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Incubation Temperature Modifies Neonatal Thermoregulation in the Lizard *Anolis carolinensis*

RACHEL M. GOODMAN* AND JUSTIN W. WALGUARNERY
Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee

ABSTRACT The thermal environment experienced during embryonic development can profoundly affect the phenotype, and potentially the fitness, of ectothermic animals. We examined the effect of incubation temperature on the thermal preferences of juveniles in the oviparous lizard, *Anolis carolinensis*. Temperature preference trials were conducted in a laboratory thermal gradient within 48 hr of hatching and after 22–27 days of maintenance in a common laboratory environment. Incubation temperature had a significant effect on the upper limit of the interquartile range (IQR) of temperatures selected by *A. carolinensis* within the first 2 days after hatching. Between the first and second trials, the IQR of selected temperatures decreased significantly and both the lower limit of the IQR and the median selected temperature increased significantly. This, along with a significant incubation temperature by time interaction in the upper limit of the IQR, resulted in a pattern of convergence in thermoregulation among treatment groups. The initial differences in selected temperatures, as well as the shift in selected temperatures between first and second trials, demonstrate plasticity in temperature selection. As a previous study failed to find environmentally induced plasticity in temperature selection in adult *A. carolinensis*, this study suggests that this type of plasticity is exclusive to the period of neonatal development. *J. Exp. Zool. 307A:439–448, 2007.*

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The thermal environment experienced during embryonic development is known to have major impacts on numerous phenotypic traits in ectothermic animals. In many oviparous reptiles, incubation temperature of eggs in natural nests and laboratory experiments affects body size and relative proportions of body parts (Shine et al., '97; reviewed in Birchard, 2004; Deeming, 2004), locomotor performance (Vanhooydonck et al., 2001; Blouin-Demers et al., 2004; reviewed in Deeming, 2004), and antipredatory behaviors (Burger, '98; Downes and Shine, '99; Flatt et al., 2001). Maternal thermal environment and selection of body temperature in viviparous reptiles have also been shown to affect the above phenotypic traits of offspring (Shine and Downes, '99; Lourdais et al., 2004; Ji et al., 2006). Indeed, a substantial portion of the disparity between some populations in neonatal phenotype, otherwise assumed to be due to genetic divergence, can be explained by differences in incubation temperature (Qualls and Shine, 1998).

The precision and accuracy with which ectotherms regulate body temperature varies widely among species, but many reptiles, and particularly lizards, are known to maintain active body temperatures within a relatively narrow range (Hertz et al., '93; Christian and Weavers, '96; van Marken Lichtenbelt et al., '97) suggested to approximate the range of optimal physiological functioning (Huey and Bennett, '87). However, preferred or selected body temperature may differ according to sex (Patterson and Davies, '78b; Sievert and Hutchison, '89; Brown and Griffin, 2005), presence or composition of ingested food (Gibson et al., '89; Geiser et al., '92; Brown and

*Correspondence to: Rachel M. Goodman, Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, 1416 Circle Drive, Knoxville, TN 37996-1610.
E-mail: rmgoodman@utk.edu
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Griffin, 2005), and reproductive condition (Brana, '93; Rock et al., 2000; Le Galliard et al., 2003). Additionally, seasonal acclimatization in nature and acclimation to different temperatures in laboratory settings may alter selected temperatures (Wilhoft and Anderson, '60; Christian et al., '83; Sievert and Hutchison, '89).

The effect of incubation temperature on thermoregulation in reptiles has received less attention than have the effects on the above postnatal factors, and has been studied in few species of lizards (Shine and Harlow, '96; Qualls and Andrews, '99; Blumberg et al., 2002; Buckley et al., 2007), despite the potential fitness benefits such a link could confer in the form of beneficial acclimation (Leroi et al., '94). A relationship between incubation temperature and temperature preference or thermal tolerance could have cascading effects on many aspects of the physiology, behavior, and life history. For example, body temperature in reptiles affects locomotion (Hertz et al., '83; Stevenson et al., '85; van Berkum, '86), predator evasion (Christian and Tracy, '81; Hertz et al., '82), and feeding and digestion (Avery et al., '82; Van Damme et al., '91; Angilletta et al., 2002). Therefore, influences of incubation environment on thermoregulatory behavior could have important fitness consequences through well-described links between thermoregulation and growth and survival.

The early thermal environment of embryos or juveniles has been suggested to alter thermal set points or tolerances in ways that may be impervious to adjustment in later life stages (Winkler, '85). However, the existence and potential persistence of causal relationships between developmental temperature and thermoregulation have been infrequently examined. Such relationships could have important consequences through lifetime behavior and resource utilization, particularly in oviparous species for which embryonic development may occur under varied environmental conditions. We tested the effects of incubation temperature on temperature selection in an oviparous lizard, *Anolis carolinensis*. In this species, eggs are deposited in and under natural or man-made objects, buried in shallow soils or leaf litter, or even left exposed (Gordon, '60; Michaud, '90). Therefore, eggs may be subject to very different thermal environments in different populations or within the same habitat over the course of a reproductive season (April through August; Gordon, '56). In this study, we examined how three incubation temperatures that span a range encountered by eggs of *A. carolinensis* in the wild and that successfully produce healthy hatchlings in the laboratory (23, 27, 30°C) affect the thermal preference of juveniles.

