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The Effects of Urbanization on Performance, Habitat Selection, and Persistence of Anolis Lizards

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THE EFFECTS OF URBANIZATION ON PERFORMANCE, HABITAT SELECTION, AND PERSISTENCE OF ANOLIS LIZARDS

BY

ANDREW CLARK BATTLES

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OF

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ABSTRACT

Urbanization is a global change phenomenon that is increasing in frequency and magnitude worldwide. As a greater proportion of the human population resides in urban areas, cities must grow, therefore exposing an increasing number of species to human-modified habitat. While some species become extirpated when their habitat is urbanized, others persist and even spread throughout cities. Furthermore, human activity increases the rates of species invasions around the world, and many introductions occur in urban areas. The objective of this dissertation is to evaluate the consequences of some major environmental changes caused by urbanization for non-human urban dwellers. Specifically, I measure the effects of changes in the structural and thermal properties of urban habitats on two species of *Anolis* lizards introduced to Miami, FL: the Cuban brown anole (*Anolis sagrei*) and the Puerto Rican crested anole (*Anolis cristatellus*). These species, and anoles in general, are arboreal and ectothermic, therefore likely sensitive to the habitat changes caused by urban development. Separate from the following manuscripts, Appendix 2 documents standing phenotypic variation in morphology, thermal traits, and behavior in an urban and natural population of *A. sagrei*.

One of the most dramatic effects of urbanization is the change in the structural habitat, the matrix of physical objects that comprise a habitat. In order to develop a landscape for human use, vegetation is removed and modified and artificial structures are added. In the first chapter, I evaluate the qualities and magnitude of differences in the structural habitat between natural and urban habitats, and how lizards express their habitat preferences, given the changes in the urban environment. First, in the lab at

URI, I assessed lizard preference for perch diameter using individuals from natural populations of both species. I allowed lizards to choose between vertical perches of three different diameters and recorded the proportion of time spent on each perch. Second, in four urban and four natural sites in the field in Miami, FL, I measured the diameter, height, and type (e.g., "tree trunk", "branch", "metal pole") of available vegetation and artificial structures upon which lizards might perch. I could therefore asses habitat selection by also recording these values for perches that lizards used. From the preference trials, I discovered that lizards of both species prefer the largest available perches. In the field, I found that both vegetation and artificial structures in urban areas were broader compared to vegetation in natural areas. Lizards expressed their preference for broad diameters by selecting broader perches than were randomly available in both habitat types, including artificial structures. Therefore, in urban habitats, lizards used broader perches than they did in natural habitats, demonstrating an expansion of the structural niche axis in urban areas.

Using the broadest perches in urban areas means that lizards often use artificial structures (the broadest available), exposing them to substrate properties they rarely encounter in natural habitats. Artificial structures, such as metal poles and painted walls, can be extremely smooth, challenging locomotion for species that primarily utilize vegetation (and man-made structures in cities) for daily activity. In the second chapter, I assess how lizard sprint performance is impacted by substrate smoothness and whether urban populations, more often exposed to smooth, artificial substrates, perform better. I measured the velocity and two-dimensional hindlimb kinematics of lizards running on three substrates of increasing smoothness (rough bark, concrete, and smooth wood) for two inclinations (inclined: 37°; vertical: 90°). I filmed lizards from urban and natural populations of both species using a high-speed video camera to capture limb positions at footrise and footfall during a sprint. I found that on vertical tracks, lizards ran slower, took shorter strides, moved their bodies shorter distances with a single step, kept their foot in contact with the substrate for longer (duty factor), and exhibited more contracted limb postures upon finishing a step than when running on the inclined track. I also observed these kinematic effects on the smooth wood substrate compared to the rough bark, though this effect was not as strong as with incline. I did not find an overall effect of habitat type, such that urban lizards did not run faster or use different gait characteristics or hindlimb positions compared to natural lizards.

An effect of the structural changes caused by urbanization evaluated in the first chapter, along with increased impervious coverage (e.g., rooftops, parking lots, roads), is increased ambient and surface temperatures in cities. Known as the urban heat island effect, warmer urban temperatures could have great impact on ectothermic organisms, such as the anoles studied in this dissertation. To regulate their internal body temperature (T_b), ectotherms depend on not only ambient conditions, but also the presence of sunny basking sites and cool, shady sites, between which they can shuttle to raise or lower their temperature. This shuttling, or active thermoregulation, incurs costs because time spent shuttling is time not available for performing other tasks, such as foraging or territory defense. In the third chapter, I asses how the structural changes of urbanization affect thermal conditions and in turn the body temperatures of lizards. I first measured operative temperatures (T_e), the body temperatures lizards would have if they did not actively thermoregulate, using copper models distributed randomly throughout each of the four urban and four natural sites used in the first chapter. Then, while the operative temperature models were recording data, I captured lizards and took internal body temperature measurements. Next, in the lab, thermal preferences were established for lizards of both species from urban and natural areas. Finally, I evaluated temperature-dependent sprint performance by measuring lizard sprint speed at six temperatures for *A. sagrei* and five temperatures for *A. cristatellus*. I found that urban areas had more open canopies compared to natural areas, which led to higher T_e in urban sites than in natural habitats. I also found that lizards actively thermoregulated, maintaining T_b higher than T_e in all sites. While thermal preference ranges and thermal performance did not differ between urban and natural populations for either species, overall, *A. sagrei* preferred warmer temperatures and sprinted faster at higher temperatures than did *A. cristatellus*. Urban sites may lower thermoregulatory costs for both species, but I found only *A. sagrei* T_b more often within their preferred temperature range in urban compared to natural habitat. Furthermore, based on available T_e within each species' preferred temperature range, urban sites with only *A. sagrei* appear less-suitable to *A. cristatellus*, while natural areas, even those that contain *A. sagrei*, are more suitable to *A. cristatellus*. While *A. sagrei* may find opportunities for dispersal in many urban locations, *A. cristatellus* is likely constrained to relatively cooler microclimates provided by forested locations and areas with higher canopy coverage.

My dissertation evaluates several important ways that urbanization alters habitats for arboreal ectotherms. Together, the first two chapters constitute a complete evaluation of one mechanism behind persistence in urban habitats. From how the urban structural habitat differs from that of the ecologically and evolutionary historical natural habitat, to how lizards express preferences for habitat elements and the resultant performance consequences. The third chapter explores another mechanism influencing persistence that is critically important for ectotherms. As urban areas grow and more species are introduced to areas outside of their native ranges, studies such as these are important to understand and predict persistence and invasion dynamics. Furthermore, a deeper understanding of the mechanisms behind persistence in urban habitats may be critical for designing cities that maintain the biodiversity of a region and preserve ecosystem function.

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The projects in this dissertation involved collecting and analyzing immense amounts of data, which I achieved with assistance from many fabulous undergraduate research assistants. First, I thank Malcom Moniz (Coastal Fellow, 2014) for his help in one of the most intense summers of field work imaginable. In Miami, we endured scorching heat, heavy downpours, and a crazy diet all while collecting the great data set presented herein (we tried to have some fun in Miami, too). I also thank Elizabeth Shadle for her work on the lizard videos, Nathan Andrews for his help with lizard care and racetrack days, Danielle Jordan for her time in the lizard room, and Amanda Merritt for her data collection.

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PREFACE

This dissertation is prepared in manuscript format. Chapter 1, entitled “Living in the big city: preference for broad substrates results in niche expansion for urban *Anolis* lizards” was submitted to *Urban Ecosystems* in January 2018 and is currently in review. Chapter 2, entitled “Effects of substrate inclination and smoothness on performance and hindlimb kinematics in two *Anolis* lizard species” is currently in preparation for submission to the *Journal of Experimental Biology*. Chapter 3, entitled “The Miami Heat: Urban areas alter thermal biology and costs of thermoregulation for two non-native *Anolis* species” is currently in preparation for submission to *Global Change Biology*. The chapters are presented as they are submitted to the journals and may be subsequently amended for publication. Additional authors for manuscripts are listed at the beginning of each chapter. Appendices are presented at the end of the dissertation and contain data conducted in support of this dissertation but were not included in the manuscripts.

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CHAPTER 1

LIVING IN THE BIG CITY: PREFERENCE FOR BROAD SUBSTRATES RESULTS IN NICHE EXPANSION FOR URBAN *ANOLIS* LIZARDS

Submitted to Urban Ecosystems, January 2018

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ABSTRACT

Persistence of animals in urban habitats, a stark environmental contrast to natural habitats, can be explained through evaluating the mechanisms behind organism-habitat interactions. One of the most notable effects of urbanization is the change in structural habitat; vegetation is removed and modified, favoring large trees and adding artificial structures in cities, which may alter how organismal preferences for aspects of the habitat are realized. We evaluated the mechanisms by which structural habitat changes associated with urbanization alter the available vegetation and substrates on which two species of *Anolis* lizards perch in urban and natural forest sites in Miami, FL. We also experimentally assessed habitat preference in the lab to establish the mechanism behind habitat selection. We found that vegetation was broader in urban areas compared to natural habitats, and artificial structures in urban areas were more than twice the diameter of available natural perches. Lizards expressed their preference for broad perches by selecting broader vegetation and artificial structures compared to their availability in both habitats. With the increased availability of broad substrates in urban areas, perch diameters selected by lizards resulted in an expansion of this aspect of the structural habitat niche for both species. The two species differed, however, in other responses to altered urban habitats. *Anolis cristatellus* tended to avoid artificial substrates, whereas *A. sagrei* used both natural and artificial structures in proportion to their availabilities. This study provides a mechanistic explanation for how urbanization alters structural habitats, leading to niche expansion for organisms living in cities.

INTRODUCTION

Most species alive today have an evolutionary history that includes persisting through environmental changes and encountering novel habitats to some degree (Thompson, 2013). However, ongoing human-induced rapid environmental change (HIREC) is unprecedented in both its rate and magnitude of environmental change on this planet (Palumbi, 2001; Hobbs et al., 2006; Sih et al., 2011; Barnosky et al., 2012). Local extinctions and range shifts demonstrate that some organisms are unable to respond successfully *in situ* to HIREC (Lynch and Lande, 1993; McKinney and Lockwood, 1999; Brook et al., 2008; Estrada et al., 2015). In contrast, other species persist, and some even thrive, when encountering novel environments produced by human activities (Kowarik 2011; Lowry et al., 2013). To better understand how changing environmental conditions challenge the persistence of populations, we need mechanistic studies that quantify changes in niche dimensions due to global change (Shochat et al. 2006; Sol et al. 2013). Such studies should evaluate changes in resource availability in altered habitats, how organisms respond through their resource use and preferences, and if individuals experience any fitness consequences. This niche-based assessment should yield valuable insight into the role of niche dynamics (e.g. niche contraction or niche expansion) in determining whether populations persist under HIREC (Wingfield et al., 2011).

Urbanization likely alters the niche space available in cities, ultimately determining whether or not populations persist there, yet we know little about the underlying dynamics of how organisms respond to this change. Urbanization is a form of HIREC where natural vegetation is removed and replaced with novel artificial structures (e.g. walls, pavement, and lamp posts) and managed vegetation assemblages (McDonnell and Pickett, 1990; Forman, 2014; McDonnell and Hahs, 2015). While the resulting environmental changes can be novel (e.g. artificial light at night), in other cases urbanization alters existing ecological niche axes, such as increased environmental temperatures due to the urban heat island effect (Rizwan et al., 2008; Imhoff et al., 2012). Whether entirely novel or not, habitat changes in cities occur at rates far greater than in natural habitats and in some cases, elicit phenotypic responses distinct from those observed in natural habitats (Winchell et al., 2016; Alberti et al., 2017). Organismal responses to urbanization vary; for some species, suitable habitat or resources will decrease and predation or competition pressures might increase leading to niche contraction (Shochat et al., 2010; Aronson et al., 2014). These and other selection pressures in urban areas may promote adaptive evolution in traits that improve fitness in cities (Nemeth and Brumm 2009; Atwell et al. 2012; Donihue and Lambert 2014; Weaving et al. 2016; Winchell et al. 2016). Conversely, new resources may benefit species with adaptations that happen to be useful in urban habitats (i.e. pre-adaptation; McDonnell and Hahs, 2015). Previous studies show that urban animal populations exploit a variety of anthropogenic resources (Lowry et al., 2013; Oro et al., 2013; Penick et al., 2015). If urban habitats increase the availability of habitat elements preferred by an organism, urban populations may experience a realized niche expansion (Pearman et al. 2008). A more mechanistic, niche-focused framework that includes organismal preferences, organism-habitat interactions, and comparison of habitat differences between urban and natural environments is needed.

To evaluate how the habitat alterations caused by urbanization influence preferences and habitat selection, we need a system with both urban and natural populations and a well-studied habitat use-performance relationship. Numerous species of *Anolis* lizards (or anoles) are found in urban and natural habitats in their native and non-native ranges (Irschick et al. 2005a,b; Marnocha et al. 2011; Kolbe et al. 2016a; Winchell et al. 2016). A key axis of diversification for anoles is the structural habitat – the diameter, height, and type of vegetation used by perching lizards (Losos, 2009). Anole structural habitat use varies interspecifically, intraspecifically, and in different environments (Irschick et al., 2005a,b). Strong habitat use-performance relationships drive habitat selection in anoles (Rodríguez-Robles et al. 2005; Johnson et al. 2006; Losos 2009). Anole habitat selection is correlated with locomotor performance, a commonly used fitness proxy in anoles (Irschick and Losos 1999; Losos 2009; Gilman and Irschick 2013; Irschick and Higham 2016). Among and within species, relatively longer limbed anoles (accounting for their body size) perform better on wide- versus narrow-diameter perches (Losos and Sinervo, 1989; Losos and Irschick, 1996; Irschick and Losos, 1998). Lizards confronted with the variability of diameters and inclines typical of arboreal habitats modulate their limb movements depending on the perch (Spezzano and Jayne, 2004; Foster and Higham, 2012). These studies of anole habitat use, performance and behavior in natural settings provide a solid foundation for predictions concerning how anoles may respond to habitat changes in urban areas.

Two *Anolis* species found in Miami, FL, USA, *Anolis cristatellus* and *Anolis sagrei,* are ideal for evaluating effects of urbanization. Both species inhabit urban and natural forest habitats that occur in close proximity to each other. In their natural forest habitats, these species are commonly found on trunks, branches, leaves, and the ground, making them likely to be sensitive to the structural habitat changes of urbanization. In this study, we first compared the structural habitat availability and use by lizards in natural forests and urban areas. After comparing availability and use in the field, we evaluated preference for a key aspect of the structural habitat – perch diameter – using a laboratory experiment.

We predict that urban areas will contain a greater proportion of broad substrates than natural areas due to the removal of smaller trees, branches, and woody debris in urban areas and the addition of artificial substrates, such as walls and posts. We expect that lizards will prefer larger-diameter perches and non-randomly select wide perches compared to their availability, leading to an expansion of the structural habitat niche in urban sites. Results from our study help to identify mechanisms behind patterns of organismal responses to urbanization that should improve predictions regarding species and population persistence in our increasingly urbanized world.

METHODS

Study species and study sites

We studied two species of *Anolis*, small insectivorous lizards found naturally in southern North America, Central and South America, and throughout the Caribbean (Losos, 2009). Several *Anolis* species have been introduced to the Miami metropolitan area (Kolbe et al., 2007), two of which are common in both natural forest and urban areas. *Anolis sagrei* is native to Cuba and the Bahamas, and non-native populations are now widely distributed in the southeastern United States with Miami area populations dating to the 1940-60s (Bell 1953; Salzburg 1984; Kolbe et al. 2004). *Anolis cristatellus* is native to Puerto Rico, and was first documented in Miami in the mid-1970s (Wilson and Porras 1983; Powell et al. 1996; Bartlett and Bartlett 1999; Kolbe et al. 2007). In contrast to the nearly ubiquitous *A. sagrei*, the distribution of *A. cristatellus* is more restricted, radiating out from two independent points of introductions in the Miami area (Kolbe et al. 2016b). Both *A. sagrei* and *A. cristatellus* are classified as trunk-ground habitat specialists, meaning they commonly occupy the ground and perches up to ~2 m (Salzburg 1984; Losos 2009). The larger *A. cristatellus* (snout-vent length, or SVL, up to 75 mm in males and 60 mm in females; mean mass is 8.5 g in males) typically perches higher than the smaller and more terrestrial *A. sagrei* (SVL up to 69 mm in males and 55 mm in females; mean mass is 4.8 g in males).

We studied lizards in four urban and four natural sites throughout the Miami metropolitan area. Generally, natural sites were closed-canopy forests on upland hammocks, consisting of hardwood-oak overstory canopy with palmettos and saplings in the understory. All natural sites were forest patches within the urban matrix of metropolitan Miami. The ‘Barnes’ natural site contains *A. sagrei* and is a designated natural area within A.D. Barnes Park, a typical city park. The ‘Montgomery’ natural site contains *A. sagrei*, and is a forest patch inside of the Montgomery Botanical Center, which features large lawns and managed gardens, insulating the site from nearby urban areas. The ‘Matheson’ natural site contains *A. cristatellus* and is a designated natural area within Matheson Hammock Park. The ‘Bear Cut’ natural site contains *A. cristatellus* and is a designated natural area within the Crandon Beach Park on Key Biscayne.

Urban sites are located within human-altered areas, generally along roadsides with bike paths, canals, and sidewalks. The ‘UM’ urban site contains *A. sagrei* and is located along a road near the University of Miami in Coral Gables. The ‘Gables’ urban site contains *A. sagrei* and is located near downtown Coral Gables, with very little vegetation and primarily artificial substrates. The ‘Red Rd.’ urban site contains both *A. sagrei* and *A. cristatellus*, and is located along a portion of Red Road in South Miami and Pinecrest. The site is a linear park along a road, bike path, and canal with a guard-rail in some parts. The ‘Crandon’ urban site contains both *A. sagrei* and *A. cristatellus*, and is located along a portion of Crandon Boulevard on Key Biscayne. We are unaware of any urban sites in Miami that contain only *A. cristatellus*.

Habitat availability, use and selection

Because structural habitat is a key niche axis for anoles, we evaluated the impact of urbanization on the availability of perch sites and perch use by lizards. To understand the relationship between perch availability and the perch use, we conducted habitat availability transects to quantify the differences between urban and natural sites, and then compared these availabilities to lizard habitat use. We measured the diameter (cm), height (cm), and substrate type of potential lizard perches, denoted further as habitat availability, which includes artificial substrates in urban sites. At all sites, we measured available vegetation (and structures in urban areas) at 0.25 m intervals, from 0-2 m vertically. Trunk-ground ecomorphs such as the two *Anolis* species in this study rarely perch higher than 2 m (Losos, 2009). We measured one potential perch at each height, within a 0.5-m radius of the sample point. We followed perches that continued outside of the 0.5-m radius but that originated from within it. If a particular height did not have an available perch, such as when vegetation was < 2 m (e.g. a low bush), we did not record data at that height. Some transect points had no vegetation within a 0.5 m radius, and in these cases no data were recorded for any height; these were considered ‘open ground’ and used to analyze percent open space. We did not include walls in the perch diameter analyses because we are uncertain on how to quantify accurately these surfaces; very large diameters would skew the results and capping measurements would be inaccurate. Because urban sites were usually along a road, we conducted 6-8 m transects every 30-50 m perpendicular to the road. Exact transect lengths and intervals between transects depended on the length of the site and the distance between the road and the edge, such as the canal edge or building. In natural sites, we conducted two separate transects beginning from haphazard locations within the study plots that followed a random compass heading. Each transect was approximately 20 m in length, with 6-m perpendicular transects at 5 m intervals along the main transect. We collected roughly 2-3 times as many habitat availability observations (not including transect points without vegetation) as lizard perch use observations at each site. We compared vegetation availabilities to perch use by lizards in the urban and natural sites to determine habitat selection. Using a telescopic pole with a noose, we captured undisturbed lizards and recorded sex, species, and SVL as well as the diameter, height, and substrate of the location where each lizard was perched. We captured males and females totaling approximately 120 adult lizards per species, per site (male and female sample sizes in table S1), evenly collected throughout their activity time during the day (0700 to 1800 hrs), and never in inclement weather.

Habitat preference

To measure perch-diameter preference, we collected 20 male *A. sagrei* and 20 male *A. cristatellus* from natural sites and shipped them to the University of Rhode Island (Kingston, RI). In a 1.8 m x 1.8 m x 1.8 m mesh enclosure in the lab, we presented individual lizards a choice of six perches (i.e., tree trunks, 1.6 m high) in three duplicated diameters of 2, 7, and 12 cm. These sizes cover the range of mean vegetation diameters observed for availability and use by both species in urban and natural habitats. After a two-minute acclimation period under an opaque cover, lizards were given 15 minutes to explore the perches. We recorded the proportion of time spent on each perch compared to the total time lizards were on a perch. Lizards usually selected a perch within the first two minutes, sometimes moving between several perches. We arranged the perches in alternating sizes in a circle. Before each trial, we randomized the location of perches in the circle to eliminate a location effect. Each lizard went through the preference experiment two times. Some lizards never selected a perch and were removed from the analysis (*A. sagrei* N = 1, *A. cristatellus* N = 4); sample sizes in Table S1.

