

# Lizard nest environments differ between suburban and forest habitats

SARIN TIATRAGUL<sup>1,\*</sup>, JOSHUA M. HALL<sup>1</sup>, NATHANIEL G. PAVLIK<sup>2</sup> and DANIEL A. WARNER<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

<sup>2</sup>Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

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Nesting success is critical for oviparous species to maintain viable populations. Many species often do not provide parental care (e.g. oviparous reptiles), so embryos are left to develop in the prevailing conditions of the nest. For species that occupy diverse habitats, embryos must be able to complete development across a broad range of environmental conditions. Although much research has investigated how environmental conditions influence embryo development, we know little about how nest conditions differ between diverse habitats. *Anolis* lizards are commonly found in various habitats including those heavily modified by humans (e.g. cities). We describe nest sites of anoles in two different habitat types: a suburban area and a nearby forest. The suburban area had less total nesting habitat but a greater variety of microenvironment conditions for females to use for nesting, compared to the forest. Suburban nests were warmer and drier with greater thermal variance compared to forest nests. Finally, we use data from the literature to predict how nest conditions may influence development. Our study provides the first quantitative assessment of anole nest sites in human-modified environments and shows how suburban habitats may generate variation in developmental rate.

ADDITIONAL KEYWORDS: *Anolis* – behaviour – nesting ecology – plasticity – urban.

## INTRODUCTION

Nesting is a critical component of fitness for oviparous organisms because females must select nest sites that facilitate successful development of offspring (Hansell, 2005). Because extended exposure of embryos to unfavourable 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 behaviour is crucial for reproductive success and population persistence (Telemeco *et al.*, 2009; Mainwaring *et al.*, 2017; Campbell *et al.*, 2018).

For oviparous species that do not provide 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 the prevailing environment (Telemeco *et al.*, 2009). Maternal choice of nest microenvironment (e.g. temperature, canopy cover, substrate, moisture) can influence egg hatching success (Deeming & Ferguson, 1991), 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 behaviour 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). Several studies provide evidence that the non-random use of microhabitats by nesting females is adaptive (Brown & Shine, 2004; Reedy *et al.*, 2013; Li *et al.*, 2018).

Anoles have been models for studies of ecology, evolution, and behaviour (Sanger *et al.*, 2008; Losos, 2009; Mitchell *et al.*, 2018b) making them ideal for research on adaptation to rapidly changing

\*Corresponding author. E-mail: [stiatragul@auburn.edu](mailto:stiatragul@auburn.edu)

environments (e.g. urbanization; Kolbe *et al.*, 2012, 2016, Winchell *et al.*, 2016; Donihue *et al.*, 2018). This group of lizards also has the potential to serve as a model for studies of nesting behaviour in anthropogenically disturbed environments even though little is currently known about the specific microhabitats that females choose for nesting in the field (Rand, 1967; Andrews, 1982). Laboratory research shows that conditions chosen by females have consequences for offspring fitness (Socci *et al.*, 2005; Reedy *et al.*, 2013; Pearson & Warner, 2016). Moreover, laboratory-based information about the effects of egg incubation conditions on egg survival and phenotypic development in anoles is accumulating (Supporting Information, Table S1). Results from these laboratory studies are insightful, as they help direct predictions about optimal microhabitats that females should choose for nesting in different environments. Information about nest microenvironments in the field will be important in understanding how embryos might respond to human-altered habitats.

We have two primary objectives for this study. The first is to characterize nest microenvironments of three non-native and one native species of *Anolis* lizards within two different habitats: a suburban area and an adjacent forest. Our approach is unique compared to existing field studies of *Anolis* nest conditions in that we made quantitative measurements of microhabitat variables, rather than describing general microhabitats of locations where eggs were found (e.g. under leaf litter, in tree hole; Sexton *et al.*, 1963; Andrews, 1982; Schlaepfer, 2003; Robinson *et al.*, 2014). To do this, we systematically searched for anole nests in suburban and forest locations and collected microenvironmental data (e.g. temperature, shade cover, soil moisture). Due to the urban heat-island effect, we predict that nest conditions will be warmer and more variable in suburban areas compared to forested areas. Our second objective is to summarize published information from laboratory egg incubation studies of *Anolis* lizards to provide insights into the consequences of maternal nesting behaviour between diverse habitats. Based on microhabitat data of nests coupled with information from the literature, we discuss how developmental rate and egg hatching success may differ between suburban and forest habitats.

