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ORIGINAL ARTICLE

Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis* based on experimental manipulations of egg size

Rachel M. Goodman

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Abstract Geographic variation in body size is of special interest because it affects nearly all aspects of an organism's life. I examined whether differences in body size among four populations of the green anole lizard, *Anolis carolinensis*, were attributable to maternal investment in egg size and/or growth rates of embryos and juveniles. Larger body size and larger egg size relative to female size in the northern part of the range have been documented in this species, and suggested to be adaptive responses to more extreme winters. The current study confirmed the trends in adult size and egg size in the north, but rejected the trend of larger egg size relative to body size in the south. To control for differences in maternal investment in egg size among populations, I performed yolk removals on eggs from two northern populations to produce comparably sized eggs relative to one southern population. This manipulation was designed to minimize the confounding effect of maternal investment in yolk, the primary energy reserves for eggs, so that intrinsic differences in embryonic growth due to metabolism could be investigated. I found that differences in juvenile and, potentially, embryonic growth rates existed among populations of *A. carolinensis*, both due to and independent of differences in egg size. Juveniles from the northernmost population were bigger not only due to larger egg size, but also due to faster juvenile growth and possibly differences in developmental stage of oviposition or conversion of egg mass to hatchling mass. Larger body size may hold a number of advantages in northern populations of this species, including starvation resistance through winters and better competitive access to food resources and warmer microhabitats.

Keywords Geographic variation · Life history · Maternal investment · Reptile · Yolk removal

Introduction

Patterns of geographic variation in morphology within a species interest biologists, because they may reflect adaptation to environmental factors that vary throughout the range of a species. Biologists have long been concerned with explaining large-scale patterns of body size in animals, dating back to Bergmann (1847). He proposed that larger-bodied endothermic vertebrates occur in cooler climates (1847), a trend that became known as Bergmann's rule and has been hotly debated since (Rensch 1938; Scholander 1955; McNab 1971; Meiri and Dayan 2003). The traditional explanation was that larger body size allowed heat conservation via a reduced surface area to volume ratio. Bergmann trends have been demonstrated in many endothermic taxa; however, selective pressures other than average environmental temperature and heat conservation have been suggested as explanations (reviewed in Blackburn et al. 1999).

In ectotherms, and especially in small-bodied species with low thermal inertia (Porter and Gates 1969), heat conservation should not apply as in endothermic species. Among reptiles, turtles comply with Bergmann's rule, whereas lizards and snakes generally oppose it (Ashton and Feldman 2003). The green anole lizard, *Anolis carolinensis* (Polychrotidae), follows an intraspecific Bergmann trend in part of its range (Michaud and Echternacht 1995), and the explanation for this is unclear. The current study examines

R. M. Goodman (✉)
Department of Ecology and Evolutionary Biology,
University of Tennessee, 569 Dabney Hall,
1416 Circle Drive, Knoxville, TN 37996-1610, USA
e-mail: rmgoodman@utk.edu

how growth and development vary among embryos and juveniles in populations along a latitudinal gradient for which adult body size is known to vary.

Anolis carolinensis occurs throughout the southeastern United States. A similar life history is found throughout the range, with most lizards hatching, growing, and overwintering in the first year and reproducing in the second, and few lizards surviving beyond the second year (Gordon 1956; King 1966; Michaud 1990; Bishop 2000). Michaud and Echternacht (1995) documented a trend of increasing body size and egg size with latitude in the eastern part of the range. Larger body size may confer greater fitness via enhanced starvation resistance in low resource periods (Schultz and Conover 1999), greater thermal inertia in thermoregulation (Porter and Gates 1969; Stevenson 1985), competitive dominance (Stamps 1984), ability to consume larger and more diverse prey (Vitt 2000), less vulnerability to smaller predators (Ferguson and Fox 1984; Vitt 2000), and increased survivorship (Andrews et al. 2000; however, see Warner and Shine 2007). Larger body size may be a consequence of increased maternal investment in eggs or initial offspring size, or increased growth rates of juveniles. These growth rates may in turn be caused by increased resource availability or exploitation, activity levels, foraging efficiency, thermoregulation, competitive ability, or inherent differences in metabolic processes.

Research in reptiles has shown that in addition to juvenile or adult growth rates, populations may differ in developmental stage at oviposition, nutritional and hormonal contents of eggs, and/or embryonic conversion of egg mass into hatchling mass which may be affected by environmental factors and intrinsic metabolic factors (Andrews and Mathies 2000; Oufiero and Angilletta 2006). Therefore, it is possible that maternal effects could differ among populations of *A. carolinensis* and contribute to subsequent observed differences in body size of offspring. Egg size is known to vary among populations of *A. carolinensis;* however, previous research indicates that nonpolar lipids per unit of egg mass (a primary indicator of reserves for embryonic growth) do not differ significantly between populations of wild collected females (Michaud 1990 and see “Discussion”).

