

# Energetics of Lizard Embryos Are Not Canalized by Thermal Acclimation

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## ABSTRACT

In some species of ectotherms, temperature has little or no effect on the amount of energy expended during embryonic development. This phenomenon can result from either of two mechanisms: (1) a shorter incubation period at higher temperatures, which offsets the expected increase in metabolic rate, or (2) a compensatory decrease in the rate at which embryos expend energy for maintenance. To distinguish the relative importance of these two mechanisms, we quantified the acute and chronic effects of temperature on embryonic metabolism in the eastern fence lizard (*Sceloporus undulatus*). First, we measured metabolic rates of individual embryos at 27°, 31°, and 34°C. Second, we examined the capacity for thermal acclimation by measuring the metabolic rates of embryos at 30°C, after a period of incubation at either 28° or 32°C. As with adult reptiles, the metabolic rates of embryos increased with an acute increase in temperature; the  $Q_{10}$  of metabolic rate from 27° to 34°C was 2.1 ( $\pm 0.2$ ). No evidence of thermal acclimation was observed either early or late in development. In *S. undulatus*, a shorter incubation period at higher temperatures appears to play the primary role in canalizing the energy budget of an embryo, but a reduction in the cost of growth could play a secondary role.

## Introduction

Despite the fact that embryos generally grow faster at higher temperatures, one cannot predict how temperature will affect

the efficiency of embryonic growth (defined as the proportion of the energy absorbed by an embryo that is deposited in tissue). In some species, temperature has little or no effect on growth efficiency (Johns et al. 1981; Leshem et al. 1991; Whitehead et al. 1992; Kamler et al. 1998; Booth et al. 2000; Oufiero and Angilletta, forthcoming). In other species, temperature exerts either positive or negative effects on growth efficiency. For example, two species of Australian turtles (*Chelodina expansa* and *Emydura signata*) grew less efficiently at 24°C than at higher temperatures (Booth 1998a, 2000). Positive relationships between temperature and growth efficiency were also observed in embryos of salmon (Hayes and Pelluet 1945) and moss frogs (Mitchell and Seymour 2000). In contrast, a species of Australian lizards (*Lampropholis guichenoti*) grew less efficiently at 30°C than at 25°C (Booth et al. 2000). Clearly, the proximate mechanisms by which temperature affects the energetics of embryos are fairly complex and poorly understood.

To understand the thermal sensitivity of energetics, one must consider the cellular processes that determine the acquisition and allocation of energy. The energy budget of an embryo can be conceptualized as two sources divided among several sinks. The chemical potential energy of yolk and albumen is either transferred to embryonic tissues or used to generate the ATP that fuels the maintenance, activity, and growth of these tissues (see Dietz et al. 1998). The maintenance of ionic gradients, the synthesis of macromolecules, and the motility of cells require ATP (Clarke 1980). The effect of temperature on the rate and efficiency of growth will depend on whether these processes respond proportionally or disproportionately (Wieser 1994).

Based on current knowledge, these processes almost certainly respond disproportionately to a change in temperature. Indeed, temperature should have independent effects on the energetic costs of growth and maintenance. In theory, the energy required to produce a quantity of embryonic tissue depends only on the composition of the tissue and the substrates used for anabolism (Parry 1983; Wieser 1994); therefore, any effect of temperature on the efficiency of embryonic growth should be mediated primarily by a change in the cost of maintenance. Among other things, maintenance includes the processes of ion transport and protein turnover. The diffusion of ions through membrane channels increases with increasing temperature, so higher temperatures should cause embryos to spend more energy to maintain ionic gradients (Hurlbert and Else 2000). The degradation of proteins increases with increasing temperature, so higher temperatures should cause embryos to spend more energy on protein turnover (i.e., the synthesis of proteins to offset their

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degradation; Clarke 1980). Overall then, we expect embryos in warmer environments to expend energy on maintenance at a higher rate.