## MATERIAL AND METHODS

### STUDY SITES

Our study areas consisted of a suburban green space and a nearby forest located 1.2 km from each other in Miami-Dade County, Florida, USA. The two sites are separated by two-lane roads, a residential area and a canal, and differ substantially in surrounding habitat

structure (Fig. 1; Supporting Information, Fig. S1). The suburban site is located along a 1-km stretch of land between a two-lane road (State Road 959, locally known as West 57<sup>th</sup> Avenue or 'Red Road') and Snapper Creek canal, which runs along the edge of a suburban neighbourhood of Pinecrest Village (Supporting Information, Fig. S1). Parallel to the road is a paved bicycle trail that is frequently used by pedestrians and cyclists. The bicycle trail is flanked by a regularly mowed lawn next to the road, and by an unkept lawn (i.e. thick leaf litter) along the canal. Fig trees (e.g. *Ficus citrifolia*, *F. aurea*) and palm trees are scattered along the lawn next to the canal. This area also has many human-made structures such as stone-walls, lamp posts, small bus stops and guard rails (Fig. 1A).

The nearby forest site is in the interior of Matheson Hammock Preserve, which is accessible via walking trails (Fig. 1B). The preserve is a fragment (0.21 km<sup>2</sup>) of rockland hammock consisting of mature canopy trees (e.g. *Bursera simaruba*, *Coccoloba diversifolia*, *Quercus virginiana*, *F. aurea*) with interspersed smaller shrubs (e.g. *Eugenia* spp., *Psychotria*



**Figure 1.** Representative photographs of our study sites in Miami-Dade County, FL. A, section of the suburban site featuring the road, bike path and canal (GPS coordinates: 25°40'53.60"N, 80°17'02.96"W). B, section of a study site in the forest (GPS coordinates: 25°40'54.93"N, 80°16'29.52"W).

*nervosa*; FNAI, 2010). The ground consists of karst and oolitic limestone with dense leaf litter covering a thin layer of highly organic soil. The preserve interior contains no human-made structures and is visited less frequently by humans than the suburban site (S. Tiatragul, pers. obs.).

#### NEST SEARCH PROTOCOLS

Five species of anoles are established at our 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*. We are 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 our plots, and *A. sagrei* was never sighted. In contrast, suburban plots contained all species. Thus, we cannot definitively determine the species of each egg, and henceforth, we use the general term anole (see Supporting Information, Table S2, for visual encounter survey data). Importantly, because *Anolis* lizards have similar nesting requirements in the laboratory (Sanger *et al.*, 2008a) and patterns of development appear highly conserved across species (Sanger *et al.*, 2008b), our data are useful given the general scarcity of information on anole nest conditions in the field.

To quantify microenvironments used by nesting females, we established four searchable blocks in each site that consisted of a chosen central tree where anoles were present (Supporting Information, Fig. S1; Table S2). Each tree was circumscribed by a circular area with a radius of 20 m (i.e. block). We randomly selected ten 1-m<sup>2</sup> plots within each block ( $N = 40$  suburban 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 and 20 m) and direction (between 0 and 359° from north) from each central tree by using a random number generator. If the randomized direction and distance (measured with a compass and measuring tape) landed in an area obviously unsuitable for nesting (e.g. asphalt, pavement, body of water, rock face), then we randomized a new location until the plot landed in a searchable area; the number of times we randomized for new plots was recorded to account for the availability of nesting locations between sites. Once we located a searchable area, we laid out a 1-m<sup>2</sup> quadrat (= plot) and searched the plot for hatched or unhatched eggs by removing all ground cover debris (e.g. leaf litter, logs, small rocks). Hatched eggshells probably represent a 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, pers. obs.). If the soil

was loose, we ran our fingers or spoons 1–3 cm from the soil surface to locate any buried eggs. For each egg found, we marked the exact location with a labelled flagging tape. We considered the location of each egg to be a nest because anoles lay single-egg clutches. Hence, there may be multiple nests within a single plot. With the above protocol, we found a total of 94 nests in 47 plots ( $N = 36$  forest;  $N = 11$  suburban).

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

#### MICROENVIRONMENT MEASUREMENTS

For each plot, we recorded hourly temperatures between 3 July and 15 August 2017 ( $N = 43$  days) with a Thermochron iButton (programmed to record hourly) wrapped in a water balloon and Parafilm ( $N = 62$  iButtons deployed in plots with nests). Because some plots contained more than one nest, we placed the iButton next to the nest with an unhatched egg. If the plot contained only nests with hatched eggs, we placed the iButton next to a random nest with a hatched egg in that plot. Hemispherical view photographs were taken above each plot using a Canon Powershot Elph 180 with a 180° fisheye lens. We calculated per cent canopy openness using Gap Light Analysis (v.2.0) software on hemispherical view photographs (Frazer *et al.*, 1999; Doody *et al.*, 2006).