Statistical Analyses

We performed all statistical analyses in R (R Core Team, 2015), and analyzed species separately for all statistical tests. We compared mean available vegetation diameters of all sites, nested within their site type (urban and natural), using an analysis of variance (ANOVA). We used ANOVA to test for a difference in diameter among vegetation in natural areas, vegetation in urban areas, and artificial structures in urban areas, for both availability and use by males and females. We compared diameters of available substrates (i.e., vegetation and artificial structures) and perch use by sex and site type (urban and natural) with an ANOVA. We also used ANOVA to test for differences in perch height use by sex and site, but did not include availability because nearly all heights were available at each site. To compare the distributions of diameters of available substrates and perches used by lizards between natural and urban environments, we used two-sample Kolmogorov-Smirnov tests. We used Simpson's index of diversity to calculate the diversity of perches used by each species and sex in both site types; values range from 0 (no diversity) to 1 (infinite diversity). We used chi-squared tests to compare the proportion of artificial and natural perches available in urban habitats to the proportion used by lizards, separately by sex. For all tests, lizards were only compared to availability in the sites that they were found (e.g. *A. cristatellus* for only two of the four urban sites where it was present).

We assessed perch diameter preference from the laboratory experiment using a multinomial mixed model, which accounts for the non-independence of response values, with lizard ID as a random effect, and compared the proportions of time spent on small, medium, and large perches for both trials combined using the MCMCglmm package (Hadfield, 2010). The model runs Markov chain Monte Carlo iterations to generate posterior distributions of the response levels. In this case, the mean distributions for time spent on medium and small perches are each compared to time spent on the large perch.

RESULTS

Habitat Availability

Urban sites had broader substrates available than natural sites ($F_{1,6} = 154.33$, $p < 0.0001$, Figs. 1a, S2); within natural sites, Montgomery and Matheson had narrower vegetation than Barnes ($F_{6,2816} = 3.26$, $p < 0.01$). Larger mean diameters in urban areas were due to both broader vegetation in urban sites as compared to natural areas and the addition of even broader artificial substrates in urban sites ($F_{2,2896} = 165.7$, $p < 0.0001$, Fig. 1a). These large differences in vegetation and substrate diameters existed despite not including measurements for walls, which account for about 3% of availability in urban areas. The distribution of available substrates in urban areas was shifted away from smaller diameters and toward larger ones compared to natural areas ($D = 0.23$, $p < 0.0001$, Fig. S2). In most instances, the full range of potential perch heights from the ground to 2 m was available in both natural and urban sites. Urban habitats also had more open ground than natural areas; about half of the urban survey points lacked vegetation (Fig. 2a, Table S2). Urban habitats had a greater variety of potential perches due to the addition of artificial substrates, such as poles, posts, and walls (Fig. 3), which accounted for about 25% of available substrates (Fig. 2b). Overall, urban areas had broader substrates available (both vegetation and artificial structures) and more open space compared to natural habitats.

Habitat Selection

Despite the ubiquity of open ground in urban habitats, lizards were almost always found on vegetation or artificial structures (Fig. 2a). Lizards used wider perches in urban compared to natural areas, and they selected wider perches than were available in both areas (*A. cristatellus:* $F_{5,1914} = 55.657$, $p < 0.001$, Figs. 1, 4a; *A. sagrei*: $F_{5,2841} = 31.435$, $p < 0.001$, Figs. 1, 4b). Lizards also used a greater diversity of perches in urban compared to natural areas (Table 1, Figs. S2, S3). The use of broader perches in urban areas was driven by lizards selecting both wider vegetation, which was almost always wider than vegetation used for perching in natural areas, and artificial substrates, which were over twice the diameter of vegetation in urban areas (*A. cristatellus:* $F_{5,517} = 10.27$, $p < 0.001$, Fig. 1b; *A. sagrei*: $F_{5,714} = 9.675$, $p < 0.001$, Fig. 1c). Neither sex of *A. cristatellus* differed in perch height use between urban and natural sites, but females perched lower than males within both sites ($F_{3,523} = 9.152$, $p < 0.0001$, Figs. 4a, S1). Male and female *A. sagrei* perched lower in urban areas than in natural areas and females always perched lower than males ($F_{3,775} = 25.53$, $p < 0.0001$, Figs. 4b, S1). Both sexes of *A. sagrei* used artificial perches at the same frequency as their availability, but female and male *A. cristatellus* used artificial perches at a lower rate (female: $X^2 = 6.80$, df= 1, $p < 0.001$; male: $X^2 = 4.93$, df= 1, $p < 0.03$, Fig. 2b), suggesting avoidance of artificial substrates by *A. cristatellus* in Miami.

Habitat Preference

In the experimental perch preference trials, *A. cristatellus* and *A. sagrei* spent an average of 53.1% and 66.0% of their time, respectively, on the largest-diameter perches (Fig. 5). *Anolis sagrei* exhibited a stronger preference for broad-diameter perches, preferring the largest perches to both small ($p < 0.001$) and medium ones ($p < 0.001$), whereas *A. cristatellus* preferred only the largest to the smallest perches ($p < 0.001$).

DISCUSSION

We found that lizard habitat preferences interact with habitat availability to drive the expansion of the structural habitat niche of anoles in urban areas (Fig. 4). Lizards selected broader diameter vegetation compared to the availability of potential perches in natural habitats (Figs. 1, S1). Urban habitats had broader substrates compared to natural areas due to a combination of broader vegetation and the addition of artificial structures (Fig. 3), and lizards still selected broader perches than available (Figs. 1, S1). Moreover, lizards used a greater diversity of perch diameters in urban sites (Table 1, Fig. S3). Other studies have observed expansion of the realized niche in response to HIREC, particularly in the context of invasive species responding to novel climates in their non-native range (Holt et al., 2005; Broennimann et al., 2007; Fitzpatrick et al., 2007; Pearman et al., 2008; Tingley et al., 2014), but observations of niche expansion in urban environments are limited. Studies of urbanization tend to focus on population- and community-level responses to habitat alterations, which document changes in abundance and diversity that ultimately emerge as a result of underlying niche dynamics (Aronson et al., 2014; Fischer et al., 2015; Boivin et al., 2016). The broad perches encountered by lizards in urban areas were often artificial substrates (i.e., smooth, vertical surfaces), amounting to roughly one-quarter of available perches (Fig. 2), and lizard locomotor performance has been shown to decrease on smooth, artificial substrates (Kolbe et al. 2016a). Consequently, preference-driven niche expansion in urban areas may result in urban lizards choosing sub-optimal substrates in terms of locomotor performance. The implications of this paradox in cities range from behavioral changes to fitness losses with the potential to alter selective regimes in urban environments (Kolbe et al. 2016a; Winchell et al. 2016).

Both *Anolis* species in our study preferred the largest diameter perches available, with *A. sagrei* having a stronger preference (Fig. 5), which likely results from a strong association between use of broad perches and increased fitness in natural habitats. Sprint speed, a common fitness proxy in anoles, is faster on wider-diameter perches for long-limbed species, such as the two species in this study (Losos and Sinervo, 1989; Irschick and Losos, 1998). Large perches, such as tree trunks, may also confer other fitness benefits. To escape predators, anoles will squirrel, or run to the opposite side of a trunk, placing them out of reach and view of a predator (Cooper, 2006). Further, flight initiation distance, or the distance between a perceived predator and an anole when the anole flees, decreases with increasing perch diameter in five anole species (Losos and Irschick, 1996), suggesting that the benefits of staying on a perch increase as its diameter increases. Tree trunks typically have fewer nearby branches, thereby increasing visibility. This can increase scanning ability to defend territories, identify prey, find and attract mates, and see predators from farther distances (Johnson et al., 2010). Therefore, for these and possibly other reasons, *Anolis* species such as these trunk-ground habitat specialists have developed an innate preference for larger-diameter perches in natural habitats.

Preference for larger-diameter perches was consistent with habitat selection by lizards in our study; lizards generally used wider perches than were available in both urban and natural habitats (Figs. 1, S2). Similarly, Wright (2009) found that male and female *A. sagrei* used broader perches added to their habitats (even though these perches were artificial). In natural habitats in our study, the largest perches were tree trunks and expressing preference for these perches likely confers higher fitness. Because the widest perches available in urban habitats were often artificial (Fig. 1), we would expect both species to occupy broad, artificial structures, especially *A. sagrei*, which had a stronger preference for large perches (Fig. 5). Indeed, in urban habitats we found *A. sagrei* using natural and artificial perches at rates equivalent to their availability (Fig. 2b), suggesting they do not differentiate between artificial and natural structures per se. In contrast, *A. cristatellus,* which exhibited a weaker preference for broad perches in lab trials and tended to avoid artificial substrates in urban areas, using them roughly 10% of the time compared to their 22% availability (Fig. 2b). While artificial structures certainly contribute to the niche expansion, lizards also select broad vegetation for perching in urban areas (Fig. 1b,c). However, smooth substrates, which are rare or absent in natural habitats, may alter the costs and benefits of using broad perches in urban environments (Kolbe et al. 2016a).

A mismatch between preference and performance could have fitness consequences, possibly resulting in an evolutionary trap in which a maladaptive behavior occurs because organisms prefer a resource that reduces their fitness (Dwernychuk and Boag, 1972; Fletcher et al., 2012; Rodewald et al., 2011; Robertson et al., 2013). Rapidly changing environments, such as those experiencing urbanization, are likely to cause evolutionary traps (Battin, 2004). Populations experience evolutionary traps when they increase their preference for a low-fitness resource, or maintain their preference for a resource whose quality has decreased (Hale and Swearer, 2016). Evolutionary traps have been observed and evaluated in urban habitats in a range of taxa. Cooper's hawks in Tucson were found to nest in greater densities in urban areas despite historical aversion to human activity, likely drawn to higher prey abundance and tall, exotic tree species for nesting (Boal and Mannan, 1998). Despite the preference of Cooper’s hawks for urban habitats, they experience decreased nest success compared to exurban areas due to nestling death from a parasitic avian disease, resulting in lower fitness (Boal and Mannan, 1999). In another example, great tits prefer to nest in the largest available cavities, which in urban habitats are much larger than those found in nature, but urban habitats likely do not provide adequate insect prey to raise hatchlings (Demeyrier et al. 2016). If lizards prefer broad habitat features that in urban areas result in decreased performance, decades of performance-fitness literature would suggest that urban lizards should have lower fitness than those in natural areas (Irschick and Higham, 2016). Aviles-Rodriguez (2015) found that lizards on walls fled sooner than those on trees in urban areas, suggesting that walls may not confer the same performance advantage for escaping as natural broad perches, such as tree trunks. However, our own observations and other urban anole studies do not indicate any major reductions in population numbers or densities in urban habitats (Kolbe et al. 2016a; Winchell et al. 2016). Future studies that evaluate a potential evolutionary trap due to urbanization should compare demographic variables (e.g. population density) between habitats to assess population-level consequences of reduced locomotor performance on artificial substrates.

If artificial structures confer reduced performance, we can think of two primary reasons why lizards use them without apparent fitness losses. First, behavioral strategies may be used to avoid suboptimal habitat. *Anolis crisatellus* demonstrates that niche expansion in urban areas can still occur without relying upon artificial substrates (Figs. 1, 2); perch diameter does not differ between artificial and natural perches for this species in urban areas (Fig. 1b). Avoidance of artificial substrates may have occurred because the fitness losses of using artificial substrates are great enough to alter habitat selection cues in urban areas (Schlaepfer et al., 2005). Variation in habitat selection behavior may be adaptive as populations encounter urban environments (Lapiedra et al., 2017). Second, evolutionary adaptation to HIREC, including urbanization, occurs across a wide range of taxa (Nemeth and Brumm, 2009; Atwell et al., 2012; McDonnell and Hahs, 2015), so specific components of the urban habitat, such as substrate composition are likely to be a selective force. For example, Winchell et al. (2016) showed predictable phenotypic differences (i.e., greater relative hindlimb length and more lamellae) between anoles in urban and natural habitats based on habitat characteristics (such as perch diameter) and demonstrated a genetic basis for these differences, which supports adaptation. Kolbe et al. (2016a) found a performance basis for this morphological variation where *A. cristatellus* with longer limbs proportional to their body size sprinted faster and were more stable on smooth, vertical substrates. Both Kolbe et al. (2016a) and Winchell et al. (2016) were conducted in the native range of *A. cristatellus*, where populations have experienced the effects of urbanization for potentially hundreds of generations, whereas the Miami populations in this study were introduced only a few decades ago, so the time exposed to urbanization may influence any potential selective pressures. The extent to which the invasion process alters phenotypes and environmental conditions for non-native populations compared to their source populations needs to be explicitly evaluated in future studies.

In this study, we explored mechanisms by which two species of *Anolis* lizards persist in urban habitats markedly different than their natural habitats, an important task in understanding how HIREC phenomena affect organism-environment interactions (Wingfield et al., 2011). We demonstrated that preference for broad perches and their increased availability in urban habitats interact to facilitate niche expansion of a key component of the structural habitat niche. However, the broadest perches in urban habitats are artificial structures, which are ecologically novel and could reduce fitness (e.g. reduced locomotor performance in Kolbe et al. 2016a). Several factors may allow lizards to minimize the performance losses associated with artificial substrates and therefore not experience population declines in cities. First, artificial structures, though significantly larger than vegetation in urban areas, are not the sole contributor to niche expansion. Lizards also use the broader vegetation, such as tree trucks, which are characteristic of urban areas and should not result in decreased performance. Second, lizards may avoid reduced-fitness artificial structures, as *A. cristatellus* does, suggesting preference cues may shift from substrate diameter to other features, such as surface roughness. Compared to *A. sagrei*, *A. cristatellus* from natural forest sites already had weaker preference from broad perches. Third, morphology may be under selection in urban areas, resulting in increased performance on artificial substrates. Finally, factors beyond the structural habitat likely contribute to fitness in urban areas. For example, increased urban temperatures may affect several traits important for fitness of these ectothermic organisms, such as metabolism, behavior, and performance (Gunderson and Leal, 2012). Urbanization, among other anthropogenic activities, may decouple long-established habitat cues from their performance-mediated fitness consequences, and may even lead some organisms into evolutionary traps (Fletcher et al., 2012; Robertson et al., 2013). This study demonstrates that the consequences of urbanization for one aspect of the ecological niche. Urban landscapes provide opportunities for researchers to study how organisms cope with environmental change at relatively accessible scales (e.g. spatial, temporal; McDonnell and Pickett, 1991), increasing the power of predictions for organismal response to future change. Future studies should follow a mechanistic framework for evaluating influences of other urban habitat changes to better understand what factors contribute to the persistence of species in cities and how those factors interact.

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COMPLIANCE WITH ETHICAL STANDARDS

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Table 1. Simpson's index of diversity for the perch diameter classes used by male and female *A. cristatellus* and *A. sagrei* and the Kolmogorov-Smirnov distances, indicating the maximum difference between cumulative distribution functions (Fig. S3), significance at $p < 0.01$ between distributions in natural and urban sites shown in bold.

	A. cristatellus		*A. sagrei*	
	Female	Male	Female	Male
Natural Site, diameter diversity (Simpson's D)	0.42	0.71	0.40	0.54
Urban Site, diameter diversity (Simpson's D)	0.62	0.76	0.62	0.76
K-S distance	**0.40**	**0.21**	**0.22**	**0.22**

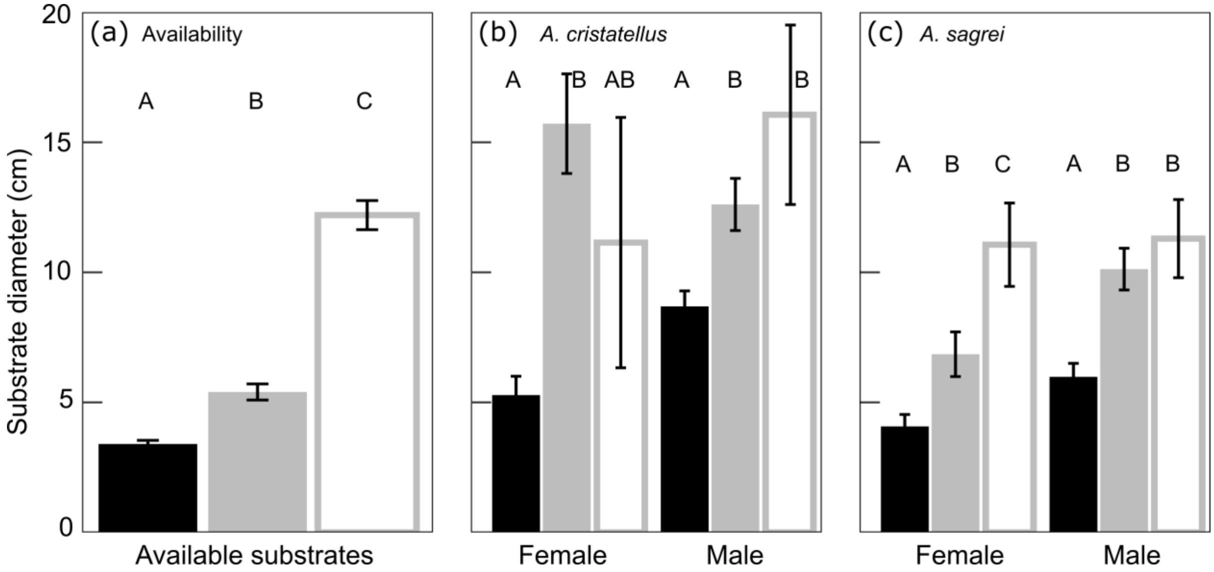

Fig. 1 Mean (±SE) for diameters of a) available vegetation and artificial substrates in natural and urban sites, b) perches used by *A. cristatellus*, and c) perches used by *A. sagrei*. Vegetation at natural sites is in black, vegetation at urban sites in grey, and artificial substrates in white, with letters indicating significant differences (*P* < 0.05).

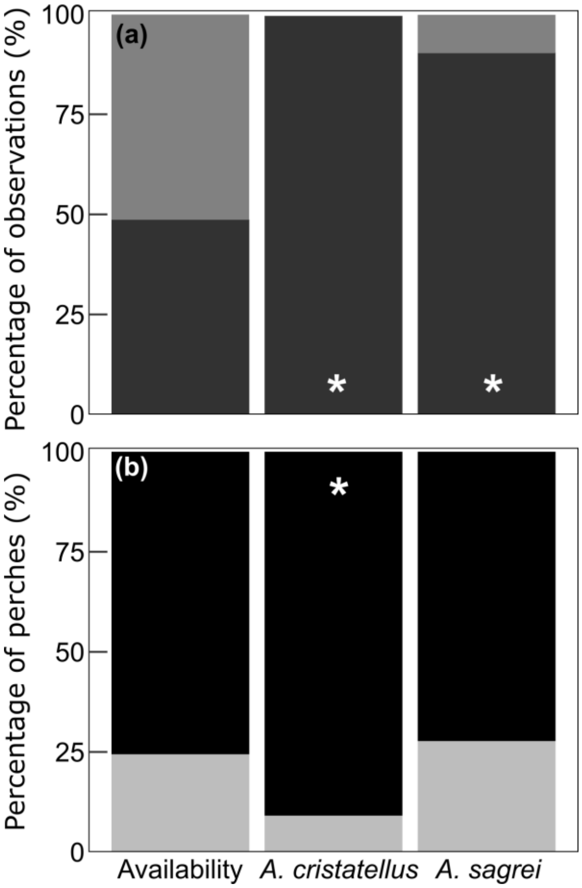

Fig. 2 Panel a) shows the proportion of total observations from habitat availability transects that were open ground (light grey) versus vegetation (i.e., potential perches, dark grey) and the proportion of the time lizards used perches (dark grey) compared to the ground (light grey) (Table S2). Panel b) shows the percentage of natural (black) versus artificial (grey) substrates available and perches used by lizards in urban sites. Sites with and without *A. cristatellus* did not differ in availability/use comparisons and so are pooled for availability columns, and female and male perch use did not differ significantly for either species and were pooled for this figure. * indicates $P < 0.05$ for the chi-squared test of availability versus perch use by lizards.

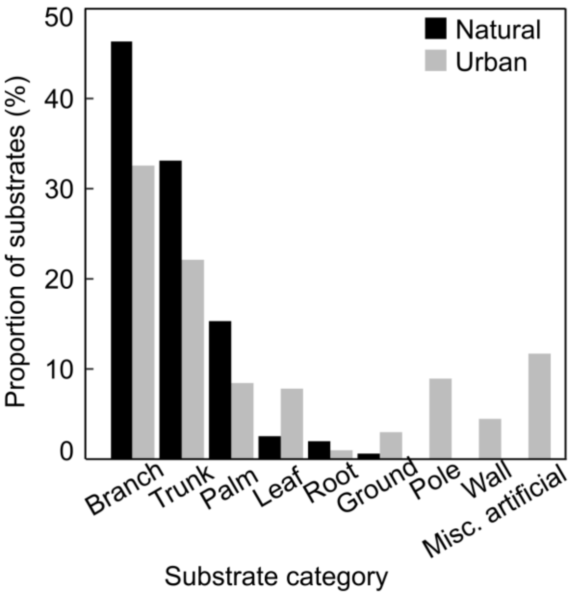

Fig. 3 Frequency of natural and artificial substrate availability at natural forest (black) and urban (grey) sites.

