


Nestled in the city heat: urban nesting behavior enhances embryo development of an invasive lizard

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Abstract

Urbanization transforms many aspects of natural landscapes and poses many new challenges for individual survival and population persistence. Thus, urbanization provides an opportunity to examine how organisms deal with novel environmental change. Many studies provide evidence of phenotypic adaptation to urban environments, but few focus on responses during early life stages. Filling this information gap is important, because early life stages are particularly sensitive to abiotic factors, and no population is sustainable without successful embryo development. We tested the hypotheses that (i) embryos tolerate warmer temperature conditions of urbanized areas and (ii) maternal nesting behavior protects embryos from potentially lethal thermal conditions in urbanized habitats. We studied introduced populations of a subtropical lizard, *Anolis cristatellus*, in suburban and forested areas in Miami, Florida. In each habitat, we measured microenvironment variables for locations that females used for nesting vs. locations they did not use. We then incubated eggs from both populations under thermal conditions that mimicked used and unused sites. Nests in the suburban site were warmer than in the forest; however, in the suburban site, locations that females used were relatively cool compared with locations that were not used. We found no evidence that embryos are adapted to their respective suburban or forested thermal environments, but rather maternal nest-site choice enhanced embryo development in the suburban habitat. Maternal nesting behavior is likely an important factor for population persistence under major environmental changes, and a key contributor to the establishment and spread of invasive organisms across urbanized landscapes.

Key words: early life, *Anolis*, maternal effect, nesting, development plasticity

Introduction

Urbanization is arguably the leading form of human-induced environmental change, as it transforms nearly all aspects of natural landscapes and often places local species in environments to which they are not adapted (Lewis and Maslin 2015; Hopkins et al. 2018). These novel urban habitats introduce new selective pressures and influence phenotypic evolution across small and large spatiotemporal scales (Rodewald, Shustack, and Jones 2011; Johnson and Munshi-South 2017; Merckx, Kaiser, and Dyck 2018; Tucker et al. 2018). Species that rapidly respond to novel conditions via plasticity (e.g. in morphology, physiology) or

exhibit flexibility in behavior are likely to thrive in novel areas (Diamond et al. 2018; Lapiedra 2018). Additionally, communities often differ between urbanized and natural landscapes due to variation in how different species cope with urbanization (Grant et al. 2011; Santini et al. 2019). Moreover, many species that thrive in urban landscapes are also non-native, suggesting a link between the ability to take advantage of urban habitat and the potential to establish outside their native range (Hufbauer et al. 2012; Suzuki-Ohno et al. 2017; Stepkovitch et al. 2019).

Many studies have sought to determine how adult phenotypes respond to urbanization and other forms of habitat

modification (Magle et al. 2012; Alberti et al. 2017). For populations to sustainably establish in novel environments, however, younger age classes must be able to develop into adults (Andrews 1998; Engel, Tollrian, and Jeschke 2011). Early life stages are important because development is sensitive to many environmental factors that are altered by urbanization, such as warmer temperature (Deeming 2004; Brans and De Meester 2018). These types of environmental change can have immediate consequences (e.g. kills the embryo) and/or have persistent influences throughout an individual's lifespan. Thus, effects at early life stages should be considered along with adult stages when drawing conclusions about the impacts of urbanization on wildlife and establishment of invasive species.

For oviparous animals without parental care (e.g. many non-avian reptiles and arthropods), developing embryos can do little behaviorally to adjust for adverse conditions (Telemeco et al. 2016). Nevertheless, nesting mothers can select microenvironments that positively influence hatching success and offspring fitness (Shine and Harlow 1996; Pike, Webb, and Shine 2012; Li et al. 2018). The literature contains many examples of parental choice of specific nest conditions that facilitate offspring development (e.g. spiders: Pike, Webb, and Shine 2012; fish: Probst et al. 2009; amphibians: Rudolf and Rödel 2005). Moreover, shifts in nesting behavior of reptiles have been documented in urbanized habitats (Ryan et al. 2008; Foley, Price, and Dorcas 2012; López-Luna et al. 2015). For example, Australian water dragons (*Itellagama lesueurii*) dig deeper nests and choose novel substrates in urban areas compared with in their natural riparian habitat, which may help them adjust to urbanization (Kent et al. 2019). In other cases, however, habitat modification could affect nesting behaviors in ways that are detrimental to offspring. For example, artificial lights and shadows of tall buildings along urban nesting beaches disorient emerging sea turtles (Salmon et al. 1995), and shade from tall buildings can modify nest temperatures in ways that influence sex ratios in species with temperature-dependent sex determination (Mrosovsky, Lavin, and Godfrey 1995).

Alternatively, if maternal nesting behavior does not shield embryos from adverse conditions, embryos may adapt their physiology in ways that enable them to cope with more extreme incubation conditions (Du et al. 2019). Variation in thermal tolerances among individuals could provide an opportunity for selection to drive adaptation to population-specific thermal environments as long as this aspect of thermal physiology is heritable. Some comparative studies of reptile embryos across thermally variable habitats provide evidence of thermal adaptation (reviewed by Du et al. 2019). Nonetheless, although both maternal nesting behavior and thermal adaptation are mechanisms that influence how successfully populations establish in urban environments, behavior is typically more flexible and can buffer organisms from physiological adaptation (Huey, Hertz, and Sinervo 2003; Buckley, Ehrenberger, and Angilletta 2015).

Anolis lizards make excellent models for studying adaptation to novel urban habitats. For example, the Puerto Rican crested anole (*Anolis cristatellus*) is a diurnal lizard that has colonized urban environments both within (Puerto Rico) and outside (Miami, Florida) its native range (Kolbe et al. 2016; Winchell et al. 2016). Many aspects of this species' life history are influenced by urban conditions (Lapiedra, Chejanovski, and Kolbe 2017), and adults have rapidly adapted their morphology and physiology to urban environments (Winchell et al. 2016, 2018b). For instance, anoles that reside in urban habitats have longer limbs and more subdigital scales (called lamellae) compared with those from natural areas, suggesting adaptation for clinging to relatively broad and smooth surfaces that are common in

cities (Winchell et al. 2016). Despite being a popular model for studying urban adaptation, few studies have addressed responses of early life stages to urban conditions (Tiatragul et al. 2017; Hall and Warner 2018). This leaves a gap in our understanding about how embryo physiology and maternal nesting behavior may contribute to their success in colonizing urban habitats. Moreover, this knowledge gap is indicative of a larger problem in urban ecology: relatively few studies are concerned with population ecology (e.g. embryo survival) and most studies are focused on few taxa (i.e. mammals, birds; Magle et al. 2012). Thus, understanding nesting behavior and its effects on embryo survival in non-avian reptiles is critical to more fully understand urbanization's influence on biodiversity.

