Nesting ecology of reptiles: Effects of maternal nesting behavior and egg incubation environments on offspring phenotypes and fitness

Jenna Elizabeth Pruett

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
August 7, 2021

Keywords: Maternal Effects, Plasticity, Nesting Ecology, Western Painted Turtle, Brown Anole

Approved by

Daniel A. Warner, Chair, Assistant Professor, Department of Biological Sciences
Wendy Hood, Associate Professor, Department of Biological Sciences
Haruka Wada, Associate Professor, Department of Biological Sciences
Matthew Wolak, Assistant Professor, Department of Biological Sciences
Alan Wilson, Professor, School Fisheries, Aquaculture, & Aquatic Sciences

Abstract

Understanding the role of the environment in shaping phenotypic variation has been a goal at the heart of evolutionary biology since its inception. Through the work that led to the modern synthesis framework, we see that the environment can act as a force in natural selection, leading to the “survival of the fittest” in a given environment. However, from more recent work, we also see that the environment can shape variation through phenotypic plasticity, or the ability of a genotype to produce more than one phenotype in response to the environment. The role of plasticity in evolution is still a complicated topic, particularly with regard to how selection can act on plastic traits and how plasticity contributes to an individual’s fitness.

My PhD work aims to understand the adaptive nature of phenotypic plasticity with a particular focus on maternal nest site choice and its effects on offspring development. An organism’s developmental environment is composed of several dynamic components (e.g. temperature, moisture) that can influence survival and phenotypic outcomes at this very critical early-life stage. While there is a significant amount of literature on developmental plasticity, there is a lack of studies that examine these topics under ecologically relevant conditions. Oviparous reptiles serve as excellent systems for studies on developmental plasticity because many species exhibit no parental care after the point of nesting. This allows for examining the effects of nest site selection and nest environment on development without the confounding effects of parental care throughout development. My work uses these systems to test the adaptive significance of nesting behavior by examining its effects on offspring fitness across life stages in reptile systems.

The first chapter of my dissertation assessed nest-site choice and its effects on offspring in the Western painted turtle (*Chrysemys picta*). I used long-term data on nest-site selection and

natural nest microclimates to design a cross-fostering experiment that tested the effects of canopy cover on egg hatching success. I found that female painted turtles choose nest sites that are relatively warm with open canopy, which increase egg hatching success. The results from this experiment support nesting as an adaptive behavior.

For the remaining chapters of my dissertation, I focused on a different study system, the brown anole (*Anolis sagrei*). My second chapter sought to assess brown anole nesting behavior in the field. Though anoles are a popular system for research across several biological disciplines, very little was known about their nesting behavior beyond chance natural history encounters and lab studies. I collected the largest data set to date on anole nest-site choice in the field, and established that females actively choose nest sites with specific environmental parameters. Once this was established, I also incubated anole eggs in the field to determine which conditions improve hatching success and hatchling condition. Taken together, the results of my experiments show that females select nest sites that improve survival probability of offspring, again suggesting that their nesting behavior is adaptive.

In my third chapter, I tested the effect of predation pressure on nesting behavior in the brown anole. Curly tailed lizards (*Leiocephalus carinatus*) overlap in their native range and are established predators for brown anoles, though they are confined to the ground and cannot prey on anoles that are perched in trees. Previous work on their relationship in the field has shown that curly tail presence leads to reduced exploration and higher perching in the brown anole. We housed a group of brown anoles in enclosures with and without curly tails and gave them a choice of a nesting container that was on vs above the ground. Females that were in enclosures with predators used above-ground nesting containers more often than females in predator-free enclosures.

Finally, for my fourth chapter, I conducted a study that examined the effects of a continuous range of constant temperature incubation treatments on offspring development and survival to adulthood in the brown anole. I incubated eggs across eight treatments and released the resulting hatchlings into the field to assess survival to adulthood. We found that optimum incubation conditions for survival varied among life stages. Though these results are novel on their own, these data have more potential to be used in theoretical models estimating environmental impact on lifetime fitness. Overall, my dissertation work supports the idea that nesting is an adaptive behavior and underlines the importance of developmental conditions in shaping offspring fitness across life stages.

Acknowledgments

I distinctly remember my childhood concept of getting a PhD. I thought it meant you had to write a book and that people would call you doctor. Well, this is my book and nobody really *has* to call me doctor, but I've come a long way in my understanding of the degree with the help and unfailing support of so many people. Words on a page can't express my gratitude for the unconditional love and encouragement of my parents and first teachers, Beth and Eddie Pruett. They gave me all of the tools I needed to succeed, all the reassurance I needed to persevere, and all the independence I needed to follow my own dreams. My late grandfathers, Ed Bonnette and Ed Pruett nurtured my love of the natural world as a child whether we were in a fishing boat or behind the wheel of a tractor. My late grandmother, Eugenie Bonnette and my grandmother, Barbara Jean Pruett have always been there to remind me how blessed I am to have the opportunity to pursue this education and tell me how proud they are. My sister, Katie, and my brother, Thompson, have also been constant sources of levity, respite, and support when the demands of academia weighed heavy.

As close to family as someone can get, Carolyn Zacharias, Jessica Weise, and Rachel Pair have brought nothing but pure joy and understanding into my life, and I wouldn't have been able to do this without them. Embrace Church has been a constant safe place to land and has became another type of family, especially my band, Amanda Hammett, Beth Cofield, and Brandy Simpkins. One of the most essential support systems for making it through a PhD are the colleagues who become friends and can commiserate and encourage from a place of empathy. I could always count on Abby Beatty, Emily Driessen, Alex Rubin, Kaitlyn Murphy, Dr. Josh Hall, Putter Tiatragul, and my Sestras group chat when I needed a listening ear or advice.

There are countless teachers who have been instrumental in helping me get to this point. All the way back in elementary school, Dr. Glenda Westbury fostered my natural abilities in science and constantly reassured me that I could do hard things. When I transitioned from my small high school to a comparatively huge university, Dr. Karen Pressprich gave me the confidence to pursue teaching opportunities in Chemistry and Dr. Nora Espinoza's animated lectures made me fall in love with Biology. They both showed me that I could pursue Science as a career. Dr. Rick Blob and Dr. Christopher Mayerl picked up where they left off and gave me the opportunity to experience the ins and outs of biological research. Their mentorship taught me what it means to be a scientist and they were both shining examples of what a mentor should be.

During my time at Auburn, several faculty members have been essential to the completion of this dissertation. I owe a special thanks to Dr. Liz Addis for her assistance in completing my first chapter. Dr. Tonia Schwartz also contributed her time and expertise to teach me molecular skill sets that will be valuable for my future career. Dr. Amelie Fargevieille was indispensable for completing the work in my second and fourth chapters. I owe my gratitude to Dr. Christian Cox for making my third chapter possible during Covid. I would also like to thank my committee members, Drs. Wendy Hood, Haruka Wada, and Matt Wolak, each of whom have challenged me to become a better scientist and provided valuable critiques to my proposed work. Finally, I would like to thank my advisor, Dr. Dan Warner, who has been infinitely patient and relentlessly positive from the moment I started this program. His wisdom and experience were crucial in shaping my work and his kindness and compassion were invaluable in making a notoriously taxing degree program enjoyable and intellectually energizing. The phrase "It takes a village" was originally intended to refer to child rearing, but it's certainly true of a PhD as well. An achievement like this can't be reached alone, and I am eternally thankful for my village.

Table of Contents

Abstract....2

Acknowledgments....5

List of Tables....9

List of Figures....10

Background Information....12

Chapter 1. The influence of maternal nesting behavior on offspring survival: evidence from correlational and cross-fostering studies....

- Introduction....17
- Methods....20
- Results....28
- Discussion....33
- Supplementary Materials....38

Chapter 2. Temporal variation in maternal nest choice and its consequences for lizard embryos....

- Introduction....41
- Methods....44
- Results....52
- Discussion....55

Supplementary Materials 63

Chapter 3. The effects of predator presence on nesting behavior and survival in the brown anole (Anolis sagrei). ....

Introduction 68

Methods 71

Results..........73

Discussion 76

Chapter 4. Spatial and temporal variation in phenotypes and fitness in response to developmental thermal environments..........

Introduction 80

Methods 85

Results 89

Discussion 96

Supplementary Materials 104

Conclusion..........108

References 114

List of Tables

Chapter 1

Table 1.1 – The effects of nest area, year, and microhabitat on hatching success 30

Table 1.2 – Effects of nest microhabitat on egg hatching success 32

Chapter 4

Table 4.1 – Test statistics for survival to hatching, juvenile, and adulthood....................................91

List of Figures

Background

Figure 0.1 – Diagram of dissertation chapters 14

Figure 0.2 – Study species used in my dissertation....................16

Chapter 1

Figure 1.1 – Map of my Idaho field site....................21

Figure 1.2 – Schematic of my experimental design....................25

Figure 1.3 – Canopy openness distributions....................26

Figure 1.4 – Canopy openness and temperature box plots....................28

Figure 1.5 – Comparisons of canopy openness, temperature, and nest number among sites....................29

Figure 1.6 – Survival probability curves....................31

Chapter 2

Figure 2.1 – Aerial photo of field site....................45

Figure 2.2 – Soil moisture and temperature box plot....................53

Figure 2.3 – Survival percentages and nest temperature distributions....................54

Figure 2.4 – Egg hatching success probability....................55

Figure 2.5 – Hatchling morphology in relation to temperature and soil moisture....................55

Chapter 3

Figure 3.1 – Nest site choice probability....................75

Figure 3.2 – Perch height and survival percentage box plots 76

Chapter 4

Figure 4.1 – Probability of hatching success and incubation duration 90

Figure 4.2 – Effects of incubation temperature on hatchling morphology 92

Figure 4.3 – Interactive effects of temperature and release date on hatchling survival 94

Figure 4.4 – Survival probability curves and optimum incubation temperature distributions 95

Conclusions

Figure 5.1 – Anolis ecomorphs 110

Background Information

Natural environments are endlessly complex with innumerable interacting elements that shape the experiences of organisms across their lifetime. Living things have a suite of responses to this environmental variation that allow them to persist in the complexity from one generation to the next. Behavior is often a first response to environmental fluctuations that allows animals to mitigate the environmental pressures they might experience (Huey et al. 2003). Environmental heterogeneity can also play a role in shaping phenotypes, including behavior, both directly, via phenotypic plasticity, and indirectly, via natural selection acting on existing variation (Pigliucci et al. 2006). These three responses to environmental variation—behavior, plasticity, and selection—are not independent of each other and can often be deeply intertwined and synergistic. Biologists still have a long way to go in parsing out the tangled web of responses to the equally complex array of environmental variables that induce them, and plasticity represents a promising point from which to enter into such integrative inquiry—plasticity generates phenotypic variation that selection acts on and behavior intervenes in, and is thus shaped by both processes (Fusco & Minelli 2010).

Phenotypic plasticity is ubiquitous across taxa and can be observed at all life stages, but early-life stages are particularly sensitive and labile (West-Eberhard 2003). Developing organisms, from eggs in a nest or seeds in the ground to juvenile animals and saplings, are at the mercy of a constantly changing environment that will interact with their genes to shape phenotypic expression. Furthermore, the environment developing organisms experience is often heavily dependent on their parents. In viviparous organisms, developing embryos are subject to the internal workings of the mother's physiology, which can be shaped by innumerable factors such as her behavior, choice in partner, and initial body condition that dictates how she might intervene in their development. Oviparous organisms that display parental care are similarly able to continuously monitor and change the conditions their eggs are developing under. Oviparous reptiles, however, often lay their eggs somewhere and leave them to develop with no care or intervention. This places a tremendous amount of importance on the initial choice of a nest site.

When a mother chooses a site for oviposition, she determines the conditions, both biotic and abiotic, her offspring will experience during development (Angilletta et. al. 2009). The choice of (1) microhabitat and (2) geographical location of a nest has tremendous impacts on offspring survival and fitness as well as potential effects on maternal survival (Refsnider & Janzen 2010). Nest microhabitat factors may include shade cover (solar transmittance), soil type, and nest temperature, all of which play a key role in development and have been shown to affect offspring phenotype (Weisrock & Janzen 1999). Furthermore, maternal nest location may affect post-hatching survival depending upon the macrohabitat and the distance of the nest to suitable habitat for newly-hatched offspring; These factors have important consequences on predation rates or success in dispersal to suitable habitat (Warner & Mitchell 2013, Møller, 1988). Of course, all of these findings are predicated on the assumption that females selected nest sites non-randomly, which is supported by numerous studies (Mitchell et. al. 2013).

These plastic responses to the environment during development have the potential to set the trajectory of an individual's life and intervene across its lifetime, with long-term implications for fitness. However, while the phenotypic effects of individual environmental variables have been quantified, the direct relationship between variation in phenotypic traits derived from phenotypic plasticity and the resulting fitness variation is still poorly understood. Furthermore, the connection between maternal nest site choice and resulting offspring fitness aren't well studied, particularly under natural field conditions where embryos experience the environmental complexity they would in the wild. My dissertation work seeks to address these gaps by examining 1) maternal nesting behavior, 2) the effects of nest conditions on offspring phenotype and survival, and 3) the long-term effects of developmental environments into adulthood.

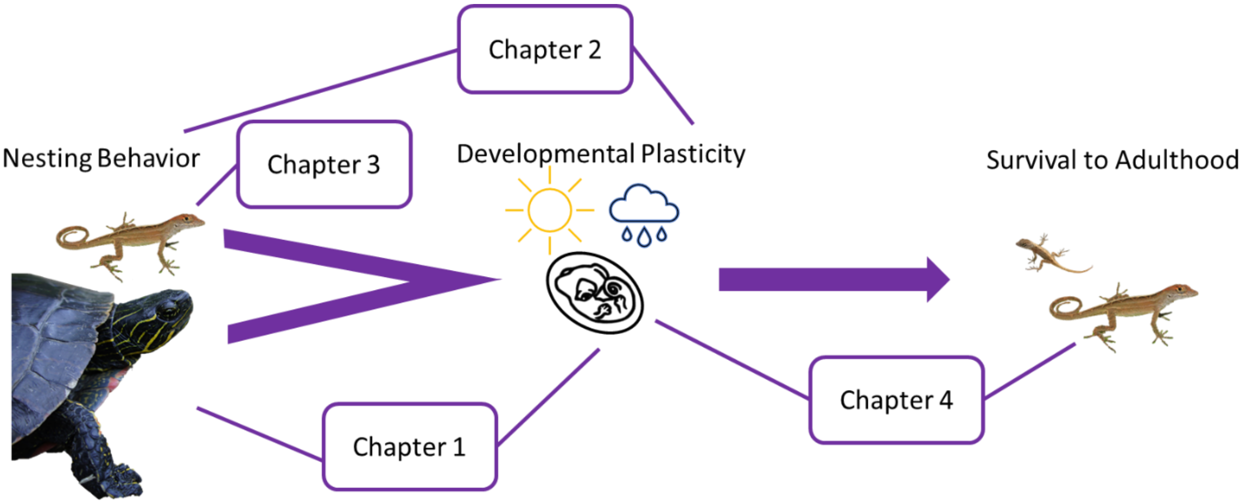

Figure 1. A diagram connecting each of my dissertation chapters to the concepts they address

Study Species

Chrysemys picta

The western painted turtle (*Chrysemys picta*) is widespread across the continental United States. The painted turtle has several qualities that are ideal for studies of maternal nesting behavior. They nest during the summer months from May-July and can lay 1-3 relatively large clutches (1-23 eggs/clutch) during a season. The large clutch size allows for cross fostering studies that are ideal for disentangling the effects of the nest environment from the effects of maternal identity. They also spend a significant amount of time constructing underground nests, meaning it is logistically easy to observe them in the act of nesting. This allows researchers to capture nesting females, mark them, and release them so maternal identity is a known factor in the experiment. Painted turtles are not suited for lab studies or longitudinal studies, but are ideal for field-based studies on nesting and maternal effects. Specific benefits of this study species are outlined in chapter 1.

Anolis sagrei

Brown anoles (*Anolis sagrei*) are native to Cuba and the Bahamas but have a continuous invasive distribution across the state of Florida. Brown anoles are relatively well studied. However, despite their increasing popularity as a study system, we know very little about their nesting ecology and the effects of natural nest conditions on development. We know that anoles lay approximately one egg every 7-10 days across a long nesting season (April-October), that females choose nest sites with high moisture levels in the lab, and that eggs have been found in aggregations in the field. This species has several qualities that are ideal for addressing the questions posed in my dissertation. First, capture and husbandry for these lizards is extremely logistically feasible. They are highly abundant in their native range, relatively easy to capture, and very hardy in captivity. Additionally, they are extremely fecund with a long reproductive season, leading to large sample sizes and an additional layer of environmental variation. There is seasonal variation in climate within the reproductive season, meaning females can alter behavior, nesting phenology, and provisioning for eggs. Within one season, the early-laid eggs can hatch and reach adulthood before the later eggs are even laid. This allows for novel approaches to questions around nesting behavior, its effects on development, and fitness consequences. Because we know so little about anole nesting in the field, research in this area has the potential to be highly influential and impactful. Finally, brown anoles are relatively short-lived and easy to uniquely mark. This makes them ideally suited for longitudinal mark-recapture studies. Specific benefits of this species are outlined in each chapter.

Figure 2. Study species used in this dissertation. (A) Western painted turtle in Round Lake, Id (B) Western painted turtle hatchlings on top of a nest (C) Female brown anole in Palm Coast, Fl (D) Brown anole eggs found in the field. All photos taken by J. Pruett.

Chapter 1

The influence of maternal nesting behavior on offspring survival: evidence from correlational and cross-fostering studies

Manuscript published in *Animal Behaviour* (Pruett et al. 2019)

Introduction

Maternal effects arise when the mother's environment or phenotype, rather than her genes, affects the phenotype of her offspring (Mousseau & Fox 1998). These effects can occur through many mechanisms (e.g., cellular and epigenetic modifications, physiological or provisioning alterations, behaviour) and play a unique role in shaping the evolutionary landscape (Roosenburg, 1996). Historically, maternal and environmental effects have been viewed mostly as a nuisance when attempting to determine the genetic basis for phenotypic variation (Falconer 1989; Mousseau & Fox 1998). In the past couple decades, however, maternal effects have been considered an important reproductive trait that is shaped by natural selection (Mousseau & Fox 1998; Marshall & Uller 2007).

Mothers can have profound influences on the phenotypes and survival of their offspring beyond the direct effects of their genetic material (Mousseau & Fox 1998; Galloway 2005; Räsänen & Kruuk 2007). Indeed, the level of nutrient provisioning for embryo development (Lipar & Ketterson, 2000; Polich et al. 2018), quality or quantity of post-natal care (Champagne 2008, Franklin et al. 2010; Curley & Champagne 2016), and several environmental factors experienced by females affect phenotypes and survival of offspring (Painter et al. 2008; Hasselquist & Nilsson 2009, Shama et al. 2016). However, maternal effects are often difficult to quantify in nature, due to several potential confounding environmental factors (Wolf & Wade 2009). Thus, several experimental approaches have been implemented to separate effects of intrinsic maternal factors from extrinsic environmental factors on offspring phenotypes and fitness (Warner & Andrews 2002; Paquet, Covas & Doutrelant 2015; Raveh et al. 2016). Cross-fostering enables researchers to quantify the relative contributions of maternal and environmental factors to variation in offspring phenotypes and survival (e.g., Van De Pol, Bakker et al. 2006; Hager et al. 2009; Mitchell et al. 2013B). By exposing offspring of one female to the developmental conditions provided by another female, this approach isolates the environmental conditions experienced by the offspring and decouples them from intrinsic maternal effects.

Oviparous animals that lack parental care after oviposition are excellent models for studying the effects of the abiotic environment and maternal provisioning on offspring phenotype (Warner 2014). Offspring are strongly influenced by the developmental conditions that their mothers select for nesting, and embryos have little to no control over these conditions (Telemeco et al. 2016; but see Du & Shine 2015). Furthermore, because maternal provisioning terminates immediately after oviposition, the effects of abiotic conditions within nests are not confounded with those of prolonged additional parental care, which facilitates experimental designs and interpretation of results.

Behavioural choice of nest site is an example of a maternal effect that greatly influences the biotic and abiotic conditions that offspring experience during development (Angilletta et al. 2009). In many reptiles, mothers choose nest sites with relatively warm and moist microhabitats with little shade cover (Janzen & Morjan 2001; Doody et al. 2006; Warner & Shine 2008), all of which can positively affect offspring phenotype and survival (Brown & Shine 2004; Weisrock & Janzen 1999; Reedy et al. 2013). Furthermore, nest-site choice may affect post-hatching survival depending upon the distance of the nest to suitable habitat for newly-hatched offspring; these factors could have important consequences on predation rates or likelihood of finding suitable habitat (Møller, 1988; but see Paitz et al. 2007; Warner & Mitchell 2013). Considering that nest conditions can have major consequences on offspring fitness, natural selection should favour mothers that choose nest sites with environments that positively affect offspring phenotypes and survival, which is supported by numerous studies (Brown & Shine 2004; Peet-Paré & Blouin-Demers 2012; Reedy et al. 2013; Mitchell et al. 2013A; Li et al. 2017).

Among many microclimate variables, temperature plays a key role in reptile development. Embryos can tolerate a broad range of incubation temperatures that affect developmental and phenotypic characteristics of offspring (Deeming 2004; Noble et al. 2018). For example, relatively high temperatures increase metabolic rates of embryos which accelerates development (Birchard & Reiber 1996; Janzen & Morjan 2002; Du et al. 2007; Du et al. 2010; Hulbert et al. 2017). Warm incubation temperatures also produce larger offspring with faster running speeds than those incubated under cool environments (Pearson & Warner 2016; Noble et al. 2018). Despite thermal tolerance for a wide array of developmental temperatures, slight departures from an optimum can produce abnormalities (Telemeco et al. 2013), and also increase egg mortality (Liang et al. 2015; Smith et al. 2015; Hall & Warner 2018). Thus, maternal control over thermal conditions experienced by embryos is critical, and is strongly influenced by canopy cover over the nest or its distance from water (via hydric differences; Morjan 2003).

Much of our current knowledge about the effects of incubation microclimate on offspring development comes from laboratory-based studies. Although this work has provided substantial contributions, ecologically-relevant interpretations can be difficult to draw because environmental parameters reproduced in the lab (such as temperature) do not mimic natural variation (Bowden et al. 2014). To overcome these issues, researchers have monitored environmental conditions inside nests and evaluated correlations between those conditions and the resultant hatching success or phenotypes produced (Warner & Shine 2009; Warner et al. 2010). In this study, we combined correlational data from the field with a cross-fostering experiment to better understand the role of maternal nesting behaviour in shaping offspring survival in a population of painted turtles (*Chrysemys picta*) from northern Idaho, USA. In addition to cross fostering eggs among maternal nests, we also placed eggs in artificial nests that span the range of maternally-chosen shade cover and distance from water to experimentally examine the importance of these variables.

Our study addressed two primary questions. First, do mothers choose nest sites nonrandomly with respect to several microhabitat variables? We predicted that females would choose relatively open, warm areas for nesting compared to randomly chosen sites, as observed previously in other *C. picta* populations (Janzen & Morjan 2002). Second, is variation in egg survival explained by maternally-chosen nest sites (natal vs non-natal), by specific microhabitat variables of the nest sites, or by both? We predicted that egg survival would be positively associated with temperature and canopy openness, and would be relatively high for eggs incubated in natal nests.

Methods

Study species and field site

The painted turtle (*Chrysemys picta*) is widespread across much of the United States. They nest during the summer months from mid May to early July, with some females nesting multiple times in one season. Clutch size ranges from 1-23 eggs. Eggs usually hatch in the fall and hatchlings usually remain in their natal nests until spring the following year when they disperse to suitable habitat (Ernst, Lovich, & Barbour 1994).

Field research was conducted at Round Lake State Park near Sagle, Idaho. Our field site consisted of three nesting areas along a stream on the west side of Round Lake (Fig. 1). From late May to early July, female *C. picta* emerge from the stream to nest on a south-facing bank immediately adjacent to the water. The three nesting areas have little canopy cover compared to the surrounding forest habitat, and they differ from each other in size and some microhabitat features.

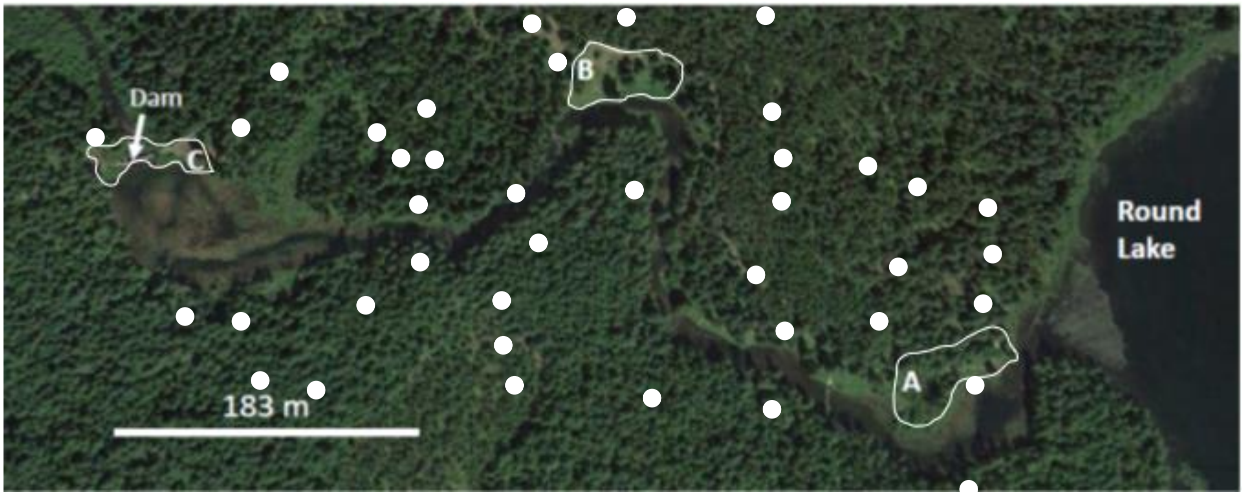

Figure 1. Map of the field site with each nesting area circled and labelled (A, B, and C) and each randomly chosen GIS site represented by a white dot. Water flows from Round Lake into a stream and collects in a small pond above the dam on the west end of the study site. Female turtles emerge from the stream and can be observed nesting near a hiking trail that runs parallel to the stream (See Fig. 2B).

Observational study from 2013-2016

Nesting activity of *C. picta* was monitored during May and June from 2013-2016 (Supplementary Table 1). The three nesting areas were patrolled daily for nesting females between 15:00 and 21:00. When a nesting female was discovered, we observed it from a distance to minimize disturbance. Upon completion of nesting, females were captured, identified by unique marks on their marginal scutes (or given a unique mark if not captured previously)

(Cagle, 1939), measured, and then released. Each nest was carefully opened at its neck using a spoon, and the depth to the top egg was measured. Each egg was removed from the nest and weighed. After all eggs were removed, the depth to the bottom of the nest was measured. After processing, eggs were returned to their nests for incubation. A Thermochron iButton (programmed to take hourly temperature readings) was placed among the eggs in each nest; iButtons were wrapped in a balloon and parafilm for waterproofing. After all eggs were replaced, the nest was covered with soil (similar to how females left the nest) and a wire mesh nest protector was staked down to deter predators. Hemispherical photographs were taken above each nest with a digital camera (Nikon Coolpix L30) and 180° fish-eye lens (Zykkor). The photographs were uploaded into Gap Light Analysis software where percent canopy openness was quantified (Doody et al. 2006).

Measurements of canopy cover and ground temperature were recorded for forty randomly selected locations around the study site (Fig. 1). The randomly selected locations (henceforth, “random nests”) were determined with GIS using unequal probability-based spatial sampling within the area around the stream and the surrounding forest (Krivoruchko & Butler 2013). Given the area in which random sites were chosen (which included the three nesting areas), the maximum distance a random could have been from the stream was ~100 m. Random nests provided a quantitative assessment of what microclimates were available in the landscape at large and served as a comparison to the microhabitats that mothers actually used for nesting. Importantly, turtles were observed in both forested and open areas while in search of nest sites, and thus both these habitats were included within the area that random sites were chosen. An iButton (programmed to record hourly temperatures) was buried at each random nest at a randomly chosen depth ranging from 6 to 12 cm, which is within the range of depths of *C. picta* nests. Canopy cover measurements were made with hemispherical photographs as described above for maternally-chosen nests. Temperature data were collected each year at the same random nest locations from 2013-2016. In September of each year, we carefully excavated nests to assess hatchling survival. At this time, all iButtons were retrieved (from random and maternal nests) and hatchlings were measured and then placed back into their nest for overwintering.

An Analysis of Variance (R Version 3.4.2) was used to quantify annual differences in habitat parameters between maternally selected nest sites and random sites, with a Tukey's HSD post-hoc test to compare among years. A linear mixed model with maternal identity as a random factor was used to quantify differences in habitat parameters (e.g., canopy openness, average temperature) across nesting areas. Finally, egg survival was assessed as a percentage of eggs that hatched out of the total number of eggs in each nest. A linear mixed model was used to quantify the effects nest temperature, nesting area, egg mass, canopy openness, year, and various 2-way interactions on egg survival; the mean value of nest temperature and egg mass for each nest was used for this analysis. Maternal identity was used as a random factor because some females had multiple nests across and within years. For the interaction terms, we were specifically interested in whether the influence of nest temperature and egg mass on egg survival differed among years (i.e., temperature x year and egg mass x year interactions).

Experimental study in 2017

In 2017, we conducted a cross-fostering experiment to decouple the effects of nest environment and intrinsic maternal effects on egg hatching success. For this experiment, all nests were located and monitored according to the 2013-16 protocol described above. Egg processing was also similar, but not identical, to the protocol in the previous years (details below). After eggs were processed, they were placed into plastic containers with moist soil from their nest, and kept temporarily in a cool, shaded area until they were partitioned into their appropriate incubation treatments. Eggs were kept for 1-4 days (mean = 2.2 days) before returning them to nests.

