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THERMAL EFFECTS ON THE ENERGETICS OF LIZARD EMBRYOS: IMPLICATIONS FOR HATCHLING PHENOTYPES

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Abstract. In many ectotherms, incubation temperature has profound effects on the timing of hatching and size of hatchlings, but the mechanisms underlying these effects are poorly understood. We studied the energetics of embryonic development and growth in the lizard *Sceloporus undulatus.* Eggs were incubated at six constant temperatures, ranging from 28° to 38°C, and embryonic metabolism, incubation period, and body size at hatching were determined. The duration of embryonic development decreased significantly from 55 d at 28° to 40 d at 32°C but did not differ significantly between 32° and 34°C. Embryos incubated at temperatures above 34°C did not survive to hatching. Metabolic rate at specific stages of development (percentage of total incubation period) did not differ among embryos incubated at 28°, 30°, and 34°C. As a result, the total amount of energy expended during the incubation period at 28°C (2.0 kJ) was greater than that at 30°–34°C (1.7–1.8 kJ). However, the difference in energy expenditure did not affect body size at hatching; neither snout–vent length nor body mass varied significantly with incubation temperature, and both were the same as those of hatchlings collected in the field. Thus, there was no apparent trade-off between hatching date and body size of lizards at hatching.

In a natural population in New Jersey, USA, we quantified soil temperatures at potential nesting sites and studied the thermoregulatory behavior of gravid females to examine the possible consequences of female behavior for hatchling phenotypes. In females and at potential nest sites, embryos would experience temperatures that resulted in high mortality in the laboratory experiment (>32°C). Gravid females had a field body temperature of 33.9°C (95% CI = 0.8°C) and selected a body temperature of 33.3°C (95% CI = 1.0°C) when placed in thermal gradients in the laboratory. Soil temperatures rose above 32°C for several hours each day. Embryos must be able to survive intermittent exposure to temperatures that were lethal under conditions of chronic exposure in the laboratory. Selection of relatively high body temperatures by gravid females, coupled with tolerance of acute exposure to relatively high soil temperatures, would reduce the incubation period without a concomitant reduction in body size at hatching.

Key words: body size; embryo energetics; hatchling size; incubation temperature; incubation period; metabolic rate; nest site choice; preferred body temperature; reptiles; Sceloporus undulatus; *temperature effects; thermoregulation by gravid females.*

INTRODUCTION

The embryonic environment can have a lasting impact on the physiology and behavior of ectotherms. Thermal and hydric conditions during incubation can influence locomotion (Miller et al. 1987, Burger 1990, 1991*a*, Van Damme et al. 1992, Elphick and Shine 1998), olfaction (Burger 1991*b*), defense (Burger 1998*a*, *b*), growth (Qualls and Shine 1996, Joanen et al. 1987) and survival of hatchlings (de March 1995). In some cases, consequences of the incubation environment for offspring quality can last months (Burger 1989, Qualls and Shine 1996) or even years (Joanen et al. 1987, Roosenburg and Kelley 1996). Over the last two decades, a sizeable literature on incubation effects has emerged from research programs investigating the ecology of embryos (e.g., Muth 1980, Overall 1994), the evolution of viviparity (e.g., Shine 1995, Qualls and Shine 1996), and patterns of environmental sex determination (e.g., Ferguson and Joanen 1983, Webb and Cooper-Preston 1989, Roosenburg 1996). Unfortunately, less attention has been directed toward understanding mechanisms by which the incubation environment alters phenotypes of embryos and hatchlings (Deeming and Ferguson 1991, Packard 1991, Bernardo 1996).

In many reptiles, individuals incubated at higher temperatures hatch earlier at a smaller body size (Packard and Packard 1988, Deeming and Ferguson 1991). Most of the evidence for this phenomenon comes from laboratory studies using constant incubation temperatures, but available data from field studies also support the notion that temperature affects incubation period and body size differently (Cagle et al. 1993, Castilla and Swallow 1996, Shine et al. 1997). Packard and Packard (1988) suggested that the negative relationship between incubation period and body size at hatching

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is caused by differential effects of temperature on development and growth. They reasoned that embryos incubated at higher temperatures exhibit higher metabolic rates, resulting in faster development, but less efficient growth. Packard and Packard's argument predicts that the energetic cost of development (= total energy expenditure during incubation) is greater at higher temperatures. The energetic cost of development depends on both the metabolic rate of embryos and the length of the incubation period. However, a greater energetic cost of development at high temperatures can arise only from a higher metabolic rate of embryos, because incubation period decreases with increased temperature. Quantitative measures of embryonic energetics are critical to test the hypothesis that the energetic cost of development is greater at high incubation temperatures.

The effect of incubation temperature on the energetic cost of development is known for only a few species of reptiles, and those data indicate that the energetic cost of development is relatively insensitive to temperature. In three species of turtles (*Trionyx triunguis*, *Emydura macquari* and *E. signata*) and the tuatara (*Sphenodon punctatus*) the energetic costs of development were similar at all temperatures (Booth and Thompson 1991, Booth 1998). In contrast, embryos of the crocodilian *Crocodylus johnstoni* had a greater energetic cost of development at 29°C than at 31°C, even though hatchlings from eggs incubated at 29°C were larger (Whitehead 1987). Thus, there is little direct support for the hypothesis that higher temperatures impose a greater energetic cost of development on reptilian embryos. However, the negative relationship between incubation temperature and hatchling size is supported largely by data on squamate reptiles (lizards and snakes), whereas the thermal sensitivity of the energetic cost of development in these species is unknown.

It is clear that a trade-off between incubation period and body size at hatching should exert a major influence on the thermoregulatory and nesting behaviors of gravid females. Large size at hatching can reduce predation risk and increase foraging success, leading to higher survival and faster growth of hatchlings (Sinervo 1993). On the other hand, early emergence enables individuals to better exploit resources in a seasonal environment (Lesage and Gauthier 1998), where availability of resources varies temporally. Also, early emergence at a small size may be associated with a greater amount of lipid in hatchlings (Gutzke and Packard 1987), enabling faster growth after hatching (Troyer 1987). Clearly, thermoregulation and nest-site selection by gravid females can have manifold and complex consequences for the growth and body size of juveniles. Prior to oviposition, females are expected to balance their own temperature preference with that of their offspring when the two are different. Also, females must be able to locate nesting sites that will provide a suitable thermal environment throughout incubation (Bernardo 1996).