We address these knowledge gaps with a field study and a laboratory experiment using *A. cristatellus*. First, we quantified maternal nest-site choice by comparing microenvironments (principally temperature) of sites females used for nesting (previously published in Tiatragul et al. 2019) with those of sites that females did not use (not previously published) in suburban and forest sites. Second, we performed an incubation experiment that reciprocally exposed eggs from suburban and forest habitat to each natural thermal regime. We also incubated eggs under conditions that mimicked thermal conditions that were used vs. not used by nesting females. With measurements of fitness-related variables (e.g. embryo survival, hatchling body size), our design provides insights into whether embryos from suburban habitat are adapted to their thermal environment or if maternal nesting behavior buffers embryos from potentially lethal temperatures of suburban habitat.

Our study tests two hypotheses: (i) embryos from suburban habitat can better tolerate suburban thermal regimes than embryos from forested habitat and (ii) females in suburban areas shield developing offspring from adverse thermal conditions via nest-site selection. These hypotheses are not mutually exclusive. If embryos have adapted to their habitat-specific thermal regimes, then eggs from suburban females should have higher survival than eggs from forest females under suburban incubation conditions and lower survival in the forest incubation condition. The opposite effect on survival is expected for eggs from forest females. If maternal nesting behavior is beneficial, eggs incubated under nest conditions used by mothers should have greater survival compared with eggs incubated under conditions that were not used. Our incubation study provides an assessment of how early life stages respond to thermal stress and how behavior might ease colonization of invasive species to urban environments.

Methods

Study system

Female *A. cristatellus* lays one egg every 8–15 days throughout the breeding season, and eggs are exposed to variable moisture and temperature conditions during incubation (Hall and Warner 2017; Tiatragul et al. 2019). Eggs are often laid on the ground surface or buried in shallow cavities (1- to 4-cm deep), and then subjected to the microenvironment that mothers have chosen. Laboratory studies show that other anoles prefer to lay eggs in microenvironments that enhance the fitness of offspring (Socci, Schlaepfer, and Gavin 2005; Reedy, Zaragoza, and Warner 2013), and that embryonic development is sensitive to surrounding incubation conditions (Warner et al. 2012; Sanger et al. 2018). At our study site in Miami-Dade County in south Florida (USA), ambient environmental conditions differ between

forested and urbanized habitats (Battles and Kolbe 2018); accordingly, ground temperatures are warmer and more variable in urban areas where lizards nest and can reach temperatures that kill developing embryos (Tiatragul et al. 2017; Hall and Warner 2018). Two incubation studies provide no evidence that embryos have physiologically adapted to the thermal environment of urbanized areas (Tiatragul et al. 2017; Hall and Warner 2018); however, incubation treatments used in these experiments did not reflect the complex thermal fluctuations of natural nests in forest or urbanized areas. Although subsequent work has more fully characterized nest microhabitats at our study sites (Tiatragul et al. 2019), we have neither examined maternal choice of nest microhabitat (i.e. compared sites used by nesting females vs. unused), nor the consequences of 'natural' thermal regimes that eggs experience in suburban and forested habitat. In this study, we directly test how embryos cope with urban thermal environments (i.e. via embryo adaptation vs. being shielded by maternal nesting behavior).

Nest environments in the field

Since anole nesting behavior is inconspicuous, we put considerable effort into finding nests in the field, rather than observing nesting directly. From 4 June to 3 July 2017, we searched for anole nests in a suburban site along a two-lane road (State Road 959 or 'Red Road') and a nearby forest (Matheson Hammock Preserve) in Miami-Dade County. The suburban site is located along a 1 km stretch between the road and Snapper Creek Canal. A paved path separated from the adjacent road by guardrails is flanked by a regularly mowed lawn lined with sporadically distributed trees (e.g. *Ficus citrifolia*, *Ficus aurea*, and *Sabal* spp.) and many human-made structures (e.g. buildings, lamp posts and bus stops). Matheson Hammock is a 0.21 km² fragment of dense forest comprising large fig trees intermixed with smaller shrubs. The preserve interior where we conducted our study contains no human-made structures other than narrow walking trails.

We have characterized nest thermal environments previously (Tiatragul et al. 2019) using the same dataset here; however, in this study, we also provide information about microenvironments of locations that were not used as nests. We briefly reiterate important details of the protocol here. We established four searchable blocks in each site. A block was defined as a 40-m diameter area where numerous *A. cristatellus* were observed on a central tree. We randomly located ten 1 m² plots within each block and searched each plot thoroughly for hatched or unhatched eggs (Fig. 1). We 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, we found a total of 44 plots with nests ($n = 31$ forest; $n = 13$ suburban) and 36 plots without a nest ($n = 9$ forest; $n = 27$ suburban). Henceforth, we refer to plots with nests as 'used plots' and plots without a nest as 'unused plots'. To increase representation of used plots in suburban areas and unused plots in the forest, we also conducted 'targeted' searches in areas that appeared suitable based on our own experience working with anoles in the laboratory and field. Because our suburban site was along the road and narrow compared with our forest site, we searched ~200 m down the road away from the original blocks and found 10 additional used plots. We assigned these plots to five new blocks to account for spatial variation, because they were not within our original blocks. All additional unused plots in the forest ($n = 15$) were found within the same blocks used previously. We located four