Acclimation to controlled laboratory temperatures can temporarily alter thermal preferences in some species of lizards (Wilhoft and Anderson, '60; Patterson and Davies, '78a). However, studies testing for plasticity in thermoregulation in lizards typically involve only adult animals. It should not be assumed that effects induced during incubation will not subsequently be altered by plasticity in juveniles, even if such plasticity is absent in adults. Studies that examine phenotypic traits such as thermoregulation immediately after hatching may only document temporary effects of the incubation environment (Qualls and Shine, 2000; Seebacher, 2005; Buckley et al., 2007). Repeated testing is needed to determine whether persistent effects are present. Thermal preferences of adult *A. carolinensis* do not undergo acclimation in the laboratory (Licht, '68), but this phenomenon has not been tested in other age classes. We examined potential plasticity in thermoregulation of juveniles by testing thermal preferences after incubation in different temperatures and after 22–27 days in a common laboratory environment. We tested the null hypothesis that *A. carolinensis* incubated at different temperatures would exhibit neither differences in selected temperature at hatching, nor differences after approximately 3 weeks of growth, versus the alternative hypothesis that plasticity in response to thermal experience would result in differences in thermal preferences.

MATERIALS AND METHODS

Seventy adult female *A. carolinensis* were purchased from a reptile supplier in LaPlace, Louisiana and shipped to Tennessee in June and July 2005. Most female *A. carolinensis* in the wild carry stored sperm at this point in the reproductive season, which they use to fertilize eggs (ovulated and oviposited singly) in the laboratory for months (Licht, '73). Upon arrival at the University of Tennessee, Knoxville, females were weighed to the nearest 0.01 g and measured for snout-vent length (SVL) and total length (TL) to the nearest 0.5 mm. Females were then housed for up to 2 months in 3.8-L glass jars with screened lids containing a perch, a cover object, and a sand substrate. Enclosures were misted with water daily, and vitamin-dusted crickets were

provided every other day. Females were kept in temperatures of 25–28°C and placed under UVB and broad-spectrum fluorescent lights on a daily 12:12 h light:dark cycle. Eggs were collected from the sand substrate in each enclosure every other day, and immediately measured for mass, length, and width. Eggs were placed in 345-mL plastic containers with 10 g of vermiculite moistened with 10 mL water and randomly assigned to one of three temperature treatments: 23, 27, and 30°C. Incubation temperatures were recorded every 60 min with Stowaway Temperature Tidbit Loggers (Onset Computer Corporation, Bourne, MA). Three incubators and a temperature-controlled room were used for incubation. Temperature treatments were initially rotated between the three incubators; however, the lowest temperature treatment had to be moved to the temperature-controlled room after failure of one incubator. Therefore, the standard deviation of the lowest temperature treatment differed from those of the other treatments (SD = 0.86, 0.47, 0.34°C for 23, 27, 30°C, respectively). However, the temperature ranges of all treatments remained entirely exclusive of each other. Also, owing to double sealing of the plastic containers housing eggs (plastic wrap and lids) and complete darkness for all incubation treatments, moisture and other factors should not have differed between any incubators and the temperature-controlled room.

Positions of eggs within incubators were rotated, and new hatchlings were collected on a daily basis. Within 24 hr of hatching, SVL, TL, and mass were measured for each hatchling. Hatchlings were housed randomly with regard to treatment in 38-L enclosures holding several perches and cover objects and each containing a total of five individuals of the same age. Enclosures were misted several times per day and received UVB and broad-spectrum fluorescent illumination on a 12:12 h light:dark cycle. Before the first temperature selection trial (described below), no food was provided to hatchlings. Many hatchling *A. carolinensis* will not eat for some days after hatching (personal observation), so withholding food equalized stomach contents among hatchlings.

The first temperature trial for each individual was conducted within 48 hr after hatching. Subsequently, individuals were housed as described above for pretrial hatchlings. Lizards were provided fruit flies, pinhead crickets, and fruit baby food ad libitum. Temperatures in enclosures followed a diurnal cycle, with daily highs of 32–34°C in light and 28–30°C in shade and nightly lows of 23–25°C. Food was replenished daily and some prey were always apparent in all enclosures. Therefore all juveniles had equal access to food, and so satiety levels (which may affect temperature preference; see Introduction) at the time of the second trial should not bias results. Positions of enclosures within the laboratory were rotated once per week. After 22–27 days, the second temperature trial was performed for each juvenile. All lizards survived and gained 47–258% (average 137%) of their initial body mass by the time of the second trial.

