

The influence of incubation temperature on offspring traits varies across northern and southern populations of the American alligator (*Alligator mississippiensis*)

Christopher Smaga¹, Samantha Bock¹, Josiah Johnson¹, Thomas Rainwater², Randeep Singh², Vincent Deem³, Andrew Letter³, Arnold Brunell³, and Benjamin Parrott¹

¹University of Georgia

²Clemson University

³Florida Fish and Wildlife Conservation Commission

August 21, 2023

Abstract

Maternal provisioning and the developmental environment are fundamental determinants of offspring traits, particularly in oviparous species. However, the extent to which embryonic responses to these factors differ across populations to drive phenotypic variation is not well understood. Here, we examine the contributions of maternal provisioning and incubation temperature to variation in hatchling morphological and metabolic traits across four populations of the American alligator (*Alligator mississippiensis*), encompassing a large portion of the species' latitudinal range. Our results show that whereas the influence of egg mass is generally consistent across populations, responses to incubation temperature show extensive population-level variation in several fitness-related traits, including mass, head length, head width and residual yolk mass. Additionally, the influence of incubation temperature on developmental rate is greater at northern populations, while the allocation of maternal resources towards fat body mass is greater at southern populations. Overall, our results suggest that responses to incubation temperature, relative to maternal provisioning, are a larger source of interpopulation phenotypic variation and may contribute to the local adaptation of populations.

INTRODUCTION:

In oviparous vertebrates, maternal provisioning of nutrients and signaling molecules is critical for proper development and can be a major determinant of offspring traits (Groothuis et al., 2005; Radder et al., 2007; Van Dyke and Griffith, 2018). Complex biological and ecological factors, including maternal diet (Royle et al., 2003; Warner and Lovern, 2014), stress (McCormick, 1998; Saino et al., 2005), and age (Beamonte-Barrientos et al., 2010; Urvik et al., 2018) can influence the quantity and quality of resources provisioned to embryos (Moore et al., 2019; Mousseau and Fox, 1998). In addition, components of the developmental environment can influence how maternal resources are utilized by developing embryos (Brown et al., 2011; Du and Shine, 2022, 2008; Mueller et al., 2015; Shine and Brown, 2002). For example, egg mass is a primary determinant of hatchling mass (Deeming and Birchard, 2007), but incubation temperature has been shown to modify the efficiency by which maternal resources are converted into somatic tissue (Bock et al., 2021; Marshall et al., 2020; Pettersen et al., 2019) and how those resources are allocated to different phenotypes (Flatt, 2001; Telemeco et al., 2010). However, despite the importance of maternal provisioning and incubation temperature in shaping hatchling phenotypes, the extent to which these factors contribute to trait variation across populations is not well resolved (but see (Bodensteiner et al., 2019; Orizaola and Laurila, 2016, 2009; Richter-Boix et al., 2015)).

Species with broad geographic ranges are likely under selective pressures to maximize fitness under population-specific ecological conditions, which can include altering embryonic responses to maternal resources and environmental variables (Conover and Schultz, 1995; Kawecki and Ebert, 2004; Merilä et al., 2000; Orizaola and Laurila, 2009). For instance, in oviparous reptiles, latitudinal differences in the influence of temperature on incubation duration have been shown to occur across populations (Du et al., 2010a; Pettersen, 2020). Whereas cooler incubation temperatures slow development, populations from northern latitudes often show counter-gradient variation in incubation duration, developing faster than populations from more southern latitudes at the same temperatures (Pettersen, 2020). Similarly, high altitude populations of wall lizards (*Podacris uralis*) have been shown to allocate more maternal resources towards somatic tissue relative to low altitude populations when raised at a common temperature (Pettersen et al., 2023). However, the extent to which populations vary in how maternal provisioning and incubation temperature shape additional fitness-related traits in taxonomically diverse species remains unclear. Such information is critical to understand how responses to developmental conditions contribute to adaptive evolutionary change.

In the present study, we test whether embryonic responses to maternal provisioning and incubation temperature show interpopulation variation by examining several fitness-related traits in a large reptile, the American alligator (*Alligator mississippiensis*). Like many turtles and some lizards, crocodilians display temperature-dependent sex determination (TSD), in which thermal signals experienced during a discrete developmental window determine sex, along with additional phenotypic traits (Allsteadt and Lang, 1995; Bock et al., 2021; Kohno et al., 2014; McCoy et al., 2016): incubations at warmer, male-promoting temperatures (MPT) result in larger hatchlings with greater residual yolk reserves than those incubated at female-promoting temperatures (FPT). Recent reports demonstrate that temperature sensitive traits, including body mass index (BMI) and snout-vent length (SVL), correlate with juvenile survival in this species (Bock et al. 2023, preprint; Johnson et al. 2023), which may contribute to the adaptive significance of TSD (Schwanz et al. 2016; Bock et al. 2023, preprint). This life history strategy, combined with the unique taxonomic position of crocodilians relative to other extant vertebrates, makes alligators particularly informative in deciphering how variation in response to the developmental environment contributes to trait diversity across populations. The alligator's range extends from southern Florida to northeastern North Carolina, providing ample opportunity for local adaptation of phenotypic responses to maternal resources and incubation temperature. Yet, the extent to which embryonic response to these components of the developmental environment vary across populations is currently unknown. Here, we use a common garden incubation and grow out design to resolve the relative influence of egg mass (a proxy for maternal provisioning) and incubation temperature on morphological and metabolic traits across two northern and two southern populations spanning a large latitudinal portion of the alligator's geographic range.