In a laboratory study, I examined growth and development of embryos and juveniles from populations along a latitudinal gradient, controlling for egg size (or initial resources) both statistically and experimentally though egg yolk removal. I performed yolk removals on eggs from two northern populations to produce comparably sized eggs relative to one southern population. This manipulation was designed to minimize the confounding effect of maternal investment in yolk, the primary energy reserves for eggs, so that intrinsic differences in embryonic growth due to metabolism could be investigated. I tested the null hypothesis that embryonic growth, incubation period, and juvenile growth would not vary in a common environment among populations of *A. carolinensis* that differ in latitudinal origin and adult body size. I predicted that juveniles from northern populations would exhibit higher intrinsic growth rates to compensate for inhabiting a colder environment with a shorter growing season associated with higher latitude.

Materials and methods

Animal collection and husbandry of adult females

I collected 31–53 adult female *A. carolinensis* from each of four populations in May–June of 2005: south of Greenback, Blount Co., TN (35°33.486′N, 84°06.210′W: TN), Augusta, Columbia Co., GA (33°32.976′N, 82°02.228′W: GA), Jacksonville, Duval Co., FL [30°15.952′N, 81°30.697′W: North Florida (NFL)], east of Orlando, Seminole Co., FL [28°37.915′N, 81°07.482′W: Middle Florida (MFL)]. Nearly all females carry sperm at this point in the reproductive season, which they store and can use to fertilize eggs (ovulated and oviposited singly) in the laboratory (Licht 1973). Females were transported to the University of Tennessee, Knoxville and processed and housed in a laboratory within 48 h of capture. They were weighed (to 0.01 g) and measured for snout-vent length and total length (SVL and TL; to 0.5 mm). Females were housed in 3.8-L glass jars with screened lids and containing a perch, cover object, and Repti-sand® substrate (ZooMed Laboratories). Enclosures were misted with water daily, and vitamin-dusted crickets were provided every other day. Females were kept in temperatures cycling from 25°C during scotophase to 28–30°C during photophase, and placed under UVB and broad-spectrum fluorescent lights on a daily 12:12 h light:dark cycle. Females were returned to their exact sites of capture after collection of eggs for this experiment ceased.

Egg collection and manipulation

Eggs were collected from the sand substrate in each enclosure every other day, or between regular egg checks if laid at the surface of the sand. Collected eggs were measured for mass to the nearest 0.05 g. Eggs were manipulated to reduce size and equalize initial resources for embryos in two of four populations through the removal of egg yolk. This technique has been used in recent years with success in several reptiles (Sinervo and McEdward 1988; Sinervo 1990; Sinervo and Huey 1990; Ji et al. 1999; Radder et al. 2004; Oufiero and Angilletta

2006). In the current study, yolk removal, egg puncture (without yolk removal) and no manipulation (control) were conducted on eggs, with treatment among first, second, and third eggs of each female randomly determined. A maximum of three eggs per female were used in the study, though not all females produced three eggs. Sterile 25 M gage syringes were used to remove an average of 0.064 g (range = 0.018–0.133 g; SD = 0.026 g), or 18.2% of total egg yolk. Hatching success in this study was 87.0% for control eggs, 81.6% for punctured eggs, and 77.2% for eggs with yolk removal ($n = 54$, 38, and 79 before manipulations, respectively). I verified that yolk removal, but not the act of egg puncture alone, affected hatchling mass and SVL in GA and TN (ANOVA models contained significant treatment effects but not population effects; Tukey Kramer MCT's showed significant differences between yolk removal versus puncture only and control, with no differences between the latter two treatments).

All eggs were incubated at 27°C in individual, sealed, 345-mL plastic containers started with 10 g vermiculite and 10 mL water, with positions of eggs within incubators rotated daily. Initial mass of each container was recorded, and water was added to maintain this mass every week after the oviposition date for each egg.

Hatchling husbandry and measurement

New hatchlings were collected daily and measured within 24 h of hatching. Mass was measured to the nearest 0.05 g, and SVL and TL were measured to the nearest 0.05 mm with digital calipers after restraining hatchlings at the bottom of a small transparent plastic bag folded over. Two measurements of length were made for each individual (and repeated if they differed noticeably), and the average of these was used in analyses. Hatchlings were housed haphazardly with regard to population in 38-L enclosures holding several perches and cover objects, and each containing three individuals of roughly the same age. Toe clipping of 1–2 toes allowed for identification of individuals. Enclosures were misted at least two times per day and received UVB and broad-spectrum fluorescent illumination on a 12:12 h light:dark cycle. Temperature profiles 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. Lizards were provided fruit flies, pinhead crickets, and fruit baby food ad libidum. Positions of enclosures within the laboratory were rotated once per week. I measured mass and SVL of juveniles weekly for 8 weeks, as described above. Growth rates were calculated as gm per week for mass and mm per week for length (Table 5). Offspring were released at capture sites of their mothers at the completion of the experiment.