We examined selection of substrate temperature in juvenile *A. carolinensis*, rather than body temperature, because of the prohibitive difficulty of measuring body temperature in such small lizards (<0.5 g) without restricting movement, disrupting behavior, or directly altering body temperature. Owing to the small body size and minimal thermal inertia of the lizards, body temperature should reflect substrate temperature in the absence of a radiant heat source (Stevenson, '85; Blouin-Demers et al., 2000). Each of four substrate thermal gradients were constructed using a 2 cm^2 section aluminum rod of 122 cm TL spanning the central long axis of a wooden box frame of inside dimensions $30 \times 30 \times 100$ cm. The aluminum rod protruded 9 cm from each end of the box frame. Polystyrene board front and rear walls were affixed to the aluminum rod along the 100 cm within the box frame and supported a ventilated, clear, acrylic plastic lid, so as to comprise an enclosed temperature gradient chamber of dimensions $2 \times 5 \times 100$ cm. The inside surface of the walls was coated with Fluon (AGS Chemicals Europe, Ltd., UK), an aqueous dispersion of polytetrafluoroethylene to which anoles cannot adhere, so that anoles in the temperature gradient chamber had to remain in contact with the rod. One end of the aluminum rod rested on a thermoelectric cold plate and the other end rested on a hot plate. By adjusting the temperatures applied to the ends of the aluminum rod via the cold and hot plates, a linear temperature gradient of approximately 18–46°C was established along the rod. The rod was marked at every 2 cm, and the ends of thermocouples were affixed to the bottom of the rod at the ends of the chamber and at marked points every 20 cm. Thermocouple temperatures were read with a six-channel digital microprocessor thermometer (Omega HH23, HH20SW, OMEGA Engineering, Inc., Stamford, CT). Because the temperature gradient was linear, temperature was directly related to position, and therefore the temperature at any point

could be accurately determined by interpolation from the temperatures measured at the two closest thermocouples. The interior of the chamber was diffusely and uniformly provided with a low level of illumination by overhead fluorescent fixtures fitted with 40-W bulbs. A mirror suspended at a 45° angle above the entire length of the temperature gradient chamber allowed the observer to view the chamber while minimizing disturbance to lizards. Lizards were assigned randomly to one of the four chambers to eliminate confounding effects of any unapparent influences besides temperature on lizard behavior in the chambers. The testing room was maintained at a constant ambient temperature of 25.5–26.5°C.

One hour before each temperature preference trial, lizard enclosures were thoroughly misted with water. For each trial, a single lizard was placed at a haphazardly selected point in the temperature gradient chamber between 10:30 and 11:00. After one half hour on the gradient, and at each subsequent half hour for 4 h, the position of the lizard in the gradient and all thermocouple temperatures were recorded. Body mass and SVL were measured at the end of the 4-hour testing period. Each lizard was included in only a single trial, and to control for any potential maternal effects, no more than one lizard from each mother was included in this study. Age of hatchlings did not differ between incubation treatments at the time of the second trial (analysis of variance [ANOVA], $F_{2,77} = 0.98$, $P = 0.378$).

At sexual maturity, *A. carolinensis* is sexually dimorphic and displays differences in temperature preference (Brown and Griffin, 2005). Therefore, we compared mass of lizards between incubation treatments and sexes using a two-way ANOVA. The temperatures selected by each individual were ranked and four metrics of thermoregulation were recorded: the median selected temperature, the interquartile range (IQR), and its lower and upper limits. The IQR for an individual was defined as the difference between the closest two observations demarcating at least the middle 50% of observations for that individual. Given 9 observations per individual, the lower and upper limits of the IQR are the third coolest and third hottest selected temperatures, respectively. We used repeated measures (RM) ANOVA models with incubation temperature and sex as potential factors explaining the four metrics of thermoregulation. We present final analyses from reduced RM ANOVA models for comparisons between incubation treatments. All analyses were conducted in SPSS (Release 14.0.0, 2005, SPSS Inc., Chicago, IL).

RESULTS

All incubation treatments produced high levels of hatching success (81.9% overall; 75.3% at 23°C; 83.3% at 27°C; 92.3% at 30°C). Hatchling mass differed between sexes at the first trial (two-factor ANOVA, temperature: $F_{2,74} = 2.77$, $P = 0.069$; sex: $F_{1,74} = 4.55$, $P = 0.036$) and among incubation temperatures and sexes at the second trial (two-factor ANOVA, treatment: $F_{2,74} = 20.68$, $P < 0.001$; sex: $F_{1,74} = 5.11$, $P = 0.027$). The magnitudes of these differences were small during the first trial (means: 23°C = 0.31 g, 27°C = 0.32 g, 30°C = 0.29 g; $M = 0.30$ g, $F = 0.32$ g), but had increased by the second trial (means: 23°C = 0.83 g, 27°C = 0.78 g, 30°C = 0.62 g; $M = 0.71$ g, $F = 0.78$ g). Across incubation treatments and in either trial, however, neither sex nor mass had a significant effect on any metric describing temperature selection, so these were not included as factors or covariates in RM ANOVA models for thermoregulation.

Incubation temperature was not a significant main effect in any of the RM ANOVAs (Table 1). However, these analyses showed a significant trial (i.e., time) × incubation temperature interaction effect on the IQR upper limit (Table 1).