Weekly substrate moisture was measured between 2 July and 18 August 2017 ( $N = 8$  weeks). To calculate substrate (soil and organic material) moisture throughout the study period, we collected weekly substrate samples (5 cm<sup>3</sup>) from each plot within 15 cm from the iButton to minimize disturbance of the temperature profile; thus, the microhabitat where substrate samples were collected was not noticeably different from the microhabitat where the iButton was located. Substrate samples were collected with a spoon ~1 cm into the ground (due to shallow layer of soil at 1–3 cm depth) and kept in a 5-mL capped

tube. We weighed each tube within 3 h of collection and dried the sample in an incubator (set at 60 °C) with the tube cap open. Dry mass was recorded after three consecutive days of weighing the tube to the nearest 0.01 g with no change in mass. Per cent 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; Geneva 2015; Fetters & McGlothlin, 2017; Tiatragul *et al.*, 2017).

For each nest, we 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 the road (at suburban plots only).

#### LITERATURE SEARCH AND DATA COLLECTION

We 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. We then performed another search with TITLE: (anol\*) AND TOPIC: (incubat\*) AND TOPIC: (temperature). We also requested data from individuals who study the common anole species at our field site. Our search returned 27 unique papers, of which 22 concerned anoles. Sixteen papers were not considered for our 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 nine unique datasets.

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

#### STATISTICAL ANALYSIS

Data analyses were performed in R (R Core Team, 2017). The availability of nest microhabitat at each site was calculated as the number of suitable nest plots (e.g. those not on a road, pavement 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. We calculated the daily average temperature mean, minimum, maximum and variance recorded by each iButton across all 43 days of the study. We also calculated substrate moisture mean, maximum, minimum and variance for each plot based on eight weekly samples collected throughout the study.

Because temperature and moisture data were correlated, we used principal component analyses (PCAs) to collapse the variation into uncorrelated axes using the *FactorMineR* package (Lê *et al.*, 2008). For the PCA, we 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% and 8% individually) (Table 1). The loading scores for PC1 indicated a negative correlation between temperature and moisture.

We used mixed-effects linear models to estimate nest microenvironment differences between sites (suburban vs. forested). Dependent variables (analysed in

**Table 1.** Loading scores on each principal component (PC) axis. Values in bold type represent traits that best represented the PC axes.

	PC axis 1	PC axis 2	PC axis 3	PC axis 4
Temperature (°C)				
Mean	<b>0.83213409</b>	-0.42079051	-0.11648997	-0.074062616
Maximum	<b>0.80728694</b>	-0.41764592	-0.20950277	-0.196018443
Minimum	0.04333977	-0.17074757	-0.86675376	0.458766800
Variance	<b>0.79331455</b>	-0.3441063	-0.07052266	<b>-0.345746725</b>
Soil moisture (%)				
Mean	<b>-0.7954266</b>	-0.28618894	-0.26721962	-0.286467206
Maximum	<b>-0.5925537</b>	-0.68895094	0.10950809	0.007670361
Minimum	<b>-0.7836874</b>	0.05969727	<b>-0.4059627</b>	-0.398672335
Variance	-0.2253097	-0.83640261	<b>0.34143124</b>	0.246975616
Proportion of variance explained	49.5%	21.9%	14.7%	8.4%

separate models) included canopy openness, distance to the closest tree, distance to the second closest tree, PC1 scores, daily temperature parameters and weekly moisture parameters. We 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. Another GLMM with a Poisson distribution was used to analyse the number of nests per plot. For all models, we included block as a random effect.

We 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). However, we did 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. Four iButtons in plots with nests ( $N = 2$  forest;  $N = 2$  suburban) failed during the study and were not included in the analysis. We also excluded another iButton from one nest in the suburban site from the analysis because the egg was suspended in grass at the base of a tree.