We examined the effect of incubation temperature on the energetics of embryonic development in the lizard *Sceloporus undulatus.* Our goals were to understand the mechanisms underlying the relationship of hatching size to incubation temperature in reptiles, and to examine the extent to which female thermoregulation and nest site availability may influence hatchling body size. Specifically, we addressed three questions. Are the energetic costs of incubation at different temperatures consistent with the pattern of variation in body size at hatching? Do gravid females select body temperatures that are favorable for embryonic development and growth? What thermal environments are embryos likely to experience during incubation at potential nesting sites? To answer these questions, we measured the energetic cost of development, embryonic survival, incubation period, and hatchling body size at six incubation temperatures. These data were compared to preferred body temperatures and field body temperatures of gravid females, and soil temperatures at potential nesting sites. We demonstrate that knowledge of embryonic energetics is critical to understanding the effects of incubation temperature on body size, especially in cases where patterns of body size do not follow the general trend.

Methods

Acquisition and care of eggs

Sceloporus undulatus in New Jersey produces two clutches of 8–9 eggs per year, the first in May/June and the second in June/July (Niewiarowski 1994; M. J. Angilletta, *personal observation*). To eliminate seasonal effects on egg size and egg composition, we obtained eggs from females collected in June/July of 1997. Gravid females were collected from Burlington County, New Jersey, and transported to the University of Pennsylvania to allow for oviposition in the laboratory. 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 10:14 were maintained. Females were fed crickets, ad libitum, and provided with water by misting daily. Terraria were inspected twice daily, and freshly laid eggs were removed and weighed to the nearest 0.01 mg.

Eggs were incubated at one of six temperatures: 28°, 30°, 32°, 34°, 36°, and 38°C. The range of incubation temperatures was chosen for two reasons: (1) previous study indicated that embryos of *S. undulatus* do not survive well below 25°C (Sexton and Marion 1974), and (2) we were particularly interested in characterizing the effect of temperature on incubation period at relatively high temperatures. Eggs of each clutch were distributed uniformly among the six temperature

groups. A total of 17–26 eggs were assigned to each treatment temperature.

Eggs were incubated in plastic containers (four eggs per container) in a medium of fine sand (~500 g) and a constant mass of water. Initially, distilled water was added to each container, 1 g per 100 g of sand, resulting in a water potential of −400 kPa. Evaporated water was replaced every other day by adding distilled water until each container was brought to its initial mass. Because eggs absorbed water throughout incubation, water potential of the soil decreased during the experiment. To estimate changes in water potential during the experiment, we constructed a standard curve for our sand by using a thermocouple psychrometer to measure water potentials of samples over a range of water content. Using the standard curve and increases in egg mass, we estimated the decrease in soil water potential to be 125 kPa at incubation temperatures below 32°C and 200 kPa at higher incubation temperatures. Changes in soil water potential probably did not affect water flux to a large degree, considering that eggs of *S. undulatus* that were incubated using identical methods experienced a linear increase in egg mass throughout incubation (M. J. Angilletta, *unpublished data*).

Metabolism and energetics of embryos

We quantified the effect of incubation temperature on metabolic rate by measuring the CO_2 produced by embryos from each treatment group at their respective temperature (e.g., eggs incubated at 28°C were measured at 28°C). Fifteen eggs, having a healthy appearance (i.e., a turgid shell with no signs of fungal growth or decay) were selected from each incubation temperature. Eggs selected from each group represented a broad range of developmental stages, from just oviposited, to within a week of hatching. Prior to measurement, eggs were removed from their incubation containers and weighed to the nearest 0.01 mg. Each egg was placed on a sterile sponge that was moistened with distilled water, and was placed in a 30-mL respirometry chamber. During the procedure, we were careful to maintain eggs in their original orientation.

Metabolic rates were measured with a flow-through respirometry system (TR-3, Sable Systems International, Henderson, Nevada). Respirometry chambers were placed in an incubator set at the treatment temperature (Model 818, Precision Scientific, Chicago, Illinois). An opening in the incubator, 5 cm in diameter, was used for incoming and outgoing tubing. Incoming air was scrubbed of H_2O and CO_2, and pushed at 50 mL/min through 20 m of copper tubing submerged in 38 L of water that was at equilibrium with the incubator temperature. As a result, the air entering the respirometry chambers was at the same temperature as the air in the incubator. Outgoing air was scrubbed of water vapor and entered a mass-flow meter (v1.0, Sable Systems), and CO_2 analyzer (Model LI-6251, LI-COR, Incorporated, Lincoln, Nebraska). Prior to the study, the mass-flow meter was calibrated using a mass-flow controller valve (Sidetrak, Sierra Instruments, Incorporated, Monterey, California) connected to a mass-flow controller electronics unit (v1.0, Sable Systems).

Metabolic rate of each embryo was measured continuously for a 10-min period between 1200 and 1600. The respirometry system ran for ~1 h prior to measurement of metabolism, to ensure that egg temperature was in equilibrium with air temperature in the chamber. Data acquisition software (DAC, Sable Systems) was used to record CO_2 concentration and flow rate each second of the 10-min period. Before and after each period, a baseline measurement of CO_2 concentration was recorded from a chamber containing only a sterile moistened sponge, identical to those on which the eggs were placed.

Metabolic data were analyzed using the computer program DATACAN (Sable Systems). For each recording, pre- and postrecording measurements of baseline concentrations of CO_2 were used to calculate proportional enrichment of CO_2 by the egg. Rate of CO_2 production (milliliters per hour), calculated as the product of proportional enrichment and flow rate at each sampling point, was averaged for the entire sampling period, resulting in a single value of CO_2 production for each embryo. We used ANCOVA to examine the effects of incubation temperature (28°–38°C) on CO_2 production. Egg mass is known to influence metabolic rate of avian embryos (Hoyt 1980, Rahn and Ar 1980), so initial egg mass was used as a covariate in the analysis. Also, it is important to control for differences in the stage of development (defined as a percentage of the total incubation period, hereafter referred to as percent incubation) when comparing metabolic rates of embryos incubated at different temperatures because metabolic rates of reptilian embryos increase throughout incubation (Vleck and Hoyt 1991). Unfortunately, percent incubation could only be calculated for embryos incubated at temperatures that produced hatchlings, but egg mass in *S. undulatus* increases linearly throughout incubation (M. J. Angilletta, *unpublished data*). Therefore, we used egg mass at the time of measurement as a second covariate to control for differences in developmental stage among eggs.