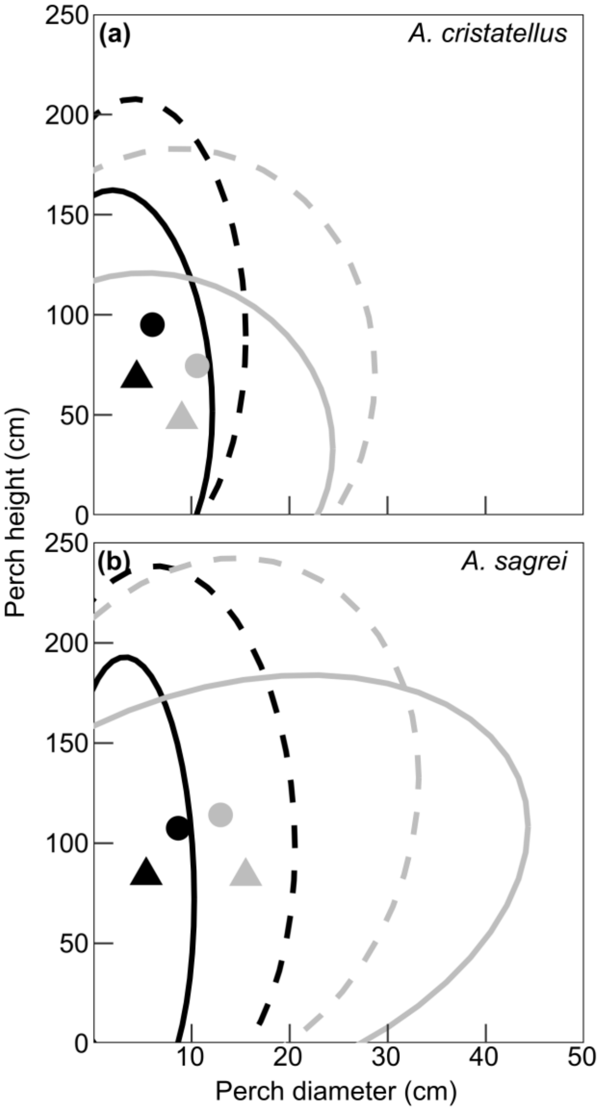

Fig. 4 Urban niche expansion of structural habitat use by a) *A. cristatellus* and b) *A. sagrei*. Plots of perch diameter against perch height show means (S.E. error bars do not exceed shape size) and 95% confidence ellipses, separate for males (circles, dashed ellipses) and females (triangles, solid ellipses). Natural sites are in black and urban sites in grey.

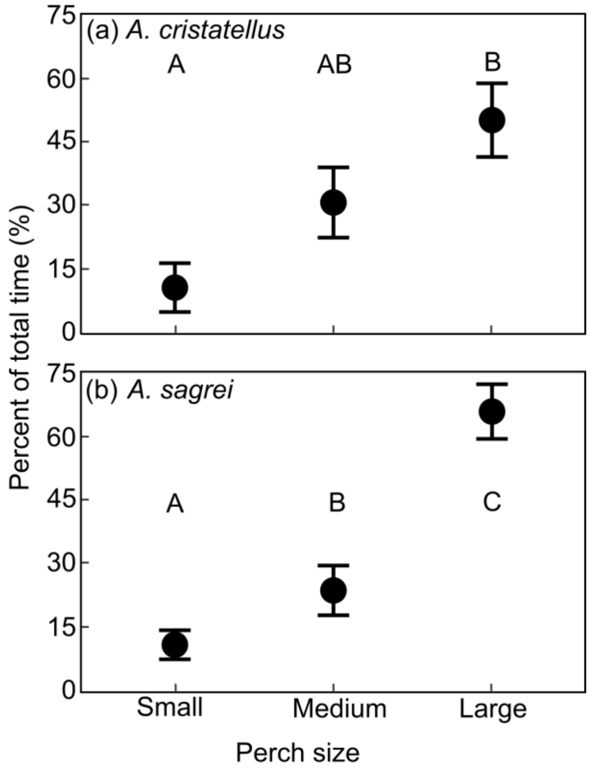

Fig. 5 Mean (±SE) percentage of total time that a) *A. cristatellus* and b) *A. sagrei* spent on small, medium, and large diameter perches during perch preference trials. Letters indicate significant differences ($P < 0.01$), separate for each species.

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SUPPLEMENTAL MATERIAL

Table S1: Morphological measurements for experimental groups

Mean (±SD) for morphological variables, sample size (n), and site information for the three different groups of lizards used in this study.

GROUP	n		SVL (mm)		Mass (g)		Hindlimb length (mm)		Lamella number		Number of sites	
Anolis sagrei	Natural	Urban	Natural	Urban	Natural	Urban	Natural	Urban	Natural	Urban	Natural	Urban
Habitat Use	M: 150 F: 111	M: 313 F: 205	M: 54.1 ± 4.42 F: 44.0 ± 1.89	M: 57.3 ± 5.24 F: 44.6 ± 2.76	--	--	--	--	--	--	2	4 (2 with *A. cristatellus*)
Preference (M only)	19	--	54.2 ± 3.69	--	4.5 ± 0.84	--					1	0
Anolis cristatellus												
Habitat Use	M: 160 F: 102	M: 176 F: 89	M: 63.7 ± 7.16 F: 46.3 ± 2.56	M: 66.1 ± 5.74 F: 46.3 ± 2.76	--	--	--	--	--	--	2	2 (overlap with *A. sagrei*)
Preference (M only)	16	--	66.8 ± 2.26	--	9.0 ± 1.28	--	--	--	--	--	1	0

Table S2. Site-specific availability data

A comparison of natural and urban sites in this study showing species present, perch diameter available (mean±SE), and percent of open space, calculated as the percentage of open points from availability transects.

Site	Species Present	Perch Diameter (cm)	Open Space (%)
Natural			
Matheson	*A. cristatellus*	2.8±0.22	2
Bear Cut	*A. cristatellus*	3.7±0.28	2
Montgomery	*A. sagrei*	2.8±0.26	0
Barnes	*A. sagrei*	4.2±0.36	0
Urban			
UM	*A. sagrei*	6.0±0.45	42
Coral Gables	*A. sagrei*	6.9±0.60	56
Crandon	*A. cristatellus* & *A. sagrei*	7.9±0.45	51
Red Road	*A. cristatellus* & *A. sagrei*	7.7±0.75	56

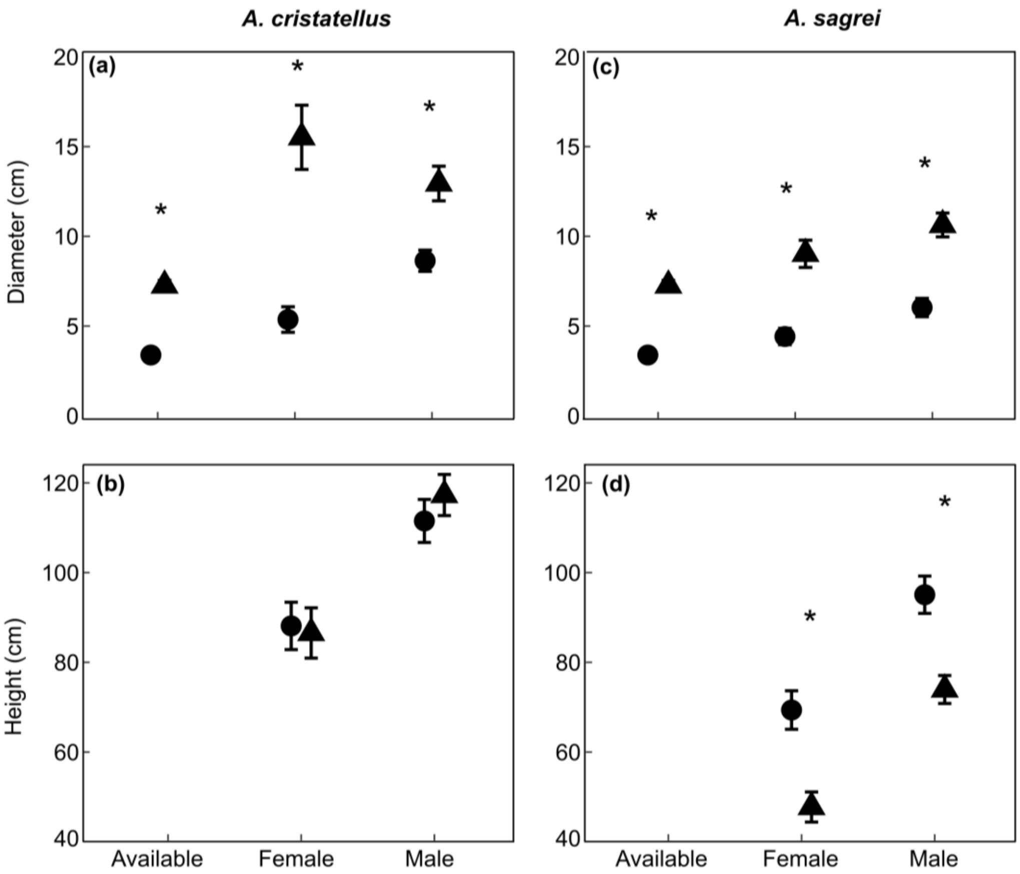

Figure S1. Mean perch diameter and height of available perches and those used by lizards

Mean (±SE) for habitat availability and habitat use by males and female lizards. Panels a & c show perch diameter, and b & d show perch height for *A. cristatellus* (a, b) and *A. sagrei* (c, d). Perch height availability was not measured because all heights are available. Asterisks denote a significant difference between urban and natural sites.

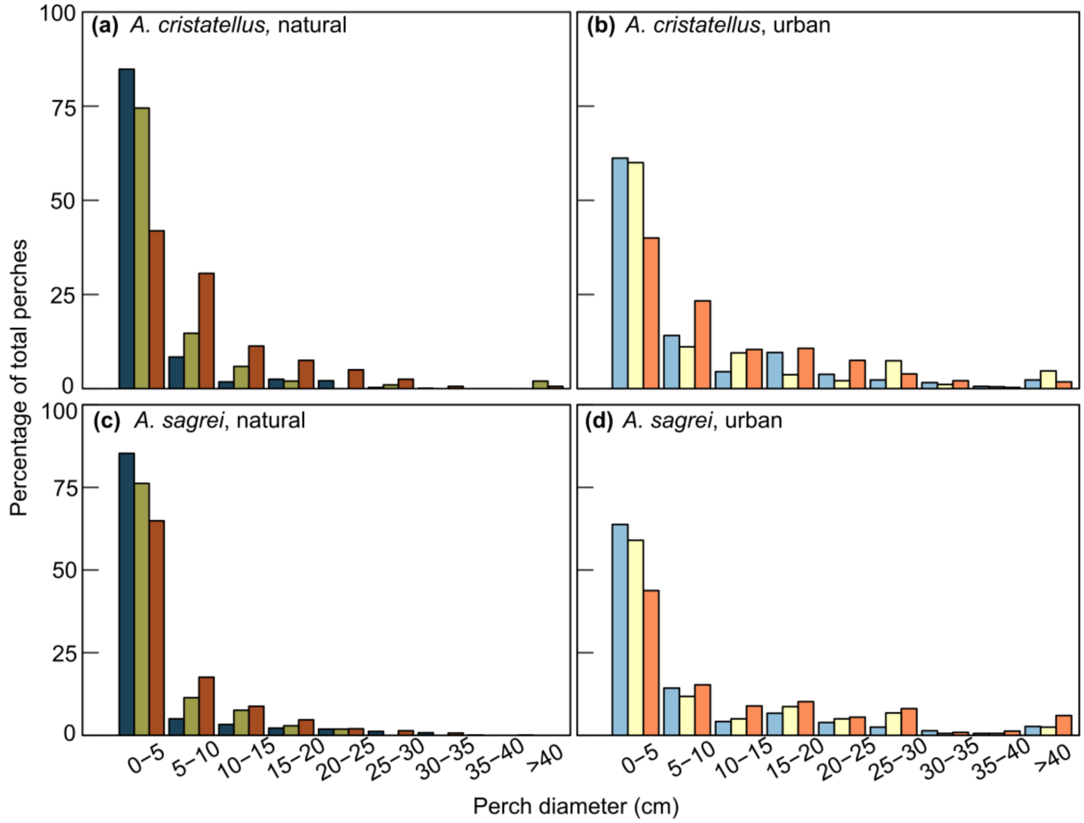

Figure S2. Histogram of perch availability and use

Frequency histograms showing perch diameter availability (blue) and perch diameter use by females (yellow) and males (red) for *A. cristatellus* in (a) natural and (b) urban sites, and for *A. sagrei* in (c) natural and (d) urban sites.

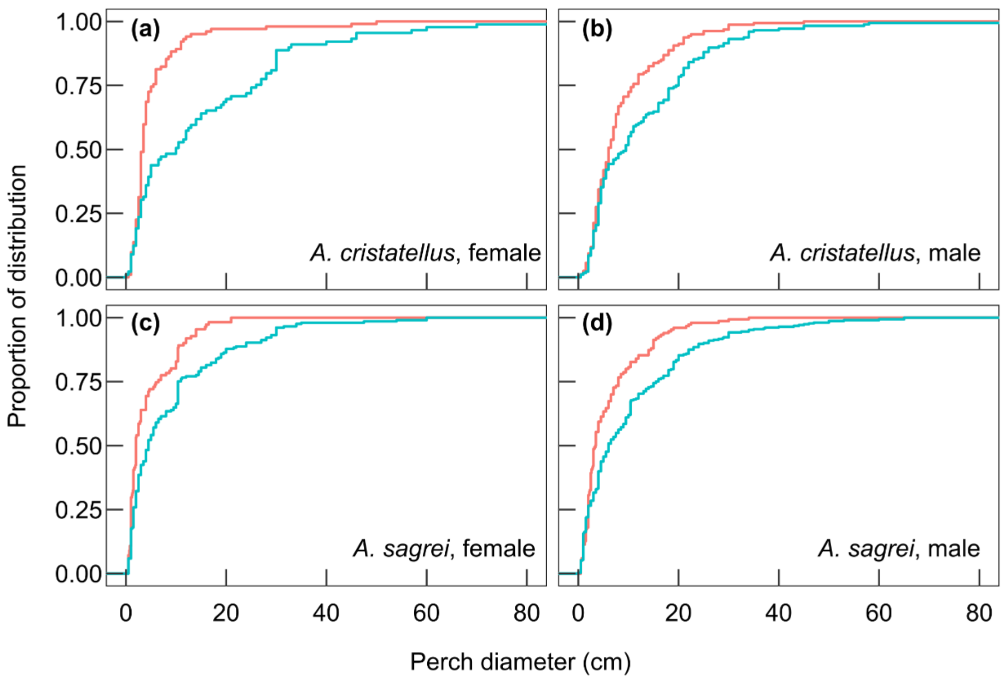

Figure S3. Differences in perch use distributions between urban and natural sites

Cumulative distribution functions illustrating differences in perch diameter niche between natural (red) and urban (blue) for *A. cristatellus* (a) females and (b) males, and *A. sagrei* (c) females and (d) males.

CHAPTER 2

EFFECTS OF SUBSTRATE INCLINATION AND SMOOTHNESS ON PERFORMANCE AND HINDLIMB KINEMATICS IN TWO *ANOLIS* LIZARD SPECIES

In preparation for submission to the Journal of Experimental Biology

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Keywords: artificial surface, urbanization, urban development, sprint speed, locomotion

SUMMARY

Animal locomotion involves maneuvering on a variety of substrates, prompting a range of responses, from morphological specialization to flexible locomotor strategies. Arboreal locomotion in particular requires solutions to challenges including variation in inclination, diameter, and substrate roughness. When humans alter habitats, they can modify the substrates available for arboreal animals. For example, urbanization increases the frequency of very smooth substrates by adding artificial objects, such as metal poles, painted walls, and wood fences, to the habitat. Despite the challenges to locomotion that smooth structures present, many species persist in cities without avoiding them. Therefore, urban animals may employ strategies to overcome these challenges that animals from natural habitats do not. We assessed locomotor performance and two-dimensional hindlimb kinematics of two species of *Anolis* lizards (*Anolis cristatellus* and *Anolis sagrei*) from both urban and natural habitats in Miami, FL, running on three substrates of increasing smoothness (rough bark, concrete blocks, and smooth, unpainted wood) on inclined (37°) and vertical tracks. We found that on vertical tracks, lizards ran slower, took shorter strides, moved their bodies shorter distances with a single step, kept their foot in contact with the substrate for longer (duty factor), and exhibited more contracted limb postures upon finishing a step than when running on the inclined track. We also observed these kinematic effects on the smooth wood substrate compared to the rough bark, though this effect was not as strong as with incline. We did not find an overall effect of habitat type on limb kinematics, such that urban lizards did not use different gait characteristics or hindlimb positions compared to natural lizards. While smooth

substrates do impose functional challenges for lizards, urban lizards, which likely have greater exposure to these substrates, did not adjust their movement to increase performance. This result, and similarity of kinematic strategies between the two species, suggests high behavioral flexibility for lizards using smooth substrates. Without better strategies for running on smooth substrates, urban lizards may require selection on morphology to improve performance.

INTRODUCTION

Substrate properties can limit the efficacy of locomotion as animals move through their habitats. For example, many animals need to sprint close to maximum velocities to flee from predators or to capture prey (Irschick and Garland, 2001; Irschick and Higham, 2016), but maximum velocity is not achievable on all types of substrates (Tulli et al., 2012). Arboreal animals in particular must overcome several challenges because of the properties of the substrates they use for locomotion. For example, movement up steep inclines can pose functional challenges, especially for larger animals, as increased body mass can result in increased gravitational forces that impede effective locomotion (Cartmill, 1985; Jayne and Irschick, 1999). In addition, variation in substrate diameter, and its interaction with other substrate properties, has been shown to influence locomotion in a variety of ways, from affecting how an animal grips a branch (Lammers, 2004; Herrel et al., 2013) to impeding movement altogether (Astley and Jayne, 2007). Finally, for some animals, falling from arboreal perches can pose the danger of injury or death, as well as temporary removal of an animal from its home range. These aspects of the arboreal habitat present trade-offs for locomotion in many animals (Losos et al., 1993; Vanhooydonck and Van Damme, 2001). For example, one trade-off involves the speed and stability of locomotion, such that specializations enhancing stability will also result in reduced speed, and vice versa. For instance, to prevent toppling backwards as incline increases, some animals hold their bodies closer to the substrate during locomotion and reduce stride lengths to increase contact time with the surface, resulting in decreased speed (Spezzano and Jayne, 2004; Lammers et al., 2006; Herrel et al., 2013; Birn-Jeffery and Higham, 2016). Narrow perches also pose a velocity-stability tradeoff (Spezzano and Jayne, 2004), resulting in decreased maximal velocity and acceleration capacity for some *Anolis* lizard species (Losos and Irschick, 1996; Vanhooydonck et al., 2006). In sum, there is a substantial body of research which shows that variables associated with arboreal habitats are likely to have strong impacts on the locomotion of animal groups, although such studies have largely focused on relatively few groups, mostly lizards and primates. However, there has been little work relating such locomotor effects to variation in habitat use in novel environments.

Urbanization is one such novel environment that significantly changes the habitat with profound effects for many organisms. Ectotherms may encounter thermoregulatory challenges in urban areas because environmental temperature increases (Ackley et al., 2015), whereas songbirds must overcome traffic noise to make their calls heard (Chace and Walsh, 2006). Arboreal animals face drastic modifications to the habitat features that they are specialized to use. Specifically, trees, branches, and other natural substrates are reduced in density by clearing vegetation, whereas artificial structures, such as metal poles, wooden fences, and painted walls, are added (Battles et al., 2018). Species with ecological and evolutionary history of moving on natural vegetation in a dense matrix of the forest must now maneuver on human-made structures and handle more open space between trees and other habitat patches (Winchell et al., 2018; Battles et al., 2018). Perhaps one of the greatest contrasts between urban and natural habitats for arboreal animals is the increased frequency of vertical, smooth surfaces in urban areas, such as poles and walls (Kolbe et al., 2016b). The high frequency of smooth substrates in cities may present novel locomotor challenges for animals that normally use rougher substrates more common in natural habitats, but we do not yet know whether or how locomotion changes in response to them.

Smooth substrates are not exclusive to urban habitats, but compared to natural habitats, smooth and vertical substrates are much more common in urban areas (Kolbe et al., 2016b; Battles et al., 2018). However, the effect of substrate smoothness on locomotion is rarely explored. From previous studies, when inclines increase, *Anolis* lizards decrease their velocity because of both changes in limb positioning (i.e., to hold the body closer to the locomotor surface) and stride lengths (Spezzano and Jayne, 2004). Velocity also decreases for anoles running on smooth surfaces (Kolbe et al., 2016b). While foot traction is generally worse on low-friction substrates (Clark and Higham, 2011), we do not yet know how gait or limb positions change for quadrupedal organisms on smooth surfaces. However, for geckos, which are notable for their ability to traverse smooth substrates of all orientations, the clinging benefits offered by highly specialized toepad morphology come with a cost of reduced maximal velocity. For geckos, increasing incline decreases velocity more than substrate smoothness (Russell and Higham, 2009). Furthermore, their specialized toes contribute to better acceleration performance on smooth substrates compared to rough substrates (i.e., wire mesh and rough cloth) because their toepads can adhere more completely, or create van der waals interactions, more often on the smooth surface (Vanhooydonck et al., 2005). Gecko locomotor performance on smooth substrates has been studied more so because of the unique combination of convenience to researchers and the physical ability of geckos rather than specific ecological relevance. Because urbanization exposes a variety of taxa to novel artificial structures, which are often smooth and vertical, we investigated locomotor performance and kinematics for organisms not particularly specialized to use these substrates.