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

## RESULTS

### GENERAL DESCRIPTIONS OF NESTS

All randomized plots in the forest were suitable for nesting compared to 64% in the suburban 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 suburban site (Supporting Information, Table S3). Considering only plots from random searches, we found ten unhatched ( $N = 7$  suburban;  $N = 3$  forest) and 84 hatched eggs ( $N = 30$  suburban;  $N = 54$  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 2). The probability of finding a nest from random searches was 7.9 [95% confidence limit (CL) 1.6–53.9] times as likely in the forest than in the suburban area ( $\chi^2 = 5.5$ ; d.f. = 1;  $P = 0.019$ ); however, nest density was greater in the suburban site: suburban plots had 1.6 times as many nests per plot (95% CL 1.0–2.4) as the forest ( $\chi^2 = 4.28$ ; d.f. = 1;  $P = 0.04$ ).

Suburban nests had  $12.7 \pm 3.09\%$  (mean  $\pm$  SE, here and below) less shade cover than forest nests ( $F_{1,11} = 16.87$ ;  $P = 0.002$ ; Fig. 2; Table 3). 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:  $\beta = 4.72 \pm 2.54$  m;  $F_{1,11} = 3.45$ ;  $P = 0.09$ ). The size (i.e. circumference) of the closest tree at the suburban site was  $0.93 \pm 0.29$  m greater than those in the forest ( $F_{1,11} = 10.47$ ;  $P = 0.008$ ), and this pattern was similar for the second closest tree ( $\beta = 0.80 \pm 0.18$  m;  $F_{1,11} = 20.55$ ;  $P = 0.001$ ; Fig. S2; Table 3).

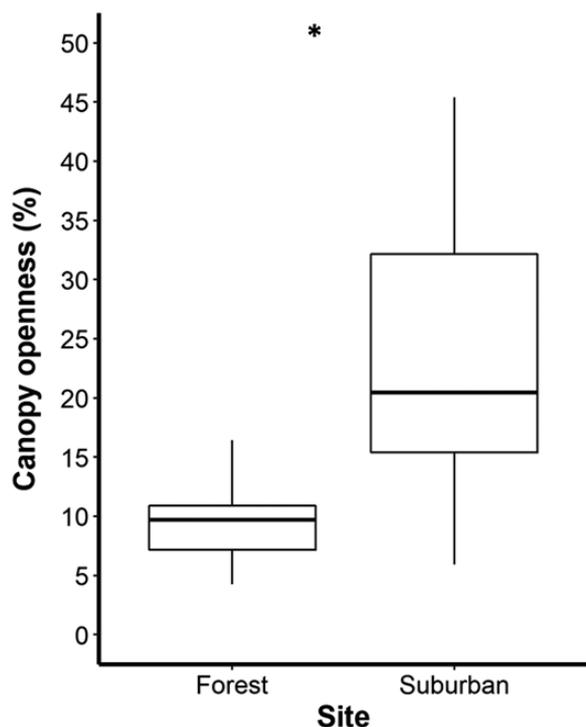
### TEMPERATURE AND MOISTURE

Nest temperatures were higher at the suburban site than at the forested site (Table 3; Supporting Information, Table S4). The mean temperatures of the coolest and warmest nests for the suburban area (27–30 °C) and the forest (26–27 °C) slightly overlapped. Mean daily temperatures in suburban nests were  $1.6 \pm 0.2$  °C higher than the forest nests ( $F_{1,10} = 41.6$ ;  $P < 0.001$ ), and daily maximum temperature in the suburban site was  $4.5 \pm 1.1$  °C higher than the forest nests ( $F_{1,10} = 17.2$ ;  $P = 0.002$ ). Average minimum temperature did not differ between the two sites ( $\beta = 0.2 \pm 0.2$  °C,  $F_{1,10} = 1.0$ ,  $P = 0.33$ ). Suburban nests showed  $1.3 \pm 0.5$  °C greater thermal variance than forest nests ( $F_{1,12} = 6.9$ ;  $P = 0.026$ ; Fig. 3).

Weekly mean, maximum and minimum substrate moisture in forest plots were  $3.42 \pm 0.93\%$  ( $F_{1,11} = 13.16$ ;  $P = 0.004$ ),  $3.28 \pm 1.31\%$  ( $F_{1,11} = 6.22$ ;  $P = 0.030$ ) and  $3.11 \pm 0.76\%$  ( $F_{1,11} = 16.58$ ;  $P = 0.0018$ ) greater than the plots in the suburban site, respectively. However,

**Table 2.** Description of nest locations for all plots (random and targeted). Data are provided for each site (suburban vs. forest) and for both sites combined. Debris is the combination of leaf litter, sticks, 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



**Figure 2.** Boxplots of canopy openness for nests between suburban and forest sites. The boxes indicate 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 ( $P < 0.05$ ).

the variance in substrate moisture did not differ between the two sites ( $\beta = 1.11 \pm 2.99\%$ ;  $F_{1,11} = 0.14$ ;  $P = 0.72$ ). The scores from PC1 indicated that nests in the suburban site were warmer, drier and had greater thermal variance compared to nests in the forest ( $\beta = 3.2 \pm 0.5$ ;  $F_{1,11} = 38.03$ ;  $P < 0.001$ ; Fig. 4).

