Stressful Body Temperatures as a Maternal Effect on Lizard Reproduction

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ABSTRACT

Understanding the relationship between the environment parents experience during reproduction and the environment embryos experience in the nest is essential for determining the intergenerational responses of populations to novel environmental conditions. Thermal stress has become commonplace for organisms inhabiting areas affected by rising temperatures. Exposure to body temperatures that approach, but do not exceed, upper thermal limits often induces adverse effects in organisms, but the propensity for these temperatures to have intergenerational consequences has not been explored in depth. Here, we quantified the effects of thermal stress on the reproductive physiology and development of brown anoles (*Anolis sagrei*) when thermal stress is experienced by mothers and by eggs during incubation. Mothers exposed to thermal stress produced smaller eggs and smaller offspring with reduced growth rates, while egg stress reduced developmental time and offspring mass. Hatchling survival and growth were negatively affected by thermal stress experienced by mothers but not by thermal stress experienced as eggs. We found mixed evidence for an additive effect of thermal stress on offspring; rather, thermal stress had specific (and most often negative) effects on different components of offspring phenotypes and fitness proxies when experienced either by mothers or by eggs. Stressful body temperatures therefore can function in a similar manner to other types of maternal effects in reptiles; however, this maternal effect has predominantly negative consequences on offspring.

Keywords: heat wave, oviparous, life history, climate, invasive species, stress.

Introduction

Maternal effects refer to the nongenetic influence of the maternal environment or phenotype on offspring traits and include factors such as diet (Sorci and Clobert 1997; Wang et al. 2017), age (Sinervo and Doughty 1996; Bleu et al. 2022), or stress (Meylan et al. 2004; Cadby et al. 2010). Multiple offspring traits, including fitness-relevant traits such as body size, body mass, sprint speed, and growth, are impacted by maternal conditions (Noble et al. 2014; Wang et al. 2017; Hao et al. 2021). In some cases, stressful maternal conditions can affect offspring phenotypes in ways that enhance offspring fitness (Bestion et al. 2022). For example, female *Lacerta vivipara* and *Sceloporus undulatus*—two distantly related lizard taxa—exhibit a common pattern where elevated stress during reproduction produces offspring that are less risk averse, potentially as an adaptive mechanism to behaviorally match offspring to the stressful environment experienced by their mothers (Uller and Olsson 2006; Ensminger et al. 2018). However, some maternal effects can also be deleterious to offspring fitness (MacLeod et al. 2021). For instance, low-quality diets and stress have negative effects on offspring performance and morphology in the Carpetan rock lizard (*Iberolacerta cyreni*; Horvath et al. 2008), highlighting that maternal effects have variable and context-dependent effects on offspring. Consequently, how maternal effects may enable populations to adaptively respond to anthropogenic environmental change is an active topic of research (Fuller et al. 2010; Meylan et al. 2012; Cooke et al. 2014).

As global temperatures rise, one commonality experienced across ecosystems is an increase in the frequency of severe weather fluctuations (Ebi et al. 2021). These events expose organisms to elevated or stressful body temperatures (T_b's), and in the case of ectothermic organisms, core T_b's can approach or surpass upper lethal thresholds (i.e., critical thermal maximum [CT_{max}]; Sinervo et al. 2010). As a result, there is a growing need to understand how thermal stress affects fitness in wild populations. If thermal stress is experienced during reproduction, then elevated T_b's may function as a parental effect on offspring phenotypes (Souza et al. 2015; Rutschmann et al. 2016). Moreover, during reproduction, adults and early embryos might be differentially affected by thermal stress. Elevated T_b's during vitellogenesis or spermatogenesis may reduce the quality of individual gametes and induce maladaptive consequences in offspring (Tveiten and Johnsen 1999). For example, simulated heat waves reduce sperm viability in multiple insect species (Gasparini et al.

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2018; Sales et al. 2018, 2021) and sperm motility in some fish species (Fenkes et al. 2017). Additionally, in Atlantic salmon, oocyte maturation is inhibited when females are exposed to elevated temperatures (King et al. 2003). During fertilization, elevated T_b's can inhibit gamete performance and reduce embryonic viability (Adriaenssens et al. 2012; Rebolledo et al. 2020). In oviparous taxa, females often impart molecular signatures of their own stress into eggs (Ensminger et al. 2018); the consequences of which have negative effects on offspring performance, growth, and survival. Following fertilization, thermally stressed females might select suboptimal nest sites for offspring (Seltmann et al. 2014). In viviparous taxa, elevated temperatures might inhibit offspring development by altering maternal behavior or physiology (Han et al. 2020). As each stage of ectotherm reproduction and development has the propensity to be affected by thermal stress, exposure to these stressors might function additively, whereby chronic exposure across generations may contribute to suboptimal offspring phenotypes and affect the ecological and evolutionary dynamics of populations (Kingsolver et al. 2013).