We estimated the energetic cost of development at each temperature from metabolic rates of embryos. We constructed regression models of metabolic rate as a function of time since oviposition, and used these models to estimate total CO_2 production over the incubation period. Total CO_2 production was converted to energy expenditure by assuming that energy was derived equally from protein and lipid, which yielded 23.1 and 27.7 J/mL, respectively (Nagy 1983). This assumption is well supported by data on metabolism of lizard embryos (Thompson and Stewart 1997, Thompson and Russell 1998, 1999*a*). The energy available to an embryo was calculated assuming a dry mass of 174 mg

per egg (Niewiarowski 1994) minus 15 mg for the mass of the eggshell (M. J. Angilletta, *unpublished data*) and an energy density of 25 kJ/g dry mass (Derickson 1976, Ballinger et al. 1981, Tinkle and Dunham 1986). Using the mean energy content of an egg, we calculated the proportion of energy available to an embryo that is used for metabolism.

Incubation period and phenotypes of hatchlings

Eggs were checked daily for freshly emerged hatchlings. At first sign of pipping, an egg was marked as hatched, but hatchlings took up to 24 h to emerge from the egg. Once hatchlings fully emerged, snout–vent length and tail length were measured to the nearest mm, and body mass was measured to the nearest 0.01 mg. Incubation period was calculated as the number of days between oviposition and pipping. Eggs that appeared to decay were kept until all other hatchlings had emerged before being scored as dead.

Pearson chi-square test was used to assess differences in hatching success among incubation temperatures. Because some clutches may have contained infertile eggs, we excluded eggs from clutches that produced no hatchlings from the analysis of hatching success. Analysis of Variance was used to determine the effect of incubation temperature on incubation period. Hatchling morphology was analyzed by MANCOVA to examine effects of incubation temperature on snout–vent length, tail length, and body mass, with initial egg mass serving as a covariate.

Thermoregulation by gravid females

In 1997 and 1998, we searched for females in Burlington County, New Jersey. Gravid females were captured by noosing, and body temperatures were recorded immediately with a quick-reading cloacal thermometer (Model T-4000, Miller and Weber Incorporated, New York, New York). Eleven females were transported to the University of Pennsylvania for use in laboratory measurements of preferred body temperature.

In 1998, we measured preferred body temperatures of gravid females in thermal gradients, which consisted of 38-L aquaria with a 60-W infrared-emitting ceramic bulb suspended above one end. Aquaria were kept in an air-conditioned room, creating a stable range of operative temperatures from 27°–39°C (measured with a hollow copper model; Bakken and Gates 1975). Lighting was available from several windows, but natural light was supplemented during photophase with fluorescent light from above. On the day of capture, gravid females were placed in gradients at ~1700 and were undisturbed until the following day. Cloacal temperature was measured at 1000 and 1400 with a cloacal thermometer. Morning and afternoon body temperatures were averaged to produce a single preferred body temperature for each individual.

Soil temperatures in a natural population

In 1997, we measured soil temperature profiles at known depths (0, 1, 2, 4, 8, and 16 cm) to determine the dampening depth of the soil (Campbell and Norman 1998). This allowed us to calculate the temperature at any depth, given the temperature at a known depth. From June through September of 1998, we monitored soil temperatures at a depth of 2 cm at nine points, evenly distributed among three microhabitats (open, partial shade, and full shade). Data were recorded every 15 min by single-channel dataloggers (Model H8, Onset Corporation, Bourne, Massachusetts). Replicate measures of soil temperature within microhabitats were averaged, and mean values were used to calculate soil temperatures at other depths (4, 8, and 16 cm). Equations derived by Campbell and Norman (1998) were used to calculate minimum and maximum temperatures at various depths in each microhabitat (open, partial shade, full shade). Daily means of soil temperatures were assumed to be the same at all depths (Campbell and Norman 1998).

Incubation period of embryos in the field

Incubation period for field-incubated hatchlings was calculated as the difference between median laying date of laboratory females and median capture date of field-incubated hatchlings. Median oviposition date was determined from dates of oviposition by females in a laboratory study of *S. undulatus* (Angilletta and Sears 2000), including only females laying their first clutch of 1998. The same year, we searched for newly emerged hatchlings in a 4-ha plot in Burlington County, New Jersey. Searches were made every 1–2 d to ensure that hatchlings were sighted as close to hatching date as possible. Hatchlings were captured by hand, and were transported to the laboratory where they were weighed to the nearest 0.01 mg within 24 h of capture. Prior to release, hatchlings were toe clipped and paint marked to prevent pseudoreplication of data resulting from the recapture of individuals. We excluded any hatchlings that weighed $\geq$0.70 g from the calculation of incubation period, because no lab-incubated hatchling of *Sceloporus undulatus* has been observed to weigh more than 0.65 g (M. J. Angilletta, M. W. Sears, and R. S. Winters, *unpublished manuscript*). This assumption is conservative in that it allows for additional mass of food contained in the guts of hatchlings collected in the field. However, there is a possibility that some of the hatchlings used to estimate hatching date had emerged several days prior to capture. Such a bias would cause an overestimation of incubation period in the field.

The use of oviposition dates of females in the laboratory instead of those of field-active females may bias our estimate of incubation period. Females used in the laboratory study were collected from a site adjacent to the 4-ha study plot from which natural hatch-

lings were caught, so laboratory and field-active females experienced similar environments during the time that ovulation and mating occurred. Also, females from the study plot were observed to be gravid over the same time interval during which we collected gravid females for the laboratory study. However, females in the laboratory may have delayed oviposition in response to the stresses of capture and handling. Delayed oviposition by females in the laboratory relative to females in the field would cause an underestimation of incubation period in the field. Importantly, the potential biases imposed by use of oviposition dates in the laboratory (underestimation) and capture dates of unmarked hatchlings (overestimation) to estimate incubation period in the field tend to cancel one another. Thus, our estimate of incubation period of field-incubated hatchlings is probably close to the actual incubation period under natural conditions.