#### INCUBATION TEMPERATURES FROM THE LITERATURE

Of the temperatures used in studies of egg incubation for the common anole species at our study site, most (80%) were within the range of mean nest temperatures measured in our study (26–30 °C) (Fig. 5). The range of nest temperatures measured at our sites have no apparent effect on survival ( $\beta = 2.3 \pm 2.5$ ;  $P = 0.36$ ); however, we estimated that incubation period decreases by  $2.1 \pm 0.4$  days for each 1 °C increase in mean nest temperature ( $P < 0.001$ ; Fig. 5). For context, eggs in the suburban site would hatch 4–12 days earlier than eggs incubated in forest nests. Moreover, due to a wider range of mean nest temperatures in the suburban habitat, the range of developmental rates is approximately three times greater in the suburban site than in the forest (Fig. 5).

## DISCUSSION

The primary goals of this study were to describe the characteristics of nests of *Anolis* lizards, determine how they differ between two diverse habitats (suburban vs. forest sites) and predict potential consequences of nest microenvironments on development based on results from previously published egg incubation studies. Nests in suburban areas differed substantially from nests in forested areas in distribution, shade cover, temperature and moisture. These differences in nest characteristics were probably a function of the overall habitat differences between our study sites and will probably 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. This study differs considerably from existing qualitative descriptions of nest habitat (e.g. Andrews, 1982) and fills gaps in our understanding of this otherwise well-studied group of vertebrates.

#### DISTRIBUTION OF NESTS

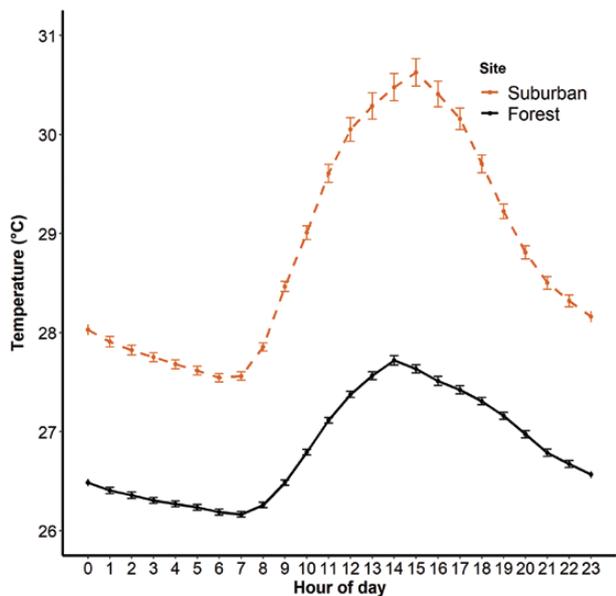
Based on random sampling, we found more nests in the forest than in the suburban site. However, the suburban site contained more nests per plot, suggesting that suburban females may clump nests (possibly due to a lack of high-quality nesting habitat; e.g. roads, pavement). Nesting aggregations or communal nesting have been described in many oviparous animals (Doody *et al.*, 2006; in anoles, see Robinson *et al.*, 2014; Godfrey *et al.*, 2018) and may be more common in suburban areas due to reduced availability of high-quality nesting habitat (Radder & Shine, 2007). For example, sea turtles (*Caretta caretta*) that nest in urban areas clump their nests in areas with conditions (e.g. low light) that might benefit their newly hatched offspring (Witherington & Bjørndal, 1991; Salmon *et al.*, 1995). However, egg aggregations may also be disadvantageous because fungal infection can easily spread due to the close proximity of eggs (Warner & Chapman, 2011). Alternatively, it is also possible that a single female may repeatedly put multiple nests in the same location.