The developmental environment of embryos can induce plasticity in offspring phenotypes that has strong effects on offspring fitness (Reedy et al. 2013; Warner 2014; Hall and Warner 2020), and species that lack parental care are particularly sensitive to early environments (Uller 2008). Exposure to elevated temperatures in the nest is a particularly powerful stressor that might cause embryos to desiccate or develop improperly, as well as constrain offspring viability (Hall and Warner 2018, 2021). In squamate reptiles in particular, conditions that embryos experience in the nest can have lifelong consequences (Mitchell et al. 2018*b*; Noble et al. 2018; While et al. 2018). Eggs deposited by stressed females might be at a disadvantage compared to eggs from unstressed females. This disadvantage could be attributed to a reduction in egg size (Mileva et al. 2011) or in the energetic content of individual eggs (Warner and Lovern 2014) or to the presence of harmful molecular markers such as stress hormones or heat-shock proteins (Saino et al. 2005; Hanafi et al. 2022). Nest site selection is the primary way females can affect the development of offspring and mitigate the effects of stressful environments on offspring, thereby buffering future generations from stressful environmental conditions (Tiatragul et al. 2020; Du et al. 2023). However, this argument hinges on nesting locations being buffered from acute shifts in temperature or moisture, which is unlikely for most oviparous taxa because of a reduced predictability of future nest conditions at the time of oviposition (Warner et al. 2023).

Thermal stress can affect adult reproduction as well as the development of embryos. As a result, to determine how thermal stress functions as a maternal effect, we need to describe how temporal variation in thermal stress (i.e., experienced by gravid mothers, developing embryos, or both) has a preeminent effect on offspring viability or maternal fitness (Massey and Hutchings 2021). If we describe the effects of thermal stress on female reproduction and offspring development simultaneously, then we can determine when thermal stress has the most significant impact on reproductive physiology. Additionally, we may also determine whether mothers are able to mitigate the potentially negative effects of their own stress on future progeny through nest site selection. In this study, we used a 2 × 2 factorial design to quantify the effects of thermal stress on the reproductive physiology of the brown anole (*Anolis sagrei*). We manipulated the timing of thermal stress to be experienced by mothers, eggs during incubation, or both. We predicted that (i) thermal stress experienced by mothers would reduce maternal investment into offspring and the rate of maternal reproduction (e.g., reduced egg mass, greater interegg intervals, and lower fecundity); (ii) thermal stress experienced by embryos would negatively affect hatchling phenotypes, reducing size at hatching, growth, and survival; and (iii) offspring that experienced thermal stress both from mothers and as embryos would exhibit the strongest negative effects, indicative of an additive effect of thermal stress on reproductive physiology.

Methods

Lizard Collection and Animal Husbandry

We collected adult brown anoles (*Anolis sagrei*; $N = 50$ of each sex) from Tomoka State Park in Florida, USA, between April 2 and 6, 2023. These small (adult body size: 39–65 mm) lizards are invasive to North America and produce single-egg clutches approximately every 7–10 d between April and October, coinciding with the most thermally stressful time of year in the southern United States. We captured adults using handheld lassos and measured snout-vent length (SVL) and body mass upon capture. Lizards were placed into individual mesh bags for no longer than 48 h during capture and measurements and then transported to laboratory facilities at Auburn University (Auburn, AL), where they were maintained in a climate-controlled room at a constant 25.5°C and a relative humidity of 51%. Males and females were randomly paired and placed into terraria (29 cm × 26 cm × 39 cm). A nesting pot (19 cm × 12 cm × 9 cm) filled with moist soil was placed into each terrarium for females to lay eggs throughout the experiment. Our terraria were not outfitted with refuges for lizards to escape elevated temperatures to reduce the effect of behavioral thermoregulation on avoiding thermal stress (Huey and Slatkin 1976). Lizards were fed twice weekly with four crickets (*Acheta domesticus*) dusted with vitamin powder and were misted with water daily.

Thermal Stress Treatments

The 50 terraria were randomly assigned to either a control treatment held at a constant temperature (25.5°C) or a thermal stress treatment. The thermal stress terraria each had a 100-W infrared bulb set on an automatic timer to expose adults to a 39°C heat wave between 12 and 2 p.m. each day. While the heat bulbs were on, lizard T_b reached a target of 37°C–39°C, which we confirmed by measuring lizard T_b with an internal cloacal probe after 1 h during the thermal stress exposure (mean T_b [±SD] of stressed lizards during exposure: 37.4°C ± 0.4°C; mean T_b [±SD] of control lizards during the same time: 25.6°C ± 0.2°C). Specific thermal profiles are provided visually in figure S1 (available online).