The experimental design consisted of 5 “blocks” of nests with eggs that were cross-fostered among nests (Fig. 2A). Each block consisted of three maternally-chosen nests and four artificial nests. Thus, eggs from the three maternally-chosen nests were distributed among seven nests in total. Our design ensured that each nest contained two eggs from three different females (i.e., six eggs per nest); thus, each block required 42 eggs (6 eggs per nest x 7 nests). This protocol required a minimum of 14 eggs produced in a clutch. For the three maternally-chosen nests (per block), eggs were cross-fostered so that each nest contained two natal eggs, and four non-natal eggs; the four non-natal eggs consisted of two from each of the non-natal mothers. Nests were assigned to blocks chronologically and irrespective of nesting area. Thus, eggs from a given nesting area could be assigned to a block with eggs from a different nesting area. For nests with less than the 14 eggs required for a full block, eggs were only crossed among the four artificial nests, and not among the three maternally selected nests; this was referred to as a “partial block”, which required a minimum of 8 eggs per nest for a total of 24 eggs per partial block. In total, the experiment contained 24 maternally-chosen nests (i.e., 3 nests x 5 full blocks + 3 nests x 3 partial blocks = 24 nests).

Artificial nests within blocks were organized into a full 2x2 factorial design of shade cover vs distance to water (Fig. 2B). These artificial nests were placed in nesting area B (Fig. 1) because the landscape in that area is a mosaic of open and shaded locations and was large enough to place nests near or far from the stream. To manipulate shade cover, we leveraged the shade variation at this site so that nests were either placed in open grass or in the shade from nearby trees. In total, we constructed 32 artificial nests, and each contained 6 eggs, 2 from each of the 3 females in a block (or partial block), where one nest was far from water and shaded, one was far from water in the open, one was close to water and shaded, and the last was close to water in the open. Each treatment contained two replicate locations, and every other block was distributed into one or the other replicate (Fig. 2B). Importantly, the locations of the artificial nests were within the range of distances that females travel from water to nest, and the shade cover mimicked the lower and upper ends of the distribution for maternally-chosen nest sites (Fig. 3).

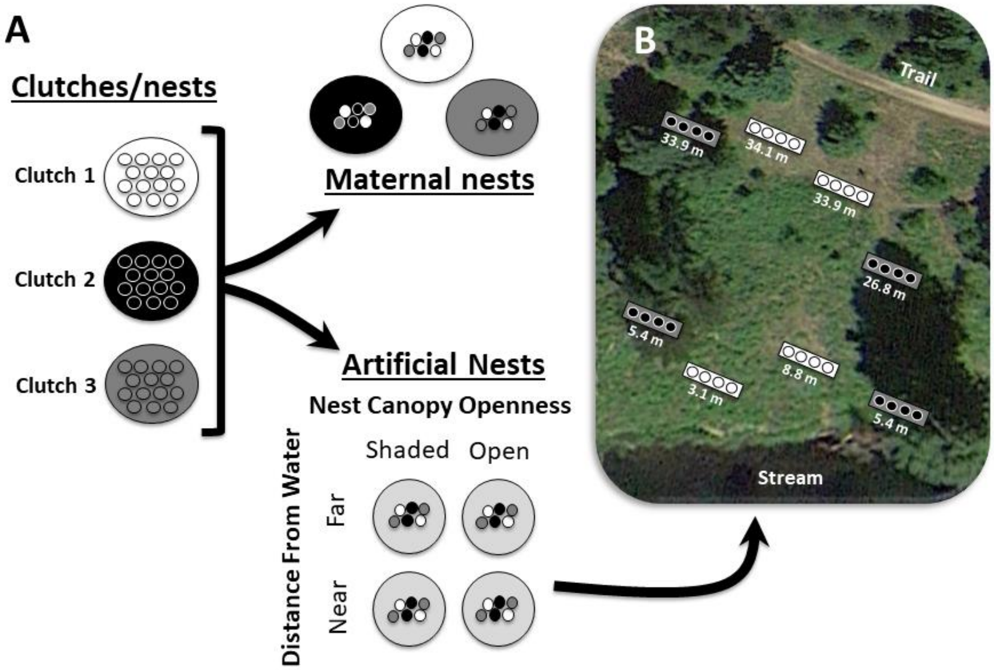

Figure 2. Schematic of the experimental design used in 2017. Panel A represents one experimental block of three maternally selected nests and four artificial nests. Two eggs from each mother were allocated to each nest within a block for a total of six eggs in each nest. Thus, a single block consisted of three clutches that were placed in three maternally-selected, and four artificially-constructed nests. Eggs with the same shading represent siblings. Panel B illustrates the layout of artificial nests at nesting site B (see Fig. 1). Each circle represents a nest. Black circles in gray boxes represent shaded nests while white nests in white boxes represent the open treatment. The exact distances of each nest set from the stream is indicated below the nests in panel B. Nests on either side of the dashed line represent two replicate locations for this experiment.

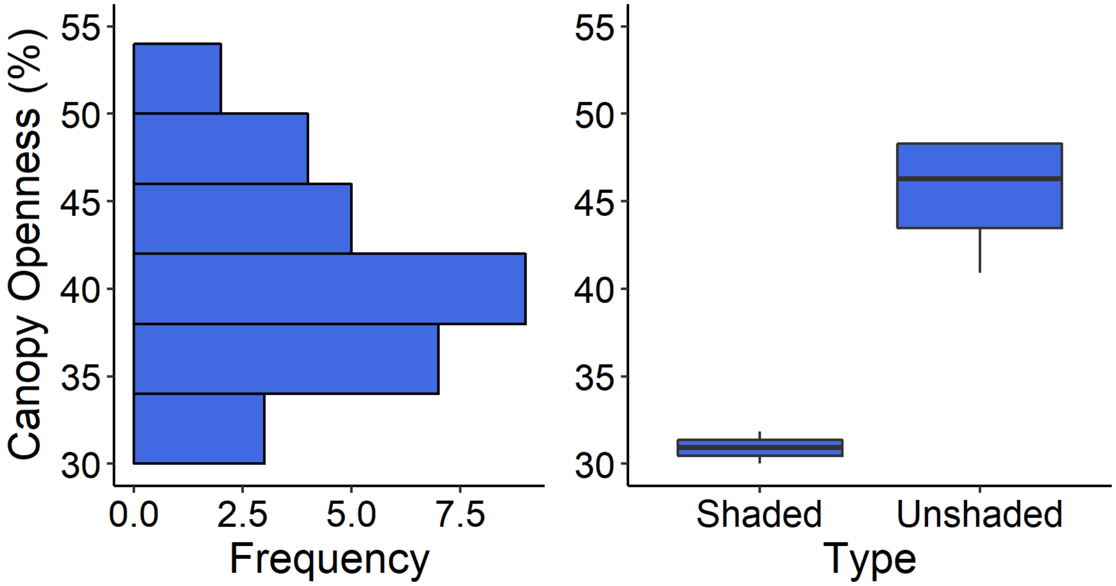

Figure 3. Distribution of canopy openness for maternally-selected nest sites (A), and a comparison of canopy openness of artificial nest sites between the shaded and unshaded treatments in the 2017 experiment (B). The bold line represents the 50th percentile lower and upper bounds represent the 1st and 3rd quartiles respectively. The whiskers extending from the boxes represent the 1st and 3rd quartiles ± 1.5 x interquartile range and the points represent outliers in the dataset. Statistical results are in the text.

Within each block or partial block, eggs were assigned to a nest using a random number generator. Artificial nests were dug to the average depth of the three maternal nests for that block. To ensure eggs could be reliably matched to hatchlings, eggs were placed into mesh nylon pouches and sealed with a colour-coded zip tie to ensure correct identification of hatchlings upon excavation in the fall. On 23 September, we returned to the field site to excavate nests, quantify egg survival, and measure hatchlings. All nests were relocated and carefully opened. All dead eggs were counted to ensure that none were missed.

Due to a correlation between nest temperature and canopy openness, a principal component analysis (PCA) was used to collapse the variation in these measurements into uncorrelated axes using the *FactorMineR* package (Lê et al. 2008). The PCA included daily average minimum temperature, daily average maximum temperature, daily average mean temperature, daily average temperature variance, and canopy openness values for each nest. The first two PC axes explained 84% of the variation in the data (between 56% and 28%, respectively) (Supplementary Table 2). PC1 loadings were related to mean, maximum, and variance of nest temperature, while PC2 loadings were related to minimum temperature and canopy openness where canopy openness correlated positively with minimum temperature of a nest. The scores for PC1 and PC2 were used in the analysis of egg survival for the cross-fostering experiment.

The cross-fostering experiment addressed three primary questions with two separate analyses. Each analysis used a generalized linear mixed model with egg survival as the dependent variable (binomial distribution) and maternal identity nested within block as a random effect. The first analysis was aimed at determining if natal status (natal vs non-natal nest) and microhabitat contribute to variation in egg survival. For this analysis, only maternally-selected nest sites were used, and independent variables included natal status, egg mass, PC1 scores, and PC2 scores. The second analysis aimed at determining if maternally-chosen vs artificial nests differed in egg survival and determined if shade cover and distance to water (treated as continuous variables) contributed to variation in egg survival. For this analysis, all nests were used, and independent variables included nest type (maternal vs artificial), egg mass, distance to water, PC1 scores, PC2 scores, as well as interactions between nest type and each of the PC scores. We were also interested with whether maternal identity (an index of intrinsic genetic or maternal effects) contributed to variation in egg survival. Thus, the effect of maternal identity (random factor in our models) was assessed with Akaike information criterion values for models with and without the random factor included.

Results

Observational study from 2013-2016

Females consistently nested in relatively open, warm microhabitats across years. Maternal nest sites were warmer than random sites (F_1=599.1, p<0.0001; Fig. 4). Similar trends were observed when comparing nesting frequency across areas; most females nested in areas B (60.4% nested) and C (34.8% nested), which are both more open and warmer than area A (4.9% nested) (All pairwise comparisons p≤0.0082; Fig. 5, Supplementary Table 3); this pattern was consistent across years ($F_{1,71}$=1.41, p=0.2511).

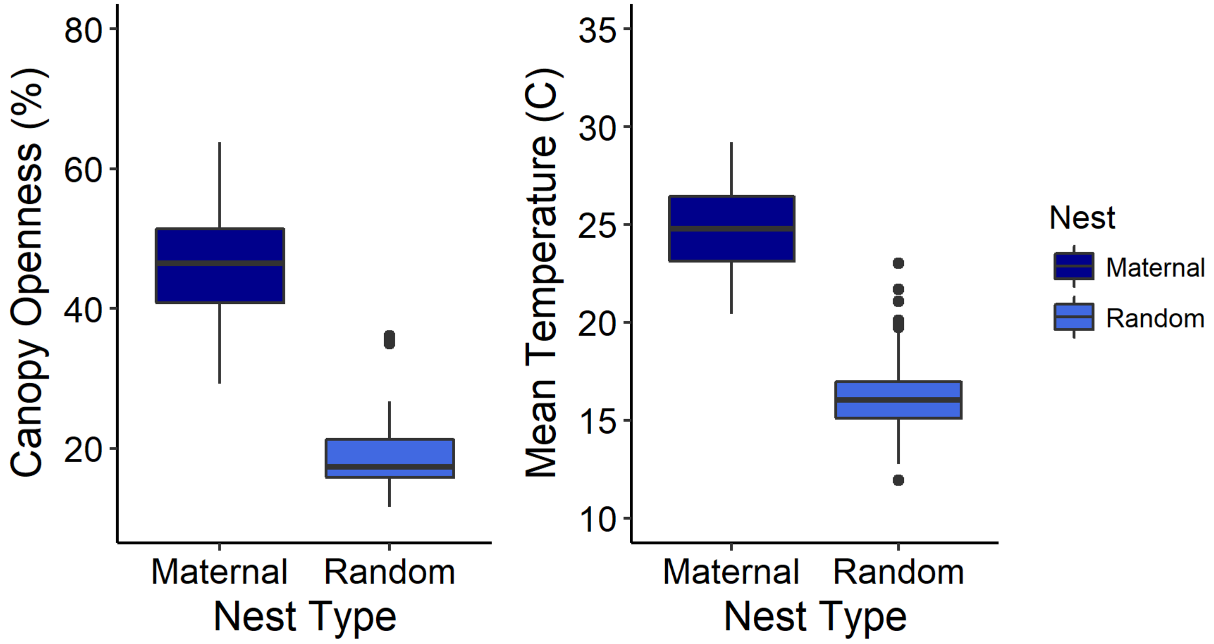

Figure 4. Canopy openness (A) and nest temperature (B) for maternally selected nest sites and randomly selected sites for the 2013-2016 nesting season (see text for statistics). The bold line represents the 50th percentile lower and upper bounds represent the 1st and 3rd quartiles respectively. The whiskers extending from the boxes represent the 1st and 3rd quartiles ± 1.5 x interquartile range and the points represent outliers in the dataset.

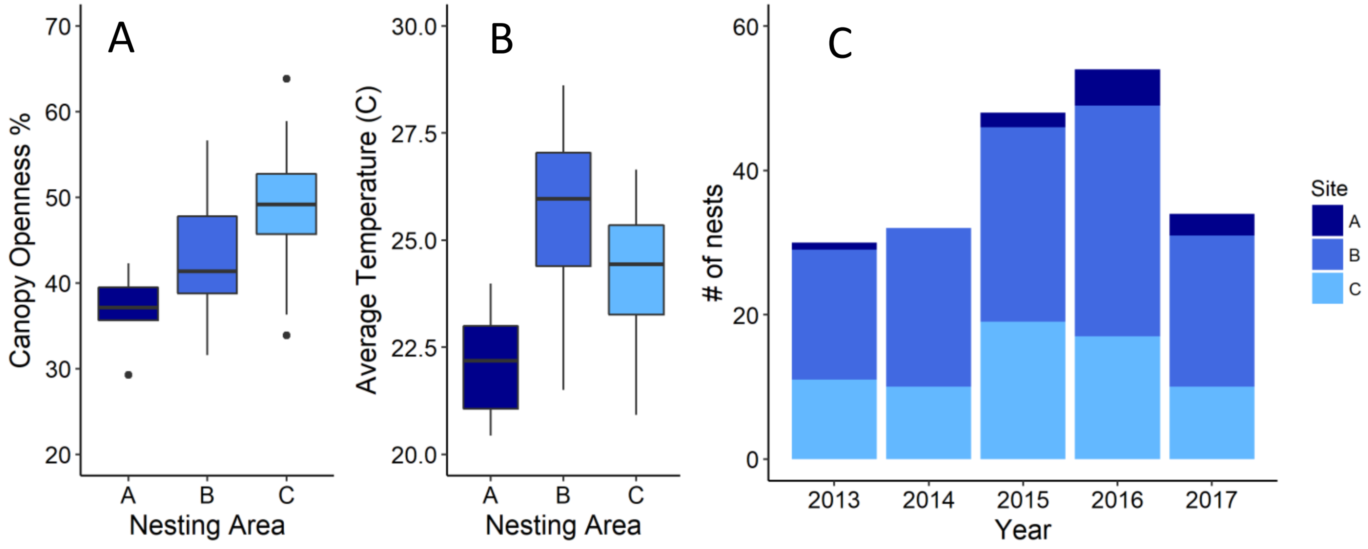

Figure 5. Comparison of (A) canopy openness and (B) temperature of nests among nesting areas and (C) number of nests in each area from 2013-2017. The bold line represents the 50th percentile lower and upper bounds represent the 1st and 3rd quartiles respectively. The whiskers extending from the boxes represent the 1st and 3rd quartiles ± 1.5 x interquartile range and the points represent outliers in the dataset. All nesting areas differed from each other for both canopy openness (P≤0.0082) and nest temperature (P≤0.013).

Egg survival varied among years (60.5%, 65.1%, 36.7%, and 73.1% for 2013-2016, respectively) but was not explained by nesting area, average egg mass, and canopy openness (Table 1). However, the influence of nest temperature on egg survival varied among years (temperature x year interaction). Egg survival was positively related to mean nest temperature in 2016 (p=0.00012, $r^2 = 0.32$), but no relationship was evident in 2013, 2014, or 2015 (Supplementary Figure 1). Importantly, nest temperature was relatively low in 2016 compared to the other years.

Table 1. Effects of nest area, year (2013-2016), and microhabitat variables on egg hatching success (dependent variable). Analyses were performed with a linear mixed model.

Independent Variable	denDF	numDF	F-Value	P-Value
Average Temperature	60	1	0.1960	0.6596
Nesting Area	60	2	0.6603	0.5204
Average Egg Mass	60	1	1.2738	0.2635
Canopy Openness	60	1	0.4616	0.4995
Year	60	3	5.2053	**0.0029***
Nesting Area×Canopy Openness	60	2	0.4857	0.6176
Average Temperature×Year	60	3	6.6481	**0.0006***
Average Egg Mass×Year	60	3	2.7139	0.0527

Experimental study in 2017

Egg hatching success in 2017 was relatively low (11.7%) compared to that in the previous four years. For eggs that were cross fostered among maternally-chosen nest sites, hatching success was not related to egg mass or any microhabitat variable measured; nor did hatching success differ between eggs that remained in their natal nest vs those cross-fostered into another maternally-chosen nest (Table 2). In the analysis that included all nests (maternal and artificial), hatching success was not influenced by egg mass or PC1 (i.e., mean, maximum, and temperature variance), but eggs in maternally-chosen nests had greater survival than those in artificial nests (Fig. 6). Additionally, egg hatching success increased with higher canopy openness and warmer minimum temperatures (variables described by PC2), particularly for eggs in artificial nests as indicated by an interaction between PC2 and nest type (maternal or artificial) (Fig. 6). Distance from water, however, had no effect on survival, and was removed from the model. These patterns were corroborated by analyses using raw data for nest temperature and canopy openness, rather than using PC scores. The inclusion of maternal identity as a random factor in the model contributed to a better fit for both the model analyzing only maternal nests (AIC with Maternal ID:80.00, without:82.252), and the model analyzing both maternal and artificial nests (AIC with Maternal ID:150.8, without:155.06), suggesting that maternal identity is an important source of variation in egg survival.

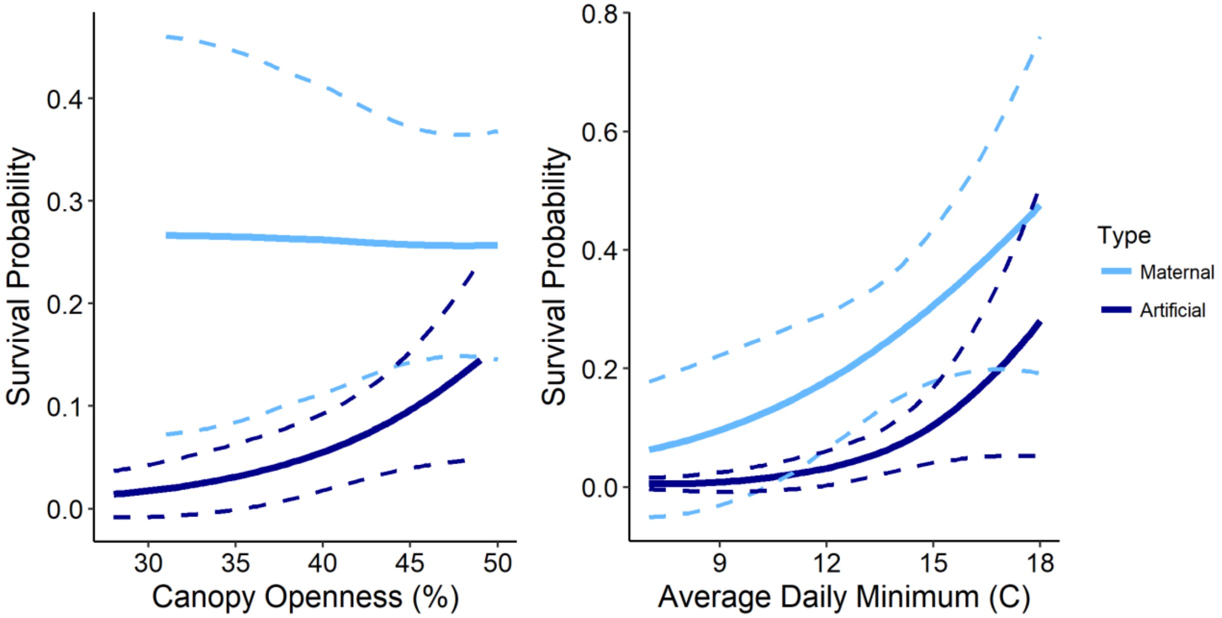

Figure 6. Probability of egg hatching success for *Chrysemys picta* in relation to (A) canopy openness and (B) average daily minimum temperature of the nest. The survival probability curves were generated using curves from generalized linear models of survival plotted against canopy openness and temperature respectively. The bold lines represent predicted survival probability and the corresponding dashed lines represent the upper and lower confidence limits.

Table 2. Effects of nest microenvironment on egg hatching success in 2017. The first analysis (generalized linear mixed model with binomial distribution) used only eggs that were cross-fostered and incubated in maternally-selected nest sites. The second analysis used all eggs from the cross-foster experiment and examined differences due to nest type (maternal vs artificial nests) rather than differences between natal and non-natal eggs.

	Independent Variable	β ± SE	F	P
Maternal Nests	PC1	-2.25±1.35	3.86	0.0955
	PC2	-1.17±0.99	1.60	0.2365
	Egg Mass	-1.90±0.40	2.98	0.1728
	Natal/Non-Natal	-0.81±1.12	0.87	0.4680
All Nests	PC1	-0.23±0.40	0.52	0.5659
	PC2	1.83±0.63	3.71	**0.0037**
	Egg Mass	-0.35±0.49	0.04	0.4655
	Nest Type	5.12±1.37	9.31	**0.0002**
	Nest Type×PC1	-1.22±0.67	3.37	0.0675
	Nest Type×PC2	-2.33±0.89	8.45	**0.0086**

Maternally selected nests did not differ from artificial nest sites in canopy openness ($F_{1,40}$=0.742, p=0.394) as the range of canopy openness overlapped greatly between these nest types (maternal range: 31.57 – 52.04%; mean= 41.01%; artificial range: 28.82 – 48.33%; mean= 37.79%). However, the average temperature for maternally selected nests (ranged from 21.87 – 27.81°C; mean= 25.22°C) was warmer than that for artificial nests (ranged from 16.76 – 25.78°C; mean= 21.33°C) ($F_{1,40}$=31.8, p<0.0001).

Discussion

The main goals of this study were to (1) determine if female turtles nest non-randomly with respect to different microhabitat variables, and to (2) quantify the effects of nest site (natal vs non-natal) and nest microhabitat features on egg survival. The first goal was addressed using four years of observational data on nesting behaviour, and the second was addressed using an experimental approach that was guided by the observational data. Our experimental design was unique in that we cross-fostered eggs into maternally-selected nests and artificial nests that were exposed to discrete differences in shade cover and distance from water; this cross-foster design minimized variation due to maternal identity and enabled us to directly examine the effects of these two environmental factors. Additionally, all aspects of this study were conducted in the field, and thus provide ecologically meaningful information about the importance of nesting behaviour in generating variation in egg survival (Paitz et al. 2010).

Females chose sites that were more open and warmer than randomly selected sites (Fig. 4). This pattern has been demonstrated in other turtles (Wilson 1998; Janzen & Morjan 2001; Mitchell et al. 2013A) and this behaviour should facilitate egg hatching success as relatively warm conditions are favourable for development in many reptiles (Warner & Shine 2008; Noble et al. 2018). Although most of our search efforts focused within three known nesting areas that are considerably more open than the surrounding forest, no turtle has ever been observed nesting in the forest habitat during five years of surveys. This supports our assertion that females prefer open habitat for nesting, likely due to favourable thermal conditions (Angilletta et al. 2009). In further support of this observation, relatively few females were observed nesting in area A, which is more shaded than the other two nesting areas (Fig. 5).

Maternally-chosen nest environments can have strong effects on egg survival and offspring phenotypes in many organisms (Refsnider & Janzen 2010; Mainwaring et al. 2017), including painted turtles (Mitchell et al. 2015). In our observational study, no nest variable had an overarching influence on egg survival. For example, the relationship between nest temperature and egg survival varied among years, which has also been shown in another population of painted turtles (Warner et al. 2010). Although some of the variation in annual egg survival may have been due to annual differences in temperature (Table 1), correlations between nest temperature and egg survival were generally weak (Fig S1). Thus, an environmental source of variation in egg survival remains unknown using these correlative data. Nevertheless, egg survival varied considerably among nests, ranging from 0% to 100% in a single season. Given that maternal identity was a significant contributor to variation in egg survival, this variation may reflect intrinsic differences that may be attributed to maternal factors.

The correlative results from 2013-2016 guided our experimental design in 2017. We indirectly manipulated nest temperature by placing artificial nests in open and shaded locations, and we manipulated the distance of nests to water. Nest distance from water is important as it affects the time (and length of exposure to predators) that hatchings travel to suitable habitat after emergence from the nest (Warner & Mitchell 2013). Additionally, distance from water can impact the hydric environment of the nest (Morjan 2003), which has several consequences for embryo development and offspring phenotypes (Packard et al. 1988). Distance, however, had no effect on egg survival and was removed from our final statistical models. We showed no variation in egg survival among maternally-chosen nests, and variation was not explained by whether eggs were in a natal or non-natal nest. This result provides evidence that microhabitats chosen by nesting females do not have family-specific consequences on survival, and that females may nest in microhabitats that have similar effects on development across the population (Mitchell et al. 2013B). Alternatively, the lack of an effect of natal status may have been due to unusually dry conditions in 2017 that reduced variance in egg survival among nests.

Despite low egg survival in 2017, eggs incubated in maternal nests had greater survival than those in artificial nests. This difference between maternal and artificial nests suggests that either mothers generally select nest sites that confer relatively high survival (Wilson 1998, Warner & Andrews 2002, Mitchell et al. 2015), or we chose poor locations for artificial nests or constructed nests in a way that reduced egg survival. Our data cannot distinguish between these alternative explanations, but we provide some possible explanations and rule out others. First, although artificial nests were placed only meters away from maternally-selected nests, minimum nest temperature was relatively cool in artificial nests, despite no difference in canopy cover. This pattern may be explained by the direction of shade cover over nests, which can impact the length and intensity of solar radiation transmitted to a nest site (a tree south of a nest blocks more solar radiation than a tree to the north despite similar values for openness). The low minimum temperatures (rather than canopy openness) in artificial nests may have contributed to reduced egg survival, which has been observed in other reptiles (Noble et al. 2018).

Second, the difference in egg survival between maternal and artificial nests may be due to differences in the distribution of canopy openness between nest types. For instance, artificial nests were constructed at the extremes of the distribution (Fig. 3), where the fewest number of nests were observed across all nesting seasons. Because we did not construct artificial nests at intermediate levels of canopy openness, we cannot compare egg survival between artificial and maternal nests under the average shade conditions chosen by mothers. Nevertheless, egg survival in artificial nests increased with canopy openness, suggesting that increased canopy openness slightly offsets the negative effect of artificial nest sites on egg survival. This pattern was similar for minimum temperature, which is positively correlated with openness (both loaded on PC2). It is possible that canopy openness or minimum temperature interact with some unmeasured variable in artificial nests in ways that reduce egg survival. Although female *C. picta* choose relatively open nest sites that confer greater hatching success (Mitchell et al. 2015) the cause of relatively low egg survival in artificial nests, but not maternal nests, under shaded conditions warrants further investigation. Lastly, the possibility of researcher-induced mortality via egg movement is unlikely (Mitchell et al. 2013A) given that survival did not differ between eggs that were moved and those that remained in natal nests. Poor artificial nest construction might explain variation in egg mortality, but we saw similarly high levels of mortality in natural nests that were not included in the 2017 experiment (n=21 eggs from 4 nests). Additionally, research on another emydid turtle that compares egg survival between artificial and natural nests located just 50 cm from each other reveals no effect of nest construction on egg survival (Lloyd & Warner 2019).

Conclusions

Nesting behaviour is an important maternal effect with consequences on offspring survival. Identifying nesting behaviour as a maternal effect is difficult, however, because the consequences can be mediated by intrinsic maternal factors (e.g., genes, egg investment) and extrinsic factors (e.g., nest temperature, shade cover) that are typically confounded. Field experiments like ours are needed to decouple these effects and identify the specific factors that generate variation in nest success in the wild, and hence will provide novel insights into the ecological relevance of variation in nesting behaviour.

Our study showed that mothers select relatively open sites for nesting that are warmer than randomly selected nest sites. However, relationships among nest canopy openness and temperature with egg survival varied among years despite considerable within-year variation in egg hatching success. These patterns suggest that the ecological consequences of maternal nesting behaviour are temporally dynamic, and that intrinsic factors also likely generate variation in hatching success. We also experimentally showed that shade cover and minimum nest temperature are important drivers of variation in egg survival in the field. Eggs that are moved to shaded nest sites with low minimum temperatures have relatively low survival, which are conditions that females tend to avoid. This result suggests that females select specific microclimate parameters that lead to improved hatching success and reinforces the importance of behavioural maternal effects in generating variation in fitness.