Statistical procedures

Prior to all statistical analyses, we examined data for violations of assumptions. Cochran's test was used to assess homogeneity of variances, and chi-square goodness of fit tests were used to examine the assumption of normality. When a covariate was used, we ensured that slopes of the relationship between the covariate and dependent variables were homogeneous among groups. Unless otherwise stated, data satisfied the assumptions required for analysis. Least significant difference (lsd) tests and Tukey's honest significant difference (hsd) tests were used to make planned and unplanned comparisons, respectively. Statistical analyses were performed with STATISTICA for Windows (release 5.0 B, Statsoft 1996). All descriptive statistics are given as mean and 95% CI.

RESULTS

Embryonic metabolism

Metabolic rate (milliliters CO_2 per hour) was not significantly related to initial egg mass ($t = -1.79$, df $= 82$, $P = 0.08$), but was highly dependent on egg mass at the time of measurement ($t = 14.36$, df $= 2$, $P < 0.000001$). Egg mass increased more rapidly during development at higher incubation temperatures (MS $= 0.11$, $F_{3,55} = 7.49$, $P = 0.0003$); however, eggs at 32° and 34°C underwent similar changes in mass (Tukey's test, $P > 0.05$; Fig. 1). Incubation temperature had a significant effect on the metabolic rate of embryos (MS $= 0.0008$, $F_{5,82} = 3.10$, $P = 0.01$). Unplanned comparisons indicated that metabolic rates at 32° and 34°C (0.072 ± 0.020 and 0.057 ± 0.020 mL/h, respectively) were significantly greater than those at 36° and 38°C (0.033 ± 0.009 and 0.040 ± 0.009 mL/h, respectively). Also, metabolic rate of embryos at 28°C (0.047 ± 0.013 mL/h) was significantly lower than that of embryos at 32°C. Embryos incubated at 28°, 30°, and 34°C had similar metabolic rates throughout incubation (Tukey's test, $P > 0.05$; Fig. 2). The relationship between percent incubation and the metabolic rate of embryos was exponential at 28°–34°C. The relationship between percent incubation and metabolic rate could not be determined for embryos incubated at 36° and 38°C, because no eggs survived to hatching at these temperatures.

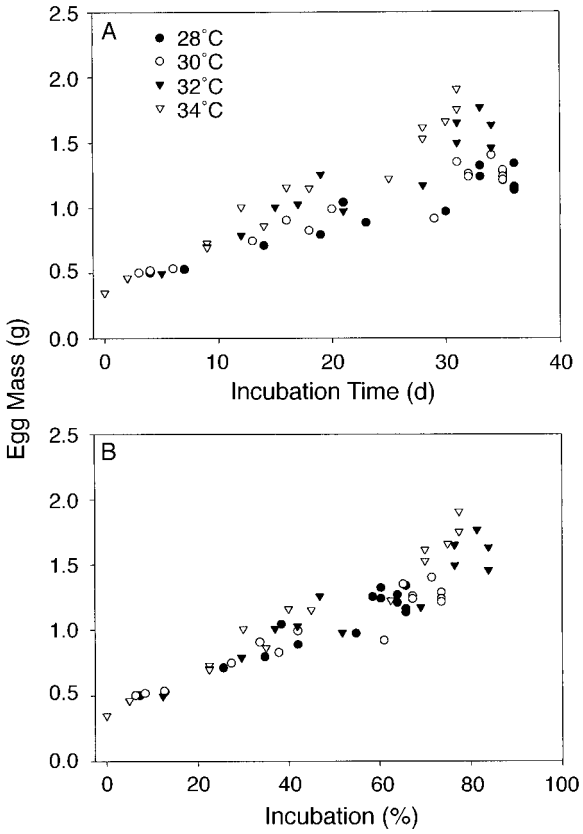

FIG. 1. Increase in egg mass during incubation as a function of (A) absolute time and (B) percent incubation for embryos incubated at constant temperatures (28°, 30°, 32°, and 34°C).

The energetic cost of development was 1.7–1.8 kJ at 30°–34°C and 2.0 kJ at 28°C. These values are equivalent to energy expenditures of 43–50% of the 4.0 kJ available to an embryo. Though regression equations explained 64–94% of the variation in metabolic rate, standard errors of beta coefficients were sufficiently great to indicate the energetic cost of development did not vary significantly among incubation temperatures (Table 1).

Hatchling phenotypes

Of the 114 fertile eggs, 49 hatched successfully. Hatching success was significantly affected by incubation temperature ($\chi^2 = 51.48$, df $= 5$, $P < 0.00001$). Survival to hatching was relatively high for embryos incubated at 30° and 32°C (86% and 71%, respectively), but was lower at 28°C (62%). Survival to hatching was

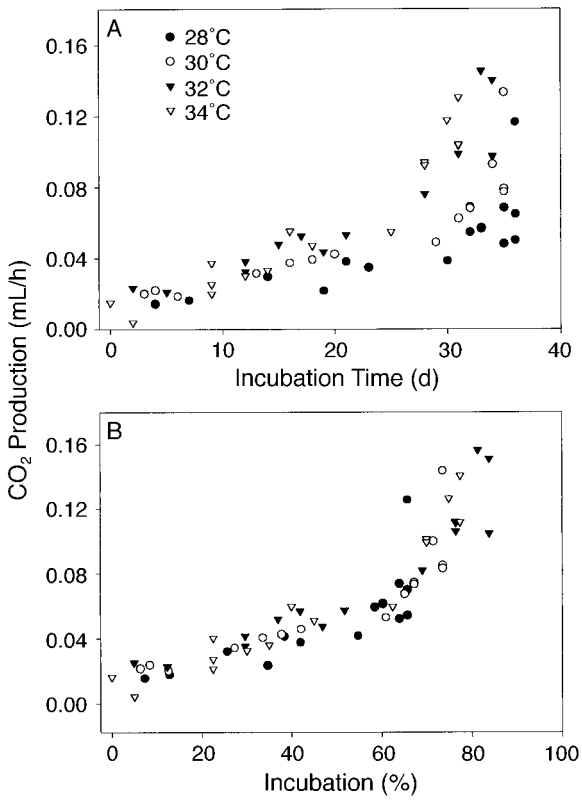

FIG. 2. Increase in metabolic rate as a function of (A) absolute time and (B) percent incubation for embryos incubated at constant temperatures (28°, 30°, 32°, and 34°C).

poor at 34°C (28%), and no embryos survived at incubation temperatures of 36° and 38°C.