An acute increase in temperature should increase embryonic metabolism, but the total energy expended during incubation may or may not be sensitive to temperature. This statement might seem paradoxical but can be explained by two phenomena. First, the incubation period decreases with increasing temperature (Booth and Thompson 1991); thus, the energy lost to an increase in metabolic rate will be offset by the energy saved from a decrease in the duration of metabolism. Second, embryos at high temperatures can compensate by consuming ATP at a lower rate. For example, the cost of maintaining ionic gradients can be reduced by modifying the structure of cellular membranes (Hazel and Williams 1990; Hazel 1995; Hurlbert and Else 2000). A further reduction in metabolic rate can be achieved by reducing protein turnover (Hawkins 1991; Bayne 2004). In summary, the effect of temperature on an embryo's energy expenditure, and hence its growth efficiency, depends on the relative thermal sensitivities of the incubation period and each of many metabolic processes.

Thermal acclimation of cellular processes should cause changes in metabolic rate that one can observe when comparing embryos from different thermal environments. For example, the embryos incubated at a high temperature usually expend energy at the same rate as embryos incubated at a low temperature, after rates are adjusted for developmental stage (Birchard and Reiber 1995; Angilletta et al. 2000). To determine the effect of thermal history on the metabolism of embryos, Booth (1998b) switched embryos from high temperature to low temperature (and vice versa) midway through development. He reported a difference in metabolic rate that was consistent with the hypothesis of metabolic compensation; immediately after switching embryos, the metabolic rate of embryos that had been incubated at the low temperature was greater than the metabolic rate of embryos that had been incubated at the high temperature. However, this difference disappeared within a few days of the switch in incubation temperature. Through a novel experiment, O'Steen and Janzen (1999) acquired convincing evidence that incubation temperature affects the metabolism of snapping turtles (*Chelydra serpentina*) via circulating levels of thyroid hormone. Despite the evidence that suggests embryos can compensate for thermal heterogeneity, we do not know (1) whether most embryos possess a capacity for thermal acclimation or (2) whether thermal acclimation occurs early enough during development to significantly affect the energy budget.

In this article, we report the acute and chronic effects of temperature on the embryonic metabolism of the eastern fence lizard (*Sceloporus undulatus*). In a previous study of *S. undulatus*, Angilletta et al. (2000) observed that the energy budget of an embryo was relatively insensitive to incubation temperature. In this study, we aimed to discover whether thermal

acclimation of metabolic rate contributed to the canalization of the energy budget. To determine the acute effects of temperature on metabolic rate, we measured metabolic rates of individual embryos over a range of temperatures experienced within natural nests. To our knowledge, we present the first direct estimate of the thermal sensitivity of metabolism in reptilian embryos. To determine whether metabolic compensation occurs during development, we compared the metabolic rates of embryos at a common temperature after they had been incubated at either a low or a high temperature. The presence or absence of metabolic compensation would distinguish its possible role in canalizing the energy budget.

### Material and Methods

We conducted two experiments to quantify acute and chronic effects of temperature on embryonic metabolism. Conceivably, one could quantify both effects in a single experiment, but such an experiment would be difficult to execute. Metabolic compensation is documented by comparing metabolic rates of individuals with different thermal histories. Each group of embryos must be incubated at a different temperature, and then the relationship between temperature and metabolic rate in these groups must be compared after some duration of incubation. Such comparisons are valid, however, only if they are made at specific stages of development because the composition of tissues and the intensity of cellular functions change throughout development (Hurlbert and Else 2000). In contrast, the thermal sensitivity of metabolic rate is best estimated through repeated measures of metabolism within individuals (e.g., Hoyt et al. 1978). These measures must affect the trajectory of development and thus make it difficult to estimate the stage of embryos during further incubation. Therefore, the procedures for measuring thermal sensitivities preclude controlled comparisons of metabolism between embryos from different incubation temperatures. To overcome this problem, we designed two experiments. In the first experiment, we estimated the thermal sensitivity of metabolic rate by repeatedly measuring the metabolism of individual embryos. In the second experiment, we incubated embryos at either a low or a high temperature and then measured their metabolic rates at an intermediate temperature during specific stages of development. These experiments were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee (protocol 701641).