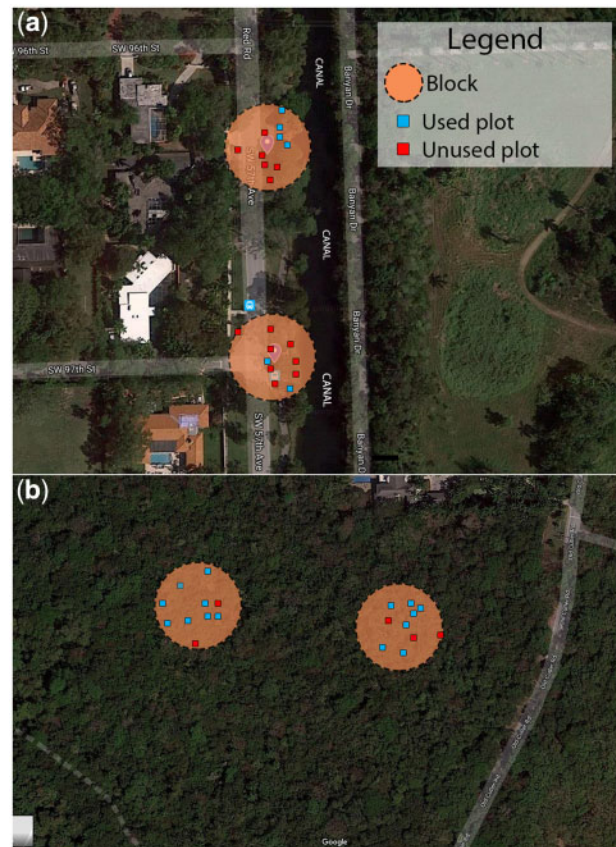


Figure 1: Examples of (a) two suburban blocks located along 'Red Road' (25° 40' 53.60" N, 80° 17' 02.96" W), and (b) two forest blocks in Matheson Hammock Preserve (25° 40' 54.93" N, 80° 16' 29.52" W). The orange circles represent two blocks with 20 m radii and squares show the distribution of plots (plot size = 1 m²). The section of the suburban site features the road, bicycle path and canal

additional used plots in the forest while searching for unused plots. Therefore, the total of random and targeted plots combined is 57 used plots ($n = 35$ forest; $n = 22$ suburban) and 52 unused plots ($n = 24$ forest; $n = 28$ suburban) from 13 blocks ($n = 8$ original; $n = 5$ targeted in suburban site).

Temperature of each plot was recorded hourly from 3 July to 15 August 2017 with a Thermochron iButton ($n = 109$) wrapped in a water balloon and Parafilm. Because most eggs are laid during this time (Hall and Warner 2017), these temperatures represent what most eggs experience in the field. For used plots that contained more than one nest, we placed the iButton next to the nest with an unhatched egg. Otherwise, the iButton was either in the middle of the plot (for unused plots) or next to a randomly selected hatched egg within that plot. iButtons were placed in the same microenvironment as the eggs (e.g. buried in the soil, on soil surface beneath leaf litter). For unused plots, iButtons were buried 2–4 cm beneath the soil surface which is a typical nest depth. For substrate moisture, we collected samples of the nest substrate once a week during the same period ($n = 8$ weeks) and calculated percent water content as [(wet mass – dry mass)/wet mass] × 100. These protocols provided temperature and moisture profiles for each plot over 43 days, which covers the typical incubation period for *A. cristatellus* (Hall and Warner 2018; Tiatragul et al. 2019). Canopy openness (inverse of shade cover) was calculated from hemispherical photographs taken above each plot (using a Canon Powershot

Elph 180 with a 180° fisheye lens) using Gap Light Analysis ver. 2.0 (Frazer, Canham, and Lertzman 1999; Doody et al. 2006). Distance from nests to closest tree was the length (nearest 0.01 m) between the actual nest site within a plot to the base of the closest tree or plant with a diameter at breast height > 0.05 m. For unused plots, we measured the distance from the center of the plot to the trunk of the tree.

Our work focused on *A. cristatellus*, despite the presence of other anoles at our study sites. Although *A. cristatellus* is very abundant at the suburban site (Tiatragul et al. 2019), three other species (*Anolis sagrei*, *Anolis distichus* and *Anolis carolinensis*) that lay similar sized eggs also occur at this site. In the forest site, however, *A. cristatellus* occurs almost exclusively and other anole species are rarely sighted (Battles and Kolbe 2018). Although the eggs found during our study could have been from any of these species, all resident *Anolis* species have similar nesting requirements in captivity and highly conserved patterns of development (Sanger et al. 2008).

Consequences of nest thermal environments

To quantify the effects of nest thermal environments on embryo development, we obtained eggs from a captive colony of *A. cristatellus* in the year after the field study. On 19 March 2018, we collected adult *A. cristatellus* from the suburban (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. Ambient room temperature (27.5–33.5°C) was within the range of field body temperatures of lizards from our sites (Battles and Kolbe 2018). Cages were outfitted with cage carpet substrate (Zoo Med Inc.), two bamboo perches, artificial plants and a nesting pot filled with moist soil to encourage oviposition. We fed each lizard three crickets (dusted with calcium and vitamins) twice per week and sprayed cages with water daily to maintain high humidity. Each male was randomly assigned to four female cages and rotated among those cages fortnightly, but males from one site were never mixed with females from the other. Lizards were kept in captivity for 2 months to acclimate before we started collecting eggs.

We collected eggs from nest pots three times per week between 4 June and 9 July 2018. Immediately after collection, we weighed eggs (to 0.0001 g) and placed each egg in a glass jar filled with moist vermiculite (−150 kPa). We covered each jar with plastic wrap (sealed with a rubber band) to prevent evaporation, and allocated eggs to one of three incubation treatments that mimicked thermal regimes measured the previous year in the field. Specifically, incubation treatments mimicked the thermal regimes of (i) used plots in the suburban site, (ii) unused plots in the suburban site and (iii) used plots in the forest. We did not have a treatment that mimicked unused plots in the forest because the thermal profiles for forest used and unused plots were virtually identical (see Results section and Supplementary Fig. S1). Therefore, our experiment consisted of a 2 × 3 factorial design (two populations and three incubation temperature treatments), which allowed us to assess the consequences of maternally selected thermal environments and whether eggs from different populations vary in their response to habitat-specific incubation temperatures. To minimize potential effects of egg order, we randomly allocated a given female's first egg to a treatment and then alternated her successive eggs among the treatments. This pattern of egg allocation to treatment ensured that no female was over-represented in our treatment groups. We expected greater mortality in the treatment that mimicked suburban unused plots

due to extreme fluctuations in temperature that characterized this treatment (Hall and Warner 2018), so we allocated more eggs to this treatment ($n = 47$, compared with 25 and 28 in the other treatments).

We used AtmoCONTROL software ver. 2.5.3.0 to create 42-day temperature programs that were uploaded to Memmert IPP 550+ incubators; the program repeated the cycle at the end of the 42-day period. As such, the incubation temperatures mimicked averaged natural thermal regimes that eggs would experience in used and unused plots in both habitats (Fig. 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 with what occurs naturally (Supplementary Fig. S2). To correct for this, we centered each daily thermal curve at the peak temperature and then calculated the mean temperature for each hour of the day. Therefore, the thermal regimes we constructed did not necessarily represent the mean temperature across all plots at a given hour, but rather, they provided a realistic estimate of the daily rise and fall of nest temperature across the sites.

