

Maternal nesting behavior in lizards enhances colonization of urban environments

by

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Abstract

Lizards occupy diverse habitats around the world from dense forests to arid deserts. The environments in which lizards exist pose different challenges for nesting females because embryo development is directly affected by nest microenvironments. For lizards that occupy urban environments, the nesting situation for females is exacerbated as ground temperature can extend beyond tolerable limits for developing embryos. Despite this, many species have established populations in urban areas outside their native ranges and habitats. Without physiological adaptations by embryos to markedly hotter conditions, maternally-selected nest sites may facilitate embryonic survival. Mothers may choose nests in microenvironments that are, on average, cooler than microenvironments that are not used for nesting. In turn, maternal choice of nest microenvironment should facilitate egg survival. To test the hypothesis, I studied the Puerto Rican crested anole (*Anolis cristatellus*) from urban area in Miami, FL. I searched random plots for nests and recorded microenvironment variables in the field. In the lab, I incubated eggs from a captive colony of wild-caught *A. cristatellus* from Miami under thermal conditions that mimic 1) maternally-selected nests in urban areas, 2) sites not used for nesting in urban areas, and 3) nests sites in a nearby forest. My results indicate that thermal regimes of maternally-selected urban nests yield higher egg survival than those of sites that were not used by nesting females. Survival did not differ between urban and forest nest treatments. However, eggs incubated under urban treatments developed faster and hatched earlier than those incubated

under forest thermal regimes. My study revealed that maternal nest sites in urban areas shield embryos from potentially lethal conditions, which enhances colonization of anoles in Miami.

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List of Abbreviations

SVL Snout-to-vent length

TL Tail length

Chapter 1: General Introduction

Urbanization is a pervasive, rising, and ecologically significant element of global change in the Anthropocene (Lewis & Maslin, 2015). As the human population continues to rise, natural areas make way for cities which dramatically transform natural landscapes. The increase in impervious surfaces, loss of vegetation cover, and increase in heat retaining materials collectively lead to elevated local temperatures due to the “urban heat island effect” (Rizwan et al., 2008; Forman, 2014). This creates novel ecological niches to which native species must adapt or acclimate, and non-native species may exploit and establish new populations (Riley et al., 2005). In particular, warmer temperatures have important consequences for ectothermic animals, many of which elicit behavioral, morphological, and physiological responses to thermal stress (Hufbauer et al., 2012). However, embryos that develop within nests have limited capacity to behaviorally thermoregulate since eggs are typically left exposed to prevailing environments (Telemeco et al., 2016). To deal with extreme environments, natural selection may favor either physiological adjustments that enable embryos to cope with new conditions, or behavioral adjustments by mothers so that eggs are laid in habitats that buffer embryos from extreme conditions. Hence, the goal of my research is to experimentally assess the role of maternal nesting behavior on the success of an invasive species in novel environments, specifically urban areas.

Nesting is a critical component of reproduction for oviparous organisms. Females select nest locations with conditions that facilitate proper development of their offspring (Hansell, 2005). Because extended exposure of embryos to conditions beyond physiological limits can

lead to abnormal development and death (Van Eenennaam et al., 2005; DuRant et al., 2013; Sanger et al. 2018), the capacity of mothers to choose suitable nest microenvironments should be under strong selection (DuRant et al., 2013; Mainwaring et al., 2017).

In oviparous species that lack parental care (e.g., most reptiles), embryos develop within nests and have limited capacity to respond to adverse conditions because eggs are left exposed to prevailing environments (Telemeco et al., 2016). In the absence of substantial behavioral responses by embryos (Shine & Du, 2018), maternal choice of nest microenvironment (e.g., temperature, canopy cover, substrate, moisture) can influence egg hatching success (Deeming & Ferguson, 1991), as well as offspring phenotypes (Deeming, 2004), sex ratios (Doody et al., 2006; Refsnider & Janzen, 2010), and fitness (Shine & Harlow, 1996; Brown & Shine, 2004, Mitchell et al., 2018). Furthermore, nesting behavior via microenvironment selection can shield embryos from adverse conditions (Doody et al., 2015; Mainwaring et al., 2017), particularly in spatially and temporally heterogeneous environments (Löwenborg et al., 2011; Carlo et al., 2018). Several studies provide evidence that the non-random use of microenvironments by nesting females is adaptive (Brown & Shine, 2004; Reedy et al., 2013; Li et al., 2018).

Study System

The focus of this research is to determine how maternal effects contribute to the success of an invasive lizard in urban environments. Some invasive species that were introduced as biological control or part of the pet trade have shown extensive phenotypic plasticity and increased ability to take over novel niches (Urban et al., 2007; Davidson et al., 2011). Due to its geographical location and history, Miami (Florida, USA) has become an area of artificially high biological diversity due the staggering number of non-native plants and animals that have been introduced.

Among the foreign residents that have successfully colonized both the urban and forest areas is the Puerto Rican crested anole (*Anolis cristatellus*), a small diurnal lizard introduced on multiple, independent occasions in Miami (Kolbe et al., 2012). Previous studies provide evidence that adults are locally adapted to urban environments: increased tolerance to lower winter temperature in Miami (introduced range), and longer limbs relative to body size with more subdigital scales suited for clinging on to impervious surfaces (Kolbe et al., 2012; Winchell et al., 2016). Yet, we know little about nesting behavior and nest conditions in this species, or anoles in general (Rand, 1967; Andrews, 1982; Schlaepfer, 2003).

Successful embryonic development is essential in a species' ability to establish a viable population (Mainwaring et al., 2017). For anoles in Miami, there is no evidence of physiological adaptation by embryos to urban thermal environments (Tiatragul et al., 2017). However, maternal behavior may play an important role in facilitating embryo survival in urban environments, but relationships among maternal nesting behavior, nest microenvironment, and offspring fitness are largely unexamined. Such information would be critical in understanding how embryos might respond to human-altered habitats.

Anoles lay eggs in the ground, where developing embryos are directly exposed to variable soil temperatures and moisture content (Deeming, 2004). These factors can vary significantly between urban and natural areas, which has the potential to affect development and subsequent hatchling performance (body conditions, survival, flight-initiation distance, etc.). One way that maternal behavior can indirectly buffer developing embryos from extreme environmental condition is through nest-site choice (Mainwaring et al., 2017). However, nest conditions of anoles are poorly understood, except that females use a variety of nest sites (Rand, 1967; Andrews, 1988) and tend to prefer relatively moist conditions for nesting (Socci et al., 2005; Reedy et al., 2013). Even though

anoles have been subject to extensive studies, we still have much to learn about two critical factors that could affect their fitness in an urban environment: embryonic development and maternal nesting behavior.

Questions and Hypotheses

Q1. What are the characteristics of nest microenvironments in urban environments.

Q2. Do nest microenvironments in urban site differ from nest microenvironments in forest?

Hypothesis 1: Microenvironments of maternally selected nest sites differ from sites females did not choose. To test this hypothesis, I will search for maternally selected sites and take pertinent microenvironment data (canopy cover, moisture, substrate type, distance to closest nest, and temperature). Then, I will compare these data with those collected from other random plots in my experimental blocks. I predict that mothers will avoid oviposition in overly hot or dry plots in urban areas to buffer eggs from lethal conditions, whereas nest sites in the forest will be widely distributed across the landscape due to cooler conditions and greater thermal homogeneity.

Q3. Does the availability suitable nesting habitat differ between the urban and forest sites?

Hypothesis 2: Availability of suitable nesting habitat differ between urban and forest sites. To test this hypothesis, I will compare the odds of finding a suitable nesting location based on randomized coordinates. Because urban areas contain substrates lizards cannot lay eggs in (e.g., roads and paved walkways), I predict that the forest will have less plots that are unsuitable for nesting compared to the urban area.

Q4. Does survival differ under conditions that mothers chose compared to conditions that mothers did not choose?

Hypothesis 3: Egg survival differ for eggs incubated under conditions mothers chose compared to conditions mothers did not choose. Natural selection should favor nesting behaviors that positively affect embryonic and post embryonic development (Fox et al., 1997). Embryonic development of anoles is robust to urban thermal environments (Tiatragul et al., 2017), but unaccounted variables such as “thermal spikes” are lethal to developing embryos (Hall & Warner, 2018). I predict that females in the urban areas select nest site that shield developing embryos from unusually hot thermal spikes.

Q5. Do eggs from urban environments have greater hatching success in an urban environment than in a forest environment, and vice versa for eggs from forest environments (environment matching)?

Q6. Do maternally selected nest sites affect hatchling phenotypes?

Hypothesis 4: Hatchling success of eggs from urban and forest population differ based on incubation thermal regime. To test this, I will subject eggs to incubators programmed to mimic natural conditions that mothers chose and did not choose from each site such that each treatment contains eggs from both populations (urban and forest). I predict embryos from urban population will have greater survival under urban incubation regime than eggs from forest population, and vice versa. Acclimation or exposure to urban thermal spikes by mothers as adults may induce differential allocation to offspring that prepare embryos from thermal stress. I predict that hatchling morphology will not differ between incubation regimes.

Chapter 2: Description of Anole Nest Sites

Introduction

Nesting is a critical factor that influence fitness for oviparous organisms because females must select nest sites that facilitate successful development of offspring (Hansell, 2005). Because extended exposure of embryos to unfavorable environmental conditions can lead to abnormal development and death (Van Eenennaam et al., 2005; DuRant et al., 2013; Sanger et al., 2018), nest site choice should be under strong selection (DuRant et al., 2013; Mainwaring et al., 2017). Moreover, due to anthropogenic factors, such as climate change (Field et al., 2014) and habitat modification (e.g., land-use change; Forman, 2014), the ability to adjust nesting behavior is crucial for reproductive success and population persistence (Telemeco et al., 2009; Mainwaring et al., 2017).

For oviparous species that lack parental care (e.g., most non-avian reptiles), embryos develop within nests and have limited capacity to respond to adverse conditions because eggs are left exposed to prevailing environments (Telemeco et al., 2016). Under predictable environmental conditions, maternal choice of nest microenvironment (e.g., temperature, canopy cover, substrate, moisture) can influence egg hatching success (Deeming & Ferguson, 1991; Mainwaring et al., 2017), offspring phenotypes (Deeming, 2004), sex ratios (Doody et al., 2006; Refsnider & Janzen, 2010), and offspring fitness (Shine & Harlow, 1996; Brown & Shine, 2004, Mitchell et al., 2018a). Furthermore, nesting behavior can shield embryos from adverse conditions (Doody et al., 2015; Mainwaring et al., 2017), and this may be most critical in spatially and temporally heterogeneous environments (Löwenborg et al., 2011; Carlo et al.,

2018). Thus, several studies provide evidence that the non-random use of microenvironments by nesting females is adaptive (Brown & Shine, 2004; Reedy et al., 2013; Li et al., 2018).

Anolis lizards can serve as an excellent model for studies of nesting behavior in heterogeneous environments. Anoles have served as models for studies of ecology, evolution, and behavior (Sanger et al., 2008; Losos, 2009; Mitchell et al., 2018b) making them ideal for research on adaptation to rapidly changing environments (Kolbe et al. 2012, Winchell et al., 2016; Donihue et al., 2018). Although little is currently known about the specific microenvironments that females choose for nesting in the field (Rand, 1967; Andrews, 1982), laboratory research shows that the conditions chosen by females have consequences for offspring fitness (Socci et al., 2005; Reedy et al., 2013). Moreover, laboratory-based information about the effects of egg incubation conditions on egg survival and phenotypic development in *Anolis* is accumulating (Table S1.1). Results from these laboratory studies are insightful, as they help direct predictions about optimal microenvironments that females should choose for nesting in different environments. Information about nest microenvironments in the field will be critical in understanding how embryos might respond to human-altered habitats.

I have two primary objectives for this study. My first is to characterize nest microenvironments of common anoles within two diverse habitats: a urban area and an adjacent forest. My approach is unique compared to existing field studies of *Anolis* nest conditions in that I made quantitative measurements of microenvironment variables, rather than describing general microenvironments of locations where eggs were found (e.g., under leaf litter, in tree hole; Sexton et al., 1963; Andrews, 1982; Schlaepfer, 2003). To do this, I systematically searched for anole nests in urban and forest locations and collected microenvironment data (e.g., temperature, shade cover, soil moisture). Due to the urban heat island effect, I predict that nest conditions will

be warmer and more variable in urban areas compared to forest areas. My second objective is to summarize published information from laboratory egg incubation studies of *Anolis* lizards to provide insights into the consequences of maternal nesting behavior between diverse habitats. Based on microenvironment data of nests coupled with information from the literature, I discuss how developmental rate and egg hatching success may differ between urban and forest habitats.

Material and Methods

Study sites

My study consisted of an urban green space and a nearby forest located 1.2 km from each other. The two sites are separated by busy roads, a residential area, and a canal, and differ substantially in surrounding habitat structure (Fig. 1.1). The urban site is located along a 1 km stretch of land between a two-lane road (57th Avenue, “Red Road”) and Snapper Creek canal, which runs along the edge of an urban neighborhood (Pinecrest) in South Miami, FL. Parallel to the busy road is a paved bicycle trail that is frequently used by pedestrians, bicyclists, and runners. The bicycle trail is flanked by a regularly-mowed lawn next to the road, and by an unkept lawn (i.e. thick leaf litter) scattered with *Ficus* and palm trees along the canal. This area also has many human-made structures like stone-walls, lamp posts, small bus stops and guard rails (Fig. 1.1A).

The nearby forest site is in the interior of Matheson Hammock Preserve, which is accessible via walking trail (Fig. 1.1B). The preserve is a large fragment (0.21 km²) of dense homogenous forest consisting of large *Ficus* trees interspersed by smaller trees. The ground consists of karst and oolitic limestone with dense leaf litter covering a thin layer of soil. The preserve interior contains no human-made structures and is infrequently visited by humans as most stay on a paved trail on the perimeter of the preserve.

Nest search protocols

Five species of anoles are established at my study sites: *Anolis cristatellus*, *A. sagrei*, *A. distichus*, *A. equestris* (all non-native), and *A. carolinensis* (native). It is difficult to distinguish among eggs of these species except for the noticeably large eggs of *A. equestris*. I am confident that most eggs found at the forest site are from *A. cristatellus* given that *A. distichus* and *A. carolinensis* were only rarely encountered within my plots, and *A. sagrei* was never sighted. In contrast, urban plots contained all species. Thus, I cannot definitively determine the species of each egg, and henceforth, I use the term anole, generally (see Table S1.2 for visual encounter survey data). Importantly, because *Anolis* lizards have similar nesting requirements in the lab (Sanger et al., 2008a) and patterns of development appear highly conserved across species (Sanger et al., 2008b), my data are useful given the general scarcity of information on anole nest conditions in the field.

To quantify microenvironments used by nesting females, I established four searchable blocks in each site that consisted of a chosen central tree where anoles were abundant. Each tree was surrounded by a circular area with a radius of 20 m (i.e. block). I randomly selected ten 1 m² plots within each block (n = 40 urban plots across four blocks; n = 40 forest plots across four blocks). The location of each plot was determined by randomly choosing a distance (between 1 - 20 m) and direction (between 0 - 359° from North) from each central tree. If the randomized direction and distance (measured with compass and measuring tape) landed in an area obviously unsuitable for nesting (i.e. asphalt, pavement, body of water, rock face), then I randomized a new location until the plot landed in a searchable area; the number of times I randomized for new plots was recorded to account for availability of nesting locations between sites. Once I located a searchable area, I laid out a 1 m² quadrat and searched the plot for hatched or unhatched eggs by

removing all ground cover debris (e.g., leaf litter, logs, small rocks). Hatched eggshells are good proxy for nest sites because hatchlings rarely move their eggs after hatching and eggshells remained stationary despite heavy rain over the study period (S Tiatragul per obs.). If the soil was loose, I ran my fingers or spoons 1 – 3 cm from the soil surface to locate any buried eggs. For each egg found, I marked the exact location with a labelled flagging tape. I considered the location of each egg to be a nest since anoles lay single-egg clutches. Hence, there may be multiple eggs within a single plot. With the above protocol, I found a total of 47 nests (n = 36 forest; n = 11 urban).

To increase representation of nests, I also conducted “targeted” searches. For targeted searches, I looked for nests in areas that appeared suitable based on knowledge gathered from my previously described sampling method as well as my own experience working with anoles in the laboratory and field. Due to the heterogeneous landscape of the urban site, I searched further away from the original blocks and found 11 additional plots with a single nest. Since targeted plots were found further away from the original searching blocks, for analysis purpose, I assigned them to four new blocks to account for landscape heterogeneity. All additional plots in the forest (n = 4) were found within the same blocks used previously. When combining random and targeted plots, I had a total of 62 plots with nests (n = 40 forest; n = 22 urban) from 12 blocks (n = 8 original; n = 4 targeted in urban site).