Increased nest clumping in suburban habitats may reflect differences in the distribution and density of adult lizards between the suburban and forest sites (Supporting Information, Table S2). This would explain why the likelihood of locating a nest was greater in the forest compared to the suburban site. In the suburban site, anoles were usually found concentrated on large trees or disjunct patches of vegetation, with many metres of unoccupied area between occupied spaces. Our data indicated that lizards in the suburban site nest close to trees as in the forest (Table 3). The

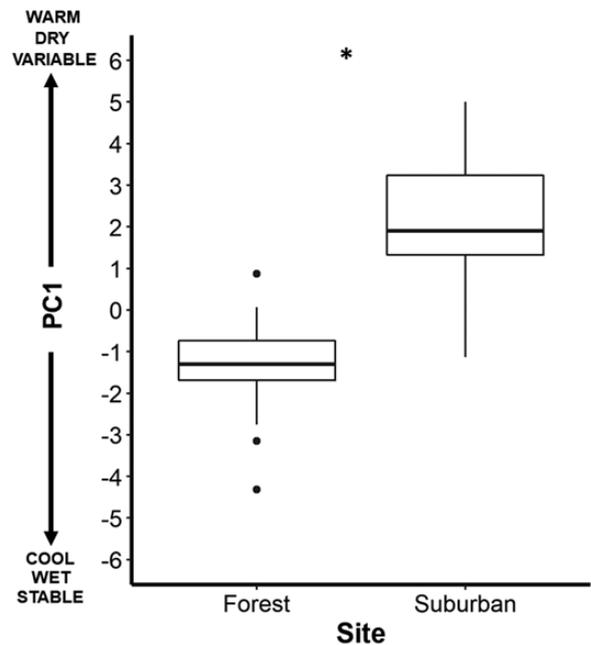
**Table 3.** Quantitative descriptions of nests in forest and suburban habitats. Nest sites were found by using both random and targeted searches in each habitat (see text for details). CBH, circumference at breast height (m).

Variables	Forest					Suburban				
	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
Distance (m) to:†										
closest tree	62	0.37	0.00	0.95	0.06	47	1.02	0.00	3.66	0.85
2 <sup>nd</sup> 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 <sup>nd</sup> 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	21	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	0.9	43	30.8	26.5	39.5	4.7
Daily mean minimum‡	43	25.9	22.0	27.5	1.1	43	27.0	22.0	30.0	2.0
Daily mean variance‡	43	0.6	0.0	5.0	0.3	43	1.6	0.00	11.5	3.6
Moisture (%)*	35	10.1	2.0	31.4	15.0	22	6.8	0.2	26.5	15.9
Weekly mean‡	8	10.1	5.3	14.3	4.7	8	6.8	3.0	11.5	6.4
Weekly maximum‡	8	15.1	7.9	31.4	16.0	8	11.8	5.7	26.5	22.0
Weekly minimum‡	8	5.6	2.0	9.8	3.4	8	2.6	0.2	9.0	5.3
Weekly variance‡	8	12.0	2.6	60.7	112.0	8	10.8	1.7	54.4	126.0

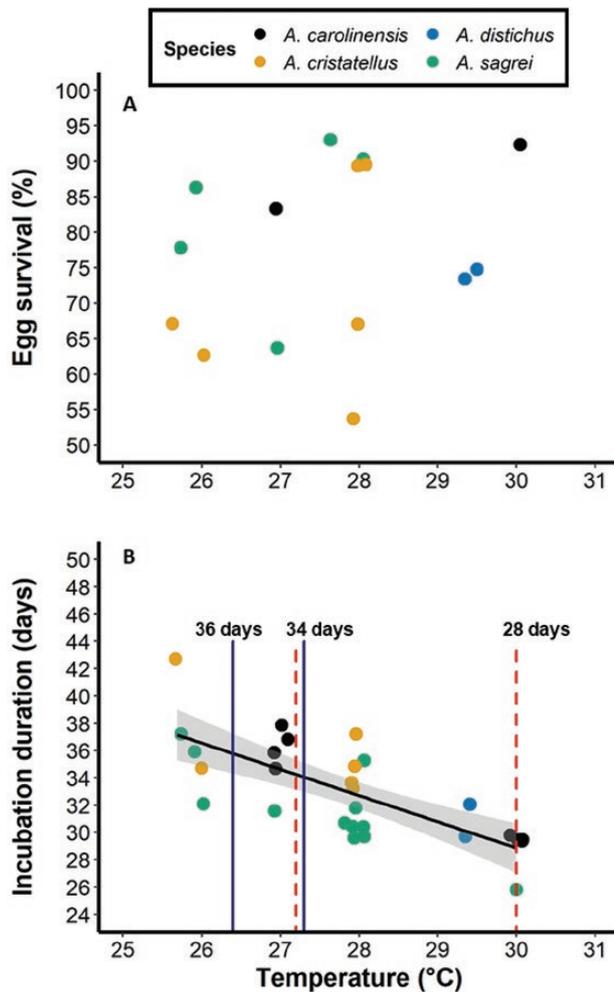
\*Sample size represents number of iButtons or plots.  
 †Sample size represents number of eggs from which trees were measured.  
 ‡Sample size represents number of days for temperature and number of weeks for moisture.