Thermal spikes are common in suburban areas and they influence embryo development and survival (Hall and Warner 2018). Therefore, we incorporated two thermal spikes in each incubation regime. Their peak temperatures were 43, 39.5 and 33°C for unused suburban plot, used suburban plot and used forest plot treatments, respectively. The magnitudes of these thermal spikes are the warmest temperatures recorded in the field for each of these categories. Thermal spikes were programmed to occur twice per 42-day cycle (18 days apart), which represents their frequency of occurrence in the field. Importantly, because oviposition is not synchronous, the date at which eggs entered the 42-day incubation regimes varied; however, all embryos experienced at least one thermal spike during their expected incubation duration of 29–40 days (Tiatragul et al. 2017; Hall and Warner 2018).

We checked incubators daily for hatchlings. For each hatchling, we recorded the date of hatching, body mass (to 0.0001 g), and measured snout-vent length (SVL) and tail length (to 0.01 mm) with a caliper. These morphological variables were measured because they each respond to incubation temperature (Pearson and Warner 2016, 2018) and are relevant to performance and survival (Warner and Lovern 2014; Hsieh 2016). We did not identify offspring sex because *A. cristatellus* hatchlings lack external sexually dimorphic characters.

Statistical analysis

Data analyses were performed in R ver 3.5.1 (<www.r-project.org>). We used mixed-effects linear models to estimate microenvironment differences between plot types (used vs. unused), site (suburban vs. forest) and their interaction. Dependent variables (analyzed in separate models) included plot temperatures and substrate moisture (mean, maximum, minimum and variance), plot percent shade cover, and mean plot distance to the closest tree. All models included block as a random effect. Due to heteroscedasticity among blocks, we fit models that allowed the variance to differ among blocks when analyzing temperature, shade cover and distance to closest tree. Ten iButtons that failed ($n = 5$ suburban; $n = 5$ forest) were not included in the analysis.

For the laboratory study, we 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 (suburban

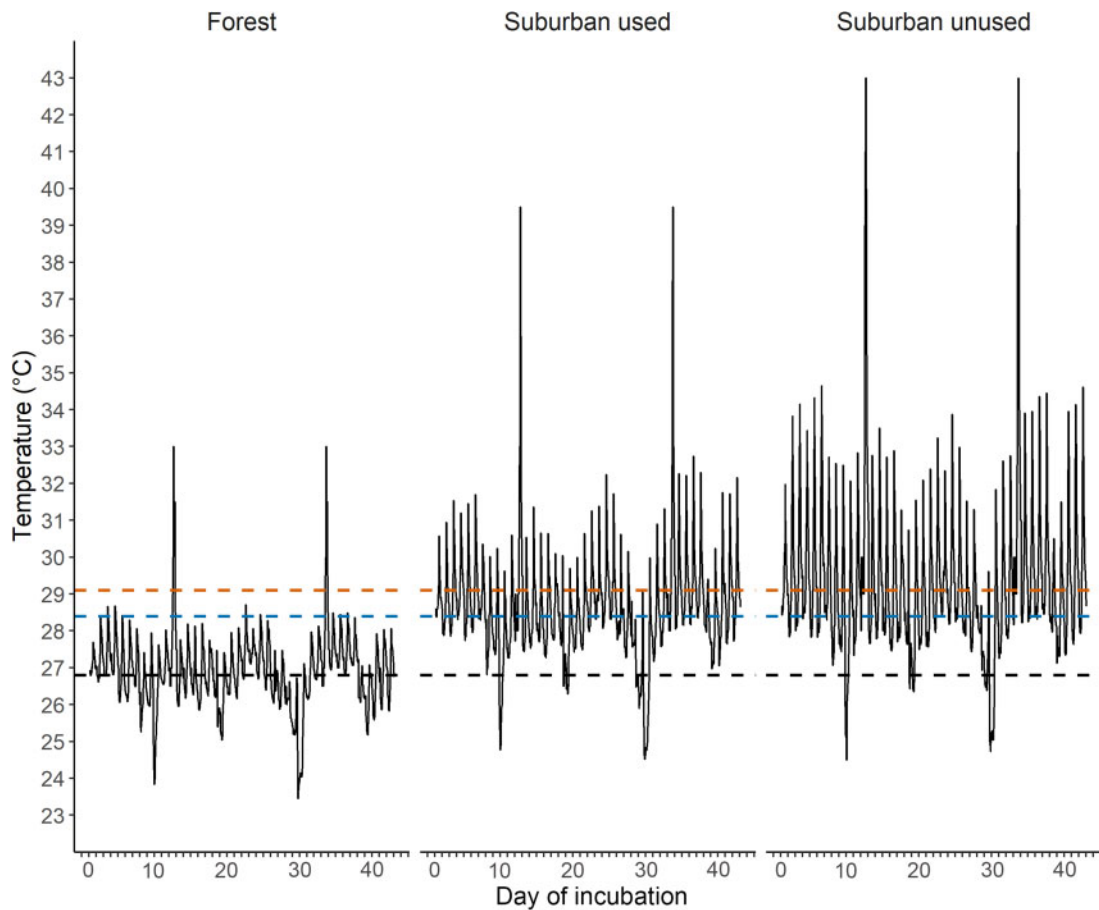


Figure 2: Temperature profiles of the incubation treatments used in this study; plots used by nesting females in the forest, used plots in the suburban habitat, and unused plots in the suburban habitat. Dashed lines represent mean temperature from each treatment (lowest/black, forest; middle/blue, suburban used; and upper/orange, suburban unused). Treatments were calculated by first correcting the data (see [Supplementary Fig. S2](#)) and then taking the mean of each hour of the day across all plots in each site. Two thermal spikes were included in each treatment as they were the highest temperatures recorded in each plot type that we mimicked in this experiment. The 42-day thermal profiles looped continuously

vs. forest), thermal treatment (used suburban plot vs. unused suburban plot vs. used forest plot) and their interaction on egg survival. Mixed-effects linear models in the *nlme* package ([Pinheiro et al. 2018](#)) quantified variation in incubation duration and hatchling morphology due to maternal origin, treatment and their interactions. We analyzed hatchling mass and SVL separately and used initial egg mass as a covariate. Hatchling body condition was assessed with body mass as a dependent variable and SVL as a covariate. Tail length was analyzed with SVL as a covariate. All maternal origin by treatment interaction terms were statistically unclear (all $P > 0.13$ with all $\alpha = 0.05$) and therefore dropped in all models. If there were clear statistical effects (i.e. $P < \alpha$) for variables that had more than one group, we report the estimates of differences between groups, degrees of freedom, and the P -values adjusted for false discovery rate (P_{adj}) using the *emmeans* package ([Lenth 2018](#)). Maternal identity was included as a random effect in all models.