TABLE 1. Results of repeated measures analysis of variance on measures of temperature selection in Anolis carolinensis subject to three treatments of incubation temperature

	Between subjects	Within subjects	
	Incubation temperature	Time	Time × incubation temperature
Median	$F_{2,\ 77} = 0.40$, $P = 0.670$	$F_{1,\ 77} = 14.06$, $P < 0.001$	$F_{2,\ 77} = 1.66$, $P = 0.198$
IQR upper limit	$F_{2,\ 77} = 1.34$, $P = 0.267$	$F_{1,\ 77} = 2.57$, $P = 0.113$	$F_{2,\ 77} = 4.56$, $P = 0.013$
IQR lower limit	$F_{2,\ 77} = 0.03$, $P = 0.971$	$F_{1,\ 77} = 13.88$, $P < 0.001$	$F_{2,\ 77} = 1.01$, $P = 0.368$
IQR	$F_{2,\ 77} = 2.42$, $P = 0.095$	$F_{1,\ 77} = 8.10$, $P = 0.006$	$F_{2,\ 77} = 0.75$, $P = 0.477$

Lizards were tested for temperature selection within 48 hr of hatchling and after 22–27 days in a common environment.
IQR, interquartile range.

This measure increased with time for juveniles incubated at 23 and 30°C, but decreased for juveniles incubated at 27°C (Table 2; Figs. 1, 2b). Tested by simple ANOVAs, there was a significant difference between incubation treatments in the IQR upper limit at the first trial; however, this difference was no longer significant at the second trial (trial 1: $F_{2,77} = 3.42$, $P = 0.038$; trial 2: $F_{2,77} = 1.49$, $P = 0.231$). For all incubation treatments, RM ANOVAs showed a significant increase between trials in both the IQR lower limit and the median selected temperature (Table 1; Figs. 1, 2a and c). The pooled median selected temperature was 29.27°C (SE = 0.38) at the first trial and 31.05°C (SE = 0.31) at the second trial. The IQR for all incubation treatments decreased from the first to second trials (RM ANOVA, Table 1; Figs. 1, 2d) with a pooled mean IQR of 5.46°C (SE = 0.37) for the first trial and 4.23°C (SE = 0.23) for the second trial.

DISCUSSION

The selected substrate temperatures in this study were similar to laboratory selected body temperatures and naturally occurring body temperatures of *A. carolinensis* in other studies. Mean preferred temperature of *A. carolinensis* (males only) in a study by Licht (1968) was approximately 31°C, with all body temperatures maintained between 28 and 36°C in a photothermal gradient. Captive-bred adult *A. carolinensis* selected body temperatures of 29.0–31.5°C (mean varied by sex and fasting status) in another laboratory thermal gradient (Brown and Griffin, 2005). In a natural population in March and April in Texas (during activity on clear days), Clark and Kroll ('74) found mean body temperatures of *A. carolinensis* of 28.0 (±0.4)°C over the whole day and 30.8 (±0.3)°C during midday when body temperatures plateau. Therefore, although we are not aware of any studies reporting body temperatures of juvenile *A. carolinensis* from natural populations, the range of temperatures reported for adults brackets our observed mean selected substrate temperatures for juveniles in the laboratory.

In this study, we monitored selected substrate temperatures in lieu of body temperatures, which under some experimental conditions could complicate interpretation of temporal shifts in thermal preferences. The viability of substrate temperature as a proxy for body temperature might change with factors such as air temperature, which was constant between trials, but also with body mass, which increased from the first to second trials. However, under our design the potential for an influence of body mass was negligible because of the small size of hatchings during both trials (<1 g) and the lack of significance of body mass as a covariate in ANOVA models comparing temperature selection measures between trials.

Incubation temperature affected thermoregulation of juvenile *A. carolinensis* in this study. Immediately after hatching, the upper limit of the IQR was highest for the group incubated at 27°C. Several changes occurred between the first and second trials. The median selected temperature increased for all groups, and the IQR decreased for all groups. The upper limit of the IQR showed an interaction between time and treatment groups, increasing with time in juveniles incubated at 23 and 30°C but decreasing for

TABLE 2. Averages and standard errors (SE) for temperature selection measures of juvenile Anolis carolinensis from three incubation temperatures after hatching and after 22–27 days in a common environment

		Median		Mean		IQR upper limit		IQR lower limit		IQR	
	N	Average	SE	Average	SE	Average	SE	Average	SE	Average	SE
<48 hr post-hatching											
23°C	27	29.12	0.68	29.00	0.57	31.34	0.64	26.79	0.79	4.56	0.61
27°C	20	29.64	0.65	29.86	0.47	33.60	0.46	27.23	0.68	6.38	0.79
30°C	33	29.16	0.65	28.91	0.56	31.67	0.64	26.40	0.79	5.27	0.72
All treatments	80	29.27	0.38	29.18	0.32	32.04	0.37	26.74	0.45	5.46	0.37
After 22–27 days in common environment											
23°C	27	30.75	0.59	30.77	0.46	32.80	0.50	28.90	0.56	3.90	0.39
27°C	20	30.29	0.58	30.16	0.53	32.44	0.60	28.16	0.54	4.28	0.49
30°C	33	31.75	0.45	31.00	0.38	33.56	0.37	29.09	0.47	4.48	0.33
All treatments	80	31.05	0.31	30.71	0.26	33.03	0.27	28.79	0.30	4.23	0.23

IQR, interquartile range.