### Acquisition and Care of Eggs

We collected gravid females from Burlington County, New Jersey, and transported them to the University of Pennsylvania. Each female was placed in a 6-L terrarium with a substrate of moist sand. Terraria were placed in an incubator in which a constant temperature of 30°C and a light cycle of 10L:14D

were maintained. Females were fed crickets ad lib. and provided water by daily misting. Terraria were examined for eggs several times each day. Immediately after discovery, eggs were removed and weighed to the nearest 0.01 mg. Each female laid an average of eight eggs, and clutches were divided evenly among incubation temperatures (thermal treatments are described below). Eggs were incubated in a medium of fine sand with a water content of 1% by mass, resulting in a water potential of  $-10$  kPa (determined by tensiometry). At least once per week, the water content of the medium was restored to its original value by replacing water that was lost to evaporation or was absorbed by eggs.

### *Respirometry*

Metabolic rate was measured with a flow-through system (Sable Systems, Henderson, NV), which was described in detail by Angilletta et al. (2000). Eggs were weighed and sealed in sterilized respirometry chambers ( $\approx 30$  mL). We flowed air through the chambers for at least 1 h before measuring metabolic rate to ensure the temperature of the egg was at an equilibrium. Carbon dioxide production was recorded continuously for a 5-min period using data acquisition software (DAC; Sable Systems). Before and after each period, a baseline measurement of  $\text{CO}_2$  concentration was recorded from an empty chamber, which was identical to the chambers that contained eggs.

Metabolic rates were analyzed with the computer program DAN (Sable Systems). The percent enrichment of  $\text{CO}_2$  by an egg was calculated from the baseline concentrations associated with each recording. The rate of  $\text{CO}_2$  production ( $\text{mL h}^{-1}$ ) at each sampling point was calculated as the product of the percent enrichment and the flow rate. Rates of  $\text{CO}_2$  production were averaged over each 5-min recording to yield a single estimate of metabolic rate. Carbon dioxide production was converted to energy expenditure by assuming energy was derived equally from protein and lipid (Thompson and Stewart 1997; Thompson and Russell 1998, 1999), which yielded 23.1 and 27.7  $\text{J mL}^{-1}$ , respectively (Schmidt-Nielsen 1997).

### *Thermal Sensitivity of Metabolic Rate*

To quantify the thermal sensitivity of metabolic rate, we made repeated measures of  $\text{CO}_2$  production by individual embryos. These embryos ( $n = 22$ ) were surplus from another experiment (Angilletta et al. 2000). The embryos had been incubated at a constant temperature ( $28^\circ$ ,  $30^\circ$ , or  $32^\circ\text{C}$ ) until they had completed an average of 54% of incubation (95% confidence interval [CI] was  $\pm 8\%$  of incubation). For each embryo, we measured the carbon dioxide production at three temperatures ( $28^\circ$ ,  $32^\circ$ , and  $36^\circ\text{C}$ ), hereafter referred to as experimental temperatures. Embryos in natural nests experience these temperatures daily (Angilletta et al. 2005). Metabolic rates were measured at one experimental temperature per day. The order of

temperatures was randomized but was the same for all individuals ( $32^\circ$ ,  $28^\circ$ ,  $36^\circ\text{C}$ ). Repeated measures were separated by 48 h to enable eggs to regain water lost during respirometry. Before each measurement, eggs were weighed to the nearest 0.01 mg.