Results

Nest environments in the field

Suburban plots were less shaded, warmer, drier, and more thermally variable than plots in the forest ([Table 1 and Fig. 3a](#)). Used and unused suburban plots differed substantially from one another; however, used and unused plots in the forest were

remarkably similar to each other. Used suburban plots had 5.2% (± 2.3 SE) more shade cover than unused suburban plots ($df = 79$, $P_{adj} = 0.03$). In contrast, shade cover in used forest plots did not differ from unused forest plots (used plots 0.7% ± 1.4 SE shadier than unused; $df = 79$, $P_{adj} = 0.59$). We observed a significant habitat by plot type interaction for distance to the nearest tree ([Table 1 and Fig. 3b](#)). In the suburban site, used plots were 2.41 m (± 0.31 SE) closer to trees than unused plots ($df = 79$, $P_{adj} < 0.0001$). In the forest, however, used and unused plots did not differ (used plots 0.25 m ± 0.28 SE closer than unused; $df = 79$, $P_{adj} = 0.56$).

Mean and maximum temperatures in suburban plots were higher than in forest plots, and thermal variance was greater in suburban plots ([Table 1 and Supplementary Fig. S1](#)). Within the suburban site, the mean temperature of used plots was 0.8°C (± 0.2 SE) cooler than unused plots ($df = 84$, $P_{adj} < 0.001$). The suburban site was characterized by greater thermal variation than the forest ([Table 1](#)), but the variation between used and unused plots in the suburban site did not differ statistically (used 0.2°C ± 0.4 SE lower than unused, $df = 84$, $P_{adj} = 0.61$; [Supplementary Fig. S3](#)). In the forest site, the maximum temperature of unused plots was on average 0.8°C higher (± 0.3 SE, $df = 84$, $P_{adj} = 0.014$) than in used plots, but this difference was driven by a single unused plot that reached 41.5°C 1 day; no other plots reached temperatures $>33^\circ\text{C}$ in the forest, whereas

Table 1: Effect of site (suburban vs. forest), plot type (used vs. unused) and their interaction on microenvironment variables^a

Variables	Site (suburban vs. forest)			Plot type (unused plot vs. used plot)			Site × plot type		
	$\beta \pm SE$	F_{df}	<i>P</i>	$\beta \pm SE$	F_{df}	<i>P</i>	$\beta \pm SE$	F_{df}	<i>P</i>
Canopy openness (%)	13.5 ± 3.8	$F_{1,11} = 12.3$	0.005	0.7 ± 1.4	$F_{1,79} = 0.3$	0.59	4.5 ± 2.7	$F_{1,79} = 2.8$	0.099
Distance to tree (m)	0.31 ± 0.51	$F_{1,11} = 0.37$	0.56	0.25 ± 0.28	$F_{1,79} = 0.4$	0.37	2.16 ± 0.4	$F_{1,79} = 26.6$	<0.001
Temperature (°C)									
Plot mean	1.7 ± 0.1	$F_{1,11} = 425.2$	<0.001	0.1 ± 0.1	$F_{1,84} = 0.61$	0.44	0.7 ± 0.2	$F_{1,84} = 17.7$	<0.001
Plot minimum	0.2 ± 0.1	$F_{1,11} = 3.4$	0.09	-0.1 ± 0.1	$F_{1,84} = 0.4$	0.52	0.3 ± 0.2	$F_{1,84} = 2.4$	0.12
Plot maximum	4.1 ± 1.1	$F_{1,11} = 14.9$	0.003	1.0 ± 0.3	$F_{1,84} = 6.7$	0.01	0.0 ± 0.7	$F_{1,84} = 0.0$	0.99
Plot variance	1.3 ± 0.4	$F_{1,11} = 9.8$	0.009	0.1 ± 0.1	$F_{1,84} = 0.7$	0.42	0.1 ± 0.4	$F_{1,84} = 0.1$	0.75
Moisture (%)									
Plot mean	-4.1 ± 0.9	$F_{1,11} = 15.7$	0.0008	-1.4 ± 0.5	$F_{1,94} = 1.4$	0.01	2.0 ± 0.8	$F_{1,94} = 6.6$	0.01
Plot minimum	-3.9 ± 0.4	$F_{1,11} = 76.5$	<0.001	-0.4 ± 0.5	$F_{1,94} = 0.7$	0.40	0.7 ± 0.6	$F_{1,94} = 1.4$	0.24
Plot maximum	-4.2 ± 1.1	$F_{1,11} = 13.9$	0.003	-1.9 ± 1.0	$F_{1,94} = 4.0$	0.047	3.1 ± 1.4	$F_{1,94} = 4.9$	0.03
Weekly variance	-2.6 ± 1.5	$F_{1,11} = 3.2$	0.10	-0.7 ± 2.2	$F_{1,94} = 0.1$	0.75	3.0 ± 2.5	$F_{1,94} = 1.4$	0.24

^aThe effect sizes represent 'Suburban minus forest' and 'unused minus used plot' for each variable. Bold text denotes statistical significance ($\alpha = 0.05$).

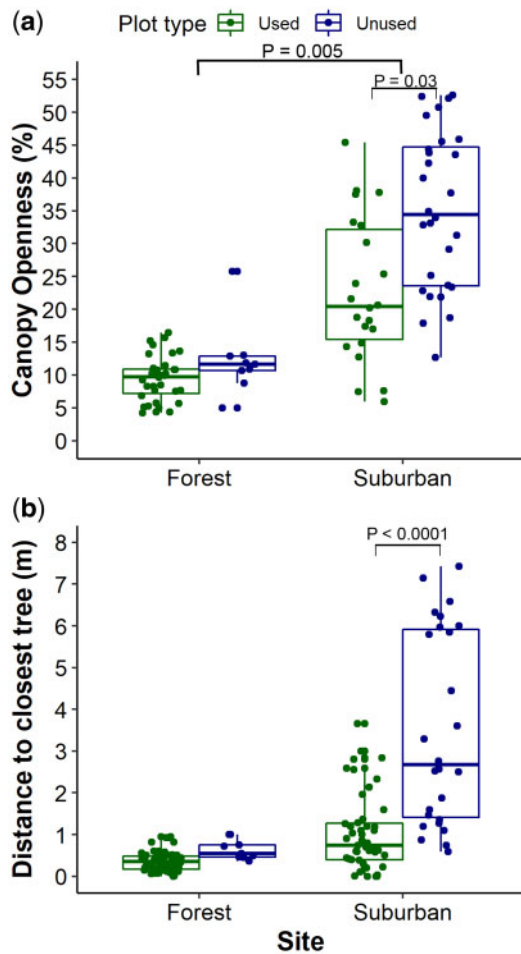


Figure 3: Percentage canopy openness (a) and distance from plots to the closest tree (b) between site and plot types. Boxes indicate interquartile range with median as the bold line. The upper/lower whiskers represent observations from the upper/lower hinge no $>1.5 \times$ interquartile range. Solid circles represent raw data. *P*-values between site represent model statistic, whereas *P*-values within site represent post hoc pairwise comparisons using false discovery rates ($\alpha > 0.05$)

daily peaks $>38^\circ\text{C}$ in unused suburban plots were common. We found no other differences in temperature between habitat and plot types (all $P_{\text{adj}} > 0.09$).

