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Latent Effects of Egg Incubation Temperature on Growth in the Lizard *Anolis carolinensis*

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ABSTRACT Varied egg incubation temperatures can result in immediate effects on the phenotype of reptiles, and also latent effects that can augment or contradict effects evident at egg hatching. I examined the effects of incubation temperature on embryonic development, hatching morphology, and subsequent growth in multiple populations of the lizard *Anolis carolinensis*. Eggs from wild-caught females in four populations were incubated at up to three temperatures, 23.5, 27, and 30°C. Measures of body size were collected immediately after hatching and weekly thereafter, while juveniles were maintained in a common laboratory environment for 8 weeks. Cooler incubation temperatures resulted in longer incubation periods but did not affect conversion of egg mass to hatchling mass. Incubation temperature did not affect hatchling mass or snout vent length (SVL), but did affect subsequent growth in both mass and SVL, which varied by population. Cooler incubation temperatures generally resulted in greater overall growth over 8 weeks of housing all juveniles in a common environment. In *A. carolinensis*, egg incubation temperature had latent effects on juvenile growth despite the absence of any detected immediate effects on hatchling phenotype. Therefore, the total impact and evolutionary importance of developmental environment should not be assessed or assumed based solely on the phenotype of reptiles at birth or hatching. *J. Exp. Zool. 309A, 2008.* © 2008 Wiley-Liss, Inc.

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Developmental conditions can have profound effects on the morphology and ecological interactions of organisms and the evolutionary trajectories of populations (Qualls and Shine, '98; Pigliucci, 2001; West-Eberhard, 2003; Fordyce, 2006). The temperature of development in many ectotherms in particular has been demonstrated to affect several aspects of growth, development, and performance (Atkinson, '94; Johnston and Bennett, '96; Spencer et al., 2006). In many oviparous reptiles, incubation temperature has been shown to affect hatchling size and body proportions (Shine et al., '97; reviewed in Birchard, 2004; Deeming, 2004), growth rates (Van Damme et al., '92; Alberts et al., '97; Deeming, 2004), locomotor performance (Vanhooydonck et al., 2001; Blouin-Demers et al., 2004; Deeming, 2004), and behaviors including thermoregulation (Burger, '98; Downes and Shine, '99; Flatt et al., 2001; Deeming, 2004). Although reaction norms may differ dramatically between populations (Niewiarowski and Roosenburg, '93; Iraeta et al., 2006), many studies of temperature-induced plasticity in reptiles focus on one population (reviewed in Deeming, 2004; however, see O'Steen, '98; Buckley et al., 2007). The current study examined temperature-induced plasticity in development and growth rates in several populations of the lizard *Anolis carolinensis* with similar life histories, but varying thermal environments.

Body size and egg size both increase with latitude in *A. carolinensis*, and the adaptive and mechanistic reasons are currently under study (Michaud and Echternacht, '95; Goodman, 2008). To contribute to this investigation, I examined how incubation temperature affects embryonic and juvenile growth in this species. The egg is an

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appropriate stage to subject to different temperatures, because variation in thermal environments of eggs must exist both within and among populations. *Anolis carolinensis* occurs throughout the southeastern United States. The mean monthly temperature differential during the time when eggs are incubated (May–August) between the northern and southern populations in this study ranged from 3.0°C (July) to 6.6°C (May) over 1995–2004 (Knoxville, TN, and Orlando, FL; NOAA, '95–2004). Female *A. carolinensis* deposit eggs in and under natural or man-made objects, in shallow soils or leaf litter, or leave eggs exposed or in vegetation (Gordon, '60; Michaud, '90; Echternacht, personal communication). Embryos within these eggs do not have the capacity to move to optimal temperatures as adults do, and are therefore subject to the thermal environments where they are deposited. A previous study found that cooler incubation temperatures produced larger hatchlings in this species but did not examine subsequent growth (Viets, '93; however, see Discussion for criticism of methods in that study).

Varied egg incubation temperatures may result in immediate effects on the phenotype of reptiles, and also latent effects that may augment or contradict effects evident at egg hatching. On the basis of previous studies in lizards, I predicted that cooler incubation temperatures would produce larger hatchlings relative to original egg size that subsequently grow faster than those incubated at warmer temperatures. Specifically, I tested the null hypothesis that juveniles from different incubation temperatures would exhibit similar incubation periods, hatchling sizes, and growth rates during 8 weeks in a common laboratory environment.