Microenvironment measurements

For each plot, I recorded hourly temperatures between 3 July–15 August 2017 (n = 43 days) with a Thermochron iButton (programmed to record hourly) wrapped in a water balloon and Parafilm. Since some plots contained more than one egg, I placed the iButton next to the nest with an unhatched egg. If the plot contained only hatched eggs, I placed the iButton next to a

random hatched egg in that plot. Hemispherical photographs were taken above each plot using a Canon Powershot Elph 180 with a 180° fisheye lens. I calculated percent canopy openness using Gap Light Analysis (ver. 2.0) software on hemispherical photographs (Doody et al., 2006).

Weekly substrate moisture was measured between 2 July–18 August 2017 (n = 8 weeks). To calculate substrate (soil and organic material) moisture throughout the study period, I collected weekly substrate samples (5 cm³) from each plot approximately 15 cm away from the iButton to minimize disturbance of the temperature profile. Substrate samples were collected from the ground surface to ~1 cm into the ground (due to shallow layer of soil between 1-3cm deep) and kept in a 5 ml capped tube. I weighed each tube within 3 h of collection and dried the sample in a 60 °C incubator with the tube cap open. The dry mass was recorded after three consecutive days of weighing the tube to the nearest 0.01 g with no change in mass. Percent water content was calculated by subtracting dry mass from wet mass divided by the wet mass multiplied by 100. These protocols provided temperature and moisture profiles for each plot over 43 days, which covers the range of a typical incubation period for these anole species (Goodman & Walguarnery, 2007; Goodman, 2008; Fetters & McGlothlin, 2017; Tiatragul et al., 2017).

For each nest, I also recorded whether eggs were hatched or unhatched, its specific location (buried under soil or on soil surface), whether it was beneath surface debris (leaf litter, sticks, other organic litter and small rocks), distance to the nearest two trees, the size of those trees (circumference at breast height), and its distance to road (at urban plots only).

Literature search and data collection

I conducted a literature search using Web of Science (v. 5.29) with ‘title’ or ‘topic’ search terms: lizard, temperature* AND incubat* AND anol* AND *embryo. I then performed another search with TITLE: (anol*) AND TOPIC: (incubat*) AND TOPIC: (temperature). I also

requested data from individuals who study the common anole species at my study site. My search returned 27 unique papers, of which 22 were about anoles or anoline lizards. Sixteen papers were not considered for my review because the studies did not report results from incubation experiments of anole eggs that were initiated within 48 h of oviposition and contained at least one incubation temperature. Three datasets were obtained from contacting authors, resulting in a total of 9 unique datasets.

The response variables of interest from each study were proportion egg survival and incubation duration. I extracted the means (or proportion survival), standard deviation (or standard errors/confidence intervals) and samples sizes for each response variable at each incubation temperature used; values were gathered from the text, tables, figures, or personal communications. I also classified studies according to whether they used constant or fluctuating incubation regimes.

Statistical analysis

Data analyses were performed in R (R Development Core Team, 2017). The availability of nest microenvironment at each site was calculated as the number of suitable nest plots (e.g., those not on a road, sidewalk, or rock) relative to the total number of plots selected at random; the proportion of suitable plots for nesting was compared between sites with a chi-square test.

I calculated the daily average temperature mean, minimum, maximum and variance recorded by each iButton across all 43 days of the study. Data from iButtons indicated that daily temperatures peaked at different times among plots (likely due to variation in the position of shade cover). To correct for the timing of thermal peaks, I centered each daily thermal curve at the peak temperature and then calculated the mean temperature for each hour of the day. Therefore, the temperature profiles I present do not necessarily represent the mean temperature

across all plots at a given hour, but rather, they provide a realistic estimate of the rise and fall of nest temperatures through the day. I also calculated substrate moisture mean, maximum, minimum and variance for each plot based on 8 weekly samples collected throughout the study.

Because temperature and moisture data were correlated, I used principal component analyses (PCA) to collapse the variation into uncorrelated axes using the *FactorMineR* package (Lê et al. 2008). For the PCA, I used temperature and soil moisture mean, maximum, minimum, and variance values for each plot. The first four PC axes explained 95% of the variation in the data (between 49% to 8% individually) (Table 1.1). The loading scores for PC1 indicated a negative correlation between temperature and moisture.

I used mixed-effects linear models to estimate nest microenvironment differences between sites (urban forest vs. forest). Dependent variables (analyzed in separate models) included canopy openness, distance to the closest tree, distance to the second closest tree, daily temperature parameters, and weekly moisture parameters. I also analyzed PC1 scores in a model separately. I used a generalized linear mixed model (GLMM) in the *lme4* package (Bates et al. 2015) with a binomial distribution to analyse the probability of finding a nest in each habitat and another GLMM with a Poisson distribution to analyse the number of nests per plot. For all models, I included block as a random effect.

I omitted data from targeted searches for the above GLMMs, analyses of nest site availability, and specific egg location (under debris, on top of soil, etc.). I did, however, include canopy openness, distance to road, distance to trees, tree size, temperature and moisture data from targeted searches to provide details of nest microenvironments based on a larger sample of nests. Eight iButtons (n = 4 urban; n = 4 forest) failed during the study and were not included in

the analysis. One nest was exclusively made of grass (egg was found suspended in grass next to a tree), so I did not collect substrate moisture data.

I present all data collected from my literature review in graphs and tables; however, for my analysis, I excluded incubation temperatures that fell outside the range of mean nest temperatures recorded in my study. I used linear regression to estimate the relationship between egg survival and incubation duration and incubation temperature.

Results

General descriptions of nests

All randomized plots in the forest were suitable for nesting compared to 64% in the urban site ($\chi^2 = 13.33$; $P < 0.001$). Of the 40 random plots in each location, 36 contained at least one nest in the forest compared to 11 in the urban site (Table S1.3). Considering only plots from random searches, I found 10 unhatched ($n = 7$ urban; $n = 3$ forest) and 84 hatched eggs ($n = 30$ urban; $n = 54$ forest). Overall, most nests were found partially or fully buried in the substrate beneath a layer of debris (combination of leaves, small sticks, rocks, and dead grass), with few buried without debris (Table 1.2). The probability of finding a nest from random searches was 7.88 (1.60 – 53.93, 95% CL) times as likely in the forest than in the urban area ($\chi^2 = 5.51$; d.f. = 1; $P = 0.019$); however, nest density in was greater in the urban site: urban plots had 1.55 times as many nests per plot (1.01 – 2.36, 95% CL) as the forest ($\chi^2 = 4.28$; d.f. = 1; $P = 0.04$). Table 1.3 provides raw means for all variables at each site.

Urban nests had 12.7% (± 3.09 SE) less shade cover than forest nests (Fig. 1.2; $F_{1,11} = 16.87$; $P = 0.002$). The distance between the nests to the closest two trees did not differ between the two sites (closest tree: $\beta = 0.36 \pm 0.39$ m; $F_{1,11} = 0.83$; $P = 0.38$; second closest tree: $\beta = 4.72$ m ± 2.54 SE; $F_{1,11} = 3.45$; $P = 0.09$). The size (i.e., circumference) of the closest tree at the urban

site was 0.93m (± 0.29 SE) greater than those in the forest ($F_{1,11} = 10.47$; $P = 0.008$), and this pattern was similar for the second closest tree (Fig. S1.1; $\beta = 0.80 \pm 0.18$ SE; $F_{1,11} = 20.55$; $P = 0.001$).

Temperature and moisture

Average daily mean, maximum, and minimum temperatures for each site are summarized in Table 1.2. The mean temperatures of the coolest and warmest nests for the urban area (27 – 30°C) and the forest (26 – 27°C) slightly overlapped. Mean daily temperatures in urban nests were 1.5°C (± 0.2 SE) warmer than the forest nests ($F_{1,12} = 47.3$; $P < 0.001$), and daily maximum temperature in the urban site was 4.1°C (± 1.1 SE) warmer than the forest nests ($F_{1,12} = 14.2$; $P = 0.0027$). Average minimum temperature did not differ between the two sites ($\beta = 0.2 \pm 0.2$ SE, $F_{1,12} = 1.3$, $P = 0.28$). Urban nests had 1.2°C (± 1.5 SE) greater thermal variance than forest nests ($F_{1,12} = 6.8$; $P = 0.023$). Hourly mean temperature values for the two sites are shown in Fig. 1.3.

Weekly mean, maximum, and minimum substrate moisture in forest plots were 3.36% (± 0.91 SE; $F_{1,11} = 13.60$; $P = 0.004$), 3.21% (± 1.30 SE; $F_{1,11} = 6.07$; $P = 0.031$), and 3.08% (± 0.76 SE; $F_{1,11} = 16.41$; $P = 0.002$) greater than the plots in the urban site, respectively. However, the variance in substrate moisture did not differ between the two sites ($\beta = 1.10 \pm 3.00$ SE; $F_{1,11} = 0.13$; $P = 0.72$). The scores from PC1 indicated that nests in the urban site were warmer, drier and had greater thermal variance compared to nests in the forest (Fig. 1.4; $\beta = 3.2 \pm 0.5$ SE; $F_{1,11} = 36.46$; $P < 0.001$).

Incubation temperatures from the literature

Of the temperatures used in studies of egg incubation for the common anole species at my study site, most (80%) were within the range of mean nest temperatures measured in my study (26°C – 30°C) (Fig. 1.5). The range of nest temperatures measured at my sites have no

apparent effect on survival ($\beta = 2.3 \% \pm 2.5 \text{ SE}$; $P = 0.36$). However, based on literature review, I estimate that incubation period decreases by 2.1 days ($\pm 0.4 \text{ SE}$) for each 1°C increase in mean nest temperature (Fig. 1.5; $P < 0.001$). For context, eggs in the urban site would hatch from 4-12 days earlier than eggs incubated in forest nests. Moreover, due to a wider range of mean nest temperatures in the urban habitat, the range of developmental rates is approximately three times greater in the urban site than in the forest (Fig. 1.5).

Discussion

The primary goals of this study were to describe the characteristics of nests of *Anolis* lizards, determine how they differ between habitats (urban vs. forest sites), and predict potential consequences of nest microenvironments based on results from previously-published egg incubation studies. Nests in urban areas differed substantially from nests in forest areas in distribution, shade cover, temperature, and moisture. These differences in nest characteristics were likely a function of the overall habitat differences between my study sites and will likely have important consequences on habitat-specific development of embryos. These results provide a rare quantitative assessment of how nest conditions of anoles differ across diverse habitats and differs considerably from qualitative descriptions of nest habitat (e.g., Andrews, 1982), and fills in a major gap in my understanding of this otherwise well-studied group of vertebrates.

Distribution of nests

Based on random sampling, I found more nests in the forests than in the urban site. However, urban nest plots contained more nests per plot, suggesting that in the urban area, an individual female may lay multiple clutches in one spot (possibly due to a lack of suitable nesting habitat; e.g., roads, sidewalk). Nesting aggregations or communal nesting have been described in many oviparous animals (Doody et al., 2009; in anoles – see Robinson et al., 2014;

Godfrey et al., 2018) and may be more common in disturbed habitats due to reduced availability of high-quality nesting habitat (Mainwaring et al., 2017). However, egg aggregations due to restricted suitable egg-laying sites may be disadvantageous because disease (e.g., fungal infection) can spread easily due to proximity (Warner & Chapman, 2011), and hatchlings will have to travel further from each other to reduce competition (Rand, 1967). Alternatively, it is also possible that a single female may repeatedly put multiple nests in the same location. I also cannot rule out the possibility of increased nest predation in the forest, which could influence nest detectability.

Increased nest clumping in urban habitats may reflect differences in distribution and density of lizards between the urban and forest sites (Table S1.2). This would explain why the likelihood of locating a nest was greater in the forest compared to the urban site. In the urban site, anoles were usually found concentrated on large trees or disjunct patches of vegetation, with many meters of unoccupied area between occupied spaces. My data indicated that lizards in the urban site nest close to trees like in the forest (Table 1.3). Likely, the habitat fragmentation that typifies urban areas restricts females from nesting relatively far from trees. Conversely, the usable habitat in the forest is continuous and allows lizards to move freely around. Females may simply use areas for nesting that are close to the trees they inhabit. Nesting close to trees may also reduce travel distances and the risk of predation (Rand, 1967). Additionally, gravid females also benefit from reduced detectability by ground-dwelling predators via crypsis in leaf litter (Schwarzkopf & Shine, 1992).

General nest characteristics

Under laboratory settings, females nearly always nest 2 to 5 cm below the soil surface, even when surface structure (e.g., a leaf) is provided (Propper et al., 1991; Sanger, 2008; JM Hall

pers. obs., but see Socci et al., 2005). However, I found most nests to be only partially buried beneath surface cover or fully buried to between 1-3 cm in the substrate layer underneath surface debris in the urban site. More than half of the nests in the forest were found exposed on the ground surface with little substrate cover. This is likely due to the shallow soil layer (often ~1 cm) over a rocky surface within Matheson Hammock Preserve. The urban site had some areas with soil deeper than 5-7 cm, but I did not find nests this deep despite potentially moist and stable conditions at greater depths (Booth, 2006; Doody et al., 2015; Andrews, 2018). These habitat differences in nest depth or exposure to the atmosphere could impact water exchange between eggs and the environment (e.g., as water vapor or liquid), and in turn influence water availability for embryos (Ackerman et al., 1985) and phenotypes of offspring (Packard et al., 1988; Brown & Shine, 2018).

All nests except for those completely buried were found under some surface debris. In the urban site, nests were found in locations with high abundance of surface debris and unkept lawn (i.e. receives no landscaping maintenance). My findings are consistent with those of Socci et al. (2005) in that anoles prefer (and benefit from) sites with leaf cover. In the wild, leaf cover also shields eggs from direct sunlight and can buffer against lethally hot conditions in open habitats. Additionally, neonates may benefit from hatching in areas with thick leaf litter due to a relatively high biomass of invertebrate prey (Heinen, 1992).

Soil moisture content in nests was statistically higher at the forest site than at the urban site, but the average difference of ~2 – 5 % may not be biologically significant considering the high precipitation and relative humidity of South Florida during the nesting season. Thus, both habitats probably contained moisture levels suitable for development (albeit, exchange of water vapor vs. liquid could be important; Ackerman et al., 1985). Substrate moisture of nests at my

sites never exceeded 32% during the study period (even with occasional heavy rain), whereas Reedy et al. (2013) show female anoles in the laboratory select nest sites that are 50% or above in soil moisture content. Nesting grounds with such high moisture levels might be rare in the wild, indicating that anoles may consistently nest in soils that are below the preferred moisture content. Alternatively, the difference in substrate moisture may be because my samples contained surface debris (roots, leaves, shells, etc.), which differ in water holding properties from the potting soil used by Reedy et al. (2013). Moreover, I observed that many nests in my study were not completely buried and may be influenced more by relative humidity in the air than by substrate moisture. I suggest future experiments with soil moisture consider natural substrates of nests and relative humidity of the microenvironment, especially for species that do not completely bury their nests (or eggs).

Nests in urban areas were warmer and experience greater thermal variation than the forest. The forest contained homogenous extensive canopy cover, which generates a relatively stable microclimate on the ground. In contrast, the urban site is patchy with large areas of open canopy. Thus, the heterogeneity in the urban site provides females with more opportunities to select among nesting conditions compared to the forest. How females choose nest microenvironments in urban and forest sites relative to the conditions available can have major consequences for embryo development (Refsnider & Janzen, 2010; Sanger et al., 2018). Studies that focus on maternal nest-site choice in urban vs. forest habitat will help researchers understand how these organisms deal with human-induced environmental changes.

Potential consequences of nest characteristics

Egg incubation studies provide insight into the potential consequences of the nest environments described above. Most studies of egg incubation conditions in reptiles focus on

temperature, and few explore other variables such as moisture or canopy openness (Warner et al., 2018). Thus, perceptions of the consequences of nest microenvironment are somewhat restricted to assumptions about the effect of temperature. Given the thermal heterogeneity in urban environments, intuition suggests this variation will generate greater variation in egg survival, incubation duration and offspring phenotypes than the homogenous conditions of forest. Indeed, nest thermal environments were more variable in the urban site, with some nests reaching high thermal maxima (up to 39.5 °C); exposure to such extremes even for short periods can significantly reduce embryo survival and influence developmental rate (Hall & Warner, 2018). However, based on published literature for the four common anole species at my study sites, the thermal environments in urban and forest habitats are unlikely to generate much variation in egg survival (Fig. 1.5). There is, however, substantial variation in incubation period between urban and forest sites (Tiatragul et al., 2017). I predict that, on average, embryos will hatch 4 – 12 days earlier in the urban habitat than in the forest. This could be a benefit because decreased incubation durations mean that embryos spend less time exposed to potentially harmful developmental conditions (e.g., extreme temperature, moisture, or depredation). Egg survival can be a significant determinant of population density in anoles (Andrews, 1988; Chalcraft & Andrews, 1999). Although egg survival *per se*, may not vary between forest and urban nests due to temperature, egg mortality may be greater in the forest if eggs take longer to develop. This may be a contributor to variation in adult density between these habitats. What is more, my linear regression with the data from the literature indicated that the urban nests have greater discrepancy in incubation duration than the forest. Embryos that develop in urban nests, thus, are subjected to a wider range of conditions during development, which may have fitness consequences early in life (Li et al., 2018; Mitchell et al., 2018).