Mean, minimum, and maximum percentage substrate moisture in the suburban site were each less than in the forest (Table 1). Mean and maximum percentage substrate moisture also differed by plot type and the interaction between site and plot type (Table 1). In the forest, the mean substrate moisture in used plots was 1.4% (± 0.5 SE) wetter than in unused plots ($df = 94$, $P_{\text{adj}} = 0.02$), but there was no difference between suburban plot types (used plots were $0.6\% \pm 0.6$ SE wetter than unused plots, $df = 94$, $P_{\text{adj}} = 0.30$). Averaged maximum substrate moisture in used forest plots was 1.9% (± 1.0 SE) wetter than unused forest plots ($df = 94$, $P_{\text{adj}} = 0.09$), and used and unused suburban plots did not differ (used plots $1.1\% \pm 1.0$ SE drier, $df = 94$, $P_{\text{adj}} = 0.31$). Variance in substrate moisture did not differ between sites, plot types, or the site by plot type interaction (Table 1 and Supplementary Fig. S4). Supplementary Table S1 provides summary statistics of each response variable according to habitat and plot type.

Consequences of nest thermal environments

Egg survival differed among the three incubation treatments and maternal origin, but there was no interaction between maternal origin and treatment (Table 2 and Fig. 4). Embryos in the forest treatment were 36.7 (6.8–197.6, 95% confidence limit (C.L)) times as likely to survive than those in the unused suburban plot treatment ($P_{\text{adj}} < 0.001$). Likewise, embryos in the used suburban plot treatment were 27.8 (5.6–136.5, 95% C.L) times as likely to survive than those in the unused suburban plot treatment ($P_{\text{adj}} < 0.001$). Eggs from suburban mothers were 3.8 (1.1–13.3, 95% C.L) times as likely to survive than those from forest mothers (Table 2). Incubation duration differed between the treatments, but not by maternal origin (Table 2 and Fig. 5). Eggs in the forest plot treatment took 3.9 days (± 0.5 SE) longer to hatch than those in the used suburban plot treatment and 4.8 days (± 0.6 SE) longer than the unused suburban plot treatment ($F_{2, 19} = 48.2$, $P < 0.001$). Incubation duration of eggs in used vs. unused suburban plot treatments did not differ (used 0.9 days ± 0.6 SE longer to hatch than unused; $df = 19$; $P_{\text{adj}} = 0.19$).

Hatchling body mass did not differ between treatments but differed between the two populations (Table 2). Body mass and SVL of offspring produced by suburban mothers were statistically greater than those of offspring from forest mothers (Fig. 6). Variation in hatchling body condition and tail length was not explained by treatment or maternal origin (Table 2).

Table 2: Effect of incubation treatment (suburban used vs. suburban unused vs. forest used) and maternal origin (suburban vs. forest) on egg survival (implies embryo survival), incubation duration and hatchling morphology of *A. cristatellus*^a

Trait	Covariate	Treatment	Maternal origin
Egg survival	Egg mass	$\chi^2 = 30.0$ df = 2 P < 0.001	$\chi^2 = 4.5$ df = 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	$F_{2,19} = 1.25$ P = 0.31	$F_{1,39} = 10.11$ P = 0.0047
Snout-vent length (SVL, mm)	Egg mass	$F_{2,19} = 0.21$ P = 0.81	$F_{1,39} = 4.96$ P = 0.033
Body condition (mass, g)	Egg mass	$F_{2,19} = 2.42$ P = 0.12	$F_{1,39} = 3.75$ P = 0.06
Tail length (mm)	Egg mass	$F_{2,17} = 2.67$ P = 0.10	$F_{1,39} = 3.89$ P = 0.056

^aOne individual that autotomized its tail was not used in analyses of tail length. The interaction terms were not significant (all $P > 0.13$), and thus removed from final models. Bold text denotes statistical significance ($\alpha = 0.05$).

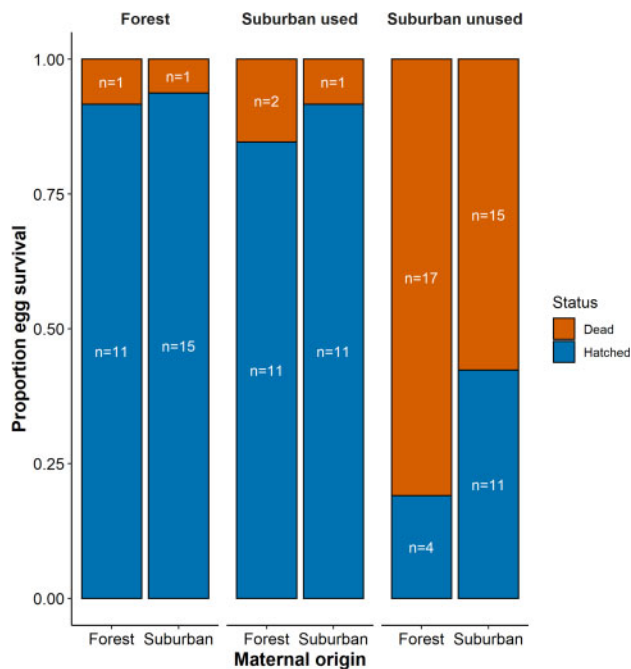


Figure 4: Hatching success of *A. cristatellus* eggs by incubation treatment (labeled above) and maternal origin (below x-axis). See Fig. 2 for graphs of incubation temperatures for each treatment

Discussion

The goal of this study was to assess two hypotheses: (i) embryos from suburban habitat can better tolerate suburban thermal regimes than embryos from forested habitat (potentially due to physiological adaptation) and (ii) maternal nesting behavior in suburban areas shield developing offspring from adverse thermal conditions. We found that suburban nest sites had reduced shade cover, higher temperatures, greater thermal range and lower moisture content compared with those in the forest. Moreover, suburban females nest in areas that shield their eggs