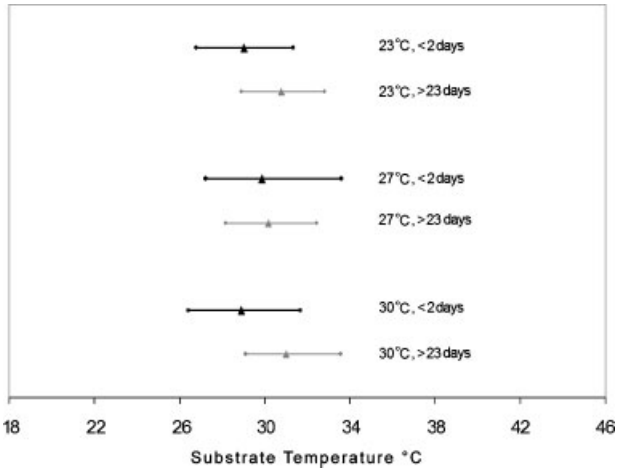

Fig. 1. Mean temperature selection measures of juvenile *Anolis carolinensis* from three incubation temperatures after hatching and after 22–27 days in a common environment. Triangles represent median selected substrate temperatures. Outer points represent the upper and lower limits of interquartile range of selected temperatures. Incubation treatment and age of lizards are identified on each group. The range of temperatures at the bottom of the figure (18–46°C) represents the approximate range available in thermal gradients at all trials. Sample sizes were 27, 20, and 33 lizards for 23, 27, and 30°C, respectively.

juveniles incubated at 27°C. At the second trial, there were no significant differences between incubation treatment groups in the median selected temperature or the lower and upper limits of the IQR. Collectively, these results show that initially disparate patterns of thermoregulation for the three incubation treatments converged after approximately 3 weeks of growth in a common environment.

The fitness consequences of differences in thermoregulation observed in this study are unknown. Shortly after hatching, individuals from 27°C were found in hotter temperatures, as indicated by the upper limit of the IQR being 2°C hotter on average than that of other treatments. However, the median selected temperature did not differ across treatments. These results combined could indicate a greater tolerance of hotter temperatures in lizards from the intermediate treatment, a temporally limited preference for hotter temperatures, or a lesser precision in thermoregulation. Given the apparently short longevity (<3 weeks) of the incubation-induced differences in thermoregulation observed in this

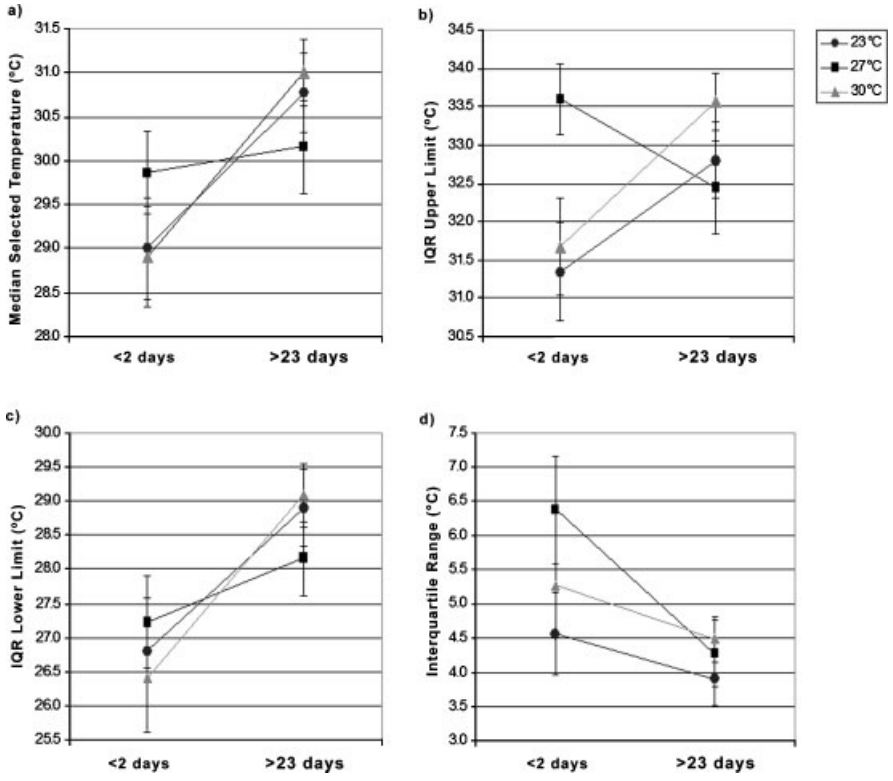

Fig. 2. Results of substrate temperature selection trials conducted with *Anolis carolinensis* juveniles from three incubation temperatures. Ages of lizards at the two trials are shown on the x-axis. Errors bars represent ±1 SE. Plots show (**a**) median selected temperature, (**b**) upper limit of interquartile range of selected temperatures, (**c**) lower limit of interquartile range, and (**d**) magnitude of interquartile range. Sample sizes were 27, 20, and 33 lizards for 23, 27, and 30°C, respectively.

study, it might be assumed that ultimate fitness consequences of any of these effects would be correspondingly limited. However, mass specific energetic demands as well as the thermal sensitivity of metabolic processes and rates in reptiles can peak at or near hatching (Booth, 2000; McCue and Lillywhite, 2002). Therefore, early post-hatching thermoregulation may actually have a disproportionately large effect on growth and survival to reproduction. Indeed, although the thermoregulatory differences among incubation temperature treatment groups were diminished by the second trial, differences in mass had actually become more pronounced.