Although some studies examine plasticity of morphology at the hatchling stage only (examples in Deeming, 2004), I chose to examine postnatal growth as well to determine whether initial differences in morphology would persist, be amplified, or be compensated for with time (e.g. Joanen et al., '87; Elphick and Shine, '98; Ji et al., 2003; Spencer et al., 2006; Buckley et al., 2007). Incubation or developmental temperature affects thermoregulation in juvenile reptiles including *A. carolinensis* (Blouin-Demers et al., 2000; Blumberg et al., 2002; Goodman and Walguarnery, 2007), which in turn may affect growth rates. This potential effect was limited in the current study by rearing juveniles in a common environment with some, but limited, opportunities for thermoregulation.

MATERIALS AND METHODS

Collection and husbandry of adult females

In May and June of 2005, I collected 31–53 adult female *A. carolinensis* from each of three populations: south of Greenback, Blount Co., TN (N 35°33.486′, W 84°06.210′: TN), Jacksonville, Duval Co., FL (N 30°15.95′, W 81°30.70′: North Florida—NFL), and east of Orlando, Seminole Co., FL (N 28°37.92′, W 81°07.48′: Middle Florida—MFL). Additionally, 69 originally wild-caught females were purchased from a reptile supplier in LaPlace, LA (approx. N 30°03.93′, W 90°29.18′: LA) and shipped to Tennessee in June and July of 2005. Females were all measured upon arrival at the laboratory and housed individually as described by Goodman and Walguarnery (2007).

Collection and incubation of eggs

Eggs were collected from the sand substrate in each female's enclosure at least every other day and immediately measured for mass, length, and width. Eggs were incubated in airtight, 345 mL plastic containers with 10 g vermiculite and 10 mL water at 23.5, 27, or 30°C. I chose experimental treatments covering a wide range of incubation temperatures known to produce viable hatchlings in the laboratory (Viets, '93). Because of additional experiments on these subjects, eggs from the three eastern populations (MFL, NFL, TN) were subject to two incubation temperatures (27 and 30°C), whereas eggs from the LA population were subject to three temperatures (23.5, 27 and 30°C). The total weight of the water and vermiculite was recorded, and water was added to maintain this weight every week after the oviposition date for each egg. Only one egg per treatment per female was allowed, and the order of eggs in all treatments was distributed evenly by random assignment of the first egg for each female (and of the second egg in LA). Incubation temperatures were recorded every 60 min with Stowaway Temperature Tidbit Loggers (Onset Computer Corporation, Bourne, MA). The standard deviation of the 23.5°C treatment (used for LA only; SD = 0.86°C) differed from those of 27 and 30°C treatments (used for all populations; SD = 0.47 and 0.34°C, respectively) owing to logistic difficulties with one incubator. However, the temperature ranges of all treatments were entirely exclusive of each other. I rotated positions of egg enclosures within incubators and collected new hatchlings daily.

Husbandry and measurement of offspring

I measured snout vent length (SVL), tail length (TL), and mass of hatchlings within 24 hr of hatching and before first feeding. Sex, which is genetically determined in this species, was ascertained by the presence (male) or absence (female) of enlarged postanal scales, as viewed under a magnifying glass. Hatchlings from the three eastern populations were housed randomly with regard to population and incubation temperature in 38 L glass aquaria holding perches and cover objects and visual barriers between adjacent aquaria. Hatchlings from LA were not included in the growth portion of this study. Each aquarium contained three individuals of roughly the same age, identified by unique combinations of one to two clipped toes. I verified that sex, population, and incubation temperature had no influence on the order of introduction into the enclosures. Aquaria were misted at least two times per day, and fruit flies, pinhead crickets, and fruit baby food were provided ad libitum. Lights provided UVB and broad-spectrum fluorescent illumination on a 12:12-hr light:dark cycle. Temperatures followed a diurnal cycle within the aquaria, with daily highs of 32–34°C in light and 28–30°C in shade and nightly lows of 23–25°C. I rotated positions of enclosures within the laboratory once per week, and measured mass and SVL of juveniles weekly for 8 weeks. For the eastern populations, females were returned to their exact sites of capture after collection of eggs for this experiment ceased, and offspring were released at capture sites of their mothers at the completion of the experiment.