The goal of this design was to expose lizards to acute thermal stress on a daily basis, which for our purposes we define as T_b's above the preferred temperature range but below the CT_{max} of populations adjacent to our focal population (Hulbert et al. 2020).

We are not using the terms "stress" or "stressful" in a context that equates our design with stress defined from an endocrinological perspective; rather, we are using "stress" as a term to denote a strain exerted on lizard physiology by temperatures approaching their CT_{max}. A 39°C heat wave was selected because, in adults, this T_b falls above what natural populations of *A. sagrei* prefer but below what they can physiologically tolerate (Corn 1971; Gunderson et al. 2020; Ryan and Gunderson 2021). Additionally, maternally chosen nest sites of *A. sagrei* in their invasive range frequently experience temperatures that exceed 39°C (Pruett et al. 2020), but temperatures beyond 39°C can induce mortality in embryos (Warner 2014; Hall and Warner 2018; Pearson and Warner 2018; Tiatragul et al. 2020). A consistent value (39°C) of thermal stress in both treatments provides a direct interpretation of the thermal effects on offspring traits and maternal reproduction, as differences in the magnitude of thermal stress experienced by mothers and embryos (i.e., selecting a different temperature at which to stress mothers vs. embryos) could lead to unclear interpretations as to how thermal stress acts as a maternal effect.

Nesting pots were checked for eggs three times weekly. When eggs were found, they were immediately weighed and randomly assigned into one of two incubation treatments: a control treatment where eggs were incubated at a constant 25.5°C (identical to the conditions of adult lizards in our control treatment) and a thermal stress treatment where eggs were incubated at a constant 25.5°C but were exposed to a 39°C heat wave between 12 and 2 p.m. each day. The first clutch found in each nesting pot was purged because some females were already gravid and gestating eggs during our field capture. Because anole lizards lay a single-egg clutch, we alternated the incubation of each egg from each female. The second egg found in each female's nesting pot was randomly assigned to either the control ($N = 105$ eggs) or the thermal stress ($N = 102$ eggs) incubation treatment, and subsequent eggs were placed in the alternative treatment.

After weighing, eggs were placed into individual jars with moist vermiculite (−150 kPa) and sealed with plastic wrap to prevent excess evaporation of substrate moisture. We did not change the substrate during incubation, as we did not want to additionally stress eggs by handling them. Incubators were checked daily for hatchlings. When a hatchling was found, we immediately measured its mass and SVL and recorded its sex by the presence or absence of postanal scales (male or female, respectively). Hatchlings were placed into individual cages (21 cm × 16.5 cm × 11 cm) and housed in a climate-controlled room (25.5°C). Hatchlings were fed ad lib. flightless fruit flies (*Drosophila melanogaster*) twice per week and misted with water daily.

Statistical Analysis

Interegg intervals were calculated as the number of days between each egg-laying event. Absolute growth rates of hatchlings were calculated as the difference in SVL divided by the hatchling's lifespan. Surviving hatchlings were euthanized on day 31 after hatching, so the remeasurement of SVL to calculate growth was conducted on the day when the hatchling naturally died, or on day 31. We used linear mixed effect models to determine how maternal and offspring traits differed between our control and thermal stress treatments, and we assessed the significance of fixed effects using log-likelihood ratio tests.

If our dependent variable was a maternal phenotype (interegg interval, egg mass, fecundity), then we used maternal treatment as a fixed effect and maternal ID as a random effect. If our dependent variable was an offspring phenotype (incubation time, SVL/mass at hatching, growth), then we included egg mass, maternal treatment, egg treatment, lay date, and interaction between maternal and egg treatments as fixed effects. We included lay date in our models for offspring phenotypes because brown anoles lay larger eggs that take longer to hatch as the breeding season progresses (Mitchell et al. 2018*a*; Hall et al. 2020). This life history pattern may be sensitive to cumulative stress, that is, later eggs might be more affected by maternal stress than early eggs because mothers experienced more stress as the experiment continued. To account for this temporal pattern, we specified lay date as a random slope in our models for offspring phenotypes and included maternal ID as a random intercept. We used a mixed effect Cox proportional hazards model to determine how the probability of hatchling survivorship was impacted by our treatments. This model included both maternal ID and lay date as random effects. Hatchlings that survived to day 31 ($N = 14$) were excluded from our survival analysis. All analyses were conducted in R (ver. 4.0.2; R Core Team 2023), with use of the lme4, coxme, survival, and ggplot2 packages (Bates et al. 2014; Therneau 2015*a*, 2015*b*; Wickham 2016).