We investigated the effects of substrate inclination and smoothness on locomotor performance and two-dimensional hindlimb kinematics for populations from natural forest habitats and urban sites, the latter of which are exposed to artificial substrates. We studied two species of *Anolis* lizards, which are an excellent system to explore novel substrate use because of extensive previous research on locomotor performance and kinematics (e.g., Losos, 1990; Foster and Higham, 2014), and several *Anolis* species are found in both urban and natural areas (Marnocha et al., 2011; Winchell et al., 2018). Two introduced species, *Anolis sagrei* and *Anolis cristatellus*, occupy both natural and urban habitats in Miami, FL (Kolbe et al., 2007; Kolbe et al., 2016a). Both species specialize in similar structural habitats, commonly occupying the ground and perches up to ~2 m (Salzburg, 1984; Losos, 2009), and therefore may share kinematic responses to variation in inclination and smoothness. Furthermore, lizards utilize broad-diameter perches in urban areas, which are frequently artificial, smooth structures (Winchell et al., 2016; Battles et al., 2018). We conducted trials in which lizards ran on two inclines (37° or inclined, and 90° or vertical) and three substrates of increasing smoothness (rough bark, concrete block, and smooth wood). We predicted that (1) maximal sprint speed and stride length will decrease on vertical structures, and (2) to overcome increased effects of gravity lizards will utilize a broader stance, bringing their body closer to the track surface (Jayne and Irschick, 1999). We also predicted that (3) velocity and stride length will decrease on smooth substrates. Finally, we predicted that (4) lizards from urban habitats, which encounter smooth, vertical substrates more often, should employ kinematic strategies (e.g. gait characteristics and limb positioning) that are more effective on smooth, vertical substrates, and will therefore perform better on these substrates than lizards from natural habitats.

MATERIALS AND METHODS

Study species and collection

We studied two species of *Anolis*, small insectivorous lizards found naturally in southern North America, Central and South America, and throughout the Caribbean (Losos, 2009). *Anolis sagrei* is widespread in Miami and throughout Florida, whereas the distribution of *A. cristatellus* is more restricted, radiating out from two independent points of introduction in the Miami area (Kolbe et al., 2016a). We collected males of *A. cristatellus* from natural (N = 13) and urban (N = 15) sites, and *A. sagrei* from natural (N = 15) and urban (N=15) sites in Miami, FL. We measured mass, SVL using a ruler, hindlimb length with digital calipers, and counted the number of toepad lamellae for each individual. We calculated relative hindlimb lengths as residuals of a non-linear, scaled-SVL index (Peig and Green, 2009).

Racetrack procedure

We ran lizards on six tracks that were combinations of three substrates, bark (very rough), cinder block (somewhat smooth; hereafter called ‘block’), and unfinished plywood (very smooth; hereafter called ‘wood’), and two inclines, 37° (inclined) and 90° (vertical). To keep lizards from jumping off the sides, both tracks had sidewalls.

Lizards were placed at the base of each track and allowed to run upwards. We encouraged movement with gentle taps near their tails when needed. To increase our chances of capturing the best performance, each lizard ran three to five times on each track within a single trial. We filmed all lizard runs at 240 frames-per-second with two digital cameras (Casio Exilim Ex-zr1000), positioned dorsally and laterally to lizards on the tracks. Such filming frequencies are effective for estimating basic movements of small lizards with the running velocities (Walker, 1998). We ran all lizards at the same temperature (~27° C) and lizards were run no more than twice a day, with a minimum of one hour between trials.

Limb kinematic measurements

Out of the total number of lizards collected from the field (N=58), we recorded data from 13 individuals of each species (seven from natural populations and six from urban populations) that had complete, steady-speed runs on all six tracks. For the longest stride within each run (multiple runs per track; see above), we measured velocity, stride length (i.e., the distance between successive footfalls), stride duration (i.e., the time for that stride), step length (i.e., the distance the body advances from when the foot is planted to when it is lifted), and duty factor (i.e., the proportion of the stride in which the foot is in contact with the surface). At two points within each complete stride, the footfall of the hindlimb and the following footrise, we measured sprawl (i.e., the angle around the hip), excursion (i.e., the angle around the knee), and step width (i.e., the single-axis distance between the ankle and the midline) (Fig. 1). We used ImageJ (Schneider et al., 2012) to measure these variables from still frames of the videos.

Statistical Analyses

We performed all statistical analyses in R (R Core Team, 2015), and analyzed both species separately. For each kinematic variable and velocity (Tables 1, 2), we performed mixed-model ANOVA with factors including incline (inclined or vertical), substrate (bark, block, or wood), population source (urban or natural), and their interactions, as well as lizard ID as a random effect. We used SVL and relative hindlimb length as covariates, even when not significant. We also calculated correlations between the kinematics variables, SVL, relative hindlimb length and velocity, and performed Pearson's t-tests to test for differences between urban and natural populations for morphological variables. We used t-tests to test for differences in morphological variables between urban and natural populations (full, 58-lizard sample).

RESULTS

Morphology

For all lizards captured, of which only a subset had full kinematics data, urban and natural *A. cristatellus* did not differ in SVL, mass, relative hindlimb length, or the number of toepad lamellae. In contrast, for all lizards captured, urban *A. sagrei* were larger (SVL: $t_{110.7} = -4.88$, $p < 0.0001$; mass: $t_{108.6} = -6.90$, $p < 0.0001$), had relatively longer hindlimbs than those from natural sites ($t_{105.5} = -2.84$, $p < 0.01$), and larger toepads ($t_{104.5} = -4.44$, $p < 0.001$).

Kinematics

Velocity

In *A. cristatellus*, velocity decreased significantly on vertical compared to inclined tracks, and on the wood tracks compared to both block and bark (Table 1, Fig. 2). There was a significant interaction between incline and population source, such that the difference in velocity between urban and natural lizards was less on vertical compared to the inclined tracks (Table 1). Urban *A. cristatellus* also tended to run faster overall, but this result was not significant (Fig. 2). For *A. sagrei*, velocity decreased significantly on vertical compared to inclined tracks as well as on the smoother tracks compared to bark (Table 2, Fig 3). Population source also had a three-way interaction with incline and substrate, such that urban lizards ran slower than natural lizards on only the vertical wood (smooth) track (Table 2).

Whole-stride characteristics

For *A. cristatellus,* both incline and substrate had highly significant effects on stride length (Table 1), such that stride length was shorter on vertical tracks, and on wood compared to bark tracks (Table 1; Fig. 4A). The interaction of substrate and habitat source shows that lizards from urban areas decrease less in stride length between block and wood substrates than do lizards from the natural site. Stride length also increased with increasing hindlimb length (Table S1). Similarly, step length significantly decreased on vertical tracks, and as tracks became smoother, but the decrease between inclined and vertical was less on the block substrate than on the other two (Table 1, Fig. 4B). Furthermore, while step lengths sometimes differed by population source on the inclined track, they did not differ on the vertical track (Fig. 4B). The duty factor increased significantly on vertical compared to inclined tracks, and with increasing smoothness, but with some variation based on population source for the differences between block and the other substrates (Table 1, Fig. 4C). Stride duration decreased on vertical compared to inclined tracks (Table 1, Fig. 4D).

For *A. sagrei*, both incline and substrate had highly significant effects on stride length (Table 2). Stride length was shorter on vertical tracks, with a more exaggerated difference for urban lizards, and as tracks became smoother, with significantly shorter stride lengths on block compared to bark, and wood compared to block (Fig. 5A). Similarly, step length significantly decreased on vertical tracks and this decrease was greater for urban lizards than natural lizards (Table 2, Fig. 5B). Step length also decreased as tracks became smoother (Table 2, Fig. 5B). Duty factor increased significantly on vertical tracks and as tracks became smoother, but for urban lizards, duty factor did not increase from block to wood tracks (Table 2, Fig. 5C). Stride duration increased on vertical compared to inclined tracks (Table 2, Fig. 5D).

Limb angles

For *A. cristatellus*, incline had a highly significant effect on sprawl angle in both foot positions, but the effects of substrate were not always significant (Table 1). With the foot down, the sprawl angle was greater on vertical compared to incline tracks, and this increase in sprawl angle was greater between inclined and vertical wood tracks than bark (Table 1, Fig. 6A). With the foot up, the sprawl angle was significantly smaller on vertical tracks compared to inclined and on wood and block tracks compared to bark (Table 1, Fig. 6B). The excursion angle was significantly influenced by both inclination and substrate (Table 1). With the foot down, excursion angles were greater on vertical tracks and smaller on wood compared to bark substrates (Fig. 6C). With the foot up, the excursion angle was smaller on the vertical track and on wood compared to the other substrates, but there was no difference among substrate types on the inclined track (Fig. 6D). With the foot down, stride width increased on vertical tracks for both populations, but more so for urban lizards (Table 1, Fig. 6E). Also with the foot down, SVL had a significant positive effect on stride with ($F_{1,8} = 10.9$ $P < 0.05$). With the foot up, stride width increased on vertical tracks (Table 1, Fig. 6F).

For *A. sagrei*, incline had a highly significant effect on sprawl angle in both foot positions, but the effects of substrate were not always significant (Table 2). With the foot down, the sprawl angle was greater on vertical compared to incline tracks (Table 2, Fig. 7A). With the foot up, the sprawl angle was significantly smaller on vertical tracks compared to inclined and on wood and block tracks compared to bark (Table 2, Fig. 7B). The decrease in angle was also greater on block and wood tracks than on the bark (Table 2, Fig. 7B). The excursion angle with the foot down was not affected by any of the factors and the model was quite poor ($R^2 = 0.07$; Table 2). With the foot up, the excursion angle was smaller on the vertical tracks and on wood compared to bark tracks, with the decrease in angle greater on the wood and block tracks than the bark (Table 2, Fig. 7D). Furthermore, for urban lizards, the excursion angle with the foot up did not differ between the inclined and vertical bark track. With the foot down, stride width increased on vertical tracks for both populations, but was greater for urban lizards (Table 2, Fig. 7E). SVL had a significant positive effect on stride width the foot down ($F_{1,9} = 38.7$, $P < 0.001$). With the foot up, stride width increased on vertical tracks (Table 2, Fig. 7F).

Leg angles may be more easily visualized with a schematic of angles with the foot up and down (*A. cristatellus* – Fig. 8; *A. sagrei* – Fig. 9).

DISCUSSION

Effects of incline and smoothness

Our study of locomotion in two species of *Anolis* lizards from urban and natural habitats revealed several key findings. As predicted, for both species, we found that on smooth, vertical substrates compared to rough, inclined substrates, velocity decreases, stride and step lengths decrease, and duty factor increases. Furthermore, matching predictions, we show that these same kinematic responses were also occurred in response to smooth substrates (also see Kolbe et al. 2016b). In addition to changes in stride characteristics, lizards overcame functional challenges through manipulation of limb kinematics as we predicted. In both species, with the foot up (step finishing), the leg angles were reduced (i.e., the legs were more contracted, and the foot positioned more laterally to the body on vertical and smooth substrates). This resulted in a greater stride width on vertical tracks, but stride width did not increase with the smoothness of the surface. With the foot down, (step/stride beginning), leg angles were larger on vertical tracks, causing stride width to be greater as well. Substrate smoothness and incline influenced leg angles less than whole-stride characteristics. We found no support for our prediction that lizards from urban areas would maneuver differently on smooth substrates compared to lizards from natural areas for either species. While there were a few interactions between population source and locomotor variables, we did not find any consistent patterns for improved performance on smooth substrates by urban lizards.

Lizards, and other taxa, often employ a range of strategies to enable them to move effectively across substrates they typically encounter (Biewener, 2003). Lizards have been shown to modulate stride length and stride frequency, their primary agents of locomotor effort, and that these characteristics change in a regular way with incline (Huey and Hertz, 1984; Irschick and Jayne, 1998; Higham et al., 2011). The decrease in velocity on inclines has been attributed to changes in stride characteristics that help lizards overcome the effects of gravity and the potential to topple backwards, such as holding the body and center of mass closer to the locomotor surface (Cartmill, 1985), which we also observed in this study. Furthermore, shortened stride lengths, likely affected by more-contracted limb positions, also contribute to reduced velocity on inclines (Irschick and Jayne, 1998; Jayne and Irschick, 1999). Our results also support previous findings that as inclines increase, step lengths decrease (Jayne and Irschick, 1999) and duty factor increases (Foster and Higham, 2012). Duty factor also increases with decreased speed (Lammers et al., 2006). In response to increasing inclines, lizards and other taxa typically cannot both run at maximal velocity and remain stable (Foster and Higham, 2012). While not evaluated here, lizards also show predictable strategies in other behavioral traits for changes to substrate diameter (e.g. Losos and Irschick, 1996). Importantly, our results show that the strategies for overcoming the challenge of increasing incline are also effective for maintaining stability on smooth substrates. Specifically, the effects of smoothness on lizard velocity and stride characteristics are in the same direction as for inclination, but the effects are not as strong.

Urbanization and Locomotion

The growth of urban areas worldwide exposes countless species to this human-modified habitat, and species respond on a spectrum from avoidance to dependence on urban habitats (Fischer et al., 2015). How animals use urban habitats is increasingly studied to better understand and predict the persistence of populations in these widespread habitats (Shochat et al., 2006; Sol et al., 2013; Winchell et al., 2018). Urban lizards encounter very smooth artificial surfaces more often than lizards from natural habitats (Kolbe et al., 2016b; Battles et al., 2018), and this work shows that there is substantial flexibility in the movement patterns of anoles, a result generally consistent with the large body of work showing that climbing lizards can move on a wide range of surfaces using the same basic strategy. For instance, in a study testing acceleration, sprint speed, and endurance on rough and smooth surfaces, Vanhooydonck et al. (2015) found that multiple species of lacertid lizards were able to attain relatively high speeds after running more than 50 cm on smooth substrates, even though they differed in morphology. Urban lizards of both species did not alter limb angles to any advantage, and the effects of population source nearly always interacted with incline, not smoothness. However, considering that multiple, phylogenetically disparate taxa respond to increased incline with shorter stride lengths and broader limb positions (anoles – Spezzano and Jayne, 2004; lizards – Bergmann and Irschick, 2010; amphibians – Herrel et al., 2013; mammals – Lammers et al., 2006), the relatively recent population divergence because of urbanization may not prompt novel strategies for moving on smooth substrates. Even the two species in this study handled smooth substrates almost identically. While our result is somewhat surprising, it would also explain in part why anole lizards are able to so effectively utilize urban habitats (Kolbe et al. 2016b). Nevertheless, further research is needed to determine if the documented submaximal performance on smooth substrates has an impact on fitness in urban areas.

Changes in morphology may allow urban populations to gain performance improvements that locomotor behavior could not realize. Animals often display a high degree of specialization in terms of morphological adaptations that enable them to move effectively on surfaces they commonly encounter (Biewener, 2003; Irschick and Higham, 2016). Anole species that use different structural habitats show considerable variation in hindlimb morphology, which allows shorter-limbed species to more easily move on, and occupy narrow perches, and longer-limbed species to move faster on broader surfaces (Losos and Sinervo, 1989; Losos, 2009). Similarly, in urban habitats, where anoles encounter and use broader perches more often compared to natural habitats (Battles et al., 2018), some anole populations have evolved longer limbs (Winchell et al., 2016), which should increase stability and velocity on broad substrates (Kolbe et al. 2016b). However, smooth substrates may disrupt the traditional relationship between morphology and habitat use, as the broadest structures in urban habitats are often artificial (Winchell et al., 2018; Battles et al., 2018), and typically much smoother than those found in natural habitats (Kolbe et al. 2016b). In our study, hindlimb length and stride length were positively correlated in *A.*

cristatellus, and greater strides resulted in faster sprint speeds across all tracks. Therefore, morphological changes that improve performance on broad structures (e.g. longer hindlimbs) may also increase performance on smooth substrates. In urban populations, we did not find greater relative hindlimb lengths in *A. cristatellus*, but did in *A. sagrei*. We can think of two potential reasons why our results did not consistently follow the predicted trend. First, *A. cristatellus* was introduced more recently than *A. sagrei*, and so may have had less time for urban and natural populations to diverge. Second, *A. cristatellus* was likely introduced to Miami from urban areas in Puerto Rico (Kolbe et al., 2007), and thus may be *preadapted* to urban habitats in Miami (Hufbauer et al., 2012). Future research should evaluate lizards from urban and natural sites in their native ranges, where populations have been exposed to urbanization for longer and potential founder effects associated with invasion are not relevant.

The relatively high frequency of smooth substrates in urban areas adds to the functional challenges faced by urban organisms. Our results show that lizards modulate strides and limb kinematics when running on vertical and smooth substrates, resulting in decreased velocity. We found much stronger effects of incline than smoothness on all locomotion response variables; smoother surfaces are certainly challenging, but they appear to magnify effects of incline rather than pose novel functional challenges. Interestingly, urban populations did not utilize different kinematic strategies than natural populations, despite likely encountering smooth substrates more often. Because locomotor performance seems more likely to improve with morphological changes, we expect that smooth, artificial structures will impose selection on urban lizards for increased limb length. We did not test effects of diameter in this study, but studies have shown strong effects of diameter on performance and kinematics (Spezzano and Jayne, 2004), therefore interactions among diameter, incline, and substrate smoothness are likely significant, particularly for urban lizards.

We also note that the ultimate fitness value of performance traits remains a topic of debate (Irschick et al., 2008), and as noted by others (Garland and Losos 1994), not all ecological situations require maximal sprint capacity. Behavioral strategies are also critical for avoiding predators, and the use of such strategies often depends on the habitat occupied (e.g. availability of nearby refuges) and the predator in question (Hopper, 2001; Templeton and Shriner, 2004). Therefore, while sprint speed has been shown in lizards to decrease on smooth, vertical substrates, anoles may utilize different antipredator behaviors when using these substrates, such as jumping instead of sprinting. When approached by a predator, they may also flee earlier when using smooth, vertical surfaces to account for their reduced velocity (Aviles-Rodriguez, 2015). Anole predators may also suffer reduced performance on smooth substrates, and future research should investigate this possibility. Alternatively, running at top sprint speeds may be less relevant for other behaviors like foraging or pursuing mates (Irschick and Garland, 2001), and as such, lower values of maximum sprint speed on smooth, vertical substrates may not have a significant negative effect on fitness for most situations. Furthermore, some *Anolis* species in natural habitats alter their behavior to use substrates that enhance locomotor performance more often than other available substrates (Irschick and Losos, 1999; Gilman and Irschick, 2013) or avoid those substrates that confer poor performance (Irschick and Losos, 1999).

Nonetheless, urbanization presents a host of ecological challenges to non-human urban dwellers, with smooth substrates as a significant challenge for locomotion. As ecologists gain understanding of the mechanisms behind habitat use and performance capability, we can better evaluate persistence of organisms in urban areas and other human-modified habitats.

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Table 1. Effects (*F*-values) from three-way ANOVAs performed separately on each variable for *A. cristatellus*. SVL and relative hindlimb length were included as covariates. Full table with F-values of covariates and variance from lizard ID in Table S1.

Anolis cristatellus	ANOVA effects			Interactions				
	Incline	Substrate	Type	Incline X Substrate	Substrate X Type	Incline X Type	3-way interaction	Model R^2 (marginal)
Velocity	194.6**	11.4**	0.0	1.4	1.1	4.0*	0.5	0.47
Stride Length	353.4**	30.7**	0.3	1.5	4.3*	0.9	2.7	0.66
Step Length	298.3**	36.4**	0.2	4.2*	4.2*	1.5	4.4*	0.63
Stride Duration	8.3*	0.1	0.4	1.6	0.4	1.4	2.0	0.18
Duty Factor	160.4**	37.5**	0.2	5.0*	5.2*	0.0	3.5*	0.51
Sprawl, foot down	77.3**	1.6	1.1	7.2**	0.1	0.0	1.6	0.29
Excursion, foot down	40.0**	6.1*	0.1	1.0	1.3	2.8	0.0	0.20
Sprawl, foot up	96.3**	8.7**	0.0	2.6	0.2	0.0	0.3	0.35
Excursion, foot up	80.2**	5.4*	2.1	5.4*	0.9	0.6	0.5	0.33
Stride Width, foot down	66.5**	0.2	0.2	1.3	1.5	6.2*	2.2	0.30
Stride Width, foot up	59.9**	1.6	0.0	2.7	1.8	1.4	1.4	0.26

**P*<0.05, ***P*<0.001

Table 2. Effects (*F*-values) from three-way ANOVAs performed separately on each variable for *A. sagrei*. SVL and relative hindlimb length were included as covariates. Full table with F-values of covariates and variance from lizard ID in Table S2.