Due to evaporative water loss, body temperatures of embryos during respirometry were lower than air temperatures. We estimated the temperature of embryos by measuring the equilibrium temperature of an egg under each experimental condition. A thermocouple was placed inside an egg, and the egg was sealed in a respirometry chamber. We flowed air through the chamber as if gas exchange was being recorded. The temperature of the egg was monitored for 2 h, but an equilibrium temperature was achieved well within the first hour. The same procedure was used to estimate the temperature of an embryo at all three experimental temperatures. Temperatures of embryos were estimated to be  $27.1^\circ$ ,  $30.8^\circ$ , and  $34.0^\circ\text{C}$  at experimental temperatures of  $28^\circ$ ,  $32^\circ$ , and  $36^\circ\text{C}$ , respectively. Calculations of thermal sensitivity (i.e.,  $Q_{10}$ ) were made using these estimates of embryonic body temperature.

We used an ANOVA for repeated measures to assess the effect of body temperature on the  $\text{CO}_2$  production of embryos. Only embryos that survived to hatching (12/22 or 54%) were included in the analysis and were used to generate descriptive statistics. Because the data did not meet the assumption of compound symmetry required by the univariate analysis ( $W = 0.19$ ,  $\chi^2 = 16.40$ ,  $df = 2$ ,  $P < 0.001$ ), we used the multivariate analysis to test for the within-subjects effect of body temperature. The multivariate analysis makes no assumption about the structure of the variance-covariance matrix (Potvin et al. 1990), and all other assumptions of this analysis were met. Statistical analyses were performed using the GLM module of Statistica for Windows (StatSoft 2003). All descriptive statistics are reported as mean  $\pm$  95% CI.

### *Thermal Acclimation of Metabolic Rate*

We conducted a second experiment to determine if thermal compensation occurs during embryonic development. Sixty-three eggs from seven clutches were randomly assigned to be incubated at either  $28^\circ$  or  $32^\circ\text{C}$  ( $n = 32$  and  $31$ , respectively). At two stages of incubation, we removed eggs from their respective thermal treatment and measured metabolic rate at  $30^\circ\text{C}$ . By using a common temperature at which to compare metabolic rates, we were able to identify any compensation that might have occurred during incubation while controlling for acute effects of temperature on metabolism.

Because growth and development proceeds more rapidly at  $32^\circ\text{C}$  than at  $28^\circ\text{C}$  (Angilletta et al. 2000), we aimed to measure metabolic rates at particular stages rather than particular ages. Based on Marr (1966), we considered the ratio of dry embryo mass to dry egg mass (embryo mass + yolk mass) to be a reliable estimate of developmental stage; this estimate is pref-

erable to those based on morphology because temperature can uncouple growth and differentiation so that developmental sequences may not be comparable for embryos at different temperatures (Hayes and Pelluet 1945). Also, this method controls for differences in metabolic rate that could be caused by variation in residual yolk. Because individuals incubated at different temperatures develop at different rates but hatch at a similar body size (Angilletta et al. 2000), we reasoned that Marr's estimate of developmental stage would correlate well with the percentage of total incubation completed (hereafter called the stage of incubation). We based our reasoning on the assumption that the pattern of growth during development is insensitive to temperature, even though the rate of growth increases with increasing temperature. This assumption is supported by the observation that the metabolic rate of *Sceloporus undulatus* increases exponentially throughout incubation, regardless of incubation temperature (Angilletta et al. 2000; C. E. Oufiero and M. J. Angilletta, unpublished data). Specifically, we aimed to measure metabolic rates at approximately 40% and 80% of incubation; based on previous work, we anticipated these stages to correspond to days 22 and 44 of incubation for embryos at 28°C and days 16 and 32 of incubation for embryos at 32°C. Based on dates of hatching in our study, measures were actually made when embryos had completed  $39.5\% \pm 0.6\%$  and  $79.1\% \pm 1.2\%$  of incubation.