Results

Effects of Thermal Stress on Maternal Reproduction

Daily exposure to acute thermal stress did not affect interegg intervals ($F_{1,44} = 0.22, P = 0.64$) or fecundity ($F_{1,44} = 0.28$, $P = 0.59$). However, thermally stressed mothers laid smaller eggs than unstressed mothers ($F_{1,45} = 6.8, P = 0.01$; fig. 1*A*). We found no effect of maternal SVL on egg mass across both treatments ($F_{1,205} = 0.38$, $P = 0.53$).

Effects of Thermal Stress on Embryonic Development

Hatching success was >97% in both egg incubation treatments. Exposure to thermal stress during development shortened incubation time ($F_{1,77} = 77.5, P < 0.001$). Embryonic stress had a stronger effect on incubation duration than on maternal stress (table 1). Hatchling body mass was negatively affected by both embryonic stress ($F_{1,198} = 52.2, P < 0.001$) and maternal stress ($F_{1,189} = 5.8$, $P = 0.01$; fig. 1*B*) independently. Hatchling SVL was not affected by maternal stress ($F_{1,194} = 0.07, P = 0.79$) or embryonic stress ($F_{1,190} = 0.83$, $P = 0.36$). Early hatchlings were smaller than later hatchlings ($F_{1,200} = 678$, $P < 0.001$). Embryonic stress did not affect hatchling growth ($F_{1,198} = 0.2$, $P = 0.65$); however, maternal stress significantly reduced hatchling growth ($F_{1,197} = 16.4$, $P < 0.001$). Late hatchlings tend to grow slower in brown anoles, but maternal stress altered this relationship, where later hatchlings of stressed mothers grew faster than early hatchlings of stressed mothers ($F_{1,199} = 16.7$, $P < 0.001$; fig. 2). Maternal stress also reduced the probability of survival for