Anolis sagrei	ANOVA effects			Interactions				
	Incline	Substrate	Type	Incline X Substrate	Substrate X Type	Incline X Type	3-way interaction	Model R^2 (marginal)
Velocity	368.9**	6.06*	1.4	1.9	1.3	0.0	3.4*	0.56
Stride Length	478.7**	26.9**	0.2	1.9	0.9	6.6*	0.3	0.67
Step Length	273.0**	36.6**	0.0	1.1	0.3	5.3*	0.2	0.59
Stride Duration	11.7**	2.8	0.6	2.1	1.0	1.2	2.3	0.15
Duty Factor	102.0**	34.8**	1.5	0.6	3.3*	2.8	0.5	0.45
Sprawl, foot down	6.9*	2.4	0.6	2.6	1.3	0.2	2.7	0.12
Excursion, foot down	3.7	2.5	0.5	0.2	0.3	0.0	3.8	0.07
Sprawl, foot up	118.3**	61.4**	0.0	5.8*	0.3	0.5	1.2	0.55
Excursion, foot up	70.2**	9.1**	0.0	6.2*	0.7	1.1	3.2*	0.32
Stride Width, foot down	59.7**	0.5	6.1*	1.3	0.1	7.0*	0.5	0.36
Stride Width, foot up	59.2**	0.0	0.2	0.5	0.4	0.8	1.6	0.24

**P*<0.05, ***P*<0.001

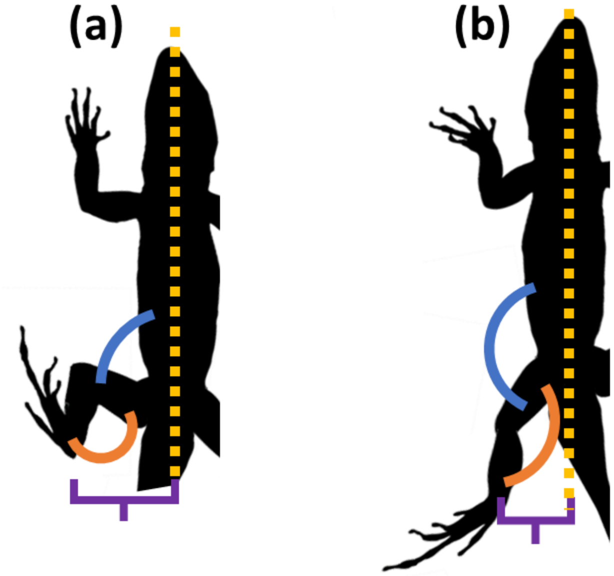

Figure 1. Diagram of measurements taken for each foot position (a: footfall, step beginning; b: footrise, step end): excursion, blue arc at hip; sprawl, orange arc at knee, step width, purple bracket between midline and ankle.

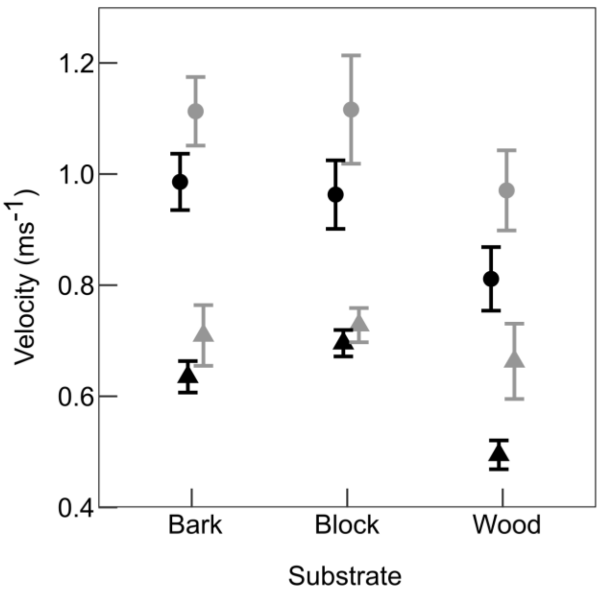

Figure 2. Velocity (mean ± S.E.) for *A. cristatellus* on each substrate, with inclined tracks represented with circles, vertical tracks represented with triangles, and colors showing the population source (black = natural, grey = urban).

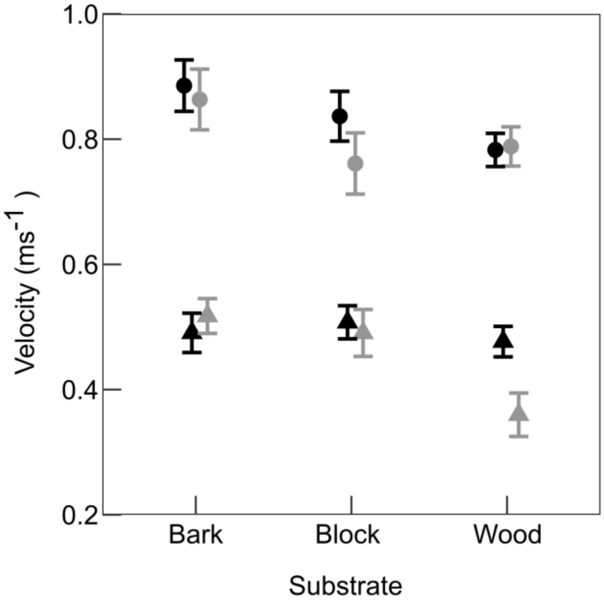

Figure 3. Velocity (mean ± S.E.) for *A. sagrei* on each substrate, with inclined tracks represented with circles, vertical tracks represented with triangles, and colors showing the population source (black = natural, grey = urban).

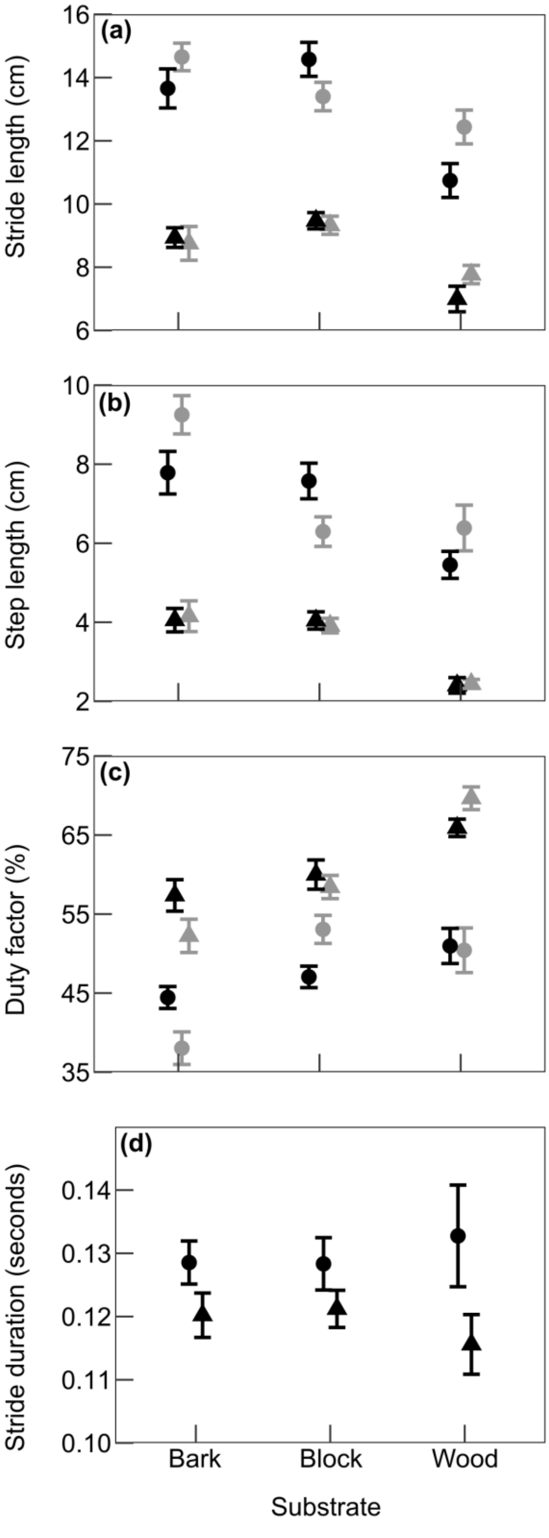

Figure 4. Gait characteristics (mean ± S.E.) by substrate for *A. cristatellus*, with inclined represented with circles and vertical with triangles. a) stride length, b) step length, c) duty factor, and d) stride duration. For gait characteristics affected by population source (a-c), natural population is in black and urban in grey.

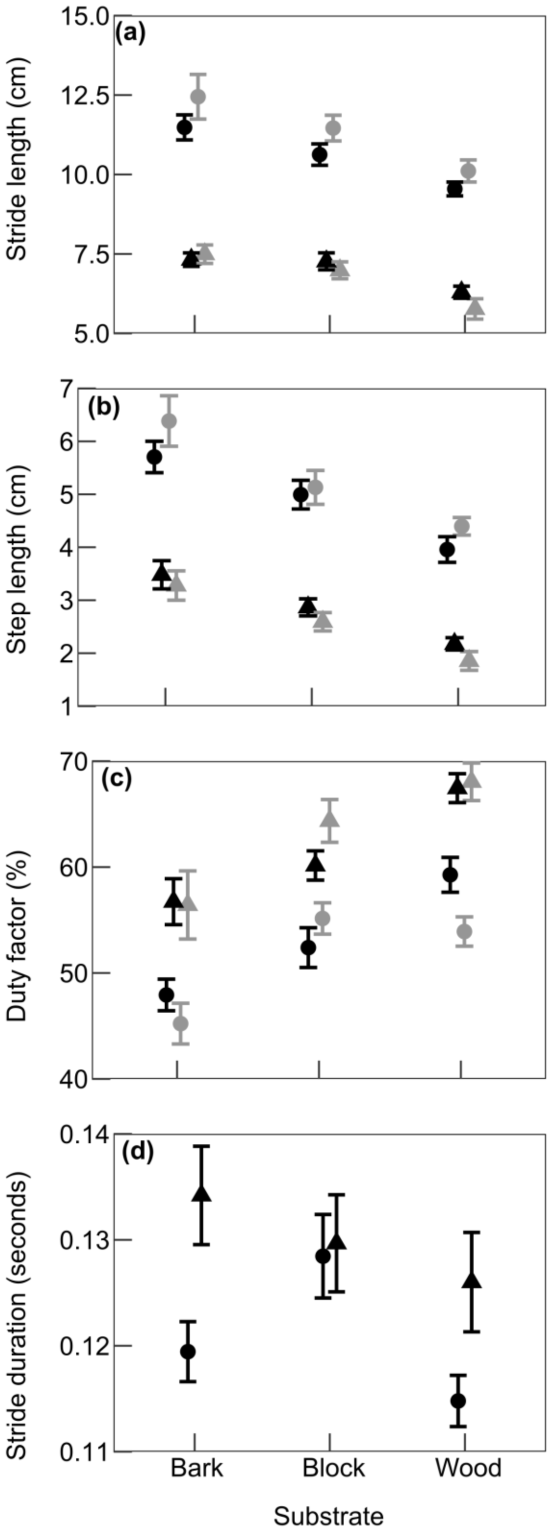

Figure 5. Whole stride characteristics (mean ± S.E.) by substrate for *A. sagrei*, with inclined represented with circles and vertical with triangles. a) stride length, b) step length, c) duty factor, and d) stride duration. For gait characteristics affected by population source (a-c), natural population is in black and urban in grey.

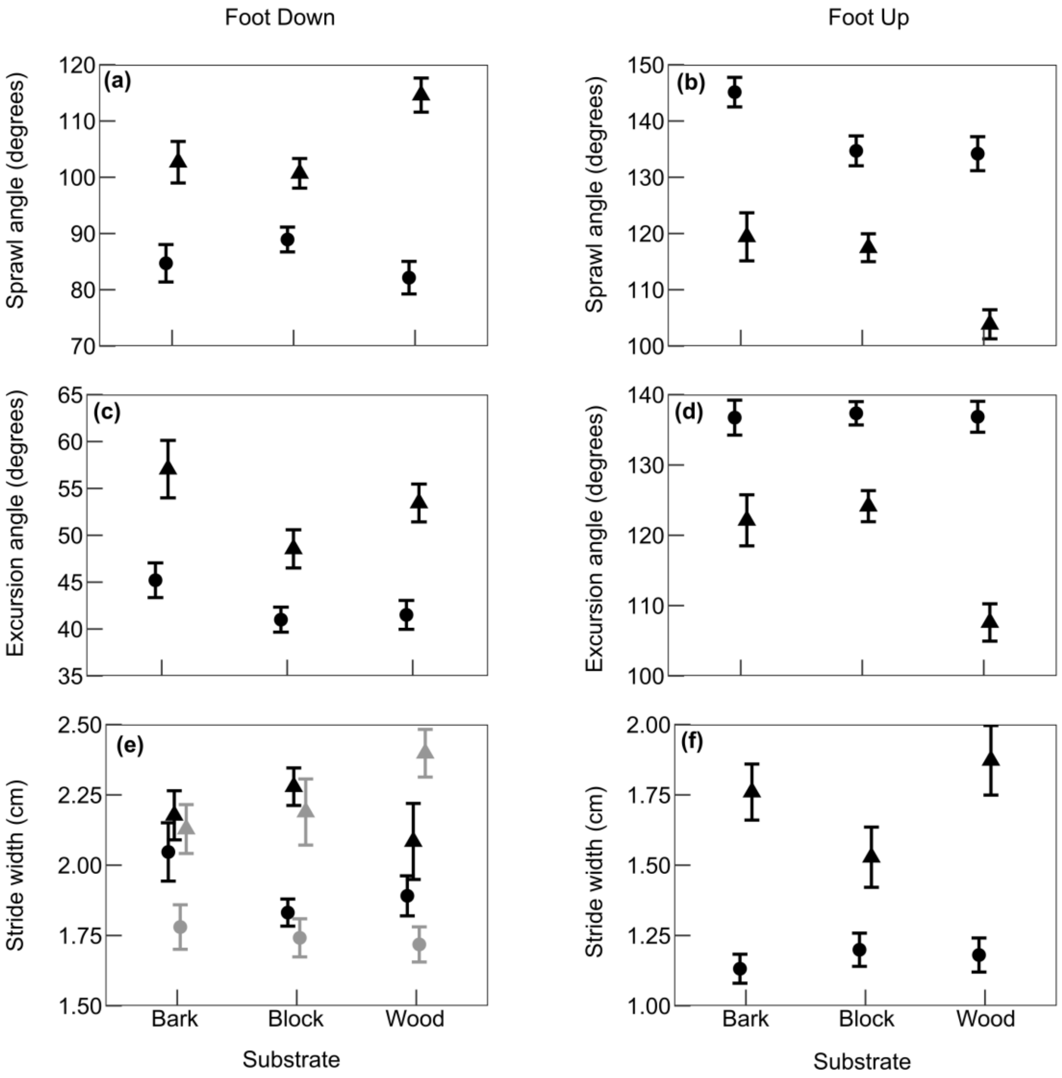

Figure 6. *Anolis cristatellus* mean limb angles with the foot down: a) sprawl; c) excursion, and with the foot up: b) sprawl; d) excursion ± S.E. Mean (±SE) stride widths with the (e) foot down and the (f) foot up. The inclined track is represented by circles and the vertical by triangles. When population source significantly affected a kinematic variable (e), natural lizards are shown in black, and urban in grey.

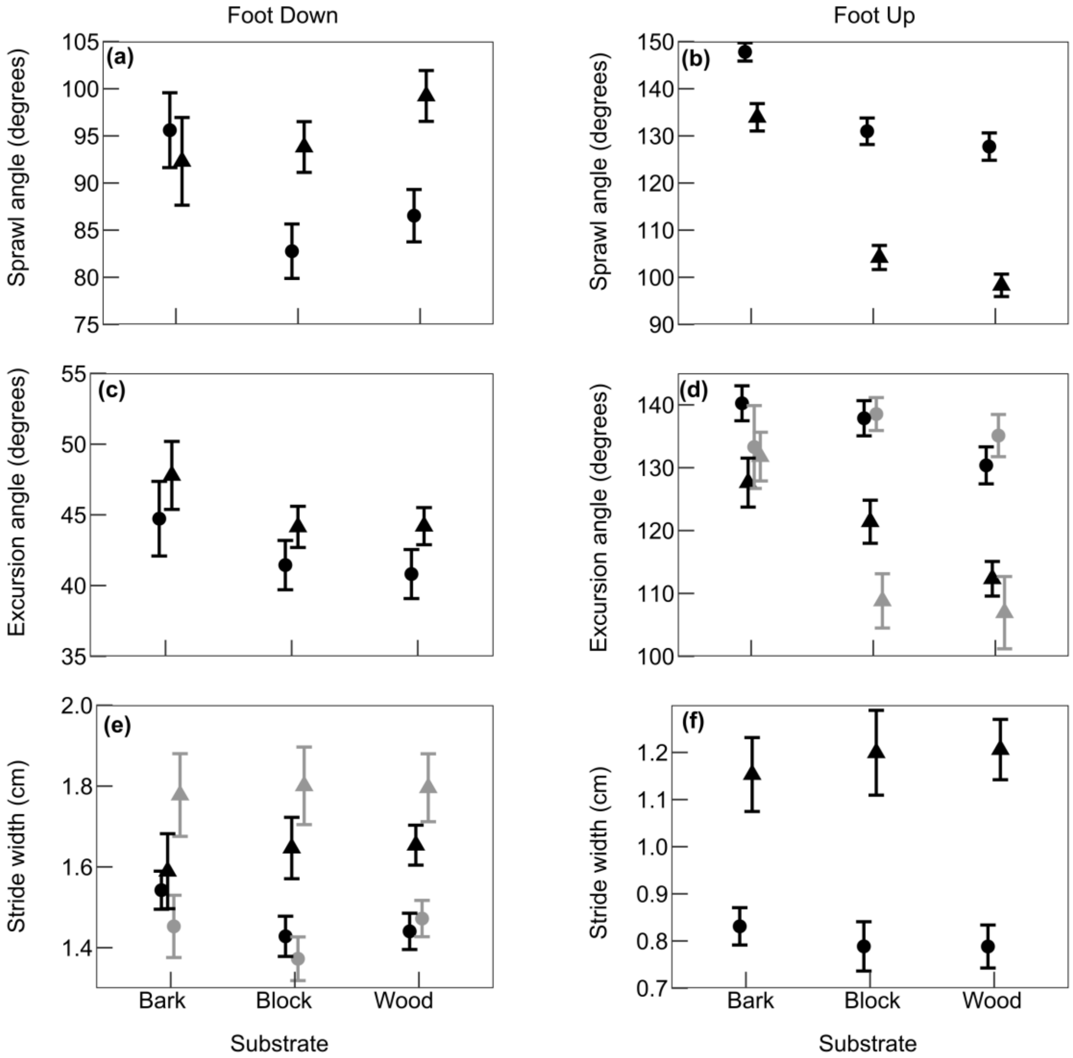

Figure 7. *Anolis sagrei* mean limb angles with the foot down: a) sprawl; c) excursion, and with the foot up: b) sprawl; d) excursion ± S.E. Mean (±SE) stride widths with the (e) foot down and the (f) foot up. The inclined track is represented by circles and the vertical by triangles. When population source significantly affected a kinematic variable (d,e), natural lizards are shown in black, and urban in grey.

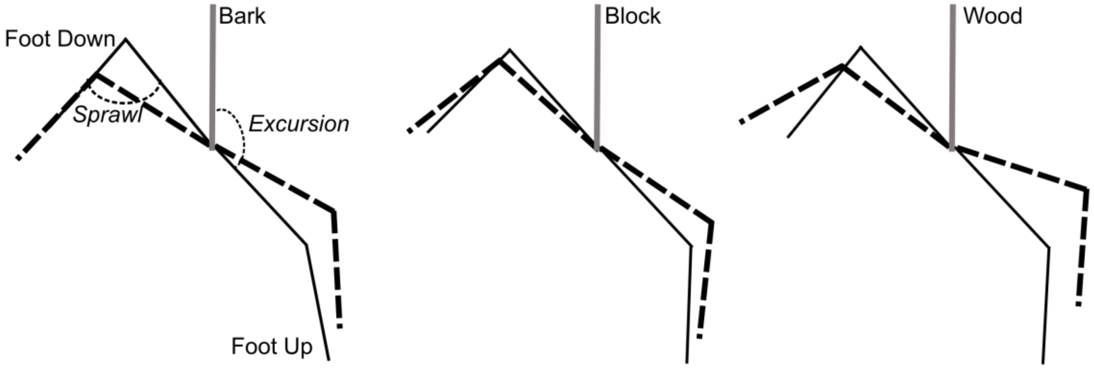

Figure 8. Schematic of mean sprawl and excursion angles when the foot is down (left side of each cartoon) and up (right side of each cartoon) on each of the substrates for *A. cristatellus*. Solid lines show leg positions on the inclined tracks and dashed lines show leg positions on the vertical tracks.

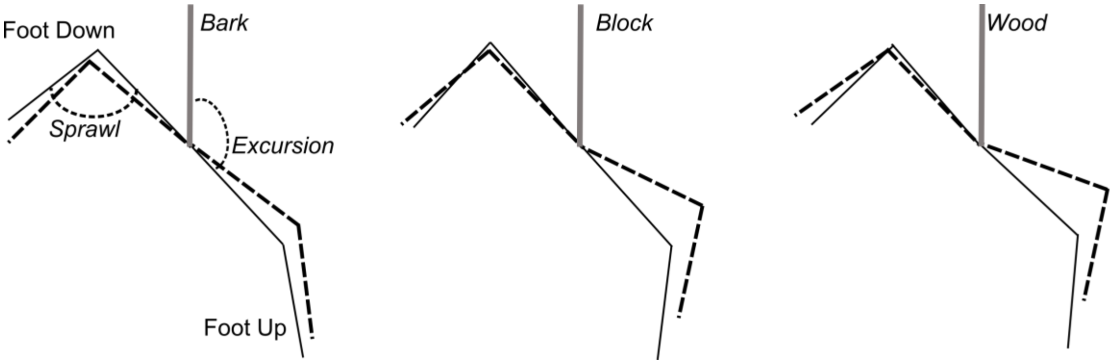

Figure 9. Schematic of mean sprawl and excursion angles when the foot is down (left side of each cartoon) and up (right side of each cartoon) on each of the substrates for *A. sagrei*. Solid lines show leg positions on the inclined tracks and dashed lines show leg positions on the vertical tracks.

