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Beating the Heat: nest characteristics of anoles across suburban and forest habitats in South Miami

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One would have to try very hard to walk down the suburban areas of Miami and not notice the abundance and diversity of anoles! Because these conspicuous lizards are literally everywhere, people rarely stop and observe their charismatic behaviors. But those that do take notice readily see that their social lives are highly complex. Indeed, these lizards are constantly patrolling territories, communicating with each other, and darting at various prey. But one aspect of their biology that is much less conspicuous is their nesting behavior. Even biologists that have devoted countless hours studying their behaviors know very little about where females lay eggs, what microhabitats they prefer, and how those nesting behaviors impact embryonic development.

Anoles occupy diverse environments from dense tropical forests to small arid islands, which poses challenges to nesting females. In addition, as more natural areas are converted into urban areas like in Miami, some species of anoles have found themselves in heavily modified habitats. Urbanized areas are hotter than the forest due to less canopy cover, more impervious surfaces, and heat retaining materials (e.g. concrete). For oviparous ectotherms that lack parental care like anoles, eggs are left to the mercy of prevailing environments (because they cannot move away to find better spots). Hotter temperatures in urban areas due to the urban heat island effect presents a challenge for developing embryos. Yet, anoles are very common in South Miami, with crested anoles (*Anolis cristatellus*) and brown anoles (*Anolis sagrei*) among the two most common species. While previous studies have shown that these species can withstand putative nest temperatures in urban areas (Tiatragul et al. 2017), we still lack data from real nest sites in the wild. Apart from a description of crested anole communal nesting behavior by Stan Rand (1967), no formal study has been conducted to describe the nest sites of crested or brown anoles.

As part of research for a MS degree at Auburn University (for Sarin Tiatragul), we designed an ambitious study that involved searching for crested anole nests in a suburban area ("Red Road" along Snapper Creek and Pinecrest Neighborhood) and a nearby forest (Matheson Hammock Preserve) in South Miami during the peak breeding season (between June and

August). With assistance from an undergraduate colleague (Nathanial Pavlik, University of New Mexico), we randomly sampled plots ($1m^2$) at both sites in search of eggs (Fig. 1).

Figure 1. A random $1m^2$ quadrat laid on the ground to demarcate where to search for nests. Microenvironment variable data were collected from each plot.

We recorded microenvironmental variables (shade cover, distance to closest tree, temperature, and substrate moisture) for every plot, whether it contained an egg or not. We then compared the microenvironment variables between plots that contained eggs (n=22 suburban; 36 forest) to those that did not have an egg (n=29 suburban; 20 forest) as a way to quantify the microhabitat that females choose for nesting. The location of each egg is considered a nest since anoles lay single egg clutches (Fig. 2).

Our data indicated that plots in the suburban site were approximately 13% less shaded than the forest. Plots with nests are usually found close to trees, perhaps indicating that anoles do not venture far to lay eggs on the ground. Mean nest temperatures in the suburban site was 28.4°C compared to 26.8°C in the forest. While maximum temperature reached by a nest in the suburban area was 39.5°C compared to 33.0°C in the forest, there was no difference between the minimum temperature. Temperatures that reach as high as some sites in the suburban area have previously been shown to reduce hatching success in anoles (Hall & Warner 2018; Sanger et al. 2018). Substrate moisture in the suburban site is about 3.4% drier than the forest, possibly due to its negative correlation with temperature and canopy openness (see table 1).

Figure 2. An anole egg found in the suburban site.

Some plots had multiple eggs in close proximity to each other (<50cm apart), particularly in the suburban habitat; this "clustering" of eggs may represent communal nesting, or may be explained by females exhibiting nest-site fidelity, or by different females using preferred microhabitat cues that are limited in suburban areas. These alternative explanations for "nest clustering" warrant further investigation. Furthermore, it is important to note that we only searched the ground for nests. There are multiple accounts (Sexton et al. 1964; Rand 1967; Andrews 1982) of eggs being found above ground (e.g., tree holes) or in areas that might have been excluded by our search protocols. We encourage those who find eggs to document their observations and report it to the community via the Anole Annals blog (or any other appropriate medium).

Table 1. Selected quantitative descriptions of nests between two sites in South Miami.

	Forest				Suburban			
	Mean	Max.	Min.	Var.	Mean.	Max.	Min.	Var.
Canopy openness (%)	9.45	16.42	4.25	11.55	22.77	45.40	5.94	121.70
Distance to closest tree (m)	0.37	0.95	0.00	0.06	1.02	3.66	0.00	0.85
Tree Size (m)	0.26	2.44	0.01	0.15	1.17	3.58	0.02	1.32
Temperature (°C)	26.8	33.0	22.0	0.9	28.4	39.5	22.0	2.4
Substrate moisture (%)	10.0	31.4	2.0	15.0	6.8	26.5	0.2	15.9

This work provides a rare quantitative assessment of anole nesting habitat. Although we do not know the species for each egg found in this study, we suspect that most are from *Anolis cristatellus* due to its relatively high abundance at the field sites compared to other anoles. Generating data on nesting behavior and egg microhabitats in the wild is challenging due to a variety of reasons (e.g., relatively small size/speed of anoles, lack of conspicuous digging marks on the ground as seen in other reptiles). This is probably why very few studies have focused on this topic, and why ours is one of the first to quantify nest sites in the wild. We hope the data we present here will be useful in designing ecologically relevant experiments, which is important considering the roles anoles have played as model organisms in ecology and evolution. Future studies from our group (Warner Laboratory at Auburn University) will focus on the consequences of maternally-selected nest sites compared to random areas in urban habitats. Using programmable incubators, we are currently incubating eggs under temperature regimes that mimic natural nest fluctuations and those in urban areas not chosen by female anoles. If the maternally-selected nest conditions yield high hatching success of eggs (relative to those exposed to conditions that females do not choose), maternal nesting behavior may be a major factor that help embryos beat the city heat and facilitate urban establishment of these lizards.

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