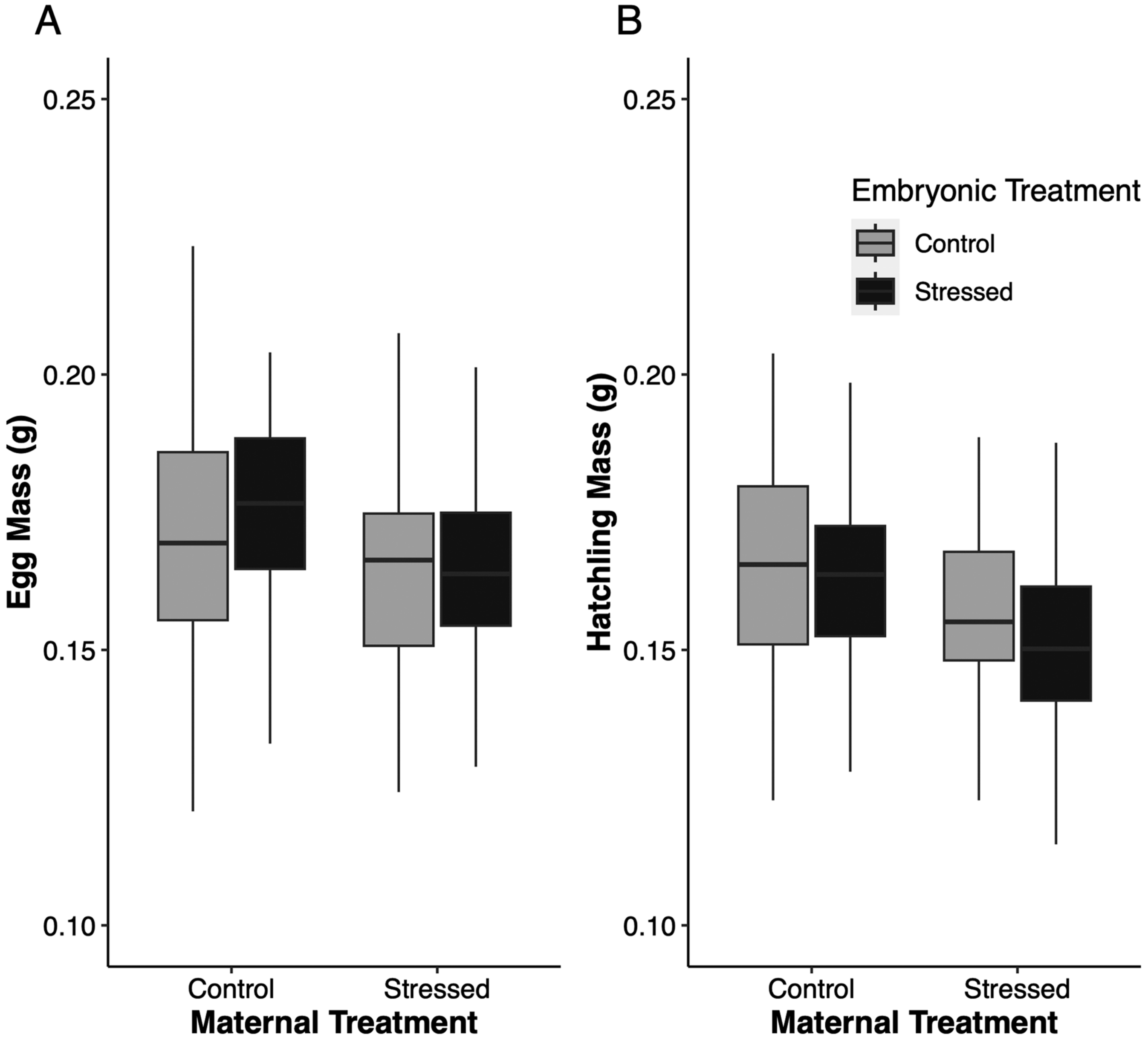

Figure 1. Effects of maternal stress on egg mass at oviposition (*A*) and hatchling mass (*B*) from each 2 × 2 combination of treatments. Eggs were assigned to either a control embryonic treatment (gray) or a stressed embryonic treatment (black). Boxplots are broken down by 25% and 75% quartiles (whiskers) and inner 50% quartiles (box), and the horizontal line within each box refers to the median.

hatchlings ($Z = 2.42$, $P = 0.02$; fig. 3*A*), but embryonic stress did not influence hatchling survival ($Z = 1.10$, $P = 0.27$; fig. 3*B*). Early hatchlings exhibited reduced survival probabilities compared to late hatchlings ($Z = 3.2$, $P = 0.001$).

Discussion

Maternal effects exert strong influences on offspring phenotypes and survival and may enable individuals to respond to environmental pressures in ways that maintain or increase fitness (Mousseau and Fox 1998). However, altered environmental conditions may change maternal physiology in such a way that an increased energetic cost of homeostatic maintenance reduces energetic investment in offspring or induces maternal transfer of harmful molecular markers that exert adverse effects on offspring (Sikkink et al. 2014). Here, we find that thermal stress experienced by adult females induces maladaptive effects in offspring, including reduced hatchling size, growth, and survival. A smaller size in brown anoles is associated with reduced survival probabilities in hatchling brown anoles (Delaney and Warner 2017). Additionally, embryonic stress induced similar effects as maternal stress, indicating that multiple life stages are sensitive to acute elevation of T_b's. These findings support the idea that thermal stress has pervasive impacts on lizard reproduction and that elevated T_b's experienced by parents can function in a similar manner to other types

Table 1: Linear mixed effect model output for offspring phenotypes

Dependent variable, fixed effect	df	*F*	*P*
Incubation time:			
Maternal treatment	1, 196	3.41	.06
Egg treatment	**1, 193**	**59.07**	**<.001**
Lay date	**1, 200**	**1,721.1**	**<.001**
Egg mass	**1, 200**	**50.72**	**<.001**
Maternal treatment × egg treatment	1, 192	.13	.71
Offspring SVL:			
Maternal treatment	1, 194	.07	.79
Egg treatment	1, 190	.83	.36
Lay date	**1, 200**	**678.1**	**<.001**
Egg mass	**1, 200**	**15.83**	**<.001**
Maternal treatment × egg treatment	1, 189	1.21	.27
Offspring mass:			
Maternal treatment	**1, 189**	**5.81**	**.01**
Egg treatment	**1, 196**	**9.29**	**.002**
Lay date	**1, 198**	**163.1**	**<.001**
Egg mass	**1, 200**	**59.31**	**<.001**
Maternal treatment × egg treatment	1, 196	.02	.88
Absolute growth:			
Maternal treatment	**1, 197**	**16.37**	**<.001**
Egg treatment	1, 198	.21	.65
Lay date	**1, 199**	**16.76**	**<.001**
Egg mass	1, 199	3.26	.07
Maternal treatment × egg treatment	1, 198	3.41	.06

Note. All models were constructed using the following structure: phenotype ~ maternal stress × embryonic stress + lay date + egg mass. Maternal ID was used as a random effect in each model, and lay date was included as a random slope to account for the life history pattern exhibited by female brown anoles, where larger eggs are laid later in the season. Values that are statistically significant at $P < 0.05$ are shown in bold. For raw values of all phenotypic traits, please see table S1. df = degrees of freedom; SVL = snout-vent length.

of well-established maternal effects (Paranjpe et al. 2013; Warner and Lovern 2014).