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SUPPLEMENTARY MATERIAL

Table 1. Extension of Table 1 (Chapter 2), showing effects of treatment variables on Velocity and other kinematic variables in the models for *A. cristatellus*.

	Incline	Substrate	Type	Incline X Substrate	Substrate X Type	Incline X Type	3-way interaction	Relative hindlimb	SVL	Lizard ID (Random)	Model R^2
Velocity	194.6**	11.4**	0.0	1.4	1.1	4.0*	0.5	1.7	3.2	0.21	0.47
Stride Length	353.4**	30.7**	0.3	1.5	4.3*	0.9	2.7	6.9*	0.5	0.03	0.66
Step Length	298.3**	36.4**	0.2	4.2*	4.2*	1.5	4.4*	2.2	0.0	0.05	0.63
Stride Duration	8.3*	0.1	0.4	1.6	0.4	1.4	2.0	0.6	7.7*	0.00	0.18
Duty Factor	160.4**	37.5**	0.2	5.0*	5.2*	0.0	3.5*	0.5	0.7	7.51	0.51
Sprawl, foot down	77.3**	1.6	1.1	7.2**	0.1	0.0	1.6	0.1	0.0	0.10	0.29
Excursion, foot down	40.0**	6.1*	0.1	1.0	1.3	2.8	0.0	0.1	0.4	0.07	0.20
Sprawl, foot up	96.3**	8.7**	0.0	2.6	0.2	0.0	0.3	0.5	0.5	0.00	0.35
Excursion, foot up	80.2**	5.4*	2.1	5.4*	0.9	0.6	0.5	0.1	1.7	0.01	0.33
Stride Width, foot down	66.5**	0.2	0.2	1.3	1.5	6.2*	2.2	1.7	10.9*	0.04	0.30
Stride Width, foot up	59.9**	1.6	0.0	2.7	1.8	1.4	1.4	1.1	1.7	0.00	0.26

*P<0.05, **P<0.001

Table S2. Extension of Table 2 (Chapter 2), showing effects of treatment variables on velocity and other kinematic variables in the models for *A. sagrei*.

	Incline	Substrate	Type	Incline X Substrate	Substrate X Type	Incline X Type	3-way interaction	Relative hindlimb	SVL	Lizard ID	Model R^2
Velocity	368.9**	6.06*	1.4	1.9	1.3	0.0	3.4*	2.8	0.2	0.15	0.56
Stride Length	478.7**	26.9**	0.2	1.9	0.9	6.6*	0.3	4.6	0.5	0.08	0.67
Step Length	273.0**	36.6**	0.0	1.1	0.3	5.3*	0.2	2.4	0.0	0.07	0.59
Stride Duration	11.7**	2.8	0.6	2.1	1.0	1.2	2.3	0.0	1.1	0.00	0.15
Duty Factor	102.0**	34.8**	1.5	0.6	3.3*	2.8	0.5	0.3	5.0	5.78	0.45
Sprawl, foot down	6.9*	2.4	0.6	2.6	1.3	0.2	2.7	0.1	3.0	0.11	0.12
Excursion, foot down	3.7	2.5	0.5	0.2	0.3	0.0	3.8	0.6	0.0	0.04	0.07
Sprawl, foot up	118.3**	61.4**	0.0	5.8*	0.3	0.5	1,2	2.7	0.9	0.01	0.55
Excursion, foot up	70.2**	9.1**	0.0	6.2*	0.7	1.1	3.2*	1.2	1.2	0.06	0.32
Stride Width, foot down	59.7**	0.5	6.1*	1.3	0.1	7.0*	0.5	4.6	38.7**	0.03	0.36
Stride Width, foot up	59.2**	0.0	0.2	0.5	0.4	0.8	1.6	0.4	4.4	0.02	0.24

*$P<0.05$, **$P<0.001$

Table S3. Correlations table for kinematic variables, Velocity, and SVL for *A. cristatellus* (all treatments pooled). Bolded values are significant

	Velocity	SVL	Rel. hindlimb	Stride Length	Stride Duration	Step Length	Duty Factor	Sprawl, foot down	Sprawl, foot up	Excursion, foot down	Excursion, foot up	Stride Width, foot down
SVL	**-0.27**											
Rel. hindlimb length	**0.31**	**-0.31**										
Stride Length	**0.74**	0.06	**0.19**									
Stride Duration	**-0.34**	**0.38**	**-0.16**	**0.15**								
Step Length	**0.68**	0.02	**0.15**	**0.91**	**0.25**							
Duty Factor	**-0.47**	0.06	-0.12	**-0.66**	**-0.19**	**-0.84**						
Sprawl, foot down	**-0.23**	0.01	**-0.14**	**-0.33**	**-0.28**	**-0.43**	**0.42**					
Sprawl, foot up	**0.40**	-0.03	0.09	**0.57**	0.05	**0.60**	**-0.56**	**-0.33**				
Excursion, foot down	**-0.18**	0.03	-0.01	**-0.26**	0.02	**-0.14**	0.10	**0.23**	**0.76**			
Excursion, foot up	**0.37**	0.08	0.08	**0.52**	0.06	**0.47**	**-0.41**	**-0.29**	**-0.31**	**-0.29**		
Stride Width, foot down	**-0.30**	**0.23**	-0.02	**-0.30**	-0.09	**-0.35**	**0.31**	**0.58**	**-0.23**	0.12	-0.10	
Stride width, foot up	**-0.37**	-0.06	-0.12	**-0.44**	-0.06	**-0.43**	**0.41**	**0.26**	**-0.25**	**0.21**	**-0.46**	**0.14**

Table S4. Correlations table for kinematic variables, Velocity, and SVL for *A. sagrei* (all treatments pooled). Bolded values are significant

	Velocity	SVL	Rel. hindlimb	Stride Length	Stride Duration	Step Length	Duty Factor	Sprawl, foot down	Sprawl, foot up	Excursion, foot down	Excursion, foot up	Stride Width, foot down
SVL	0.04											
Rel. hindlimb length	**0.13**	**-0.27**										
Stride Length	**0.85**	0.03	**0.15**									
Stride Duration	**-0.51**	**0.16**	-0.02	**-0.21**								
Step Length	**0.72**	-0.06	**0.13**	**0.85**	**-0.13**							
Duty Factor	**-0.57**	**0.14**	-0.03	**-0.63**	**0.26**	**-0.80**						
Sprawl, foot down	-0.06	**0.17**	-0.05	-0.07	-0.06	-0.09	0.12					
Sprawl, foot up	**0.47**	-0.01	-0.09	**0.54**	-0.09	**0.58**	**-0.62**	-0.02				
Excursion, foot down	**-0.15**	0.04	-0.04	-0.08	**0.17**	0.00	-0.10	**0.24**	-0.01			
Excursion, foot up	**0.42**	-0.05	-0.08	**0.48**	**-0.13**	**0.37**	**-0.52**	**-0.16**	**0.69**	**-0.18**		
Stride Width, foot down	**-0.25**	**0.39**	-0.03	**-0.29**	0.03	**-0.22**	**0.21**	**0.56**	**-0.18**	0.08	**-0.24**	
Stride width, foot up	**-0.39**	**0.16**	0.02	**-0.36**	**0.16**	**-0.30**	**0.37**	**0.20**	**-0.28**	**0.21**	**-0.54**	**-0.30**

CHAPTER 3

THE MIAMI HEAT: URBAN AREAS ALTER THERMAL BIOLOGY AND COSTS OF THERMOREGULATION FOR TWO NON-NATIVE *ANOLIS* SPECIES

In preparation for submission to Global Change Biology

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ABSTRACT

Urbanization is a worldwide phenomenon that alters community assemblages, facilitates species introductions, and causes public health problems. One dimension of urbanization is the urban heat island effect, where urban areas experience higher temperatures than less-developed suburban and natural habitats. The resulting altered thermal environments can have important implications for the persistence and dispersal of organisms in cities. Ectotherms are particularly sensitive to environmental changes that affect thermal conditions, and therefore increased urban temperatures may pose significant challenges to thermoregulation and alter temperature-based activity. To evaluate whether these changes to the thermal quality of available habitat affect the persistence and dispersal of ectothermic species in urban areas, we studied two species of *Anolis* lizards (*A. cristatellus* and *A. sagrei*) introduced to Miami-Dade County, FL, USA, where they occur in both urban and natural habitats. We took canopy photographs and measured operative temperature (T_e), which estimates the body temperature of a non-thermoregulating lizard, using copper lizard models in random locations in four urban and four natural sites. While these models were recording T_e, we captured lizards between 0700 hrs and 1800 hrs and recorded their internal body temperature (T_b). We found that urban areas had more open canopies compared to natural habitats, which led to higher T_e in urban sites. We also found that lizards of both species actively thermoregulated, maintaining T_b higher than T_e in all sites. We found in lab trials that *A. cristatellus* preferred lower temperatures than *A. sagrei*. Urban sites appear to lower thermoregulatory costs for both species, but only *A. sagrei* had field T_b that were more often within their preferred temperature range in

urban habitats compared to natural areas. Furthermore, based on available T_e within each species' preferred temperature range, urban sites with only *A. sagrei* appear less suitable for *A. cristatellus*, and natural sites with only *A. cristatellus* are less suitable for *A. sagrei*. While *A. sagrei* may find opportunities for dispersal in many urban locations, *A. cristatellus* is likely constrained to forest remnants and other areas with high canopy cover. Consequently for *A. cristatellus*, dispersal may depend on human activities and the connectivity of the relatively cooler microhabitats that they prefer.

INTRODUCTION

The local and landscape-scale changes of urbanization have profound impacts on the temperature of local areas. Habitat fragmentation and local-scale habitat modifications associated with urbanization result in a phenomenon called the urban heat island effect, in which urban and developed areas are warmer than less developed and nearby natural habitats (Imhoff, Zhang, Wolfe, & Bounoua, 2010; Rizwan, Dennis, & Liu, 2008). When humans construct and expand cities, vegetative cover is reduced and replaced with impervious, heat-absorbing artificial surfaces such as roads, parking lots, and buildings (Forman, 2014; Oke, 1982; Yuan & Bauer, 2007). At larger scales, once-continuous ecosystems become habitat fragments with increased surface area exposed to solar radiation (Delgado, Arroyo, Arévalo, & Fernández-Palacios, 2007; Mcdonald, Kareiva, & Forman, 2008). At smaller scales, the distribution of warm and cool microclimates can be influenced by single trees (Georgi & Zafiriadis, 2006; Kolbe et al., 2016) and can be drastically different from that of natural habitats. For example, adding vegetative landscaping in a desert landscape increased temperature heterogeneity for lizards (Ackley, Angilletta, DeNardo, Sullivan, & Wu, 2015). Although the urban heat island effect is well studied from the perspective of its physical characteristics, very little is known about the consequences for organisms that inhabit cities (but see Angilletta et al., 2007). For ectothermic organisms in particular, whether the altered distribution of thermal microhabitats and increased temperatures of urban areas benefit or prove costly for these organisms is an open question.

Environmental temperature increases in urban areas may have a direct impact on ectotherm fitness. Enzymatic activity that drives metabolism, movement, reproduction, and growth is usually positively correlated with body temperature up to an optimal functional temperature, after which performance sharply decreases as temperature increases (Angilletta, 2009; Huey & Kingsolver, 1989). Activity rates, such as for mating or foraging, change continuously with body temperature and reach their highest levels within the preferred temperature range (Gunderson & Leal, 2015, 2016). Because maximal performance, such as locomotion, can be critical for escaping predators, capturing prey, and defending territories (Irschick & Losos, 1998), the environmental temperature can have a strong impact on fitness. For example, when *Anolis* lizards were transplanted to a hotter habitat, only those with maximal performance at higher temperatures and greater performance breadth survived (Logan, Cox, & Calsbeek, 2014). Urban environments may be too extreme, with temperatures regularly above thermal tolerances or lethal temperatures (Kappes, Katzschner, & Nowak, 2012; Menke et al., 2011); without enough cool refuges, some species will be excluded from these areas. However, ectotherms have been shown to increase thermal tolerance in cities, urban ants in Sao Paulo, Brazil could tolerate high temperatures

(42°C) for longer than ants from rural areas (Angilletta et al., 2007). Alternatively, if urban areas are warmer but temperatures are within physiological tolerances, access to optimal and preferred body temperatures may increase, and constraints on activity time and performance may be reduced (Gunderson & Leal, 2015). For example, shade from landscaping vegetation increased activity time by nearly 400% for lizards in an arid ecosystem (Ackley et al., 2015). Whether increased urban temperatures improve or reduce fitness depends on how well ectotherms can use their habitat to regulate body temperatures.

Thermoregulatory costs may change for ectotherms in urban areas where the structural and thermal landscapes differ significantly from natural habitats. To regulate their body temperatures, ectotherms exchange heat with the surrounding environment (Angilletta, 2009), depending not only on ambient conditions (e.g. air and ground temperatures, and access to solar radiation), but also the spatial distribution of thermally variable microclimates (Sears & Angilletta, 2015; Sears, Raskin, & Angilletta, 2011). Some ectothermic organisms actively thermoregulate, moving between cool and warm microhabitat to achieve a preferred body temperature (Huey, Hertz, & Sinervo, 2003). Alternatively, the body temperature of an ectotherm in a thermally homogeneous habitat, such as a dense forest, conforms to the ambient temperature (Huey et al., 2009). The costs of each strategy, whether energetic requirements (e.g. shuttling between basking sites) or opportunistic (e.g. metabolic or performance losses when outside their preferred temperature range), depend on both the amount of available sunny and shady microhabitats and their distribution in the habitat (Huey & Slatkin, 1976; Sears et al., 2016; Sears & Angilletta, 2015). For example, a population of *Anolis cristatellus* in warmer, more arid habitats in southwestern Puerto Rico actively thermoregulates, whereas lizards in cooler forested habitats with fewer basking sites thermoconform (Gunderson & Leal, 2012). Because the thermal landscape (i.e., the spatial distribution of thermal microclimates) determines the relative ease of achieving optimal and preferred temperatures (i.e., cost of thermoregulation) (Sears et al., 2016), maximal performance capacity and activity time, important components of fitness, could vary between habitats that differ in thermal quality. If warmer urban areas are more favorable thermal environment, this may reduce thermoregulatory costs, allowing organisms to expend less effort to reach preferred temperatures (Gunderson & Leal, 2012). Alternatively, thermoregulatory costs may increase if urban areas are too hot and ectotherms spend more time and energy seeking cooler microhabitats (Lagarde et al., 2012; Scheffers et al., 2013). Few studies have evaluated thermal ecology of ectotherms in cities, which will factor heavily into persistence in these widespread and growing environments.

Changes to the costs of temperature-dependent activity may influence the persistence of ectotherms in cities and determine the ability of non-native ectothermic species to expand their ranges. Due to habitat modification and extirpation of native species, among other urban phenomena, urbanized areas can function as points of entry and centers of population growth for introduced species (Blair & Johnson, 2008; Hufbauer et al., 2012). Furthermore, human activity and land development often contribute positively to invasion success (Roura-Pascual et al., 2011; Shochat et al., 2010). Because abiotic factors (e.g. temperature) play a major role in invasion success (Menke & Holway, 2006), when the thermal qualities of an urban area benefit an introduced species, such as those that prefer warmer conditions, urbanization may facilitate their spread (Menke et al., 2011; Piano et al., 2017). Alternatively, urban temperatures may exceed thermal tolerances, which may preclude introduced ectotherms from portions of urban habitats (e.g. Kolbe et al., 2016). Because invasive species can cause environmental damage and economic losses, understanding the mechanisms behind their spread and persistence is critical (Zenni & Nuñez, 2013).

Anolis lizards are an excellent system for evaluating the impact of urbanization on temperature-mediated traits in introduced species. Anoles have been used extensively in research on thermoregulation (Hertz, Huey, & Stevenson, 1993; Huey et al., 2003; Huey, 1974) . Because of their relatively small size and small home ranges, their body temperatures can be compared to nearby models that represent their body temperature if they were not actively thermoregulating (i.e., the operative temperature: T_e), allowing for an assessment of habitat choice and estimates of the costs of thermoregulation (Gunderson & Leal, 2012; Huey et al., 2003). The temperature dependence of locomotion (thermal performance curve: TPC) is well-studied in anoles (Gunderson & Leal, 2012; Huey, Niewiarowski, Kaufmann, & Herron, 1989), and warmer conditions have been shown to impose selection on the thermal performance curve for anoles (Logan et al., 2014) as well as other lizard taxa (Gilbert & Miles, 2017). We studied the effects of urban environments on the thermal biology of two species, *Anolis cristatellus* and *Anolis sagrei*. Both species use similar portions of the structural habitat and are found in urban and natural habitats in both their native and non-native ranges. Where they co-occur in the Miami area, they have been shown to compete and affect each other’s habitat use (Salzburg, 1984). *Anolis* *cristatellus* has lower reported thermal preferences and tolerances than *A. sagrei*, and appears constrained to areas in Miami that have high canopy cover (Corn, 1971; Kolbe et al., 2016). *Anolis sagrei* is widespread throughout urban areas of Miami as well as some natural forest locations (Battles et al. 2018).

We predict that the structural habitat changes because of urbanization will result in more open canopies in urban compared to natural areas. We also predict that urban areas will be warmer than natural areas (T_e), demonstrating an urban heat island effect at a scale relevant to lizards. Because thermal traits can acclimate or adapt to local conditions (Clusella-Trullas & Chown, 2014), we predict that urban lizards in both species will have higher thermal preferences (T_{pref}), higher optimal performance temperatures (T_{opt}), and greater performance breadths than lizards from natural sites. Lizards may benefit in urban areas if they can maintain T_b within T_{pref} range for more time during the day compared to natural areas. Alternatively, urban areas may present more extreme conditions (i.e., warmer overall and fewer cool spots) than natural areas, reducing or eliminating these potential benefits. Finally, we predict that the thermal characteristics of a site largely dictate which of our two species is present, lending support to the hypothesis that abiotic factors influence the presence and spread of these invasive species.

MATERIALS AND METHODS

Study species and study sites

We studied two *Anolis* species, small insectivorous lizards found naturally in southern North America, Central and South America, and throughout the Caribbean (Losos, 2009). Several *Anolis* species have been introduced to the Miami metropolitan area (Kolbe et al., 2007), two of which are common in both natural forest and urban areas. *Anolis sagrei* is native to Cuba and the Bahamas, and non-native populations are now widely distributed in the southeastern United States with Miami area populations dating to the 1940-60s (Bell, 1953; Kolbe et al., 2004). *Anolis cristatellus* is native to Puerto Rico and was first documented in Miami in the mid-1970s (Bartlett & Bartlett, 1999; Kolbe et al., 2007; Powell, Henderson, Adler, & Dundee, 1996; Wilson & Porras, 1983). In contrast to the nearly ubiquitous *A. sagrei*, the distribution of *A. cristatellus* is more restricted, radiating out from two independent points of introductions (Kolbe et al., 2016).

We conducted our study in four urban and four natural sites throughout the Miami metropolitan area. Generally, natural sites were closed-canopy forests on upland hammocks, consisting of hardwood-oak overstory canopy with palmettos and saplings in the understory. All of the natural sites were forest patches within the urban matrix of metropolitan Miami. The ‘Barnes’ natural site contains *A. sagrei* and is a designated natural area within A.D. Barnes Park, a typical city park. The ‘Montgomery’ natural site contains *A. sagrei*, and is a forest patch inside of the Montgomery Botanical Center, which features large lawns and managed gardens, insulating the site from nearby urban areas. The ‘Matheson’ natural site contains *A. cristatellus* and is a designated natural area within Matheson Hammock Park. The ‘Bear’ natural site contains *A. cristatellus* and is a designated natural area within the Crandon Beach Park on Key Biscayne.

Urban sites are located within human-altered areas, generally along roadsides with bike paths, canals, and sidewalks. The ‘UM’ urban site contains *A. sagrei* and is located along a road near the University of Miami in Coral Gables. The ‘Gables’ urban site contains *A. sagrei* and is located near downtown Coral Gables, with limited vegetation and primarily artificial substrates. The ‘Red’ urban site contains both *A. sagrei* and *A. cristatellus* and is located along a portion of Red Road in South Miami and Pinecrest. The site is a linear park along a road, bike path, and canal with a guard-rail in some parts. The ‘Crandon’ urban site contains both *A. sagrei* and *A. cristatellus* and is located along a portion of Crandon Boulevard on Key Biscayne. We are unaware of any urban sites in Miami that contain only *A. cristatellus*.