Wilhoft and Anderson (1960) observed selection of a lower mean body temperature in adult *Sceloporus occidentalis* acclimated from nature to a high laboratory temperature (35°C) and suggested that this response was a behavioral precaution against metabolic burnout. Acclimation from nature to lower temperatures (15°C, 25°C) produced no change in preferred body temperatures in that study, further suggesting that physiological effects of extreme high temperatures were the primary influence on adjustment of thermal preferences. Metabolic rate is correlated positively with temperature (Jenssen et al., '96). An optimal range in metabolic rate is expected to exist because of tradeoffs among resource consumption, growth rate, developmental rate, and performance (Cossins and Bowler, '87; Cano and Nicieza, 2006). Therefore, homeostatic adjustment of metabolism via changes in preferred body temperature during activity might be expected as an immediate compensatory response to long-term environmental change (in lieu of or complementary to acclimation in standard metabolic rate during inactivity; Wheeler, '86). It could be hypothesized then that our observation of a depression in the upper limit of preferred temperature in the hottest incubation group relative to that in the next hottest incubation group is indicative of a thermoregulatory correction to pre-hatching metabolism. A constant incubation temperature of 30°C might cause relatively rapid and excessive consumption of embryonic resources in *A. carolinensis*, a species that while preferring active temperatures near 30°C as adults, would nevertheless in all developmental stages typically experience lower temperatures during a large portion of the daily cycle in nature.

Studies of developmental temperature effects on thermoregulation in other species show mixed results, so that no general trends have yet emerged. Studies with snapping turtles (*Chelydra serpentina*) found that juveniles from cooler incubation temperatures select warmer water temperatures (O'Steen, '98; Rhen and Lang, '99). Spotila et al. ('94) found no effect of incubation temperature on thermal preference in another turtle, *Gopherus agassizii*. In contrast, studies of juvenile crocodilians show the opposite trend wherein higher incubation temperatures result in selection and maintenance of higher body temperatures among juveniles (Lang, '87). Studies of snakes have failed to show significant effects of incubation temperature on thermoregulation or have shown idiosyncratic effects (Arnold et al., '95; Burger, '98; Blouin-Demers et al., 2000). Among lizards, one study by Blumberg et al. (2002) examined thermoregulatory behavior (shuttling between two substrate temperatures on a hotplate) in hatchlings of the nocturnal gecko *Paroedura pictus*. Incubation at higher temperatures resulted in significantly higher temperatures when exiting the cold portion of the plate, and a trend for higher temperatures when exiting the hot portion. A study of juvenile skinks, *Bassiana duperreyi*, showed no significant effect of incubation temperature at 1 week of age, but at 1 month of age suggested a similar trend with juveniles from a hotter incubation temperature spending more time basking (Shine and Harlow, '96). In contrast, Qualls and Andrews ('99) found that hatchling *Sceloporus virgatus* from a colder incubation treatment chose warmer temperatures in a thermal gradient, and maintained those temperatures more precisely (lower SD of selected temperature).

Juvenile *A. carolinensis* in this study changed temperature selection behavior after being held for 22–27 days in common laboratory conditions and showed a trend across treatments for an increase in median selected temperature. This change in thermoregulation may have been an ontogenetic effect or an acclimation response to the temperature in the laboratory, which included temperatures warmer than those experienced in any of the incubation treatments. Licht ('68) tested for acclimation of selected body temperature in adult *A. carolinensis* kept in 20 and 32°C at 0, 6, and 14 hr of light per day for 4 months. He found no influence of maintenance temperature on selected body temperatures. Although no acclimation response was found for adults in that study, the possibility of acclimation and adaptive adjustment of selected temperatures in

A. carolinensis should not be discounted, considering the results of this study. We suggest that this species may exhibit differing levels of plasticity in thermal preference at different life stages. Collectively, the lack of an incubation treatment effect at the second temperature selection trial and the changes in measures of selected temperature between the first and second trials suggest that thermal acclimation occurs in neonatal *A. carolinensis*. However, the extent to which the observed pattern is an adaptive acclimation response to thermal experience versus an effect of other factors must be considered. On the basis of these results, we cannot determine whether the significant shift in selected temperatures was an adjustment toward the mean operative temperature of the laboratory environment, the maximum available temperature, or some optimal set point that may or may not change intrinsically with growth and development.

In summary, incubation temperature of *A. carolinensis* had a short-lived effect on thermoregulation in a laboratory thermal gradient. When juveniles from different temperatures were held in a common thermal environment with opportunities for thermoregulation, initial differences in temperature selection were diminished within 22–27 days. These results indicate the existence of plasticity in early age that is not observed in adults. This study also suggests that with age there is increased precision in selection of temperatures around a higher median and indicates a need for further work to distinguish acclimatory responses from ontogenetic shifts in thermoregulatory set points. Additional work is also required to address the functional consequences of incubation-induced shifts in thermoregulation at the physiological and organismal levels, which may in turn impact fitness.