Chapter 1 Supplemental Material

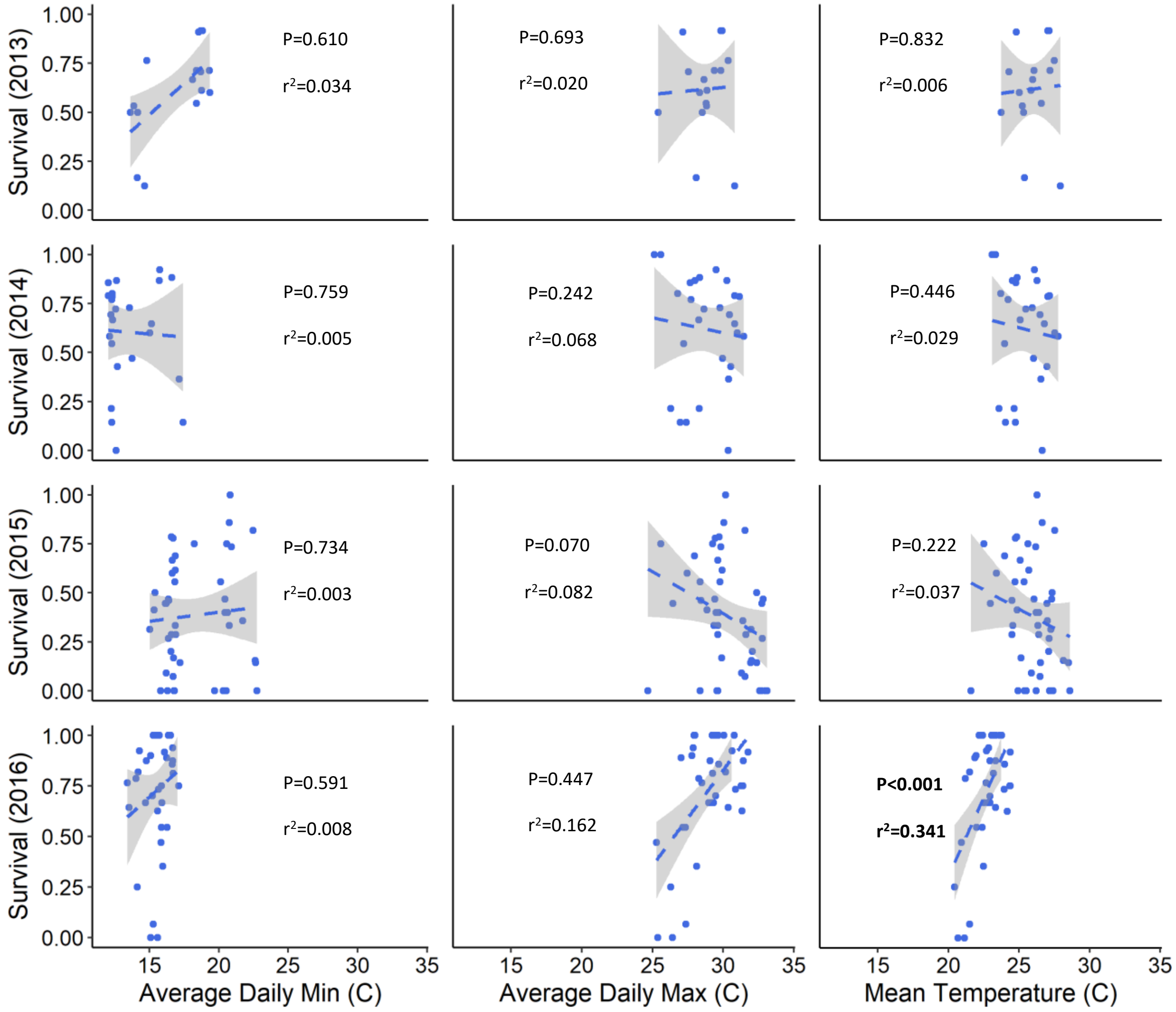

Figure S1. Regressions of egg hatching success (% of eggs that survived to hatching in a nest) in relation to average daily minimum temperature (℃), average daily maximum temperature (℃), and overall mean temperature (℃) for nests in the 2013-2016 seasons. Each point represents a nest, the dashed line represents the line of best fit, and the grey cloud represents standard error.

Table S1. Dates of field work and number of nests in each nesting area (A-C) from 2013-2017. Each field season consists of a summer nesting season and a fall trip to excavate and measure hatchlings.

Year	Nesting season observed	Egg excavation	Number of nests at each nesting area		
			A	B	C
2013	10 June – 8 July	21 Sept	1	18	11
2014	7 June – 2 July	13 Sept	0	22	10
2015	21 May – 29 June	4 Sept	2	27	19
2016	21 May – 30 June	16 Sept	5	32	17
2017	20 May – 30 June	23 Sept	3	20	10

Table S2. Loading scores on each principal component (PC) axis.

	PC axis 1	PC axis 2	PC axis 3
Mean temperature	0.9260	0.2499	0.1760
Maximum temperature	0.9842	-0.0373	0.1417
Minimum temperature	-0.1245	0.8907	0.4136
Temperature variance	0.8616	-0.4426	0.0537
Canopy Openness	0.4803	0.6196	-0.6187
Proportion of Variance Explained	56%	28%	12%

Table S3. Pairwise comparisons of canopy openness (%) and average nest temperatures (°C) between each of the three nesting areas in our field site. Pairwise values were obtained using a linear mixed model with maternal identity as a random factor coupled with a pairwise lease-squares means test.

Dependent Variable	Contrast	Estimate	P-Value
Canopy Openness (%)	C -A	12.58±2.41	<0.0001*
	C - B	6.21±1.10	<0.0001*
	A - B	-6.37±2.35	0.0082*
Average Temperature (°C)	C - A	1.89±0.64	0.0130*
	C - B	-1.14±0.30	0.0003*

Chapter 2

Temporal variation in maternal nest choice and its consequences for lizard embryos

Manuscript published in *Behavioral Ecology* (Pruett et al. 2020)

Introduction

Maternal effects play a critical role in generating phenotypic variation and determining the fitness of offspring (Moore et al. 2019). Because early life stages are often very sensitive to developmental environments, mothers have the potential to impact survival and phenotypes of their progeny via variation in their own physiology or behavior beyond the effects of heritable genetic material (Mousseau & Fox 1998). Although maternal effects can be energetically costly to females (e.g. increased provisioning to embryos, long-term parental care), they can enhance fitness by improving offspring survival and perpetuating the mother's genetic material into future generations (Marshall & Uller 2007). Parental care is a prime example of how behavior can impact the fitness of offspring. In many birds, for example, parental attendance of eggs or food provisioning to offspring can influence a suite of fitness-relevant traits of offspring, such as growth and immunocompetence (Saino et al. 1997, Tinne et al. 2005). In oviparous organisms, maternal nesting behavior can be viewed as another form of parental care because it determines the environmental conditions offspring will experience during development (Mainwaring et al. 2016).

Nesting is common across vertebrate and invertebrate taxa (Hansell 2005), and much of our understanding of nesting behavior and its consequences comes from studies of avian systems (Refsnider 2016). Birds provide extensive post-laying parental care, and invest considerable energy towards maintaining consistent nest temperatures through brooding and caring for their young post-hatching (Deeming & Reynolds 2015). In species that exhibit extended post-laying parental care, mothers have continued opportunities to modify the developmental conditions experienced by their offspring. In many species, however, females lay eggs and leave them unattended for the rest of development (Shine 2005), which limits opportunities for parents to modify the nest environment during the development of their offspring. Therefore, the timing of nesting, environmental conditions chosen by females, and design of nests are particularly important at the time of oviposition (Deeming & Ferguson 1991, Telemeco et al. 2009).

Evidence that females make active choices of oviposition sites is needed to classify nesting behavior as a true "maternal effect" (Wolf & Wade 2009). This evidence is observed in many reptiles whereby females actively select specific microclimates for nests (Brown & Shine 2004, Mitchell et al. 2013a, Li et al. 2017). Furthermore, nesting is a plastic behavior that is dependent on prevailing conditions. For example, the onset of nesting is influenced by winter temperatures in painted turtles (Schwanz & Janzen 2008), and nest site choice can vary to match local climates (Refsnider et al. 2013) or change depending on predation risk (Spencer 2002; but see Refsnider et al. 2015). Aspects of nest construction by three-lined skinks, such as nest depth, correlate with changes in ambient temperatures (Telemeco et al. 2009). Several lab-based studies reveal that females choose nesting conditions that are favorable for offspring fitness, suggesting that this behavior is adaptive (Warner & Andrews 2002, Pike et al. 2010, Reedy et al. 2013). Importantly, most species inhabit temporally heterogeneous environments, and consequently, available nesting conditions shift through the reproductive season (Warner & Shine 2008). This heterogeneity could influence nesting behavior and its consequences for offspring development and survival. Few studies have examined the importance of temporally-shifting environments because many organisms have a relatively short window for reproduction due to seasonal constraints.

Anolis lizards provide a unique and understudied system for examining adaptive nesting behavior. Anoles produce one egg at a time, approximately once a week (Smith et al. 1973) over a reproductive season that can extend 6 or more months. This means that energy allocation toward reproduction is temporally extended in anoles compared to species that produce large, but infrequent clutches. Moreover, this reproductive pattern could enable females to spread their eggs across multiple sites that experience different conditions. This aspect of *Anolis* life history leads to seasonal variation in nesting conditions across the long reproductive season, thereby providing females an opportunity to adjust their nest choices to changes in ambient conditions.

Although *Anolis* lizards have been models for testing concepts in behavior, ecology, and evolution (Losos 2009), and are emerging as a model for developmental biology (Sanger et al. 2007) and plasticity (Warner 2014), nearly nothing is known about their nesting behavior in nature. Anecdotal reports of anole nesting suggest that these lizards lay eggs in a variety of habitats (Delaney et al. 2013) and might nest communally (Rand 1967, Swierk et al. 2019), and lab studies show that females prefer moist substrate with leaf cover for nesting (Reedy et al. 2013, Socci et al. 2005). Empirical work on anole nesting in the field is, however, limited (Tiatragul et al. 2019). Relatively more is known about the effects of developmental conditions on anole embryos (Goodman & Heah 2010, Warner et al. 2012, Pearson & Warner 2016, 2018), but much of this work has also been done in lab settings and does not fully capture the environmental fluctuations embryos would experience in the field. Nevertheless, these studies have provided valuable insight into this crucial life stage of a very prolific model system, but we need a better understanding of nesting behavior in the field to advance research on their reproduction and development. Furthermore, the unique nature of anole reproductive biology enables us to answer new questions that will advance understanding of maternal investment in nesting.

We sought to address these knowledge gaps using a two-part study on the brown anole, *Anolis sagrei*. The first objective was to quantify the nest microhabitats chosen by females in the field and to characterize general qualitative patterns of anole nesting behavior. We did this by searching for anole eggs using randomized and targeted approaches in the field. Furthermore, because females lay approximately one egg per week from April to October, we repeated our approach three times to examine seasonal changes in nesting behavior. We predicted that females would choose relatively cool and moist nesting sites because these microhabitats would prevent desiccation and limit exposure to extreme thermal fluctuations that might be lethal to embryos (Hall & Warner 2018). The second objective was to examine the effects of microclimate conditions on embryo development in the field. To do this, we planted anole eggs across our field site at two times during the nesting season; our approach exposed eggs to a broad range of temperature and moisture conditions, and enabled us to assess the effects on hatching success and offspring phenotype. We predicted that cooler sites with more moisture would be beneficial for both hatching success and fitness-relevant hatchling phenotypes, which would suggest that nesting behavior is adaptive (according to our first prediction). Lastly, given that ambient conditions change through the season, we expect that patterns of maternal nesting behavior and egg hatching success will also shift seasonally.

Methods

Study Species and Field Site

The Cuban brown anole (*Anolis sagrei*) is native to Cuba and the Bahamas, and invasive across the southeastern United States. This species produces about one egg per week from April to early October, and eggs hatch continuously throughout the season (Mitchell et al. 2018). Embryos exhibit considerable plasticity in response to incubation conditions over the season (Pearson & Warner 2018).

This study was conducted on an island in the Intracoastal Waterway located within the Guana Tolomato Matanzas National Estuarine Research Reserve near Palm Coast, Florida (Fig. 1A). Based on continuous surveys since 2011, the numbers of adult lizards on this island has varied from about 73 to 486 individuals (unpubl. data). The island also has a diversity of habitat types including a central area of dense tree canopy (cedar trees and palm trees) surrounded by open areas. The north, south, and west ends of the island are densely covered in needlerush (*Juncus roemerianus*). To facilitate habitat measurements and egg searches for our study, we installed a permanent grid system across the island using PVC stakes that were spaced 5 m from each other, which created a 9x11 grid of 5x5 m squares across the island (Fig. 1B).

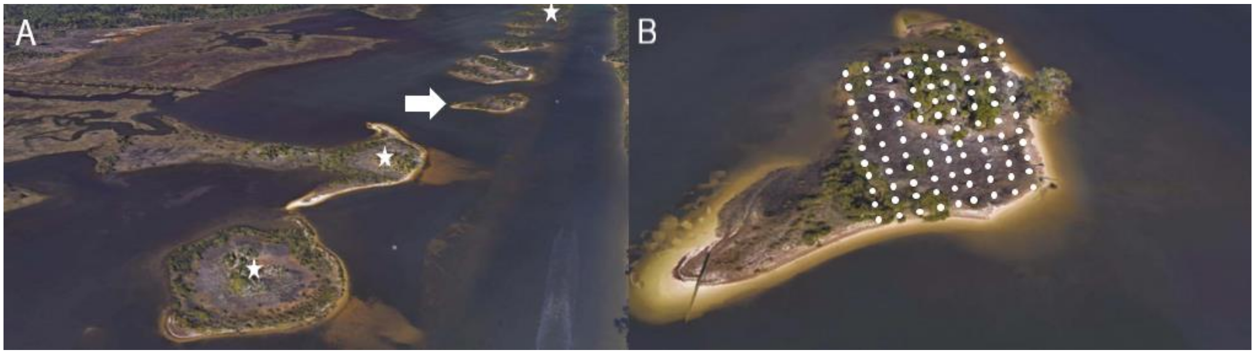

Figure 1. Aerial photographs of the field site. The island marked with a white arrow (panel A) was used to quantify nesting microhabitat and for the egg placement experiment, and the islands with white stars (in addition to the mainland) are locations used to collect the breeding colony. The permanent grid system is represented by white dots (panel B).

Quantifying maternal nesting behavior

To quantify maternal nesting behavior, we searched for *A. sagrei* eggs at our field site (Fig. 1) during early, middle, and late periods of the nesting season in 2018 (June 25-27, July 21-24, and October 3-5, respectively). We randomly located plots to search for eggs to ensure that our sampling was not biased towards any specific microhabitat. During each trip, a random number generator was used to select 20 stakes in the grid and to determine a distance (1-500 cm) and direction (0-359°) from each stake. At each of the 20 randomly-selected locations, a flag was placed at the designated distance and direction from the stake, and a 1x1 m quadrat was placed around it. The quadrat was searched thoroughly for eggs by turning up 1-2 cm of soil with a spoon and searching under any rocks or debris within the quadrat. We also conducted targeted searches for eggs in locations that appeared to be suitable incubation habitat based on information from laboratory studies (Reedy et al. 2013) and anecdotal field observations (Delaney et al. 2013). Targeted searches were conducted independently of the grid system and were opportunistic in nature, with each potential nest site being searched as we came upon a suitable location based on our previous anecdotal observations of nests; the key factors that led us to choose targeted sites were presence of leaf litter, nearby trees, and cover objects (e.g. rocks, fallen palm fronds). When an egg was located, a 1x1 m quadrat was placed around it, and the quadrat was searched thoroughly for eggs as described above for the random searches. A search was only counted as a targeted search when an egg was found, but we searched more areas that did not contain eggs.

When an egg was found using either search method, a soil sample (~2 tablespoons) was collected at the location of the egg, hemispherical photographs were taken with a digital camera (Nikon Coolpix L30) with a 180° fish-eye lens (Zykkor), and Thermocron iButtons (programmed to take hourly temperature readings) were placed next to the egg. When multiple eggs were found near each other (within 25 cm), a single soil sample, photograph, and iButton were used to quantify microhabitat of that site. We conducted 20 random searches and 20 targeted (until we found 20 sites with eggs present) searches in June, July, and October to capture potential temporal variation in nesting behavior. However, in October only 7 maternal nest sites were found, likely because this was the end of the nesting season. Therefore, only 7 successful targeted searches were conducted for this final time period.

Microhabitat measurements were taken at every other stake (n=50 stakes) across the grid (henceforth, “available nest site”) to compare to maternally-selected sites. iButtons were buried under ~2 cm of soil at the base of the stake, and soil samples (~2 tablespoons) were collected. These samples were taken concurrently with samples from maternally selected sites. Temperature was recorded hourly for 15 days for each period after loggers were placed in the ground. Soil moisture was determined by weighing samples before and after drying (to a consistent mass) and calculating the percent water content (Warner & Andrews 2002).

An analysis of variance (R Version 3.4.2) was used to quantify temporal differences in microhabitat parameters between maternally selected and available nest sites. Temperature, canopy openness, and soil moisture were the dependent variables in three separate models; month (June, July, and October), nest type (Maternal or Available), and their interaction were the independent variables. A Least Squares Means test was used for pairwise comparisons among months and nest type. Analyses of variance were also used to quantify differences in microhabitat variables (temperature, soil moisture, and canopy openness) between eggs found individually versus those in aggregations, using the microhabitat variable as the dependent variable and aggregation status as the independent variable.

Animal Husbandry and Egg Collection

In addition to egg searches, we planted eggs across the island landscape to assess the effects of a broad range of microclimate variables on egg survival and embryo development. To obtain eggs for this study, we housed a captive breeding colony of *A. sagrei* at Auburn University. Adult lizards for the breeding colony (321 females and 46 males) were collected from 1-3 April 2018 from nearby islands (Fig. 1A) and the mainland of Palm Coast, Florida. Adults were housed outdoors at Auburn University in screen cages (0.61 m long x 0.61 m wide x 1.22 m high) (~9 females and one male per cage) that contained an upright bamboo perch (1.37 m) wrapped in artificial leaves for hiding and climbing. Each cage contained a plastic nesting pot (19 cm long x 12 cm wide x 9 cm high) filled with potting soil and placed at the base of the bamboo perch. Lizards were fed crickets (dusted with calcium and vitamins) twice per week and misted with water daily if there was no rain.

Nest pots were checked for eggs twice weekly. Eggs found were weighed and placed in petri dishes (radius: 26 mm; height: 15 mm) filled halfway with moist vermiculite (-150 kPa) and sealed with parafilm. Eggs were incubated (Peltier-cooled Incubator IPP55 Plus, Memmert GmbH, Schwabach, Germany) under fluctuating incubation regimes that mimicked conditions measured from the early season (Figure S1). This cool, early-season temperature regime was chosen because it slows development and thus minimized the amount of development that occurred prior to being placed in the field for incubation. All eggs were between 1-14 days (Stage 4-10; Sanger et al. 2007) from oviposition at the time of their placement, and, on average, only 18.6% of the incubation period occurred in the lab prior to egg placement in the field (Figure S2).

Development of embryos under field conditions

On June 22 and August 22, 2018, eggs were transported to the field in compartmentalized tackle boxes with moist vermiculite (-300 kPa), and were weighed before being placed on the island. To control for possible effects of transportation, 40 additional eggs traveled to the field site and then immediately back to Auburn for incubation; hatching success was 92.5%, suggesting minimal, if any, impact of transportation. Four eggs were placed at every other stake on the island grid system starting with the south-westernmost stake (4 eggs at 50 stakes=200 eggs). Eggs at each stake were randomly assigned one of the cardinal directions and buried 25 cm from the stake under ~2 cm of soil. A small piece of hardwire cloth (3x3 cm square of 0.5 cm mesh size) was placed over each egg and buried just under the soil surface to narrow the search area for relocation when eggs were recovered for assessment of survival. This cloth was not in physical contact with the egg and had no measurable impact on the nest environment. Microclimate data (e.g. temperature, percent soil moisture, and canopy cover) were collected from each egg placement location; similar to methods outlined above, ibuttons were buried 2 cm at the base of each stake, and soil samples and canopy photos were taken at the time of egg placement for measurements of substrate moisture and canopy openness, respectively.

Because of the importance of substrate moisture for development (Packard & Packard 1988) and due to its correlation with other microhabitat variables (i.e., open/warm sites tend to be drier than shaded cooler sites), we performed an additional experiment to isolate the effects of moisture on egg hatching success from other variables. To do this, an additional 30 eggs were placed in moisture-controlled chambers and buried at randomly selected stakes. These chambers were petri dishes filled halfway with moist vermiculite (-150 kPa) and sealed with parafilm and duct tape, which controlled moisture level and allowed comparisons with eggs that were subjected to natural moisture conditions. This comparison enabled us to determine the relative importance of moisture vs other microhabitat variables (temperature, substrate) towards egg survival and offspring phenotypes.

Eggs incubated on the island for 18 days (percentage of incubation prior to collection: min: 51.3%; mean: 68.9%; max: 85.7%), at which point they were collected, weighed, and returned to the laboratory where they were placed in fresh petri dishes with moist vermiculite (-150 kPa) and placed in a programmable incubator set at a natural fluctuating incubation regime (Figure S1) until hatching. Collecting eggs prior to hatching was necessary to ensure that hatching success was reliably scored and offspring phenotypes measured. Because several eggs hatched within 24 hours of collection, this confirmed that the timing of collection was appropriate. Moreover, the 18-day incubation period in the field subjected eggs to predation and extreme thermal and hydric conditions, which led to some eggs not being recovered on each trip (though signs of predation were never observed as hardwire cloth always remained in position). Rather than counting those eggs as dead, they were removed from the analyses to take the most conservative approach. Hatchlings were measured (mass, snout-vent length (SVL), and tail length) before being euthanized according to our animal care protocol. We calculated individual body condition as residuals from a regression of hatchling mass against SVL. This protocol was performed in June and August to capture temporal changes in the effect of nest conditions on embryo development and survival.

A general linear model was used to determine differences in microclimate parameters between June and August. A generalized linear mixed model with a binomial distribution (R package lme4; Bates et al. 2015) was used to determine the effect of average daily mean and maximum temperatures, canopy cover, month (June or August), treatment (petri dish or no petri dish), and a month by treatment interaction on hatching success with egg mass as a covariate and stake as a random factor. A similar model was used to analyze survival using only eggs contained within petri dishes. This model provided an analysis of survival for eggs under constant moisture conditions known to be suitable for development. To analyze hatching success of eggs placed in the ground in August (low survival in June prevented further analysis), a generalized linear mixed model was used with average daily mean and maximum temperature, canopy cover, and percent soil moisture as fixed effects, egg mass as a covariate, and a random effect of stake.

Mass gained during incubation for eggs with no petri dish was analyzed using a linear mixed model with initial egg mass, average daily mean temperature, canopy openness, and soil moisture as fixed effects and stake as a random effect. Incubation duration was analyzed by using the number of days before hatching after eggs were removed from the field as the dependent variable and estimated egg stage (see figure S2), average daily mean temperature, canopy cover, percent soil moisture, and initial egg mass as independent variables. Hatchling body condition was analyzed using a linear mixed model with mass as the dependent variable, and with SVL, soil moisture, canopy cover, and average daily mean temperature as independent variables, and stake as a random variable. Similar models were used to analyze these traits for eggs in petri dishes, but soil moisture was not included in these models. A principal component analysis (PCA) was conducted (loadings in Table S1) to collapse the variation in microhabitat variables into uncorrelated axes, but produced the same results as the raw values, so we elected to use raw values for ease of interpretation.

Results

Nest Microhabitat

Females consistently used relatively cool and moist sites to lay their eggs. Maternally selected sites were significantly cooler (β=-1.43±0.19, $F_{1,190}$=53.80, $p<0.0001$; Fig. 2) with higher soil moisture (β=5.36±1.21, $F_{1,199}$=43.47, $p<0.0001$; Fig. 2), and lower canopy openness (β=-6.12±2.66, $F_{1,193}$=8.79, p=0.0222) than what was available across the island. This pattern remained consistent between time periods for temperature ($F_{2,190}$=2.81,p=0.063; Fig. 2). Additionally, temperature for both maternally selected and available sites declined as the season progressed with June being the warmest and October being the coolest (Fig. 2, Table S2). Although soil moisture of maternally-selected sites in June and July differed from that available across the island, this pattern was not evident in October ($F_{2,199}$=9.10, $p<0.0001$; Fig. 2). Canopy openness was consistent in June and October, but a significant interaction was driven by lower canopy openness for maternally selected sites in July ($F_{2,193}$=6.20, p=0.002). Of the 80 eggs that we found, 3.75% were found using the random search method, and 96.25% were found using the targeted method. Most eggs (68.75%) were found under cover objects (e.g., rocks, palm fronds) or in aggregations (21 sites that were searched contained 2 or more eggs; the largest aggregation was 5 eggs). Nests with aggregations versus those with a single egg did not differ in temperature (β=0.16±0.32, p=0.609), moisture (β= -0.26±3.30, p=0.938) or canopy openness (β=4.35±4.52, p=0.341).

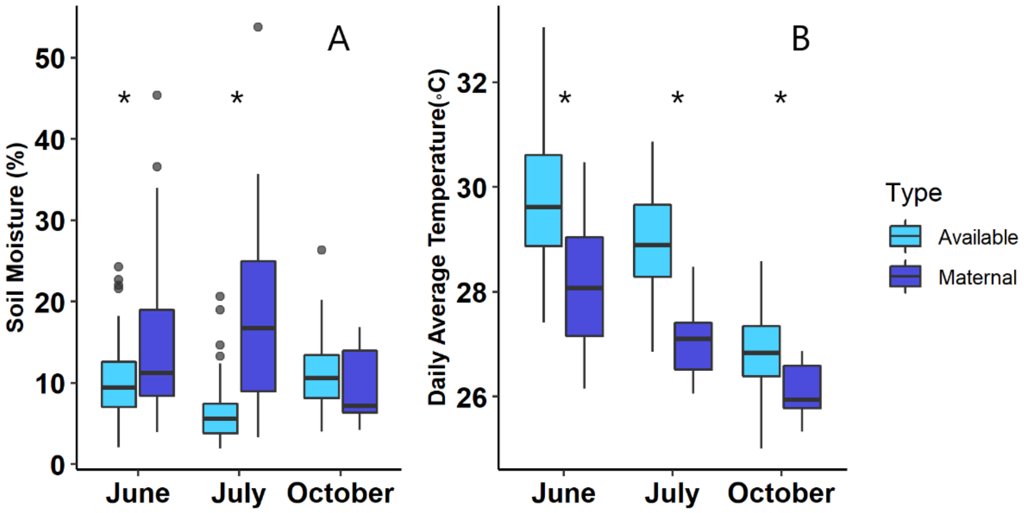

Figure 2. Soil moisture (A) and average daily mean nest temperature (B) for maternally selected nest sites and available sites for each month of egg searches (see text for statistics). The bold line represents the 50th percentile lower and upper bounds represent the 1st and 3rd quartiles respectively. The whiskers extending from the boxes represent the 1st and 3rd quartiles ± 1.5 x interquartile range and the points represent outliers in the dataset.

Effect of Nest Microclimate on Development

Microclimate conditions and egg survival differed between the two egg placement trials (Fig. 3). Eggs placed in June experienced warmer mean ($\beta=1.02\pm0.23$, $p<0.001$) and maximum temperatures ($\beta=4.24\pm0.91$, $p<0.001$) than those in August with no significant difference in soil moisture ($\beta=1.08\pm0.96$, $p=0.263$). Of the 200 eggs placed in the June trial, 138 were recovered, and only 4 hatched (2.89% survival). In the August trial, 182 eggs were recovered and 118 eggs hatched (64.83% survival). Furthermore, hatching success of the eggs placed in the field within sealed petri dishes showed similar temporal trends with 43.3% and 93.3% survival for June and August, respectively (Fig. 3). Eggs incubated in petri dishes were 10.68±2.23 times more likely to survive than eggs incubated with no petri dish ($p=0.003$), and eggs from both treatments had lower survival in June than in August ($F_{1,376}=26.57$, $p<0.001$, Table S3).

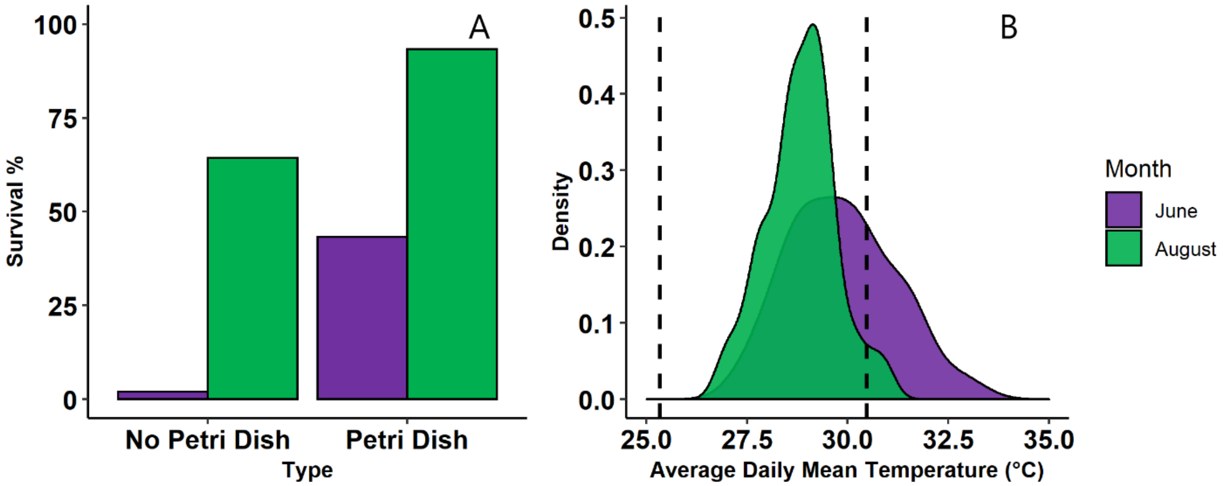

Figure 3. Survival percentages for eggs with and without a petri dish in June and August (A), and a density distribution of average daily mean temperatures for egg placement locations in June and August (B). The vertical dashed lines in panel B represent the range of the average daily mean temperatures selected by females across the season.