Although laboratory studies of egg incubation can be used to predict potential consequences of nest environments, one caveat is that most lab studies do not simulate the complex nest environments in nature (Carter et al., 2018; While et al., 2018; Sanger et al., 2018). Indeed, only 36% of anole egg incubation studies use fluctuating incubation temperatures, and only one of those studies simulated the complex day-to-day thermal variation (Pearson & Warner, 2016; Table S1.1). Moreover, although most thermal treatments are within the minimum and maximum temperature ranges of natural nests, some experiments use thermal treatments that fall outside the range experienced by natural nests in the field. These observations call attention to the need for more studies that simulate natural nest environments and their importance in making ecologically-meaningful interpretations. Data on nest microenvironments is available for many reptiles (e.g., Janzen & Morjan, 2001; Brown & Shine, 2004; Doody et al., 2006; Warner & Shine, 2008), but such information is greatly lacking for anoles. Thus, natural experiments, such as mine, are valuable for informing future hypothesis-driven experiments.

Conclusion

Nesting behavior is a critical component of reproduction that has serious consequences for fitness. The nest microenvironment that females use can differ substantially among habitats (Doody et al., 2006), particularly in areas that are modified by human activity (Mainwaring et al., 2017). Oviparous reptiles offer fascinating models to study how maternal nesting behavior may be modified by aspects of global change (e.g., urbanization). I demonstrate significant differences in microenvironment conditions between anole nests in urban and natural forest habitat, and that these differences have the potential to generate variation in egg hatching success and developmental rate. I also emphasize the importance of descriptive studies in providing valuable information for guiding hypothesis-driven experiments. Indeed, data from nest sites

reported here will be critical in understanding how organisms respond to urbanization at early life-history stages.

Chapter 3: Effects of Nest Sites on Egg Survival and Hatchling Phenotype

Introduction

Urbanization is a pervasive driver of global change (Lewis & Maslin, 2015). To accommodate human population growth, natural areas are converted to towns and cities, creating novel environments for local species (Lowry et al., 2012; Johnson & Munshi-South, 2017). The conversion of native habitats to urban landscapes alters habitat structure (e.g., more impervious surfaces, loss of vegetation), changes hydrological cycles (e.g., increased runoff), and increases ambient temperature due to the urban heat island effect (Pickett et al., 2001; Rizwan et al., 2008; Forman, 2014). Such factors can introduce new selective pressures (Rodewald et al., 2011) and influence phenotypic evolution across small and large spatiotemporal scales (Hargeby et al., 2004; Yeh, 2004). Indeed, phenotypic change can occur more rapidly in human-modified habitats than in undisturbed areas (Alberti et al., 2017) and may be strongly influenced by plasticity (Hendry et al., 2008). As such, the alteration of habitats by humans should not solely be considered a threat to species persistence, but also a generator of phenotypic variation (Brady, 2012; Sparkman et al., 2018). Thus, species that rapidly respond to novel conditions via inherent plasticity (in morphology, physiology and behavior) are likely to thrive in novel areas (Ord et al., 2015; Levis & Pfenning, 2016). This may be why communities often differ between urban and natural landscapes since some species struggle in urban habitats (“urban sensitive”) while others thrive (“urban exploiters”) (Blair, 1996; Grant et al., 2011).

Many urban-exploiters are also non-natives and there may be a link between the ability to exploit urban habitat and the potential to establish outside the native range (Latella et al., 2011;

Hufbauer et al., 2012; Gaertner et al., 2017; Suzuki-Ohno et al., 2017). Non-native species that have become invasive often exhibit greater levels of behavioral flexibility or phenotypic plasticity which may increase their potential for establishment in urban habitats both within and outside the native range (Davidson et al., 2011; Knop & Reusser, 2012). Although many studies have sought to determine how wildlife responds to urbanization (Mockford & Marshall, 2009; Lowry et al., 2012; Møller & Ibáñez-Álamo, 2012), much of this work has been focused on adult phenotypes, and very little has considered the importance of earlier life-stages (i.e. larvae; Kaiser et al., 2016). However, early stages are critically important due to their extreme sensitivity to many environmental factors that are altered by urbanization (Deeming, 2004; Esquerré et al., 2014). Furthermore, no population is sustainable in a novel habitat unless the habitat is suitable for reproduction, which includes successful embryo development (Ehrlich, 1986; Engel et al., 2011). As such, early-life stages should be considered along with adult stages when drawing conclusions about the impacts of urbanization on wildlife.

For egg laying animals, developing embryos within nests are not capable of moving away from adverse conditions (Telemeco et al., 2016a; Shine & Du, 2018). Yet, nesting females can selectively use microenvironments (e.g., shade cover, nest depth, substrate, temperature, and moisture) that influence hatching success and offspring fitness (Deeming & Ferguson, 1991; Shine & Harlow, 1996; Brown & Shine 2004; Mitchell et al., 2018). Indeed, several studies found that non-random nesting behavior by females is adaptive (Reedy et al., 2013; Li et al., 2018). However, whether nesting behavior in response to novel environments can facilitate the colonization of urban habitats remains to be investigated.

A few lizard species commonly called anoles make excellent models for studying how animals colonizes urban landscapes. Among others, the Puerto Rican crested anole (*Anolis*

cristatellus), a diurnal lizard native to Puerto Rico, has colonized urban environments both within their native range and outside their native range in South Florida (Kolbe et al., 2004; Kolbe et al., 2016; Winchell et al., 2016). Many aspects of this species' life history are influenced by conditions in urban areas (Tyler et al., 2016; Chejanovski et al., 2017), and adults have rapidly adapted their morphology and physiology to urban environments (Winchell et al., 2016; 2018). Nonetheless, relatively little is known about their nesting behavior in the wild (i.e., no observations recorded) and the consequence of maternal nesting behavior on hatchlings.

Observations of captive Puerto Rican crested anoles show that females lay eggs on the ground surface or will bury eggs in shallow cavities in the ground (between 1 – 4 cm deep). After laying eggs, females abandon their nests and eggs are subjects to the conditions of the microhabitat mothers have chosen. Laboratory studies show that related species of anoles prefer to lay eggs in microenvironments that enhance the fitness of offspring (Socci et al., 2005; Reedy et al., 2013), and embryonic development is sensitive to surrounding incubation conditions (Warner et al., 2011; Pearson & Warner, 2016; Hall & Warner, 2018; Sanger et al., 2018). Puerto Rican crested anoles produce one egg about every 8 – 15 days throughout the breeding season, and eggs are exposed to variable moisture and temperature conditions. These factors differ considerably between urban and forest habitats (Tiatragul et al., 2017; chapter 1) and affect embryo development, hatchling morphology and performance (Pearson & Warner, 2016; Hall & Warner, 2018; Sanger et al., 2018). Importantly, brief exposure to extreme soil temperatures (~43°C, “thermal spike”) measured in urban areas can reduce egg survival (Hall & Warner, 2018), and there is no evidence that embryos have physiologically adapted to putative urban nest conditions (Tiatragul et al., 2017). To buffer eggs from lethally warm temperatures, females in urban areas may choose nest sites nonrandomly with respect to thermal conditions (Doody et al.,

2006; Refsnider & Janzen, 2010; Telemeco et al., 2016b). Testing this hypothesis is difficult, however, because eggs of the Puerto Rican crested anole are small, and females can quickly lay eggs and move away from nests undetected. To address this, I put considerable effort into finding nests in the field and compared the components of microenvironments used by nesting females to unused conditions that are available to nesting females. If microenvironments that females choose are beneficial to developing embryos (i.e., greater survival), then maternal nesting behavior could partially explain how widespread lizards like anoles establish in urban habitats.

The goal of this study is to quantify nest temperatures in urban and forest landscapes to determine how nesting behavior impacts egg survival and hatchling phenotypes in urbanized areas. I use the microenvironmental data from chapter 1 to determine the difference between plots with and without nest within urban and forest sites. I expected the urban site to be warmer and drier on average and shade cover to be more heterogenous compared to the forest due to fragmented tree cover. Consequently, I predicted that in the urban site, plots with nests will be relatively cool, dry and shaded compared to what is available. In contrast, females in the forest will choose sites that are relatively warm compared to what is available. To quantify the effects of maternal nest conditions on egg survival and hatchling phenotypes, I subjected eggs of *A. cristatellus* from urban and forest populations to conditions that mimicked thermal microenvironments used by nesting females (i.e., plots with nests) in both urban and forest sites, as well as conditions that mimicked urban microenvironments that were not used by nesting females (i.e., plots without nests). I predicted that eggs exposed to maternal nest conditions will have greater survival compared to eggs exposed to conditions that mothers do not use for nesting (Tiatragul et al., 2017). I also expect eggs incubated under warm urban conditions to hatch

sooner than those incubated under cool forest conditions due to the positive relationship between temperature and developmental rate (Tiatragul et al. 2017; chapter 1; Sanger et al. 2018).

Additionally, my experimental design allowed me to assess whether the effect of the thermal environment on egg survival and hatchling morphology differs between source populations (i.e., forest vs. urban). If urban eggs have higher survival than forest eggs under urban nest conditions, and vice versa, then embryos may be locally adapted. My incubation study employed natural thermal fluctuations measured from the field to assess the consequences of maternal nesting behavior on embryo survival, which will advance my understanding of the factors that enable these organisms may persist in urban environments.

Materials and Method

Study site and nest search protocols

From 4 June to 3 July 2017, I searched for anole nests in an urban site (residential area along 57th Avenue, “Red Road”) and a nearby dense forest (Matheson Hammock Preserve) in south Miami; these sites are located 1.2 km from each other (Fig. 2.1). The urban site is located along a 1 km stretch between a two-lane road and Snapper Creek Canal. The pavement (regularly used by pedestrians and cyclists) separated from the adjacent road by guard rail is flanked by a regularly-mowed lawn lined with sporadically distributed trees (*Ficus* and palm) and many human-made structures including buildings, lamp posts and guardrails. Matheson Hammock Preserve is a large fragment (0.21 km²) of dense homogenous forest comprising large *Ficus* trees intermingled with smaller hardwood trees. The preserve interior where I conducted my study is constantly shaded and contains no human-made structures other than a few narrow walking trails.

Eggs of five species of anoles (*Anolis cristatellus*, *A. sagrei*, *A. distichus*, *A. equestris*, and *A. carolinensis*) that are established at my study sites are indistinguishable except for *A. equestris*' outstandingly large eggs. In the forest site, *A. cristatellus* occur almost exclusively in and other anole species are rarely sighted (Tiatragul et al., chapter 1). All five species occur sympatrically at the urban site. Nonetheless, because *Anolis* lizards have similar nesting requirements in captivity (Sanger et al., 2008a) and have similar developmental patterns (Sanger et al., 2008b), my comparisons between urban and forest sites are still meaningful in the context of the study goals.

My protocols for nest searches and quantifying microenvironment variables have been discussed previously in a study that describes anole nest microenvironments (Tiatragul et al. chapter 1), but I briefly reiterate important details here. To quantify microenvironments used and not used by nesting females, I established four searchable blocks in each site that consisted of a chosen central tree. Each tree was surrounded by a circular area with a radius of 20 m (i.e. block). I randomly located ten 1 m² plots within each block by randomly choosing a distance (between 1 - 20 m) and direction (between 0 - 359° from North) from each central tree (Fig 1.1). If the randomized direction and distance landed in an area obviously unsuitable for nesting (i.e., asphalt, pavement, body of water), I then randomized a new location until the plot was searchable. I searched each random plot thoroughly for hatched or unhatched eggs by removing all leaf litter, logs and small rocks, and digging up to 4 cm in the ground. I considered the location of each egg found to be a nest since anoles lay single-egg clutches. Hence, there may be multiple nests within a single plot. With the above protocol, I found a total of 44 plots with nests (n = 31 forest; n = 13 urban) and 36 plots without nests (n = 9 forest; n = 27 urban). To increase representation of plots with nests in urban areas and plots without nests in the forest, I also

conducted “targeted” searches in areas that appeared suitable based on my own experience working with anoles in the laboratory and field. Because my urban site is stretched along the road and is narrow compared to my forest site, I searched ~200 m down the road away from the original blocks and found 11 additional plots with a nest. Since targeted plots were found further away from the original searching blocks, I assigned them to four new blocks to account for landscape heterogeneity (for analytical purposes). All additional plots without nests in the forest (n = 14) were found within the same blocks used previously. I also found three additional plots with nests in the forest while searching for plots without nests. Therefore, the total of random and targeted plots combined is 58 plots with nests (n = 34 forest; n = 24 urban) and 50 plots without nests (n = 23 forest; n = 27) from 12 blocks (n = 8 original; n = 4 targeted in urban site).

For each plot, I recorded hourly temperatures between 3 July – 15 August 2017 (n = 43 days) with a Thermochron iButton (programmed to record hourly) wrapped in a water balloon and Parafilm. Most of the population should be reproductive during this time (Hall & Warner, 2017); thus, these temperatures should represent what most eggs experience in the field. For plots that contained more than one nest, I placed the iButton next to the nest with an unhatched egg. Otherwise, the iButton was either in the middle of the plot (for plots without nests) or next to a hatched egg. For substrate moisture, I collected samples of the nest substrate once a week during the same period I recorded temperature (n = 8 weeks). I weighed and then dried the sample, and the percent water content was calculated by dividing the change in mass between dry and wet samples by the wet mass multiplied by 100. These protocols provided temperature and moisture profiles for each plot over 42 days, which covers the range of a typical incubation period for Puerto Rican crested anoles (Tiatragul et al., 2017; Hall & Warner 2018; chapter 1). Shade cover was determined from hemispherical photographs taken above each plot using a

Canon Powershot Elph 180 with a 180° fisheye lens. I calculated percent canopy openness (the inverse of shade cover) using Gap Light Analysis (ver. 2.0) software on hemispherical photographs (Doody et al., 2006). Distance from nest to closest tree was obtained by measuring the distance to the nearest 0.01 m from the actual nest site within a plot to the base of the closest tree or plant with a diameter at breast height (DBH) greater than 0.05 m. For plots without nests, I measure the distance to tree from the center of the plot.

Lizard collection and husbandry

Between 18-19 March 2018, I collected adult *A. cristatellus* from the urban (53 females, 14 males) and forest sites (56 females, 14 males). Females were housed individually in cages (29 × 26 × 39 cm; height × width × depth) illuminated with Reptisun 5.0 UVB bulbs (Zoo Med Inc.) with 12:12 h light/dark cycle. The ambient room temperature ranged between 27.5 – 33°C. Cages were outfitted with two bamboo perches, artificial plants, a reptile cage carpet (Zoo Med Inc.) and a nesting pot filled with moist soil to encourage oviposition. I fed each lizard three crickets (dusted with calcium and vitamins) twice per week and sprayed cages with water daily to maintain high humidity. Because I had one male per four females, each male was rotated among four cages about every two weeks. I randomly assigned males to the four females, but males from one site were never mixed with females from the other. Lizards were kept in captivity for two months to acclimate before I started collecting eggs.

I collected eggs from nest pots three times per week from 4 June – 9 July 2018. Immediately after collection, I weighed the egg (to 0.0001 g) and placed each egg in a glass jar filled with moist vermiculite (-150 kPa). I covered each jar with plastic wrap (sealed with a rubber band) to prevent evaporation. I allocated eggs to one of three incubation treatments that mimicked thermal regimes measured in plots with and without nests from urban and forest

habitats (details below). Because *A. cristatellus* lays an egg every 8 – 15 days, for a given female I randomly allocated her first egg to a treatment and then alternated successive eggs among the treatments. This minimized potential biases associated with order of egg production. I expected greater mortality in the treatment that mimicked urban plots without nests, so I allocated more eggs to this treatment (n = 47, compared to 25 – 28 in the other treatments) after I reached my target sample sizes in the other treatments.

I checked incubators daily for hatchlings. For each hatchling, I recorded the date of hatching, body mass to 0.0001 g with an electronic scale, and measured snout-vent length (SVL) to 0.01 mm and tail length (TL) to 0.01mm with a caliper. One hatchling autotomized its tail before tail measurement and was excluded in the analysis for tail length. I did not determine the sex of hatchlings because *A. cristatellus* hatchlings lack external sexually dimorphic characters at this stage.