Maternal Stress Reduces Offspring Mass, Growth, and Survival

Consistent with our hypothesis, maternal thermal stress had negative effects on egg mass and negatively affected offspring mass, growth rates, and probability of hatchling survival. In addition, the rate of egg production and total offspring produced did not differ between maternal treatments. Given that gravid females in both treatments experienced the same diet, our stressed females likely experienced an increased metabolic demand because of their exposure to elevated T_b's. Metabolic rates scale exponentially with increasing temperature across taxa (Gillooly et al. 2001), and short-term exposure to elevated temperatures can increase resting metabolic rates and energetic requirements and induce life history trade-offs (Cano and Nicieza 2006; Schulte 2015; Berger et al. 2017; Hao et al. 2021). However, the metabolic responses to thermal stressors are not consistent across taxa (Kovacevic et al. 2019). Endothermic species, such as pigs (Fausnacht et al. 2021), reduce their metabolic expenditures when exposed to stressful temperatures, presumably to conserve energy for a cellular stress response (Tabh et al. 2023).

As a consequence of thermally induced increases in metabolic demands, available energy for vitellogenesis may be reduced (Sinervo and Adolph 1994); this could have resulted in a reduction in egg mass, explaining the reduced egg size produced by thermally stressed females in our experiment. This hypothesis could be confirmed by measuring the energetic content of eggs (e.g., yolk quantity or caloric content; Vitt 1978). Additionally, maternal stress reduced offspring growth for those produced early in the season while growth rates of offspring from both maternal treatments tended to converge on similar values toward the end of the experiment (fig. 2), suggesting that stressed females may somehow compensate for the effects of their elevated T_b's on offspring. Because later hatchlings tended to survive better than early hatchlings (see "Results"), thermal stress on mothers might have a more pronounced effect on early offspring than on late offspring. The ability to adjust reproductive phenology through plasticity, for example, may provide a reliable means of avoiding the deleterious effects of thermal stress (Olsson et al. 2018)

The downstream effects of maternal thermal stress on offspring growth and survival require further examination. Our study design leaves us unable to determine whether the effects of maternal stress we describe are a true maternal effect or whether they represent a direct effect of thermal stress on embryos before