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LITERATURE CITED

Angilletta MJ, Hill T, Robson MA. 2002. Is physiological performance optimized by thermoregulatory behavior? A case study of the eastern fence lizard, *Sceloporus undulatus*. J Therm Biol 27:199–204.

Arnold SJ, Peterson CR, Gladstone J. 1995. Behavioral variation in natural populations. 7. Maternal body temperature does not affect juvenile thermoregulation in a garter snake. Anim Behav 50:623–633.

Avery RA, Bedford JD, Newcombe CP. 1982. The role of thermoregulation in lizard biology: predatory efficiency in a temperate diurnal basker. Behav Ecol Sociobiol 11:261–267.

Birchard GF. 2004. Effects of incubation temperature. In: Deeming DC, editor. Reptilian incubation: environment, evolution, and behaviour. UK: Nottingham University Press. p 103–123.

Blouin-Demers G, Kissner KJ, Weatherhead PJ. 2000. Plasticity in preferred body temperature of young snakes in response to temperature during development. Copeia 2000:841–845.

Blouin-Demers G, Weatherhead PJ, Row JR. 2004. Phenotypic consequences of nest-site selection in black rat snakes (*Elaphe obsoleta*). Can J Zool 82:449–456.

Blumberg MS, Lewis SJ, Sokoloff G. 2002. Incubation temperature modulates post-hatching thermoregulatory behavior in the Madagascar ground gecko, *Paroedura pictus*. J Exp Biol 205:2777–2784.

Booth DT. 2000. Incubation of eggs of the Australian broad-shelled turtle, *Chelodina expansa* (Testudinata: Chelidae), at different temperatures: effects on pattern of oxygen consumption and hatchling morphology. Aust J Zool 48: 369–378.

Brana F. 1993. Shifts in body temperature and escape behavior of female *Podarcis muralis* during pregnancy. Oikos 66:216–222.

Brown RP, Griffin S. 2005. Lower selected body temperatures after food deprivation in the lizard *Anolis carolinensis*. J Therm Biol 30:79–83.

Buckley CR, Jackson M, Youssef M, Irschick DJ, Adolph SC. 2007. Testing the persistence of phenotypic plasticity after incubation in the western fence lizard, *Sceloporus undulatus*. Evol Ecol Res 9:169–183.

Burger J. 1998. Effects of incubation temperature on hatchling pine snakes: implications for survival. Behav Ecol Sociobiol 43:11–18.

Cano JM, Nicieza AG. 2006. Temperature, metabolic rate, and constraints on locomotor performance in ectotherm vertebrates. Funct Ecol 20:464–470.

Christian KA, Tracy CR. 1981. The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. Oecologia 49: 218–223.

Christian KA, Weavers BW. 1996. Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. Ecol Monogr 66:139–157.

Christian KA, Tracy CR, Porter WP. 1983. Seasonal shifts in body temperature and use of microhabitats by Galapagos Land Iguanas (*Conolophus pallidus*). Ecology 64:463–468.

Clark DR, Kroll JC. 1974. Thermal ecology of anoline lizards: temperate versus tropical strategies. Southwest Nat 19: 9–19.

Cossins AR, Bowler K. 1987. Temperature biology of animals. London: Chapman & Hall.

Deeming DC. 2004. Post-hatching phenotypic effects of incubation in reptiles. In: Deeming DC, editor. Reptilian incubation: environment, evolution, and behaviour. UK: Nottingham University Press. p 229–251.

Downes SJ, Shine R. 1999. Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? Oecologia 120:9–18.

Flatt T, Shine R, Borges-Landaez PA, Downes SJ. 2001. Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. Biol J Linnean Soc 74: 339–350.

Geiser F, Firth BT, Seymour RS. 1992. Polyunsaturated dietary lipids lower the selected body temperature of a lizard. J Comp Physiol B 162:1–4.

Gibson AR, Smucny DA, Kollar J. 1989. The effects of feeding and ecdysis on temperature selection by young garter snakes in a simple thermal mosaic. Can J Zool 67:19–23.

Gordon RE. 1956. The biology and biodemography of *Anolis carolinensis carolinensis* Voigt. [Unpublished Ph.D. dissertation] New Orleans, Louisiana: Tulane University.

Gordon RE. 1960. The influence of moisture on variation in the eggs and hatchlings of *Anolis carolinensis*. Voigt Nat Hist Misc 173:1–6.

Hertz PE, Huey RB, Nevo E. 1982. Fight versus flight: body temperature influences defensive responses of lizards. Anim Behav 30:676–679.

Hertz PE, Huey RB, Nevo E. 1983. Homage to Santa Anita: thermal sensitivity of sprint speed in Agamid lizards. Evolution 37:1075–1084.

Hertz PE, Huey RB, Stevenson RD. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. Am Nat 142:796–818.

Huey RB, Bennett AF. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution 41:1098–1115.