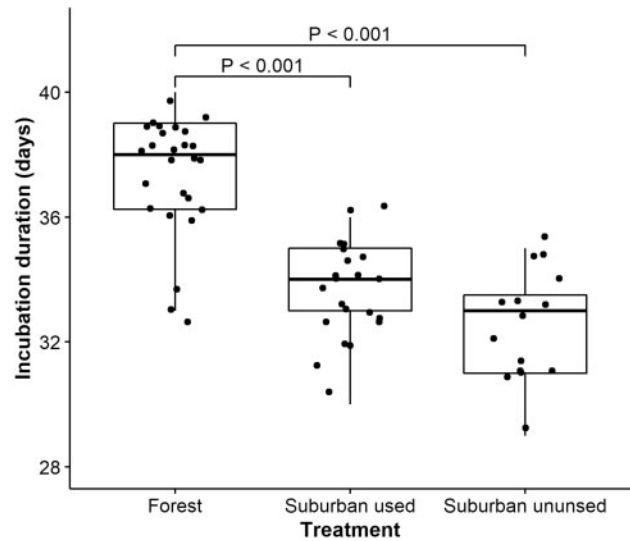


Figure 5: Incubation duration of *A. cristatellus* eggs by incubation treatment. The effect of maternal origin on incubation duration was not statistically clear ($P > 0.05$). Boxes indicate interquartile range with median as the bold line. The upper/lower whiskers represent observations from the upper/lower hinge no $> 1.5 \times$ interquartile range. Solid circles represent raw data

from adverse thermal conditions. Thus, our data support the hypothesis that maternal nesting behavior reduces exposure of embryos to suboptimal incubation temperatures in the suburban site. Non-significant maternal origin by incubation treatment interactions indicates that embryos are not adapted to their population-specific thermal environment.

Nesting habitat use

Apart from adaptation to urban environments (Johnson and Munshi-South 2017), behavioral flexibility can enable populations to overcome the challenges of novel urban conditions (Sol, Lapedra, and González-Lagos 2013; Mainwaring et al. 2017). A suite of behavioral differences between urbanized and natural environments have been documented in a variety of taxa (e.g. Kralj-Fišer, Hebets, and Kuntner 2017; Minias, Jedlikowski, and Włodarczyk 2018; Halfwerk et al. 2019) including anoles (Lapedra et al. 2017; Stroud et al. 2019). Differences in nesting behavior between these environments have also been documented in birds (Yeh, Hauber, and Price 2007; Lambrechts et al. 2017), mammals (O'Donnell and DeNicola 2006), turtles (Kolbe and Janzen 2002; Foley, Price, and Dorcas 2012) and other lizards Kent et al. 2019). Taken together, behavioral plasticity enables a range of taxa to overcome the challenges of urbanization and the results from our study suggest that nest-site choice plays a crucial role.

Because nest-site choice largely determines the conditions that embryos experience during development, it can generate substantial variation in embryo survival and fitness-relevant phenotypes of offspring (Dayananda and Webb 2017; Li et al. 2018). Consequences of maternal nest-site choice should vary among habitats that differ widely in microhabitat conditions, such as in suburban vs. forested areas. Although we did not directly observe females nesting, the presence/absence of eggs is a good proxy for this behavior. Across the suburban site, females use nests that expose embryos to relatively cool, shaded and more thermostable conditions compared with what is generally available. However, microhabitats are more variable

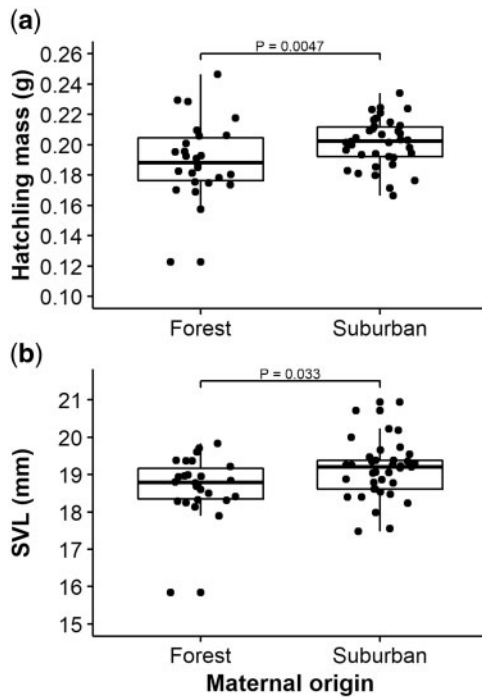


Figure 6: Hatchling mass (a) and SVL (b) of *A. cristatellus* by maternal origin. Incubation treatment effect on hatchling mass and SVL were statistically unclear ($P > 0.05$). Boxes indicate interquartile range with median as the bold line. The upper/lower whiskers represent observations from the upper/lower hinge no $> 1.5 \times$ interquartile range. Solid circles represent raw data

and extreme in the suburban site than in the forest. In this case, the suburban environment is more thermally heterogeneous than the forest, and females have a broader range of conditions to choose among when nesting. Urbanization often results in ecological homogenization (McKinney 2006), but this depends on a scale that is relevant to the organism and habitat. For example, although our suburban site is structurally less complex than the forest, the thermal environment for nesting females is considerably more heterogeneous. This is most evident when we consider that used plots in the suburban site were relatively shaded and cool compared with unused plots, whereas used and unused plots in the forest had the same level of canopy cover and were thermally similar (Supplementary Figs S1 and S4c). Although we demonstrate differences in nest environments between suburban and forest sites, our study was limited to a single suburban and a single forest site, which weakens our ability to make general comparisons between these two types of habitat. Future studies should use multiple replicates of each habitat type and/or make comparisons across natural-urban gradients. It is worth noting, however, that measuring nesting behavior in squamates is uniquely challenging compared with other oviparous reptiles (e.g. birds, turtles) because squamate nesting behavior is comparatively inconspicuous and nests are difficult to locate in the field (Li et al. 2018). This is likely one reason why squamate nesting behavior and egg/nest survival are underrepresented in studies of urbanization.