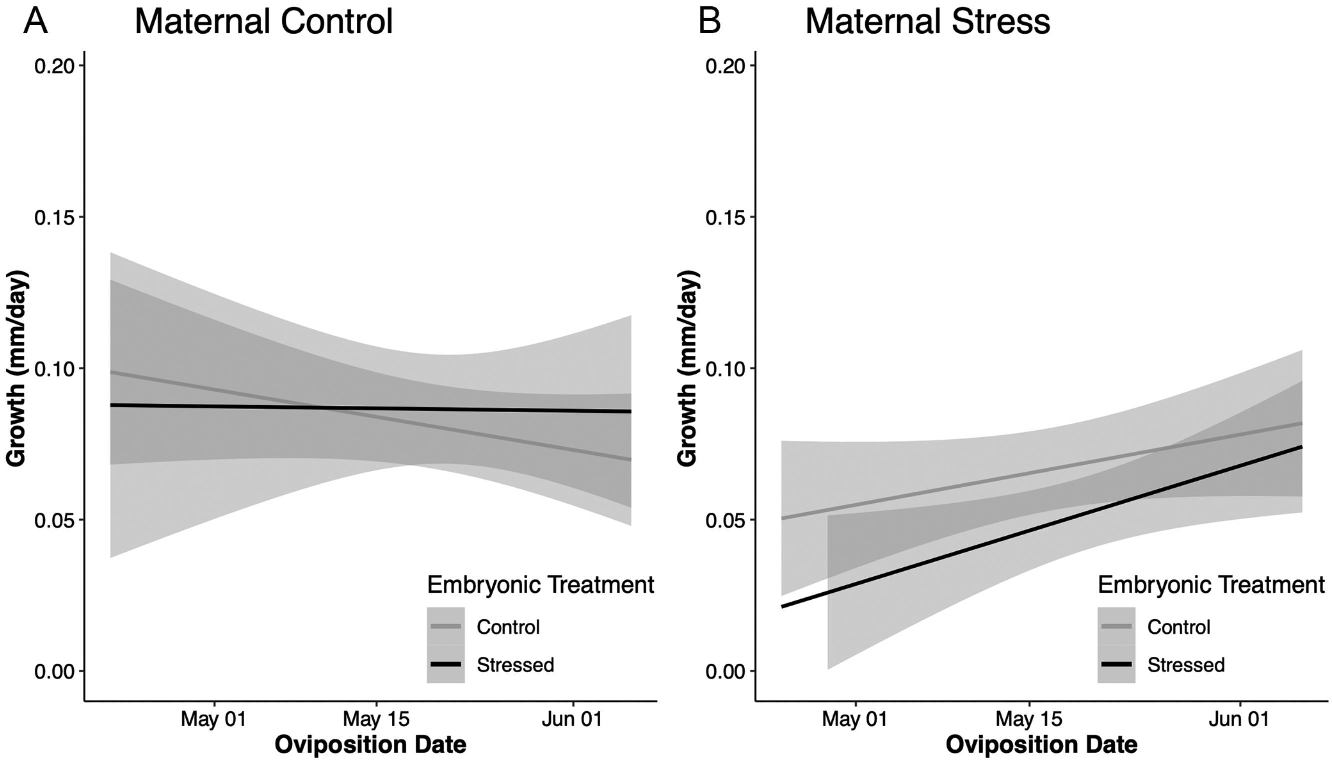

Figure 2. Hatchling growth rates (mm/d) as a function of oviposition date. Shaded regions overlapping trend lines correspond to the 95% confidence intervals for fitted values. *A*, Growth of hatchlings produced by control mothers declined as the reproductive season progressed. *B*, Growth of hatchlings produced by stressed mothers increased as the reproductive season progressed. Growth of hatchlings produced by stressed mothers was significantly lower than growth of hatchlings produced by control mothers.

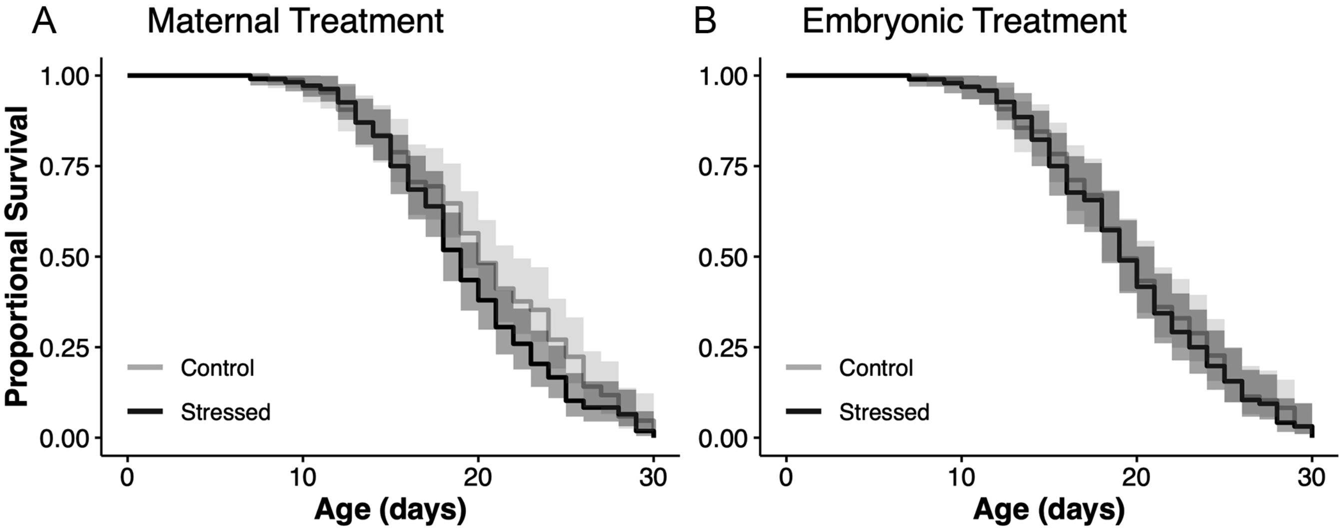

Figure 3. Percentage of hatchlings surviving over time as a function of maternal treatment (*A*) and embryonic treatment (*B*). Shaded regions correspond to the 95% confidence intervals for fitted values. Hatchling survival was significantly affected only by maternal treatment, where stressed mothers produced hatchlings that were less likely to survive to day 31.

oviposition by females. This caveat highlights the difficulties in quantifying the effects of thermal stress on organismal physiology during reproduction because multiple stages of embryonic development, including those dependent on or independent of mothers (e.g., follicle development or development following oviposition), can be affected by acutely elevated T_b's (Domínguez-Castanedo et al. 2023). Brown anole embryos before oviposition are at stage 4 of embryonic development (i.e., the "early limb-bud stage"; Sanger et al. 2008), indicating that the vast majority of embryonic development occurs after oviposition. As a result, thermal stress experienced by embryos after oviposition should have stronger effects than the thermal stress potentially experienced by embryos during the first four stages of development. Isolating the temporal effects of thermal stress on development (e.g., using a design that exposed anole embryos to acutely elevated temperatures at specific points during the developmental process) could resolve when and whether thermal stress has the strongest effects on offspring development.