Experimental design and constructing incubation programs

Temperature data recorded from each plot (described above) indicated that the urban site was warmer than the forest, and the degree of this difference varied between plot types (i.e., those with a nest vs. those without nest) (see Results). However, forest plots with nests did not differ in temperature from forest plots without nests (see Results). Thus, I constructed incubation temperature treatments that mimicked 1) plots with nests in the urban site (“urban nest treatment”, 2) plots without nests in the urban site (“urban without-nest treatment”), and 3) plots with nests in the forest (“forest nest treatment”). I did not have a treatment that mimicked forest plots without nests because this thermal profile was identical to the forest plots with a nest. Thus, my experiment consisted of a 2 × 3 experimental design (two populations and three incubation

temperature treatments), which allowed me to assess if eggs from different populations differ in their response to incubation temperatures from different locations.

I used AtmoCONTROL software (ver. 2.5.3.0) to create temperature programs that were uploaded to Memmert IPP 550 incubators. Thus, the incubation temperatures mimicked averaged natural thermal regimes eggs would likely experience in plots with nests and plots without nests (Fig. 2.2). Data from iButtons indicated that daily temperatures peaked at different times across plots (likely due to variation in the position of shade cover). Thus, simply averaging hourly temperatures would reduce the magnitude of daily fluctuations in nest temperatures compared to what occurs naturally (Fig. 2.3). To correct for this, I centered each daily thermal curve at the peak temperature and then calculated the mean temperature for each hour of the day (Fig. S2.1). Therefore, the thermal regimes I constructed do not necessarily represent the mean temperature across all plots at a given hour, but rather, they provide a realistic estimate of the rise and fall of nest temperatures throughout the day. Although this approach reduces sample size for cooler hours of the day, my temperature data indicate that minimum temperatures do not differ between habitats ($\beta = 0.2^{\circ}\text{C} \pm 0.1 \text{ SE}$; $F_{1,12} = 1.52$; $P = 0.24$) nor plot types in the urban site (estimate $0.2^{\circ}\text{C} \pm 0.2 \text{ SE}$; $P=0.35$).

Unusually high temperatures (i.e. thermal spikes) are common in urban areas and they potentially influence embryo development and survival (Hall & Warner 2018). Therefore, I incorporated two thermal spikes where temperatures rise to 43°C , 39.5°C , and 33°C for urban without-nest, urban nest, and forest nest treatments, respectively. The magnitudes of these thermal spikes represent the warmest temperatures recorded from the field for each habitat and plot type. Thermal spikes were programmed to occur twice per 42-day cycle (excluded one day for practical reasons) on days with unusually high temperatures (Fig. 2.2). Since my data

indicated that there were two thermal spikes in 42-days, I programmed thermal spikes 18 days apart so that most embryos will experience at least one during their expected incubation duration of 29 – 40 days under similar conditions (Tiatragul et al., 2017; Hall & Warner, 2018). The 42-day thermal regime was programmed to repeat the cycle once it ends.

Statistical analysis

Data analyses were performed in R (R Development Core Team 2018). For the field data, I used mixed-effects linear models to estimate microenvironment differences between plot types (with nest vs. without nest), site (urban vs. forest), and their interaction. Dependent variables (analyzed in separate models) included mean plot temperature, mean plot moisture, and percent shade cover. Eight iButtons (n = 4 urban; n = 4 forest) failed during the study and were not included in the analysis.

For the laboratory incubation study, I used a generalized linear mixed model in the *lme4* package (Bates et al., 2015) with a binomial distribution to quantify the effects of maternal origin (urban vs. forest), thermal treatment (urban nest vs. urban without-nest vs. forest nest) and their interaction on egg survival. Mixed-effects linear models in the *nlme* package (Pinheiro et al., 2018) were used to quantify variation in incubation duration and hatchling morphology due to maternal origin, treatment and their interactions. I analyzed hatchling mass and SVL separately and used initial egg mass as a covariate for these models. Hatchling body condition was assessed with body mass as the dependent variable and SVL as the covariate. Tail length was analyzed with SVL as a covariate. I found no interaction between maternal origin and treatment in any models (all $P > 0.13$). Thus, for all models described, I dropped the interaction term. If there was statistical significance for variables that had more than one group, I report the estimates of

differences between groups, degrees of freedom, and the Tukey-adjusted P-values (“P_{adj}”) using the *emmeans* package (Lenth 2018). Maternal identity was a random effect in all models.

Results

Microenvironments

Urban plots had 12.97% (± 3.89 SE) less shade cover than forest plots, but the degree of this difference varied by plot type (Fig. 2.3A; Table 2.1). Urban plots with a nest had 10.2% (± 2.4 SE) more shade cover than plots without a nest (d.f. = 79; P_{adj} = 0.035). In contrast, forest plots with a nest versus those without nest did not differ in shade cover (2.0% ± 3.1 SE; d.f.= 79; P_{adj} = 0.51). I observed a habitat by plot type interaction for distance to the nearest tree (Fig. 2.3B, Table 2.1). In the urban site, plots with a nest were 2.25 m (± 0.39 SE) closer to trees than plots without a nest (d.f. = 80, P_{adj} < 0.001; Fig. 2.3B). However, plots with and without nests in the forest did not differ in distance to the closest tree (-0.19 m ± 0.48 SE; d.f. = 80, P_{adj} = 0.98).

Mean temperatures in urban plots were 1.6°C (± 0.2 SE) warmer than the forest plots and the average maximum temperature from plots in the urban site was 4.3°C (± 0.9 SE) warmer than plots in the forest (Table 2.1; Fig. S2.2). Plot temperatures in the urban site were more variable than in the forest site, but the degree of this difference varied by plot type (Table 2.1; Fig S2.2). Plots with nest in the urban site had less temperature variation than plot without nests. Plot types in the forest did not differ in any temperature measurements (Table 2.1). Also, mean plot substrate moisture in the forest was greater than in the urban site (mean 3.6% ± 1.0 SE, minimum 3.3 ± 0.9 SE, maximum 3.34 ± 1.2 SE; Table 2.1). Substrate moisture did not differ between plot types, nor was the interaction between site and plot type (Table 2.1; Fig S2.2).

Egg survival and incubation period

Egg survival differed among the three incubation treatments and maternal origin (Fig. 2.4; Table 2.2; Table S2.2 for raw means). Eggs in the urban without-nest treatment were 37 (7 – 197, 95% C.L) times as likely to die compared to forest with nest treatment ($P_{\text{adj}} < 0.001$), and 28 (6 – 136, 95% C.L) times as likely compared to the urban nest treatment ($P_{\text{adj}} < 0.001$). Eggs from forest mothers were 3 (1 – 13, 95% C.L) times as likely to die compared to eggs from urban mothers (Table 2.2). Incubation duration differed between the treatments (Fig. 2.5), but not by maternal origin (Table 2.2). Eggs in the forest-with-nest treatment took 3 – 4 days longer to hatch than those in the urban-with-nest treatment and 4 – 5 days longer than urban without-nest treatments ($P < 0.001$; Table 2.2). Post-hoc comparison of incubation duration from eggs in urban with vs. without nests treatments did not statistically differ (0.8 ± 0.6 SE; d.f. = 19; $P_{\text{adj}} = 0.41$).

Hatchling phenotypes

Hatchling body mass did not differ between treatments but differed between maternal origin (Table 2.2; Table S2.2 for raw means). Hatchling body mass from urban mothers were 0.0120 g (± 0.0043 SE) greater than hatchlings from forest mothers (Fig. 2.6A). Similarly, SVL of hatchlings from urban mothers were 0.4 mm (± 0.2 SE) larger than those in the forest (Fig. 2.6B). Body condition and tail length did not differ between treatment nor maternal origin (Table 2.2).

Discussion

The goals of this study were to identify how embryos deal with urban environments, and to determine the consequences of urban conditions on egg survival and hatchling phenotypes. I

found that nest sites in urban areas were warmer, had greater thermal variability, had less shade cover, and lower moisture content compared to those in the forest. Thus, urban habitats are more heterogenous than the forest with respect to shade, temperature and moisture conditions for nesting anoles. This finding implies that urban females have a broader range of conditions to choose among when selecting nest sites. This is most evident when I consider that plots with a nest in the urban area were relatively cool compared to plots without a nest, but in the forest, plots with and without a nest were essentially identical. Accordingly, my prediction in the urban site was supported (that females nest in relatively shaded and cool microenvironments), but the prediction for the forest site was not (that females use relatively warm nesting sites). Moreover, eggs that incubated under urban-with-nest temperatures had greater survival than those exposed to urban conditions that were not used by nesting females and develop faster than those in the forest.

Nesting habitat use

Maternal nest-site choice largely determines the ambient conditions that embryos experience during development, which in turn, can generate substantial variation in embryo survival and fitness-relevant phenotypes of offspring (Mitchell et al., 2013; Dayananda et al., 2016; Li et al., 2017). Consequences of maternal nest-site choice should vary among habitats that differ widely in microhabitat conditions, such as urban or forest areas. While I did not test for maternal nest-site choice *per se* by observing females nesting, my microenvironment data indicate that in urban environments, females chose nests that expose embryos to relatively cool, shaded and stable conditions compared to what is generally available across the urban landscape. Although many studies of lizards show that females generally prefer relatively open, warm nest sites in cooler environments (Warner & Shine, 2008; Li et al., 2017), my data indicate that

females in warmer urban habitats benefit from nesting in shaded locations because this behavior may buffer embryos from lethal temperatures. In contrast, the highly homogenous forest habitat may limit nesting choices for females, resulting in nest conditions that reflect what was available. I found that lizards' nest close to trees in the urban site (like in the forest), rather than further away from trees (Fig. 2.3B). This is expected since the urban habitat is fragmented by human disturbance and lizards are concentrated in areas with vegetation. In contrast, the forest consists of complex vegetation structure that allows lizards to move freely around. Rather than actively choosing more shaded and cooler nest sites in urban areas, females may simply use areas for nesting that are close to the trees they inhabit. Consequently, eggs incubate in relatively shaded, cool areas in the urban site, which are favorable for egg survival. Thus, maternal nesting behavior likely facilitate colonization of urban environments.

Consequence of nest conditions

Even if there is no selection on maternal nesting behavior, the incubation conditions in the urban area are warmer than the forest, which significantly reduces incubation duration (Tiatragul et al., 2017; chapter 1). A shorter incubation period may benefit lizards in two ways. First, in seasonal environments, offspring survival is enhanced for individuals that hatch relatively early because they have more time to grow prior to the onset of winter or the dry season (Shine & Olsson, 2003; While et al., 2015). Second, the chances of egg predation or exposure to unfavorable conditions are reduced when embryos develop quickly and hatch earlier (Doody, 2011; Doody & Paull, 2013). Though egg survival *per se*, did not vary between forest and urban nests due to temperature, egg mortality may be greater in the forest if eggs take longer to develop. Furthermore, egg survival can be a significant determinant of population density in anoles (Andrews, 1988; Chalcraft & Andrews, 1999). As such, the urban landscape may provide

a more suitable developmental environment for some species of lizards (i.e., urban exploiters). Indeed, the spread of invasive anoles is associated with faster life-history traits (e.g., rapid reproduction; Fetters & McGlothlin, 2017) and with urban sprawl (Suzuki-Ohno et al., 2017). Rapid development in urban areas may decrease the time from oviposition to maturity, enhancing spread in a newly colonized landscape.

The forest provides females with cooler, more homogenous nesting conditions. The urban habitat, conversely, offered more variation in nest conditions and warmer temperatures accelerate development at little cost (Tiatragul et al., 2017; Hall & Warner, 2018). It appears, however, that most of the forest nesting habitat is suitable for successful embryo development. In contrast, much of the urban landscape may be unsuitable for embryo development and this may limit the spread of non-native species throughout the urban matrix. At Matheson Hammock (the forest site), crested anoles inhabit virtually every corner of the forest and their densities are strikingly high. Across the urban landscape, however, densities are much lower and this species appears to be restricted to cooler microenvironments (Table S1.2). Kolbe et al (2016) found that the spread of Puerto Rican crested anoles through the urban matrix was associated with dense vegetation and closed canopies. They seem to be dependent on such cooler microclimates, and it is reasonable that this is at least in part the result of the large portions of the urban matrix that are inhospitable to embryo development. I recommend that this be considered in future studies that seek to understand how wildlife invade and persist in urban habitats.

Despite greater variability in nest conditions in urban nests, I did not observe variation in morphological traits via developmental plasticity in hatchlings. The effects of temperature treatment hatchling morphology are consistent with other studies with the same species (Tiatragul et al., 2017; chapter 1). However, eggs from urban vs. forest mothers differed

statistically in survival, hatchling mass, and SVL. Under the urban without-nest treatment, a higher proportion of eggs from urban mothers survived compared to eggs from forest mothers (Fig. 2.4). Survival under forest and urban nest treatments did not differ between eggs from urban vs. forest mothers, which is consistent with the finding in an experiment that used putative nest temperatures from urban and forest sites (Tiatragul et al., 2017). Eggs that incubate under the urban without-nest treatment also hatch out about a day sooner than those under urban nest treatment, but this difference was not statistically significant (Fig. 2.5). Incubation duration observed in my study is consistent with another study that showed survivors of thermal extremes hatch out sooner than those that did not experience thermal extremes (Hall & Warner, 2018). With respect to initial hatchling size, I found that hatchlings from urban mothers are about 5.8% heavier in mass and 2.1% longer in SVL than hatchlings from forest mothers, even though mothers were fed the same diet in captivity. Heavier initial hatchling size of urban lizards may be a consequence of generally larger adult in urban areas (Hall & Warner, 2017). However, the study was not designed to determine if large body size is adaptive in urban environments.

Apart from adaptation to urban environments (Johnson & Munshi-South, 2017), behavioral plasticity also helps many species to overcome the challenges of novel urban conditions (Sol et al., 2013; Wong & Candolin, 2015; Pellitteri-Rosa et al., 2017). Studies of birds show that urban-dwelling females select and build nests differently than in natural habitats (Hanmer et al., 2017; Lambrechts et al., 2017). This behavioral plasticity in nesting can facilitate colonization of novel environments (Yeh et al., 2007; Minias, 2015). Studies on how nesting behavior affects colonization is lacking in other oviparous taxa. In anoles, plasticity in feeding, thermoregulation, risk-taking, and habitat selection is often induced by urbanization (Lapiedra et al., 2016; Chejanovski et al., 2017; Winchell et al., 2018). There should be no reason, then, to

doubt that plasticity in nesting behavior is also important for establishment of anoles in urban areas. I found that microenvironments that nesting females select in urban areas yield greater survival than what is generally available. Therefore, I suggest that nesting behavior plays an important role in population establishment and naturalization in urban habitats.

Conclusion

How populations respond to urbanization varies considerably among taxa. Oviparous ectotherms are frequently introduced to non-native areas, such as urban environments, which provide a unique opportunity to study the mechanisms that enable successful colonization of novel habitats. The results from my study support the hypothesis that urban-dwelling lizards use relatively cool and shaded sites for nesting. Maternal nesting behaviour by which females actively select nest sites also yield greater egg survival than randomly nesting, which likely contributes to the establishment in urban environments. Nesting strategies that evolved in forest habitat appear to also be adaptive in urban areas as they limit the exposure of embryos to potentially lethal conditions. In many cases, human-modified habitats often present an “ecological trap” and can reduce fitness (e.g., Kolbe & Janzen, 2002), but urban environments seemingly facilitate many aspects of reproduction. Such benefits may be widespread in taxa that exploit urban environments, and research that assess how nesting females behave when introduced in a new ecological setting could provide new insights into urban adaptation and biological invasion.

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colonisation of a novel environment by a passerine bird. *Oikos* 116:1473–1480.

Tables

Table 1.1. Loading scores on each principal component (PC) axis of temperature and substrate moisture of urban and forest plots sampled. Boldfaced values represent traits that explain most of the PC axis.

	PC axis 1	PC axis 2	PC axis 3	PC axis 4
Temperature (°C)				
Mean	0.83213409	-0.42079051	-0.11648997	-0.074062616
Maximum	0.80728694	-0.41764592	-0.20950277	-0.196018443
Minimum	0.04333977	-0.17074757	-0.86675376	0.458766800
Variance	0.79331455	-0.3441063	-0.07052266	-0.345746725
Soil Moisture (%)				
Mean	-0.7954266	-0.28618894	-0.26721962	-0.286467206
Maximum	-0.5925537	-0.68895094	0.10950809	0.007670361
Minimum	-0.7836874	0.05969727	-0.4059627	-0.398672335
Variance	-0.2253097	-0.83640261	0.34143124	0.246975616
Proportion of variance explained	49.50%	21.90%	14.7	8.40%

Table 1.2. Description of anole nest locations for all plots (random and targeted). Data are provided for both sites (urban vs forest) and for both sites combined. Debris is combination of leaf litter, stick, dead grass and small rocks.