Statistical analysis

I analyzed the effects of incubation temperature on incubation period, conversion of egg mass to hatchling mass, hatchling mass, SVL, body proportion (TL/SVL), and body condition (mass/SVL) using analysis of covariance (ANCOVAs) with temperature and population as factors and egg mass as the covariate (samples size of 44, 50, and 25 for TN, NFL, and MFL, respectively; 58 and 61 for 27 and 30°C, respectively). Similar analyses excluding the factor of population were conducted for the LA population, wherein eggs were incubated at three temperatures (sample sizes of 50, 32, and 37 for 23.5, 27, and 30°C, respectively). Sex had no significant effects in the above analyses, and therefore reduced models are presented in Table 1. I examined growth rates by analyzing the effects of incubation temperature on mass and SVL during 8 weeks in the laboratory with repeated measures (RM) ANCOVAs (samples size of 44, 50, and 25 for TN, NFL, and MFL, respectively; 58 and 60 for 27 and 30°C, respectively). Temperature, population, sex, and hatchling mass or SVL were between subjects factors, and within subjects factors were time and time interactions with temperature, population, sex, and hatchling mass or SVL. Because significant effects of incubation temperature on growth and final size were demonstrated, I analyzed the effects of temperature, sex, and hatchling mass on final mass, and temperature, sex, and hatchling SVL on final SVL, within each of the three eastern populations using ANCOVAs.

I verified assumptions of normality of residuals and homogeneity of variance for ANCOVAs. Additionally, Greenhouse–Geisser corrections are included in *P*-values for the within subjects effects tests in RM ANCOVAs, because of violations of sphericity as indicated by Mauchly's test. I performed all statistical analyses in SPSS (Release 14.0.0, 2005, SPSS Inc., Chicago, IL) with a critical α of 0.05.

RESULTS

Cooler incubation temperatures resulted in longer incubation periods in all populations (Table 1, Fig. 1). However, incubation temperature did not affect conversion of egg mass to hatchling mass in any population (Table 1). No temperature-induced plasticity was detected in any of the four populations with respect to hatchling mass or SVL after controlling for egg mass (Table 1). Incubation temperature also had no effect on body proportion (TL/SVL) or condition (mass/SVL) in any of the populations (Table 1).

During 8 weeks of growth in a common laboratory environment, incubation temperature affected growth in mass among hatchlings from the three eastern populations after accounting for the effects of sex, population, and hatchling mass (between subjects effect, Table 2, Fig. 2a–c). Temperature, sex, and hatchling mass interacted with time in their effect on growth in mass (within subjects effect, Table 2). The same results were evident for *length* of hatchlings for all between subjects factors: temperature, population, sex, and hatchling SVL (Table 2, Fig. 3a–c). Males generally grew faster than females in terms of mass and length (Table 2). Within subjects effects on growth

TABLE 1. Results of ANCOVAs examining the effects of incubation temperature, population, and egg mass on length of incubation period, egg to hatchling mass conversion, mass, snout vent length (SVL), body proportion, and body condition of Anolis carolinensis hatchlings