METHODS:

Experimental design and data collection:

In June and July of 2021, 7-8 clutches of alligator eggs were collected from each of four, geographically distinct populations, including Par Pond on the United States Department of Energy's Savannah River Site in Aiken, South Carolina (SR), Tom Yawkey Wildlife Center in Georgetown, South Carolina (YK), Lake Woodruff National Wildlife Refuge in De Leon Springs, Florida (WO), and Lake Apopka in Apopka, Florida (AP; Figure 1A). After locating nests by helicopter or airboat, all eggs were removed from a nest cavity shortly after oviposition. Eggs were placed in plastic bus pans with natal nesting material for transport back to the University of Georgia's Savannah River Ecology Laboratory in Aiken, SC, where they were individually weighed and 1-2 eggs from each clutch were staged according to Ferguson (1985). The remaining eggs were transferred into new bus pans with dampened sphagnum moss and kept in commercial incubators (model I36NLC, Percival Scientific, Perry, IA, USA) at 32°C, an intermediate temperature that produces mixed sex ratios (Lang and Andrews, 1994). During this period, eggs were misted twice daily, and bins were rotated once daily within each incubator to limit the effect of intra-incubator temperature variation. Incubator temperatures were also monitored with HOBO TidbiT® v2 Temp Loggers (Onset, Bourne, MA, USA) placed within bus pans and incubator set temperatures were adjusted daily to maintain a constant 32°C.

At embryonic stage 15, just prior to the opening of the thermosensitive period of sex determination (McCoy et al., 2015), eggs from each population were randomly assigned in a split-clutch design to one of two temperature treatments: a constant MPT (33.5°C) or a constant FPT (29.5°C). Since full clutches were collected for multiple studies, a random subset of 3-10 eggs/clutch/temperature/site were chosen at this time to raise until hatch for this experiment. Throughout the entire incubation period, eggs were continually monitored and incubator temperature set points were adjusted to maintain consistent temperatures.

Once hatchlings pierced the eggshell (“pipped”), the date was recorded, and eggs were placed in glass Mason jars (one egg/jar) with damp sphagnum moss. Embryos were given 48 hours to hatch from the egg before being assisted if they did not hatch on their own. Once fully hatched, neonates were weighed using a digital balance ($\pm 0.01\text{g}$) and SVL and tail girth at the cloaca (TG) were measured using a flexible ruler ($\pm 0.1\text{ mm}$), and head length (HL) and head width (HW) were measured using calipers ($\pm 1\text{ mm}$). Hatchlings were then individually marked using unique, numbered toe tags and transported to large, indoor, fiberglass holding tanks where they were maintained under common-garden conditions and natural light cycles for 10-days. During this period, hatching alligators relied on maternal yolk reserves and were not fed. Water was changed daily, and hatchlings were monitored twice daily for overall health and survival. At 10-days post-hatch (10-DPH), hatchlings were remeasured, euthanized via cervical severance and pithing, and dissected to obtain residual yolk mass and fat body mass. Phenotypes analyzed included morphological traits of mass, BMI, SVL, TG, HL, and HW at hatch, and metabolic traits including incubation duration (measured in days from stage 15 to pip), change in morphological traits between 10-DPH and hatch ([?] mass [?] BMI, [?] SVL, [?] TG), residual yolk mass, and fat body mass. All experiments were approved by the University of Georgia Animal Care and Use Committee (A2021 05-007-Y3-A0) and collections were carried out under permits from the South Carolina Department of Natural Resources (SC-08-2021) and Florida Fish and Wildlife Conservation Commission (SPGS-18-33).

Statistical analysis:

All statistical analyses were conducted in RStudio (R Core Team 2021, version 4.1.2) and all models were built using the *lme4* package (Bates et al. 2015). Model assumptions of residual normality and homoscedasticity were checked visually via residual vs fitted and Q-Q plots, with log transformations made as necessary to best meet assumptions. To compare initial egg mass across populations, we used a linear mixed-effects model (LMM) including a fixed-effect of site, while controlling for clutch as a random effect. To determine whether hatch probability or survival to 10-DPH differed across temperatures or sites, we used a generalized linear-mixed model with a binomial distribution including temperature, site, and their interaction as fixed effects with clutch nested within site as a random effect. Post-hoc pairwise comparisons were conducted using the *emmeans* package (Lenth et al. 2023) with Kenward-Roger degrees of freedom and correcting for multiple testing using Tukey’s method.

To test for differences in the relative contributions of egg mass and incubation temperature to phenotypic traits across populations, we constructed separate LMMs for every phenotype at each site. In every model, we included fixed effects of egg mass and incubation temperature, with the exception of incubation duration, in which we only included a fixed effect of incubation temperature, while controlling for clutch as a random effect. We then compared model estimates across populations by extracting beta values and 95% confidence intervals (CIs) using the *confint* function in R. Model beta estimates in which CIs did not overlap zero or another population were considered statistically significant.

To further examine how embryos respond to temperature and maternal provisioning across populations, we used the *ggeffects* package (Ludecke 2018) to predict temperature-specific mean values of each phenotype at a common egg mass, corresponding to the average egg mass across the dataset (83g), from each population-specific model. By comparing egg mass-corrected mean phenotypes across temperatures and populations, we were able to determine whether populations differed in mean trait values irrespective of egg mass at either or both temperatures and whether variation in the influence of incubation temperature was driven by phenotypic differences at 29.5degC, 33.5degC, or both. Mean values in which 95% CIs did not overlap were considered statistically significant.

Given that populations can also vary in how maternal resources are allocated towards particular phenotypes, we compared ratios of SVL, TG, HL, HW, residual yolk mass and fat body mass to hatchling mass across populations within and across temperatures using LMMs. For this analysis, we included temperature, site, and their interaction as predictors, along with egg mass as a covariate, while controlling for clutch nested within site as a random effect. We then compared predicted mean values from the model within and among temperatures across populations using the *emmeans* package. Values in which CIs did not overlap were considered statistically significant. We used ratios of traits to hatchling mass instead of egg mass for this analysis because there were significant differences in temperature-specific mass across populations (see below), and as a result, differences in the ratio of traits to egg mass would be confounded by population-specific effects of temperature on mass and may not represent differences in the allocation of maternal resources towards specific phenotypes. All figures were created using the R package *ggplot2* (Wickham 2016).