	On top of soil (%)		Partially buried in soil (%)		Completely buried (%)	
	With debris	Without debris	With debris	Without debris	With debris	Without debris
Forest	54.4	0	45.6	0	0	0
Suburban	18.9	0	67.6	0	0	13.5
Combined	40.4	0	54.3	0	0	5.3

Table 1.3. Quantitative descriptions of anole nests between forest and urban habitats. Nest sites were found by using both random and targeted searches in each habitat (see text for details). CBH = Circumference at Breast Height (m). *Sample sizes represent number of iButtons or plots, **Sample sizes represent number of days for temperature and number of weeks for moisture, † Sample size represent number of eggs from which trees were measure.

Variables	Forest					Urban				
	n	Mean	Min	Max	Var	n	Mean	Min	Max	Var
Canopy openness (%)*	35	9.45	4.25	16.42	11.55	22	22.77	5.94	45.40	121.70
Distances (m) †										
to closest tree	62	0.37	0.00	0.95	0.06	47	1.02	0.00	3.66	0.85
to 2 nd closest tree	62	0.63	0.17	1.43	0.059	47	3.22	0.20	15.00	9.19
Tree size (m) †										
CBH closest tree	62	0.26	0.01	2.44	0.15	48	1.17	0.02	3.58	1.32
CBH 2 nd closest tree	62	0.23	0.01	2.44	0.16	48	1.08	0.01	3.53	1.16
Nest density per plot*	31	1.8	1	5	1.1	13	2.8	1	6	3.8
Temperature (°C)*	32	26.8	22.0	33.0	1.0	22	28.4	22.0	39.5	2.6
Daily mean**	43	26.8	24.4	28.6	0.5	43	28.4	25.3	31.8	1.1
Daily mean maximum**	43	28.0	26.0	33.0	0.8	43	30.7	26.5	39.5	4.6
Daily mean minimum**	43	25.9	22.0	27.5	1.1	43	27.0	22.0	30.0	1.9
Daily mean variance**	43	0.6	0.0	5.0	0.3	43	1.6	0.00	11.5	3.5
Moisture (%)*	36	10.0	2.0	31.4	15.0	22	6.8	0.2	26.5	15.9
Weekly mean**	8	10.0	5.3	14.3	4.6	8	6.8	3.0	11.5	6.4
Weekly maximum**	8	15.0	7.9	31.4	15.6	8	11.8	5.74	26.5	22.0
Weekly minimum**	8	5.5	2.0	9.8	3.3	8	2.6	0.2	9.0	5.3
Weekly variance**	8	12.0	2.59	60.7	109.0	8	10.8	1.7	54.4	126

Table S1.1. Literature review summary. Egg survival (proportion) and incubation duration (mean \pm SE) were gathered from published studies or personal communications with the corresponding author. Not all studies reported egg survival or incubation duration.

Species	Year of study	Locality of anole population	Mean temp. (°C)	Thermal regime	Range of fluctuation (°C)	Egg survival (%)	Incubation duration (days)
<i>A. carolinensis</i> ¹	2005	Greenback, Tennessee	27	constant	27-27	-	34.7 \pm 2.6
<i>A. carolinensis</i> ¹	2005	Greenback, Tennessee	30	constant	30-30	-	29.5 \pm 1.6
<i>A. carolinensis</i> ¹	2005	Jacksonville, Florida	27	constant	27-27	-	35.8 \pm 2
<i>A. carolinensis</i> ¹	2005	Jacksonville, Florida	30	constant	30-30	-	29.5 \pm 2.5
<i>A. carolinensis</i> ¹	2005	LaPlace, Louisiana	23.5	constant	23.5-23.5	-	55 \pm 3.5
<i>A. carolinensis</i> ¹	2005	New Orleans, Louisiana	27	constant	27-27	-	36.8 \pm 4.5
<i>A. carolinensis</i> ¹	2005	New Orleans, Louisiana	30	constant	30-30	-	29.8 \pm 1.9
<i>A. carolinensis</i> ¹	2005	Orlando, Florida	27	constant	27-27	-	37.9 \pm 2.1
<i>A. carolinensis</i> ¹	2005	Orlando, Florida	30	constant	30-30	-	29.4 \pm 1
<i>A. carolinensis</i> ²	2005	LaPlace, Louisiana	23	constant	23.9-22.1	75.3	-
<i>A. carolinensis</i> ²	2005	LaPlace, Louisiana	27	constant	27.4-26.5	83.3	-
<i>A. carolinensis</i> ²	2005	LaPlace, Louisiana	30	constant	30.3-29.7	92.3	-
<i>A. cristatellus</i> ³	2013	Mayagüez (forest), Puerto Rico	28	constant	28-28	89.6	33.2 \pm 0.2
<i>A. cristatellus</i> ³	2013	Mayagüez (urban), Puerto Rico	28	constant	28-28	89.3	33.6 \pm 0.3
<i>A. cristatellus</i> ⁴	2015	Miami, Florida	26	fluctuating	25.5-26.4	62.6	34.7 \pm 0.7
<i>A. cristatellus</i> ⁴	2015	Miami, Florida	28	fluctuating	26.4-29.6	53.8	37.2 \pm 0.5
<i>A. cristatellus</i> ⁵	2017	Miami, Florida	25.7	fluctuating	25-26.4	67	42.7 \pm 0.3
<i>A. cristatellus</i> ⁵	2017	Miami, Florida	28	fluctuating	26.4-30	67	34.9 \pm 0.2
<i>A. distichus ignigularis</i> ⁶	2011-12	Baní, Dominican Rep.	29.4	constant	29.4-29.4	74.7	32.1 \pm 0.2
<i>A. distichus ravitergum</i> ⁶	2011-12	Baní, Dominican Rep.	29.4	constant	29.4-29.4	73.4	29.7 \pm 0.2

<i>A. sagrei</i> ⁷	2008	Ormond Beach, Florida	26	constant	26-26	-	35.9±0.3
<i>A. sagrei</i> ⁷	2008	Ormond Beach, Florida	28	constant	28-28	-	30.4±0.2
<i>A. sagrei</i> ⁷	2008	Ormond Beach, Florida	30	constant	30-30	-	25.8±0.2
<i>A. sagrei</i> ⁸	2012	Palm Coast, Florida	20.4	fluctuating	17.0-23.8	64.7	76.2±1
<i>A. sagrei</i> ⁸	2012	Palm Coast, Florida	21.5	fluctuating	16.1-26.8	86.7	61±1
<i>A. sagrei</i> ⁸	2012	Palm Coast, Florida	25.7	fluctuating	22.7-28.7	77.8	37.2±0.9
<i>A. sagrei</i> ⁸	2012	Palm Coast, Florida	26.9	fluctuating	22.6-31.2	63.6	31.6±0.9
<i>A. sagrei</i> ⁹	2015	Clearwater, Florida	28	constant	28-28	-	30.4±0.3
<i>A. sagrei</i> ³	2015	Miami, Florida	26	fluctuating	25.5-26.4	86.3	32.1±0.5
<i>A. sagrei</i> ³	2015	Miami, Florida	28	fluctuating	26.4-29.6	90.4	31.8±0.6
<i>A. sagrei</i> ⁹	2015	New Orleans, Louisiana	28	constant	28-28	-	29.6±0.3
<i>A. sagrei</i> ⁹	2015	North Andros, Florida	28	constant	28-28	-	35.3±0.2
<i>A. sagrei</i> ¹⁰	2015	Palm Coast, Florida	27.7	fluctuating	25.5-32.1	93	30.7±0.1
<i>A. sagrei</i> ⁹	2015	Valdosta, Georgia	28	constant	28-28	-	29.7±0.3

References: ¹Goodman (2008); ²Goodman & Walguarnery (2007); ³Winchell et al. (2016); ⁴Tiatragul et al. (2017); ⁵Hall & Warner (2017); ⁶Geneva (2015); ⁷Warner et al. (2012); ⁸Pearson & Warner (2016); ⁹Fetters & McGlothlin (2017); ¹⁰Mitchell et al. (2018b)

Table S1.2. Number of total individual Anolis lizards observed over four visual encounter surveys on a 25 m transect that stretch each searching block. Females/unsexed juveniles numbers are in parentheses. Visual encounter survey was done by walking slowly (~10 minutes) one way on 25 m transect and counting every lizard visible within 10m from the transect. Surveys were repeated four times throughout the study period. Block 7 and 8 are in adjacent areas so only block 8 was not surveyed.

Block	Site	<i>A. cristatellus</i>	<i>A. sagrei</i>	<i>A. distichus</i>	<i>A. carolinensis</i>
1	Urban	21 (3)	3 (1)	7 (1)	1 (0)
2	Urban	6 (1)	18 (1)	2 (0)	0
3	Urban	24 (3)	16 (2)	0	1 (0)
4	Urban	16 (3)	17 (2)	4(0)	5 (0)
5	Forest	122 (22)	0	0	0
6	Forest	194 (27)	0	0	0
7	Forest	111 (15)	0	0	0

Table S1.3. Summary of eggs found in each plot type through random and targeted searches.

		Forest		Urban		
		Nests	No nests	Nests	No nests	Total
	N	36	4	11	29	80
Random	Hatched eggs	54	-	30	-	84
	Unhatched eggs	3	-	7	-	10
	N	4	14	11	0	29
Targeted	Hatched eggs	4	-	10	-	14
	Unhatched eggs	0	-	1	-	1

Table S1.4. Effect of site on microenvironment variables. The effect size and standard error represents means for urban minus forest for each variable.

Variables	DF	$\beta \pm SE$	F	P
Canopy openness (%)	11	12.70±3.09	16.87	0.0017
Distances (m)				
To closest Tree	11	0.36±0.39	0.83	0.38
To 2 nd closest Tree	11	4.72±2.54	3.45	0.09
Tree size (m)				
CBH closest tree	11	0.93±0.29	10.47	0.0079
CBH 2 nd closest tree	11	0.80±0.18	20.55	0.0009
Principal Component (PC) 1	11	3.22±0.53	36.46	0.0001
Temperature (°C)				
Daily mean	12	1.5±0.2	47.3	<0.001
Daily maximum	12	4.1±1.1	14.2	0.0027
Daily minimum	12	0.2±0.2	1.3	0.28
Daily variance	12	1.2±0.5	6.8	0.023
Moisture (%)				
Weekly mean	11	-3.36±0.91	13.60	0.0036
Weekly max	11	-3.21±1.30	6.07	0.031
Weekly min	11	-3.08±0.76	16.41	0.0019
Weekly variance	11	-1.10±3.00	0.13	0.72

Table 2.1 Effect of site (urban vs forest), plot type (with a nest vs without a nest), and their interaction on microenvironment variables. The effect sizes represent “Urban minus forest” and “Without a nest minus With a nest” for each variable. There was one less block for moisture than temperature (i.e. one less degree of freedom when using block as a random effect) because one plot contained an egg that was not in substrate (it was suspended in grass next to a tree).

Variables	Site (Urban vs Forest)			Plot type (Without a nest vs With a nest)			Site × Plot type		
	$\beta \pm SE$	F_{df}	P	$\beta \pm SE$	F_{df}	P	$\beta \pm SE$	F_{df}	P
Canopy openness %	12.97±89	$F_{1,11}=11.09$	0.0067	2.02±3.07	$F_{1,79}=0.43$	0.51	8.23±3.92	$F_{1,79}=4.40$	0.039
Distance to tree (m)	0.77±0.66	$F_{1,12}=1.37$	0.26	0.19±0.48	$F_{1,80}=0.15$	0.69	2.06±0.62	$F_{1,80}=11.12$	0.0013
<i>Temperature (°C)</i>									
Plot mean	1.6±0.2	$F_{1,12}=50.3$	<0.001	0.01±0.2	$F_{1,84}=0.001$	0.96	0.6±0.3	$F_{1,84}=4.7$	0.03
Plot minimum	0.2±0.1	$F_{1,12}=1.5$	<0.24	0.3±0.2	$F_{1,84}=0.1$	0.71	0.2±0.2	$F_{1,84}=2.2$	0.14
Plot maximum	4.3±0.7	$F_{1,12}=20.8$	<0.001	1.0±0.7	$F_{1,84}=1.9$	0.18	0.6±1.1	$F_{1,84}=1.4$	0.59
Plot Variance	1.3±0.6	$F_{1,12}=5.3$	0.04	0.1±0.5	$F_{1,84}=0.04$	0.84	1.9±0.7	$F_{1,84}=7.0$	0.001
<i>Moisture (%)</i>									
Plot mean	-3.6±1.0	$F_{1,11}=9.6$	0.01	-1.0±0.5	$F_{1,95}=0.7$	0.41	1.5±0.8	$F_{1,95}=3.5$	0.06
Plot min	-3.3±0.9	$F_{1,11}=12.1$	0.005	-5±0.5	$F_{1,95}=0.2$	0.62	0.8±0.8	$F_{1,95}=1.0$	0.32
Plot max	-3.3±1.2	$F_{1,11}=6.3$	0.03	-1.8±1.1	$F_{1,95}=1.0$	0.32	2.2±1.6	$F_{1,95}=1.9$	0.17
Weekly Variance	-1.2±2.7	$F_{1,11}=0.2$	0.63	-1.5±2.6	$F_{1,95}=0.3$	0.58	0.9±3.8	$F_{1,95}=0.05$	0.82

Table 2.2. Effect of treatment and maternal origin on survival, incubation duration and hatchling morphology of Puerto Rican crested anoles (*Anolis cristatellus*). Interactions terms were not significant (all $P > 0.13$), thus I removed it from the final models.

Trait	Covariate	Treatment	Maternal origin
Survival	Egg mass	$\chi^2 = 19.1$ d.f. = 2 P < 0.001	$\chi^2 = 4.5$ d.f. = 1 P = 0.033
Incubation duration (days)	Egg mass	$F_{2,19} = 49.5$ P < 0.0001	$F_{1,39} = 0.29$ P = 0.59
Hatchling mass (g)	Egg mass	$F_{2,19} = 1.78$ P = 0.20	$F_{1,39} = 9.0$ P = 0.0047
Snout-vent length (SVL, mm)	Egg mass	$F_{2,19} = 0.12$ P = 0.89	$F_{1,39} = 4.86$ P = 0.033
Body condition (mass, g)	Egg mass, SVL	$F_{2,19} = 2.42$ P = 0.12	$F_{1,39} = 3.75$ P = 0.06
Tail length (TL, mm)	Egg mass, SVL	$F_{2,17} = 2.44$ P = 0.11	$F_{1,39} = 4.09$ P = 0.05

Table S2.1. Summary of mean, maximum, minimum, and variance of microenvironment variables measured during my study period.

All values are raw values.

FOREST	With nest					Without nest				
	N	Mean	Max	Min	Var	N	Mean	Max	Min	Var
Canopy openness (%)*	35	9.5	16.4	4.3	11.6	9	12.3	25.8	5.0	31.7
Distances (m) †										
To closest tree	62	0.370	0.95	0.00	0.06	9	0.64	1.00	0.36	0.06
Temperature (°C)*	31	26.8	33.0	22.0	0.9	3	26.8	41.5	26.0	1.0
Plot mean	43	26.8	28.6	24.4	0.4		26.8	27.6	25.0	0.4
Plot minimum	43	28.0	33.0	26.0	0.8		28.0	41.5	26.0	1.5
Plot maximum	43	26.0	27.5	22.0	1.0		25.9	27.5	21.5	1.0
Plot variance	43	0.5	3.7	0.0	0.2		0.6	11.3	0.04	0.6
Moisture (%)*	36	10.0	31.4	2.0	15.0	24	9.8	25.8	1.5	13.4
Plot mean	8	10.0	12.2	6.3	5.5	8	9.8	13.5	5.8	6.3
Plot min	8	19.1	31.4	10.2	38.5	8	16.8	25.8	10.1	26.9
Plot max	8	3.9	7.3	2.0	2.7	8	8.5	7.7	1.5	5.0
Plot variance	8	10.5	21.0	3.3	27.4	8	12.0	36.3	4.1	111.3
URBAN										
Canopy openness (%)*	22	22.77	45.40	5.94	121.70	28	35.14	52.61	12.80	143.85
Distances (m) †										
To Closest Tree	48	1.00	3.66	0.00	0.85	27	3.47	7.43	0.59	5.22
Temperature (°C)*	22	28.4	39.5	22.0	2.4	23	29.2	43.0	22.5	6.2
Plot mean	43	28.4	32.6	25.3	1.0	43	29.2	33.7	25.1	2.5
Plot minimum	43	30.7	39.5	26.5	4.6	43	33.0	43.0	26.5	14.0
Plot maximum	43	27.1	30.0	22.0	1.7	43	27.3	30.5	22.5	1.7

Plot variance	43	1.4	13.0	0.00	2.8	43	3.9	21.4	0.04	15.7
Moisture (%)*	22	6.8	26.5	0.2	15.9	28	7.0	21.6	0.0	14.3
Plot mean	8	6.8	9.1	2.9	4.0	8	7.0	8.8	3.1	4.1
Plot min	8	15.9	26.5	10.2	27.5	8	15.8	21.6	13.0	8.5
Plot max	8	1.8	3.5	0.2	1.6	8	2.0	3.9	0.0	2.5
Weekly Variance	8	12.8	32.3	6.3	74.5	8	11.1	17.2	6.9	11.5

Table S2.2. Summary statistics of egg survival, incubation duration and hatchling morphology of Puerto Rican crested anole (*Anolis cristatellus*) from forest (Matheson Hammock Preserve) and urban (57th Ave, Red Road) populations of South Miami, FL under three experimental incubation treatments.