Because of low egg survival in June (2% survival for eggs not in petri dishes) further analyses that examined relationships between egg survival and microhabitat variables were restricted to the August trial. Survival of eggs incubated in August decreased with increasing canopy openness ($F_{1,173}$=12.19; p=0.001; Table S3) and increasing temperatures (mean temperature: $F_{1,173}$=3.52, p=0.007; max temperature: $F_{1,173}$=3.03, p=0.014; Fig. 4; Table S3). Mass gained by eggs during incubation in the field increased significantly with average daily mean incubation temperature (β=0.03±0.005, p<0.001; Fig. 5A), but had no relationship with soil moisture or canopy openness. Incubation duration decreased with increasing average daily mean temperature (β=-0.82±0.25, p=0.002), stage at placement (β=-1.01±0.10, p<0.001), and decreased 1.9 days with each 0.1 g increase in egg mass (p=0.020). Hatchling body condition increased with soil moisture (β=0.001±0.0004; $F_{1,35}$=5.76, p=0.022; Fig. 5B).

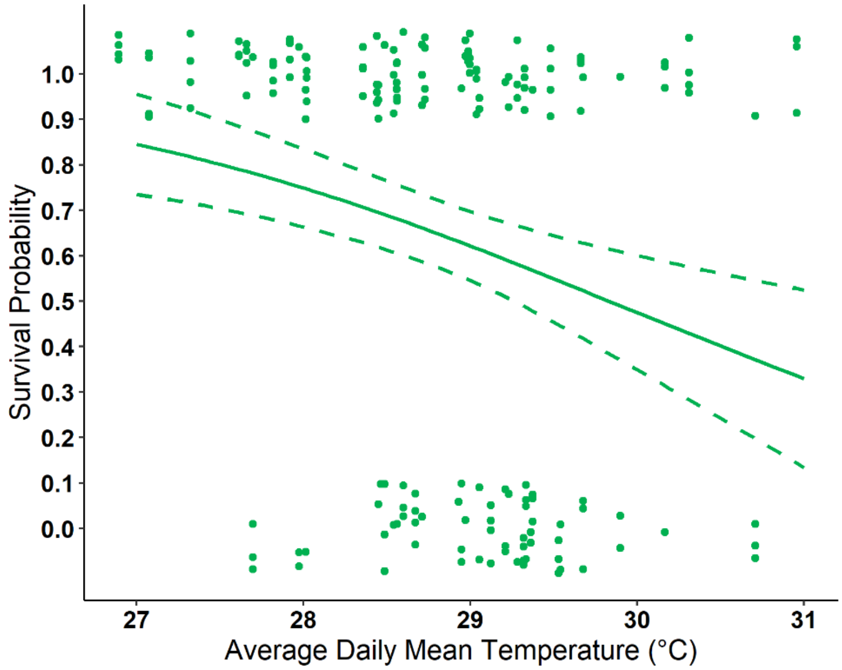

Figure 4. Probability of egg hatching success in relation to average daily mean temperature of the egg placement location. The survival probability curves were generated using generalized linear models of survival plotted against temperature. The bold line represents survival probability and the dashed lines represent the upper and lower (95%) confidence limits. The raw survival data (1=alive, 0=dead) is plotted and jittered.

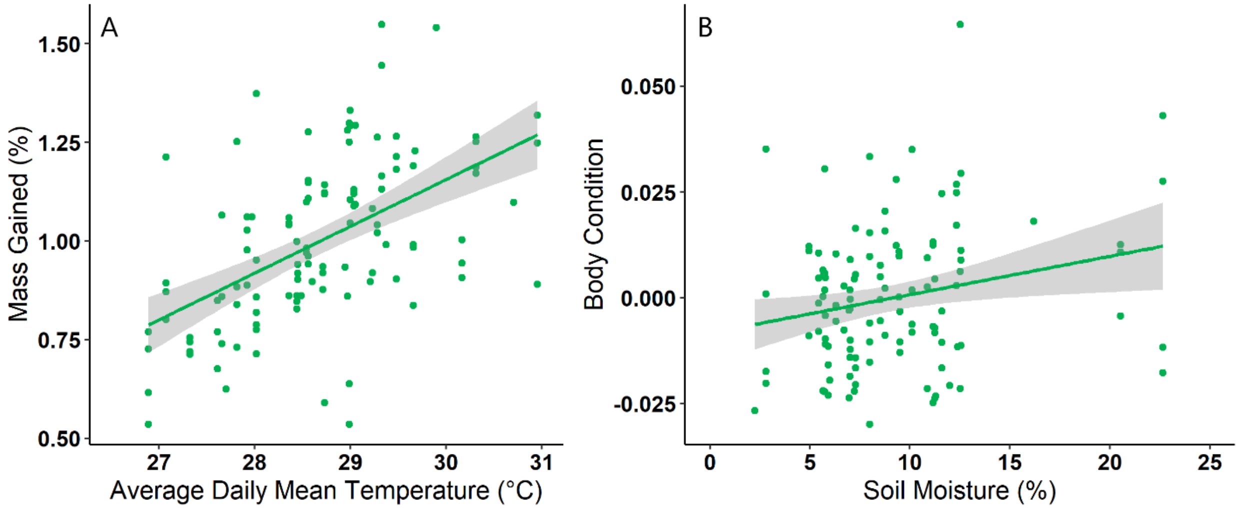

Figure 5. (A) The effect of average daily mean incubation temperature on percent egg mass gain during incubation in the field. Each dot represents an individual egg from the August placement experiment. The grey cloud represents the standard error. **(B)** A linear regression of hatchling body condition (residuals of snout-vent length plotted against body mass) against soil moisture for August hatchlings. Each dot represents an individual hatchling. The grey cloud represents the standard error.

For eggs incubated within petri dishes, egg survival probability was higher in August than in June ($F_{1,51}$=5.69; p=0.004) and decreased with increasing average daily maximum temperatures ($F_{1,35}$=2.75; p=0.045). Body condition of hatchlings from eggs incubated in petri dishes was not affected by temperature or canopy cover (p>0.335). Incubation duration of eggs in petri dishes was not affected by stage at placement (β=0.785±0.530; $F_{1,8}$=3.39, p=0.103), average daily mean temperature (β= -0.416±0.857; $F_{1,8}$=0.77, p=0.407), canopy cover (β=-0.014±0.045; $F_{1,26}$=0.022, p=0.884), or egg mass (β=-62.108±28.96; $F_{1,8}$=4.60, p=0.064).

Discussion

The consequences of maternal nesting behavior on offspring are well documented via correlative and experimental studies on a wide range of organisms (Morse 1990, Lloyd & Martin 2004, Mitchell et al. 2013b, Pruett et al. 2019). Importantly, temporally changing micro-environments impose challenges to reproductive females when selecting nest sites, since the consequences of a particular nest site may be unpredictable. In this study, we quantified maternal nesting behavior in the brown anole by assessing microhabitat of nest sites across three periods during the reproductive season. We also performed an experiment to determine the effects of nest microhabitat on egg survival under field conditions. To our knowledge, we gathered the most comprehensive data set on anole nesting in the field and provide critical insights into this poorly understood aspect of *Anolis* biology. We show that nest sites are relatively cool compared to available microhabitat across the landscape, and that nest sites used by mothers facilitate embryo development and increase egg hatching success. These patterns of microhabitat choice remained consistent across the reproductive season as ambient temperature changed, but eggs suffered substantially greater mortality early in the season.

Maternal Nesting Behavior

In line with our predictions, females chose sites that were relatively cool and moist compared to what was broadly available on the island across the season. In addition, maternally selected sites generally followed the seasonal ground temperature trends observed in the available sites, which steadily decreased across the season. This seasonal decrease in ground temperature contrasts past work at our field site that shows increases in temperature as the season progresses (Pearson & Warner 2018). However, average air temperature in June and July in 2018 did not differ substantially from each other (27.8 vs 28.6ºC), and average October air temperature was considerably lower (25.2ºC) during our study (Fig. S3). This seasonal decline in air temperature in 2018 parallels the decline in ground temperature that we measured. The decline in ground temperature from June to July can also occur due to increased rainfall over the season (a common weather pattern in Florida), which has been shown to dampen nest temperatures in other reptiles (Warner & Shine 2008; Lolavar & Wyneken 2015). Importantly, even with the seasonal decline in temperature, females were still able to find nest sites that were significantly cooler than other sites available across the island, even during the cooler part of the season. Indeed, nearly all nests found were in shaded areas and under cover objects (rocks, leaf litter), which remain cooler than the average ground temperature across the island landscape.

The similar seasonal trends of maternal and available nest temperatures suggest that females choose similar sites across the season rather than changing their behavior, and the thermal characteristics of those sites change with ambient conditions. Importantly, average daily mean temperatures of nest sites used by females are within the range of temperatures that are favorable for development (Warner et al. 2012). Females also nested in sites with relatively high levels of substrate moisture, which has been previously observed in lab studies (Socci et al. 2005, Reedy et al. 2013). However, the consequences of substrate moisture in the field are difficult to interpret for several reasons. First, our index of nest moisture was a snapshot at the time when eggs were found. Given that substrate moisture changes temporally (Robbins & Warner 2010) we can neither directly determine the moisture conditions female select for nesting, nor examine how nest hydration changes during incubation. Second, although percent water content of substrate moisture provides some index of moisture availability to eggs, this can vary depending on substrate type. Indeed, quantifying substrate water potential would provide a better indication of water availability for eggs; this might explain why substrate moisture from the field (mix of sand, shell, soil) was consistently lower than what females prefer in the lab (potting soil; Reedy et al. 2013). Moreover, soil type might be another factor used by females when choosing nest microhabitats (Tiatragul et al. 2019). Despite these caveats, we still detected differences in substrate moisture between nest sites and available sites in the expected direction. Although the difference was consistent among all sampling periods, only October exhibited no statistically significant difference; this may be due to a relatively small sample size at this period, or the cooler temperatures could have reduced variation in moisture between maternally selected and available nesting locations.

Most eggs were found under cover objects (e.g., rocks, palm fronds, seashells, dense leaf litter) and close to the soil surface, suggesting that females prefer areas that conceal their nest. This strategy, along with egg aggregations, has been observed in other species (Doody 2009, Telemeco et al. 2009) and could reduce the amount of time and energy spent during nesting activities (thereby reducing female exposure to predators; Warner & Shine 2008, Huang & Pike 2012). These nesting sites could also provide similar benefits to developing embryos than those of relatively deep nests (e.g., protection from predators, directly sunlight, or moisture loss).

Whether this nesting behavior is favored by selection as a result of reducing threats to nesting females or due to benefits for embryo development is unknown, but both may synergistically explain female preference for cover objects when nesting.

Eggs were often found in aggregations which has been observed previously in other anoles (Robinson et al. 2014, Godfrey et al. 2018, Tiatragul et al. 2019, Swierk et al. 2019). These egg aggregations may be explained by either (1) nest site philopatry where individual females repeatedly choose the same site to nest or (2) communal egg-laying where multiple females choose the same site to lay their eggs. Our study does not directly address egg aggregation behavior, but the variation in egg size suggests that both these explanations are likely. For example, some aggregations contained eggs of similar size; given that egg size substantially increases over time, the low variation in egg size suggests that eggs were of similar ages (Table S4), and hence, laid by multiple females. However, we also observed aggregations with large variation in egg size (Table S4), suggesting that one female could have repeatedly returned to that site to lay eggs over multiple weeks. Further investigation is needed to determine if egg aggregation is due to nest philopatry, communal nesting or both, as well as the fitness consequences of these aggregations (Doody 2009, Mateo & Cuadrado 2012). Finally, while the random search method forced us to look for eggs across the landscape, and hence reduced bias in our search area, this method did not locate many nests. In contrast, the success of our targeted searches suggests that eggs were largely where we predicted them to be.

Effect of nest microclimate on development

The results of our egg placement experiment generally support our predictions that nest microhabitats used by females are beneficial for offspring. Moreover, incubation of a subset of eggs within moisture-controlled conditions (i.e., within sealed petri dishes) enabled us to decouple the effects of moisture with other environmental factors, such as temperature. First, we show that eggs that experienced controlled moisture conditions consistently had higher survival than those buried directly in the soil, suggesting that sustained moisture to eggs conveys a survival advantage (Reedy et al. 2013). Thus, females that select moist nest sites could improve the success of her offspring. Secondly, eggs within and outside petri dishes had higher survival in August than in June, which suggests that temperature may have also influenced variation in egg hatching success. For instance, the distribution of August nest temperatures was more similar to the temperature range selected by mothers across the season (c.f., Fig. 2b and 3b) than the June temperature distribution – this pattern coupled with higher egg survival in August provides evidence that nesting females select thermal conditions that facilitate hatching success. Indeed, maximum temperatures at many of the stakes during the June experiment were considerably higher than the lethal limit for brown anole embryos (Hall & Warner, 2019). Moreover, substrate conditions appeared very dry when eggs were collected during the June trial, which also could have contributed to the low egg survival at this time. Although we did not detect differences in substrate moisture between June and August trials, these measurements were taken at the time eggs were placed in the field, and subsequent nest moisture was not monitored.

Although relatively high mean and maximum temperatures negatively affected egg hatching success, maximum temperatures likely drove most of the variation in embryo survival (Angilletta et al. 2013, Hall & Warner, 2018). Thus, females may improve offspring survival by choosing sites that are less likely to reach high maximum temperatures. Because these two metrics are tightly correlated, avoiding sites with high maximum temperatures would reduce overall mean nest temperature and increase incubation duration (Noble et al. 2018). Consistent with this prediction, we show that incubation duration in the field increases with decreasing temperatures. Such an effect has potential fitness consequences, as prolonged incubation time might increase the risk of exposure to predators or adverse weather conditions (Doody 2011). Consequently, this presents a major challenge for nesting females because they must select thermal conditions that optimally balance those that favor hatching success (cooler temperature) vs those that shorten the length of incubation (warmer temperature). This is particularly critical late in the nesting season when temperatures are comparatively low; not only are embryos developing slowly, but late-produced eggs hatch late in the season, which puts hatchlings at a further survival disadvantage (Pearson & Warner 2018). Thus, the shift in ambient temperature across the nesting season can generate significant temporal changes in how selection may operate on maternal nesting behavior. Temporal shifts from small to large egg size over the season, as reported previously (Mitchell et al. 2018), could enable offspring to cope with late-season nest temperatures, as larger eggs had shorter incubation durations. However, stochasticity in ambient conditions across time makes this a complex system that may sometimes constrain female nest choice.

The phenotypic consequences of the nest environment on offspring (Noble et al. 2018) adds another level of complexity to understanding how selection operates on maternal nesting behavior. Higher temperatures also increased the percentage of mass eggs gained during incubation (via water uptake), which could have been due to increased metabolic rate at higher incubation temperatures (Warner et al. 2012; Hall & Warner 2019). This effect on mass gain of eggs, however, did not have phenotypic influences on hatchlings, and thus, may not be biologically meaningful. Consistent with lab experiments (Warner et al. 2012), however, substrate moisture positively influenced hatchling body condition but the effect size was extremely small. Thus, this small range of body conditions may not generate much variation in offspring survival (Pearson & Warner 2018). Given these minimal phenotypic effects of nest conditions in the field, most variation in fitness due to nesting behavior is likely driven by environmentally-induced variation in survival during embryonic development, rather than at post-hatching stages.

Conclusions

Maternal nest-site choice has an important influence on development and early life of offspring in oviparous species. While nesting behavior and its consequences are relatively well studied in some organisms (e.g., turtles; Kolbe & Janzen 2002, Janzen & Morjan 2002), the reproductive biology of anoles (single egg clutches produced over a long season) provides a unique opportunity for examining temporal variation in this important maternal effect. Our large data set on anole nests in the field shows that females consistently select cool and moist sites for nesting across the season, and that these microhabitat choices enhance egg survival. In addition, eggs are almost exclusively laid under cover objects and commonly found in aggregations. These aspects of nest-site choice may further facilitate egg survival by retaining moisture and reducing exposure to temperature extremes, which are two factors that we demonstrated to positively impact hatching success in the field. Thus, this study adds to a growing literature demonstrating that maternal nesting behavior enhances fitness in the wild populations, and provides evidence that this important maternal effect is under selection in temporally-changing environments.

Chapter 2 Supplemental Material

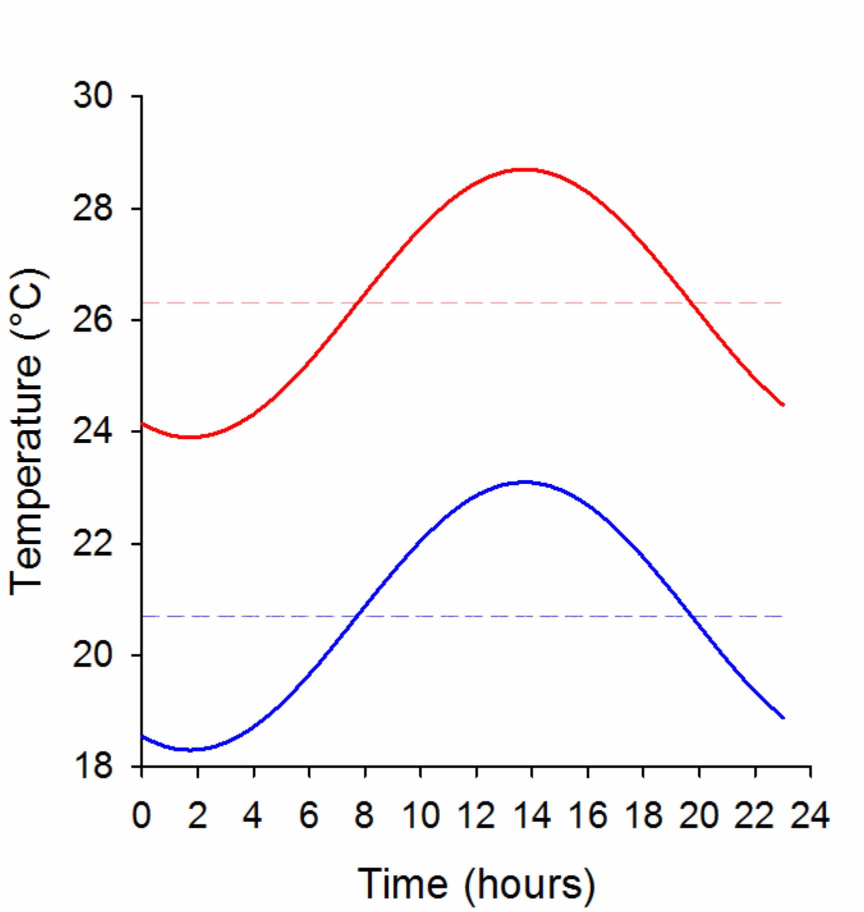

Figure S1. The two incubation regimes used before and after eggs were placed in the field. The blue curve represents the cool regime that eggs experienced before being placed in the field. The red curve represents the warmer regime that eggs experienced after being recovered from the field. Horizontal dashed lines represent the mean temperature of each regime (20.7 and 26.3ºC), and the amplitude of each curve is 2.4°C.

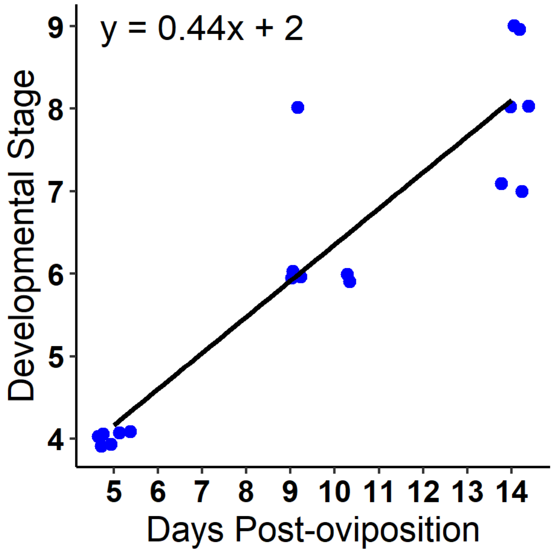

Figure S2. To determine how much development occurred before eggs were placed in the field, we incubated a subset of 18 eggs for 5, 10, and 14 days (6 eggs each) and staged embryos according to the guidelines outlined in Sanger et al. (2007). Embryo stage was plotted against number of days post-oviposition; the equation for the regression line was used to estimate the stage of each egg that was placed in the field. Points are jittered (0.1) so each point is visible. At oviposition, embryos are stage 4, so all eggs under 5 days old were assigned a stage of 4.

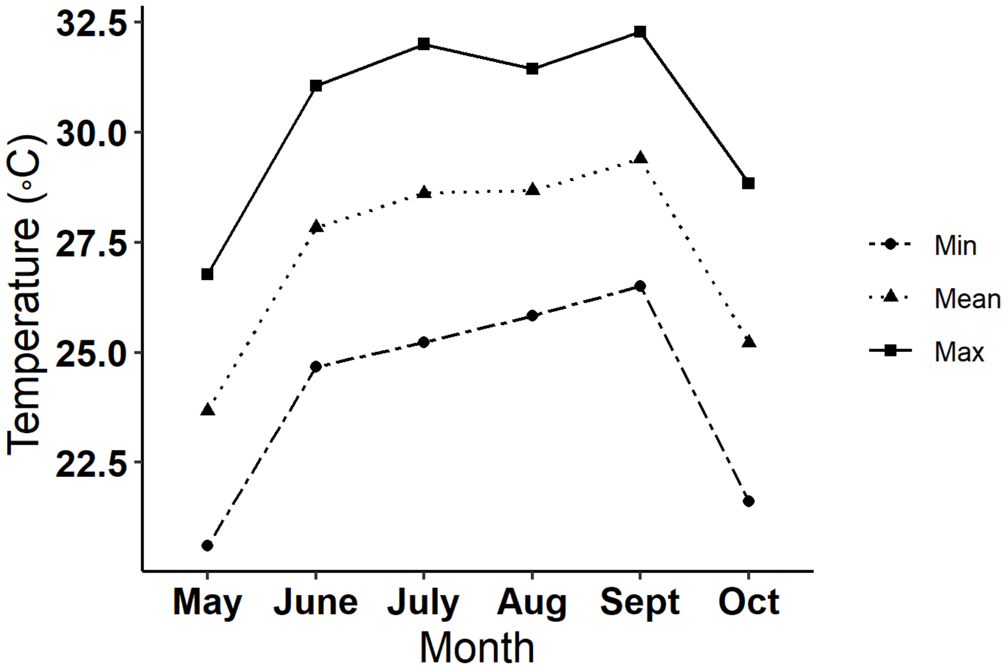

Figure S3. Average daily minimum, mean, and maximum air temperatures across the nesting season for our field site. Temperature data were obtained from the National Climatic Data Center for Palm Coast, FL.

Table S1. Loading scores on each principal component (PC) axis. Only the first two PC axes were used in statistical analyses. A principal component analysis (PCA) was used to collapse the variation in microhabitat variables into uncorrelated axes using the *FactorMineR* package (Lê et al. 2008). The PCA included daily average minimum temperature, daily average maximum temperature, daily average mean temperature, and canopy openness values for each nest.

	PC axis 1	PC axis 2	PC axis 3
Mean temperature	0.9365	0.1379	-0.2860
Maximum temperature	0.9347	-0.2532	-0.1984
Minimum temperature	-0.0455	0.9717	-0.2240
Canopy openness	0.5701	0.2661	0.7772
% Variance explained	**52%**	**27%**	**19%**

Table S2. Quantitative descriptions of nests found in each month. Nests were found using both random and targeted searches (see main text for details). The slightly lower sample sizes for thermal data was due to 4 failing ibuttons.

	June				**July**				**October**			
Variables	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max
Canopy Openness (%)	22	42.22	21.56	63.86	21	24.62	5.30	58.92	6	39.03	25.51	64.45
Daily Mean Temp	20	28.23	26.15	30.47	27	27.05	26.05	28.48	6	26.10	25.32	26.87
Daily Min Temp (◦C)	20	24.30	23.50	25.00	27	24.13	22.50	25.00	6	19.67	18.50	20.50
Daily Max Temp (◦C)	20	36.38	29.00	44.50	27	33.26	29.00	44.00	6	32.67	29.00	39.00
Soil Moisture (%)	22	15.69	3.31	53.77	28	18.33	3.94	45.39	7	9.83	4.18	16.84

Table S3. Summary results for (1) a binomial GLM analyzing the effects of canopy openness, daily mean temperature, daily max temperature, soil moisture, and initial egg mass on egg hatching success during the August trial, and (2) a binomial GLM analyzing the effects of canopy openness, daily mean temperature, daily max temperature, month (June or August), treatment (petri dish or no petri dish), a treatment by month interaction, and initial egg mass on egg hatching success. The effect size for "month" was calculated with August as a reference, and effect size for treatment was calculated as "no petri dish" as a reference.

	Effects during August placement			Effects of month (June vs August) and treatment (eggs in petri dish vs not in petri dish)		
	Estimate ±	Z value	p-value	Estimate ± SE	Z value	p-value
(Intercept)	3.07±2.12	1.45	0.147	0.73±0.25	2.94	0.003
Canopy Openness	-0.11±0.03	-3.28	0.001	-0.04±0.02	-2.52	0.012
Daily Mean Temp	-2.01±0.74	-2.71	0.007	-0.72±0.34	-2.12	0.034
Daily Max Temp	0.57±0.23	2.47	0.014	0.05±0.09	0.53	0.597
Soil Moisture (%)	-0.10±0.09	-1.12	0.264	-	-	-
Month	-	-	-	-4.74±0.66	-7.23	<0.001
Treatment	-	-	-	2.37±0.80	2.95	0.003
Month x Treatment	-	-	-	1.61±1.08	1.49	0.135
Initial Egg Mass	-5.24±9.24	-0.57	0.571	-0.50±6.74	-0.07	0.941

Table S4. Descriptive statistics of each egg aggregation found throughout the nesting season.

	# of Eggs	Mean Mass (g)	Min-Max (g)	Variance
Aggregation 1	2	0.315	0.19-0.44	0.0156
Aggregation 2	2	0.230	0.21-0.25	0.0004
Aggregation 3	2	0.310	0.28-0.34	0.0009
Aggregation 4	2	0.320	0.30-0.34	0.0004
Aggregation 5	2	0.215	0.15-0.28	0.0004
Aggregation 6	2	0.245	0.21-0.28	0.0012
Aggregation 7	2	0.315	0.27-0.36	0.0020
Aggregation 8	2	0.315	0.28-0.35	0.0012
Aggregation 9	2	0.245	0.22-0.27	0.0006
Aggregation 10	2	0.385	0.25-0.52	0.0182
Aggregation 11	2	0.195	0.19-0.20	<0.0001
Aggregation 12	2	0.325	0.27-0.38	0.0030
Aggregation 13	3	0.457	0.26-0.67	0.0282
Aggregation 14	3	0.290	0.26-0.34	0.0013
Aggregation 15	3	0.333	0.19-0.46	0.0123
Aggregation 16	3	0.333	0.30-0.35	0.0006
Aggregation 17	3	0.353	0.33-0.38	0.0004
Aggregation 18	3	0.245	0.23-0.26	0.0002
Aggregation 19	4	0.273	0.16-0.43	0.0010
Aggregation 20	5	0.316	0.18-0.48	0.0145
Aggregation 21	5	0.375	0.21-0.50	0.0124

Chapter 3

The effects of predator presence on nesting behavior and survival in the brown anole (*Anolis sagrei*)

Manuscript to be submitted to *Proceedings of the Royal Society B*

Introduction

Evolutionary theory predicts that an individual's fitness will be optimized by balancing the payoff of current reproductive efforts with that of future reproductive prospects (Lack 1947). The cost of reproduction creates a trade-off between proximate and potential reproductive success where animals must adjust to different pressures to increase their reproductive output and overall fitness (Linden & Moller 1989). For example, predation risk can impose costs across multiple reproductive stages that females must balance to optimize fitness (Magnhagen 1991). In garter snakes, gestation impairs locomotor performance and puts gravid females at greater risk for predation (Seigel et al. 1987). Adult common redshanks adjust the frequency of their nest attendance during offspring incubation to reduce their own risk of predation when predator density is high (Cervencl et al. 2011). Regardless of reproductive status, predator presence and density can shape the phenotypes and behaviors of prey species, but these phenotypic adjustments may also incur some fitness costs (Ydenberg & Dill 1986; Biro et al. 2004). These phenotypic and behavioral modifications can be seen across taxa during reproductive bouts when animals are particularly sensitive to predation pressures (Grostal & Dicke 1999; Creel et al. 2009; Lima 2009; Mukherjee et al. 2014; Dulude-de Brion et al. 2020).

The nest site chosen by a female has important impacts on offspring, but it can also influence maternal survival by increasing vulnerability to predators while she is searching for and preparing a nest site (Refsnider et al. 2015). Though predation pressure is more often examined through the lens of predation on eggs (Spencer 2001; Wesolowski 2002), nesting behavior has also been shown to change in the presence of predators to reduce risk to the mother (Delaney & Janzen 2020). Such risk may be particularly pronounced in species that exhibit parental care (e.g., most birds), as parents often defend nests from predators at the potential expense to themselves and their offspring (Huang, 2006; Huang et al. 2013). Indeed, some species, particularly those that are relatively long-lived, will modify their reproductive phenology (Michel et al. 2020), alter investment (Clutton-Brock 1984), or even forgo reproduction altogether (Scheuerlein et al. 2001) in response to conditions that are unfavorable for survival to future reproductive bouts.