RESULTS:

Egg mass and survival:

Egg masses at the two southern populations (AP and WO) were greater relative to the two northern populations (SR and YK), but only a nearly significant difference was observed between SR and WO ($\beta = -11.98$, $t = -2.57$, $p = 0.073$; Figure 1B). Whereas hatch rates were lower at 29.5°C (59.2%) compared to 33.5°C (82.9%; $\beta = 1.12$, $z = 2.08$, $p = 0.038$), differences were not observed between sites at either temperature (all pairwise $p > 0.23$). There were also no differences in survival between sites (all pairwise $p = 1$) or temperatures ($p = 1$) during the 10-day growth period, with 79 (94%) and 114 (94%) animals surviving at 29.5°C and 33.5°C, respectively. Final sample sizes of surviving individuals by temperature, clutch, and site are shown in Table 1.

Morphological traits:

Both egg mass and temperature exerted positive effects on hatchling mass across all populations (Table 2). However, whereas the influence of egg mass did not differ across sites (Figure 2A), temperature more strongly affected hatchling mass at YK compared to the other three populations (Figure 2B). In addition, there was a trend for a greater influence of incubation temperature on SVL at the northern populations relative to the southern populations, with the influence of temperature on SVL not significant in the latter (Figure 2C). Across other morphological traits, the influence of temperature was variable in both direction and magnitude, differentially affecting TG, HL, and HW in at least one population, while the influence of egg mass was not (Table 2).

We next examined the extent to which morphological phenotypes varied across populations within a temperature, including whether differences in the influence of incubation temperature were driven by variation at 33.5°C, 29.5°C, or both by comparing model means under a common egg mass. There were significant differences in trait values between at least two populations for all morphological traits after controlling for egg mass differences, with interpopulation variation in morphological traits occurring primarily at 29.5°C (Appendix 1). For instance, the influence of incubation temperature on mass of YK hatchlings was primarily driven by a reduction in mass at 29.5°C relative to the other populations (Figure 2B). This pattern was mostly consistent across additional traits that were differentially impacted by incubation temperature in at least one population (TG, HL, and HW), with the exception of SVL, which appeared to involve differences at both 29.5°C and 33.5°C (Figure 2C). Ratios of morphological traits to hatchling mass showed no significant differences across populations at either temperature.

Metabolic traits:

As with morphological traits, we also examined the effect of egg mass and incubation temperature on metabolic traits across populations. As egg mass increased, [?]mass decreased at the two southern populations, but had no effect in northern populations (Table 2). However, comparison of beta values across sites showed only a significant difference between AP and SR. A positive influence of egg mass on residual yolk mass was observed across all populations except for AP, but differences across populations were not significant.

Incubation temperature did not affect [?]*mass* or [?]*BMI* at any population, but exerted negative influences on [?]*SVL* at YK and fat body mass at all populations (Table 2, Figure 3A). On the other hand, there was a significantly positive influence of incubation temperature on [?]*TG* and residual yolk mass (Table 2, Figure 3B) in at least one population. Whereas the effect sizes of temperature on [?]*SVL*, [?]*TG* and fat body mass did not differ across sites, the influence of temperature on residual yolk mass did, showing a reduction at YK (Table 2, Figure 3B).

When comparing phenotypes across populations after correcting for egg mass, we found significant differences in fat body mass between at least two populations at both 29.5degC and 33.5degC, with a trend for smaller fat body masses at the northern populations (Figure 3A; Appendix 1). Consistent with the decreased influence of incubation temperature on residual yolk mass at YK, animals from 29.5degC at YK had significantly higher residual yolk mass compared to WO (Figure 3B, Appendix 1). Upon examination of the mass-corrected allocation of maternal resources towards metabolic phenotypes, there were significant differences for both residual yolk mass and fat body mass across populations. Animals from the southern populations tended to allocate more resources towards fat body mass than the northern populations (Figure 3C), and animals from YK at 29.5degC allocated more resources towards residual yolk mass relative to other populations (Figure 3D).

Incubation temperature had a negative influence on incubation duration across all sites (Figure 4). The influence of temperature was greater at the northern populations than at southern populations, driven by comparatively shorter incubation periods at 33.5degC and longer incubation periods at 29.5degC (Figure 4). However, differences across sites within temperatures were not significant.

DISCUSSION:

Patterns of population-level variation in embryonic responses to maternal provisioning and environmental factors have the potential to inform how the developmental environment contributes to evolutionary change. We observed that, generally, the influence of maternal provisioning on hatchling traits did not vary across populations; however, incubation temperature exerted population-specific effects on both morphological and metabolic traits. This may be explained by a constrained relationship between egg mass and hatch mass (Deeming and Birchard, 2007), which is expected to be maximized as hatchling mass is often an important component of survival and fitness (Ronget et al., 2018; Stearns, 2000). Thus, selection instead tends to act on aspects of maternal allocation, such as egg size and number, to best match population-specific conditions (Angilletta et al., 2004; Sinervo, 1990). On the other hand, responses to incubation temperature may be in part the result of differences in natural nest temperatures across populations, which has been shown in several species (Du et al., 2019), including the alligator (Bock et al., 2020). Such differences likely select for embryonic responses to temperature that match population-specific conditions. Our results suggest that plastic responses to incubation temperature, but not maternal provisions, are a source of interpopulation trait variation and may be more likely to be modified by selection.

The four populations examined in this study encompassed a large proportion of the alligator’s latitudinal range, with two populations from the northern extent and two populations from the southern extent. While not statistically significant, we observed a trend for smaller egg masses at the northern populations relative to the southern populations. In crocodilians, egg mass scales with maternal body size (Larriera et al., 2004), and differences in maternal size might underlie population differences observed here. In mammals, animals from high latitudes tend to be larger than those from low latitudes in a pattern known as Bergmann’s rule (Blackburn et al., 1999), and while this seems to hold in turtles and birds, it does not in other reptiles (Ashton, 2002; Ashton and Feldman, 2003) and has not been examined in crocodilians. Nonetheless, larger egg sizes at southern populations might suggest the opposite pattern. Interestingly, however, allometric relationships between maternal size and egg mass can be altered by environmental conditions, such as salinity (Murray et al., 2013). Whether differences in egg size observed here are the result of differences in maternal size across populations (maximum size or age at reproduction) or population-specific allometric relationships is unknown and an interesting area of future research.