Studies of marine and terrestrial ectotherms suggest that thermal stress induces oxidative damage in parents and that harmful reactive oxygen species can be passed from parents to offspring, which can have deleterious intergenerational and transgenerational effects (Domínguez-Castanedo et al. 2023; Vargas-Abúndez et al. 2023). Other molecular markers indicative of stress, including heat shock proteins, can also be inherited by offspring (Norouzitallab et al. 2014; Shanker et al. 2020). These molecular markers might not have immediate effects on offspring traits at hatching; body size or mass may be more sensitive to the developmental environment of embryos (Warner 2014; Pearson and Warner 2018). However, as hatchlings emerge from the nest, a greater-than-normal amount of reactive oxygen species or heat shock proteins may reduce future growth and viability (Sikkink et al. 2014; Roth and Landis 2017) during early life stages. However, transgenerational effects of thermal stress are highly variable across populations and taxa (Norouzitallab et al. 2014; Penney et al. 2021). Additional studies that vary the timing (i.e., at discrete reproductive stages) and magnitude of thermal stress can reveal the prominence of phenotypic and fitness responses to thermal stress and allow us to better generalize on the fitness responses of populations to this pervasive environmental stressor.

Embryonic Thermal Stress Alters Development and Offspring Phenotypes

Thermal stress experienced by eggs reduced both incubation time and hatchling body mass. The ecological relevance of the ~2-d decrease in incubation length of eggs exposed to thermal stress is questionable, but even this small difference reduces the amount of time eggs are continually exposed to thermal stress or other factors (e.g., predators). With little, if any, capacity for embryos to behaviorally thermoregulate (Cordero et al. 2018), those that develop faster might avoid stressful nest temperatures more efficiently than eggs that require longer to hatch (Massey and Hutchings 2021). Reproductive manipulations in tree frogs have demonstrated a similar pattern when embryos are exposed to stressful environments (Albecker and McCoy 2019). Thermal stress in the nest also reduced hatchling body mass, so while the fitness benefits of early hatching are present (Du and Shine 2015; Pearson and Warner 2018; Pruett and Warner 2021), these individuals may also experience reduced fitness later in life because of their poorer physiological condition.

Brown anole embryos are thermally robust to nest temperatures and can successfully hatch—without negative phenotypic consequences—at thermal spikes that reach greater magnitudes than those used in this study (Hall and Warner 2018, 2021). However, we still detected negative effects of this relatively small amount of thermal stress, indicating that thermal stress in the nest is likely a major selective factor on offspring (McLean et al. 2005; Yilmaz et al. 2022). In addition, unpredictable nesting environments may further reduce a female's ability to improve offspring fitness via nest site selection (Warner and Shine 2008; Du and Shine 2015; Du et al. 2023; Warner et al. 2023). Assessing the effects of thermal stress across generations is required to understand how variable nest environments interact with maternal effects to shape variation in offspring fitness. Our prediction that the combined effects of thermal stress on mothers and embryos are additive was not supported by any other phenotype than hatchling body mass. Rather, thermal stress had specific effects on different offspring traits, and these effects were due to the presence of both maternal and embryonic stress. This suggests that thermal stress has disproportionate effects when experienced at specific reproductive stages (e.g., vitellogenesis) and that seasonally shifting these reproductive processes to avoid thermal extremes may provide a means to mitigate the harmful effects of thermal stress. A more detailed understanding of how specific physiological markers of thermal stress (e.g., corticosterone, heat shock proteins) alter vitellogenesis and are shared between mothers and offspring would improve our ability to generalize the effects of thermal stress on reproductive physiology across taxa.

Clarifying the role of elevated T_b as a maternal effect is needed given the predicted increases on severe weather fluctuations worldwide. Given that maternal stress impacted offspring mass and growth more than embryonic stress, the T_b distribution of the mother plays a role comparable to that of the other maternal effects in reptiles, such as diet (Lorioux et al. 2013). Nevertheless, whether embryos experienced thermal stress directly in utero—or indirectly through the T_b of mothers—it is plausible that elevated T_b distributions experienced by adults are already inducing maladaptive effects in progeny. Our findings are consistent with this idea, and future studies should be cautioned not to assume the ecological or evolutionary benefits of the presence of maternal effects. To that end, experimental manipulations of adult reproduction using ecologically relevant metrics of environmental change can determine how novel conditions impose fitness costs on routine biological functions and how these conditions induce intergenerational effects in populations (Madliger et al. 2021; Domínguez-Castanedo et al. 2023).

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