Jenssen TA, Congdon JD, Fischer RU, Estes R, Kling D, Edmands S, Berna H. 1996. Behavioural, thermal, and metabolic characteristics of a wintering lizard (*Anolis carolinensis*) from South Carolina. Funct Ecol 10:201–209.

Ji X, Lin LH, Luo LG, Lu HL, Gao JF, Han J. 2006. Gestation temperature affects sexual phenotype, morphology, locomotor performance, and growth of neonatal brown forest skinks, *Sphenomorphus indicus*. Biol J Linnean Soc 88: 453–463.

Lang JW. 1987. Crocodilian thermal selection. In: Webb GJW, Manolis SC, Whitehead PJ, editors. Wildlife management: crocodiles and alligators. Chipping Norton: Surrey Beatty and Sons Ltd. p 301–316.

Le Galliard JF, Le Bris M, Clobert J. 2003. Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. Funct Ecol 17: 877–885.

Leroi AM, Bennett AF, Lenski RE. 1994. Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. Proc Natl Acad Sci USA 91:1917–1921.

Licht P. 1968. Response of the thermal preferendum and heat resistance to thermal acclimation under different photoperiods in the lizard *Anolis carolinensis*. Am Midl Nat 79:149–158.

Licht P. 1973. Influence of temperature and photoperiod on annual ovarian cycle in lizard *Anolis carolinensis*. Copeia 1973:465–472.

Lourdais O, Shine R, Bonnet X, Guillon M, Naulleau G. 2004. Climate affects embryonic development in a viviparous snake, *Vipera aspis*. Oikos 104:551–560.

McCue MD, Lillywhite HB. 2002. Oxygen consumption and the energetics of island-dwelling Florida cottonmouth snakes. Physiol Biochem Zool 75:165–178.

Michaud EJ. 1990. Geographic variation of life history traits in the lizard *Anolis carolinensis*. [Unpublished Ph.D. dissertation] Knoxville: Univ. Tennessee.

O'Steen S. 1998. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. J Exp Biol 201:439–449.

Patterson JD, Davies PMC. 1978a. Thermal acclimation in temperate lizards. Nature 275:646–647.

Patterson JW, Davies PMC. 1978b. Preferred body temperature: seasonal and sexual differences in lizard *Lacerta vivipara*. J Therm Biol 3:39–41.

Qualls FJ, Shine R. 1998. Geographic variation in lizard phenotypes: importance of the incubation environment. Biol J Linnean Soc 64:477–491.

Qualls CP, Andrews RM. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. Biol J Linnean Soc 67:353–376.

Qualls FJ, Shine R. 2000. Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, *Lampropholis guichenoti*. Biol J Linnean Soc 71:315–341.

Rhen T, Lang JW. 1999. Temperature during embryonic and juvenile development influences growth in hatchling snapping turtles, *Chelydra serpentina*. J Therm Biol 24: 33–41.

Rock J, Andrews RM, Cree A. 2000. Effects of reproductive condition, season, and site on selected temperatures of a viviparous gecko. Physiol Biochem Zool 73:344–355.

Seebacher F. 2005. A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? J Comp Physiol B 175:453–461.

Shine R, Downes SJ. 1999. Can pregnant lizards adjust their offspring phenotypes to environmental conditions? Oecologia 119:1–8.

Shine R, Elphick MJ, Harlow PS. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. Ecology 78:2559–2568.

Shine R, Harlow PS. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. Ecology 77:1808–1817.

Sievert LM, Hutchison VH. 1989. Influences of season, time of day, light and sex on the thermoregulatory behavior of *Crotaphytus collaris*. J Therm Biol 14: 159–165.

Spotila JR, Zimmerman LC, Binckley CA, Grumbles JS, Rostal DC, List A, Beyer EC, Phillips KM, Kemp SJ. 1994. Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. Herpetol Monogr 8: 103–115.

Stevenson RD. 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. Am Nat 125: 102–117.

Stevenson RD, Peterson CR, Tsuji JS. 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. Physiol Zool 58:46–57.

van Berkum FH. 1986. Evolutionary patterns of the thermal sensitivity of sprint speed in *Anolis* lizards. Evolution 40: 594–604.

Van Damme R, Bauwens D, Verheyen RF. 1991. The thermal dependence of feeding behavior, food consumption and gut passage time in the lizard *Lacerta vivipara* Jacquin. Funct Ecol 5:507–517.

van Marken Lichtenbelt WD, Vogel JT, Wesselingh RA. 1997. Energetic consequences of field body temperatures in the green iguana. Ecology 78:297–307.

Vanhooydonck B, Van Damme R, Van Dooren TJM, Bauwens D. 2001. Proximate causes of intraspecific variation in locomotor performance in the lizard *Gallotia galloti*. Physiol Biochem Zool 74:937–945.

Wheeler PE. 1986. Thermal-acclimation of metabolism and preferred body-temperature in lizards. J Therm Biol 11: 161–166.

Wilhoft DC, Anderson JD. 1960. Effect of acclimation on the preferred body temperature of the lizard, *Sceloporus occidentalis*. Science 131:610–611.

Winkler P. 1985. Persistent differences in thermal tolerance among acclimation groups of a warm spring population of *Gambusia affinis* determined under field and laboratory conditions. Copeia 1985:456–461.