	MFL, NFL, TN			LA		
Factor/covariate	df	*F*	*P*	df	*F*	*P*
Incubation period						
Temperature	1, 114	738.65	< **0.001**	2, 116	895.72	< **0.001**
Population	2, 114	11.83	< **0.001**	–	–	–
Egg mass	*1, 112*	*2.26*	*0.136*	*1, 113*	*0.85*	*0.358*
Temperature × Population	2, 114	17.46	< **0.001**	–	–	–
Temperature × Egg mass	*1, 109*	*0.10*	*0.757*	*2, 113*	*0.40*	*0.672*
Population × Egg mass	*2, 209*	*1.78*	*0.173*	–	–	–
Egg to hatchling mass conversion (hatchling mass/egg mass)						
Temperature	*1, 113*	*0.06*	*0.813*	2, 117	1.99	0.142
Population	2, 116	8.49	< **0.001**	–	–	–
Temperature × Population	*2, 113*	*0.71*	*0.496*	–	–	–
Hatchling mass						
Temperature	*1, 109*	*0.18*	*0.675*	*2, 114*	*0.78*	*0.460*
Population	2, 113	1.41	0.249	–	–	–
Egg mass	1, 113	59.45	< **0.001**	1, 118	100.80	< **0.001**
Temperature × Population	*2, 109*	0.56	0.571	–	–	–
Temperature × Egg mass	*1, 109*	0.33	0.568	*2, 114*	*0.56*	*0.572*
Population × Egg mass	2, 113	7.38	**0.001**	–	–	–
Hatchling SVL						
Temperature	*1, 109*	*0.05*	*0.830*	*2, 114*	*0.11*	*0.892*
Population	*2, 109*	*0.64*	*0.531*	–	–	–
Egg mass	1, 117	353.43	< **0.001**	1, 118	48.01	< **0.001**
Temperature × Population	*2, 109*	*0.69*	*0.502*	–	–	–
Temperature × Egg mass	*1, 109*	*0.02*	*0.877*	*2, 114*	*0.09*	*0.912*
Population × Egg mass	*2, 109*	*2.22*	*0.114*	–	–	–
Body proportions: TL/SVL						
Temperature	*1, 109*	*1.46*	*0.230*	2, 113	1.30	0.276
Population	*2, 109*	*0.13*	*0.875*	–	–	–
Egg mass	1, 117	0.25	0.621	1, 113	0.61	0.438
Temperature × Population	*2, 109*	*0.32*	*0.725*	–	–	–
Temperature × Egg mass	*1, 109*	*1.26*	*0.264*	2, 113	1.51	0.226
Population × Egg mass	*2, 109*	*0.42*	*0.655*	–	–	–
Hatchling condition: mass/SVL						
Temperature	*1, 109*	*0.33*	*0.567*	*2, 114*	*0.82*	*0.445*
Population	*2, 113*	*0.20*	*0.818*	–	–	–
Egg mass	1, 117	420.07	< **0.001**	1, 118	101.65	< **0.001**
Temperature × Population	*2, 109*	*0.91*	*0.405*	–	–	–
Temperature × Egg mass	*1, 109*	*0.58*	*0.448*	*2, 114*	*0.51*	*0.601*
Population × Egg mass	*2, 113*	*2.66*	*0.075*	–	–	–

Eggs from MFL, NFL, and TN populations were incubated at 27 and 30°C, whereas those from the LA population were incubated at 23.5, 27 and 30°C. Factors with test statistics in italics were not significant and were removed from the model before calculating test statistics for other factors. ANCOVA, analysis of covariance; MFL, Middle Florida; NFL, North Florida; TL, tail length.

in SVL were also similar; however, there was no temperature by time interaction effect, suggesting that the effect of incubation temperature remained constant through time (within subjects effect, Table 2).

By the end of 8 weeks in a common environment, incubation temperature had affected final mass and length of juveniles after controlling for hatchling mass and SVL, population, and sex (Table 2, Figs. 2 and 3). Within MFL, the 27°C incubation treatment produced greater final mass compared with 30°C (Table 3, Fig. 2a). However, there was only a nonsignificant trend for longer hatchlings resulting from 27°C as compared with

30°C, after adjusting for hatchling SVL (Table 3, Fig. 3a). In NFL, cooler incubation produced greater final mass and length of hatchlings, after adjusting for hatchling mass and SVL (Table 3, Figs. 2b and 3b). In TN, the cooler incubation treatment led to greater final mass relative to hatchling mass, but had no effect on final length (Table 3, Figs. 2b and 3c).

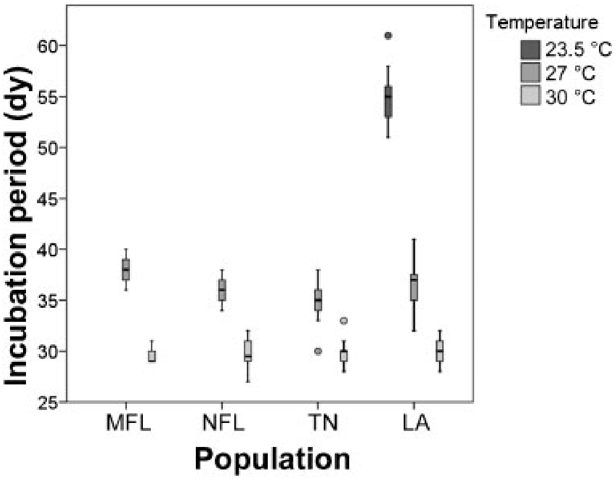

Fig. 1. Incubation periods for embryos of *Anolis carolinensis* from four populations (MFL, NFL, TN, and LA) incubated at up to three temperatures (23.5, 27, and 30°C). Boxplot shows the median, interquartile range, and outliers for each population and treatment. MFL, Middle Florida; NFL, North Florida.