While we expected to find responses to incubation temperature consistent with latitudinal differences between our population pairs, only a few traits showed such patterns. Namely, incubation duration was more strongly influenced by incubation temperature at the northern populations relative to the southern populations. Latitudinal differences in incubation duration have been shown in several species and generally follow one of two patterns: co-gradient variation, in which cooler populations development more slowly relative to warmer populations and counter-gradient variation, in which cooler populations development more quickly than warmer populations (Conover and Schultz, 1995; Pettersen, 2020). Our results show embryos from northern populations develop slightly slower at cooler temperatures and faster at warmer temperatures compared to southern populations. While differences within temperatures were not significant, they followed patterns of both co-gradient variation (at 29.5degC) and counter-gradient variation (at 33.5degC). Similar results have been shown in Asian pond turtles (*Mauremys mutica* ; Zhao et al., 2015) and may suggest that the mechanisms responsible for variation in incubation duration across populations may be temperature specific. On the other hand, increased plasticity in developmental rate at the northern populations may be driven by more variable thermal environments, which have been associated with increased levels of physiological plasticity (Seebacher et al., 2015). Additional experiments incorporating more incubation treatments and populations are needed to more completely discern how the relationship between temperature and developmental rate differs across populations as well as the underlying mechanisms responsible. Interestingly, we also observed that southern populations tended to allocate more resources towards fat body mass than northern populations at both incubation temperatures. The role of the fat body in alligators is not known, and further work examining its function, including how fat body size/mass early in life might impact survival and later life fitness, is needed to more fully appreciate the potential consequences of these patterns.

Apart from latitudinal trends, there were several differences in the influence of incubation temperature between population pairs, specifically between YK and other populations. In alligators, animals at 33.5degC are generally larger in mass than those at 29.5degC (Bock et al., 2021), which was upheld across all populations. However, at YK, the reduction of hatchling mass at 29.5degC appeared particularly pronounced and appeared to drive additional phenotypic differences. Hatchling mass relative to egg mass reflects the efficiency by which maternal resources are converted into hatchling tissue and is likely a product of the energetic cost of embryonic development (Pettersen et al., 2019). The reduction in mass at YK at 29.5degC relative to the other sites suggests that development at 29.5degC at YK was particularly inefficient. Interestingly, however, animals at 29.5degC at YK tended to have residual yolk reserves that were larger or equivalent to other populations after controlling for mass. This may suggest that alligator embryos preferentially allocate resources towards residual yolk mass at the cost of reduction in overall size under sub-optimal conditions, which has also been shown in other reptiles (Murphy et al., 2020; Radder et al., 2004).

The lack of latitudinal trends in several of the morphological and metabolic traits examined here suggests that latitude may not be the best or only microclimatic proxy within which to understand variation in responses to the developmental environment, particularly incubation temperature. A similar lack of latitudinal patterns in response to incubation temperature was shown across several populations of painted turtles (*Chrysemys picta*), another TSD species (Bodensteiner et al., 2019). These results may be driven by too broad a resolution of the relationship between temperature and latitude, making it impossible to discern subtle population differences, or other population-specific microclimatic variables that put selective pressure on thermal reaction norms. For instance, of the four populations examined, YK is the only coastal site, surrounded by brackish water, which may impose unique selective pressures on embryos and breeding females, resulting in differences in response to incubation temperature (e.g., Hudak and Dybdahl 2023). Additionally, other maternal effects, such as yolk composition and deposition of hormones and anthropogenic contaminants, may, in addition to temperature, influence phenotype (Bae et al., 2021; Du et al., 2010b; Groothuis et al., 2005), but were not considered here. Further, since our design focused on incubation temperatures that produce 100% males or females, population variation in response to incubation temperature may have been driven by sex differences that would not be explained by latitude. While previous work has shown that phenotypic differences between incubation temperatures are the result of temperature and not sex (Bock et al., 2023, preprint), whether sex differences exist across populations irrespective of temperature is not known.

Future work examining the latter and the role of additional aspects of the developmental environment as potential drivers of variable responses to temperature across populations and the consistency of such effects across years will be particularly informative.

CONCLUSIONS:

Overall, we found extensive variation in the influence of incubation temperature on morphological and metabolic phenotypes across populations of alligators. In contrast, the influence of maternal provisioning on hatchling traits was mostly consistent across populations. While the adaptive value of variable plastic responses to incubation temperature was not explicitly tested, latitudinal patterns of some traits (i.e., incubation duration) may imply local adaptation. Nonetheless, variation in the influence of incubation temperature on other traits suggests that selection can act on those relationships, allowing populations to become locally adapted and respond to changing environmental conditions. However, the mechanisms responsible for differential responses to incubation temperature are not known, including the extent to which they are driven by genetic differences or other components of the developmental environment. Determining the causes of these differences, including the developmental mechanisms involved, would provide important insight into how components of the developmental environment and embryonic responses to them influence intraspecific variation and may contribute to adaptive evolutionary change.

ACKNOWLEDGMENTS:

This work was made possible through support from the National Science Foundation (Award 1754903 (B.B.P)) and the United States Department of Energy cooperative agreement (DE-EM0004391). We are also grateful to the South Carolina Department of Natural Resources and Tom Yawkey Wildlife Center staff for permitting and assistance in the field. We thank members of the Parrott Lab for insightful conversations regarding this manuscript. This paper represents technical contribution number 7209 of the Clemson University Experimental Station.

COMPETING INTERESTS STATEMENT:

Authors declare no competing interests.