Consequences of suburban habitat and nest conditions

Contrary to studies showing that female lizards prefer relatively open, warm nest sites in cooler climates (Warner and Shine 2008; Li et al. 2018), our data indicate that females in suburban habitats benefit from nesting in shaded locations. Similar

shade-seeking behavior by nesting females have been previously observed in reptiles that live in warmer environments (Doody et al. 2006; Refsnider and Janzen 2012)—a behavior that our results suggest can also buffer embryos from lethally high temperatures in urbanized areas. In contrast, the homogenous thermal conditions in the forest provide females with an abundance of suitable nesting habitat with relatively little microclimate variation among nest sites. We found that anoles nest close to trees in the suburban site (like in the forest), rather than further away from trees (Fig. 3b). This is expected since the urbanized areas are fragmented and *A. cristatellus* are concentrated in greenspaces with relatively dense canopy cover (Battles and Kolbe 2018). The forest, however, consists of complex vegetation structure that allows lizards to move freely around. Rather than actively choosing more shaded and cooler nest sites in suburban areas; however, females may simply use areas for nesting that are close to the trees they inhabit. Thus, even if nesting behavior itself does not differ between suburban and forest habitats, the consequences of this behavior (e.g. nesting close to trees) can be beneficial for embryo development in suburban environments.

Warmer suburban nest temperatures reduce incubation duration by several days, and this may be beneficial in two ways. First, individuals that hatch relatively early enter environments with relatively low competition and have more time to grow prior to the onset of winter or the dry season (While et al. 2015; Pearson and Warner 2018). Second, the chances of egg depredation or exposure to unfavorable conditions are reduced when embryos develop and hatch quickly (Doody 2011). Egg mortality may be greater in the forest than was observed in our laboratory experiment if eggs take longer to develop and are, therefore, more susceptible to depredation. Field studies of egg survival between urban and forest areas are lacking for non-avian reptiles but would greatly increase our understanding of the consequences of nest-site selection in urbanized environments. Variation in egg mortality due to depredation can be a significant determinant of population density in anoles (Andrews 1988). Hence, the positive effect of warmer urban nest temperatures on development coupled with increased fecundity in urban populations (Hall and Warner 2017) may enhance the spread of anoles through urbanized landscapes (Suzuki-Ohno et al. 2017). However, increased fecundity of urban populations may have associated costs such as decreased immune function and longevity (Lucas and French 2012).

Despite differences in nest conditions between suburban and forest plots, morphological traits examined did not differ. Suburban hatchlings were, however, about 5.8% heavier and 2.1% longer than forest hatchlings regardless of incubation treatment. This difference in hatchling size corresponds with differences in adult body sizes for these sites (Supplementary Fig. S5), which is consistent with past work on these introduced populations (Hall and Warner 2017; but see Thawley et al. 2019). These differences in adult body size could be driven by a variety of factors (e.g. differences in resource availability, habitat-specific selection on body size), but given that offspring from urban eggs hatch at a larger size than those from forest eggs suggests that either maternal or genetic factors contribute to this size variation. Although differences in maternal diet could affect offspring body size (Warner and Lovern 2014), we minimized this source of variation by acclimating females from both populations to a standard diet in captivity. Additionally, the influence of incubation treatment on egg survival and hatchling size did not differ between eggs from suburban vs. forest mothers. This pattern does not support our hypothesis that embryos

are adapted to their local thermal environments, which is also consistent with previous work (Tiatragul et al. 2017). However, egg survival was generally greater for suburban vs. forest females, which could be due to local adaptation or habitat-specific maternal effects (i.e. plasticity); our data cannot distinguish between these mechanisms. Overall, we show that females from the suburban site produce relatively large offspring compared with females in the forest, and increase their egg survival indirectly through nesting behavior.

Urban invasion

Although *A. cristatellus* is abundant in both the suburban and forest habitats, their distribution is much more continuous in the forest at both adult (Battles and Kolbe 2018) and early life stages (Tiatragul et al. 2019). Studies of population density and habitat use in urbanized vs. natural habitats are generally restricted to adults (Lapiedra, Chejanovski, and Kolbe 2017; Winchell et al. 2018a; Battles and Kolbe 2018), and rarely consider the importance of suitable habitat for reproduction and offspring development (French et al. 2018). The distribution of suitable nesting sites in the urban areas might influence the spread of non-native species like *A. cristatellus* (Hall and Warner 2019). Although *A. cristatellus* readily uses a diversity of structures in urban areas (Winchell et al. 2018a; Stroud et al. 2019), females appear to restrict nest sites to relatively shaded areas, and egg survival is high under these conditions. The spread of *A. cristatellus* through urban habitats is associated with dense vegetation and closed canopies (Kolbe et al. 2016); this pattern may be partly explained by the large portions of the urban landscape that are inhospitable to embryo development. Other anole species (e.g. *A. sagrei*) are less confined to cooler green spaces (Kolbe et al. 2016), and their nest temperatures are potentially even warmer (Sanger et al. 2018) than those measured for *A. cristatellus* in this study. Comparative approaches across species with different embryo physiologies and maternal nesting behaviors could provide key insights into the factors that shape the distribution of invasive species across urbanized environments.

Conclusion

Oviparous ectotherms frequently encounter novel habitats due to urbanization, and their responses vary considerably, from morphological adaptation to behavioral shifts. Shifts in behavior and physiology are thought to enable many taxa to cope with changing environments (Buckley, Ehrenberger, and Angilletta 2015; Kralj-Fišer, Hebets, and Kuntner. 2017; Miranda 2017). The results from our study support the hypothesis that maternal nesting behavior by the invasive *A. cristatellus* in the suburban area positively affects embryo survival. This behavior shields embryos from suboptimal thermal environments in urbanized areas and may partly explain why embryos have not physiologically adapted to suburban thermal conditions; this finding is similar to the view that adult thermoregulatory behaviors inhibit evolutionary change in thermal physiology (see Huey, Hertz, and Sinervo 2003) except that, in our case, the behavior is maternal nesting. Intriguingly, nesting strategies of *A. cristatellus* that likely evolved in forest habitats may be adaptive in urbanized areas. In many cases, human-modified habitats present an 'ecological trap' (Kolbe and Janzen 2002) and can reduce fitness, but urbanized environments may enhance reproduction in *A. cristatellus*. Such benefits may be widespread in taxa that exploit urbanized environments and nesting

preferences of parents and physiological tolerances of embryos likely play an important role in the distribution and spread of introduced species. Studies that incorporate reproductive behaviors and embryo development will provide a more complete understanding of adaptation to novel environments and biological invasion in urban habitats.

Supplementary data

Supplementary data are available at JUECOL online.

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Data accessibility

Data set associated with analyses in this research article is available from Zenodo data repository: Tiatragul, S., Hall, J. M., and Warner, D. A. (2019) 'Data Set for Nestled in the City Heat: Urban Nesting Behavior Enhances Embryo Development of an Invasive Lizard', <http://doi.org/10.5281/zenodo.2577816>.

Ethical note

This research was conducted with approval by the Auburn University IACUC (No. 2018-3233).

Conflict of interest statement. None declared.

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