12$ eggs) and a cool cycle that ranged from 20° to 30°C with a mean of 24°C ($n = 12$ eggs). These thermal cycles were based on the temperatures of nests constructed by females in artificial thermal gradients and in natural environments (Warner and Andrews 2002; Angilletta et al. 2009). Thermal cycles were created with two programmable incubators (KB 115, Brinkmann Instruments, Westbury, NY) controlled by commercially available software (APT-COM, Binder, Tuttlingen, Germany).

Except for temperature, the environments during incubation were similar for all eggs. Eggs were placed in plastic containers (0.5 L) filled with approximately 450 g of fine sand mixed with 4.5 g of distilled water. This mixture resulted in a soil water potential of -10 kPa, as determined with a tensiometer (2725A, Soilmoisture Equipment, Santa Barbara, CA). The water lost from the medium was replaced every few days by adding distilled water until each container was brought to its original mass. Although this procedure permitted small changes in water potential, embryos incubated under these conditions absorbed water at a relatively constant rate throughout incubation (Angilletta et al. 2000). Each time we added water to the containers, their positions within the incubators were rotated to preclude spatial effects on embryonic development. Two eggs were incubated in each container; pairs of eggs were never from the same clutch to avoid systematic covariation between environmental and maternal factors.

Respirometry

We estimated the energetic cost of development from repeated measures of respiration. At set intervals throughout incubation,

we recorded the oxygen consumption of embryos by closed-system respirometry (TR-3, Sable Systems, Las Vegas, NV). These recordings began 1 d after oviposition and were repeated every 4 d until each egg hatched.

Each recording lasted 24 h so that respiration could be summed over an entire thermal cycle. Before a recording, eggs were removed from their containers, cleaned of adhering sand, and weighed to the nearest 0.01 mg. Each egg was placed on a piece of sterile cotton in a plastic dish. To prevent dehydration during respirometry, 1 mL of distilled water was added to the cotton. The dish was then positioned inside a clean glass chamber (250 mL). Sealed chambers were placed in the same programmable incubators that housed the incubation containers. To ensure that embryonic temperatures equaled the air temperature of the incubator, chambers were placed in the incubators at least 1 h before measures of respiration. During this time, we calibrated the oxygen analyzer (FC-1, Sable Systems) with a gas of known concentration. Recordings always commenced at 1600 hours. Initially, all chambers were flushed sequentially with atmospheric air purged of water and carbon dioxide by a gas generator (75-45, Parker Balston). Each chamber was flushed again at 12 and 24 h after the initial flush. At each flush, all air from the chamber was passed through the oxygen analyzer at a known rate. A computer controlled the sequential flushing of chambers to ensure that each chamber was sealed for a precise duration between recordings. Given our measures of oxygen concentration and flow rate, we could calculate the total volume of oxygen consumed while the chamber was sealed. Daily energy expenditure was calculated as the sum of oxygen consumption during the sequential 12-h periods. Although each recording ended at 0400 hours, eggs were not returned to their incubation containers until 0800–1000 hours. Thus, eggs spent about 40 h within chambers during each recording. Nevertheless, these eggs developed similarly to eggs incubated without measures of respiration (Oufiero and Angilletta 2006).

Each recording was analyzed by a computer program (CONVOL, Sable Systems) to generate a daily rate of oxygen consumption (mL d^{-1}). These rates were converted to energy expenditure (J d^{-1}) by assuming embryos catabolized protein and lipid in equal quantities ($19.05 \text{ J mL}^{-1} \text{ O}_2$; Nagy 1983)—an assumption supported by the energy densities of eggs (Oufiero et al. 2007) and the respiratory quotients of embryos (Thompson and Stewart 1997; Thompson and Russell 1998, 1999).

Cost of Development

We estimated the energetic cost of development by integrating rates of energy expenditure over the period of incubation. Previously, researchers have used a variety of statistical models to describe ontogenetic changes in energy expenditure (Leshem et al. 1991; Birchard and Reiber 1995; Marsh 1999; Booth et al. 2000; Tullis and Peterson 2000). Instead of arbitrarily choosing one of these models, we used an information-theoretic

approach (Burnham and Anderson 2002) to identify the model that best described changes in energy expenditure. Six models were considered: one linear model, two exponential models, and three sigmoidal models (Table 1). Because we made repeated measures of respiration, we fitted all six models to each embryo's change in daily respiration. All models were fitted using TableCurve 2D (ver. 5.01; SYSTAT Software, Chicago).