Operative Temperature

We measured operative temperature (T_e), the body temperature of a lizard not thermoregulating, which represents the available thermal environment for lizards. We made copper lizard models out of 28-gauge (0.32 mm) copper sheet, rolled into a cylinder and fitted with a cap from the same material on one end and flattened and folded to close the other end, and painted light brown to match lizard skin color and reflectance. Inside each model, we placed an iButton temperature logger (Thermochron model DS1921G-F5) that was wrapped in parafilm to increase waterproofing and then wrapped in cloth medical tape to buffer the iButton from directly touching the side of the model. The iButtons recorded temperatures every fifteen minutes for the duration of time at a site. To place models at the natural sites, starting from near the center of the plot, we followed a random compass heading and distance and affixed a model on the nearest substrate at this location at a random height between 0 and 200 cm. This resulted in model placement on random orientations on tree trunks and branches. In urban sites, we followed a transect parallel to the road, placing models evenly along the length of the transect, but a random distance from the road and facing a random compass heading when on the substrate. For all models, we recorded the substrate type and diameter. In both site types, we placed models randomly but only in microhabitat lizards could conceivably use (e.g., not in the middle of the road). We calibrated the temperatures recorded by the models to more closely represent lizard T_b following Dzialowski (2005). We exposed models and lizards to a range of temperatures, sun, and wind conditions, plotted model temperatures against body temperatures, and corrected model readings with the regression equation from their relationship.

Body Temperature

While the models were deployed at a site, we captured lizards and recorded their internal body temperature (T_b) with a small thermocouple (K-type, 36-gauge, 0.13 mm-diameter) briefly inserted into their cloaca. We captured 12 lizards (mixed males and females) per hour without resampling the same individuals, between 0700 and 1800, for a total of at least 132 lizards per site. At sites with both species (Red and Crandon), we captured 132 individuals of each species.

Canopy Openness

We measured canopy openness by taking hemispherical photos facing upward from model locations and lizard capture locations with a handheld camera (10-megapixel Canon® Powershot SD1200 IS) and attached fish-eye lens. We analyzed these photos with Gap Light Analyzer version 2.0 (Frazer, Canham, & Lertzman, 1999), calculating the percentage of pixels that were open sky.

Thermal Preference

We measured the preferred temperature range, the central 50% of body temperatures measured from lizards allowed to choose body temperatures in a thermal gradient free of other environmental constraints (Hertz et al., 1993), for male lizards caught in urban and natural sites (*A. cristatellus*: natural *N* = 24, urban *N* = 21; *A. sagrei* natural *N* = 14, urban *N* = 15; table SX for site and morphology details). Lizards were housed at the University of Rhode Island under a 12L:12D cycle for five to 42 days after capture, fed crickets every three days (except 24 hrs before a trial) and misted three times per day. We determined thermal preferences by placing individual lizards in a thermal gradient comprised of a series of eight visually and physically separated lanes. At one end of each lane, we placed an incandescent heat lamp to allow for basking at high temperatures, while the opposite end of the gradient contained a small plastic container filled with ice. The average temperature at the warm end of the gradient was 46.6 °C (SE=0.30; range=44-52°C) and 15.4 °C (SE=0.28; range=7-17°C) at the cold end. Temperatures in all gradients always included the range of preferred temperatures previously reported in the literature for these species and allowed lizards to select temperatures up to their critical thermal limits for both species. We measured internal body temperatures of lizards using a thermocouple (K-type, 36-gauge, 0.13 mm-diameter) inserted in the cloaca and taped to the body, leaving lizards free to move throughout the thermal gradient. We connected thermocouples to a controller that recorded temperature every ten seconds, allowing continuous monitoring of body temperature without disturbance by observers. Lizards acclimated to the lanes for 30 minutes, and then trials ran between three and four hours. We excluded lizards from the experiment if thermocouples became detached or lizards showed abnormal behavior, such as hiding. In these cases, lizards were given one additional trial, but were completely excluded from analyses if they never performed.

Thermoregulatory Efficiency

To determine how accurately a lizard achieves a preferred body temperature, given the available thermal habitat, we calculated thermoregulatory efficiency (E) for adult lizards observed in the wild with the following equations: $E = 1 - d_b/d_e$, where d_b and d_e refer to the mean deviation of T_b and T_e from the preferred temperature range, respectively (Hertz et al., 1993). Values of E approaching one signify a highly-efficient thermoregulator, whereas values of E approaching zero represent a thermoconformer or an organism behaviorally passive in terms of temperature regulation. A negative E indicates avoidance of preferred thermal habitat. We set confidence intervals on E values through 1000 replicates of bootstrap resampling of our field measurements of T_e and T_b for each site. We computed d_b and d_e by randomly drawing samples (with replacement) of n observations (total number of observations) of T_e and T_b. We also calculated the percentages of models and lizards below, within, and above the preferred T range.

Thermal Performance

We generated thermal performance curves by recording maximum sprint velocity on a standard racetrack, a 8.6 cm-wide board covered in window screen at a 37° angle to encourage quadrupedal movement, at five temperatures for *A. cristatellus* (15°C, 20°C, 25°C, 30°C, and 35°C) and six temperatures for *A. sagrei* (*A. cristatellus* temperatures and 40°C). We collected males of both *A. cristatellus* and *A. sagrei* from urban sites, Red (N = 15) and Gables (N = 15), respectively, and natural sites, Matheson (N = 13) and Montgomery (N = 15), respectively. These were a different set of lizards than those used for the thermal preference experiment and were held under the same conditions. We kept lizards in an incubator for at least 30 minutes to reach the target temperature. Lizards were placed at the base of each track and allowed to run upwards. We encouraged movement with gentle taps near their tails when needed. We filmed all lizard runs at 240 frames-per-second with a digital camera (Casio Exilim Ex-zr1000), and used ImageJ (Schneider, Rasband, & Eliceiri, 2012) to analyze maximum velocity. For *A. sagrei* thermal performance curves, we added mean critical thermal maximum (CT_{max}) temperatures calculated from a different data set for nearby urban (41.1 °C) and natural (40.6 °C) locations in Miami (Appendix 2). For *A cristatellus*, we added CT_{max} values for urban and natural *A. cristatellus* populations in Miami, which did not differ from each other (39.0°C; Leal & Gunderson 2012). To estimate thermal performance curves, we fitted data with 21 asymmetrical peak curves using TableCurve 2D (SysStat Software Inc, San Jose, CA). For each individual, we calculated AIC scores of the generated models and chose the best fit. When AIC values were too close to identify a single model, we chose the model with fewer parameters, and when models with the fewest parameters were indistinguishable, we chose the model with the highest R^2 value.

Statistics

We performed all statistical analyses in R (R Core Team, 2015), and analyzed both species separately, except when specified otherwise. We performed an ANOVA of available canopy openness by site nested within site type (natural or urban), followed by Tukey-HSD post-hoc analysis to determine differences among sites. To test whether the two species use different microhabitats from availability and each other when they co-occur at the same urban sites (i.e., Crandon and Red), we compared the availability of canopy openness to locations used by lizards using ANOVA and Tukey-HSD post-hoc tests. In urban sites with only *A. sagrei* (i.e., Gables and UM), we compared canopy openness availability and use with a t-test. Natural sites lacked variation in canopy cover, so we did not test for differences between availability and use by lizards. To measure the effect of canopy openness on T_e, we performed a mixed-model ANCOVA with site, model ID, and time of day as random effects. Then, for only the urban sites, where canopy openness had an effect on temperature, we performed a mixed-model ANCOVA of T_e by canopy openness with site and model ID as random effects, for each hour of the day.

To analyze thermal availability, we performed a mixed-model ANOVA testing for differences in T_e by site type with time of day and model ID as random effects. To test for differences between T_b and T_e, we performed a mixed-model ANOVA of temperature by type (T_e (copper model) or T_b (*A. cristatellus*, and *A. sagrei*)) and site, and their interaction, with ID (lizards and model) and time of day as random effects. We used post-hoc Tukey-HSD tests to test for differences between models and lizards in each site.

Following estimation of thermal performance curves (see above), we used t-tests to compare moments on TPC: optimal performance temperature (T_{opt}), maximal sprint speed (P_{max}), and performance breadth (95% T_{Br} and 80% T_{Br}). Next, we used chi-square tests to compare portions of the TPC (95% T_{Br}, and 80% T_{Br}) available and used in urban versus natural sites.

RESULTS

Canopy Openness

Urban areas had more open canopies than natural areas ($F_{1, 251} = 256.5$, $P < 0.001$; Fig. 1), with significant variation among urban sites, but not natural ones (Fig. 1; $F_{6, 251} = 10.0$, $P < 0.001$). Canopy openness drove T_e differences within urban areas ($F_{1,1276} = 349.6$, $P < 0.001$), overall explaining 13.6% of temperature variation not accounted for by variation between the sites, the time of day, and the model locations. The strength of the effect of canopy openness on temperature in urban areas changed throughout the day, with the strongest effects from late morning until mid-afternoon (Table 1). Because natural areas had more closed canopies and less variation in canopy cover, we did not find a canopy cover-temperature relationship in natural sites. In the urban sites with both species (i.e., Crandon and Red), *A. cristatellus* used locations with significantly more closed canopies than both *A. sagrei* and those available at random (Crandon: $F_{2, 276} = 24.4$, $P < 0.001$; Red: $F_{2, 294} = 18.3$, $P < 0.001$; Fig. 2). In the urban site Gables, which had the most open canopy of all sites, *A. sagrei* used significantly more closed canopy locations than randomly available ($t = 2.6$, $df = 47.2$, $P < 0.05$; Fig. 2). Both species had the similar mean canopy openness values in both sites, regardless of the available canopy cover (Fig. 2). Variation in canopy openness did not affect T_e in natural areas, which had uniformly closed canopies.

Field Operative and Body Temperatures

Operative temperatures (T_e) in urban areas were 1.5 °C warmer on average than in natural areas ($F_{1,\ 264.7} = 120.3$, $P < 0.001$; Fig. 3). *Anolis cristatellus* T_b were on average 0.9 °C higher in urban as compared to natural areas ($F_{1,\ 529} = 20.4$, $P < 0.001$), however, lizard T_b at the Bear natural site did not differ from the urban sites. *Anolis sagrei* T_b were on average 2.5 °C higher in urban compared to natural sites ($F_{1,793} = 181.0$, $P < 0.001$; Fig. 3). The only urban sites that differed were Gables and Crandon ($t = 3.2$, $df = 793$, $P < 0.05$), with lizard T_b at Gables being 1.0 °C higher.

Thermal Preference

The preferred temperature range for *A. cristatellus* was between 28.2°C and 31.7°C, and the preferred temperature range for *A. sagrei* was between 30.2°C and 35.7°C. Neither species differed in preference between natural and urban populations. These preferences are consistent with previously reported preferred temperature ranges of *A. cristatellus* (Hertz et al., 1993; Huey & Webster, 1976) and *A. sagrei* (Corn, 1971).

Thermoregulatory Efficiency

Overall, lizard body temperatures (T_b) exceed T_e for both species ($F_{2,1722} = 186.8$, $P < 0.001$; Figs. 3, 4). For each species at each site, T_b were significantly higher than T_e, except for *A. sagrei* at Gables (Figs. 3, 4; Tables 2, 3).

For both species, in urban and natural areas, body temperatures were closer to the preferred temperature range than were operative temperatures ($d_b < d_e$), with exception of *A. cristatellus* at Bear, suggesting that lizards actively thermoregulate at most sites (Tables 2, 3). *Anolis cristatellus* at urban and natural sites differ little in their thermoregulatory efficiency (*E*), in contrast, *A. sagrei* appears to thermoregulate more efficiently in urban areas than natural areas (Tables 2, 3). The negative *E* value for *A. cristatellus* in the Bear natural site suggests that these lizards avoid available microhabitats within the preferred temperature range (Table 2). Furthermore, *A. sagrei* generally thermoregulates more efficiently than *A. cristatellus*, which means that *A. sagrei* maintains T_b within T_{pref} despite T_e being further outside the T_{pref} range of this species.

For *A. cristatellus,* the Matheson natural site had a higher percentage of models below the preferred temperature range than the other sites, and no models were above it (Fig. 5a). As shown by the *E* values, *A. cristatellus* in natural and urban sites have similar percentages of lizards within their preferred temperature range (Fig. 5, Table 2). Compared to *A. sagrei*, *A. cristatellus* at urban sites and natural site Bear have higher percentages of lizard T_b above the preferred temperature range (Figs. 5b-d, 6).

For *A. sagrei,* urban sites Crandon and Red were similar to natural sites in that they had a high percentage of T_e below the preferred temperature range (Fig. 6a-d). Despite having similar T_e profiles as natural sites, lizards in the Crandon and Red urban sites are most often found within their preferred temperature range (Fig. 6c, d). In contrast, the more urbanized sites of Gables and UM had low percentages of T_e (and T_b) below the preferred range (Fig. 6e, f). The percentage of lizard T_b within their preferred range was similar across all urban sites (Fig. 6c-f).

Habitat Limitations

Both species co-occur at urban sites Red and Crandon. Where the species occur alone, a greater portion of T_e values are within their preferred range compared to the preferred range of the other species, except for the natural sites where

A. sagrei are found (Table 4). Additionally, *A. cristatellus* would encounter it's CT_{max} nearly twice as often as does *A. sagrei* in *A. sagrei* urban sites (Table 4).

Thermal Performance

Optimal performance temperature, maximal sprint speed, and thermal performance breadth did not differ between urban and natural lizards for either species. However, *A. sagrei* had a higher optimal performance temperature and greater maximal sprint speed than *A. cristatellus* (Table 5).

For both species, compared to natural areas, urban areas provide more access to the temperatures at which lizards can achieve 95% optimal performance (*A. cristatellus*: X^2= 39.1, df= 1, $p < 0.001$; *A. sagrei:* X^2= 105.3, df= 1, $p < 0.001$) (Table 6). Urban areas provide more access to the temperatures at which lizards can achieve 80% optimal performance for *A. sagrei* (X^2= 100.3, df= 1, $p < 0.001$), but urban and natural sites are equal for *A. cristatellus*.

Lizards were found at temperatures allowing access to 80% of optimal performance or greater more often in urban areas than in natural areas (80% *A. cristatellus*: X^2= 5.1, df= 1, $p < 0.05$; 80% *A. sagrei:* X^2= 72.1, df= 1, $p < 0.001$; 95% *A. cristatellus*: X^2= 32.2, df= 1, $p < 0.001$; 95% *A. sagrei:* X^2= 55.1, df= 1, $p < 0.001$; Table 6).

DISCUSSION

Urbanization converts natural habitats into landscapes dominated by open space and human-made structures, altering the thermal environment for small ectotherms. As predicted, we found that canopies were over three times more open in urban areas (32%) than natural areas (9%; Fig. 1), contributing to increased operative temperatures in urban habitats. These findings support patterns of higher temperatures in urban areas, which are strongly influenced by reduced tree cover (Georgi & Zafiriadis, 2006; Zhou, Huang, & Cadenasso, 2011). We further demonstrated this relationship at a scale relevant to small ectotherms, highlighting the variation in both canopy cover and thermal availability within urban areas. Not only were operative temperatures higher in urban areas, but lizard body temperatures were also higher in cities. Urban and natural areas represent distinct thermal microhabitats in which ectotherms may experience shifts in thermoregulatory costs and changes in the constraints on temperature-dependent activity and performance. Our study provides a mechanistic perspective on the urban heat island effect, showing how changes to the structural habitat because of urbanization influence the thermal biology of small ectotherms and contribute to their persistence and spread in cities.

Urban areas likely reduce the costs of thermoregulation for the *Anolis* species in our study, but in some circumstances, costs may be higher. Because urban areas increase the availability of warm, sunny patches, lizards will experience reduced thermoregulatory costs compared to natural habitat (Angilletta, 2009; Huey, 1974; Huey & Slatkin, 1976). This is supported by lower d_e values in urban sites (with exception in Bear natural site, discussed below), indicating reduced mean deviation of operative temperatures from the preferred temperature range. In this sense, energetic costs of moving to warm patches will be lower when the frequency of sunny patches is increased (Gunderson & Leal, 2012). Thermoregulation is also used to decrease body temperature, and therefore ectotherms incur costs when operative temperatures exceed thermal preferences (common in our study) or tolerances (rare in our study). In these cases, such as for *A. sagrei* at the Gables urban site where lizards used locations with more canopy cover than randomly available, thermoregulatory costs may increase in urban areas, as lizards seek out scarce or widely separated cool, shaded spots to reduce T_b (Vickers, Manicom, & Schwarzkopf, 2011). In addition to the presence of sunny and shady microhabitats, their spatial distribution also determines thermoregulatory costs (Sears & Angilletta, 2015). Our study prioritized comparing operative and body temperatures, rather than their arrangement, but future studies could evaluate how the spatial distribution of sunny and shady patches in urban areas influences thermoregulatory costs. We expect that buildings have a strong impact on thermoregulatory costs related to moving between patches, such that a single side of a building can be entirely shaded for several hours, while just a short distance away, perhaps around a corner, lizards could access full sun or a mixture of sunny and shady locations. Thermoregulatory costs are important to consider because they determine the ease at which ectotherms can achieve optimal temperatures for performance and maintain preferred temperatures, which should ultimately influence fitness (Gunderson & Leal, 2015; Huey & Berrigan, 2001).

For *A.* s*agrei*, urban areas increase access to preferred body temperatures, which should result in higher rates of activity. Temperature is one of the most important drivers of ectotherm activity (e.g., foraging, territory defense, and mating) and occurs at its highest levels when organisms are within their range of preferred body temperatures (Grant & Dunham, 1988; Gunderson & Leal, 2016). Despite increased mean temperatures in urban areas, urban populations of our study species did not have warm-shifted thermal performance curves or higher thermal preferences. Because *A. sagrei* can spend more time within T_{pref} in urban sites, this species may benefit from the thermal microhabitats of urban areas, likely reproducing at higher rates than in natural habitat (Huey & Berrigan, 2001). For example, in more open, warmer habitat compared to cooler, close-canopied forest, female *A. cristatellus* in Puerto Rico were more likely to be reproductive year-round, likely due to increased basking opportunities to achieve higher body temperatures (Otero, Huey, & Gorman, 2015). Furthermore, higher *E* values for *A. sagrei* in urban sites indicate that even when operative temperatures deviate far from preferred temperatures, lizards still precisely thermoregulate to maintain body temperatures within the preferred range (Hertz et al., 1993). Therefore, urban habitats are more favorable for *A. sagrei*, which may have trouble persisting in more close-canopied sites, such as forested habitats, that restrict their ability to achieve higher body temperatures. It is important to note that while costs of thermoregulation may be lower in urban sites, Basson, Levy, Angilletta, and Clusella-Trullas (2017) showed that even in a low-quality thermal habitat with high thermoregulatory costs in the lab, *Cordylus* lizards prioritized maintaining T_b within T_{pref}. It may be necessary to maintain warmer body temperatures that confer higher activity in urban habitats in the Miami area, even if costly, to successfully compete with multiple other introduced and native anoles (Kolbe et al., 2007), or manage urban predation pressure (Chejanovski, Avilés-Rodríguez, Lapiedra, Preisser, & Kolbe, 2017).

In contrast to *A. sagrei*, *A. cristatellus* may not find urban sites more favorable than natural habitat. The thermoregulatory efficiency (*E*) for *Anolis cristatellus* was not significantly different between urban and natural sites (Table 2). These values are similar to estimates for *A. cristatellus* in xeric habitats (warm and dry) in their native range, where lizards actively thermoregulate (Gunderson & Leal, 2012). However, thermoregulatory efficiency was negative in the Bear natural site. Negative *E* values suggest lizards are avoiding or restricted from using sites with preferred temperatures, perhaps due to predation pressure or competitors (Hertz et al., 1993), but unfortunately we could not identify any obvious factors causing this at the Bear site. In urban areas, operative and *A. cristatellus* body temperatures were less often within preferred temperature range, which suggests that urban areas may constrain *A. cristatellus* activity. Similarly, Kaiser, Merckx, and Van Dyck (2016) found that a more-thermophilic butterfly species had increased survival and greater body size in urban areas than did a woodland species adapted for cooler conditions. Furthermore, if operative temperatures too frequently exceed thermal tolerances, or if the habitat lacks enough cool refuges, certain ectotherms could be excluded from urban areas altogether (Chown & Duffy, 2015). Hall and Warner (2017) found that female *A. cristatellus* from one of our urban sites, Red, had greater body condition and fecundity than lizards from one of our natural sites, Matheson. Our findings on operative and body temperatures suggest that factors other than temperature likely contribute to body condition and fecundity increases. Certainly, animals in urban areas have been found to express a longer reproductive period than in their natural habitat, but other determinants, such as food availability, may be more important (Lowry, Lill, & Wong, 2013). However, Hall and Warner (2017) found that fecundity increased in urban areas because females began laying eggs earlier in their lab setting. We conducted our study in the summer, but urban areas may be more favorable to *A. cristatellus* during other seasons if urban areas reach preferred temperatures more often than natural areas, such as earlier in the spring when reproductive activity is beginning (Gorman & Licht, 1974; Hall & Warner, 2017; Lee, Clayton, Eisenstein, & Perez, 1989). The different reactions to urban habitats of the lizard species in our study show that warmer urban habitats will not benefit all ectotherms equally.