Dependent variables	Forest mothers			Urban mothers		
	N	Mean	SE	N	Mean	SE
Proportion egg survived						
Forest with nest	11	0.92	-	15	0.93	-
Urban with nest	11	0.85	-	11	0.92	-
Urban without nest	4	0.19	-	11	0.42	-
Incubation duration (days)						
Urban With nest	11	37.6	0.4	15	37.4	0.4
Urban Without nest	11	33.7	0.4	11	33.4	0.5
Forest	4	32.9	0.6	11	32.6	0.5
Snout-vent length (SVL)						
Urban With nest	11	18.68	0.19	15	19.13	0.17
Urban Without nest	11	18.79	0.19	11	19.23	0.20
Forest	4	18.68	0.26	11	19.12	0.21
Hatchling mass (g)						
Urban With nest	11	0.1948	0.0040	15	0.2077	0.0036
Urban Without nest	11	0.1882	0.0041	11	0.2011	0.0043
Forest	4	0.1861	0.0055	11	0.1990	0.0043
Tail length (mm)						
Urban With nest	11	27.06	0.49	15	28.17	0.44
Urban Without nest	11	28.06	0.49	10	29.16	0.49
Forest	4	26.75	0.65	11	27.86	0.53

Figures



Figure 1.1. Representative photographs of my study sites. (A) A section of the urban site featuring the road, bike path, and canal (GPS coordinate: 25.681637, -80.284705). (B) A section of a study site in the forest (GPS coordinate: 25.681926, -80.274866).

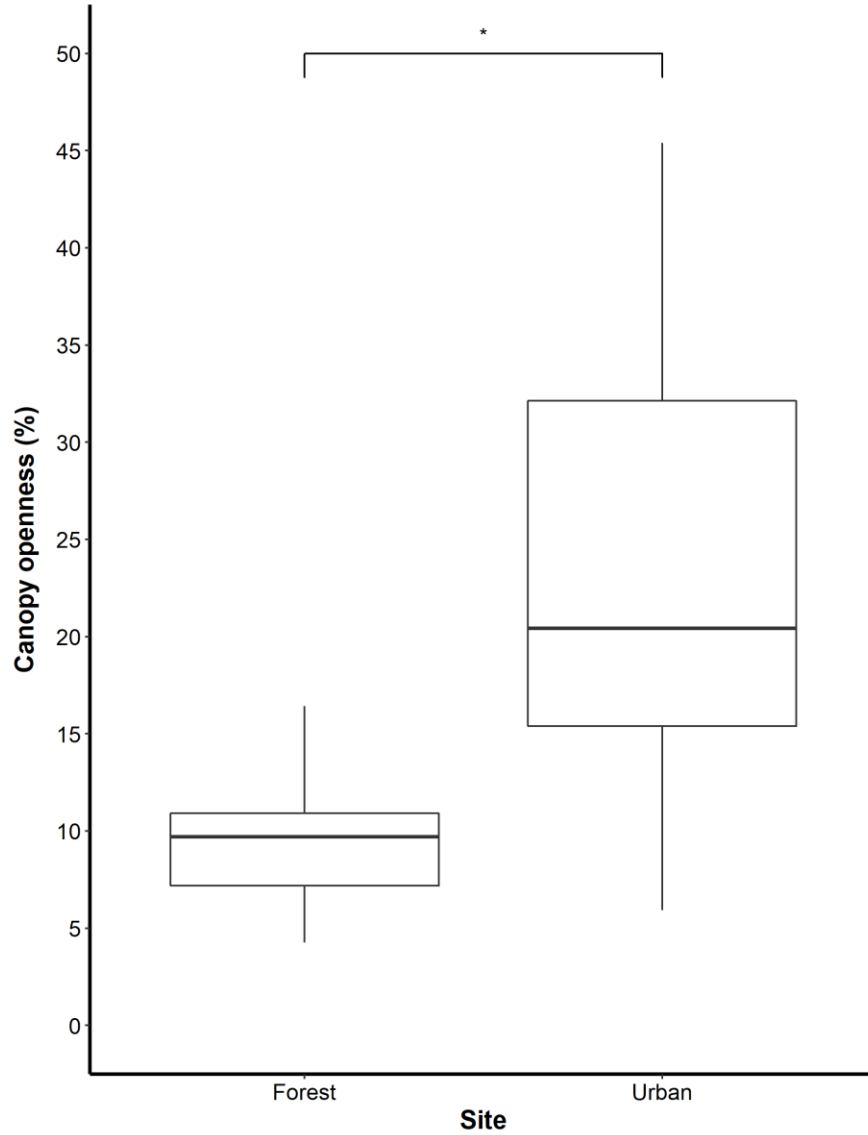


Figure 1.2. Boxplots of canopy openness for nests between urban and forest sites. The box indicates interquartile range with median as the bold line. The upper and lower extended lines show maximum and minimum values, respectively. The asterisk indicates statistical difference between groups ($P < 0.05$).

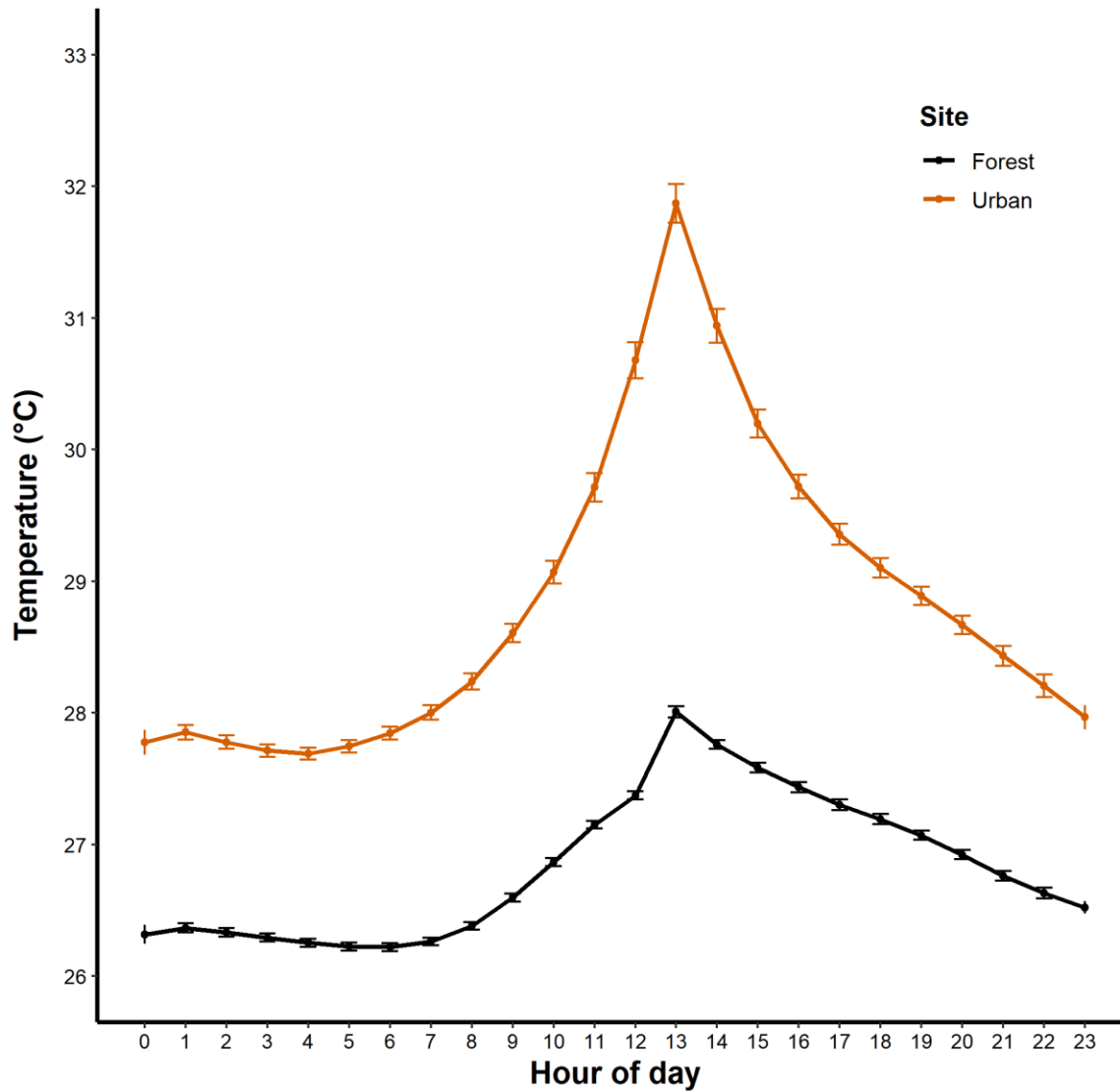


Figure 1.3. Mean daily temperature fluctuations for each site. On average, nest temperatures peak at 1300 hours each day. Daily temperatures were aligned by their maximum temperature before calculating the mean for each hour of the day. Thus, these are the temperatures that embryos likely experience throughout a typical day in each habitat. Error bars show $\pm 95\%$ confidence interval.

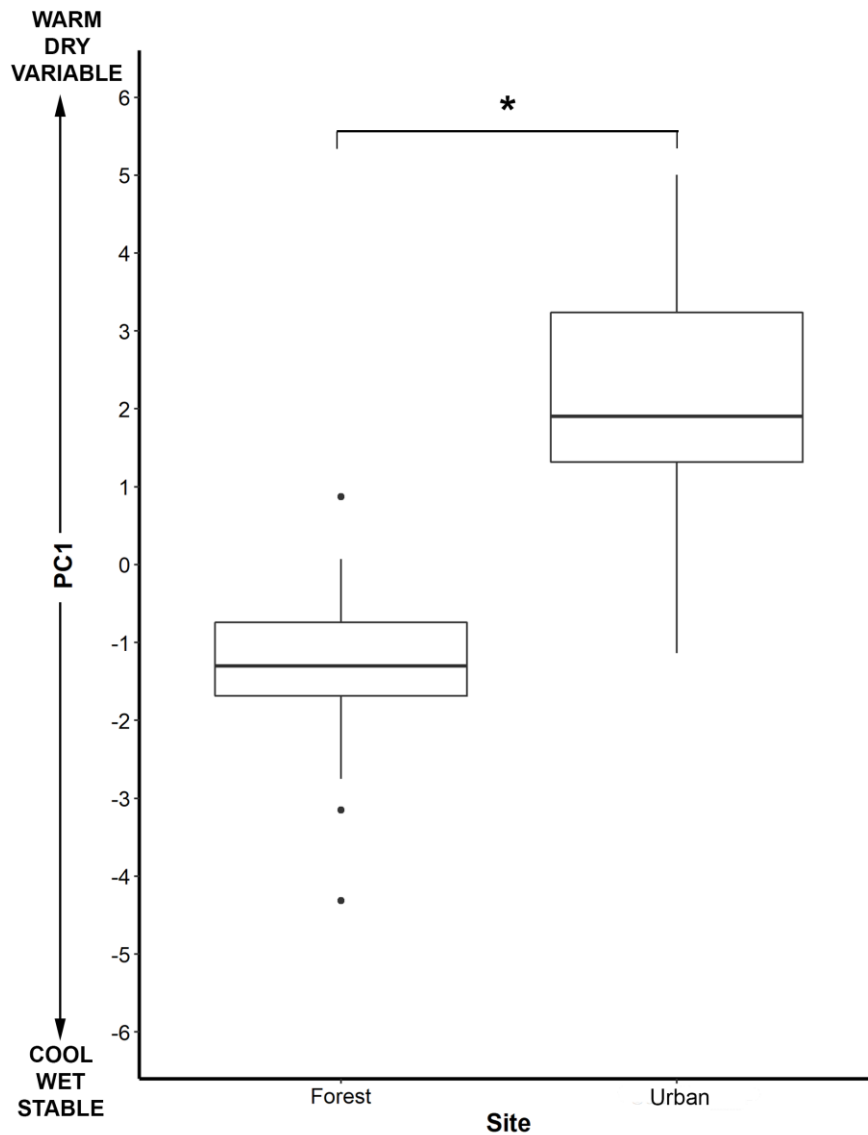


Figure 1.4. Comparison of nest conditions in urban versus forest habitat. Principal component scores from the first axis (PC1) represent thermal and moisture conditions, as well as temperature variability (Table 1). “Variable” and “stable” refer to daily temperature variance only. The asterisk indicates statistical difference between groups ($P < 0.05$).

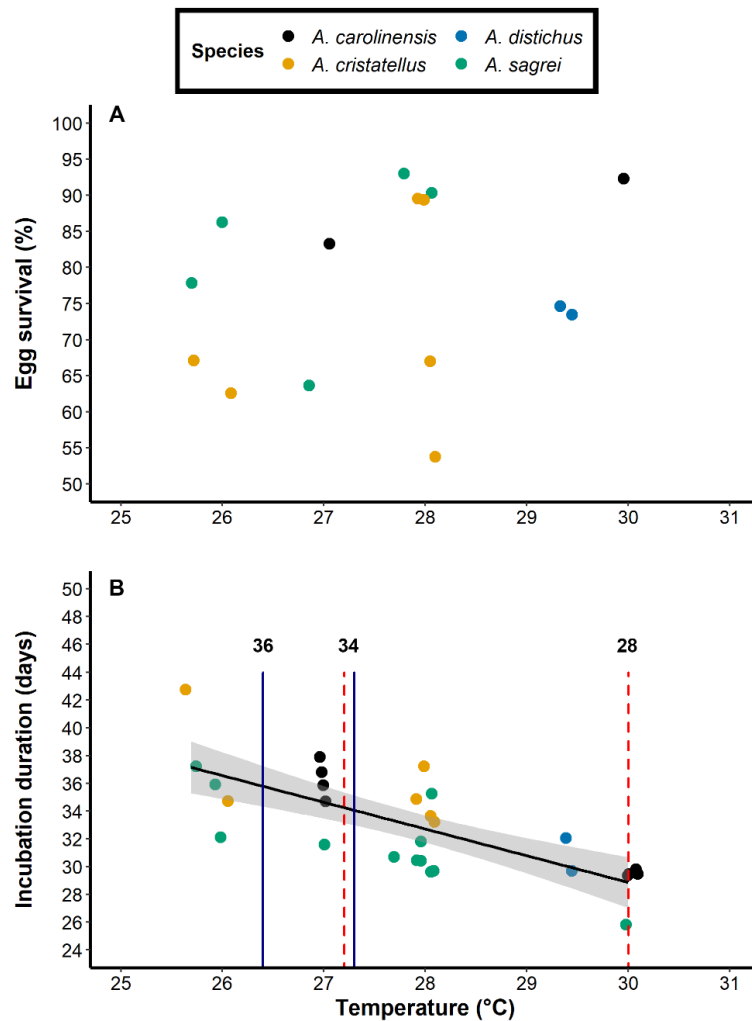


Figure 1.5. (A) Proportion of egg survival and (B) mean incubation duration at nest temperatures for common *Anolis* species at my study sites (below 25 °C not shown). Data were extracted from published studies or personal communications (Table S1.1). Regression line (black with grey shade 95% confidence interval) is shown between mean temperature of the coolest nest (26 °C) and warmest nest (30 °C) recorded in this study. Reference solid vertical lines (blue = forest) and dashed vertical lines (red = urban) represent mean temperatures for the coolest and warmest nest

temperatures for each site. Number above vertical lines show estimated incubation duration at that temperature.