Variances in incubation period among the groups were not homogeneous, and log transformation did not eliminate heterogeneity of variances. Inspection of data revealed that a single point at 34°C (= 50 d) caused the heteroscedasticity. Because results of ANOVA are usually robust to violations of homoscedasticity (Lindman 1974), we proceeded with the ANOVA. Incubation period decreased significantly with increased incubation temperature (MS = 488.18, $F_{3,45} = 92.23$, $P < 0.000001$). However, no significant difference in incubation period was found between embryos incubated at 32°C and those incubated at 34°C (Tukey's test, Table 1).

Incubation temperature significantly influenced tail length of hatchlings ($F_{9,99} = 2.66$, Wilk's $\lambda = 0.59$, $P = 0.008$). Mean tail lengths of individuals incubated at 32°C and 34°C were significantly greater than those of individuals incubated at 28°C (Tukey's test). Neither snout–vent length (28 mm for all) nor body mass (0.50–0.52 g) varied significantly with incubation temperature (Fig. 3). Egg mass at laying was positively correlated with tail length ($t = 2.88$, df = 43, $P = 0.006$, $r^2 = 0.16$), but was not correlated with snout–vent length ($t = 0.06$, df = 43, $P = 0.95$, $r^2 = 0.00$), or body mass ($t = 1.74$, df = 43, $P = 0.09$, $r^2 = 0.07$).

TABLE 1. Regression models of metabolic rate, as a function of the number of hours since oviposition (mL/h = $a \times e^{xb}$), of embryos ($n = 15$) incubated at constant temperatures.

Temperature (°C)	Incubation time (d)	a	b	1 SE of b	r^2
28	54.7 ± 0.9	0.01015	0.00222	0.00064	0.64
30	47.6 ± 1.0	0.01398	0.00221	0.00043	0.80
32	40.5 ± 0.7	0.01682	0.00246	0.00029	0.91
34	40.0 ± 5.2	0.01299	0.00295	0.00030	0.94

Note: Mean incubation time ± 95% CI at each temperature is given.

Thermoregulation by gravid females

Between mid-May and early July and from 10:00 to 18:00, we obtained body temperatures of 38 gravid females during their activity in the field. Mean body temperature was 33.9° ± 0.7°C.

Lizards in thermal gradients behaved as expected

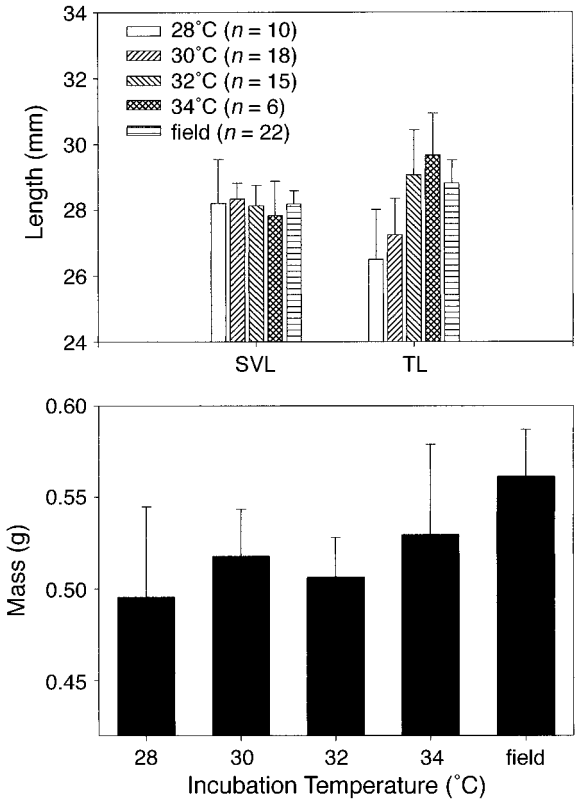

FIG. 3. Effects of incubation temperature on snout–vent length (SVL), tail length (TL), and body mass of hatchlings. Error bars represent the 95% confidence intervals of the means. Data on field-incubated hatchlings were collected upon emergence in August 1996 (M. J. Angilletta, *unpublished data*). Field-incubated hatchlings appear to be greater in mass than some laboratory-incubated hatchlings, but the difference may be caused by food present in the guts of hatchlings collected in the field.

TABLE 2. Soil temperatures (°C) in potential nesting sites of *Sceloporus undulatus.*

Microhabitat	Daily mean	Daily minimum	Daily maximum
Open			
2 cm	25.5 ± 0.6	15.7 ± 0.7	35.2 ± 1.2
4 cm		18.1 ± 0.6	32.8 ± 1.0
8 cm		20.1 ± 0.5	30.8 ± 0.9
16 cm		21.3 ± 0.5	29.7 ± 0.8
Partial shade			
2 cm	23.6 ± 0.5	20.8 ± 0.5	26.3 ± 0.5
4 cm		21.5 ± 0.5	25.6 ± 0.5
8 cm		22.0 ± 0.5	25.1 ± 0.5
16 cm		22.4 ± 0.5	24.8 ± 0.5
Full shade			
2 cm	20.9 ± 0.4	18.5 ± 0.5	23.3 ± 0.4
4 cm		19.1 ± 0.5	22.7 ± 0.4
8 cm		19.6 ± 0.4	22.2 ± 0.4
16 cm		19.9 ± 0.4	21.9 ± 0.4

Notes: Data are means ± 95% CI of daily mean, minimum, and maximum temperatures. Values for depths >2 cm were calculated using temperatures measured at a depth of 2 cm (see *Methods: Soil temperature in a natural population*).

from observations of field-active lizards. During scotophase individuals remained buried beneath the sand substrate. However, during photophase they remained on the surface basking near the heat source, and moved occasionally. Mean body temperature selected by females in thermal gradients, 33.3° ± 1.0°C (n = 11), was not significantly different from mean body temperature of field-active lizards (t = 0.76, df = 47, P = 0.45).