Statistical analysis of adult female size and egg size

To test the reported trend of adult body size, I compared SVL and mass of adult females among populations using analysis of variance (ANOVA). Post hoc comparisons were then conducted with multiple comparison *t* tests with Bonferonni corrections of *P* values. Egg size before yolk manipulation was compared among populations using analysis of covariance (ANCOVA) with population as the factor, and maternal mass as the covariate ($n = 47$, 40, 24, and 11 for TN, GA, NFL, and MFL, respectively). Throughout this and the following analyses, all factors and interactions were included in the original model, and any non-significant terms were dropped from subsequent models. Test statistics for non-significant terms from original models are presented in the tables, along with test statistics for all significant factors and interactions in reduced models. Post hoc comparisons following all significant ANCOVAs were conducted as *t* tests on estimated marginal means with Bonferonni corrections of *P* values for multiple comparisons. Within each population, egg mass (average mass of the first three eggs per female) was regressed against maternal mass using linear regression.

Comparison of egg size for TN and GA eggs subject to yolk manipulations [TN(R) and GA(R), respectively: (R) denotes yolk removal] and unmanipulated eggs from NFL was conducted using ANCOVA with maternal mass as the covariate [sample sizes of 24, 16, and 24 for TN(R), GA(R), and NFL, respectively]. I used these three treatments for comparison because egg yolk removals in northern populations aimed at the size of MFL eggs might have caused excessive mortality, and therefore northern eggs were only reduced to NFL egg size.

Statistical analysis of development and growth of offspring

Incubation periods of unmanipulated eggs from the four populations and from eggs from T(R), GA(R), and NFL were compared in separate ANCOVAs, with egg mass as the covariate. Egg mass conversion (hatchling mass/egg mass), and hatchling mass and SVL were compared among the four populations (unmanipulated eggs) and among TN(R), GA(R), and NFL using ANOVAs or ANCOVAs where appropriate.

I compared growth rates in mass and SVL using Repeated Measures ANCOVAs with population and sex as between subject effect, hatchling mass or SVL (as appropriate) as the covariate, and time (or age of juveniles) as the repeated measure. Full models with all interactions were conducted, and non-significant factors and interactions were removed. (Reduced models are presented in Tables 3 and 4; below.) Final mass and SVL were compared separately for the four populations (unmanipulated eggs) and for TN(R), GA(R), and NFL using ANCOVAs, with population and sex as factors and hatching mass or SVL as the covariate.

Only one egg per female per treatment was included in all analyses, except where noted otherwise. For all analyses, I verified assumptions of normality of data and homogeneity of variances. All analyses were conducted in SPSS (Release 14.0.0, 2005, SPSS, Chicago, IL) with a critical alpha of 0.05.

Results

Adult female size and egg size

Body size differed among adult females from the four populations (ANOVA: mass—$F_{3,104} = 23.35$, $P < 0.001$; SVL—$F_{3,\ 104} = 17.61$, $P < 0.001$), and followed a latitudinal trend with increasing body size in the north (Fig. 1a, b). Egg mass differed among populations, and was also influenced by maternal mass (Table 1; Fig. 2a). A lack of interaction between female mass and population in the ANCOVA model indicated no geographic variation in the relationship between female body size and egg size (Table 1). Egg size followed a latitudinal trend, with larger eggs in northern populations (Fig. 2a). Larger females produced larger eggs (unmanipulated) in all populations; slopes of linear regressions of egg mass against female mass and SVL were significant and positive in each population (Table 2). After yolk manipulations, eggs from GA(R) were smaller than those from NFL; eggs from TN(R) were of an intermediate size and did not differ from either group (Table 1; Fig. 2b).

Embryonic development and growth of offspring

Incubation period of unmanipulated eggs differed due to population of origin, but not due to egg size (Table 1). Eggs from MFL took longest to incubate, with those from NFL, GA, and TN taking 2.2, 2.6, and 3.2 days less to hatch, respectively (Fig. 3a). After yolk manipulations, incubation periods did not differ between NFL, GA(R), and TN(R), possibly due to the large variance in the TN(R) group and the exclusion of MFL eggs from this analysis (Table 1; Fig. 3b). Egg to hatchling mass conversion (hatchling mass/egg mass) differed among populations (Table 1). Hatchlings from TN were heaviest relative to their original egg mass compared to those from other populations, though only significantly more so than those from NFL (Fig. 4a). Comparison of manipulated eggs from TN(R) and GA(R) and eggs from NFL yielded similar results (Table 1; Fig. 4b).

Hatchling mass and SVL differed among populations after adjusting for egg mass (Table 1; Fig. 5a). Larger eggs produced heavier and longer hatchlings. Controlled for egg size, hatchling SVL still differed among populations and followed a trend of increasing size with latitude (though no pairwise comparisons with adjusted marginal means were significant with respect to mass). This trend was mirrored in a comparison of manipulated eggs from GA(R) and TN(R) and eggs from NFL. Despite similarly sized eggs after yolk removal, hatchlings from TN(R) were longer and heavier than those from NFL and GA(R) (Table 1; Fig. 5b).