To find the best model for each embryo, we calculated the Akaike Information Criterion (AIC) for each of the six models. The value of AIC was calculated as follows:

$$\text{AIC} = -2L + 2K + \frac{2K(K+1)}{N-K-1}, \quad (1)$$

where L equals the maximized log-likelihood value of the model, K equals the number of parameters (including the error term), and N equals the sample size. The maximized log-likelihood value of a model was computed from the model's residual sum of squares (RSS):

$$L = \log\left(\frac{\text{RSS}}{N}\right) \times \frac{-N}{2}. \quad (2)$$

Because AIC estimates the information lost when using a particular model to describe the data (Burnham and Anderson 2002), the best model would have the lowest value of AIC. By integrating the best model for each embryo, we obtained its energetic cost of development.

Statistical Analyses

We used ANCOVA to compare costs of development, incubation periods, and hatchling sizes between the two thermal treatments. Egg mass at oviposition was used as the covariate. In each treatment, values for eggs of the same clutch were averaged to avoid pseudoreplication. Therefore, we analyzed only eight values for each thermal treatment even though we had 12 eggs in each treatment. These analyses were performed with Statistica (ver. 6.0; Statsoft, Tulsa, OK).

Results

By adopting an information-theoretic approach, we determined the best function to describe the change in energy expenditure for each embryo (individual model). Some form of sigmoidal model best described changes in metabolic rate for 75% of the embryos in each thermal treatment (Table 1). Some form of exponential model provided the best fit for the remaining embryos in each treatment. The linear model provided relatively poor descriptions of metabolic rate for all embryos. Given the superior fit of a particular sigmoidal model (model D; Table 1), we fitted this model to data pooled among embryos in each thermal treatment (population model). Collectively, these mod-

Table 1: Six functions used to model the relationship between the hour of incubation and the rate of oxygen consumption

Model	Equation	Warm Treatment	Cool Treatment
A. Linear	$y = a + bx$		
Proportion of embryos		.000	.000
Median w_i		.000	.000
Range of w_i		.000–.001	.000–.045
B. Exponential 1	$y = ae^{(-x/b)}$		
Proportion of embryos		.000	.250
Median w_i		.056	.101
Range of w_i		.001–.219	.000–.616
C. Exponential 2	$y = a + be^{(-x/c)}$		
Proportion of embryos		.250	.000
Median w_i		.038	.042
Range of w_i		.000–.802	.000–.282
D. Sigmoidal 1	$y = a + (4be^n)/(1 + e^n)^2$, where $n = -(x-c)/d$		
Proportion of embryos		.417	.750
Median w_i		.268	.489
Range of w_i		.063–.951	.042–.985
E. Sigmoidal 2	$y = a + b/[1 + (x/c)^d]$		
Proportion of embryos		.000	.000
Median w_i		.045	.062
Range of w_i		.002–.308	.007–.133
F. Sigmoidal 3	$y = a + b/(1 + e^{-(x-c)/d})$		
Proportion of embryos		.333	.000
Median w_i		.155	.126
Range of w_i		.034–.578	.008–.311

Note. For each model, we list the proportion of embryos for which the model provided the best description of the data. We also provide the median and the range of Akaike Information Criterion weights.

els enabled us to compare energetic costs of development estimated at the individual and population levels.

Throughout the course of incubation, embryos in the warm treatment expended about the same quantity of energy as did embryos in the cool treatment (Table 2). As expected, embryos in the warmer treatment exhibited a greater metabolic rate throughout incubation (Fig. 2). Nevertheless, faster development in the warmer treatment compensated for the higher metabolic rate such that total energy expenditures were the same under warm and cool conditions. This conclusion held regardless of whether we compared energy expenditures estimated from individual models or population models. The pattern of hatchling size was consistent with the pattern of energy expenditure; neither snout-vent lengths nor masses differed significantly between thermal treatments (Table 2). The egg mass at oviposition affected the body mass at hatching, with larger eggs producing larger hatchlings ($\beta = 0.669$, $MS = 0.0086$, $F_{1,12} = 11.12$, $P = 0.006$). However, egg mass did not affect the cost of development, duration of incubation, or length at hatching ($P > 0.05$ for all three traits).