The predator-prey relationship between the ground-dwelling curly tailed lizard (*Leiocephalus carinatus*) and its prey, the arboreal brown anole (*Anolis sagrei*) has been relatively well studied, and serves as a useful system to examine the effects of predation pressure on nesting behavior and reproduction. Previous studies demonstrate that exploratory behavior of *A. sagrei* is favored by natural selection in the absence of these predators and ground avoidance is favored when they are present (Lapiedra et al. 2018). Additionally, *A. sagrei* increases perch height and they spend significantly less time on the ground when ground predators are present than when they are absent (Losos et al. 2004). This behavioral shift also shapes the evolution of limb morphology as brown anoles become more arboreal in the presences of predators. Importantly selection on exploratory behaviors of *A. sagrei* in response to *L. carinatus* are more pronounced in females than males, and females have greater mortality than males when these predators are present (Lapeiadra et al. 2018). These sex differences in selection on behavior have been attributed to the possibility of females having more difficult time obtaining food resources when predators are present (Lapeiadra et a. 2018). However, an alternative explanation is that females must spend time on the ground in search of a nest sites, which can increase the risk of predation. Importantly, given the long reproductive season of *A. sagrei* where females lay an egg approximately once per week, individuals must repeatedly expose themselves to ground predators. Thus, females must weigh the cost of risky nesting behaviors with the benefits for their offspring. Ground nesting habitats provide more stable microclimates for developing embryos, but they also risk exposure to more predators for females. Anoles typically nest on the ground under rocks or debris (Pruett et al. 2020), but curly tail presence has the potential to shift these behaviors to avoid predation while nesting. Furthermore, in studies examining the relationship between brown anoles and curly tailed lizards, females had significantly higher rates of mortality in the presence of these predators (Lapeiadra et a. 2018).

Additionally, in the invasive range of anoles throughout Florida, they have moved to latitudes far beyond the northern border of the curly tail invasive range in Southern Florida. This means that Northern brown anoles have been isolated from the threat of curly tails for several decades. These species have overlapped relatively recently in their evolutionary history, but the prey species now thrives outside of the predator’s natural and invasive range. Additionally, nesting habitats could vary between the native and invasive ranges of anoles. In the native Bahamian range of anoles, nesting habitats are mostly confined to ground sites due to low, scrubby vegetation (Losos et al. 1997). This constrains females and forces them to descend from the safety of their perching habitat to nest. Conversely, trees are present across their invasive range in Florida, providing a potential option for nesting. However, studies of anole nesting behavior in the invasive range of brown anoles show that they frequently nest on the ground, even in habitats where ground predators are present (Pruett et al. 2020; Tiatragul et al. 2020). With more options for nesting habitat, females have the ability to modulate facets of their reproduction to minimize the risk of predation. They could choose nest sites that reduce their risk of predation but provide less microclimate stability for developing embryos. They could maintain the behaviors dictated by habitat constraints (ground nesting) and reduce the number of reproductive bouts, thereby reducing predator exposure. This presents a unique opportunity to examine nest site preference in the brown anole from a mechanistic perspective to determine the respective roles of evolution and plasticity in shaping anti-predator behavior in this species.

In this study, we examined the effects of predator (curly tail lizard) presence on anole nesting behavior, survival, and reproductive investment. We gave anoles two nesting options, one on the ground and one above ground, to assess their natural preference before adding predators to the experiment to test how predation pressure might shape that preference. We hypothesized that curly tail presence would lead to shifts in anole nesting behavior, perching behavior, fecundity, and adult survival. We predicted that anoles would prefer to nest on the ground in the absence of curly tails, but would display a preference for nesting above ground when curly tails were present. Similarly, we predicted that anoles in enclosures with curly tails would perch higher than those without curly tails. Finally, we predicted that adult survival and reproductive output would be lower in enclosures with curly tails present.

Methods

Anolis sagrei were collected from Palm Coast, Florida from March 6-9, 2020 and housed indoors at Auburn University prior to our experiment. During this time, females were housed in a common environment and laid eggs for a different experiment until they were weighed, measured, and transferred to an outdoor facility on July 24, 2020. Adults anoles were distributed into eight outdoor, seminatural walk-in enclosures (1.8 m long x 2.4 m deep x 2.4 m tall; 0.5 cm mesh) that were lined with large sheets of corrugated plastic to visually isolate anoles from neighboring enclosures. The plastic sheets were marked from the floor to the ceiling at 25 cm increments to aid in observations of perching height. Each enclosure contained two artificial trees (2 m tall) and 3 bamboo perches (3 m long) that were affixed to the walls (two at diagonals and one going from the ceiling to the floor).

A smaller mesh cage for curly tail lizards was placed within each of the large enclosures. These enclosures contained cinder blocks and artificial foliage for perching and hiding. The mesh size for these enclosures was small enough to prevent curly tail lizards from escaping, but large enough for anoles to enter; this cage kept the curly tailed lizards confined to the ground and prevented them from eating the anoles unless anoles entered their cage. Each enclosure contained two nesting containers filled with a mixture of moist potting soil and peat moss; these nesting containers were covered in hardwire mesh to prevent curly tails from accessing eggs or anoles in the process of laying eggs. Curly tail lizards (*L. carinatus*) were captured in Miami, Florida and transferred to Auburn University facilities where one adult was added to four of the eight smaller cages within the walk-in enclosures on August 10, 2020 (henceforth predator enclosures). The four enclosures without curly tails served as a control group (henceforth predator-free enclosures).

In each enclosure, there was one nest pot on the ground within the curly tail cage and one mounted to a bamboo perch above the ground. Each enclosure also contained two 1.8-m artificial *Ficus* trees. Nest pots were checked twice weekly for eggs (starting on July 27, 2020) to assess nest pot choice in each cage. Nest checks were done for three weeks before curly tails were added to the experiment, five weeks after they were added, and three weeks after they were removed. All eggs were binned into a “week” of the experiment to assess temporal shifts and were weighed and incubated for another experiment. Additionally, we conducted three surveys (on 13, 19, and 20 August) to quantify the perch height of *A. sagrei* in each enclosure. The observer spent three minutes per enclosure observing lizards and counting the number of individuals perched at each of the levels marked on the enclosure wall. Observers made special effort (e.g., walking slowly, keeping maximum possible distance) to minimize disturbance to the lizards during these surveys. When the experiment was terminated on 31 September 2020, all anoles were collected from October 1st to October 12th to quantify survival. Individuals that were not found were assumed dead (possibly depredated by *L. carinatus* if they entered its cage). All surviving lizards were euthanized according to approved protocols and deposited in the Auburn University Museum of Natural History.

We used a Chi-squared test to determine nest site preference (ground vs arboreal nest sites) before the predator treatments were applied. Generalized linear mixed models with a binomial error distribution were used to analyze the effects of predator presence and week on nesting behavior (with enclosure as a random effect) before curly tails were added, after curly tails were added, and after curly tails were removed. A generalized linear model was also used to analyze the effects of treatment on adult survival. Perching height and egg quantity (eggs per week) were analyzed using a general linear mixed model with predator presence and week as dependent independent variables. An interaction between week and predator presence were tested but removed from all models due to lack of significance.

Results

Prior to predator introduction, anoles preferred to nest in the ground containers (82% ground) (χ^2=37.37; df=1, p<0.001). After predator introduction, females in predator enclosures were 3.8 times as likely to lay eggs in the above ground container than those in predator-free enclosures (±1.34 SE; df=262; p<0.001). Additionally, anoles in both treatments were 1.21 times more likely to nest above ground with each passing week (±1.06 SE; df=262; p=0.002). After curly tails were removed, the difference between predator and predator-free enclosures was no longer present (β=0.73± 1.56 SE; df=46; p=0.641). There was also no significant effect of week on nest choice (β=0.73±0.51 SE; df=46; p=0.157). Anoles that were in predator enclosures perched significantly higher than anoles in predator free enclosures (β=14.31±6.97 SE; df=226; p=0.041), and this pattern remained consistent across each of the three observation dates (β=-1.63±1.13 SE; df=226; p=0.151). Predator presence did not affect the number of eggs produced per cage each week (β=-1.50±0.88 SE; df=37; p=0.098). The number of eggs produced each week decreased by 0.14 eggs per week, but this relationship was marginally non-significant (±0.31 SE; df=79; p=0.052). Finally, anoles in predator-free enclosures were 3.06 times more likely to survive to the end of the experiment (±1.35 SE; df=191; p<0.001) than those in the predator enclosures.

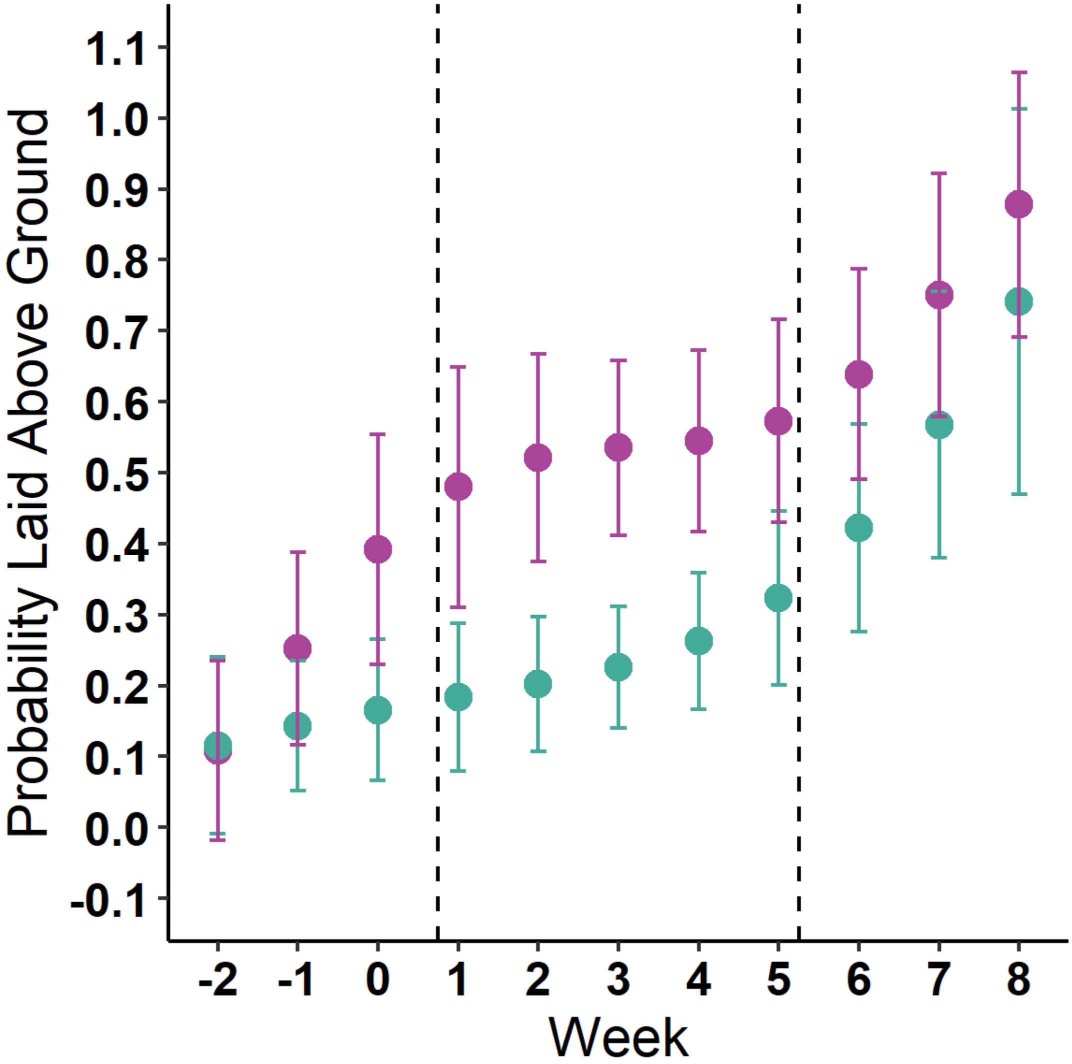

Figure 1. Probability that eggs would be laid in the above-ground container during each week of the experiment in predator enclosures (magenta) and predator-free enclosures (teal). Each point represents the probability that eggs would be laid above ground, and the error bars represent the 95% confidence interval. Probabilities were generated using a generalized linear models of nest site choice against week, broken down into the two treatments. The vertical dashed lines represent the time points when curly tails were added and removed from the experiment.

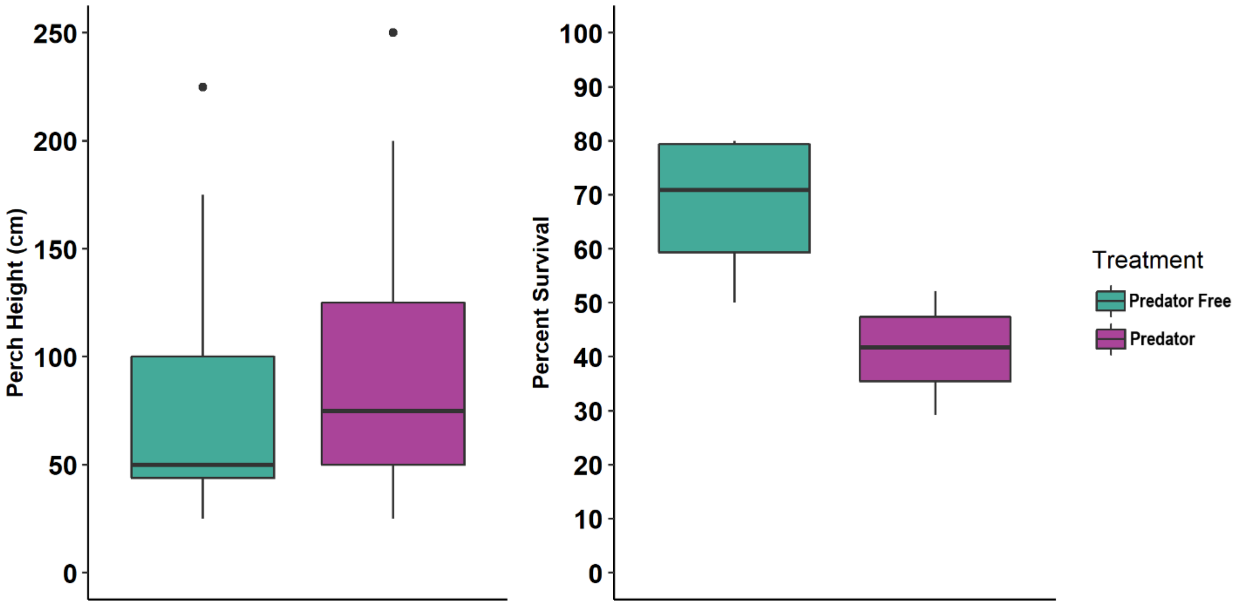

Figure 2. Perch height (A) and adult survival percentage (B).The bold line represents the 50th percentile while lower and upper bounds represent the 1st and 3rd quartiles respectively. The whiskers extending from the boxes represent the 1st and 3rd quartiles± 1.5 x interquartile range and the points represent outliers in the dataset.

Discussion

An animal's behavior is an integral component of its fitness, and can determine their ability to acquire resources, maintain territory, and even reproduce successfully (Huey et al. 2003). In this study, we examined nesting behavior in the brown anole lizard in response to the presence of a predator, and hypothesized that curly tail presence would shape nesting behavior, perching behavior, fecundity, and adult survival. Our results support the predicted response to predator presence in nesting behavior, perching behavior, and adult survival.

Before predators were introduced into the experiment, anoles showed a strong preference for the nesting containers on the ground. However, once curly tails were added, there was a sharp increase in preference for the above-ground container in enclosures with a predator. Additionally, anoles in enclosures without predators increasingly showed preference for the above ground pot, though not at the same rate as those in the predator treated enclosures. There are two possible explanations that we propose for this trend over time regardless of treatment. First, it is conceivable that the above ground pots have environmental factors not measured in this experiment that anoles find more suitable for embryo development. In the field, anoles frequently use ground nesting habitat, typically under solid cover objects (Pruett et al. 2020). However, this may be due to a lack of suitable habitat available for above-ground nesting in the wild. In this experiment, we provided identical nesting substrate on and above the ground, so that constraint no longer existed. A second possible explanation is that anoles in enclosures without predators may have been able to sense that the predator species was nearby. Though the enclosures were separated physically with barriers to prevent the possibility of visual cues, it is possible that anoles could sense the curly tails another way (e.g. olfactory). Regardless, we noticed a marked increase in preference for arboreal containers, which we believe warrants further investigation.

Despite both predator treatment groups exhibiting a similar temporal trend in nest site preference towards the above-ground pot, anoles in the enclosures with predators exhibited significantly higher preference for these pots away from the risk of predation during the time when curly tails were present. This result paired with the general trend of preference for all anoles suggests that there is some degree of behavioral plasticity in nest-site preference based on the proximity of predation threat. This idea is also supported by the perching behavior during the experiment. Anoles perched higher in enclosures with curly tails, suggesting that they were able to adjust their behavior in the presence of a predator and that individuals who were isolated from predators showed no such behavioral adjustments. This perching behavior shift has also been shown for anoles in the presence of curly tails in the field (Losos et al. 2004). Studies have also shown that anoles are less likely to exhibit “risky” behavior such as ground exploration in the presence of curly tails (Lapiedra et al. 2018). Furthermore, selection against exploratory behavior was stronger for females than it was for males. Bahamian sites are shrubbier with fewer trees, meaning that any arboreal sites would be extremely limited. This habitat constraint would push females to nest on the ground, making them more likely to be preyed upon.

Despite these behavioral shifts in favor of above-ground nesting and higher perching, there was still an effect of predator presence on adult anole survival to the end of the experiment. There are a few possible explanations for lower anole survival in the presence of curly tails. First, and most directly, curly tails could have eaten anoles that entered their enclosures. Though we did not observe any evidence of this during the experiment, we know it happens in the field. We observed higher perching and higher prevalence of above-ground nesting in these enclosures, but this may have been due to selection against individuals whose behaviors were poorly suited for predation pressures. Another possibility is that anoles in these enclosures died due to stress effects (not measured in this experiment) of being in a confined space with a natural predator, even though the enclosures were large enough for them to perch safely. One result that favors the former explanation is that for fecundity comparisons between the two treatments. There was no significant difference in egg production between anoles in enclosures with curly tails and without. Despite being subjected to stressful predation pressures, anoles were still capable of producing eggs at the same capacity as those without predation pressure.

Conclusions

The study system used in this experiment provides a unique perspective for behavioral plasticity in response to predation pressure. Because anoles nest several times across a long season, we were able to detect behavioral responses at a higher resolution than would be possible for species that lay fewer, larger clutches. Our results show that predation pressure shapes reproductive behavior in anoles and supports previous work that shows the effects on perching and exploratory behavior. Despite these behavioral responses to predator presence, we still observed reduced survival in the predator enclosures when compared to predator-free enclosures. Whether the survival costs were due to stress or direct predation, this result suggests that selection is acting through predation and individuals that exhibit behavioral plasticity in response to predator presence are likely to have higher fitness.

Chapter 4

Spatial and temporal variation in phenotypes and fitness in response to developmental thermal environments

Manuscript in revision in *Functional Ecology*

Introduction

The environment can shape distributions of phenotypes within populations by acting as a determinant of phenotypic variation (via plasticity) or as an agent of selection on phenotypic variation (via natural selection) (Lewontin 2001). Both pathways can generate phenotypes that are suited for a given environment, but the pace at which these mechanisms give rise to suitable phenotypes can differ. For example, phenotypes produced by natural selection are shaped over multiple generations depending on the strength and consistency of selection as well as the amount of heritable variation for the phenotype of interest (Kingsolver et al. 2001). In contrast, plasticity can rapidly change phenotypic values of a trait at different points within an individual's lifetime (Pigliucci 2005). These processes are not independent of one another, as natural selection can also shape variation in plasticity so that organisms respond to their environment in adaptive directions. Indeed, theoretical and empirical studies demonstrate that plasticity is an adaptation (shaped by selection) to heterogeneous environments that enables suitable phenotypes to develop in response to environmental cues (Pigliucci et al. 2010).

Environmentally induced phenotypic variation, or phenotypic plasticity, is observed across taxa and can occur at all life stages (West-Eberhard, 2003). Developmental stages are of particular importance due to the sensitivity and lability of embryos and the potential for environmental effects to persist throughout an individual's life (Duffy et al. 2002; Clutton-Brock, 2007; Galloway & Etterson, 2007). For example, many insects develop into a winged (dispersing) or unwinged (non-dispersing) morph in response to early-life environmental cues such as crowding or declining nutrition (Roff 1990; Roff & Fairbairn, 1991; reviewed by Brisson, 2010). This phenotypic effect during development determines the trajectory of an individual's life and future reproduction as winged individuals often have lower fecundity than their unwinged counterparts. Such phenotypic changes induced by developmental environments can have different consequences at different life stages due to energetic trade-offs (e.g., effects on morphology and dispersal at one stage, which has later consequences on reproduction). This intersection of plasticity and life history generates tradeoffs that can determine an individual's fitness in a given environment. Thus, studies that examine costs and benefits of developmental plasticity across life stages will provide key insights into how selection has shaped organismal responses to environmental variation.

Plastic responses to temperature, a critical component of the developmental environment, can influence variation in many traits such as circadian clocks (Gil & Park 2018) and morphogenesis (Casal and Balasubramanian 2019) in plants, color morphs (Zverev et al.,2018) and phenology (Buckley et al. 2017) in insects, locomotor performance in vertebrates (Johnston & Temple, 2002), and many other fitness-relevant phenotypes. While not a new subject of research, our understanding of the effects of temperature on development has several noticeable gaps. First, the limited number of treatments used in most studies cannot establish continuous thermal reaction norms for traits (Régnière, Powell, Bentz, & Nealis, 2012; While et al., 2018; Wickander, Rasmussen, Marteinsdóttir, Ehrlén, & Tack, 2020), and consequently, these studies lack resolution for identifying the effects of developmental temperatures on different phenotypes or thermal maxima for successful development and survival (Mueller et al., 2019). Importantly, because selection operates on phenotypic variation, well-resolved reaction norms for different phenotypes will provide insight into the evolution of plasticity. Second, studies that examine the effects of developmental conditions across life stages are rare, mainly due to logistical difficulties associated with following individuals through their lifespans (Uller, 2008; Mitchell et al. 2018), particularly under natural conditions (Warner & Shine 2008, Pearson & Warner 2018). Studies that address these challenges would provide rare insight into the evolution of plasticity, because the phenotypic effects of the developmental environment are offset from the time when selection might act on those phenotypes at later stages (Schulte et al., 2011). Related to these points, the optimal environment for a trait at one life-history stage might be very different from that at another life stage (Marshall & Uller 2007). These differences in the optimal environment across life stages may be due to changes in age-specific energetic demands for different functions or due to shifts in habitat use across life stages. This variation over an individual's lifetime is important because it can influence a population's response to developmental temperature.

Lastly, the extent of, and the contributors to, variation in reaction norms across populations are not well understood (Scheiner 1993). Thermal reaction norms for different traits can vary significantly across broad geographic ranges (Conover & Heins, 1987; Sinervo & Adolph, 1994; Du et al. 2010; van de Pol, 2012; Gunderson & Stillman 2015; Bodensteiner et al. 2019; Carter et al. 2019) as well as across smaller spatial scales (Fairbairn, 1984; Tucker & Warner, 1999; Orizaola & Laurila, 2009); these differences may imply genetic divergence in plasticity possibly due to local adaptation. Although isolated populations that are in close proximity to each other could differ in developmental responses to temperature (Blackenhorn, 1991), variation across finer geographic scales remains relatively understudied (Doody 2009; Riddell & Sears, 2015). Furthermore, many studies that examine geographic variation in thermal responses lack the context of each population's evolutionary history. That is, direct knowledge of the relationships between natural populations and their history of geographic isolation is rare, but is valuable for understanding shifts in plasticity across space. Population comparisons in plasticity in the context of geographic history would provide key insight into the factors that shape developmental responses in different environments.

Our study organism, the brown anole (*Anolis sagrei*), is well suited for addressing the above knowledge gaps. Lab protocols for husbandry for this species are well established (Sanger et al. 2008; Warner, Moody, Telemeco, & Kolbe, 2012), and individuals can be easily marked, released, and recaptured for longitudinal studies in the field. Additionally, brown anoles lay approximately one egg per week across a long nesting season from April to October (Smith et al. 1973), and individuals can reach adulthood in less than a year. These characteristics facilitate assessments of developmental effects on individuals into adulthood and enable researchers to quantify how optimal developmental conditions may vary across life stages. Furthermore, our study populations enable us to examine small-scale geographic variation in thermally-induced developmental plasticity. For instance, several small islands in the Intracoastal Waterway of Florida were seeded with adult brown anoles from the mainland (near Palm Coast, Florida), which allows us to compare reaction norms among descendant island populations and their common mainland ancestor (about 8 generations removed). Population differences in plasticity are plausible given the substantial variation in nest temperatures within (Pruett et al. 2020) and potentially among, these islands due to variation in microhabitat variables (e.g. shade cover, soil moisture).

In this study, we test three hypotheses that will provide insight into the ecology and evolution of organismal responses to developmental environments: 1) variation in embryo development and offspring morphology is influenced by developmental temperature, 2) developmental thermal environments have lasting effects on survival into adulthood, and 3) reaction norms differ among ancestral and descendant populations. Our first hypothesis was addressed by quantifying thermal reaction norms for embryo and offspring phenotypes using a wide range of developmental temperatures. Given that temperature extremes negatively affect egg hatching success (Maximum: Hall et al. 2019; Minimum: Pruett et al. 2019), we predicted that embryo survival would have a curvilinear relationship with incubation temperature (an inverted u-shape curve with lower survival values at the extremes and higher values at intermediate temperatures). We also predicted that morphological traits of offspring would follow a similar pattern (Noble et al. 2018).

Our second hypothesis was addressed by quantifying fitness consequences of incubation temperature via measuring survival across life stages into adulthood in the field. We predicted that patterns of survival would resemble the curvilinear expectation for hatching success, but that the post-hatching optimum incubation temperature would differ from that at the embryonic stage. Lastly, we predicted that individuals that hatched and were released early in the season would have higher survival than those released later as they would enter an environment with low competition and have more time to grow before winter (Pearson & Warner 2018; Hall et al. 2020).

Our third hypothesis aimed to infer evolution of plastic responses to developmental conditions by comparing reaction norms across isolated populations. To do this, we quantified thermal reaction norms for fitness-related morphological variables (SVL, mass, and body condition) and survival and compared the ancestral mainland and descendent island populations. We did not have *a priori* predictions for how reaction norms for phenotypes and survival would vary among populations, but because habitats differ among the mainland and island sites (e.g., available shade cover, vegetation structure; Warner et al. 2014; Warner, unpubl. data) it is plausible that reaction norms would differ among populations.

Materials and Methods

Animal Collection and Husbandry

From 1-3 April 2018, we collected 367 adult *A. sagrei* (321 females, 46 males) from four geographically distinct populations in Palm Coast, FL. These individuals were collected from a mainland population and three islands in the Intracoastal Waterway (Supplemental Table 1) that were populated with adults from the mainland in 2011. Individuals were weighed and measured upon capture and transported to Auburn University. Adults were housed in outdoor screen cages (0.61 m x 0.61 m x 1.22 m) (~9 females and one male per cage) and individuals from different populations were never mixed. Cages contained an upright bamboo perch (1.37 m) wrapped in artificial leaves for hiding and climbing. Each cage contained a plastic nesting pot (19 cm long x 12 cm wide x 9 cm high) filled with potting soil and placed at the base of the bamboo perch for cover during nesting. Lizards were fed crickets (dusted with calcium and vitamins) twice per week and misted with water daily if there was no rain.

Incubation Experiment

Nesting pots were checked twice weekly for eggs. Eggs were weighed, and then randomly allocated to one of eight incubation temperature treatments ranging from 21-35°C at 2°C increments (21, 23, 25, 27, 29, 31, 33, & 35°C). These temperatures fall within the range of thermal conditions measured in natural nests at our study site (Pruett et al. 2020). Eggs from each population were evenly distributed across all treatments. Although thermal regimes in natural nests fluctuate daily, we chose to use constant temperatures to simplify interpretations of continuous reaction norms. For example, different components of thermal fluctuations such as maximum (Hall et al. 2019), minimum (Pruett et al. 2019), and variance in temperature (Du & Shine 2010) can affect different aspects of development in reptiles, making it difficult to determine which component is inducing the observed effect. Thus, while thermal fluctuations are more ecologically relevant (Les, Paitz, & Bowden, 2007; Hall & Warner 2020), these conditions in an experiment like ours with 8 temperature treatments and 4 populations would be difficult to interpret.

Eggs were incubated individually in petri dishes (35 mm in diameter) half-filled with moist (-150 kPa) vermiculite and sealed with parafilm. For eggs incubated at high temperatures (31, 33, 35°C), lab tape was used to reinforce the parafilm as it was more likely to break at these temperatures. Incubators were checked daily for hatchlings, and when found they were weighed, measured (snout-vent length [SVL] and tail length [TL]), given a unique toe clip for identification, and housed individually in a cage (21 cm long x 16.5 cm wide x 11 cm high). Importantly, our body size measurements have previously been shown to be influenced by incubation temperature (Pearson & Warner 2018; Hall & Warner 2020) and are associated with survival (Warner & Lovern 2014; but see Pearson & Warner 2018). Hatchlings were kept under common thermal conditions that fluctuated daily from about 26-30 ºC (due to the 12 hr cycle of overhead lights), watered daily, and fed five fruit flies twice weekly while in captivity.