Growth rates and final size of hatchlings

Final mass and SVL of juvenile lizards in the four populations (unmanipulated eggs) were affected by age, reflecting overall growth (Tables 1 and 3, within subjects effects). Also, interactions were found between age and population, sex, and hatchling mass (but not hatchling SVL; Table 3, within subjects effects). After 8 weeks of growth in a common laboratory environment, juveniles differed in mass and SVL based on their population of origin, sex, and hatching mass or SVL (Table 1; Table 3, between subjects effects). Males grew faster than females. Juveniles from TN were heavier (relative to original mass) than those from all other populations (Table 1; multiple comparison tests with Bonferonni correction; Fig. 6a).

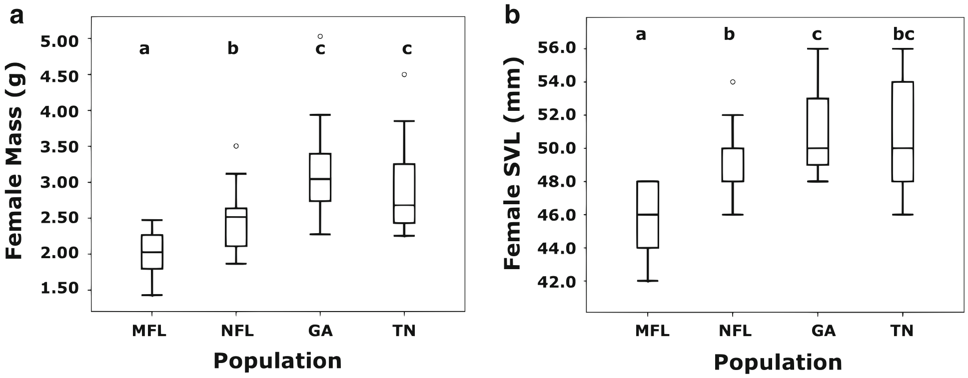

Fig. 1 **a** Mass and **b** snout vent length (SVL) of adult female *Anolis carolinensis* from four study populations (TN, GA, NFL, MFL; see text for details). *Boxplots* show the median, interquartile range, and outliers for each population. *Letters* denote significantly different groups, according to multiple comparison tests with Bonferonni correction

Table 1 Results of ANOVAs and ANCOVAs comparing egg size and characteristics of development and growth in juveniles from four populations of *Anolis carolinensis*

Factor/covariate	TN, GA, NFL, MFL			TN(R), GA(R), NFL		
	df	*F*	*P*	*df*	*F*	*P*
Egg mass						
Pop	3, 117	13.48	**<0.001**	2, 60	9.77	**<0.001**
Maternal mass	1, 117	105.34	**<0.001**	1, 60	33.30	**<0.001**
Maternal mass × pop	*3, 114*	*0.85*	*0.471*	*2, 58*	*0.98*	*0.381*
Incubation period						
Pop	3, 119	16.53	**<0.001**	2, 62	1.85	0.165
Egg mass	*1, 117*	*0.85*	*0.360*	*1, 60*	*0.29*	*0.591*
Egg mass × pop	*3, 114*	*1.32*	*0.272*	*2, 58*	*0.15*	*0.858*
Egg to hatchling mass conversion (hatchling mass/egg mass)						
Pop	3, 118	7.10	**<0.001**	2, 61	6.65	**0.002**
Hatchling mass						
Pop	3, 117	2.90	**0.038**	2, 60	7.42	**0.001**
Egg mass	1, 117	56.91	**<0.001**	1, 60	55.87	**<0.001**
Egg mass × pop	*3, 114*	*0.48*	*0.700*	*2, 58*	*2.19*	*0.121*
Hatchling SVL						
Pop	3, 117	12.13	**<0.001**	2, 60	13.60	**<0.001**
Egg mass	1, 117	41.97	**<0.001**	1, 60	27.99	**<0.001**
Egg mass × pop	*3, 114*	*0.35*	*0.788*	*2, 58*	*1.94*	*0.154*
Hatchling condition (hatchling mass/hatchling SVL)						
Pop	3, 119	16.38	**<0.001**	2, 62	2.09	0.132
Final mass						
Pop	3, 116	7.92	**<0.001**	2, 60	11.60	**<0.001**
Sex	1, 116	1.75	0.188	1, 60	13.07	**0.001**
Hatchling mass	1, 116	31.38	**<0.001**	1, 60	20.59	**<0.001**
Sex × Hatchling mass	1, 116	5.99	**0.016**	*1, 55*	*0.21*	*0.652*
Pop × sex	*3, 110*	*1.66*	*0.179*	*2, 55*	*0.89*	*0.416*
Pop × hatchling mass	*3, 110*	*1.79*	*0.153*	*2, 55*	*2.02*	*0.143*
Final SVL						
Pop	*3, 117*	*1.77*	*0.157*	*2, 60*	*1.55*	*0.220*
Sex	1, 120	21.47	**<0.001**	1, 62	9.83	**0.003**
Hatchling SVL	1, 120	85.37	**0.000**	1, 62	35.57	**<0.001**
Pop × sex	*3, 110*	*0.68*	*0.566*	*2, 55*	*0.74*	*0.484*
Pop × hatchling SVL	*3, 110*	*1.64*	*0.184*	*2, 55*	*2.85*	*0.067*
Sex × hatchling SVL	*1, 110*	*0.34*	*0.562*	*1, 55*	*0.84*	*0.364*

Left column: TN, GA, NFL, MFL, unmanipulated eggs; right column: from eggs from NFL and from TN and GA eggs subjected to yolk removals, GA(R) and TN(R); see text for details

Factors and interaction terms with test statistics in italics were not significant and were therefore removed from the models before calculating test statistics for other factors. Significant *P* values in bold

However, at 8 weeks of age, juveniles from the four populations did not differ in length when adjusted for sex and hatching length (Table 1).