AUTHOR CONTRIBUTIONS:

Christopher R Smaga: conceptualization (equal), investigation (lead), formal analysis, writing – original draft preparation. Samantha L. Bock: Investigation (supporting), writing – review & editing. Josiah M. Johnson: Investigation (supporting), writing – review & editing. Thomas Rainwater: Investigation (supporting), writing – review and editing. Randeep Singh: Investigation (supporting), writing – review and editing. Vincent Deem: Investigation (supporting), writing – review and editing. Andrew Letter: Investigation (supporting), writing – review and editing. Arnold Brunell: Investigation (supporting), writing – review and editing. Benjamin Parrott: conceptualization (equal), investigation (supporting), funding acquisition, supervision, writing – review and editing.

DATA ACCESSIBILITY :

Data for the manuscript are available on dryad (<https://doi.org/10.5061/dryad.280gb5mvk>).

DISCLAIMER:

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or

any agency thereof.

REFERENCES:

- Allsteadt, J., Lang, J.W., 1995. Incubation Temperature Affects Body Size and Energy Reserves of Hatchling American Alligators (*Alligator mississippiensis*). *Physiological Zoology* 68, 76–97. <https://doi.org/10.1086/physzool.68.1.30163919>
- Angilletta, M.J., Oufiero, C.E., Sears, M.W., 2004. Thermal adaptation of maternal and embryonic phenotypes in a geographically widespread ectotherm. *International Congress Series, Animals and Environments. Proceedings of the Third International Conference of Comparative Physiology and Biochemistry* 1275, 258–266. <https://doi.org/10.1016/j.ics.2004.07.038>
- Ashton, K.G., 2002. Patterns of within-species body size variation of birds: strong evidence for Bergmann’s rule. *Global Ecology and Biogeography* 11, 505–523. <https://doi.org/10.1046/j.1466-822X.2002.00313.x>
- Ashton, K.G., Feldman, C.R., 2003. Bergmann’s Rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* 57, 1151–1163. <https://doi.org/10.1111/j.0014-3820.2003.tb00324.x>
- Bae, J., Bertucci, E.M., Bock, S.L., Hale, M.D., Moore, J., Wilkinson, P.M., Rainwater, T.R., Bowden, J.A., Koal, T., PhamTuan, H., Parrott, B.B., 2021. Intrinsic and extrinsic factors interact during development to influence telomere length in a long-lived reptile. *Molecular Ecology* mec.16017. <https://doi.org/10.1111/mec.16017>
- Bates, D., Machler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67, 1–48. doi:10.18637/jss.v067.i01.
- Beamonte-Barrientos, R., Velando, A., Drummond, H., Torres, R., 2010. Senescence of Maternal Effects: Aging Influences Egg Quality and Rearing Capacities of a Long-Lived Bird. *The American Naturalist* 175, 469–480. <https://doi.org/10.1086/650726>
- Blackburn, T.M., Gaston, K.J., Loder, N., 1999. Geographic gradients in body size: a clarification of Bergmann’s rule. *Diversity and Distributions* 5, 165–174. <https://doi.org/10.1046/j.1472-4642.1999.00046.x>
- Bock, S.L., Hale, M.D., Rainwater, T.R., Wilkinson, P.M., Parrott, B.B., 2021. Incubation Temperature and Maternal Resource Provisioning, but Not Contaminant Exposure, Shape Hatchling Phenotypes in a Species with Temperature-Dependent Sex Determination. *The Biological Bulletin* 241, 43–54. <https://doi.org/10.1086/714572>
- Bock, S.L., Loera, Y., Johnson, J.M., Smaga, C.R., Haskins, D.L., Tuberville, T.D., Singh, R., Rainwater, T.R., Wilkinson, P.M., Parrott, B.B., 2023. Differential early-life survival contributes to the adaptive significance of temperature-dependent sex determination in a long-lived reptile. Preprint. <https://doi.org/10.1101/2023.05.24.542140>.
- Bock, S.L., Lowers, R.H., Rainwater, T.R., Stolen, E., Drake, J.M., Wilkinson, P.M., Weiss, S., Back, B., Guillette, L., Parrott, B.B., 2020. Spatial and temporal variation in nest temperatures forecasts sex ratio skews in a crocodilian with environmental sex determination. *Proceedings of the Royal Society B: Biological Sciences* 287, 20200210. <https://doi.org/10.1098/rspb.2020.0210>
- Bodensteiner, B.L., Warner, D.A., Iverson, J.B., Milne-Zelman, C.L., Mitchell, T.S., Refsnider, J.M., Janzen, F.J., 2019. Geographic variation in thermal sensitivity of early life traits in a widespread reptile. *Ecol Evol* 9, 2791–2802. <https://doi.org/10.1002/ece3.4956>
- Brown, C.A., Gothreux, C.T., Green, C.C., 2011. Effects of temperature and salinity during incubation on hatching and yolk utilization of Gulf killifish *Fundulus grandis* embryos. *Aquaculture* 315, 335–339. <https://doi.org/10.1016/j.aquaculture.2011.02.041>
- Conover, D.O., Schultz, E.T., 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology & Evolution* 10, 248–252. [https://doi.org/10.1016/S0169-5347\(00\)89081-3](https://doi.org/10.1016/S0169-5347(00)89081-3)