Soil temperatures

Soil temperatures varied considerably among microhabitat classes (Table 2). As expected, daily mean temperature and daily amplitude of temperature were highest at open sites and lowest at fully shaded sites. Soil temperatures in full shade were too low to permit survival of embryos to hatching (Sexton and Marion 1974). Two combinations of site and depth provided suitable conditions for incubation: (1) partially shaded sites at all depths, and (2) open sites at 4–16 cm depth (Table 2). Though either partial shade or open habitat would result in successful hatching, embryos would develop more rapidly and experience greater survival in open sites because soil temperatures reached 30°–32°C for several hours each day. Also, soil temperature increased gradually throughout the summer. As a result, eggs from the second clutch of the season incubated at temperatures that were consistently higher than those experienced by eggs from the first clutch (Table 3).

Incubation period of embryos in the field

In 1998, oviposition dates of females in the laboratory were between 22 May and 9 June (Angilletta and Sears 2000). That same year, hatchlings in the field began to emerge on 9 August and new hatchlings continued to appear until 5 September. A total of 104 hatchlings was marked, but 19 of these were excluded from the analysis of incubation period because their body mass indicated that they had not hatched recently (body mass > 0.70 g). Median date of oviposition (3 June) and median date of emergence (16 August) were separated by 74 d. This estimate of incubation period does not depend on whether individual eggs or clutches were used to determine the median date of oviposition (Fig. 4).

TABLE 3. Soil temperatures (°C) at a depth of 2 cm during incubation of the first (June–August) and second (July–September) clutches.

Habitat class	Daily mean	Daily minimum	Daily maximum
Open			
First clutch	25.1 ± 0.7	15.2 ± 0.8	35.0 ± 1.5
Second clutch	26.6 ± 0.6	16.1 ± 0.7	37.1 ± 1.3
Partial shade			
First clutch	23.3 ± 0.6	20.6 ± 0.6	26.0 ± 0.6
Second clutch	24.4 ± 0.4	21.6 ± 0.5	27.2 ± 0.4
Full shade			
First clutch	20.6 ± 0.5	18.2 ± 0.5	22.9 ± 0.5
Second clutch	21.5 ± 0.4	19.0 ± 0.5	24.1 ± 0.4

Note: Data are means ± 95% CI. In all microhabitats, the daily mean, daily minimum, and daily maximum temperature experienced by the second clutch are higher than those experienced by the first clutch.

DISCUSSION

Embryonic metabolism

Metabolic rates of embryos increased exponentially throughout incubation, regardless of incubation tem-

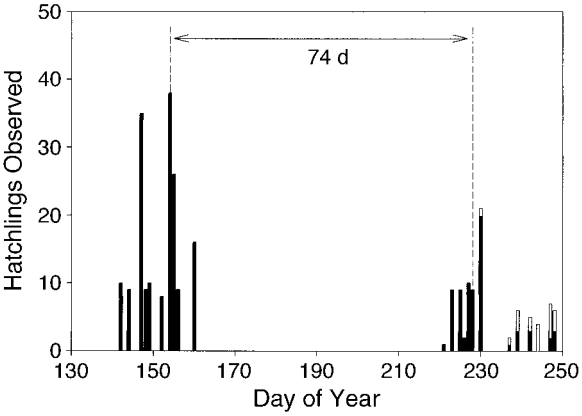

FIG. 4. Dates of oviposition and hatching for *Sceloporus undulatus* in Burlington County, New Jersey. Solid bars represent the number of eggs laid by females in the laboratory or the number of hatchling captured on a 4-ha study plot (see *Methods* for details). The open bars represent the number of hatchlings that were not captured close to the date of their emergence. The incubation time of embryos in the natural population was estimated as the number of days between the median date of oviposition by females in the laboratory and the median date of emergence of hatchling in the field (first and second dashed lines, respectively). Day one equals 1 January.

perature. Although we did not make repeated measures of metabolic rate within individuals, such measures on embryos from the same population of *S. undulatus* also indicated an exponential increase in metabolic rate during incubation (M. J. Angilletta, *unpublished data*). The relationship between percent incubation and metabolic rate in *S. undulatus* differs from that found in many other reptiles (Vleck and Hoyt 1991). For example, change in metabolic rate during incubation is sigmoidal in turtles, crocodilians, and some snakes. More importantly, metabolic rate increases sigmoidally during incubation in other lizards that have been studied (Vleck and Hoyt 1991, Thompson and Stewart 1997, Thompson and Russell 1999*b*). It is possible that we did not measure metabolic rate at a point close enough to hatching to observe a decrease in the rate of change in metabolic rate of *S. undulatus* embryos. For example, *Eumeces anthracinus* does not exhibit a plateau in metabolic rate until after 85% of the incubation period has passed (Thompson and Stewart 1997). However, metabolic rate does increase exponentially during incubation in the majority of snakes that have been studied (Vleck and Hoyt 1991). The ontogeny of metabolic rate is thought to be associated with the pattern of growth in the embryo (Vleck and Hoyt 1991). Faster growth imposes greater costs to produce new tissue and to maintain the mass of the embryo. Therefore, an increase in metabolic rate, whether exponential or sigmoidal, implies that embryos are growing continuously throughout most of the incubation period. We assume that differences in metabolic rate among embryos incubated at different temperatures reflect different rates of development and growth.

The effect of incubation temperature on the metabolic rate of embryos was more complex than we expected. Metabolic rate increased more rapidly with time at 32° and 34°C than it did at 28° and 30°C, probably due to the greater rate of mass gain in embryos at the higher temperatures. However, metabolic rate at a given percent incubation was similar at all incubation temperatures (Fig. 2), suggesting that embryonic metabolism is insensitive to temperature. An alternative hypothesis is that thermal acclimation was responsible for the lack of variation in stage-specific metabolic rates of embryos. If the temperature-specific metabolic rate of embryos is affected by past incubation environment, a down-regulation of metabolism in warmer environments or an up-regulation of metabolism in cooler environments would result in similar metabolic rates across a range of incubation temperatures. An evaluation of this hypothesis requires repeated measures of the metabolic rate of individual embryos to quantify the thermal sensitivity of metabolic rate in embryos from different incubation environments. Such data have been collected for *S. undulatus* (M. J. Angilletta, *unpublished data*), and they confirm the capacity for thermal acclimation in embryos of this species; although metabolic rate increased with increasing temperature within individuals, embryos incubated at 34°C had higher metabolic rates at all temperatures than those incubated at 28°C.