Discussion

Few researchers have examined the effects of fluctuating temperatures on the development of embryos, much less their ef-

fects on energetics. When embryos have been incubated at fluctuating temperatures, an increase in the mean temperature always reduced the duration of incubation (Qualls and Shine 1996, 1998; Elphick and Shine 1998; Qualls and Andrews 1999; Shine 1999; Lu et al. 2009). Although warmer conditions increase the rate of development, they do not necessarily increase the energetic cost of development. In our experiment, both embryos in warm and cool environments consumed nearly identical amounts of energy during incubation. This canalization of energy expenditure likely resulted from a balance between the rate and the duration of embryonic metabolism (Angilletta et al. 2006); higher temperatures speed the rate of metabolism but also reduce the duration of incubation. If these factors balance precisely, the energetic cost of development would vary little with temperature (Booth and Thompson 1991; Booth et al. 2000).

The energetic costs of development reported here compare favorably with those quantified at constant temperatures. In a previous study, Angilletta and colleagues (Angilletta et al. 2000) estimated costs of development for embryos of *Sceloporus undulatus* from the same population that we sampled for our study. These researchers reported costs ranging from 1.7 to 2.0 kJ at constant temperatures ranging from 28° to 34°C (Angil-

Table 2: Phenotypes (mean \pm SD) of lizards incubated at warm and cool cycles

Trait	Warm Treatment	Cool Treatment	MS	F	P
Incubation period (d)	68.8 \pm 2.8	81.8 \pm 3.6	687.698	77.27	.000001
Hatchling mass (g)	.59 \pm .02	.61 \pm .05	.002	2.08	.17
Hatchling length (mm)	26.36 \pm .63	26.06 \pm 1.29	.261	.27	.61
Cost of development (kJ):					
Individual models	1.61 \pm .10	1.63 \pm .12	.002	.13	.73
Population models	1.71	1.79

Note. Costs of development were estimated from statistical models fitted to the data for each embryo (individual models) and from statistical models fitted to the data pooled among embryos in each treatment (population models). For variables estimated at the level of the individual, we used ANCOVA to compare means between treatments.

letta et al. 2000). In comparison with these values, our estimates of energy expenditure averaged 1.6 kJ based on the individual models and 1.7–1.8 kJ based on the population models (see Table 2). Additionally, the embryonic respiration of *S. undulatus* at fluctuating temperatures (85 mL O₂) was similar to that recorded for a congeneric species, *Sceloporus virgatus*, at a constant temperature (82 mL O₂; Vleck and Hoyt 1991). Finally, the cost of growth, defined as the amount of respiration per unit of growth, fell within the range of values determined at constant temperatures. Specifically, Booth and colleagues (Booth et al. 2000) concluded that the cost of growth for embryonic lizards should not exceed 3.0 kJ g⁻¹; in line with this

prediction, the cost of growth was 2.7 kJ g⁻¹ for embryos in both of our thermal treatments. Overall, the energetics of embryos at fluctuating temperatures did not differ qualitatively from their energetics at constant temperatures.

Researchers routinely choose from a variety of nonlinear functions that could describe changes in metabolic rate during embryonic development (Leshem et al. 1991; Birchard and Reiber 1995; Marsh 1999; Booth et al. 2000; Tullis and Peterson 2000). Unfortunately, no mechanistic justification exists for the choice of one function over others. Complex functions can be problematic because errors in the data can heavily influence the parameters of these functions (Burnham and Anderson