Similar results were obtained for the three treatments manipulated to attain similar egg sizes (TN(R), GA(R), NFL; Tables 1 and 4). Growth rates of mass and SVL differed according to population and hatchling size (between subject effects in Table 4; absolute growth rates in Table 5). After adjusting for hatching mass, TN(R) juveniles were similar in mass to regular TN juveniles, and GA(R) were heavier than regular GA juveniles (Fig. 6b). Therefore, juveniles from both TN(R) and GA(R) were heavier at the end of the experiment than those from NFL, after adjusting for hatching mass (Table 1; multiple comparison tests with Bonferonni correction; Fig. 6b). Juveniles from the TN(R), GA(R), and NFL did not differ in final length, however, when adjusted for sex and hatching length (Table 1).

Discussion

This study found differences in juvenile, and potentially, embryonic growth rates among populations of *A. carolinensis*, both due to and independent of differences in the starting point of egg size. Removing yolk from the northern TN and GA produced eggs that were similar in size and slightly smaller than those from the southern NFL population. This manipulation demonstrated that juveniles from the north (in particular, TN) were bigger not only because of larger egg size, but also due to faster juvenile growth and possible difference in developmental stage at oviposition or conversion rate of egg mass to hatchling mass. Convergent evolution of more efficient embryonic growth among northern populations was recently demonstrated in another lizard, *Sceloporus undulatus* (Oufiero and Angilletta 2006). Perhaps eggs in northern populations of *A. carolinensis* have a more efficient developmental process or spend less energy on maintenance in the shorter egg stage, thus explaining the increase in conversion of egg mass to hatchling mass in TN.

Since I did not evaluate embryonic stages of development in freshly oviposited eggs, I could not detect whether larger eggs from the north may have been at advanced developmental stages, possibly accounting for differences in incubation period and egg to hatchling mass conversion. Hormonal and nutritional quality of eggs may have also differed among populations, which could be further examined in future research. However, previous research demonstrated that percentage of nonpolar lipids per unit of egg mass did not differ significantly between wild collected females from a northern and a southern population of *A. carolinensis* (although movement to alternate environments affected lipid quantity; Michaud 1990). Also, lipid mass and egg mass were positively correlated within the

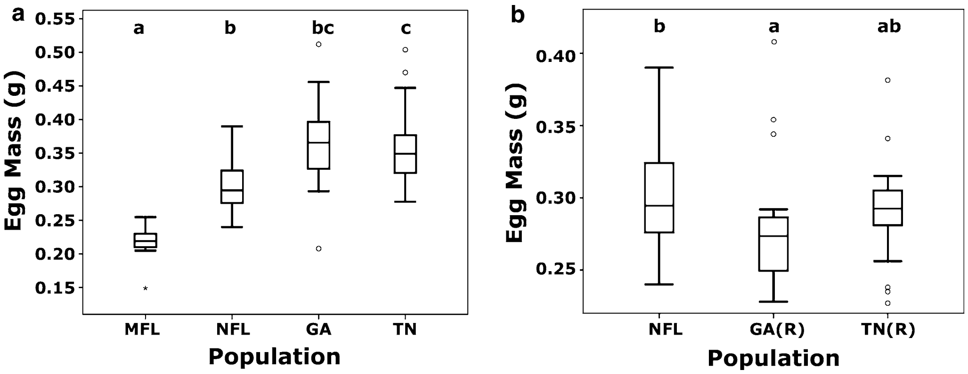

Fig. 2 Egg masses for **a** unmanipulated eggs originating from four study populations (TN, GA, NFL, MFL; see text for details) and **b** for NFL eggs as well as TN and GA eggs subjected to yolk removals [GA(R) and TN(R)]. *Boxplots* show the median, interquartile range, and outliers for each group. *Letters* denote significantly different groups, according to multiple comparison tests with Bonferonni correction. *Letters a–c* are in order of increasing means, which may not match graphical trends because tests are performed on estimated marginal means taking into account covariates (see Table 1)

Table 2 Linear regressions of egg size on mass and snout vent length (SVL) of wild adult females from four populations of *Anolis carolinensis* (TN, GA, NFL, MFL; unmanipulated eggs)