What mechanism would produce thermal acclimation of metabolic rate in embryonic reptiles? One potential cause would be differences in water flux caused by incubation temperature. It is thought that eggs in cooler environments experience more favorable water fluxes at a given soil water potential (Packard 1991). If so, embryos at cooler temperatures would experience greater rates of growth and metabolism because of the well-documented effects of water availability on embryos (Packard 1991). However, evidence that low temperatures actually result in greater amounts of water influx during incubation comes from changes in egg mass at different incubation temperatures. For example, eggs of the turtle *Chelydra serpentina* lost more mass during incubation at high temperatures than they did at low temperatures, regardless of the hydric environment (Packard et al. 1987). Packard (1991) interpreted these data as evidence for greater water loss at high temperatures, but they are also consistent with the interpretation that the energetic cost of development for *C. serpentina* is greater at high temperatures. In fact, we found that eggs incubated at 32° and 34°C gained more mass during the incubation period than did eggs incubated at lower temperatures (see Fig.1). Presumably, the difference in mass gain between groups reflects a difference in rates of water flux because body size of hatchlings did not vary significantly with incubation temperature. If water flux was greater in eggs incubated at higher temperatures, we should have observed higher metabolic rates in embryos incubated at 32°C and 34°C, but we observed the opposite. Therefore, we do not believe that the indirect effect of temperature on water flux caused the pattern of metabolic rate observed in our study. It is more plausible that direct effects of temperature (e.g., changes in protein expression, see Whiteley et al. 1997) are mechanisms of thermal acclimation in this case.

Energetics, incubation period, and hatchling size

Within limits of thermal tolerance, embryos at high incubation temperatures develop and grow more rapidly than those at low incubation temperatures (Deeming and Ferguson 1991). Several lines of evidence suggest that this is also the case for *S. undulatus.* Although the initial metabolic rates of embryos at all temperatures were similar, egg mass and metabolic rate quickly diverged among embryos at different incubation temperatures. Embryos at 32° and 34°C had a greater metabolic rate and greater egg mass on any given day of incubation than those at 28° and 30°C. Additionally, embryos at the two higher temperatures completed incubation earlier. In fact, metabolic rate, egg mass, and incubation temperature were the same for embryos at 32° and 34°C. Thus, there is a very strong covariation

between egg mass and metabolic rate that is associated with the total duration of incubation. Taken together, data on egg mass, metabolic rate, and incubation period suggest that embryonic development in *S. undulatus* is faster under warmer conditions.

The energetic cost of development can be influenced by metabolic rate during development and total duration of incubation. Both of these traits were affected by incubation temperature in *S. undulatus.* As mentioned above, metabolic rate of embryos was greater at higher incubation temperatures, but incubation period was shorter. The net result was that the energetic cost of development was similar from 30° to 34°C (1.7 to 1.8 kJ). Though embryos at 28°C had a slightly higher energetic cost of development (2.0 kJ), the standard errors of *b* in the regression models of metabolic rate indicate that actual costs of development may be much higher or lower than those estimated from metabolic data (Table 1). In either case, our results are consistent with those of other studies of reptiles. When the energetic cost of development has been measured at multiple temperatures, it either did not depend on incubation temperature (Booth and Thompson 1991, Booth 1998) or was greater at the lower temperature (Whitehead 1987).

Our results suggest that the energetic cost of development is relatively insensitive to incubation temperature in *S. undulatus* because shorter incubation periods compensate for the higher metabolic rate at higher incubation temperatures. This conclusion is consistent with the pattern of body size observed in hatchlings. Neither snout–vent length nor body mass of hatchlings was affected by incubation temperature (Fig. 3). However, hatchlings from eggs incubated at 32° and 34°C had significantly longer tails than those from eggs incubated at 28°C. It is not clear whether the greater tail length in hatchlings from high incubation temperatures was related to the lesser energetic cost of development at these temperatures.

In part, the congruence between the energetic cost of development and hatchling phenotypes relies on our ability to accurately estimate the total metabolism of embryos. Our estimate of the energetic cost of development at 30°C for *S. undulatus* (1.8 kJ) compares favorably with that reported for *S. virgatus* (1.6 kJ; D. Vleck, *unpublished data*; cited in Vleck and Hoyt 1991), which lays an egg of approximately the same mass as that of *S. undulatus* (0.36 g). Despite the longer incubation period of *S. undulatus* (48 d vs. 36 d), the energetic cost of development is only slightly greater than that of *S. virgatus,* because *S. undulatus* embryos have a lower metabolic rate at the time of oviposition (0.51 J/h vs. 0.77 J/h, assuming an respiratory quotient of 0.76). Given the concordance in energetic cost of development between these congeners, we have confidence in our measures of metabolic rate and the energetic cost of development in *S. undulatus.*

Maternal thermoregulation, soil temperatures, and hatchling phenotypes

We observed no trade-off between incubation period and hatchling body size in *S. undulatus,* because temperature influenced primarily the rate of development rather than the energetic cost of development. Assuming that this is true for embryos in the field (see Fig. 3), the consequences of incubation temperature for post-hatching body size are relatively straightforward. Individuals incubated at higher temperatures would emerge earlier at the same body size as individuals incubated at lower temperatures. Thus, high incubation temperatures promote a longer duration for post-hatching growth in the first active season. Females should make every effort to expose embryos to the highest temperatures that embryos can survive, to maximize the potential for post-hatching growth. We can test this prediction by comparing the maximum temperature for successful hatching in the laboratory to temperatures selected by females in the laboratory and field.

Hatchling survival was highly dependent on incubation temperature. Embryos incubated at 30° and 32°C experienced the greatest survival, and no embryos survived constant exposure to temperatures above 34°C. Although it is possible that our measures of metabolic rate reduced survival of embryos, hatching success in our study was higher than that observed in a previous study of *S. undulatus* (Sexton and Marion 1974). Mortality at high temperatures was not caused by energetic stress on the embryos. Metabolic rates of embryos at 36° and 38°C were lower than those of embryos at 32° and 34°C, and dissection of dead eggs revealed moderate to large quantities of yolk remaining. Furthermore, we observed 100% mortality in embryos exposed to a temperature of 41°C for a period of $<$12 h, due to an incubator failure during one of our studies. Therefore, we conclude that the certain mortality of embryos exposed to constant temperatures above 34°C was caused by developmental or functional defects.