DISCUSSION

Incubation temperature affected the length of incubation period, as predicted for lizards and ectotherms in general. Cooler developmental temperatures typically slow development in ecotherms (Atkinson, '94; Deeming, 2004), and this was the case for *A. carolinensis*. Despite this extended embryonic stage and contrary to my prediction, I detected no temperature-induced plasticity in embryonic growth in *A. carolinensis* incubated over 23.5–30°C. Conversion of egg mass to hatchling mass shows variation among populations in this species (Goodman, 2008), indicating some evolutionary flexibility if not immediate plasticity for this trait. Interestingly, Viets ('93), who also used lizards from LaPlace, LA, found that cooler incubation temperatures (21.5–25°C) produced significantly larger hatchlings than warmer temperatures (28–32°C). His results may actually have reflected desiccation of the substrate in warmer temperatures, as drier incubation substrates have been shown to produce smaller hatchlings in *A. carolinensis* (Michaud, '90). Whereas I used airtight containers to incubate eggs, and refilled lost or used moisture weekly, Viets flushed the air in his containers daily and did not add moisture during the incubation period.

Although the temperature treatments used in this study may not be those experienced in nature, they cover the range of constant temperatures under which *A. carolinensis* can be successfully

TABLE 2. Results of RM ANCOVAs examining the effects of incubation temperature (27 and 30°C), sex, population (MFL, NFL, and TN), and hatchling mass or SVL on mass and SVL of Anolis carolinensis juveniles during 8 weeks of growth in a common laboratory environment

	Mass (to 8 weeks age)			SVL (to 8 weeks age)		
	df	*F*	*P**	df	*F*	*P**
Between subjects						
Temperature	1, 112	9.85	**0.002**	1, 112	10.75	**0.001**
Population	2, 112	3.78	**0.026**	2, 112	5.14	**0.007**
Sex	2, 112	3.15	**0.047**	2, 112	3.64	**0.029**
Hatchling mass	1, 112	68.39	< **0.001**			
Hatchling SVL				1, 112	79.71	< **0.001**
Within subjects						
Time	7, 784	1.19	0.305	7, 784	2.65	0.065
Time × Temperature	7, 784	4.64	**0.012**	7, 784	0.67	0.531
Time × Population	14, 784	1.48	0.210	14, 784	2.35	**0.047**
Time × Sex	14, 784	7.62	< **0.001**	14, 784	8.78	< **0.001**
Time × Hatchling mass	7, 784	14.48	< **0.001**			
Time × Hatchling SVL				7, 784	0.85	0.441

**P*-values include Greenhouse–Geisser correction for sphericity. RM ANCOVA, repeated measures analysis of covariance; MFL, Middle Florida; NFL, North Florida; SVL, snout vent length.