We used ANCOVA to assess the effect of incubation temperature (28° or 32°C) on metabolic rate at 30°C. The stage of incubation at the time of measurement—calculated from the actual incubation period of each embryo—was used as a covariate. This approach was necessary because the actual stages of incubation differed slightly between embryos from the two treatments despite our effort to standardize these stages (see "Results"). Only embryos that survived to hatching (49/63 or 77%) were included in the statistical analysis and were used to generate descriptive statistics. Because maternal identity was included as a random factor, we had to exclude embryos from one clutch in which all survivors were from one incubation temperature. Levene's test was used to assess homogeneity of variances (all  $P > 0.05$ ), and the  $\chi^2$  goodness-of-fit test was used to examine the assumption of normality (all  $P > 0.05$ ).

To verify that embryos at similar stages of incubation did not differ in mass, we dissected 8–10 eggs from each incubation temperature at approximately 40% and 80% of incubation. Eggs were frozen at  $-80^\circ\text{C}$  and were dissected after the experiment was completed. Embryos were carefully separated from their yolk and shell. Embryos, yolk, and shells were dried for 72 h at  $50^\circ\text{C}$  and then weighed in a dry chamber. For each stage of incubation, we compared masses of embryos from the two thermal treatments using ANCOVA; the covariate was egg mass at oviposition. To avoid inflating degrees of freedom through pseudoreplication, we also included maternal identity (or clutch) as a random factor in our analysis (see Potvin 2001).

If the masses of embryos differed between the two incubation groups, they might explain a difference in metabolic rate.

## Results

### *Thermal Sensitivity of Metabolic Rate*

The body temperature of an embryo had a pronounced effect on its metabolic rate (Wilks's  $\lambda = 0.22$ ,  $F = 18.06$ ,  $df = 2, 10$ ,  $P < 0.001$ ), regardless of the embryo's thermal history (Fig. 1). The mean  $Q_{10}$  of metabolic rate between 27° and 34°C was  $2.1 \pm 0.2$ . These data were based exclusively on individuals that completed incubation successfully ( $n = 12$ ). Hatching success (54%) was within the range of 46%–68% reported by Sexton and Marion (1974) but was below the range of 62%–86% reported by Angilletta et al. (2000). Possibly, hatching success was reduced by evaporative water loss during respirometry; during the experiment, eggs that did not hatch ( $n = 10$ ) lost an average of  $10\% \pm 8\%$  of their initial mass, whereas eggs that hatched ( $n = 12$ ) gained an average of  $3\% \pm 11\%$  of their initial mass.

### *Thermal Acclimation of Metabolic Rate*

Using published estimates of incubation period (Angilletta et al. 2000), we tried to standardize the stages of incubation at which metabolic rates were compared. Unfortunately, incubation periods observed in our experiment differed slightly from those observed previously. As a consequence, embryos incubated at 28°C were measured at stages very close to our targets ( $40.5\% \pm 0.6\%$  and  $81.0\% \pm 1.3\%$  of incubation), but embryos incubated at 32°C were measured at slightly earlier stages than we had expected ( $38.1\% \pm 1.1\%$  and  $76.3\% \pm$

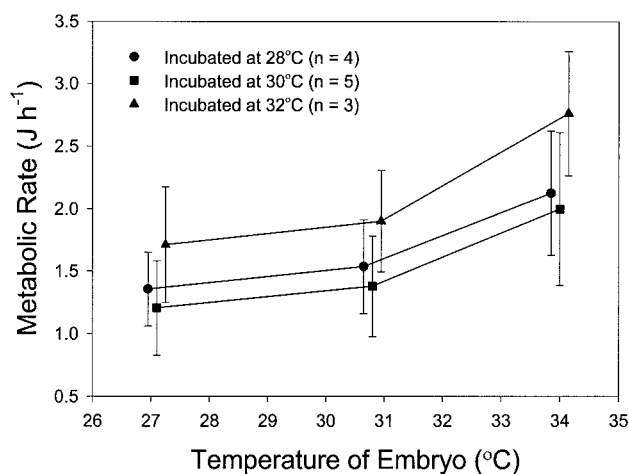


Figure 1. Metabolic rates of embryos increased nonlinearly with increasing body temperature (27°, 31°, and 34°C), regardless of prior incubation temperature. Error bars denote standard errors, and means are offset for clarity.