**Figure 3.** Mean daily temperature fluctuations for each site. On average, nest temperatures peaked at 1300 h. Error bars show ± 95% confidence interval.



**Figure 4.** Comparison of nest conditions in suburban vs. 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 5.** The proportion of egg survival (A) and mean incubation duration (B) at nest temperatures for common *Anolis* species at our study sites (below 25 °C not shown). Data were extracted from published studies or personal communications (Supporting Information, Table S1). The grey shading around the regression line (black) represents the 95% confidence interval. Reference solid vertical lines (blue = forest) and dashed vertical lines (red = suburban) represent mean temperatures for the coolest (26.4 °C) and warmest (30 °C) nests for each site. Numbers above vertical lines show estimated incubation duration at that temperature.

habitat fragmentation that typifies suburban areas probably restricts females from nesting relatively far from trees. Conversely, the usable habitat in the forest is continuous with complex structures and allows lizards to move freely. 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).

#### GENERAL NEST CHARACTERISTICS

In the laboratory, females typically nest 2–5 cm below the soil surface, even when surface structure (e.g. a leaf) is provided (Propper *et al.*, 1991; Sanger, 2008; J. M. Hall, pers. obs., but see Socci *et al.*, 2005). However, we found most nests to be only partially buried beneath surface cover or fully buried to between 1 and 3 cm in the substrate layer underneath surface debris in the suburban site. More than half of the nests in the forest were found exposed on the ground surface with little substrate cover. This is probably due to the shallow soil layer (often ~1 cm) over a rocky surface within Matheson Hammock Preserve. The suburban site had some areas with soil deeper than 5–7 cm, but we 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. Due to direct exposure to the atmosphere, water vapour conductance of partially buried eggs differs from that of completely buried eggs (Ackerman *et al.*, 1985; Deeming, 2004) and this difference in water availability for embryos may influence offspring phenotypes (Packard *et al.*, 1988; Brown & Shine, 2018).

All nests except for those completely buried were found under some surface debris. In the suburban site, nests were found in locations with high abundance of surface debris and unkept lawn (i.e. receives no landscaping maintenance). Our 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).

Substrate moisture content in nests was greater at the forest site than at the suburban site, but the average difference of ~2–5% may not be biologically significant considering the high precipitation and relative humidity of Miami-Dade County during the nesting season. Thus, both habitats probably contained moisture levels suitable for development. Substrate moisture of nests at our sites never exceeded 32% during the study period (even with occasional heavy rain), whereas *A. sagrei* females select nest sites with >50% soil moisture content in the laboratory (Reedy *et al.*, 2013). 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. This difference in field and laboratory results may be explained by the substrate available; field samples consisted of

surface debris (roots, leaves, shells, etc.), which differ in water-holding properties from potting soil (designed to hold moisture) used in the laboratory study (Reedy *et al.*, 2013). Moreover, we observed that many nests in our study were not completely buried and may be influenced more by relative humidity in the air than by substrate moisture. We suggest that future egg incubation experiments should consider both the influence of relative humidity of the microenvironment as well as substrate water potential for species that do not completely bury their eggs.

Nests in suburban areas were warmer and experienced greater thermal variation than those in the forest. The forest contained homogeneous, extensive canopy cover, which generates a relatively stable microclimate on the ground. Eggs could therefore successfully incubate in shallow or exposed nest locations. In contrast, the suburban site is patchy with large areas of open canopy and a relatively unstable microclimate. The heterogeneity with respect to temperature and shade in the suburban site provides females with more opportunities to select among nesting conditions compared to the forest. Thus, nest site selection in suburban sites can have a greater range of consequences on embryonic development; for instance, nest temperatures occasionally exceed 36 °C in the suburban habitat, whereas temperatures are less variable and less extreme in the forest (Table 3). Even short-term heat stress at this temperature can reduce egg survival and retard embryonic development in *A. sagrei* (Sanger *et al.*, 2018).