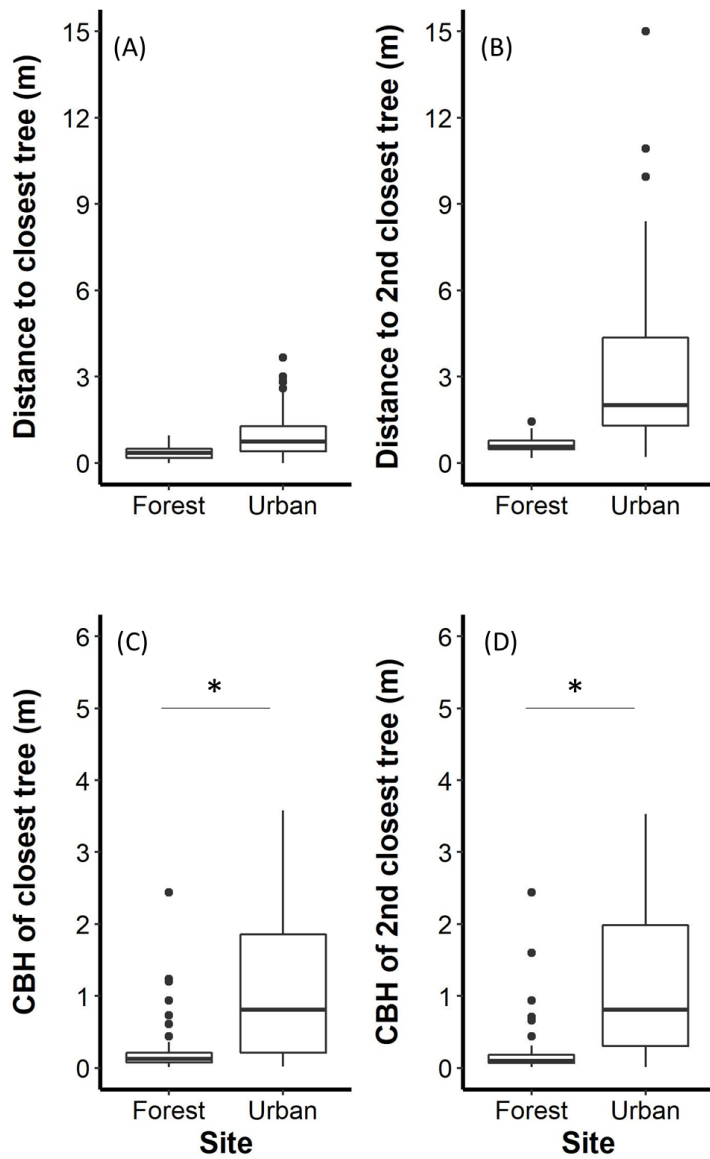


Figure S1.1. Boxplots of (A) Distance between the closest tree to the plot, (B) distance between the second closest tree to nests, (C) average size of the closest trees to nests, and (D) average size of second closest tree to nests. The box indicate interquartile range with median as the bold line. The upper and lower extended lines show maximum and minimum values, respectively. Single asterisk indicate significant difference between groups ($P < 0.05$).

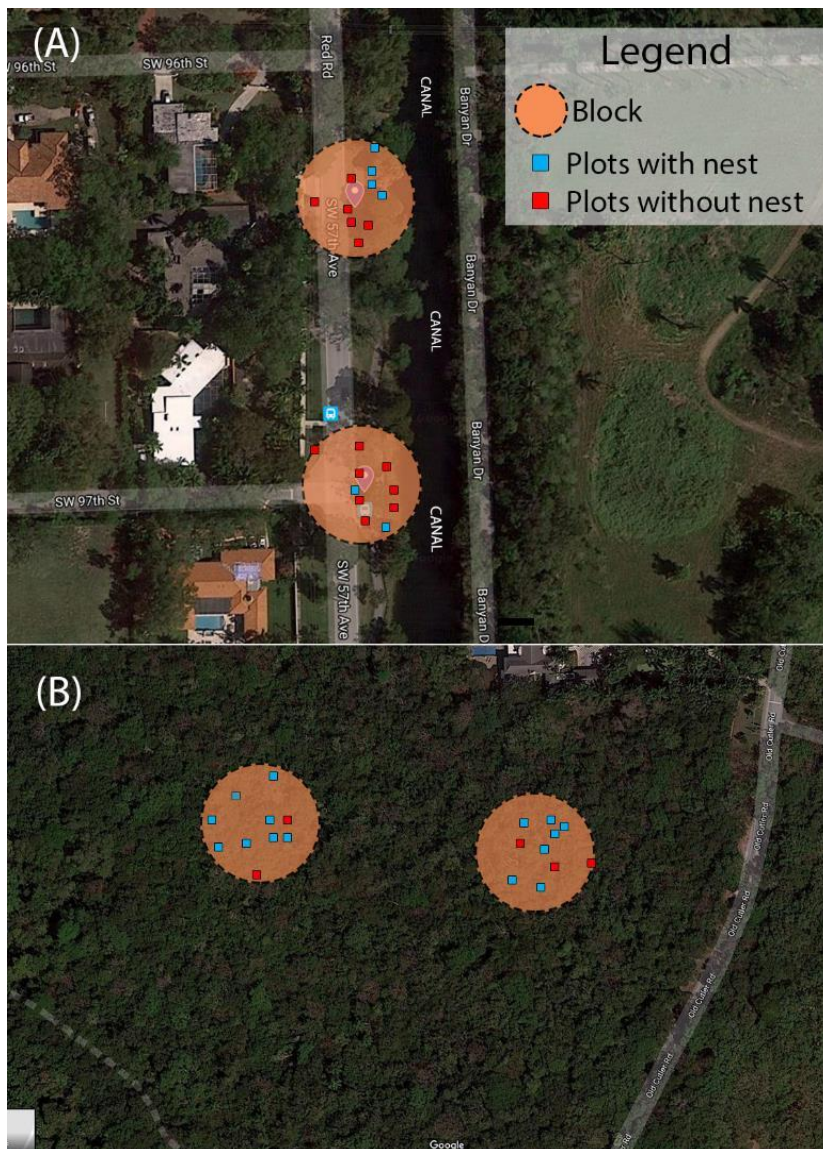


Figure 2.1. Photographs and diagram of two urban blocks and two forest blocks. (A) Example of blocks and plots in the urban site; the orange circles represent two blocks with 20m radii and squares show the distribution of plots. (B) A section of the urban site featuring the road, bike path, and canal.

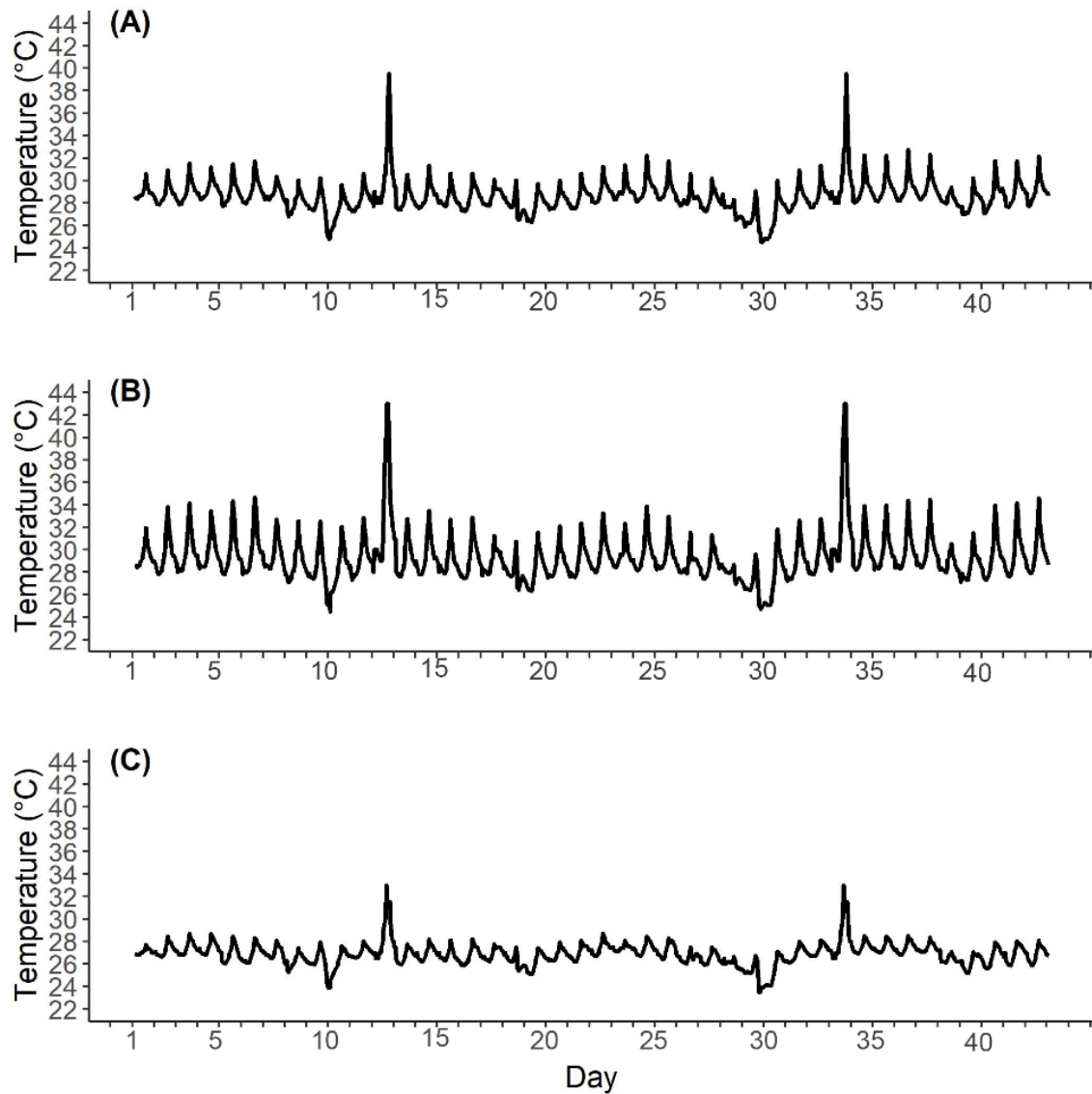


Figure 2.2. Incubation treatments used in this study (A = Urban with nest; B = Urban without nest; C = Forest with nest). Treatments were calculated by first correcting the data (see Fig. S2.1) and then taking the mean of each hour of the day across all plots in each site. Two extreme temperatures were included in each treatment as they the highest temperatures recorded in each plot type.

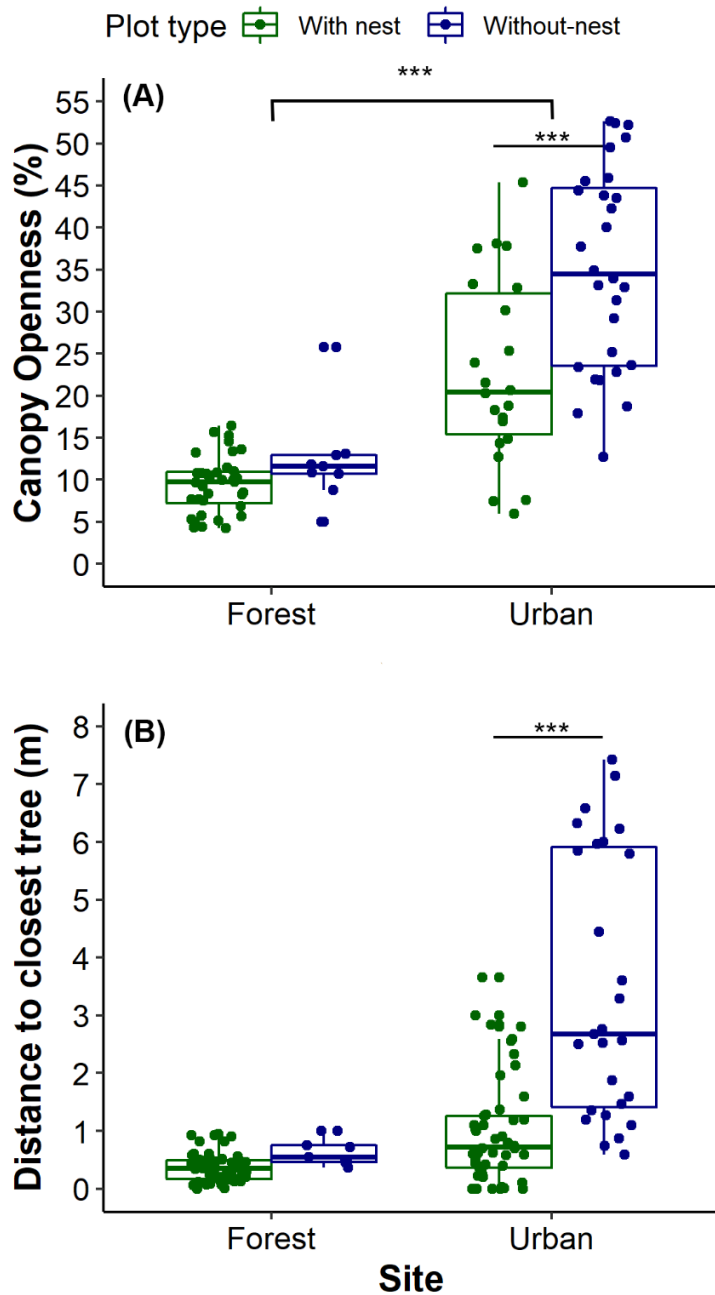


Figure 2.3. Boxplots of (A) canopy openness and (B) distance from plots to the closest tree between site and plot types. The box indicates interquartile range with median as the bold line. The upper and lower extended lines show maximum and minimum values, respectively, without outliers. Asterisks indicate statistical significance between groups ($P < 0.001$).

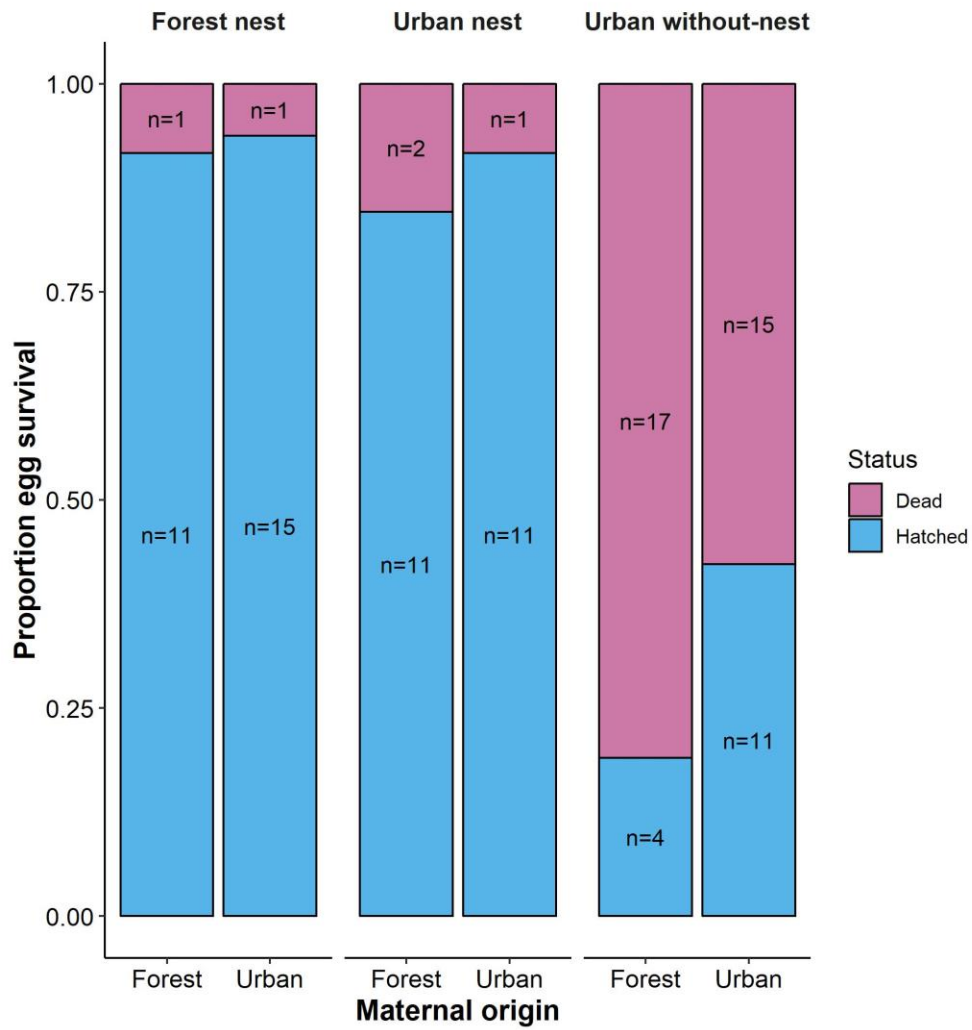


Figure 2.4. Proportion of Puerto Rican crested anole (*Anolis cristatellus*) egg survival by treatment and maternal origin along with sample sizes (n).