Release Experiment

Hatchlings were released on a small island in the Intracoastal Waterway of Florida (29°45’53”N; 81°15’28”W) over the summer of 2018 (release dates: 20 Jun, 10 Jul, 18 Jul, 24 Jul, 24 Aug, 10 Sept; Supplemental Table 2). The release island allowed us to assess survival consequences of developmental temperatures under common environmental conditions. Hatchlings were 1-29 days post-hatch when they were released, but age at release had no significant effect on survival (β=-0.06± 0.05 SE p=0.246). This island was 15.1 km north of the closest collection site of the parental lizards. The island (~5000 m^2) was already populated with *A. sagrei* and consisted of a central cluster of dense canopy surrounded by open habitat with low brush. Although this location was different from those where the hatchlings originated, this island provided a common setting that had a diversity of microhabitats and pressures similar to those at all collection sites. The first recapture effort was conducted 1-2 and 4 October 2018. Four people collected lizards for 13 h (52 person hours; 215 lizards captured). Of those captured, 27 were experimental lizards and 188 were field-hatched resident individuals. The second recapture effort was conducted 21-22 March 2019, which is a time when most offspring would have reached adulthood. Four people collected lizards for 8.5 h (34 person hours; 187 lizards captured; 17 experimental and 172 residential). Based on surveys of a nearby island population, detection probability is 73% with 75 total person hours (Warner, unpublished data). Because the combined person hours for these two recapture efforts exceed that, we are confident that sampling was thorough. For both recapture efforts, all individuals were measured (SVL and TL) and weighed. Two individuals captured in the second recapture effort that were not captured in the first effort were retroactively marked as survivors for the first recapture.

Statistical Methods

A generalized linear mixed model with a binomial error distribution (R package lme4; Bates et al. 2015) was used to determine the effects of incubation temperature, population, their interaction, and egg mass on hatching success. Because temperature has a non-linear effect on hatching success (Hall & Warner 2019), the model included both linear and second-degree polynomial (Quadratic) functions of temperature. For other phenotypic measures, AIC (Akaike Information Criterion) scores were used to determine whether a linear or quadratic representation of incubation temperature best fit the data. General linear mixed models were used to determine the effects of incubation temperature, population, and egg mass on incubation duration, hatchling mass, and SVL. Interactions between population and temperature were tested but were removed from final models because they were not statistically significant. Body condition was analyzed using a general linear mixed model with hatching mass as the dependent variable and SVL, incubation temperature as a second-degree polynomial, and population as independent variables. We use residual scores from the regression of body mass on SVL to represent body condition. An interaction between population and incubation temperature was tested but was non-significant, and therefore was removed from the final model. Maternal cage was used as a random effect in all models.

Hatchling survival was analyzed using a generalized linear model with incubation temperature as a second-degree polynomial, hatchling release date, hatchling mass, population, and an interaction between temperature and release date as independent variables. The same model was used for both recapture efforts. The recapture sample sizes were not large enough to allow for a random effect of maternal cage, but a likelihood ratio test confirmed that excluding the random effect did not significantly change the fit of the model. Additionally, little to no variance for some temperature/population combinations prevented analyses of temperature x population interactive effects on survival. Finally, to determine if the optimal temperatures for survival differed among life stages (hatching success vs post-hatching survival to October and March), we bootstrapped survival data for eggs and the two recapture events. To do this, we resampled data with replacement one thousand times, created a survival probability curve using these data, and extracted the temperature at which survival probability was the highest (the peak of the curve) for each iteration. We then calculated the mean value and the 95% confidence limits from the distribution optimal temperatures.

Results

The relationship between incubation temperature and egg hatching success was non-linear, but still generally declined with increasing incubation temperature (Fig. 1, Table 1). More specifically, hatching success was slightly reduced at the lowest temperatures, peaked at 25.1℃, and decreased steadily as incubation temperature increased. Incubation duration declined with increasing temperatures in an asymptotic fashion (linear: $\beta=-204.6\pm 3.03$ SE; df=150; $p<0.001$; quadratic: $\beta=71.6\pm 3.09$ SE; df=150; $p<0.001$, Fig. 1). Incubation duration also decreased by 4.1 days (±0.97 SE; df=150; $p<0.001$) with every 0.1 g increase in egg mass. All of these effects were consistent across populations (i.e., no significant temperature x population interaction).

Incubation temperature significantly influenced hatchling morphology, but variation among populations was minimal (Fig. 2). Hatchling SVL decreased linearly by 0.05 mm (± 0.014 SE) with each one degree increase in incubation temperature (F=12.79; df=151; $p<0.001$). Additionally, SVL increased by 1.2 mm (±0.234 SE; df=151; $p<0.001$) with each 0.1 g increase in egg mass. Hatchling mass was non-linearly related to incubation temperature (quadratic: $\beta=-0.056\pm0.013$ SE; df=150; $p<0.001$), but the linear component was not statistically significant ($\beta=-0.010 \pm0.013$ SE; df=150; $p=0.458$). Additionally, hatchling mass did not differ among the four populations (F=0.987, $p=0.401$). Hatchling mass increased by 0.0395 g (±0.004 SE; df=143; $p<0.001$) with each 0.1 g increase in egg mass. Body condition had significant linear ($\beta=0.027\pm0.013$ SE; df=150; $p<0.001$) and non-linear ($\beta=-0.041\pm0.013$ SE; df=150; $p=0.041$) relationships with incubation temperature. Though the interaction between population and temperature was not significant for all traits, two island populations differed from each other in hatchling body condition (β=0.009±0.003 SE; df=150; p=0.011).

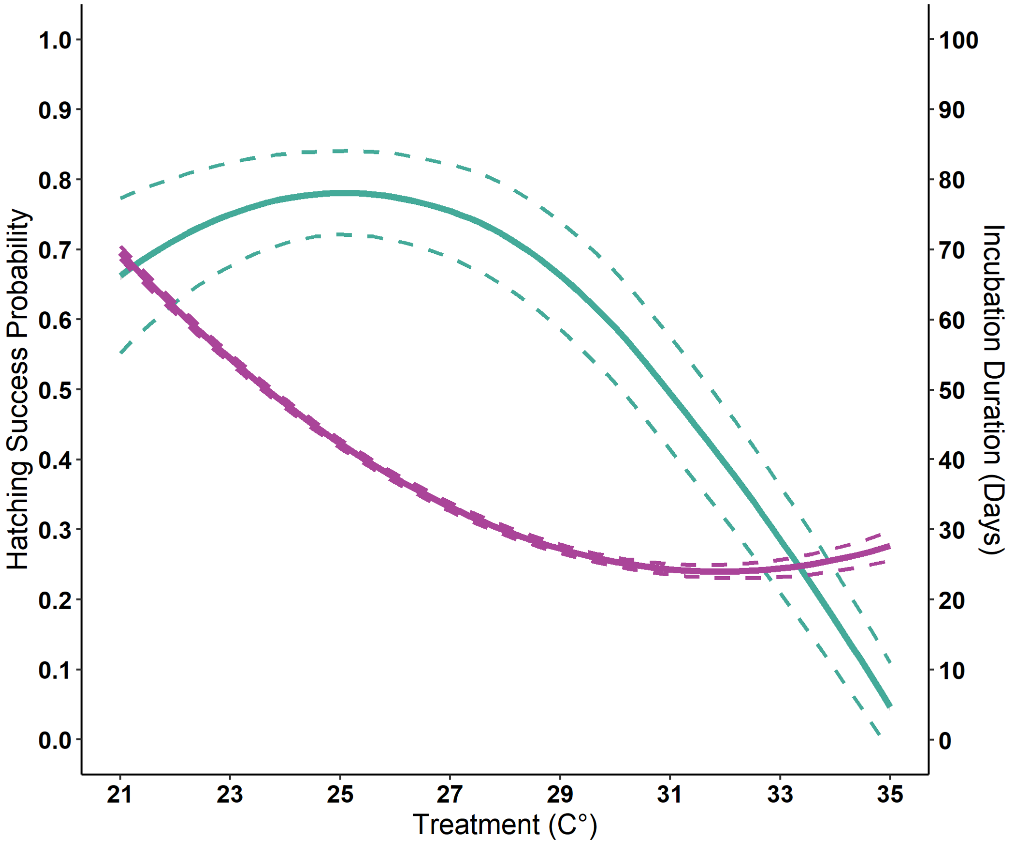

Figure 1. Probability of egg hatching (teal; left y-axis) and incubation duration (magenta ; right y-axis) in relation to incubation temperature. The survival probability curve was generated using a generalized linear model of survival plotted against temperature, and the incubation duration curve was generated using a linear model of incubation duration plotted against temperature. The dashed lines represent the upper and lower confidence limits for each curve. Descriptive statistics for each incubation temperature are in supplementary table 3.

Table 1. Test statistics for the three survival assessments. Statistically significant values are presented in bold. For population comparisons, effects sizes were calculated using the mainland population as the reference.

Independent variable	Egg hatching success	Offspring survival to October	Offspring survival to March
Temperature			
Linear	β= -20.11±2.66, **p<0.001**	β= 4.98±5.97, p=0.40	β= -10.29±8.71, p=0.24
Quadratic	β= -13.22±2.58, **p<0.001**	β= 15.41±7.01, **p=0.03**	β= 5.67±6.62, p=0.39
Population			
Island F	β= -0.54±0.38, p=0.16	-	-
Island L	β= -0.44±0.34, p=0.20	-	-
Island M	β= -0.10±0.37, p=0.77	-	-
Egg mass	β= 18.38±5.00, **p<0.001**	-	-
Hatchling mass	-	β= 2.86±13.49, p=0.83	β= -6.09±16.41, p=0.71
Release date	-	β= -1.45±0.47, **p=0.002**	β= -0.04±0.02, p=0.08
Release date x temperature (linear)	-	β= -5.67±6.63, p=0.40	β= 0.32±0.27, p=0.25
Release date x temperature (quadratic)	-	β= -14.95±6.45, **p=0.02**	β= -0.31±0.25, p=0.22

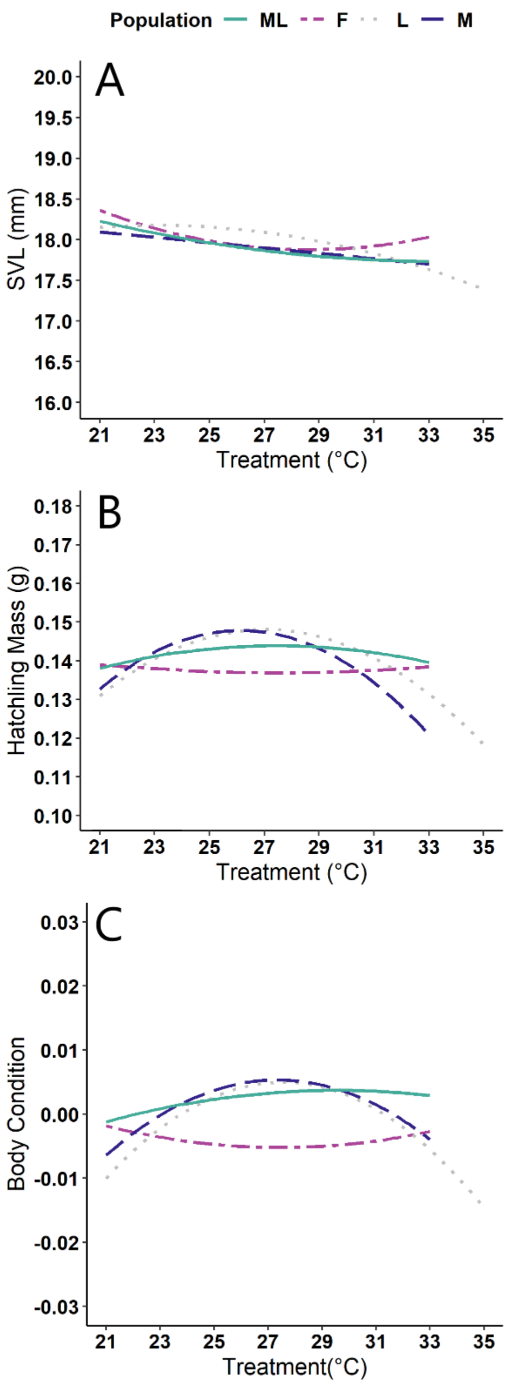

Figure 2. The effect of incubation temperature on (A) snout-vent length (SVL), (B) hatchling mass, and (C) body condition.The population labels refer to the names that we have given the islands (i.e., island F, L, M) and the mainland (ML). Descriptive statistics for each incubation temperature are in supplementary table 3.

Hatchling survival was influenced by incubation temperature and the timing of hatching/release. In October, the relationship between survival and incubation temperature was curvilinear (Table 1, Fig. 3). Hatchlings released relatively early had a greater probability of survival to October than those that hatched late, but this relationship was not present for survival to March (Table 1). The interaction between the quadratic component of incubation temperature and release date also explained variation in hatchling survival (Fig. 3, Table 1), such that the relationship between incubation temperature and survival became more curvilinear as release date progressed. Survival to March was not associated with any of the variables measured (Table 1). Lastly, based on values calculated from bootstrapping the survival data, the peak incubation temperature for hatchling survival to October (29.62; 95% CI [27.25, 35]) was significantly greater than that for egg hatching success (24.71; 95% CI [22.75, 26]; Fig. 4a); however, confidence intervals for peak incubation temperature for hatchling survival to March (28.62, 95% CI [25.75,35]) overlapped those for egg hatching success and for hatchling survival to October (Fig. 4b).

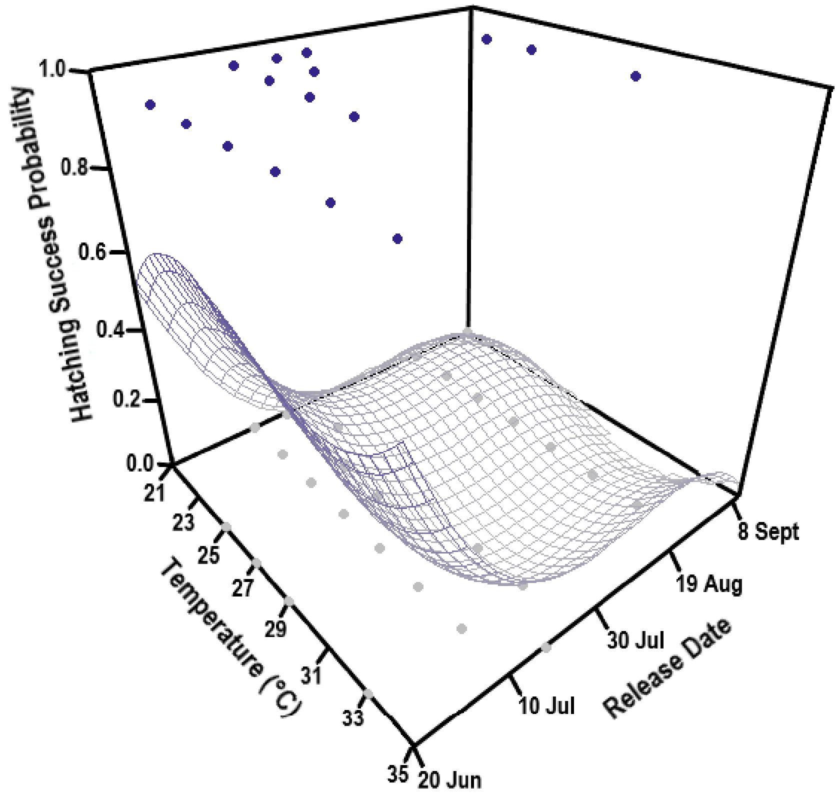

Figure 3. The interactive effect of incubation temperature and release date on probability of survival to October. Blue points represent individuals that were recaptured, gray points represent individuals that were not recaptured, and the surface grid represents the probability of survival across incubation temperatures and release dates.

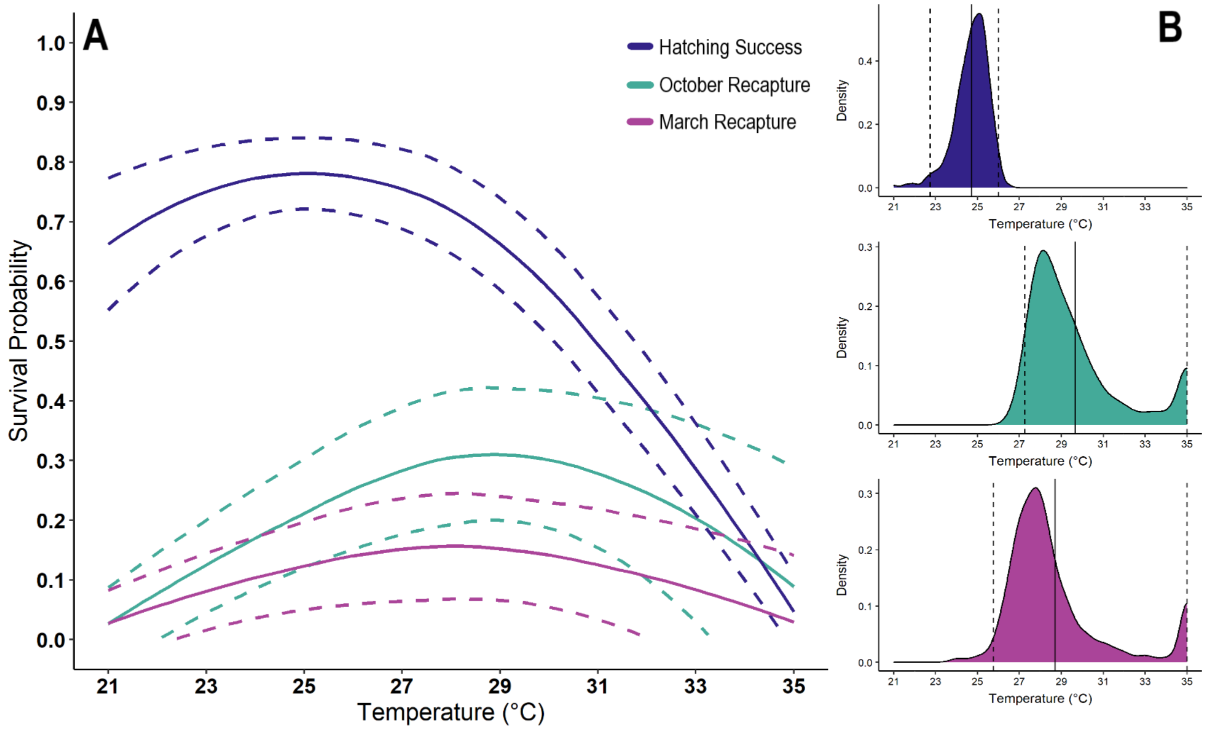

Figure 4. (A) Probability curves of egg hatching success, survival to the October recapture, and survival to the March recapture in relation to incubation temperature. The survival probability curve was generated using a generalized linear model of survival plotted against temperature. The dashed lines represent the upper and lower confidence limits for each curve. **(B)** Density distributions of the bootstrap results for the peak temperature of each survival curve. The solid vertical lines represent the mean of the 1000 iterations for each, and the dashed vertical lines represent the confidence limits. A single individual from the highest incubation temperature survived to both recapture events, which gives the recapture distributions a bimodal appearance.

Discussion

Phenotypic variation is driven by both plasticity and natural selection. Additionally, selection can shape plasticity in ways that enable phenotypes to match conditions across heterogeneous environments (DeWitt & Scheiner, 2004). Consequently, reaction norms can vary among populations, especially when they experience different environmental conditions (Fairbairn, 1984; Tucker & Warner, 1999; Orizaola & Laurila, 2009). Moreover, in the case of developmental plasticity, fitness consequences may be temporally offset from environmental cues that induce phenotypic variation (e.g., early life environments affect reproduction at adult stages; Warner & Shine, 2008). Thus, quantifying among-population variation in reaction norms, coupled with measurements of environmental effects on fitness across different life stages will provide important insights into the evolution of plasticity. In this study, we examined variation in developmental reaction norms across four nearby, but isolated, populations, as well as variation in survival (an important fitness component) extending into the adult life stage. The lack of variation in developmental thermal reaction norms for the traits we measured provides no evidence of divergence in developmental plasticity among populations. By quantifying survival at different life stages, we showed that the optimal incubation temperature differs between embryo and post-hatching stages. Moreover, the relationship between developmental temperature and post-hatching survival changed across the hatching season.

Effect of incubation temperature on embryo development and offspring morphology

Thermal reaction norms for development of different phenotypes often have a curvilinear shape with lower response values at the coldest and hottest temperatures (Arnold & Peterson, 2002; Noble et al. 2018). The reaction norm we found for hatching success was consistent with past studies and was relatively similar among our four populations. Although the optimal temperature for hatching success was 25.1℃, field-based experiments show that female brown anoles often select nest sites with average temperatures between 26-28℃ (Pruett et al. 2020). Although our confidence limits for egg survival slightly extend above 26℃ (Fig. 4b), this relatively small overlap suggests that females use nest sites with slightly suboptimal temperatures for hatching success. We provide two possible explanations for this disparity in our laboratory study of embryo development and field studies of maternal nesting behavior. First, constant incubation temperatures used in the current study may generate reaction norms with peaks at relatively low temperatures compared to a natural fluctuating regime because there is no relief from warmer, thermally-stressful conditions (Hall & Warner, 2020). Consequently, we would expect the optimal incubation temperature for hatching success to be slighter higher than that reported here had eggs experienced naturally fluctuating conditions, which would correspond with our observed maternal choices of nest microhabitat.

A second possible explanation for why females select thermal conditions that are warmer than the optimal constant temperature for hatching success may relate to thermal effects on other fitness-related variables. For example, the shape of the curvilinear relationship between incubation temperature and hatching success suggests that brown anole embryos have a relatively large range of temperatures that are suitable for development. This broad range of suitable temperatures is expected for species that do not modulate nest temperatures via maternal care (Shine 2005). The negative relationship between temperature and incubation duration (Fig. 1) combined with the broad range of suitable developmental temperatures suggests that females may select nest temperatures that optimize the balance between embryo survival and incubation duration. That is, although warmer temperatures decrease hatching success, they benefit offspring by enabling embryos to develop relatively quickly (Le Henanff, Meylan, & Lourdais, 2013). Indeed, hatching earlier improves hatchling survival probability in a broad range of taxa (Landa, 1992; Ludsin & DeVries, 1997; Warner & Shine, 2007; Tüzün & Stoks, 2018), including *A. sagrei* (Pearson & Warner 2018) and can minimize the amount of time exposed to nest predation or adverse weather (Doody, 2011). Thus, while maternal choice of slightly warmer nest temperatures may only slightly decrease hatching success, it can enhance survival at post-hatching stages. Similarly, warmer temperatures produce hatchlings with better performance (e.g. sprint speed, endurance; Hall & Warner 2020; Pearson & Warner 2016) that could increase maternal fitness even if hatching success is slightly decreased. In support of these patterns, we also show that the optimal temperature for post-hatching survival is greater than that for hatching success; this pattern is discussed further below.

Beyond hatching success, we also observed effects of temperature on hatchling phenotype. Incubation temperature had a positive linear relationship with SVL, body mass, and body condition, though the effect was minimal and may not be biologically significant. Such patterns for hatching success and morphology would have not been evident had we used only a few treatments, which is typical in most studies of developmental plasticity (Murren et al., 2014). For example, two incubation treatments can only reveal linear relationships and could miss important aspects of variation associated with the developmental environment such as non-linear relationships and resulting peaks for survival and phenotypic traits (Scheiner & Lyman, 1989). Thus, we strongly urge researchers to increase the number of treatments to generate reaction norms across a more continuous range of temperatures.

Effects of incubation temperature on post-hatching survival

The continuous production of eggs across a long reproductive season means that female anoles must repeatedly navigate temporally and spatially heterogeneous environments to select conditions for nesting (Pearson & Warner 2016; Pruett et al. 2020). Additionally, the long nesting season (April-October) (Smith 1973) can generate temporal shifts in selection on offspring and maternal traits. Indeed, not only do early-produced offspring hatch sooner than late eggs, but they also experience different pre- and post-hatching environments (Pearson & Warner 2018). This seasonal trend in reproduction and environmental conditions creates opportunity for early-season offspring to grow and gain significant survival advantages over their late-season counterparts (Ludsin & DeVries, 1997; Tüzün & Stoks, 2018), which is evident in brown anoles (Pearson & Warner 2018). While our results are consistent with these findings, we show additional complex interactions with incubation temperature on offspring survival in the field.

The effect of incubation temperature on offspring survival was more pronounced for offspring produced late in the season, than those that hatched early. Specifically, individuals released earlier in the season were more likely to survive to our first recapture event in October. Additionally, the interaction between release date and the quadratic component of incubation temperature demonstrates that the probability of survival was relatively high and nearly even across incubation temperatures for individuals released early in the season, but as release date progressed, survival probability decreased and the shape of the relationship with incubation became curvilinear. This interaction suggests that incubation temperature has little influence on survival for early-season hatchlings, but becomes more influential as the season progresses. Reproductive phenology has been shown to interact with developmental conditions to shape fitness-relevant traits such as growth and morphology in other taxa (Laurel, Hurst, Copeman, & Davis, 2008; Bentz, Logan, & Amman, 1991).

The complex interaction between incubation temperature and timing of hatching supports our notion that fitness would be enhanced if females choose nest conditions that optimize more than just hatching success of eggs. For example, if females choose warm nest microhabitats that lead to early hatching, then their offspring have more time to grow with only a small decline in the probability of hatching. This effect may be most critical for offspring that are produced late in the season because of greater competition with conspecifics at that time of year. For example, densities of brown anoles are often extremely high (Stroud, 2017) and increase even further as eggs continually hatch over the season. This creates a highly competitive environment for hatchlings, which compete with each other through aggressive displays and form hierarchies from the time of hatching (Stamps, 1978). Thus, late-hatched individuals are likely competitively inferior (and have increased mortality; Pearson & Warner 2018) due to their small size compared to early-hatched individuals that had a head start in growth. Indeed, while body size at hatching was not associated with survival in our study, large body size likely becomes important for survival and during competitive interactions as individuals grow, which is facilitated by hatching earlier in the season. By accelerating developmental rate, warm incubation temperatures may further facilitate hatching into the population before hatchling density has peaked later in the season; thus, the effect of incubation temperature on offspring survival is likely most critical at mid to late times of the reproductive season. These results emphasize the importance of seasonality on each aspect of reproduction and the degree to which fitness can vary as a result of when eggs are laid and hatch (Olsson & Shine, 1997; Warner & Shine 2007; Le Henanff, Meylan, & Lourdais, 2013; Harriman, Dawson, Bortolotti, & Clark, 2017). While competition may contribute to variation in survival among individuals that experienced different developmental temperatures, several aspects of the hatchling/juvenile environment may favor different phenotypic components that are shaped by developmental conditions, particularly in the field where the environment is very heterogeneous.

Complex interactions among multiple factors can shape variation in offspring survival, and the timing of those effects can vary among life stages (Mitchell, Janzen, & Warner, 2018). As briefly discussed above, we show that the optimal incubation temperature varies across life stages. For example, the optimal temperature for embryos was 5ºC cooler than it was for post-hatching offspring in the field. This pattern may be explained by a balance between the benefits of cooler optimal temperature for successful development versus warmer temperatures that facilitate early hatching for offspring. Indeed, individuals incubated at the optimal temperature for hatching success would take almost two weeks longer to hatch than those incubated at the optimal incubation temperature for survival to the first recapture. Importantly, the effects of incubation temperature on offspring phenotypes can further complicate interpretations about optimal temperatures for incubation. Additional research on survival to later life stages is warranted, but our comparisons between embryo and post-hatching stages (up to adulthood) suggest that the optimal temperature for embryo survival is likely driven by physiological tolerances to different temperatures, whereas for post-hatching survival the pattern may be driven (at least partially) by ecological factors (e.g., shifts in competitive environments) associated with temperature effects on the timing of hatching. Thus, understanding both the physiology and ecology of different life stages will provide critical insights into how selection has shaped responses to developmental environments.

Population comparisons of reaction norms

Populations differed very little in hatchling morphology, as well as in developmental and phenotypic responses to incubation temperature. Body condition differed very slightly between hatchlings from two islands, and no descendant island populations differed in phenotypes or reaction norms from the ancestral mainland population. This lack of divergence from the ancestral population may be explained by the relatively short period of time that these populations have been isolated from each other (~8 generations). However, previous work on *Anolis* lizards has demonstrated relatively rapid evolutionary change in morphology (Losos, Warheit, & Schoener, 1997; Losos, 2009) with even more evolutionary lability displayed in their thermal physiology (Hertz et al., 2013). These documented instances of rapid divergence were likely associated with environmental differences experienced by populations (e.g., perch characteristics associated with adaptive changes in limb length; Losos et al. 1997). In our study, however, it is possible that there was not enough environmental variation among island and mainland habitats for genetic divergence among populations, especially when their isolation from one another was very recent.

Conclusions

Developmental temperature shapes several fitness components that interact in ways that are poorly understood, but ultimately influences variation in organismal fitness (Uller, 2008). This study shows the interrelated nature of several of these components and their potential to impact individual fitness beyond immediate post-hatching life stages into adulthood. Although we did not detect phenotypic divergence of the island populations from the mainland source population, identifying local adaptation in developmental reaction norms remains an important challenge and should be examined in other systems with known histories of geographic isolation.

The implications of these results on natural populations are especially poignant in light of contemporary environmental change. With increasing temperature or even more stochastic conditions, the seemingly delicate balance of optimizing different fitness components for offspring could generate complex challenges for females when choosing nest microhabitats (Refsnider & Janzen, 2010). In spatially and temporally heterogeneous environments, maternal fitness would be enhanced if mothers choose nest conditions that are suitable not only for egg hatching, but also positively impact later life phenotypes and survival. Overall, both physiological and ecological factors can influence how developmental environments affect survival across different life stages, and this has implications for maternal reproductive behaviors. Untangling the components of this complexity will provide further insight into how selection has shaped developmental plasticity in heterogeneous environments and how organisms might respond to future environmental change.