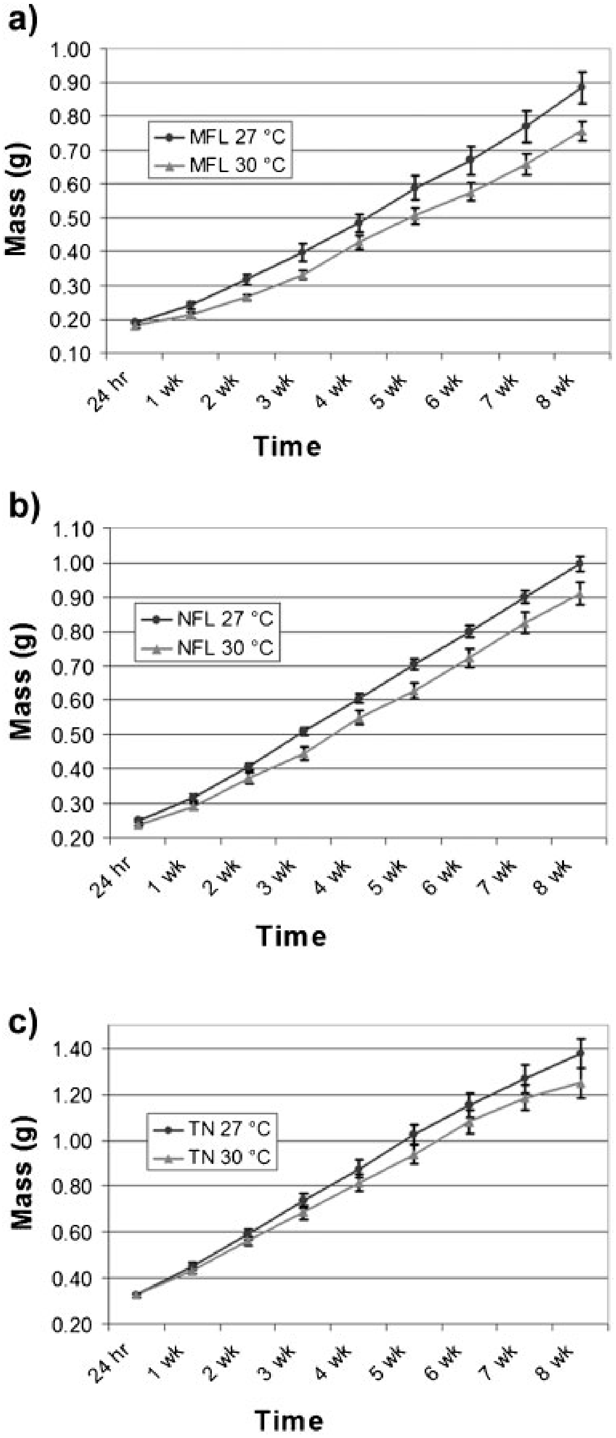

Fig. 2. Growth in mass of juvenile *Anolis carolinensis* incubated at two temperatures (27 and 30°C) and then reared in a common laboratory environment for 8 weeks. Average mass is shown for juveniles from three populations: MFL (**a**), NFL (**b**), and TN (**c**). Error bars are ±1 SE. MFL, Middle Florida; NFL, North Florida.

TABLE 3. Results of ANCOVAs examining the effects of incubation temperature, sex, and hatchling size on the final mass and snout vent length (SVL) of Anolis carolinensis hatchlings from three populations (MFL, NFL, TN)

Factor/covariate	df	*F*	*P*
Final mass—MFL			
Temperature	1, 23	5.64	**0.026**
Sex	*1, 18*	*0.74*	*0.400*
Hatchling mass	*1, 22*	*2.31*	*0.143*
Temp × Sex	*1, 18*	*0.44*	*0.517*
Temp × Hatchling mass	*1, 21*	*2.54*	*0.126*
Sex × Hatchling mass	*1, 18*	*0.42*	*0.527*
Final mass—NFL			
Temperature	1, 46	8.25	**0.006**
Sex	1, 46	3.56	0.065
Hatchling mass	1, 46	9.34	**0.004**
Temp × Sex	*1, 44*	*0.06*	*0.814*
Temp × Hatchling mass	1, 46	7.20	**0.010**
Sex × Hatchling mass	*1, 44*	*0.08*	*0.779*
Final mass—TN			
Temperature	1, 39	4.79	**0.035**
Sex	1, 39	4.00	0.052
Hatchling mass	1, 39	7.91	**0.008**
Temp × Sex	*1, 37*	*0.70*	*0.407*
Temp × Hatchling mass	*1, 37*	*0.72*	*0.402*
Sex × Hatchling mass	1, 39	5.07	**0.030**
Final SVL—MFL			
Temperature	1, 21	2.81	0.108
Sex	1, 21	8.98	**0.007**
Hatchling SVL	1, 21	6.71	**0.017**
Temp × Sex	*1, 18*	*0.04*	*0.847*
Temp × Hatchling SVL	*1, 18*	*0.49*	*0.494*
Sex × Hatchling SVL	*1, 18*	*0.39*	*0.540*
Final SVL—NFL			
Temperature	1, 47	5.88	**0.019**
Sex	*1, 45*	*3.38*	*0.073*
Hatchling SVL	1, 47	7.81	**0.007**
Temp × Sex	*1, 44*	*0.31*	*0.578*
Temp × Hatchling SVL	1, 47	5.57	**0.022**
Sex × Hatchling SVL	*1, 45*	*3.09*	*0.086*
Final SVL—TN			
Temperature	1, 39	1.21	0.277
Sex	1, 39	7.46	**0.009**
Hatchling SVL	1, 39	5.09	**0.030**
Temp × Sex	*1, 37*	*0.10*	*0.753*
Temp × Hatchling SVL	*1, 37*	*0.00*	*0.961*
Sex × Hatchling SVL	1, 39	7.96	**0.007**