- Deeming, D.C., Birchard, G.F., 2007. Allometry of egg and hatchling mass in birds and reptiles: roles of developmental maturity, eggshell structure and phylogeny. *Journal of Zoology* 271, 78–87. <https://doi.org/10.1111/j.1469-7998.2006.00219.x>
- Du, W., Warner, D.A., Langkilde, T., Robbins, T., Shine, R., 2010a. The Physiological Basis of Geographic Variation in Rates of Embryonic Development within a Widespread Lizard Species. *The American Naturalist* 176, 522–528. <https://doi.org/10.1086/656270>
- DU, W., Ji, X., Zhang, Y., Lin, Z., Xu, X., 2010b. Geographic variation in offspring size of a widespread lizard (*Takydromus septentrionalis*): importance of maternal investment. *Biological Journal of the Linnean Society* 101, 59–67. <https://doi.org/10.1111/j.1095-8312.2010.01492.x>
- Du, W., Shine, R., 2022. The behavioural and physiological ecology of embryos: responding to the challenges of life inside an egg. *Biological Reviews* 97, 1272–1286. <https://doi.org/10.1111/brv.12841>
- Du, W., Shine, R., 2008. The influence of hydric environments during egg incubation on embryonic heart rates and offspring phenotypes in a scincid lizard (*Lampropholis guichenoti*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 151, 102–107. <https://doi.org/10.1016/j.cbpa.2008.06.005>
- Du, W., Shine, R., Ma, L., Sun, B.-J., 2019. Adaptive responses of the embryos of birds and reptiles to spatial and temporal variations in nest temperatures. *Proceedings of the Royal Society B: Biological Sciences* 286, 20192078. <https://doi.org/10.1098/rspb.2019.2078>
- Ferguson, M. W. J., 1985. The reproductive biology and embryology of the crocodilians. C. G ans, F. S. Billet, and P. F. A. Maderson. *Biology of Reptilia*. New York, Wiley. 329–491
- Flatt, T., 2001. Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. *Biological Journal of the Linnean Society* 74, 339–350. <https://doi.org/10.1006/bijl.2001.0581>
- Groothuis, T.G.G., Muller, W., von Engelhardt, N., Carere, C., Eising, C., 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience & Biobehavioral Reviews* 29, 329–352. <https://doi.org/10.1016/j.neubiorev.2004.12.002>
- Hudak, A., Dybdahl, M., 2023. Phenotypic plasticity under the effects of multiple environmental variables. *Evolution* 77, 1370–1381. <https://doi.org/10.1093/evolut/qpaa049>
- Johnson, J.M., Bock, S.L., Smaga, C.R., Lambert, M.R., Rainwater, T.R., Wilkinson, P.M., Parrott, B.B., 2023. Relationships between maternally-transferred mercury and hatchling development, behavior, and survival in the American alligator (*Alligator mississippiensis*). *Science of The Total Environment* 870, 162010. <https://doi.org/10.1016/j.scitotenv.2023.162010>
- Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. *Ecology Letters* 7, 1225–1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Kohno, S., Parrott, B.B., Yatsu, R., Miyagawa, S., Moore, B.C., Iguchi, T., Guillelte, L., 2014. Gonadal Differentiation in Reptiles Exhibiting Environmental Sex Determination. *Sex Dev* 8, 208–226. <https://doi.org/10.1159/000358892>
- Lang, J.W., Andrews, H.V., 1994. Temperature-dependent sex determination in crocodilians. *Journal of Experimental Zoology* 270, 28–44. <https://doi.org/10.1002/jez.1402700105>
- Larriera, A., Pina, C.I., Siroski, P., Verdade, L.M., 2004. Allometry of Reproduction in Wild Broad-Snouted Caimans (*Caiman latirostris*). *hpet* 38, 301–304. <https://doi.org/10.1670/145-03A>
- Lenth, R.V., Bolker, B., Buerkner, P., Gine-Vazquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., Singmann, H., 2023. Emmeans: estimated marginal means, aka least-square means. <https://CRAN.R-project.org/package=emmeans>.

- Ludecke, D., 2018. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *Journal of Open Source Software* 3(26), 772. doi:10.21105/joss.00772.
- Marshall, D.J., Pettersen, A.K., Bode, M., White, C.R., 2020. Developmental cost theory predicts thermal environment and vulnerability to global warming. *Nat Ecol Evol* 4, 406–411. <https://doi.org/10.1038/s41559-020-1114-9>
- McCormick, M.I., 1998. Behaviorally Induced Maternal Stress in a Fish Influences Progeny Quality by a Hormonal Mechanism. *Ecology* 79, 1873–1883. [https://doi.org/10.1890/0012-9658\(1998\)079\[1873:BIMSIA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[1873:BIMSIA]2.0.CO;2)
- McCoy, J.A., Hamlin, H.J., Thayer, L., Guillette, L.J., Parrott, B.B., 2016. The influence of thermal signals during embryonic development on intrasexual and sexually dimorphic gene expression and circulating steroid hormones in American alligator hatchlings (*Alligator mississippiensis*). *General and Comparative Endocrinology* 238, 47–54. <https://doi.org/10.1016/j.ygcen.2016.04.011>
- McCoy, J.A., Parrott, B.B., Rainwater, T.R., Wilkinson, P.M., Guillette, L.J., 2015. Incubation history prior to the canonical thermosensitive period determines sex in the American alligator. *REPRODUCTION* 150, 279–287. <https://doi.org/10.1530/REP-15-0155>
- Merila, J., Laurila, A., Laugen, A.T., Rasanen, K., Pakkala, M., 2000. Plasticity in age and size at metamorphosis in *Rana temporaria* - comparison of high and low latitude populations. *Ecography* 23, 457–465. <https://doi.org/10.1111/j.1600-0587.2000.tb00302.x>
- Moore, M.P., Whiteman, H.H., Martin, R.A., 2019. A mother’s legacy: the strength of maternal effects in animal populations. *Ecology Letters* 22, 1620–1628. <https://doi.org/10.1111/ele.13351>
- Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. *Trends in Ecology & Evolution* 13, 403–407. [https://doi.org/10.1016/S0169-5347\(98\)01472-4](https://doi.org/10.1016/S0169-5347(98)01472-4)
- Mueller, C.A., Eme, J., Manzoni, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y., 2015. Embryonic critical windows: changes in incubation temperature alter survival, hatchling phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). *J Comp Physiol B* 185, 315–331. <https://doi.org/10.1007/s00360-015-0886-8>
- Murphy, K.M., Radder, R.S., Shine, R., Warner, D.A., 2020. Lizard Embryos Prioritize Posthatching Energy Reserves over Increased Hatchling Body Size during Development. *Physiological and Biochemical Zoology* 93, 339–346. <https://doi.org/10.1086/710053>
- Murray, C., Crother, B., Merchant, M., Cooper, Easter, M., 2013. Can Reproductive Allometry Assess Population Marginality in Crocodilians? A Comparative Analysis of Gulf Coast American Alligator (*Alligator mississippiensis*) Populations. *Copeia* 2013. <https://doi.org/10.1643/CH-11-136>
- Orizaola, G., Laurila, A., 2016. Developmental plasticity increases at the northern range margin in a warm-dependent amphibian. *Evolutionary Applications* 9, 471–478. <https://doi.org/10.1111/eva.12349>
- Orizaola, G., Laurila, A., 2009. Microgeographic variation in temperature-induced plasticity in an isolated amphibian metapopulation. *Evol Ecol* 23, 979–991. <https://doi.org/10.1007/s10682-008-9285-x>
- Pettersen, A.K., 2020. Countergradient Variation in Reptiles: Thermal Sensitivity of Developmental and Metabolic Rates Across Locally Adapted Populations. *Frontiers in Physiology* 11.
- Pettersen, A.K., Ruuskanen, S., Nord, A., Nilsson, J.F., Minano, M.R., Fitzpatrick, L.J., While, G.M., Uller, T., 2023. Population divergence in maternal investment and embryo energy use and allocation suggests adaptive responses to cool climates. *Journal of Animal Ecology* n/a. <https://doi.org/10.1111/1365-2656.13971>
- Pettersen, A.K., White, C.R., Bryson-Richardson, R.J., Marshall, D.J., 2019. Linking life-history theory and metabolic theory explains the offspring size-temperature relationship. *Ecology Letters* 22, 518–526. <https://doi.org/10.1111/ele.13213>