Population	x = female mass					x = female SVL				
	Equation	n	t	P	R^2	Equation	n	t	P	R^2
MFL	$y = 0.138 + 0.041x$	20	2.270	**0.036**	0.223	$y = -0.070 + 0.063x$	20	2.295	**0.034**	0.226
NFL	$y = 0.211 + 0.036x$	30	3.231	**0.003**	0.272	$y = -0.038 + 0.068x$	30	2.935	**0.007**	0.229
GA	$y = 0.159 + 0.063x$	25	5.037	**<0.001**	0.525	$y = -0.151 + 0.099x$	25	2.972	**0.007**	0.277
TN	$y = 0.194 + 0.053x$	31	5.350	**<0.001**	0.497	$y = -0.045 + 0.078x$	31	3.899	**<0.001**	0.344

The coefficient of determination (R^2) and the sample size (n), t test statistic (t) and P value (P) for t tests of H_o: slope = 0 are shown. Significant P values in bold

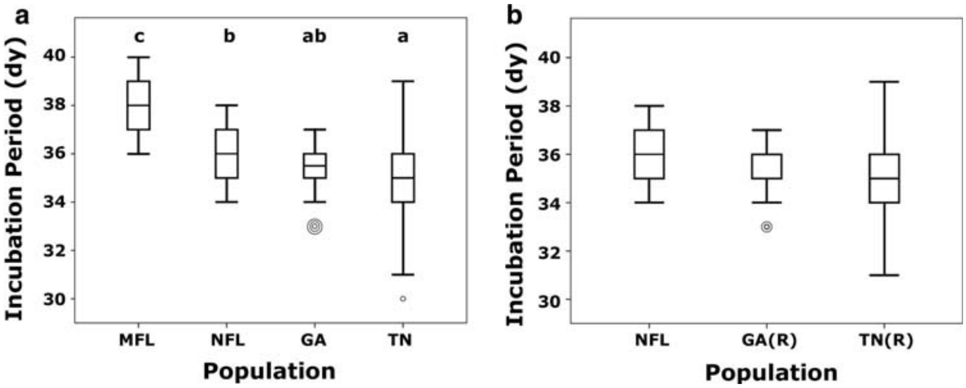

Fig. 3 Incubation period for hatchlings **a** from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL; see text for details) and **b** from NFL eggs as well as TN and GA eggs subjected to yolk removals [GA(R) and TN(R)]. See Fig. 2 legend for details of *boxplot* construction. Multiple overlain *circles* indicate number of outliers at the same value

two populations in that study, so egg mass was considered to be a reliable indicator of lipid quantity.

This study confirmed the previously described latitudinal gradient in female body size and egg size in *Anolis carolinensis*. However, I did not find a latitudinal trend in the relationship between female body size and relative egg size, as reported by Michaud and Echternacht (1995). That study found a significant positive relationship between female size and egg size in three northern populations, but not in five southern ones. The authors suggested that an optimal egg size exists in the south regardless of female body size, whereas larger eggs are advantageous in the north though a potential optimal egg size is constrained by the body size and pelvic aperture width in females. The contradictory results of the current study might be explained by my inclusion of different study populations or possible plasticity in life history traits between years that is currently unknown (Nussey et al. 2007).

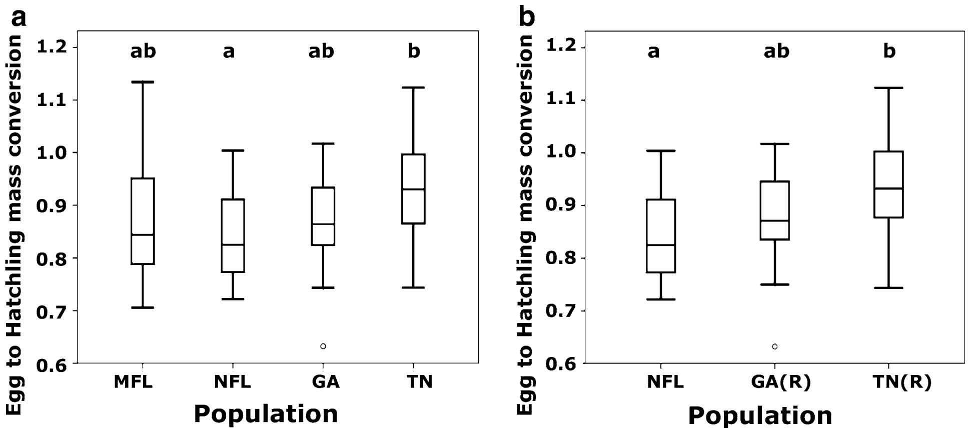

Fig. 4 Egg mass to hatchling mass conversion (g/g) for hatchlings **a** from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL) and **b** from NFL eggs as well as TN and GA eggs subjected to yolk removals [GA(R) and TN(R)]. See Fig. 2 legend for details of *boxplot* construction