Chapter 4 Supplemental Material

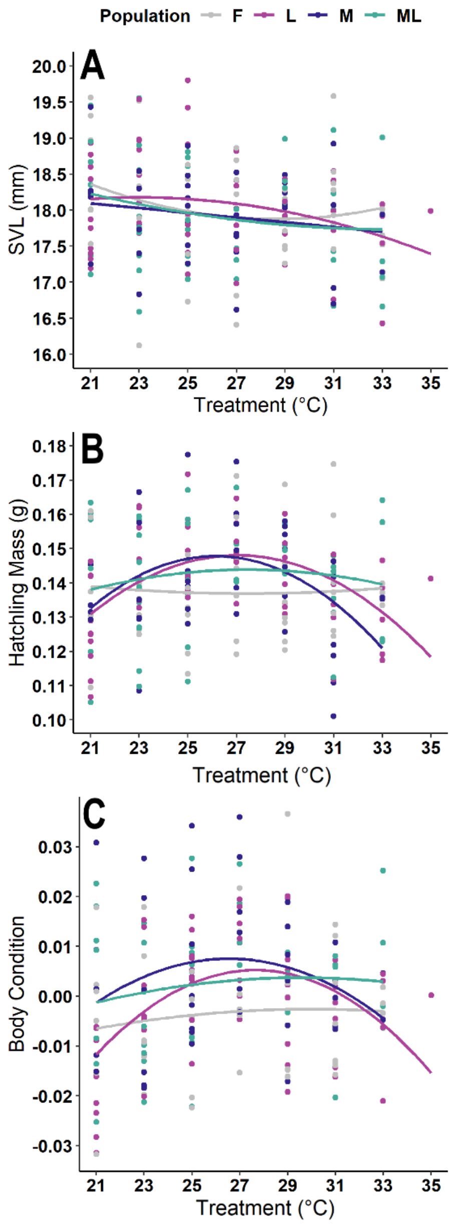

Figure S1. The effect of incubation temperature on (A) snout-vent length (SVL), (B) hatchling mass, and (C) body condition (residuals from the regression of body mass on SVL). Each point represents an individual hatchling. The population labels refer to the names that we have given the islands (i.e., island F, L, M) and the mainland (ML). Descriptive statistics for each incubation temperature are in Supplementary Table 3.

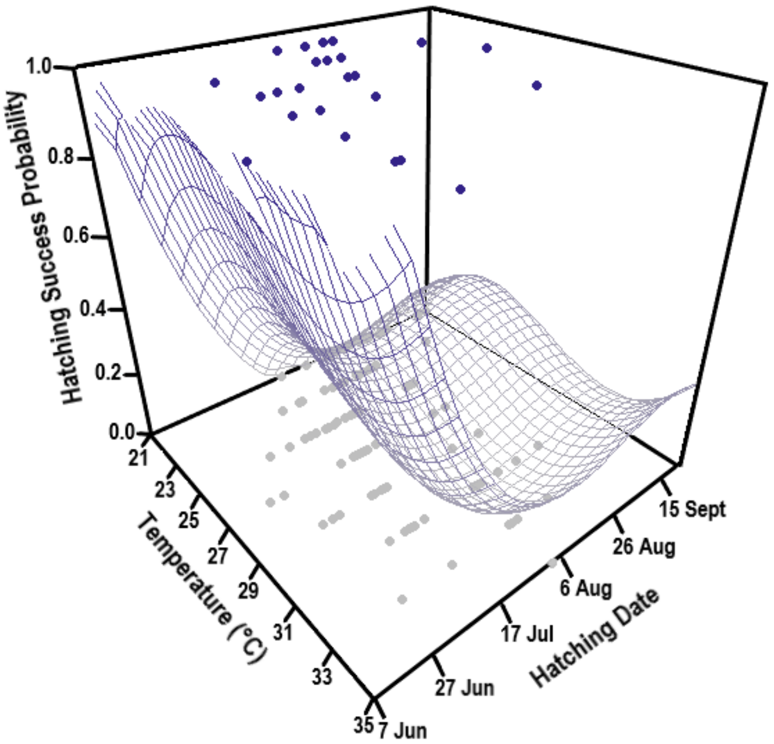

Figure S1. The interactive effect of incubation temperature and hatch date on probability of survival to the October recapture. Blue points represent individuals that were captured, grey points represent individuals that were not, and the surface grid represents the probability of survival across temperatures and release dates. Hatch day was calculated as a day of the year out of 365 to create a continuous range of days. The pattern remains consistent when release date is used (rather than hatch date), as shown in Fig. 3 of the main text.

Supplemental Table 1. The coordinates for the locations of the four populations used in this study and the number of individuals collected. The island names were given to the islands by the authors, and refer to their relative order along a chain of islands in the Intracoastal Waterway (Island F is the northern-most island of the three islands studied here).

Population	Coordinates	# of individuals (Total; female, male)
Mainland	29°36’05”N; 81°11’51”W	n=74; 61 female, 13 male
Island M	29°37’15”N; 81°12’39”W	n=93; 85 female, 8 male
Island L	29°37’24”N; 81°12’38”W	n=104; 92 female, 12 male
Island F	29°37’51”N; 81°12’44”W	n=96; 83 female, 13 male

Supplemental Table 2. The numbers of individuals released at each date, and the number of individuals recaptured at each recapture event.

Date	# of individuals released	# of individuals recaptured
20 June 2018	22	---
10 July 2018	52	---
18 July 2018	23	---
24 July 2018	11	---
24 August 2018	52	---
1-2 & 4 October 2018	---	28
21-22 March 2018	---	17

Supplemental Table 3. Summary statistics for hatching success, incubation duration, hatchling snout-vent length (SVL), body mass, tail length, and post hatching survival at each incubation temperature. Survival values include raw survival values as well as a percentage. For hatching success, the percentage was calculated out of the total number of eggs incubated at a given temperature. October and March survival percentages were calculated based on the number of eggs that hatched successfully. An asterisk (*) represents a value for which the standard error could not be calculated due to a sample size of one individual

Temperature (ºC)	N	Hatching Success Raw, Percentage	Incubation Duration (days) Mean±SE	SVL (mm) Mean±SE	Mass (g) Mean±SE	Tail Length (mm) Mean±SE	October Survival Raw, Percentage	March Survival Raw, Percentage
21	43	28, 65.12%	69.71±0.98	18.25±0.14	0.14±0.004	27.77±0.70	0, 0.00%	0, 0.00%
23	38	32, 84.21%	55.19±0.43	18.09±0.15	0.14±0.003	29.23±0.30	4, 12.50%	4, 12.50%
25	44	34, 77.27%	40.65±0.63	18.01±0.12	0.14±0.003	29.52±0.44	7, 20.59%	3, 8.82%
27	45	26, 57.78%	33.19±0.48	17.81±0.14	0.15±0.003	29.07±0.44	8, 30.77%	3, 11.54%
29	41	27, 65.85%	28.56±0.41	17.96±0.11	0.14±0.003	29.23±0.36	5, 18.52%	3, 11.11%
31	40	27, 67.5%	24.33±0.33	17.70±0.23	0.13±0.003	28.56±0.50	5, 18.52%	3, 11.11%
33	51	15, 29.41%	24.27±0.34	17.52±0.16	0.13±0.003	27.82±0.53	2, 13.33%	1, 6.67%
35	48	1, 2.08%	22*	17.99*	28.85*	0.14*	1, 100%	0, 0.00%

General Conclusions

Nest site choice in reptiles

One requirement for nest site choice to be considered a true maternal effect, there has to be evidence that females are actually choosing nest sites in the habitat matrix that is available to them. In my first three chapters, I provide robust evidence that both painted turtles and brown anoles are actively choosing nest sites for their eggs. Though the reproductive strategies of these species are vastly different, they both possess the capacity to assess aspects of their environment and choose a nest site accordingly. Furthermore, these chapters also support the idea that nesting behavior in these species is adaptive. Females are able to choose microclimates that shape development in ways that are advantageous for their offspring. Brown anoles choose nest sites that are relatively cool, painted turtles choose sites that are relatively warm, and both of these preferences lead to improved hatching success for their eggs. Additionally, they seem to prefer sites with higher levels of soil moisture which can lead to improved offspring quality.

In chapter 1, I found that painted turtle eggs incubated in maternally selected nests fared better than those incubated in artificial nests. This finding highlights the need for more studies on nesting in the field. Even though we had an idea of which conditions were favorable and chose sites within a few meters of maternally selected sites, eggs in the nest sites we chose had reduced survival. Obviously, there are things that females are honing in on and selecting that we have not detected. Nest microclimates are really *micro*climates; the conditions experienced by eggs and the resulting survival outcomes can vary significantly within a few meters. Future studies could move beyond the established determinants of offspring survival, such as temperature and moisture, and examine other aspects of nest sites to gain a fuller understanding of how females choose a place to lay their eggs.

Anolis nesting

Though *Anolis* nesting behavior is understudied, the search methods outlined in this dissertation provide a promising avenue for future research. Finding anole eggs in the field outside of chance encounters was previously considered logistically difficult, but the work outlined here shows efficient ways in improving our ability to find nests. We were able to successfully locate anole nests in a complex habitat matrix, and our methods were reliable enough to produce sample sizes large enough to test empirical questions and gain a robust understanding of which habitat characteristics female brown anoles select for in the field. The methods outlined chapter 2 were extremely effective but has been modified slightly to further reduce search bias in my more recent work that is not included in this dissertation. This work will serve as a methodological foundation that can be continually modified and improved upon to build an empirical understanding of how *Anolis* lizards nest.

Brown anoles are one species among many in a group that encompasses an immense amount of life history diversity. Anole biologists categorize species into “ecomorphs”, or groups of species with similar morphology that occupy a similar ecological niche (Williams, 1972). Species within an ecomorph group are not necessarily closely related phylogenetically, but they share many traits that make aspects of their biology similar, including their behavior. Thus, the methods for locating nests outlined in this dissertation are more likely to be generalizable to species in the same ecomorph group as the brown anole. Brown anoles are trunk-ground anoles meaning they spend much of their time on trees, but also frequently descend to the ground where we found all of the nests included in this study. However, other anoles with vastly different behavior and ecology could make use of alternative nesting habitats. For examples, crown-giant and trunk-crown anoles are typically found in the tops of trees and rarely spend time on the ground. Future work on nesting behavior in anoles should take these ecological differences into account and modify their methodology accordingly.

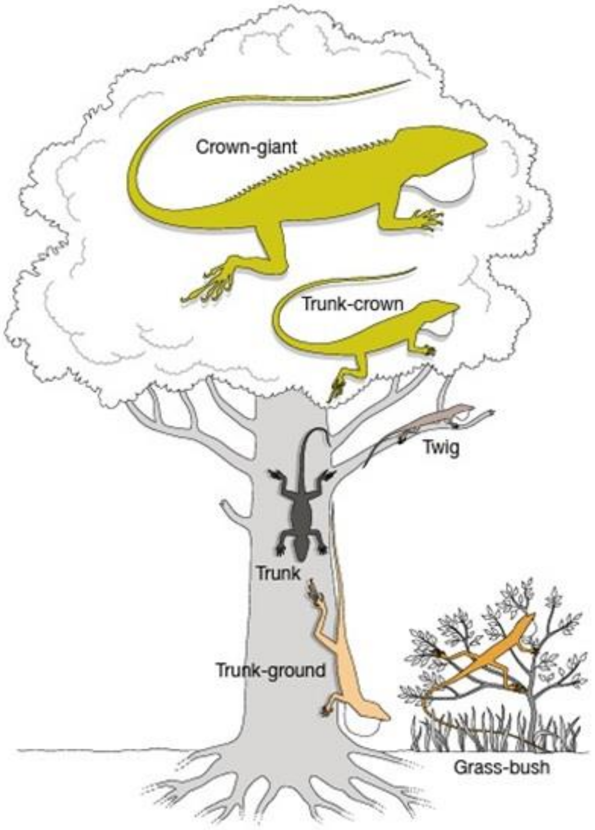

Figure 1. Diagram of *Anolis* ecomorphs from Losos 2009 adapted from Williams 1972

Though some aspects of nesting behavior may vary across species, one theme that seems generalizable is that females are able to assess their surroundings and choose a nest site accordingly. Not only are they able to choose based on microclimate characteristics, they are able to choose based on risk of predation. Anoles are particularly interesting in this regard because they lay one egg at a time across a long reproductive season. This means that females are constantly assessing and reassessing their surroundings that are changing throughout the season. A nest site that is ideal at the beginning of the reproductive season may not be ideal at the end of the season. Nesting in *Anolis* lizards needs to be explored further and I believe my dissertation work will serve as a useful starting point.

Finally, more studies on nesting behavior in anoles is critical for their continued use as model systems. If you want to study how environments affect development in anoles, it would be imminently sensible to start with studying the developmental environments they experience in the wild. Without this information, how can we relate findings to the real world? Studies on thermal developmental plasticity in reptiles have come a long way in this regard by incorporating fluctuating thermal regimes into lieu of more simplified constant temperatures regimes. Once we have more data on natural nest sites, we can replicate those conditions more closely in the lab. Indeed, there have been several recent studies in the Warner lab that have used the hourly nest temperature data from my work (chapter 2) to design incubation regimes for brown anole eggs. Studies that use rigorous methods like ours and attempt to quantify natural nest conditions are essential to designing more ecologically relevant incubation experiments in the future.

The importance of long- term field based studies in fully assessing effects of nesting ecology

In my first two chapters, I focused on nest site choice in relation to nest microclimate and how those factors affect development and early life survival in two reptile species. In my third chapter, I examined the ability of females to continuously monitor their nesting environment and modulate their nesting behavior accordingly. Each of those three chapters highlight the short-term effects of nesting behavior, but one would assume that the effects of the developmental environment continue far beyond hatching. Mitchell et al. (2018) highlighted the need for long-term studies on the effects of developmental phenotypic plasticity in reptile systems by pointing out the lack of literature on the topic and the complex findings of the few studies that do exist. The fourth chapter of this dissertation, though slightly different in focus, has a conceptual thread that connects to each of the other three chapters by measuring the long-term effects of the developmental environment and, by extension, nesting behavior, on survival.

In my fourth chapter, I show that the developmental environments that are ideal for survival to one life stage are not necessarily ideal for survival to another life stage. This emphasizes the importance of developmental conditions in shaping the trajectory of an organism's life, but also underscores the complexity of nest site choice. Particularly in anoles, there is a delicate balance of conditions that needs to be struck for embryos to develop successfully, for hatchlings to grow quickly, and for juveniles to survive to adulthood. For example, eggs laid later in the season have much less time to catch up with their cohort in terms of growth and preparation for the winter. This means that it is more crucial for eggs laid later in the season to develop quickly, which requires a relatively warm nest site. However, nest sites can also be too warm, leading to decreased hatching success. As if this is not complicated enough, the push and pull of the demands of each life stage change across the reproductive season, meaning that each egg has a different set of conditions that would optimize its fitness. Female anoles must then locate a site with that set of conditions while also assessing the risk to their own survival.

With such a complex web of behavior, plasticity, and selection, it seems like there are an endless number of questions that could be asked and experiments that could be run to understand the importance of nesting ecology in reptiles, but one thing is certain: long-term studies across life stages are absolutely necessary for understanding the role of nest site choice in evolutionary ecology. Study systems like the brown anole are ideal for these studies because they are relatively short lived, easy to mark, and easy to maintain in captivity or monitor in the field. However, there is already a bias towards short-lived animals in studies that assess long-term effects of early life conditions. Long-lived species often have very different life history traits, so work on animals like the painted turtle will go a long way in filling those knowledge gaps. Additionally, conducting at least part of these studies in the field adds to their ecological relevance and reveals additional details about how plasticity operates in natural environments. The field-based release experiment included in my fourth chapter gave us more nuanced findings than what we might have seen in a more controlled lab setting. We were able to detect interactive effects of incubation temperature and release date on survival to adulthood, which gives us insight into how the importance of nest site choice might shift throughout the season.

Final Thoughts

The more I learn about biological systems, the more I realize how much we don't know. When friends and family ask questions about my work I often find myself saying, “It depends,” or “It's a bit complicated.” Though the “messiness” of biological research can be daunting at times, making sense of the convoluted world around us is what I consider to be the most interesting part of my work as a biologist. Through the process of completing my dissertation, I have learned to love the complexity of natural environments and the myriad ways living things interact with their surroundings. E. O. Wilson once said, “The love of complexity without reductionism makes art; the love of complexity with reductionism makes science.” I don't know if it makes me an artist or a scientist to say that I hope to continue embracing the complexity of evolutionary ecology for the rest of my career.

References

Andrews, R. M. (1982). Spatial variation in egg mortality of the lizard *Anolis limifrons*. *Herpetologica* 38(1): 165–171.

Angilletta, M. J., Sears M. W., and Pringle R. M. (2009). Spatial Dynamics of Nesting Behaviour: Lizards Shift Microhabitats to Construct Nests with Beneficial Thermal Properties. *Ecology*, 90(10), 2933-939. https://doi.org/10.1890/08-2224.1

Angilletta, M. J., Zelic, M. H., Adrian, G. J., Hurliman, A. M., & Smith, C. D. (2013). Heat tolerance during embryonic development has not diverged among populations of a widespread species (*Sceloporus undulatus*). *Conserv. Physiol*. 1(1): 1-9.

Arnold, S. J., & Peterson, C. R. (2002). A model for optimal reaction norms: The case of the pregnant garter snake and her temperature-sensitive embryos. *The American Naturalist*, *160*(3), 11.

Badyaev, A. V., & Uller, T. (2009). Parental effects in ecology and evolution: Mechanisms, processes and implications. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1520), 1169–1177. https://doi.org/10.1098/rstb.2008.0302

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *ArXiv:1406.5823 [Stat]*. Retrieved from http://arxiv.org/abs/1406.5823

Bentz, B. J., Logan, J. A., & Amman, G. D. (1991). Temperature-dependent development of the mountain pine beetle (Coleoptera:Scolytidae) and simulation of its phenology. *The Canadian Entomologist*, *123*(5), 1083–1094. https://doi.org/10.4039/Ent1231083-5

Birchard, G. F., & Reiber, C. L. (1996). Heart rate during development in the turtle embryo: effect of temperature. *Journal of Comparative Physiology B*, 166(8), 461-466. https://doi.org/10.1007/s003600050033

Biro, P. A., Abrahams, M. V., Post, J. R., & Parkinson, E. A. (2004). Predators select against high growth rates and risk–taking behaviour in domestic trout populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1554), 2233–2237. https://doi.org/10.1098/rspb.2004.2861

Blanckenhorn, W. U. (1991). Life-history difference in adjacent water strider populations: Phenotypic plasticity or heritable responses to stream temperature? *Evolution*, *45*(6), 1520. https://doi.org/10.2307/2409899

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. *Ecology and Evolution*, *9*(5), 2791–2802. https://doi.org/10.1002/ece3.4956

Bowden, R. M., Carter, A. W., & Paitz, R. T. (2014). Constancy in an inconstant worls: moving beyond constant temperatures in the study of reptilian incubation. *Integrative and Comparative Biology*, 54, 830-840. https://doi.org/10.1093/icb/icu016

Brisson, J. A. (2010). Aphid wing dimorphisms: Linking environmental and genetic control of trait variation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1540), 605–616. https://doi.org/10.1098/rstb.2009.0255

Brown, G.P., Shine, R. (2004). Maternal nest-site choice and offspring fitness in a tropical snake (*Tropidonophis mairii*, Colubride). *Ecology* 85(6): 1627–1634.

Buckley, L. B., Arakaki, A. J., Cannistra, A. F., Kharouba, H. M., & Kingsolver, J. G. (2017). Insect development, thermal plasticity and fitness implications in changing, seasonal environments. *Integrative and Comparative Biology*, *57*(5), 988–998. https://doi.org/10.1093/icb/icx032

Cagle, F. R. (1939). A system for making turtles for future identification. *Copeia*, 1939, 170-173.

Carter, A. L., Bodensteiner, B. L., Iverson, J. B., Milne-Zelman, C. L., Mitchell, T. S., Refsnider, J. M., … Janzen, F. J. (2019). Breadth of the thermal response captures individual and geographic variation in temperature-dependent sex determination. *Functional Ecology*, *33*(10), 1928–1939. https://doi.org/10.1111/1365-2435.13410

Casal, J. J., & Balasubramanian, S. (2019). Thermomorphogenesis. *Annual Review of Plant Biology*, *70*(1), 321–346. https://doi.org/10.1146/annurev-arplant-050718-095919

Cervencl, A., Esser, W., Maier, M., Oberdiek, N., Thyen, S., Wellbrock, A., & Exo, K.-M. (2011). Can differences in incubation patterns of Common Redshanks Tringa totanus be explained by variations in predation risk? *Journal of Ornithology*, *152*(4), 1033–1043. https://doi.org/10.1007/s10336-011-0696-z

Champagne, F. A. (2008). Epigenetic mechanisims and the transgenerational effects of maternal care. *Fronteirs in Neuroendocrinology*, 29(3), 386-397. https://doi.org/10.1016/j.yfrne.2008.03.003

Clutton-Brock, T. H. (1984). Reproductive Effort and Terminal Investment in Iteroparous Animals. *The American Naturalist*, *123*(2), 212–229. https://doi.org/10.1086/284198

Conover, D. O., & Heins, S. W. (1987). Adaptive variation in environmental and genetic sex determination in a fish. *Nature*, *326*(6112), 496–498. https://doi.org/10.1038/326496a0

Creel, S., Winnie, J. A., & Christianson, D. (2009). Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. *Proceedings of the National Academy of Sciences*, *106*(30), 12388–12393. https://doi.org/10.1073/pnas.0902235106

Curley, J. P., & Champagne, F. A. (2016). Influence of maternal care on the developing brain: mechanisms, temporal dynamics and sensitive periods. *Frontiers in Neuroendocrinology*. 40,52-66. https://doi.org/10.1016/j.yfrne.2015.11.001

Deeming, D.C., Ferguson, M.W.J. (1991). Incubation and embryonic development in reptiles and birds. In: Tullett, S.G. (ed), *Avian incubation*. Butterworths-Heinemann, London, pp. 3–37.

Deeming, D. C. (2004). Reptilian incubation: environment, evolution, and behaviour. Nottingham, UK: Nottingham University Press.

Deeming, D.C., & Reynolds, S.J. (2015). Nests, eggs, & incubation: new ideas about avian reproduction. Oxford University Press.

Delaney, D.M., Reedy, A.M., Mitchell, TS, Durso, AM, Durso, KP, Morrison, AJ, Warner, DA. (2013). *Anolis sagrei*. nest-site choice. *Herpetol. Rev.* 44(2): 314.

Delaney, D. M., & Janzen, F. J. (2020). Risk-sensitive maternal investment: An evaluation of parent–offspring conflict over nest site choice in the wild. *Animal Behaviour*, *163*, 105–113. https://doi.org/10.1016/j.anbehav.2020.03.004

DeWitt, T. J., & Scheiner, S. M. (Eds.). (2004). *Phenotypic plasticty: Functional and conceptual approaches*. Oxford University Press.

Doody, J. S., Guarino, E., Harlow, P., Corey, B., & Murray, G. (2006). Quantifying nest site choice in reptiles using hemispherical photography and gap light analysis. *Herpetological Review*, 37(1), 49-52.

Doody, J. S., Freedberg, S., & Keogh, J. S. (2009). Communal egg-laying in reptiles and amphibians: evolutionary patterns and hypotheses. *Q. Rev. Biol.* 8(3): 229-252.

Doody, J. Sean. (2009). Superficial lizards in cold climates: Nest site choice along an elevational gradient. *Austral Ecology*, *34*(7), 773–779. https://doi.org/10.1111/j.1442-9993.2009.01983.x

Doody, J. S. (2011). Environmentally cued hatching in reptiles. *Integrative and Comparative Biology*, *51*(1), 49–61. https://doi.org/10.1093/icb/icr043

Du, W., Hu, L., & Zhu, L. (2007). Effects of incubation temperature on embryonic development rate, sex ratio and pos-hatching growth in the Chinese three keeled pond turtle, Chinemys reevesii. *Aquaculture*, 272(1), 747,753. https://doi.org/10.1016/j.aquaculture.2007.09.009

Du, W., Warner, D. A., Langkilde, T., Robbins, T., & Shine, R. (2010). The physiological basis of geographic variation in rates of embryonic development within a widespread lizard species. *The American Naturalist*, *176*(4), 522–528. https://doi.org/10.1086/656270

Du, W.-G., & Shine, R. (2010). Why do the eggs of lizards (Bassiana duperreyi: Scincidae) hatch sooner if incubated at fluctuating rather than constant temperatures?: B. Duperreyi egg-hatching with fluctuating temperature.. *Biological Journal of the Linnean Society*, *101*(3), 642–650. https://doi.org/10.1111/j.1095-8312.2010.01525.x

Du, W.-G., & Shine, R. (2015). The behavioural and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature: Thermal response of reptile and bird embryos. *Biological Reviews*, *90*(1), 19–30. https://doi.org/10.1111/brv.12089

Dufty, A. (2002). Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evolution*, *17*(4), 190–196. https://doi.org/10.1016/S0169-5347(02)02498-9

Dulude-de Broin, F., Hamel, S., Mastromonaco, G. F., & Côté, S. D. (2020). Predation risk and mountain goat reproduction: Evidence for stress-induced breeding suppression in a wild ungulate. *Functional Ecology*, *34*(5), 1003–1014. https://doi.org/10.1111/1365-2435.13514

Ernst, C.H., Lovich, J.E., & Barbour, R.W. (1994). Turtles of the United States and Canada. Smithsonian Institution Press, Washington.

Fairbairn, D. J. (1984). Mierogeographic variation in body size and development time in the waterstrider, Limnoporus notabilis. *Oecologia*, *61*, 126–133.

Falconer, D. S. (1989). Introduction to Quantitative Genetics, Ed. 3. Longman, Essex.

Franklin, T. B., Russig H., Weiss, I. C., Graff, J., Linder, N., Michalon, A., Vizi, S., & Mansuy, I. M. (2010). Epigenetic transmission of the impact of early stress across generations. *Biological Psychiatry*, 68(5), 408-415. https://doi.org/10.1016/j.biopsych.2010.05.036

Fusco, G. & A. Minelli, (2010). Phenotypic plasticity in development and evolution: facts and concepts. *Philos Trans R Soc B*, 365 (1540), 547-556.

Galloway, L. F. (2005). Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist*, 166, 173, 383-392. https://doi.org/10.1111/j.1469-8137.2004.01314.x

Galloway, L. F., & Etterson, J. R. (2007). Transgenerational plasticity Is adaptive in the wild. *Science*, *318*(5853), 1134–1136. https://doi.org/10.1126/science.1148766

Gil, K., & Park, C. (2019). Thermal adaptation and plasticity of the plant circadian clock. *New Phytologist*, *221*(3), 1215–1229. https://doi.org/10.1111/nph.15518

Godfrey, S. T., Duberstein, J. A., Mota, J., & Moore, W. (2018). *Anolis carolinensis* (green anole) nest sites and communal nesting. *Herp. Rev.* 49(1): 115.

Goodman, R. M., & Heah, T.P. (2010). Temperature-induced plasticity at cellular and organismal levels in the lizard *Anolis carolinensis*. *Integr. Zool.* 5(3): 208–217.

Grostal, P., & Dicke, M. (1999). Direct and indirect cues of predation risk influence behavior and reproduction of prey: A case for acarine interactions. *Behavioral Ecology*, *10*(4), 422–427. https://doi.org/10.1093/beheco/10.4.422

Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1808), 20150401. https://doi.org/10.1098/rspb.2015.0401

Hager, R., Cheverud, J. M., & Wolf, J. B. (2009). Change in maternal environment induced by cross-fostering alters genetic and epigenetic effects on complex traits in mice. *Proceedings of the Royal Society B: Biological Sciences*, 276(1669). https://doi.org/10.1098/rspb.2009.0515

Hall, J. M., & Warner, D. A. (2018). Thermal spikes from the urban heat increase mortality and alter physiology of lizard embryos. *Journal of Experimental Biology*. https://doi.org/10.1242/jeb.181552

Hall, J. M., & Warner, D. A. (2019). Thermal tolerance in the urban heat island: Thermal sensitivity varies ontogenetically and differs between embryos of two sympatric ectotherms. *The Journal of Experimental Biology*, *222*(19), jeb210708. https://doi.org/10.1242/jeb.210708

Hall, J. M., Mitchell, T. S., Thawley, C., Stroud, J., & Warner, D.A. (2020). Adaptive seasonal shift towards investment in fewer, larger offspring: Evidence from field and laboratory studies. *Journal of Animal Ecology*, 89:1242-1253. https://doi.org/10.1111/1365-2656.13182

Hall, J. M., & Warner, D. A. (2020). Thermal sensitivity of lizard embryos indicates a mismatch between oxygen supply and demand at near-lethal temperatures. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, jez.2359. doi: https://doi.org/10.1002/jez.2359

Hansell, M.H. (2005). Animal Architecture. Oxford University Press, Oxford.

Harriman, V. B., Dawson, R. D., Bortolotti, L. E., & Clark, R. G. (2017). Seasonal patterns in reproductive success of temperate-breeding birds: Experimental tests of the date and quality hypotheses. *Ecology and Evolution*, *7*(7), 2122–2132. https://doi.org/10.1002/ece3.2815

Hasselquist, D., & Nillson, J. (2009). Maternal transfer of antibodies in vertebrates: trans-feneerational effects on offspring immunity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364,51-60. https://doi.org/10.1098/rstb.2008.0137

Hertz, P. E., Arima, Y., Harrison, A., Huey, R. B., Losos, J. B., & Glor, R. E. (2013). Asynchronous evolution of physiology and morphology in *Anolis* lizards. *Evolution*, *67*(7), 2101–2113. https://doi.org/10.1111/evo.12072

Huang, W.,-S, Pike, D. A. (2012). Effects of intraguild predators on nest-site selection by prey. *Oecologia*. 168 (1): 35-42.

Huang, W. (2006). Parental care in the long-tailed skink, Mabuya longicaudata, on a tropical Asian island. *Animal Behaviour*, *72*(4), 791–795. https://doi.org/10.1016/j.anbehav.2005.12.011

Huang, W.-S., Lin, S.-M., Dubey, S., & Pike, D. A. (2013). Predation drives interpopulation differences in parental care expression. *Journal of Animal Ecology*, *82*(2), 429–437. https://doi.org/10.1111/1365-2656.12015

Huey, R. B., P.E. Hertz, & B. Sinervo, (2003). Behavioral drive versus behavioral inertia in evolution: A null model approach. *Am Nat*, 161, 357-366.