2.1% of incubation). In light of these data, we based our conclusions strictly on comparisons of means adjusted for the stage of incubation.

Contrary to our hypothesis, metabolic rates of embryos in the two groups did not diverge significantly during the course of incubation (Fig. 2). Early in development (<50% of incubation), metabolic rate at 30°C was not related to the stage of incubation ( $MS = 0.00002$ ,  $F = 0.50$ ,  $df = 1, 30$ ,  $P = 0.49$ ), and rates were similar for embryos incubated at high and low temperatures ( $MS = 0.000004$ ,  $F = 0.14$ ,  $df = 1, 20.0$ ,  $P = 0.71$ ). Later in development (>60% of incubation), metabolic rate at 30°C was strongly related to the stage of incubation ( $MS = 0.002$ ,  $F = 31.50$ ,  $df = 1, 30$ ,  $P < 0.0001$ ). Metabolic rates differed between embryos incubated at high and low temperatures, but this difference was caused by variation in the stage of incubation (see Fig. 2). Metabolic rates were similar after they were adjusted for the stage of incubation ( $MS = 0.000003$ ,  $F = 0.10$ ,  $df = 1, 31.8$ ,  $P = 0.76$ ).

Both early and late in development, embryos incubated at 28°C tended to be heavier than embryos incubated at 32°C (Table 1). The difference in embryonic dry mass was statistically significant early in development ( $MS = 0.00004$ ,  $F = 8.76$ ,  $df = 1, 15$ ,  $P < 0.01$ ) but was not statistically significant later in development ( $MS = 0.0003$ ,  $F = 0.45$ ,  $df = 1, 12$ ,  $P = 0.52$ ). Three facts suggest that these differences in mass were an artifact of our inability to standardize stages of incubation. First, embryos incubated at 28°C were sampled at later stages than embryos incubated at 32°C (see above); hence, small differences in embryonic mass between incubation groups should not be surprising. Second, metabolic rates adjusted for the stage of incubation did not differ significantly between incubation groups. Finally, the mean wet mass of hatchlings incubated at 28°C ( $0.55 \pm 0.02$  g) did not differ significantly ( $MS = 0.01$ ,  $F = 3.37$ ,  $df = 1, 5.3$ ,  $P = 0.12$ ) from that of hatchlings incubated at 32°C ( $0.52 \pm 0.04$  g).

## Discussion

Given that adult ectotherms respire faster at higher temperatures, biologists have assumed that embryos respond similarly to changes in temperature (Packard et al. 1977; Packard and Packard 1988). Before our study, however, little direct evidence was available to verify this assumption (reviewed by Deeming and Ferguson [1991]). Through repeated measures of individuals, we demonstrated conclusively that temperature influences the metabolism of embryonic lizards. Yet, we noted important differences between the thermal sensitivities of embryos and adults. Contrary to what has been observed in adult reptiles (reviewed by Bennett and Dawson [1976]), the thermal sensitivity of metabolic rate was greater in the higher range of temperatures (31°–34°C) than in the lower range (27°–31°C). In *Sceloporus undulatus*, the  $Q_{10}$  of metabolic rate in embryos was slightly less than the  $Q_{10}$  of metabolic rate in juveniles and