- R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>."
- Radder, R.S., Shanbhag, B.A., Saidapur, S.K., 2004. Yolk partitioning in embryos of the lizard, *Calotes versicolor*: Maximize body size or save energy for later use? *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 301A, 783–785. <https://doi.org/10.1002/jez.a.95>
- Radder, R.S., Warner, D.A., Cuervo, J.J., Shine, R., 2007. The functional significance of residual yolk in hatchling lizards *Amphibolurus muricatus* (Agamidae). *Functional Ecology* 21, 302–309. <https://doi.org/10.1111/j.1365-2435.2006.01238.x>
- Richter-Boix, A., Katzenberger, M., Duarte, H., Quintela, M., Tejedo, M., Laurila, A., 2015. Local divergence of thermal reaction norms among amphibian populations is affected by pond temperature variation. *Evolution* 69, 2210–2226. <https://doi.org/10.1111/evo.12711>
- Ronget, V., Gaillard, J.-M., Coulson, T., Garratt, M., Gueyffier, F., Lega, J.-C., Lemaitre, J.-F., 2018. Causes and consequences of variation in offspring body mass: meta-analyses in birds and mammals. *Biological Reviews* 93, 1–27. <https://doi.org/10.1111/brv.12329>
- Royle, N.J., Surai, P.F., Hartley, I.R., 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. *Functional Ecology* 17, 472–481. <https://doi.org/10.1046/j.1365-2435.2003.00752.x>
- Saino, N., Romano, M., Ferrari, R.P., Martinelli, R., Moller, A.P., 2005. Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 303A, 998–1006. <https://doi.org/10.1002/jez.a.224>
- Schwanz, L.E., Cordero, G.A., Charnov, E.L., Janzen, F.J., 2016. Sex-specific survival to maturity and the evolution of environmental sex determination: The evolution of sex determination in reptiles. 70, 329–341. <https://doi.org/10.1111/evo.12856>
- Seebacher, F., White, C.R., Franklin, C.E., 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Clim Change* 5, 61–66. <https://doi.org/10.1038/nclimate2457>
- SHINE, R., BROWN, G.P., 2002. Effects of seasonally varying hydric conditions on hatchling phenotypes of keelback snakes (*Tropidonophis mairii*, Colubridae) from the Australian wet-dry tropics. *Biological Journal of the Linnean Society* 76, 339–347. <https://doi.org/10.1046/j.1095-8312.2002.00068.x>
- Sinervo, B., 1990. The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 44, 279–294. <https://doi.org/10.1111/j.1558-5646.1990.tb05198.x>
- Stearns, S.C., 2000. Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* 87, 476–486. <https://doi.org/10.1007/s001140050763>
- Telemeco, R.S., Radder, R.S., Baird, T.A., Shine, R., 2010. Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard: Thermal effects on reptile reproduction. *Biological Journal of the Linnean Society* 100, 642–655. <https://doi.org/10.1111/j.1095-8312.2010.01439.x>
- Urvik, J., Rattiste, K., Giraudeau, M., Okuliarova, M., Horak, P., Sepp, T., 2018. Age-specific patterns of maternal investment in common gull egg yolk. *Biology Letters* 14, 20180346. <https://doi.org/10.1098/rsbl.2018.0346>
- Van Dyke, J.U., Griffith, O.W., 2018. Mechanisms of reproductive allocation as drivers of developmental plasticity in reptiles. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 329, 275–286. <https://doi.org/10.1002/jez.2165>
- Warner, D.A., Lovern, M.B., 2014. The Maternal Environment Affects Offspring Viability via an Indirect Effect of Yolk Investment on Offspring Size. *Physiological and Biochemical Zoology* 87, 276–287.

<https://doi.org/10.1086/674454>

Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, <https://ggplot2.tidyverse.org>.

Zhao, B., Chen, Y., Lu, H.-L., Zeng, Z.-G., Du, W.-G., 2015. Latitudinal differences in temperature effects on the embryonic development and hatchling phenotypes of the Asian yellow pond turtle, *Mauremys mutica*. *Biological Journal of the Linnean Society* 114, 35–43. <https://doi.org/10.1111/bij.12400>

FIGURE LEGENDS:

Figure 1: Geography and egg size of sampled populations. (A) Map showing the geographic range of the American alligator and sampled populations. (B) Egg mass variation across populations.