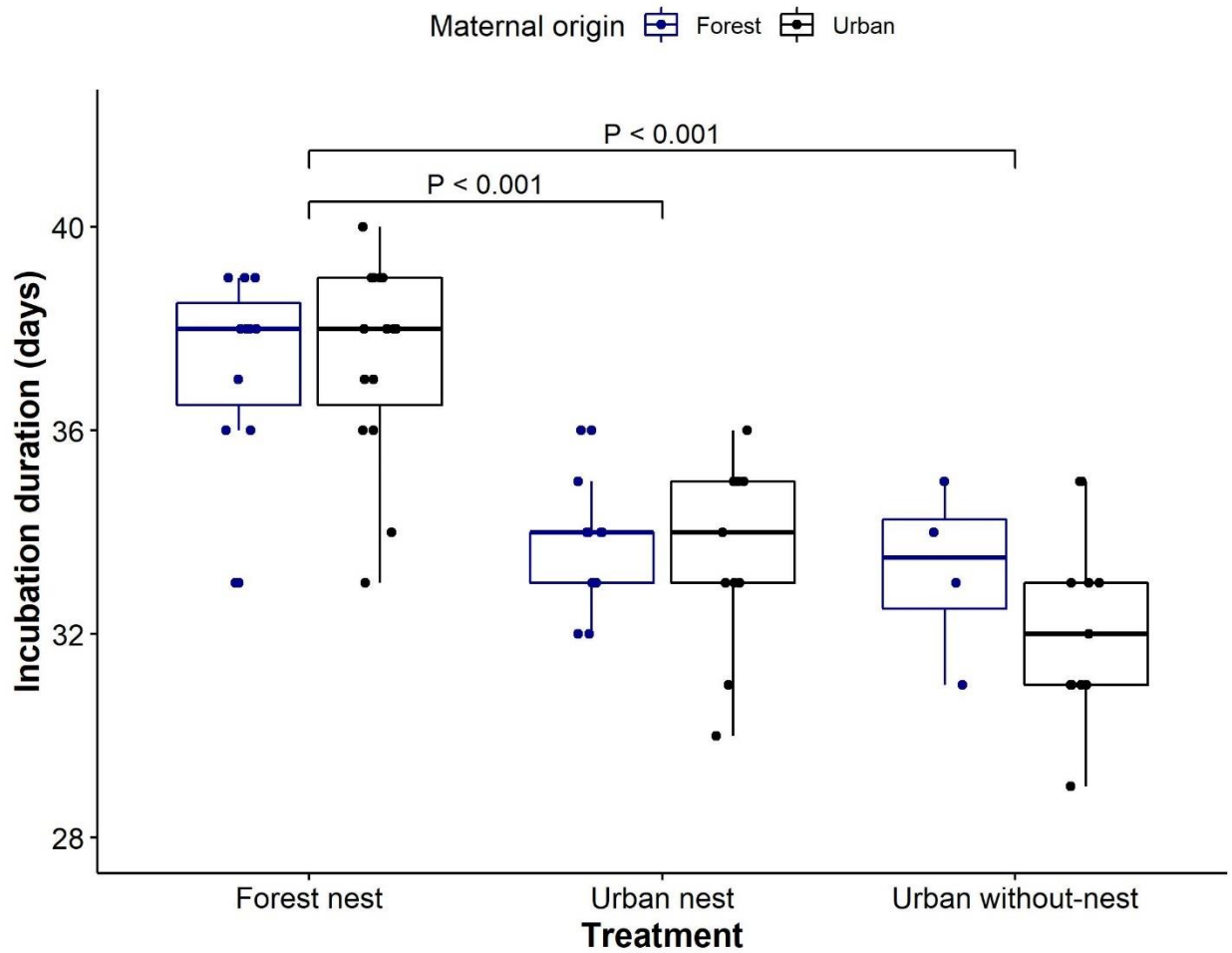


Figure 2.5. Boxplots show incubation duration of Puerto Rican crested anole (*Anolis cristatellus*) by treatment and maternal origin. The box indicate interquartile range with median as the bold line. The upper and lower extended lines show maximum and minimum values, respectively, without outliers.

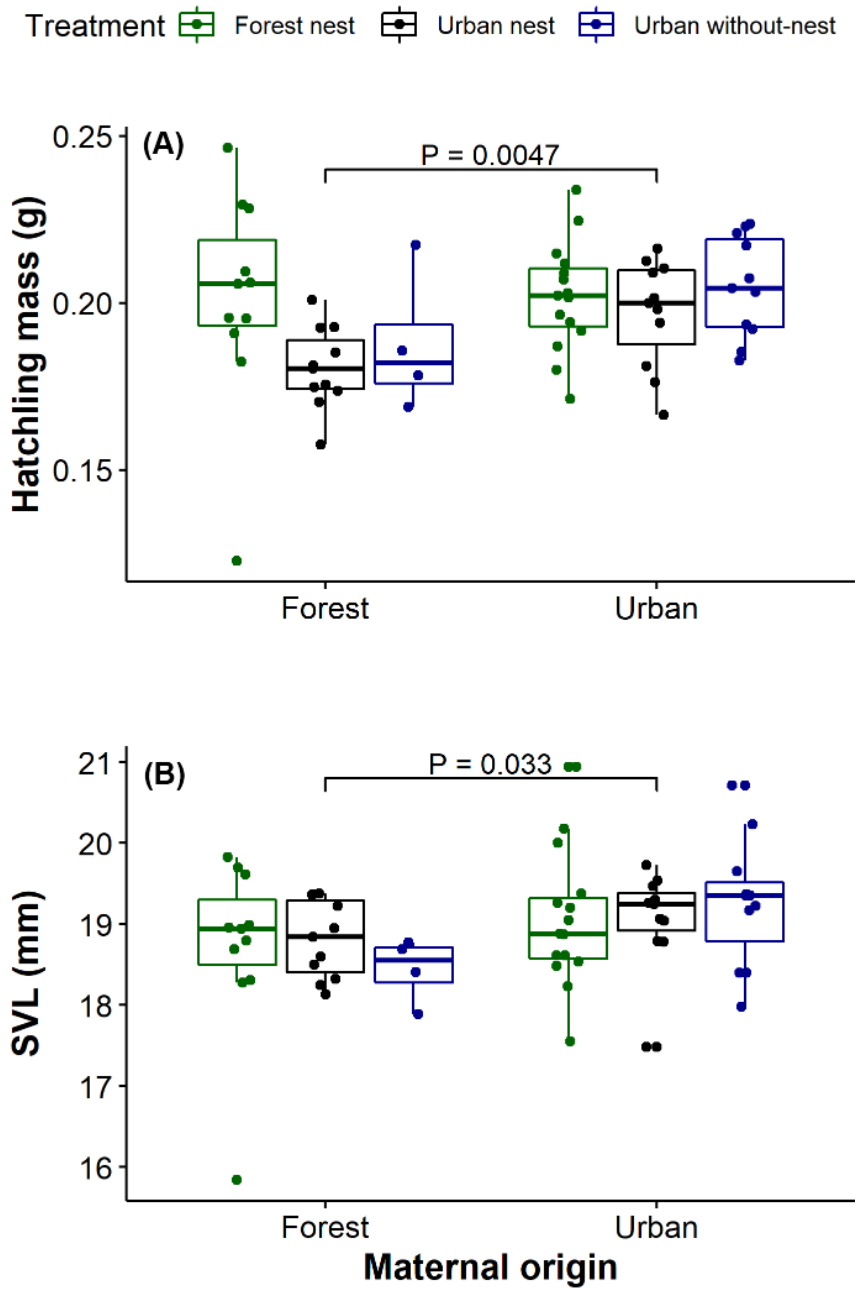


Figure 2.6. Boxplots show hatchling mass and snout-vent length (SVL) of Puerto Rican crested anole (*Anolis cristatellus*) by maternal origin and treatment. The box indicate interquartile range with median as the bold line. The upper and lower extended lines show maximum and minimum values, respectively, without outliers.

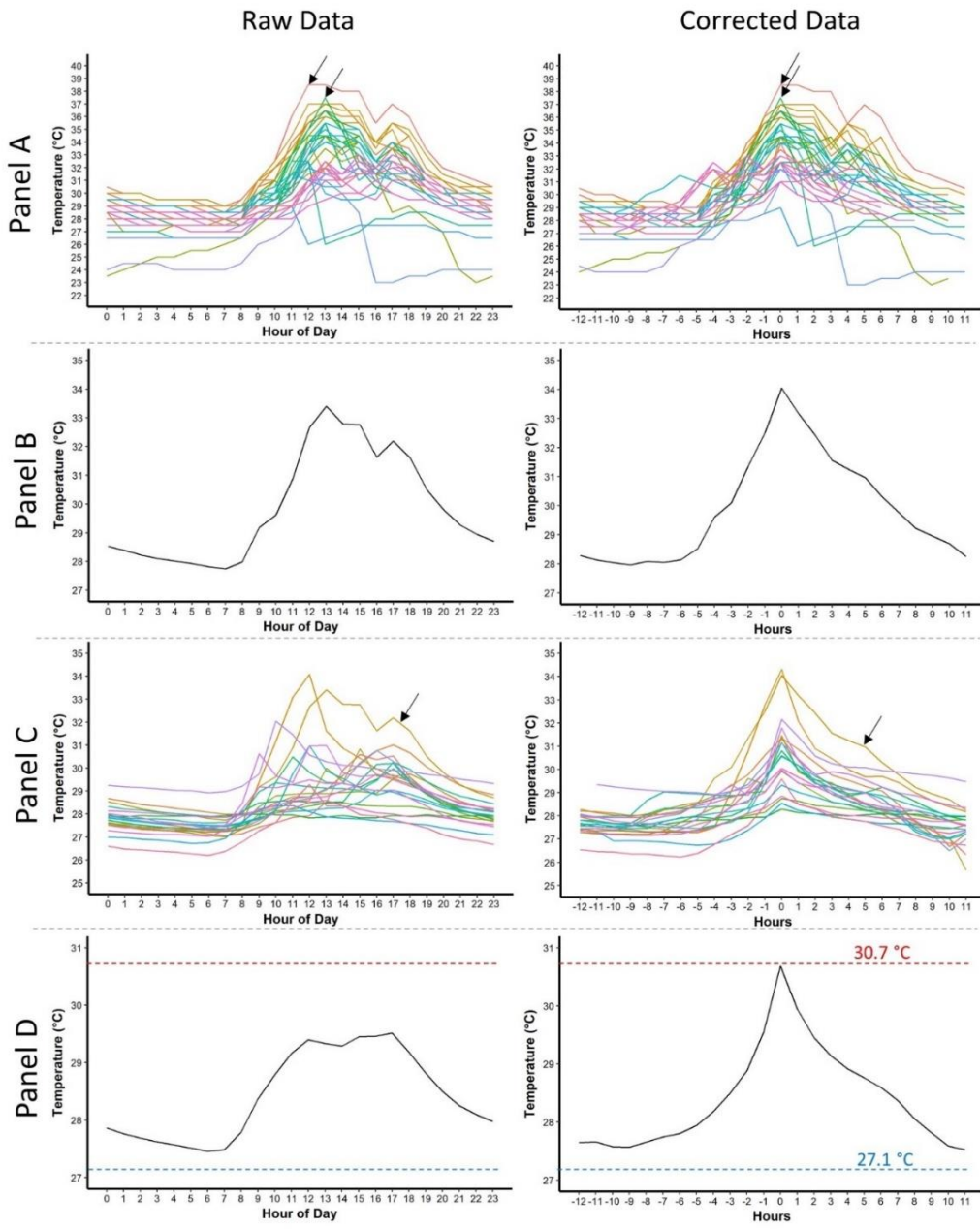


Figure S2.1. Daily thermal profiles of nest temperatures. The left and right side of each panel (panels A-D) show the raw and corrected nest data, respectively, for a single nest in the urban site. To get the corrected data, I shifted each daily thermal curve so that the maximum

temperature on that day was at time zero. Panel A shows both the raw and corrected daily temperatures of a single nest. Each colored line shows a single day ($n = 42$ days) during the study period. For the raw data, note that the nest temperature may peak at different times depending on the day of the study (see arrows). Panel B shows the mean temperature at each hour across all 42 days. Note that the mean peak temperature is lower for the raw data than the corrected data. This is caused by the variation in the time of day when the nest temperature reaches its maximum. Panel C shows the hourly mean for this nest (denoted with an arrow) along with all other nests from the urban site. For the raw data, note the wide variation in the time of day that each nest reaches its mean maximum temperature. Panel D shows the mean temperature at each hour across all urban nests. The red and blue broken horizontal lines denote the mean maximum and mean minimum temperatures, respectively, across all nests and across all days during the study. Centering the daily thermal curves according to their maximum temperature creates a daily summary curve (Panel D) that more closely approximates the mean range of temperatures that eggs experience in the field and likely better represents how rapidly temperatures rise and fall on a typical day. Due to the extreme variation in the time of day when nest temperatures peak, simply taking a raw average of each hour generates a summary curve that fails to capture the usual conditions of nests in the field.

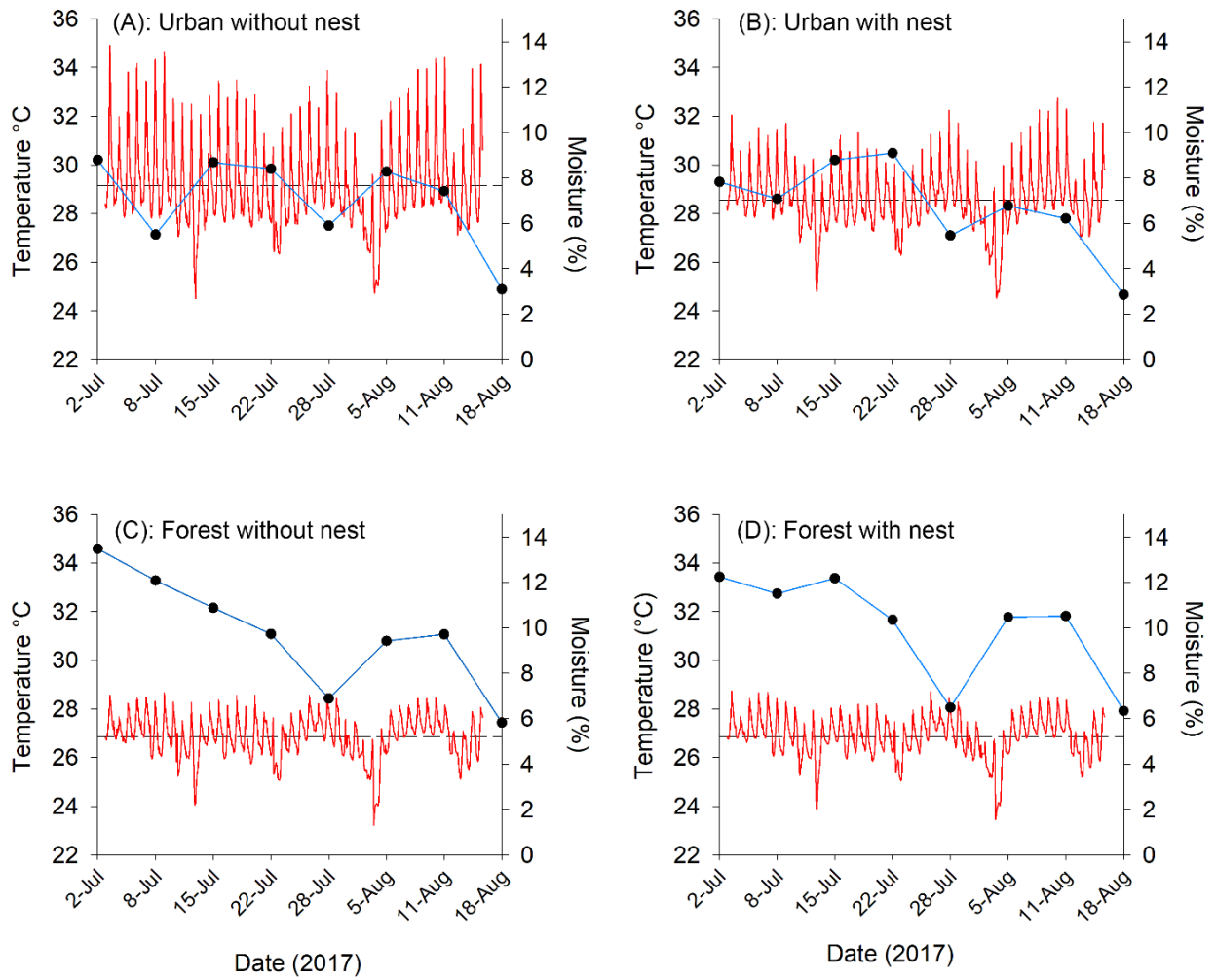


Fig S2.2 Mean temperature and moisture across plot types for forest and urban habitats (Red line = mean hourly temperatures, closed circles and blue line = mean weekly moisture). A) Urban plots without a nest, B) urban plots with a nest, C) forest plots without a nest, D) forest plots with a nest. Broken lines represent the mean temperature throughout the season in each graph (see Table S2.1). Note that A and B show remarkable variation in temperature, while C and D do not show variation in temperature. Mean moisture is greater in the forest than in the urban site, however, there is no difference in mean moisture between plot type.