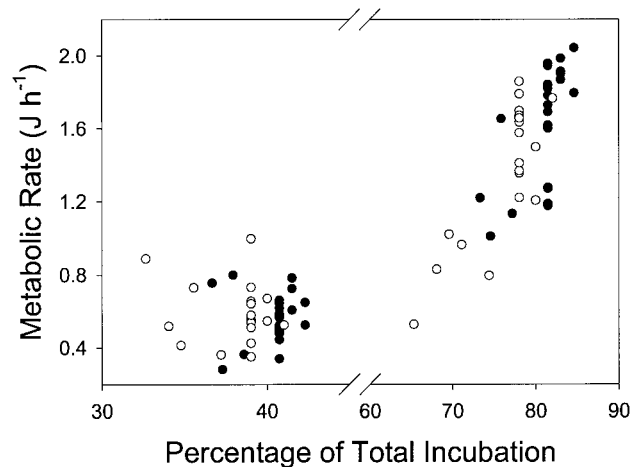


Figure 2. Metabolic rates at 30°C during two periods of embryonic development: (left) less than 50% of total incubation and (right) greater than 60% of total incubation. Before these measures, embryos had been incubated at either 28°C (solid circles) or 32°C (open circles). After adjusting for the stage of incubation, metabolic rates did not differ significantly between embryos incubated at 28°C and those incubated at 32°C.

adults ( $Q_{10} = 2.4$ , from 30° to 36°C; Angilletta 2001). Very likely, neither estimate of  $Q_{10}$  was affected by thermal acclimation because both studies comprised repeated measures of individuals over a few days.

Angilletta et al. (2000) hypothesized that, despite fluctuations in temperature, the energy budget of an embryo is canalized by thermal acclimation. Based on a pilot study conducted in 1997, they concluded that embryos probably underwent thermal acclimation, yet their conclusion was based on a sample of  $\leq 5$  individuals per incubation temperature. Our results indicate that thermal acclimation played no role in canalizing the energy budget. If acclimation did occur in our experiment, it was in the opposite direction of our expectation; in other words, embryos incubated at 32°C tended to have higher metabolic rates (at 30°C) than embryos incubated at 28°C (see Fig. 2). This observation contrasts the observations of other investigators, who presented indirect evidence of metabolic compensation in reptilian embryos. In *Chelydra serpentina*, the metabolic rate of embryos incubated at 30°C was not different from that of embryos incubated at 24°C, after adjusting for stage of incubation (Birchard and Reiber 1995). Also, metabolic rates of *Crocodylus johnstoni* did not differ between embryos incubated at 29° and 31°C (Whitehead 1987). Nonetheless, Angilletta et al. (2000) observed a similar phenomenon in embryos of *S. undulatus*, which appear to be incapable of metabolic compensation. Thus, other factors might explain (1) how similarly staged embryos at different temperatures can respire at the same rate, and (2) how the total energy expended during

Table 1: Dry masses of egg components after approximately 40% and 80% of incubation

Incubation Temperature	<i>n</i>	Mass of Embryo (mg)	Mass of Yolk (mg)	Mass of Shell (mg)	Adjusted Mass of Embryo (mg)
40% of incubation:					
28°C	10	14 (± 2)	129 (± 4)	25 (± 2)	13 (± 2)
32°C	10	11 (± 2)	133 (± 5)	25 (± 4)	10 (± 1)
80% of incubation:					
28°C	8	65 (± 22)	52 (± 22)	28 (± 7)	65 (± 21)
32°C	8	59 (± 16)	61 (± 14)	29 (± 6)	56 (± 21)

Note. Mean masses (± 95% confidence intervals) are reported. Embryo masses adjusted for egg mass at oviposition are also reported.

incubation can remain constant (or even decrease) when temperature increases.