Figure 2: Population variation in the influence of egg mass and temperature on morphological traits, showing (A) the relationship between egg mass and hatchling mass, (B) hatchling mass and (C) snout-vent-length (SVL). In (B) and (C), plotted values are model means under a common egg mass (83g). Error bars represent 95% confidence intervals.

Figure 3: Population variation in metabolic traits and the influence of incubation temperature, showing (A) fat body mass, (B) residual yolk mass, (C) mass-specific fat body mass, and (D) mass-specific residual yolk mass. Error bars represent 95% confidence intervals.

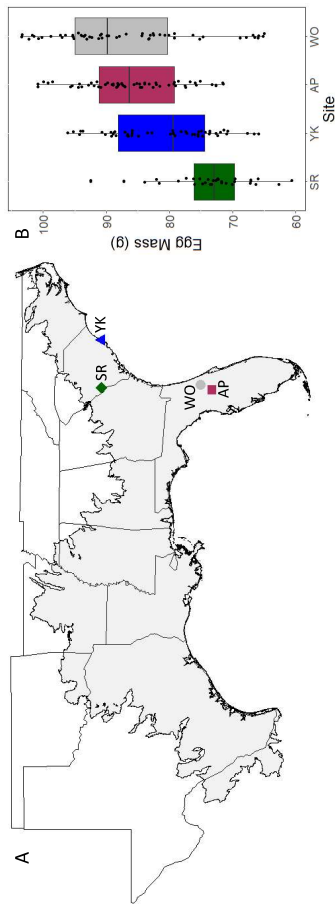
Figure 4: Population variation in the influence of incubation temperature on incubation duration. Error bars represent 95% confidence intervals.

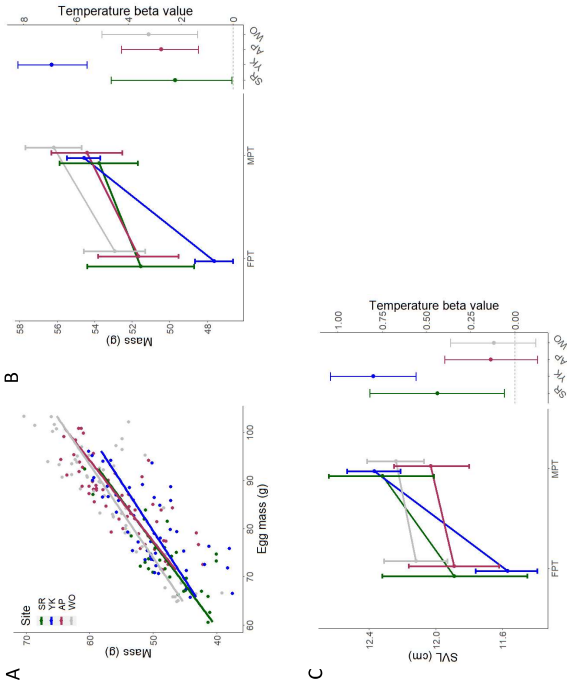
Hosted file

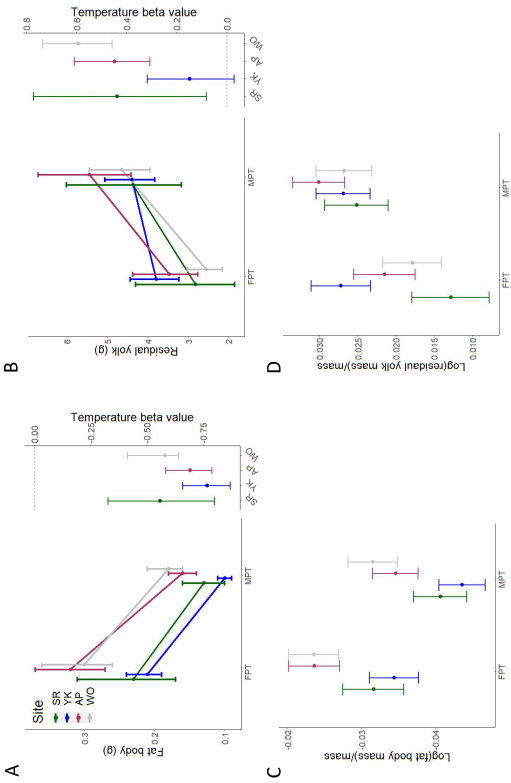
TABLE 1.docx available at <https://authorea.com/users/653978/articles/660884-the-influence-of-incubation-temperature-on-offspring-traits-varies-across-northern-and-southern-populations-of-the-american-alligator-alligator-mississippiensis>

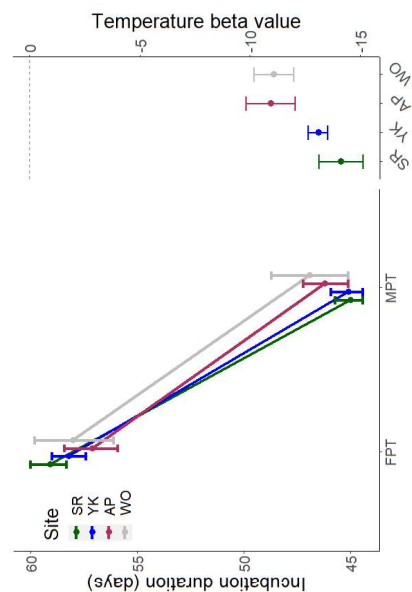
Hosted file

TABLE 2.docx available at <https://authorea.com/users/653978/articles/660884-the-influence-of-incubation-temperature-on-offspring-traits-varies-across-northern-and-southern-populations-of-the-american-alligator-alligator-mississippiensis>









Hosted file

APPENDIX 1.docx available at <https://authorea.com/users/653978/articles/660884-the-influence-of-incubation-temperature-on-offspring-traits-varies-across-northern-and-southern-populations-of-the-american-alligator-alligator-mississippiensis>