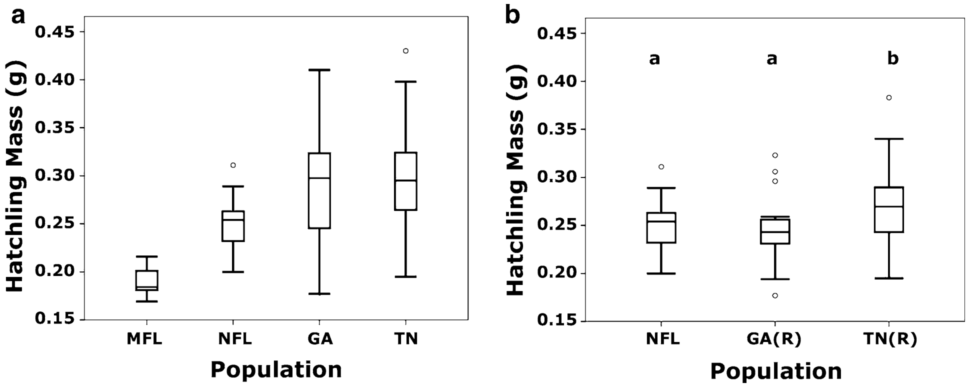

Fig. 5 Hatchling mass for hatchlings **a** from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL) and **b** from NFL eggs as well as TN and GA eggs subjected to yolk removals (GA(R) and TN(R)). See Fig. 2 legend for details of *boxplot* construction

Table 3 Results of repeated measures ANOVAs comparing weekly juvenile growth among *Anolis carolinensis* juveniles from four populations (TN, GA, NFL, MFL; unmanipulated eggs). Age (or time) is the repeated measure

	Mass (to 8 weeks age)			SVL (to 8 weeks age)		
	df	*F*	*P*	*df*	*F*	*P*
Between subjects						
Population	3, 115	13.04	**<0.001**	3, 115	5.60	**0.001**
Sex	1, 115	10.52	**0.002**	1, 115	7.00	**0.009**
Hatch mass	1, 115	72.98	**<0.001**			
Hatch SVL				1, 115	109.00	**<0.001**
Within subjects						
Age	7, 805	16.91	**<0.001**	7, 805	10.77	**<0.001**
Age × Population	21, 805	4.58	**<0.001**	21, 805	2.17	**0.002**
Age × Sex	7, 805	32.28	**<0.001**	7, 805	23.55	**<0.001**
Age × hatch mass	7, 805	8.29	**<0.001**			
Age × hatch SVL				7, 805	0.98	0.444

P values include Greenhouse–Geisser correction for sphericity. Significant *P* values in bold

Among all populations, TN had significantly larger hatchlings relative to their original egg size, suggesting that embryos more efficiently converted egg resources into hatchling body length. Eggs from these populations might also be of higher quality per unit mass; however, previous research does not necessarily support this suggestion (Michaud 1990). In the current study, eggs from northern populations, which were larger on average, took less time to incubate than those from the southern populations; however, there was no effect of egg mass on incubation period. Differences in incubation period have been shown to translate to fitness consequences in the wild in other lizards (Sinervo and Doughty 1996; Warner and Shine 2007). However, the consequences of the roughly 3-day difference in incubation period between populations in the current study are unknown.

Absolute and size-adjusted growth rates of juveniles in the common laboratory environment varied among individuals from unmanipulated eggs from all populations. Juveniles from TN and GA were heavier at the end of the experiment after adjusting for initial hatching size, indicating a difference in growth rate independent of maternally-conferred resources. However, their greater length relative to other populations was only due to their larger hatching SVL. I attempted to control for starting size or maternally-conferred resources of juveniles in the northern populations (TN and GA) through yolk removal manipulations to produce similarly sized eggs relative to a southern population (NFL). However, despite producing smaller manipulated egg size than the average for NFL eggs, both TN(R) and GA(R) eggs still resulted in larger

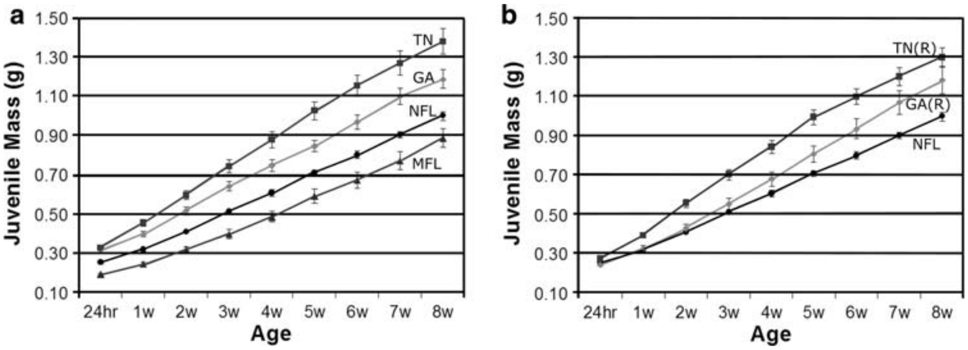

Fig. 6 Growth in mass of juvenile *Anolis carolinensis* in a common laboratory environment over 8 weeks. Juveniles from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL; see text for details) are included in (**a**). Juveniles from unmanipulated eggs from NFL and from TN and GA eggs subjected to yolk removals [GA(R) and TN(R)] are included in (**b**). *Error bars* ±1 SE