Hulbert, A. C., Mitchell, T. S., Hall, J. M., Guiffre, C. M. Douglas, D. C., Warner, D. A. (2017). The effects of incubation temperature and experimental design on heart rates of lizard embryos. *Journal of Experimental Zoology Part A*, 327(7), 466-476. https://doi.org/10.1002/jez.2135

Janzen, F. J., and Morjan, C. L. (2001). Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Animal Behaviour*, 62(1), 73-82. https://doi.org/10.1006/anbe.2000.1732

Janzen, F. J., and Morjan C. L. (2002). Egg Size, Incubation Temperature, and Posthatching Growth in Painted Turtles (Chrysemys Picta). *Journal of Herpetology*, 36(2),308-311. https://doi.org/10.1670/0022-1511(2002)036[0308:ESITAP]2.0.CO;2

Johnston, I. A., & Temple, G. K. (2002). Thermal plasticity of locomotion. *The Journal of Experimental Biology*, *205*, 2305-2322. https://doi.org/10.1007/s00360-014-0869-1

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., … Beerli, P. (2001). The strength of phenotypic selection in natural populations. *The American Naturalist*, *157*(3), 245–261. https://doi.org/10.1086/319193

Krivoruchko K., & Butler K. (2013). Unequal Probability-Based Spatial Mapping. Redlands, CA, USA

Kolbe, J. J., & Janzen, F. J. (2002). Impact of nest-site selection on nest success and next temperature in natural and disturbed habitats. *Ecology*. 83: 772-781.

Lack, D. (2008). The Significance of Clutch-size. *Ibis*, *89*(2), 302–352. https://doi.org/10.1111/j.1474-919X.1947.tb04155.x

Landa, K. (1992). Seasonal declines in offspring fitness and selection for early reproduction in nymph-overwintering grasshoppers. *Evolution*, *46(1)*, 121–135.

Lapiedra, O., Schoener, T. W., Leal, M., Losos, J. B., & Kolbe, J. J. (2018). Predator-driven natural selection on risk-taking behavior in anole lizards. *Science*, *360*(6392), 1017–1020. https://doi.org/10.1126/science.aap9289

Laurel, B. J., Hurst, T. P., Copeman, L. A., & Davis, M. W. (2008). The role of temperature on the growth and survival of early and late hatching Pacific cod larvae (Gadus macrocephalus). *Journal of Plankton Research*, *30*(9), 1051–1060. https://doi.org/10.1093/plankt/fbn057

Lê, S., Josse, J., Husson, F. (2008) FactoMineR: An *R* package for multivariate analysis. J. Stat Softw, 25,1-18.

Le Henanff, M., Meylan, S., & Lourdais, O. (2013). The sooner the better: Reproductive phenology drives ontogenetic trajectories in a temperate squamate (*Podarcis muralis*): Hatching Date Drives Individual Trajectories. *Biological Journal of the Linnean Society*, *108*(2), 384–395. https://doi.org/10.1111/j.1095-8312.2012.02005.x

Les, H. L., Paitz, R. T., & Bowden, R. M. (2007). Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *307A*(5), 274–280. https://doi.org/10.1002/jez.374

Li, S. R., Hao, X., Wang, Y., Sun, B. J., Bi, J. H., Zhang, Y. P., Janzen, F. J., & Du, W. G. (2017). Female lizards choose warm, moist nests that improve embryonic survivorship and offspring fitness. *Funct. Ecol.* 32(2): 416–423.

Liang, L., Sun, B., Ma, L., & Du, W. (2015). Oxygen-dependent heat tolerance and developmental plasticity in turtle embryos. *Journal of Comparative Physiology B*, 185(2), 257-263. https://doi.org/10.1007/s00360-014-0874-4

Lima, S. L. (2009). Predators and the breeding bird: Behavioral and reproductive flexibility under the risk of predation. *Biological Reviews*, *84*(3), 485–513. https://doi.org/10.1111/j.1469-185X.2009.00085.x

Linden, M., & Pape Moller, A. (1989). Cost of reproduction and covariation of life history traits in birds. *Trends in Ecology & Evolution*, *4*(12), 367–371. https://doi.org/10.1016/0169-5347(89)90101-8

Lipar, J. L., & Ketterson, E. D. (2000). Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-winged blackbird Agelaius phoeniceus. *Proceedings of the Royal Society B:Biological Sciences*, 267, 2005-2010. https://doi.org/10.1098/rspb.2000.1242

Lloyd, J. D., & Martin, T. E. (2004). Nest-site preference and maternal effects on offspring growth. *Behav. Ecol*. 15(5): 816-823.

Lloyd, R. B., & Warner, D. A. (2019). Maternal nest-site choice does not affect egg hatching success in an invasive turtle population. *Behaviour,* 156(3): 265-285.

Lolavar, A., & Wyneken J. (2015). Effect of rainfall on loggerhead turtle nest temperatures, sand temperatures and hatchling sex. *Endanger. Species Res.* 28:235-247.

Losos, J. B., Warheit, K. I., & Schoener, T. W. (1997). Adaptive differentiation following experimental island colonization in Anolis lizards. *Nature*, *387*, 4.

Losos, J. B., Schoener, T. W., & Spiller, D. A. (2004). Predator-induced behaviour shifts and natural selection in field-experimental lizard populations. *Nature*, *432*(7016), 505–508. https://doi.org/10.1038/nature03039

Losos, J. B. (2009). *Lizards in an evolutionary tree: Ecology and adaptive radiation of anoles*. University of California Press.

Ludsin, S. A., & DeVries, D. R. (1997). First-year recruitment of largmouth bass: The interdependency of early life stages. *Ecological Applications*, *7*(3), 1024–1038. https://doi.org/10.1890/1051-0761(1997)007[1024:FYROLB]2.0.CO;2

Magnhagen, C. (1991). Predation risk as a cost of reproduction. *Trends in Ecology & Evolution*, *6*(6), 183–186.

Mainwaring, M. C., Barber, I., Deeming, D. C., Pike, D. A., Roznik, E. A., & Hartley, I. R. (2017). Climate change and nesting behaviour in vertebrates: a review of the ecological threats and potential for adaptive responses. *Biological Reviews*, 92, 1991-2002. https://doi.org/10.1111/brv.12317

Marshall, D. J., & Uller, T. (2007). When is a maternal effect adaptive? *Oikos* 116(12): 1957–1963.

Mateo, J. A., & Cuadrado, M. (2012). Communal nesting and parental care in oudri's fan-footed gecko (*Ptyodactylus oudrii*): field and experimental evidence of an adaptive behavior. *J. Herpetol.* 46(2): 209-212.

Michel, E. S., Strickland, B. K., Demarais, S., Belant, J. L., Kautz, T. M., Duquette, J. F., Beyer, D. E., Chamberlain, M. J., Miller, K. V., Shuman, R. M., Kilgo, J. C., Diefenbach, D. R., Wallingford, B. D., Vreeland, J. K., Ditchkoff, S. S., DePerno, C. S., Moorman, C. E., Chitwood, M. C., & Lashley, M. A. (2020). Relative reproductive phenology and synchrony affect neonate survival in a nonprecocial ungulate. *Functional Ecology*, *34*(12), 2536–2547. https://doi.org/10.1111/1365-2435.13680

Mitchell, T. S., Maciel J. A., & Janzen F. J. (2013A). Does Sex-ratio Selection Influence Nest-site Choice in a Reptile with Temperature-dependent Sex Determination? *Proceedings of the Royal Society B: Biological Sciences*, 280:20132460. https://doi.org/10.1098/rspb.2013.2460

Mitchell, T. S., Warner D. A., and Janzen F. J. (2013B). Phenotypic and Fitness Consequences of Maternal Nest-site Choice across Multiple Early Life Stages. *Ecology*, 94(2),336-345. https://doi.org/10.2307/23435981

Mitchell, T. S. Maciel, J. A., Janzen, F. J. (2015). Maternal effects influence phenotypes and survival during early life stages in an aquatic turtle. *Functional Ecology*, 29, 268-276. https://doi.org/10.1111/1365-2435.12315

Mitchell, T. S., Janzen, F. J., & Warner, D. A. (2018). Quantifying the effects of embryonic phenotypic plasticity on adult phenotypes in reptiles: A review of current knowledge and major gaps. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4–5), 203–214. https://doi.org/10.1002/jez.2187

Møller, A. P. (1988). Nest Predation and Nest Site Choice in Passerine Birds in Habitat Patches of Different Size: A Study of Magpies and Blackbirds. *Nordic Society Oikos*, 53(2), 215-221. https://doi.org/10.2307/3566065

Moore, M. P., Whiteman, H. H., & Martin, R. A. (2019). A mother's legacy: the strength of maternal effects in populations. *Ecol. Lett.* 22(10): 1620-1628.

Morjan, C. L. (2003). Variation in nesting patterns affecting nest temperatures in two populations of painted turtles (Chrysemys picta) with temperature-dependent sex determination. *Behavioural Ecology and Sociobiology*, 53, 254-261. https://doi.org/10. 1007/s00265-002-0570-3

Morse, D. H. (1990). Leaf choices of nest-building crab spiders (*Misumena vatia*). *Behav. Ecol. Sociobiol.* 27(4): 265-267.

Mousseau, T. A., & C. W. Fox. (1998). Maternal Effects as Adaptations. New York: Oxford UP.

Mueller, C. A., Bucsky, J., Korito, L., & Manzanares, S. (2019). Immediate and persistent effects of temperature on oxygen consumption and thermal tolerance in embryos and larvae of the Baja California Chorus Frog, Pseudacris hypochondriaca. *Frontiers in Physiology*, *10*, 754. https://doi.org/10.3389/fphys.2019.00754

Mukherjee, S., Heithaus, M. R., Trexler, J. C., Ray-Mukherjee, J., & Vaudo, J. (2014). Perceived Risk of Predation Affects Reproductive Life-History Traits in Gambusia holbrooki, but Not in Heterandria formosa. *PLoS ONE*, *9*(2), e88832. https://doi.org/10.1371/journal.pone.0088832

Murren, C. J., Maclean, H. J., Diamond, S. E., Steiner, U. K., Heskel, M. A., Handelsman, C. A., … Kingsolver, J. (2014). Evolutionary change in continuous reaction norms. *The American Naturalist*, *183*(4), 453–467. https://doi.org/10.1086/675302

Noble, D. W. A., Stenhouse, V., & Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: A systematic review and meta-analysis: Incubation temperature and plasticity. *Biological Reviews*, *93*(1), 72–97. https://doi.org/10.1111/brv.12333

Nussey, D. H., Kruuk, L. E. B., Morris, A., & Clutton-Brock, T. H. (2007). Environmental conditions in early life influence ageing rates in a wild population of red deer. *Current Biology*, *17*(23), R1000–R1001. https://doi.org/10.1016/j.cub.2007.10.005

Olsson, M., & Shine, R. (1997). The limits to reproductive output: offspring size versus number in the sand lizard (Lacerta agilis). *The American Naturalist*, *149*(1), 179–188. https://doi.org/10.1086/285985

Orizaola, G., & Laurila, A. (2009). Intraspecific variation of temperature-induced effects on metamorphosis in the pool frog (Rana lessonae). *Canadian Journal of Zoology*, *87*, 581–588. https://doi.org/10.1139/Z09-045

Packard, G. C., Packard, M. J., Miller, K., & Boardman, T. J. (1988). Effects of temperature and moisture during incubation on carcass composition of hatchling snapping turtles (Chelydra serpentine). *Journal of Comparative Physiology B*, 158(1), 117-125. https://doi.org/10.1007/BF00692735

Painter, R. C., Osmond, C., Gluckman, P., Hanson, M., Phillips, D. I. W., & Roseboom, T. J. (2008). Transgenerational effects of prenatal exposure to the dutch famine on neonatal adiposity and health in later life. *BJOG An International Journal of Obstetrics and Gynaecology*, 115(10), 1243-1249. https://doi.org/10.1111/j.1471-0528.2008.01822.x

Paitz, R. T., Harms, H. K., Bowden, R. M., & Janzen, F. J. (2007). Experience pays: offspring survival increases with female age. *Biology Letters*, 3(1), 44-46. https://doi.org/10.1098/rsbl.2006.0573

Paitz, R. T., Gould, A. C., Holgersson, M. C. N., & Bowden, R. M. (2010). Temperature, phenotype, and the evolution of temperature-dependent sex determination: how do natural incubations compare to laboratory incubations? *Journal of Experimental Zoology*, 314(1), 86-93. https://doi.org/10.1002/jez.b.21312

Paquet, M. Covas, R., & Doutrelant, C. (2015). A cross-fostering experiment reveals that prenatal environment affects begging behaviour in cooperative breeder. *Animal Behaviour*, 102, 251-258. https://doi.org/10.1016/j.anbehav.2015.01.034

Pearson, P. R., & Warner, D. A. (2016). Habitat- and season-specific temperatures affect phenotypic development of hatchling lizards. *Biology Letters*, *12*(20160646), 5. https://doi.org/10.1098/rsbl.2016.0646

Pearson, P. R., & Warner, D. A. (2018). Early hatching enhances survival despite beneficial phenotypic effects of late-season developmental environments. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1874), 20180256. https://doi.org/10.1098/rspb.2018.0256

Peet-Paré, C. A., & Blouin-Demers, G. (2012). Female eastern hog-nosed snakes (Heterodon platirhinos) choose nest sites that produce offspring with phenotypes likely to improve fitness. *Canadian Journal of Zoology*, 90(10), 1215-1220. https://doi.org/10.1139/z2012-091

Pigliucci, M. (2005). Evolution of phenotypic plasticity: Where are we going now? *Trends in Ecology & Evolution*, *20*(9), 481–486. https://doi.org/10.1016/j.tree.2005.06.001

Pigliucci, M., C. J. Murren, & C. D. Schlichting. (2006). Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol*, 209, 2362-2367.

Pigliucci, M., & Muller, G. (Eds.). (2010). *Evolution- the extended synthesis*. MIT Press.

Pike, D. A., Webb, J. K., & Shine, R. 2010. Nesting in a thermally challenging environment: Nest-site selection in a rock-dwelling gecko, *Oedura lesueurii* (Reptilia: Gekkonidae). *Biol. J. Linn. Soc.* 99(2): 250–259.

Polich, R. L., Bodensteiner, B. L., Adams, C. I. M., Janzen, F. J. (2018). Effects of augmented corticosterone in painted turtle eggs on offspring development and behaviour. *Physiology & Behaviour*, 183(1), 1-9. https://doi.org/10.1016/j.physbeh.2017.10.004

Pruett, J. E., Addis, E. A., & Warner, D. A. (2019). The influence of maternal nesting behaviour on offspring survival: Evidence from correlational and cross-fostering studies. *Animal Behaviour*, *153*, 15–24. https://doi.org/10.1016/j.anbehav.2019.04.010

Pruett, J. E., Fargevieille, A., & Warner, D. A. (2020). Temporal variation in maternal nest choice and its consequences for lizard embryos. *Behavioral Ecology*, *31*(4), 902–910. https://doi.org/10.1093/beheco/araa032

Rand, A. S. 1967. Communal egg laying in anoline lizards. *Herpetologica.* 23(3): 227–230.

Räsänen K., & Kruuk L. E. B. (2007). Maternal effects and evolution at ecological time-scales. *Functional Ecology*, 21(3), 408-421. https://doi.org/10.1111/j.1365-2435.2007.01246.x

Raveh, S., Vogt, D., & Kölliker, M. (2016). Maternal programming of offspring in relation to food availability in an insect (Forficula auricularia). *Proceedings of the Royal Society B: Biological Sciences*, 283(1828). https://doi.org/10.1098/rspb.2015.2936

Reedy, A. M., Zaragoza, D., & Warner, D. A. (2013). Maternally chosen nest sites positively affect multiple components of offspring fitness in a lizard. *Behav. Ecol.* 24(1), 39–46.

Refsnider, J. M., & Janzen, F. J. (2010). Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics*, *41*(1), 39–57. https://doi.org/10.1146/annurev-ecolsys-102209-144712

Refsnider, J. M., Bodensteiner, B.L. , Reneker, J.L., & Janzen, F. J. (2013). Experimental field studies of species' responses to climate change: Challenges and future directions. *Anim. Conserv.* 16(5): 498–499.

Refsnider, J. M., Reedy, A. M., Warner, D. A., & Janzen, F. J. (2015). Do trade-offs between predation pressures on females versus nests drive nest-site choice in painted turtles? *Biological Journal of the Linnean Society*, *116*(4), 847–855. https://doi.org/10.1111/bij.12671

Refsnider, J. M. (2016). Nest-site choice and nest construction in non-avian reptiles: Evolutionary significance and ecological implications. *Avian Biol. Res.* 9(2): 76–88.

Régnière, J., Powell, J., Bentz, B., & Nealis, V. (2012). Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modeling. *Journal of Insect Physiology*, *58*(5), 634–647. https://doi.org/10.1016/j.jinsphys.2012.01.010

Richard C. Lewontin. (2000). *Triple helix: Gene, organism, and environment: A new introduction*. Cambridge, MA: Harvard University Press.

Riddell, E. A., & Sears, M. W. (2015). Geographic variation of resistance to water loss within two species of lungless salamanders: Implications for activity. *Ecosphere*, *6*(5), art86. https://doi.org/10.1890/ES14-00360.1

Riddell, E. A., Odom, J. P., Damm, J. D., & Sears, M. W. (2018). Plasticity reveals hidden resistance to extinction under climate change in the global hotspot of salamander diversity. *Science Advances*, *4*(7), eaar5471. https://doi.org/10.1126/sciadv.aar5471

Riddell, E. A., Roback, E. Y., Wells, C. E., Zamudio, K. R., & Sears, M. W. (2019). Thermal cues drive plasticity of desiccation resistance in montane salamanders with implications for climate change. *Nature Communications*, *10*(1), 4091. https://doi.org/10.1038/s41467-019-11990-4

Roff, D. A. (1990). The evolution of flightlessness in Insects. *Ecological Monographs*, *60*(4), 389–421. https://doi.org/10.2307/1943013

Roff, D. A., & Fairbairn, D. J. (1991). Wing dimorphisms and the evolution of migratory polymorphisms among the insecta. *American Zoologist*, *31*(1), 243–251. https://doi.org/10.1093/icb/31.1.243

Roosenburg, W. M. (1996). Maternal Condition and Nest Site Choice: An Alternative for the Maintenance of Environmental Sex Determination? *American Zoologist,* 36(2), 157-168. https://www.jstor.org/stable/3884192

Saino, N., Calza S., & Moller A. P. (1997). Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *J. Anim. Ecol.* 66:827-836.

Sanger, T. J., Losos, J. B., & Gibson-Brown, J. J. (2007). A developmental staging series for the lizard genus anolis: a new system for the integration of evolution, development, and ecology. *J. Morphol.* 269: 254–274.

Sanger, T. J., Hime, P. M., Johnson, M. A., Diani, J., & Losos, J. B. (2008). Laboratory protocols for husbandry and embryo collection of Anolis lizards. *Herpetological Review*, *39*(1), 58–63.

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, *24*, 35–68. https://doi.org/10.1146/annurev.es.24.110193.000343

Scheiner, S. M., & Lyman, F. (n.d.). *The genetics of phenotypic plasticity I. Heritability*. 13.

Scheuerlein, A., Van't Hof, T., & Gwinner, E. (2001). Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*(1476), 1575–1582. https://doi.org/10.1098/rspb.2001.1691

Schulte, P. M., Healy, T. M., & Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, *51*(5), 691–702. https://doi.org/10.1093/icb/icr097

Schwanz, L. E., & Janzen, F. J. (2008). Climate change and temperature-dependent sex determination: Can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiol. Biochem. Zool.* 81(6): 826–834.

Seigel, R. A., Huggins, M. M., & Ford, N. B. (1987). Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia*, *73*(4), 481–485. https://doi.org/10.1007/BF00379404

Shama, L. N. S., Mark, F. C., Strobel, A., Lokmer, A., John, U., & Wegner, K. M. (2016). Transgenerational effects persist down the maternal line in marine sticklebacks: gene expression matches physiology in a warming ocean. *Evolutionary Applications*, 9(9), 1096-1111. https://doi.org/10.1111/eva.12370

Shine, R. (2005). Life-history evolution in reptiles. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 23–46. https://doi.org/10.1146/annurev.ecolsys.36.102003.152631

Sinervo, B., & Adolph, S. C. (1994). Growth plasticity and thermal opportunity in sceloporus lizards. *Ecology*, *75*(3), 776–790. https://doi.org/10.2307/1941734

Smith, H. M., Sinelnik, G., Fawcett, J. D., & Jones, R. E. (1972). A survey of the chronology of ovulation in Anoline lizard genera. *Transactions of the Kansas Academy of Science (1903-)*, *75*(2), 107. https://doi.org/10.2307/3627160

Smith, C., Telemeco, R. S., Angilletta, M. J., & VendenBrooks, J. M. (2015). Oxygen supply limits the heat tolerance of lizard embryos. *Biology Letters*. https://doi.org/10.1098/rsbl.2015.0113

Socci, A. M., Schlaepfer, M. A., & Gavin, T. A. (2005). The importance of soil moisture and leaf cover in a female lizard's (*Norops polylepis*) evaluation of potential oviposition sites. *Herpetologica* 61(3): 233–240.

Spencer, R. J., Thompson, M. B., & Banks, P. B. (2001). Hatch or wait? A dilemma in reptilian incubation. *Oikos*, *93*(3), 401–406. https://doi.org/10.1034/j.1600-0706.2001.930305.x

Spencer, R. J. (2002). Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles. *Ecology* 83(8): 2136–2144.

Stamps, J. A. (1978). A field study of the ontogeny of social behavior in the lizard anolis aene Us. *Behaviour*, *66*(1–2), 1–30. https://doi.org/10.1163/156853978X00396

Stroud, J. T. (2017). Establishment of Anolis sagrei on bermuda represents a novel ecological threat to critically endangered Bermuda skinks (Plestiodon longirostris). *Biological Invasions*, *19*, 1723–1731. https://doi.org/10.1007/s10530-017-1389-1

Swierk, L., Carriere, A., Delfin, M., Fondren, A., Lopera, D., & Putnam, B. J. (2019). *Anolis aquaticus* (=*Norops aquaticus*) (Water Anole), egg size and oviposition site. *Herpetol. Rev.* 50(2): 360.

Telemeco, R. S., Elphick, M. J., & Shine, R. (2009). Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology* 90(1): 17–22.

Telemeco, R. S., Warner, D. A., Reida, M. K., Janzen, F. J. (2013). Extreme developmental temperatures results in morphological abnormatlities in painted turtles (Chrysemys picta): a climate change perspective. *Integrative Zoology*, 8,198-209. https://doi.org/10.1111/1749-4877.12019

Telemeco, R. S., Gangloff, E. J., Cordero, G. A., Mitchell, T. S., Bodensteiner, B. L., Holden, K. G., Mitchell, S. M., Polich, R. L., & Janzen, F. J. (2016). Reptile embryos lack the opportunity to thermoregulate by moving within the egg. *American Naturalist*, 188, E13-E37. https://doi.org/10.1086/686628

Tiatragul, S., Hall, J. M., Pavlik, N.G., & Warner, D. A. (2019). Lizard nest environments differ between suburban and forest habitats. *Biol. J. Linn. Soc*. 126(3): 392–403.

Tiatragul, S., Hall, J. M., & Warner, D. A. (2020). Nestled in the city heat: Urban nesting behavior enhances embryo development of an invasive lizard. *Journal of Urban Ecology*, *6*(1), juaa001. https://doi.org/10.1093/jue/juaa001

Tinne, S., Rianne, P., & Marcel, E. (2005). Experimental removal of the male parent negatively affects growth and immunocompetence in nestling great tits. *Oecologia* 145: 165-173.

Tucker, J. K., & Warner, D. A. (1999). Microgeographic variation in response of red-eared slider (Trachemys scripta elegans) embryos to similar incubation environments. *Journal of Herpetology*, *33*(4), 549–557. https://doi.org/10.2307/1565571

Tüzün, N., & Stoks, R. (2018). Pathways to fitness: Carry-over effects of late hatching and urbanisation on lifetime mating success. *Oikos*, *127*, 949–959. https://doi.org/10.1111/oik.05033

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends in Ecology & Evolution*, *23*(8), 432–438. https://doi.org/10.1016/j.tree.2008.04.005

Van de Pol, M., Bakker, T., Saaltink, D., Verhulst, S. (2006). Rearing conditions determine offspring survival independent of egg quality: a cross-foster experiment with

Oystercatchers Haematopus ostralegus. *International Journal of Avian Science*, 148(2), 203-210. https://doi.org/10.1111/j.1474-919X.2006.00479.x

Van de Pol, M. (2012). Quantifying individual variation in reaction norms: How study design affects the accuracy, precision and power of random regression models: *Individual variation in reaction norms*. *Methods in Ecology and Evolution*, *3*(2), 268–280. https://doi.org/10.1111/j.2041-210X.2011.00160.x

Warner, D. A., & Andrews, R. M. (2002). Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biological Journal of the Linnean Society*, 76 (1), 105-124. https://doi.org/10.1111/j.1095-8312.2002.tb01718.x

Warner, D. A., Andrews, R. M. (2002). Nest-site selection in relation to temperature and moisture by the lizard *Sceloporus undulatus*. *Herpetologica* 58(4) 399–407.

Warner, D. A., & Shine, R. (2005). The adaptive significance of temperature-dependent sex determination: Experimental tests with a short-lived lizard. *Evolution*, *59*(10), 2209–2221. https://doi.org/10.1111/j.0014-3820.2005.tb00929.x

Warner, D. A., & Shine, R. (2007). Fitness of juvenile lizards depends on seasonal timing of hatching, not offFrrspring body size. *Oecologia*, *154*, 65–73.

Warner, D. A., & Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile. *Nature*, *451*(7178), 566–568. https://doi.org/10.1038/nature06519

Warner, D. A., Shine, R. (2008). Maternal nest-site choice in a lizard with temperature-dependent sex determination. *Anim. Behav.* 75(3): 861–870.

Warner, D. A., & Shine, R. (2009). Maternal and environmental effects on offspring phenotypes in an oviparous lizard: do field data corroborate laboratory data? *Oecologia*, 161, 209-220. https://doi.org/10.1007/s00442-009-1366-1

Warner, D. A., Jorgensen, C. F., & Janzen, F. J. (2010). Maternal and abiotic effects on egg mortality and offspring size of turtles: temporal variation in selection over seven years. *Functional Ecology*, 24, 857-866. https://doi.org/10.1111/j.1365-2435.2010.01714.x

Warner, D. A., Moody, M. A., Telemeco, R. S., & Kolbe, J. J. (2012). Egg environments have large effects on embryonic development, but have minimal consequences for hatchling phenotypes in an invasive lizard: Environmental effects on development in lizards. *Biological Journal of the Linnean Society*, *105*(1), 25–41. https://doi.org/10.1111/j.1095-8312.2011.01778.x

Warner, D. A., & Mitchell, T. S. (2013). Does maternal oviposition site influence offspring dispersal to suitable habitat? *Oecologia*, 172(3), 679-688. https://doi.org/10.1007/s00442-012-2533-3

Warner, D. A. (2014). Fitness consequences of maternal and embryonic responses to environmental variation: Using reptiles as models for studies of developmental plasticity. *Integrative and Comparative Biology*, *54*(5), 757–773. https://doi.org/10.1093/icb/icu099

Warner, D. A., & Lovern, M. B. (2014). Maternal environments affect 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

Weisrock, D. W., & Janzen F. J. (1999). Thermal and Fitness-related Consequences of Nest Location in Painted Turtles (Chrysemys Picta). *Functional Ecology*, 13(1), 94-101. https://doi.org/10.1046/j.1365-2435.1999.00288.x

Wesolowski, T. (2002). Anti-predator adaptations in nesting Marsh Tits Parus palustris: The role of nest-site security. *Ibis*, *144*, 593–601.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. New York: Oxford University Press.

While, G. M., Noble, D. W. A., Uller, T., Warner, D. A., Riley, J. L., Du, W.-G., & Schwanz, L. E. (2018). Patterns of developmental plasticity in response to incubation temperature in reptiles. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4–5), 162–176. https://doi.org/10.1002/jez.2181

Wickander, N. J., Rasmussen, P. U., Marteinsdóttir, B., Ehrlén, J., & Tack, A. J. M. (2020). Ecological and evolutionary responses of an arctic plant to variation in microclimate and soil. *Oikos*, oik.07794. https://doi.org/10.1111/oik.07794

Williams, Ernest E. (1972). "3. The Origin of Faunas. Evolution of Lizard. Congeners in a Complex. Island Fauna: A Trial Analysis". In T. Dobzhansky; et al. (eds.). *Evolutionary Biology*. *Meredith Corporation.* pp. 47–89. refer page 72

Wilson, D. S. (1998). Nest-site selection: Microhabitat variation and its effects on the survival of turtle embryos. *Ecology*, 79, 1884-1892. https://doi.org/10.1890/00129658(1998)079[1884:NSSMVA]2.0.CO;2

Wolf, J. B., & Wade, M. J. (2009). What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1520), 1107-1115. https://doi.org/10.1098/rstb.2008.0238.

Ydenberg, R. C., & Dill, L. M. (1986). The Economics of Fleeing from Predators. In *Advances in the Study of Behavior* (Vol. 16, pp. 229–249). Elsevier. https://doi.org/10.1016/S0065-3454(08)60192-8

Zverev, V., Kozlov, M. V., Forsman, A., & Zvereva, E. L. (2018). Ambient temperatures differently influence colour morphs of the leaf beetle Chrysomela lapponica: Roles of thermal melanism and developmental plasticity. *Journal of Thermal Biology*, *74*, 100–109. https://doi.org/10.1016/j.jtherbio.2018.03.019