Factors with test statistics in italics were not significant and were removed from the model before calculating test statistics for other factors. ANCOVA, analysis of covariance; MFL, Middle Florida; NFL, North Florida.

incubated, and are thus well-suited to test the existence of temperature-induced plasticity in this species. Temperature-induced plasticity in hatchling morphology has been found in many other species of lizards using a range of temperatures similar to this study (Deeming, 2004). However, some species appear unaffected within a range of

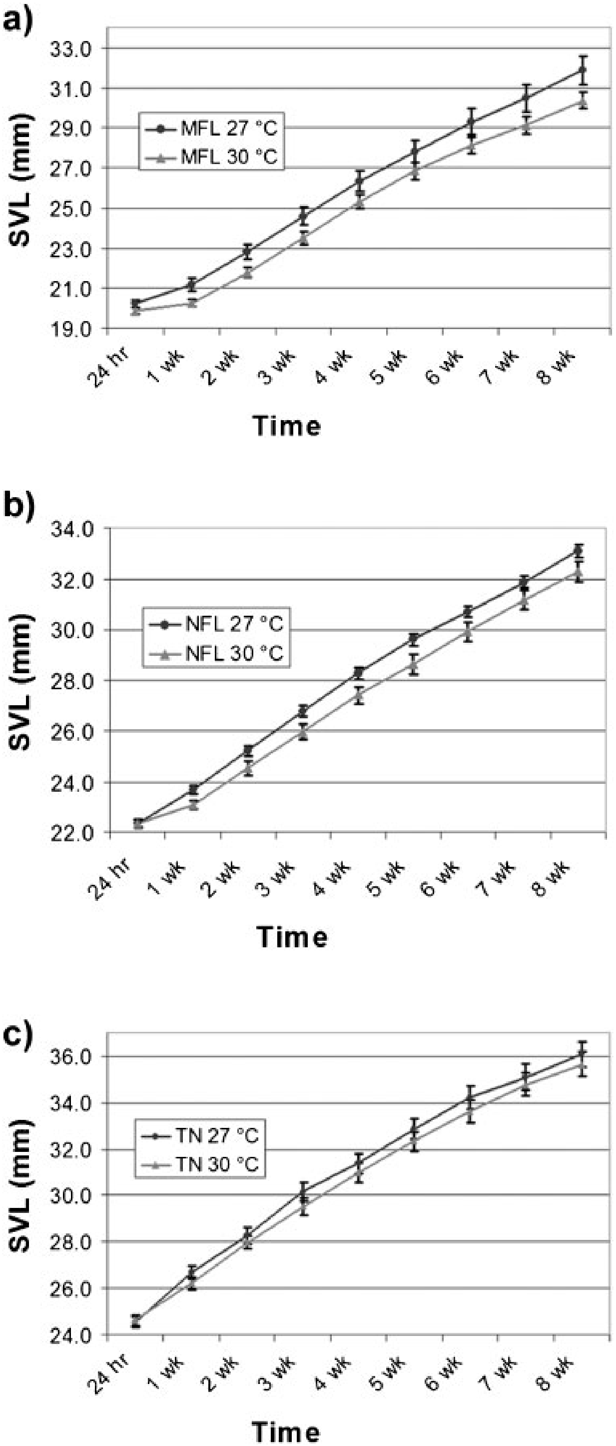

Fig. 3. Growth in snout vent length (SVL) of juvenile *Anolis carolinensis* incubated at two temperatures (27 and 30°C) and then reared in a common laboratory environment for 8 weeks. Average SVL is shown for juveniles from three populations: MFL (**a**), NFL (**b**), and TN (**c**). Error bars are ±1 SE. MFL, Middle Florida; NFL, North Florida.