Table 4 Results of repeated measures ANOVAs comparing weekly juvenile growth among *Anolis carolinensis* juveniles from two northern populations that were products of yolk removal manipulations [TN(R) and GA(R)] and from one southern population (NFL)

	Mass (to 8 weeks age)			SVL (to 8 weeks age)		
	df	*F*	*P*	*df*	*F*	*P*
Between subjects						
Population	2, 59	20.30	**<0.001**	2, 59	11.79	**<0.001**
Sex	1, 59	3.34	0.073	1, 59	2.70	0.106
Hatch Mass	1, 59	49.17	**<0.001**			
Hatch SVL				1, 59	49.34	**<0.001**
Within subjects						
Age	7, 413	6.26	**<0.001**	7, 413	34.21	**<0.001**
Age × population	14, 413	7.40	**<0.001**	14, 413	2.84	**<0.001**
Age × sex	7, 413	12.55	**<0.001**	7, 413	6.65	**<0.001**
Age × hatch mass	7, 413	5.63	**<0.001**			
Age × hatch SVL				7, 413	1.13	0.342

Age (or time) is the repeated measure

P values include Greenhouse–Geisser correction for sphericity. Significant *P* values in bold

hatchlings, creating a disparity in the growth experiment thenceforth. Hatchlings from manipulated TN eggs were significantly larger than those from NFL, and they subsequently grew at a greater absolute rate. However, after correcting statistically for starting size, TN still had higher juvenile growth rates in mass than all other populations in the experiment. This increased growth may be attributable to metabolic processes adaptive to the northern environment, in support of the latitudinal compensation hypothesis. However, because experimental enclosures housed more than one juvenile, I cannot rule out the possibility that larger size resulted in dominance within enclosures that then affected resource access and growth rates.

Sex affected growth rates in the laboratory, but not hatchling mass, SVL, or egg mass conversion. These results are in accord with findings of Michaud (1990) and Gordon (1956) but contradict the reports by Viets (1993) of slight sexual size dimorphism in hatchings, with males being larger. Although the GA population contained the biggest females (though average size was not significantly bigger than TN), juveniles from GA had slower growth rates in the laboratory, lower egg to hatchling mass conversion, and were smaller than TN juveniles at the end of the experiment. How females from the GA population get to as large as those from TN is unclear, because the TN juveniles outgrow them both as embryos and as juveniles. However, growth subsequent to the first 2 months may not follow the same patterns as above, in which case size at maturity (7+ months of age) may not be accurately predicted by early juvenile growth. Also, the laboratory environment in this experiment might not have been natural or optimal for the GA population, so more natural conditions may yield different results.

Larger body size may hold a number of advantages in lizards (see “Introduction”). In *A. carolinensis*, male body size has been shown to be related to home range size, number of resident females, and dominance in male–male interactions (Greenberg and Noble 1944; Jenssen and Nunez 1998). For green anoles in particular, larger body size may be advantageous in the north because it aids overwinter survival (Michaud 1990). Green anoles do not hibernate, but remain active on warm days throughout the winter though eating and growing little (Jenssen et al. 1996; Bishop and Echternacht 2004). Lipids in fat bodies are used primarily for maintenance energy during the winter in *A. carolinensis*, in contrast to many temperate lizards whose lipids are used for reproduction (Dessauer 1955; Greenberg and Gist 1985). In other organisms, this potential explanation for Bergmann trends in body size has been supported and termed the “starvation resistance” hypothesis (Brown and Brown 1998; Schultz and Conover 1999; Arnett and Gotelli 2003). A starvation study of fish demonstrated that strong size-dependent winter mortality in a northern (but

Table 5 Absolute growth rates of *Anolis carolinensis* juveniles housed in a common laboratory environment and measured weekly for 8 weeks

	TN	GA	NFL	MFL	TN(R)	GA(R)
Mass (g/week)	0.131 (0.007)	0.108 (0.006)	0.093 (0.003)	0.087 (0.006)	0.127 (0.006)	0.117 (0.008)
SVL (mm/week)	1.44 (0.06)	1.32 (0.05)	1.34 (0.04)	1.46 (0.08)	1.44 (0.04)	1.44 (0.07)

Mean weekly change in mass and SVL (SE in parentheses) are shown for juveniles from four populations (TN, GA, NFL, MFL; unmanipulated eggs), as well as juveniles from two populations that were products of yolk removal manipulations [TN(R) and GA(R)]

not southern) population was due to proportionally greater energy depletion in small relative to large fish (Schultz and Conover 1997, 1999). Another adaptive suggestion for larger anoles in the north is that larger juveniles may be better competitors if resources are limited, including warmer, non-freezing overwintering sites that are a subset of the available microhabitats in northern populations (Bishop and Echternacht 2004). Future research should examine overwinter survival of juvenile *A. carolinensis* in northern and southern populations in relation to body size, and determine if any relationship is attributable to starvation resistance through lipid stores or competition for food resources or overwintering sites.

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