Thermal preference of gravid females, 33.9° $\pm$ 0.8°C, was equivalent to the maximum incubation temperature that produced hatchlings in the laboratory (34°C). Although survival was still lowest at this temperature, lizard embryos survive acute exposure to temperatures that cause 100% mortality during chronic exposure (Christian et al. 1986, Overall 1994, Andrews et al. 1997). *Sphenodon punctatus* hatches successfully in nests that provide temperatures in excess of 7°C above the upper lethal limit in laboratory studies at constant temperatures (Thompson et al. 1996). In *S. undulatus,* gravid females are active at high body temperatures for a maximum of 8 h/d (M. J. Angilletta, *unpublished data*), so embryos should have higher survival than is indicated by our laboratory study at constant temperatures. In nature, gravid females maintained mean body temperature of 33.3° $\pm$ 1.0°C during activity, which was not significantly different from their preferred

body temperature. Therefore, thermoregulation by gravid females facilitates the most rapid development possible, given thermal tolerances of embryos. Interestingly, gravid females do not alter their thermoregulatory behavior to achieve this result. The preferred body temperature of male and nongravid females measured in June was 34.1° ± 1.3°C (M. J. Angilletta, *unpublished data*). Though viviparous reptiles may select lower or higher body temperatures when pregnant (reviewed by Shine 1980, Mathies and Andrews 1997, Dorcas and Peterson 1998), oviparous species typically maintain the same body temperatures when gravid and nongravid (reviewed by Shine 1980, Werner 1990). The close match between the thermal optimum for embryonic development and the preferred body temperature of females precludes a parent–offspring conflict over thermoregulatory behavior (Evans et al. 1995).

Choice of nesting sites by females can have a major influence on the phenotypes of their offspring (reviewed by Bernardo 1996, Roosenburg 1996, Shine et al. 1997, Wilson 1998). Although soil temperature varies hourly and daily within a site, females can improve their own fitness by laying eggs at sites that provide a favorable mean and variance in temperature. One strategy is to lay eggs at a site that provides a mean temperature close to the optimal temperature for development and a very small variance in temperature (Rand 1972). For *S. undulatus* in New Jersey, such sites were simply unavailable; mean daily soil temperature in three microhabitats ranged from 20.9° to 25.5°C, which is well below the incubation temperature that maximized survival and minimized incubation period in the laboratory. Alternatively, females can choose nesting sites that offer a high variance in temperature, with temperatures in the upper range equal to those that result in short incubation periods without exceeding the thermal tolerance of embryos. Open sites at depths of ≥4 cm provided soil temperatures that met these criteria (Table 2). Therefore, females that lay eggs at open sites may simultaneously optimize the survival, emergence time, and body size of their offspring.

Despite the fact that embryos in natural populations experience variable temperatures, timing of emergence depends on the mean soil temperature experienced throughout incubation (Cagle et al. 1993, Shine et al. 1997). Nesting sites used by *S. undulatus* can be inferred from soil temperatures and the incubation period of embryos in nature. Based on oviposition dates of females and emergence dates of hatchlings, we estimated the incubation period of embryos in field to be 74 d (Fig. 4). This duration is almost identical to the incubation period of 75 d estimated for *S. undulatus* in Colorado (Gillis and Ballinger 1992). Shaded sites in our New Jersey population, averaging 20.9° ± 0.4°C, were too cool to produce successful hatchlings in any time interval (Sexton and Marion 1974). Assuming an increase in incubation period of 3.5 days per °C (Table 1), we estimated that eggs in partial shade would hatch in 69 days. However, eggs at these sites would have a low probability of survival (Sexton and Marion 1974). Consequently, females should lay eggs in open sites at depths ≥4 cm, which offered temperatures between 30° and 32°C for a minimum of 6 h/d facilitating rapid development without detriment to survival. Nighttime temperatures at open sites dropped well below the lower lethal limit in laboratory studies (20°–25°C), but *S. undulatus* embryos incubated at a high temperature (30°C) can survive intermittent exposure to low temperatures; temporary exposure (<6 d) of embryos to 15°C extended incubation period by the duration of exposure, without altering hatching success (Christian et al. 1986, Andrews et al. 1997). Based on mean soil temperature, embryos at open sites would be expected to hatch in 66 d, but apparently require an additional 8 d because their temperature cycles between high temperatures that enhance development and low temperatures that retard development.

Because soil temperatures increase throughout the summer (Table 3), incubation period will depend on the timing of oviposition. In New Jersey, seasonal changes in soil temperature should result in differences in incubation period between embryos from the first and second clutches of the year. We predict that embryos from the second clutch would have an incubation period four days shorter than that of embryos from the first clutch. If so, the difference in incubation period would help to compensate for the time interval between the laying dates of the two clutches (~29 d). Hatchlings from the second clutch have less time available for activity, but only 86% less than they would have if they experienced the same soil temperatures as embryos from the first clutch. The exact degree of compensation in activity time can be determined by carefully observing the oviposition dates of females and the emergence dates of hatchlings in the field. Currently such data are unavailable because of the difficulty of making daily observations of gravid females. However, eggs from first and second clutches had similar incubation periods at 30°C (M. J. Angilletta, *unpublished data*). Thus, there is good reason to believe that some degree of compensation in activity time would result from the seasonal variation in soil temperature.

Very few studies have considered the effects of diel variation in incubation temperature on phenotypes of hatchlings (Georges et al. 1994, Shine et al. 1997). Yet, data reported here suggest that embryos experience large diel cycles in temperature during incubation. This raises the question of whether mean and variance in temperature are equally important in determining embryonic survival, incubation period, and hatchling body size. Studies that examine the effects of temperature cycles on the energetics and survival of embryos will be invaluable to understanding thermoregulatory and nesting behaviors of females. Additionally, such studies will strengthen our comprehension of the mechanisms that generate phenotypes of hatchlings. Given

the potential magnitude of temperature variation experienced by reptilian embryos, our ignorance of this subject offers a clear opportunity to make significant contributions to our understanding of the ecology of embryos.

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