#### POTENTIAL CONSEQUENCE OF NEST CHARACTERISTICS ON DEVELOPMENT

Egg incubation studies provide insight into the potential consequences of nest environments on embryo development and survival. The thermal heterogeneity in suburban environments may generate greater variation in egg survival and incubation duration than the homogeneous thermal conditions of the forest (Supporting Information, Tables S1 and S4). Variability in the thermal environments of nests in the suburban site resulted in brief exposure to 39.5 °C (Table 3), which could reduce embryo survival and influence developmental rate (Hall & Warner, 2018). However, based on published literature for the four common anole species at our study sites, the mean thermal environments (not accounting for brief thermal extremes) in suburban and forest habitats may not lead to much variation in egg survival (Fig. 5).

We predict that, on average, embryos will hatch 4–12 days earlier in the suburban habitat than in the forest. This could be beneficial if decreased incubation duration reduces the time that embryos are exposed to predators or potentially harmful environmental

conditions. Egg depredation is a primary determinant of population densities for some tropical anole species (Andrews, 1982; Chalcraft & Andrews, 1999). Accordingly, habitat differences in egg survival may contribute to variation in adult density between suburban and forested habitats. Moreover, the wider range of nest temperatures in suburban habitats must result in greater variation in incubation duration than in the forest. Thus, embryos that develop in suburban nests 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.*, 2018a).

One caveat to most laboratory studies is that they do not simulate the complex nest environments that exist in nature (Carter *et al.*, 2018; Sanger *et al.*, 2018; While *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, 2018; Supporting Information, Table S1). 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 our study. 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 microhabitats are available for many reptiles (e.g. Janzen & Morjan, 2001; Brown & Shine, 2004; Doody *et al.*, 2006; Warner & Shine, 2008), but such information is lacking for anoles. Moreover, most studies of egg incubation conditions in reptiles focus on temperature and comparatively fewer explore other variables such as moisture or substrate (Warner *et al.*, 2018). Thus, perceptions of the consequences of nest microhabitat are somewhat restricted to assumptions about the effect of temperature. Hence, observational studies, such as ours, are valuable for informing future hypothesis-driven experiments.

#### CONCLUSIONS

Nesting behaviour is a critical component of reproduction in oviparous reptiles and has profound consequences for fitness. The nest microhabitat 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 behaviour and developmental environments may be modified by aspects of global change (e.g. urbanization). We demonstrated that anole nests in suburban habitat are less shaded, warmer, drier and more thermally variable than nests in forest habitat, and that these

differences have the potential to generate variation in embryo development. This variation largely reflects greater thermal heterogeneity of suburban areas relative to that of the densely forested habitat. Studies like ours that focus on nest microhabitats in suburban vs. forest habitat offer valuable insights into how organisms respond to human-induced environmental changes at early life-history stages that are sensitive to environmental variation.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

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

**Table S2.** Number of total individual *Anolis* lizards observed during four visual encounter surveys on a 25-m transect of each searching block. The number of females/unsexed juveniles is given in parentheses. Visual encounter surveys were performed by walking slowly (~10 min) one way on the 25-m transect and counting every lizard visible within 10 m from the transect. Surveys were repeated four times throughout the study period. Blocks 7 and 8 are in adjacent areas so only block 8 was not surveyed.

**Table S3.** Summary of eggs found in each plot type through random and targeted searches at both sites. Numbers under the 'Nests' column indicate the number of plots with a nest present, while numbers under the 'No nest' column indicate plots that did not have nest.

**Table S4.** Effect of site (forest vs. suburban) on microenvironment variables. The effect size and standard error represents means for suburban minus forest for each variable.

**Figure S1.** Google Earth image with polygons indicating the area of the suburban (purple) and forest site (light blue) in Miami-Dade County, FL (GPS coordinates: 25°40'49.48"N; 80°16'46.71"W). The approximate location of the random blocks is shown (urban = blue pins; forest = green pins), while the targeted blocks are not shown. The scale and direction are shown in the bottom right corner. The two sites are separated by a suburban neighbourhood and Snapper Creek canal.

**Figure S2.** Boxplots of (A) distance of the closest tree to the plot, (B) distance of the second closest tree to nests, (C) average size of the closest trees to nests, and (D) average size of the second closest tree to nests. The box indicates interquartile range with median as the bold line. The upper and lower extended lines show maximum and minimum values, respectively. A single asterisk indicates a significant difference between groups ( $P < 0.05$ ).