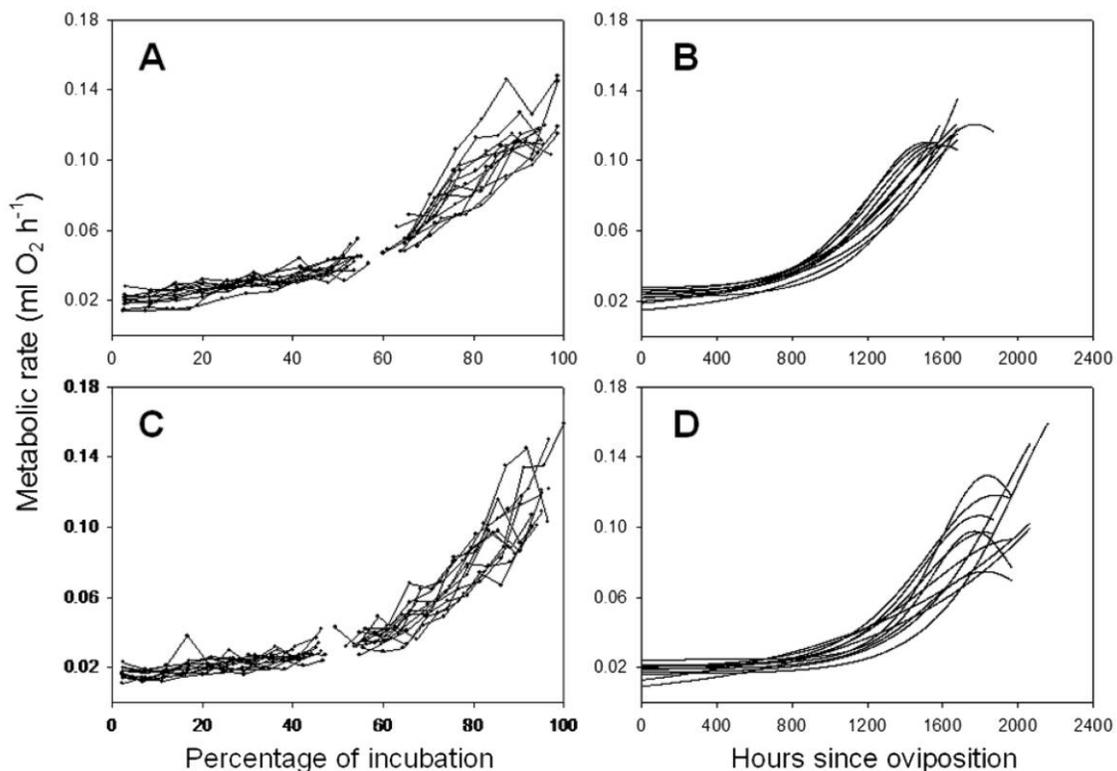


Figure 2. Metabolic rates of most embryos increased sigmoidally during development in either warm (A, B) or cool (C, D) environments. Plots on the left show the raw data for each embryo. Plots on the right show the best models selected according to the Akaike Information Criterion (Table 1).

2002). An information-theoretic approach enables one to evaluate multiple functions while guarding against the possibility of fitting error in the data. Of the six models that we evaluated, a sigmoidal model provided the best description of the ontogeny of embryonic metabolism. We find this result encouraging because embryonic metabolism decelerates before hatching in other species of lizards (reviewed by Thompson and Speake 2003). Interestingly, an exponential model was used previously to describe changes in the embryonic metabolism of *S. undulatus* (Angilletta et al. 2000). In our study, exponential models were best for a very small proportion of embryos (Table 1). Most likely, previous measurements of metabolic rates in *S. undulatus* were inadequate to characterize a sigmoidal relationship because few measurements were made toward the end of incubation, when metabolism begins to decelerate (cf. Fig. 2 with Fig. 2 of Angilletta et al. 2000). By adopting an information-theoretic approach, such as the one used here, researchers might obtain more accurate estimates of the energetic cost of development. In particular, researchers might uncover different strategies of growth and development among embryos, which could lead to studies of the evolutionary significance of this variation. Nevertheless, some chance exists that the differential fit of the models among embryos reflects error in our measures of metabolic rate rather than variation in the processes of growth and development that require oxygen consumption. In this case, the true cost of development was likely closer to the 1.7–1.8 kJ estimated from the population models than it was to the 1.6 kJ estimated from the individual models (Table 2).

Although studies of embryonic development have generally involved constant temperatures, biologists have used the results of those studies to answer questions about patterns in natural environments that can undergo dramatic fluctuations in temperature. Patterns such as maternal nesting behavior and environmental sex determination presumably stem from selective pressures imposed by embryonic physiology. If the metabolic rate of an embryo acclimates to thermal change, growth and development would depend on the interaction of the mean and the variance of environmental temperature. Consequently, phenotypes expressed in constant environments might not reflect those expressed in fluctuating environments (Casagrande et al. 1987; Taylor and Shields 1990; Worner 1992). Recent technological advances have prompted some researchers to create more natural thermal environments in their experiments. From these experiments, we have learned that thermal fluctuations can impose greater costs of growth (Olson et al. 2006) and alter phenotypes at hatching (Shine and Elphick 2001; Ashmore and Janzen 2003; Du and Ji 2006) compared with constant temperatures. Nevertheless, our results indicate that patterns observed among fluctuating environments do not always differ from those observed among constant environments; specifically, the energetic cost of development appears similar between warm and cool environments regardless of whether these environments fluctuate or remain constant throughout development. Because the thermal fluctuations in our experiment mimicked natural variation within and among nests, we should

be able to extend our inference about energetics to a natural environment. Still, the paucity of information about development in fluctuating environments warrants additional studies. Such studies will either increase our confidence in current ideas about embryonic physiology or generate new questions about developmental processes.

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