Assuming thermal acclimation did not occur, a shorter incubation period at higher temperatures partially compensated for a higher metabolic rate. The incubation period of *S. undulatus* decreased from 55 d at 28°C to 40 d at 34°C (Angilletta et al. 2000), yielding a  $Q_{10}$  of 0.6. Given the thermal sensitivity of the incubation period, a  $Q_{10}$  of 1.7 for metabolic rate would stabilize the total energy expended during incubation. Our results indicate that this mechanism cannot entirely explain the pattern of energy use by embryos. First, the mean  $Q_{10}$  of metabolic rate was greater than 2, which suggests higher metabolic rates at higher temperatures could not have been offset completely by shorter incubation periods. Second, the  $Q_{10}$  of metabolic rate was unrelated to the stage of incubation over a wide range of stages ( $F = 0.03$ ,  $df = 1, 10$ ,  $r^2 = 0.003$ ,  $P = 0.86$ ), meaning our estimate of  $Q_{10}$  was not biased by measuring metabolism at particular stages. Taken together, these facts lead us to conclude that some other factor helps to canalize the energy budget of an embryo.

A decrease in the cost of growth with increasing temperature could explain the residual energetic savings that occur at high temperatures. Although models of energy allocation by embryos assume the mass-specific cost of maintenance remains constant (Vleck et al. 1980; Vleck and Hoyt 1991; Tullis and Peterson 2000), the composition of tissues and the intensity of cellular functions certainly change during development (Hurlbert and Else 2000). These changes can alter the efficiency with which cells transcribe DNA, translate mRNA, and fold proteins, as well as the pathways by which cells synthesize structural lipids and carbohydrates. Dietz et al. (1998) argued a reduction in the cost of growth could save energy during avian development. A similar phenomenon might occur during saurian development if, as Booth and Thompson (1991) suggested, growth requires less energy at higher temperatures. Direct estimates of the cost of growth at different temperatures are needed to test this hypothesis. Wieser (1994) suggested the cost of growth be estimated as the slope of the relationship between the amount of embryonic tissue and the total energy expended. Such measures can be made through a combination of calo-

rimetry and respirometry but would require one to sacrifice embryos at specific stages of incubation.

Importantly, thermal sensitivities of the incubation period and the cost of growth could function to canalize the body size at hatching. If the growth efficiency of an embryo was constant over a range of temperatures, size at hatching would be insensitive to temperature (Birchard and Reiber 1995). Data for *S. undulatus* support this functional hypothesis. Not only were energy expenditures during incubation similar for embryos incubated over the range of 28°–34°C, body mass and snout-vent length at hatching were also unaffected by temperature (Angilletta et al. 2000). Likewise, we observed no effect of incubation temperature on body mass in our experiment. Undoubtedly, factors other than thermal acclimation minimize variation in the energetics of embryos and consequently canalize size at hatching.

Are the patterns of metabolism observed in the laboratory relevant to our understanding of embryonic ecology? Over a decade ago, Packard and Packard (1988) invited biologists to broaden studies of embryos from the constant temperatures of laboratories to the fluctuating temperatures of nests. Natural nests can fluctuate by more than 10°C each day (Cagle et al. 1993; Shine et al. 1997; Andrews 2000); at our study site, embryos of *S. undulatus* are exposed to daily fluctuations over the range of 20°–39°C (Angilletta et al. 2005). Still today, we know almost nothing about the development and energetics of embryos at fluctuating temperatures. Christian et al. (1986) found that embryos of *S. undulatus* exposed to 15°C paused development until the environment warmed. Embryos in natural nests, however, probably develop continuously because they never reach temperatures as low as 15°C. Although the energy budgets of embryos in nature might seem difficult to predict, our results should extend to natural conditions for two reasons. First, patterns observed at constant temperatures appear to hold at fluctuating temperatures as well; a recent study of *S. undulatus* under seminatural thermal cycles revealed that embryos incubated at warm cycles grew and respired the same amount as embryos incubated at cool cycles (Oufiero and Angilletta, forthcoming). Second, the incubation period depends more on the mean temperature than it does on the variance (reviewed

by Angilletta and Sears [2003]; exceptions are discussed by Georges et al. [2005]). Therefore, the primary mechanism that canalizes the energy budget of an embryo in the laboratory—a change in the incubation period—should also canalize the energy budget of an embryo in the wild.

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