incubation temperatures such as that used to test *A. carolinensis* (Deeming, 2004; Angilletta et al., 2006). Cooler temperatures produce larger hatchlings in most species of reptiles that exhibit temperature-induced plasticity, and this is usually accompanied by an increase in the length of incubation period (Birchard, 2004; Deeming, 2004). This pattern is one demonstration of the ''temperature size rule'' common to ectotherms, characterized as slower growth and development but greater final size in cooler temperatures (Ray, '60; Atkinson, '94). The current study does not fit this pattern, however, in that (1) embryonic growth in *A. carolinensis* does not appear to be affected by temperature in the range tested, and (2) postnatal growth is greater (in absolute rate) in cool-reared individuals. The possible adaptive explanation for the ''temperature size rule'' is still debated in the literature (Berrigan and Charnov, '94; Van der Have and De Jong, '96; Angilletta and Dunham, 2003); the exceptions to the rule in this study are also of uncertain significance.

As predicted, cool-incubated *A. carolinensis* displayed higher growth rates in the laboratory, in terms of mass in all populations and body length in one population. Higher growth rates may have been attributable to behavioral advantages in speed or dominance caused by cool incubation (which have been noted for other species, but were not examined in this study) that then caused differential access to food in the group housing situation. However, prey of diverse sizes were available ad libitum throughout the study. A more likely explanation for differences in growth rate is that the metabolism and physiology of lizards were somehow adjusted in the embryonic stage upon exposure to cooler temperatures. Metabolism, digestion, and growth rates are positively related to temperature in reptiles (within limits; Andrews, '82; Sinervo and Adolph, '89; Avery et al., '93; Wang et al., 2002), and incubation temperature has been shown to affect thermoregulation in *A. carolinensis* from the Louisiana population used in this study (Goodman and Walguarnery, 2007). The upper limit (but not median or lower limits) of selected temperatures was greater in hatchlings from 27°C than those from 30°C, although these differences had disappeared by around 23 days of age. Therefore, even if some selection of warmer temperatures within aquaria accounted for increased growth of cool-incubated individuals in the first 3 weeks after hatching, additional factors would have to explain the continued differences in growth during the last 5 weeks of the current study.

Larger body size in reptiles might enhance fitness through many ecological interactions, including competitive dominance (Stamps, '84), ability to eat larger and more diverse prey (Vitt, 2000), decreased predation vulnerability (Ferguson and Fox, '84; Vitt, 2000), greater thermal inertia in thermoregulation (Porter and Gates, '69; Stevenson, '85), and starvation resistance in low resource periods (Schultz and Conover, '99). Therefore, developmental conditions that affect body size can have important consequences for the evolutionary trajectories of populations. Differences among populations in these conditions, including egg incubation temperatures, could thus lead to differentiation among populations in reaction norms. The effects of incubation temperature on growth rates of juveniles differed among the three eastern populations of *A. carolinensis*. This result is not surprising considering differences in egg size, adult size, and embryonic growth and developmental rates among these populations (Michaud and Echternacht, '95; Goodman, 2008). However, this study does serve to caution those who would characterize reaction norms of growth and development in a species by experimentation in one population.

Many studies of temperature-induced plasticity in reptiles examine immediate effects only in hatchlings (reviewed in Deeming, 2004; however, see O'Steen, '98; Buckley et al., 2007). However, studies must be extended beyond this life stage to determine any long-term effects that may not be initially apparent. Although different incubation temperatures did not produce initial differences in body size in hatchling *A. carolinensis*, latent effects of this developmental condition were evident in growth rates and body size at 8 weeks of age. This stands in contrast to a recent, similar study with the lizard *Sceloporus undulatus*, wherein different incubation temperatures produced notable differences in morphology at hatching, but differences did not persist to 7 weeks in a common environment (Buckley et al., 2007). These studies indicate that environmentally shaped traits in reptiles must be studied on a species by species basis, using multiple populations that may vary in reaction norms, and using different life stages to understand the potential evolutionary importance of developmental conditions.

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