Beyond the thermal suitability for a single species, competition on thermal niche axes can further limit persistence. For instance, in the Matheson natural site, which had the lowest mean operative temperatures, 40% of operative temperatures are within the preferred range of *A. cristatellus*, whereas only 3% are within the preferred range of *A. sagrei* (Table 4). Similarly, in Puerto Rico, *A. cristatellus* and *A. gundlachi* both occupy forest habitats, but only *A. gundlachi* is found above ~300 m elevation (Gorman & Hillman, 1977). The mean available temperature in the forest above 300 m is at the low threshold for activity of *A. cristatellus*, but in the middle of activity range for *A. gundlachi* (Gunderson & Leal, 2016; Gorman & Hillman, 1977). Both species in our study co-occur along the edges of the Matheson forest patch, which is < 1 km away at our urban site Red, but *A. sagrei* was never found in the forest at Matheson. With conditions in almost the entire forest below its T_{pref}, *A. sagrei* cannot reach activity levels to effectively forage, mate and defend territories. This likely puts *A. sagrei* at a competitive disadvantage, and it may be excluded from large, forested areas with *A. cristatellus* in Miami. Similarly, at the Gables urban site, 62% of T_e are within the preferred range of *A. sagrei*, but only 26% are within the preferred range of *A. cristatellus* (Table 4). With greater potential for higher activity rates, *A. sagrei* has a substantial competitive advantage. Yet, not all types of urban habitat exclude *A. cristatellus*. In the sites with both species (i.e., Crandon and Red), *A. sagrei*, which preferred warmer temperatures, selected microhabitats with more open canopies and achieved higher T_b than did *A. cristatellus* (Fig. 2). These sites differed from the two urban sites with only *A. sagrei* in that the operative temperature distributions encompassed both species' T_{pref} ranges. Because these species do not occur in sympatry in their native ranges, competition with one another did not cause their divergent T_{pref}, but the divergence allows them to partition the thermal niche, at least in some habitats (Magnuson, Crowder, & Medvick, 1979; Paterson & Blouin-Demers, 2017). Interestingly, the natural sites that *A. sagrei* occupies, Barnes and Montgomery, seem more favorable to *A. cristatellus* than for *A. sagrei* (Table 4). If *A. cristatellus* is ever transported to these natural sites, *A. sagrei* may be out-competed and displaced by *A. cristatellus*, which is better suited thermally for these forested areas (Kolbe et al. 2016).

The success and spread of introduced species will rely upon the contribution of temperature to persistence. Abiotic factors, such as temperature, play an important role in where invasive species can or cannot persist (Bomford, Kraus, Barry, & Lawrence, 2009; Ulrichs & Hopper, 2008; Zenni & Nuñez, 2013). Because urbanization greatly increases species introductions (Shochat et al., 2010), the concurrent temperature increases associated with urban areas may enhance ectotherm invasion success worldwide. In our case, numerous populations of *A. sagrei* have been documented outside their native range, often associated with human activity (Campbell, 1996; Godley, Lohrer, Layne, & Rossi, 1981; Kolbe, Ehrenberger, Moniz, & Angilletta, 2014; Norval, Mao, Chu, & Chen, 2002), and the favorability of urban thermal conditions for *A. sagrei* is likely a key factor in this species' invasion success

(Angetter, Lötters, & Rödder, 2011). Outside of anoles, warmer conditions and human habitat modification improve the invasion success of the argentine ant in many locations around the world (Roura-Pascual et al., 2011). However, even ectotherms that benefit from warmer temperatures are still limited by low temperatures due to seasonality or elevation (Angilletta, 2009; Sunday, Bates, & Dulvy, 2012). Urban areas may increase mean daily temperatures overall, but the magnitude and effect on other environmental variables depends on the regional climate (Imhoff et al., 2010; Roth, Oke, & Emery, 1989). Yet, anole species have been shown to decrease their low-temperature tolerance by acclimation (Kolbe, VanMiddlesworth, Losin, Dappen, & Losos, 2012), and *A. sagrei* CT_{min} decreases with increasing latitude (Kolbe et al., 2014). Flexibility in low temperature tolerance combined with increased activity time and reduced thermoregulatory costs may make cities more favorable for species like *A. sagrei*.

Alternatively, *A. cristatellus* represents an example of the opposite effect of urbanization on invasion. In a study exploring the spread of *A. cristatellus* in Miami, Kolbe et al. (2016) found that this species is positively associated with dense vegetation, high canopy cover, and forest patches, thus limiting its dispersal through the city. Our study corroborates these habitat requirements and explains a possible mechanism for why very warm urban sites, where *A. sagrei* are common, may exclude *A. cristatellus*. Hourly temperature (T_e) for the urban site Gables, for example, reveal that *A. cristatellus* could achieve preferred temperatures easily in the early mornings and evenings (Fig. 4), but this would be difficult or impossible throughout the day because of the lack of canopy cover (Fig. 1). Therefore, high-temperature environments, such as urban areas, can limit activity time and reduce persistence (Lara-Reséndiz, Gadsden, Rosen, Sinervo, & Méndez-De la Cruz, 2015). Furthermore, *A. cristatellus* in Gables and similar urban locations throughout Miami would have the additional stress of more often avoiding its upper thermal tolerance (CT_{max}) than at urban sites where it is found (Crandon and Red). If high temperatures limit persistence, then dispersal of introduced species with similar thermal traits throughout urban areas will be restricted. Urbanization often exists as a gradient of intensity (Luck & Wu, 2002; McDonnell & Pickett, 1990), and some levels of urban development are suitable for introduced species while others are not (Crooks, Suarez, & Bolger, 2004; Grarock, Tidemann, Wood, & Lindenmayer, 2014). Research on the urban heat island effect shows a similar thermal gradient of intensity because of urban development (Rizwan et al., 2008), and variation in thermal quality may affect invasive ectotherms in a similar manner (Chown & Duffy, 2015).

Temperature, and other abiotic factors, likely play a major role in the persistence and spread of introduced ectotherms. Under the right thermal conditions, ectotherms can be more competitive and reproduce at greater rates than other species (Huey & Berrigan, 2001; Otero et al., 2015). In this study, we show that urbanization significantly alters thermal habitats for ectotherms, increasing both ambient temperature and the availability of warm microhabitat. Urban thermal habitat may confer decreased costs of thermoregulation for ectotherms, but urban areas in Miami impact the persistence and spread of two introduced species in opposing ways. With reduced thermoregulatory costs and increased time spent within T_{pref}, *A. sagrei* likely finds urban areas thermally superior to natural habitat in Miami. In contrast, because

A. cristatellus T_b are within T_{pref} less often in urban habitats and *A. cristatellus* is at a disadvantage to *A. sagrei* from a thermal perspective, they are likely excluded from much of urbanized Miami. Many other factors, such as competition, predation, prey abundance, and disease, could influence persistence in urban areas. However, the thermal quality of urban habitats is certainly a fundamental aspect of urban environments for ectotherms. Our study provides a foundation for studying how the thermal characteristics of urban habitats influence ectothermic organisms. Future studies should consider these findings when evaluating and predicting the spread of introduced species.

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Table 1 For each hour of lizard activity, effect of canopy openness on T_e in urban areas, degrees of freedom in the denominator (df of numerator = 1), the coefficient estimate, and the percent of variance explained by the canopy openness, without the random effects (marginal R^2).

Hour	F	Denominator df	Coefficient estimate	Variance explained (marginal R^2)
0700	1.76	112.7	0.00	0.01
0800	23.49**	114.0	0.06	0.16
0900	46.65**	113.9	0.10	0.28
1000	82.70**	114.0	0.11	0.41
1100	63.37**	113.6	0.10	0.32
1200	60.01**	112.9	0.10	0.29
1300	43.18**	112.1	0.07	0.18
1400	50.38**	115.6	0.07	0.23
1500	57.68**	66.5	0.05	0.33
1600	37.90**	115.9	0.04	0.20
1700	9.45*	114.9	0.03	0.04

$^{**}P < 0.001$, $^{*}P < 0.01$

Table 2 Summary statistics showing behavioral thermoregulation in *A. cristatellus* in two natural (N) and two urban sites (U).

Site	*t*-value	d_b	d_e	$d_e - d_b$	*E* (95% CI)
Matheson (N)	**5.57**	1.18	1.68	0.50	0.296 (0.290 - 0.302)
Bear (N)	**4.70**	0.45	0.33	-0.12	-0.369 (-0.382 - -0.356)
Crandon (U)	**4.10**	0.54	0.99	0.45	0.454 (0.450 - 0.458)
Red (U)	**6.10**	0.77	1.11	0.34	0.304 (0.299 - 0.309)

t-statistic = post-hoc comparison from T_b vs T_e model (significant values, $P < 0.05$, in bold), d_b = mean absolute deviation of T_b from preferred temperature range, d_e = mean absolute deviation of T_e from the preferred temperature range, E = effectiveness of behavioral thermoregulation (Hertz et al., 1993) and 95% confidence interval.

Table 3 Summary statistics showing behavioral thermoregulation in *A. sagrei* in two natural (N) and four urban (U) sites.

Site	*t*-statistic	d_b	d_e	d_e- d_b	E (95% CI)
Barnes (N)	**6.64**	0.84	1.37	0.53	0.391 (0.386 - 0.397)
Montgomery (N)	**6.40**	1.00	2.45	1.45	0.587 (0.584 - 0.589)
Crandon (U)	**12.26**	0.49	1.96	1.47	0.750 (0.747 - 0.753)
Red (U)	**11.68**	0.48	1.65	1.17	0.711 (0.706 - 0.715)
Gables (U)	1.36	0.31	0.94	0.63	0.667 (0.663 - 0.672)
UM (U)	**6.13**	0.24	0.61	0.37	0.588 (0.582 - 0.594)

t-statistic = post-hoc comparison from T_b vs T_e model (significant values, $P < 0.05$, in bold), d_b = mean absolute deviation of T_b from preferred temperature range, d_e = mean absolute deviation of T_e from the preferred temperature range, *E* = effectiveness of behavioral thermoregulation (Hertz et al., 1993) and 95% confidence interval.

Table 4 At sites with only *A. cristatellus* (Matheson and Bear) or only *A. sagrei* (Barnes, Montgomery, Gables, and UM), the percent of operative temperatures within the preferred temperature range (T_{pref}), the 95% performance breadth (T_{Br}), and at or above the CT_{max} for the species that is present in that site and, in parentheses, the species absent from the site.

	A. cristatellus		*A. sagrei*			
Temperature Range	Matheson	Bear	Barnes	Montgomery	Gables	UM
T_{pref} (absent species)	40.07 (3.13)	68.75 (34.10)	36.72 (65.85)	14.52 (28.65)	62.43 (26.11)	68.08 (38.74)
95% T_{Br} (absent species)	0.87 (0.17)	24.69 (0.15)	0.00 (16.34)	0.35 (9.03)	21.30 (50.56)	7.16 (48.54)
CT_{max} (absent species)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.66 (6.17)	0.17 (1.36)

Table 5 Thermal performance curve (TPC) values for each species, with urban and natural lizards pooled.

TPC value	*A. crisatellus*	*A. sagrei*
P_{max} (ms^{-1})	1.40	1.74
$\mathrm{T}_{\mathrm{opt}}$ (°C)	33.96	37.99
95% Breadth (°C)	30.85 – 34.95	34.38 – 38.73
80% Breadth (°C)	25.66 – 35.58	29.70 – 39.22

Table 6 Percentages of operative and body temperatures at within the 95% and 80% performance ranges estimated from the thermal performance curves of lizards from natural and urban areas.

Site Type	Operative Temperature		Body Temperature	
A. cristatellus	95% T_{Br}	80% T_{Br}	95% T_{Br}	80% T_{Br}
Natural	13.5	76.2	39.0	90.2
Urban	22.3	75.6	63.9	95.5
A. sagrei				
Natural	0.25	31.0	4.2	60.2
Urban	8.9	48.0	26.4	86.9

95% T_{Br} = range of temperatures that confer 95% T_{opt}; 80% T_{Br} = range of temperatures that confer 95% T_{opt} .

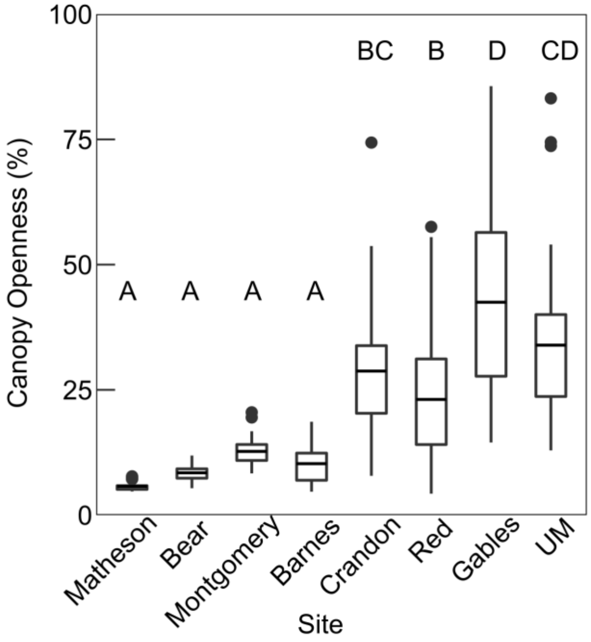

Figure 1 Mean percentage canopy openness (horizontal line), middle 50% of values (box), values within 1.5 times the interquartile range (whiskers), and outlying values (points) for each site. Letters denote post-hoc significance differences in means.

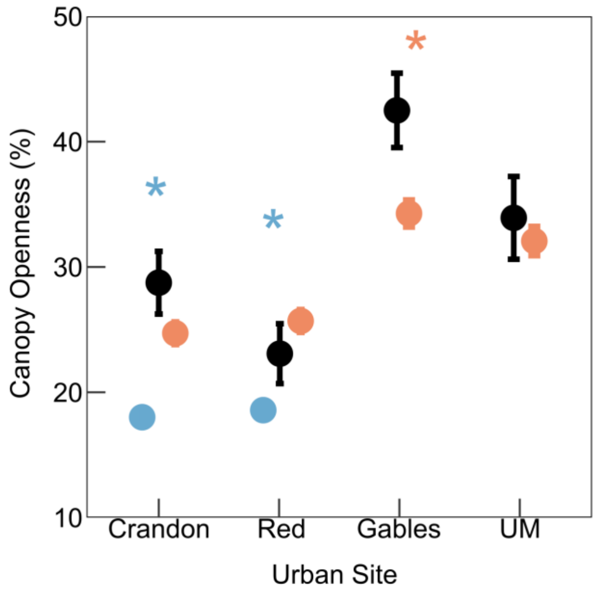

Figure 2 Mean canopy openness (± S.E.) of model locations (random availability; black) and microhabitats used by *A. cristatellus* (blue) and *A. sagrei* (red) in urban sites. Blue asterisks indicate that *A. cristatellus* uses significantly shadier microhabitats than those randomly available. Red asterisks indicate that *A. sagrei* uses significantly shadier microhabitats than those randomly available. Error bars are not visible in some cases when smaller than symbol for the mean value. Natural sites are not included because the variation in canopy cover was too low for meaningful interpretation.

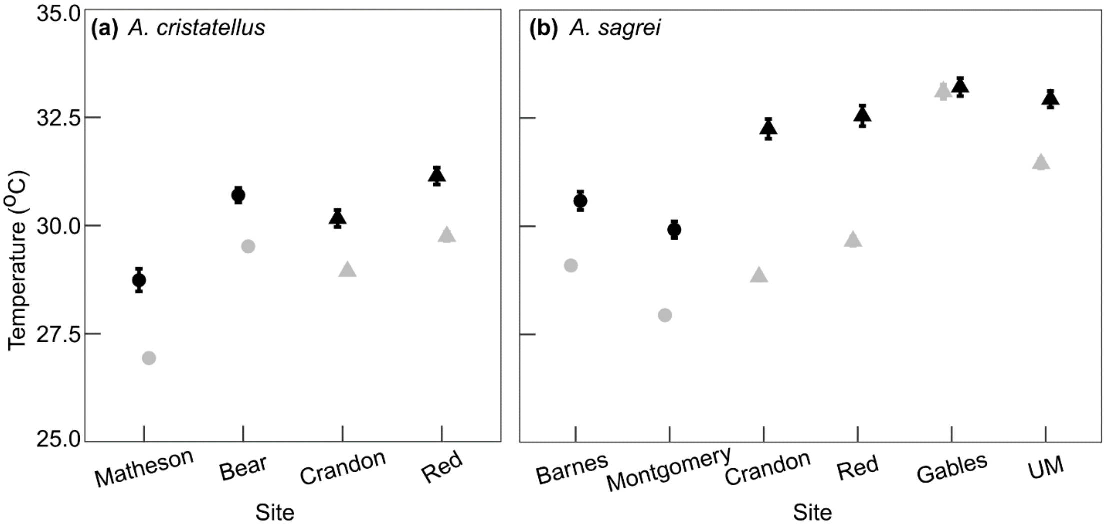

Figure 3 Mean (± S.E.) operative (grey) and lizard body (black) temperatures in natural (circles) and urban (triangles) sites for A) *A. cristatellus* and B) *A. sagrei*. Data points are mean temperatures for lizards captured during all times of the day sampled (0700–1800). Error bars are not visible in some cases when smaller than symbols for mean values.

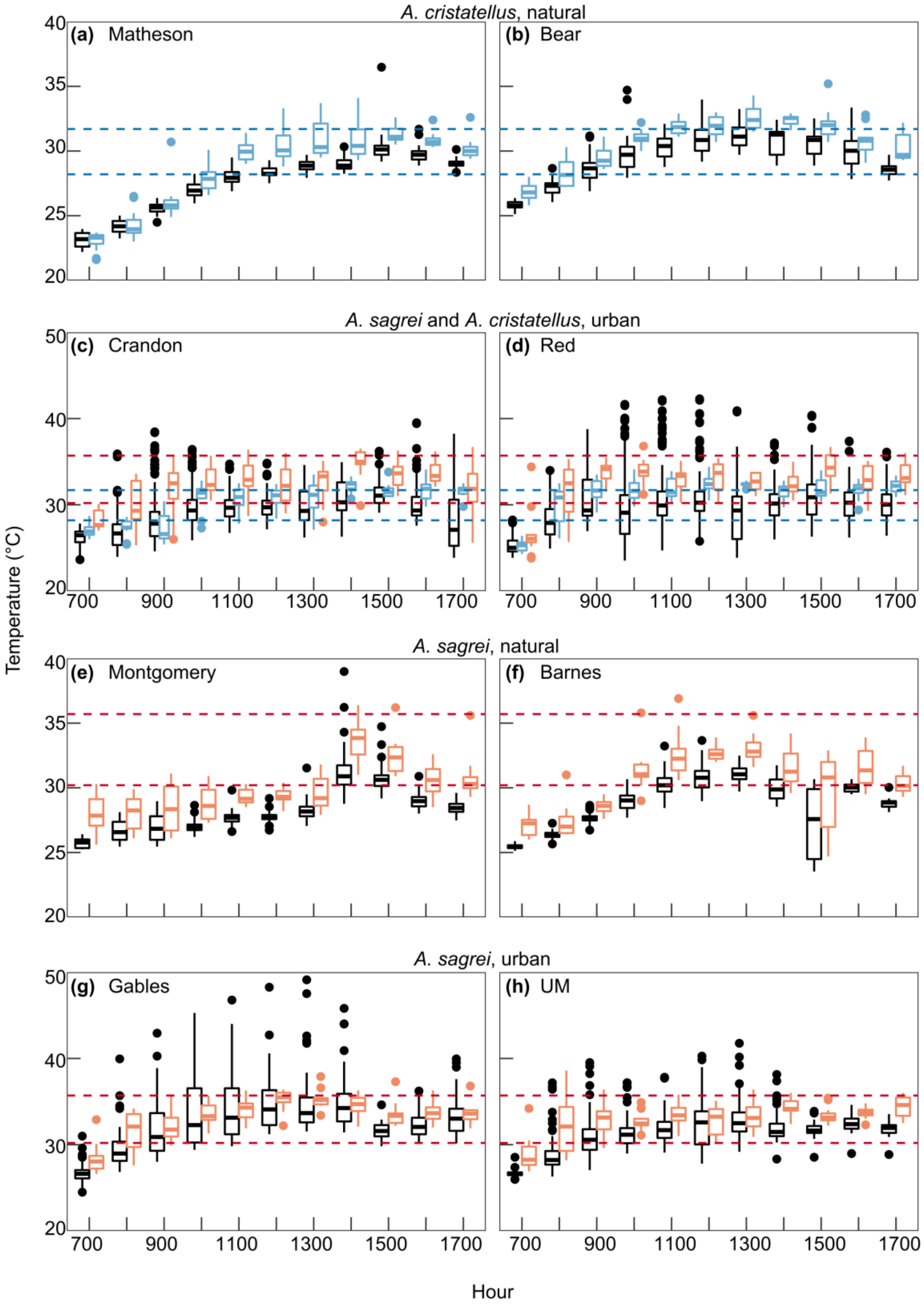

Figure 4 Boxplots showing operative temperatures (black) and lizard body temperatures (blue: *A. cristatellus*, red: *A. sagrei*) throughout the day in each site. Area

between the dashed lines denotes the preferred temperature range as measured in a thermal gradient (blue: *A. cristatellus*, red: *A. sagrei*). For each hour of the day sampled, box plots show the median value (line within box), middle 50% of data (box), and values within 1.5 times the interquartile range (whiskers), with individual points as outlying data. Note that the range of temperatures on the y-axis differs for urban and natural sites.

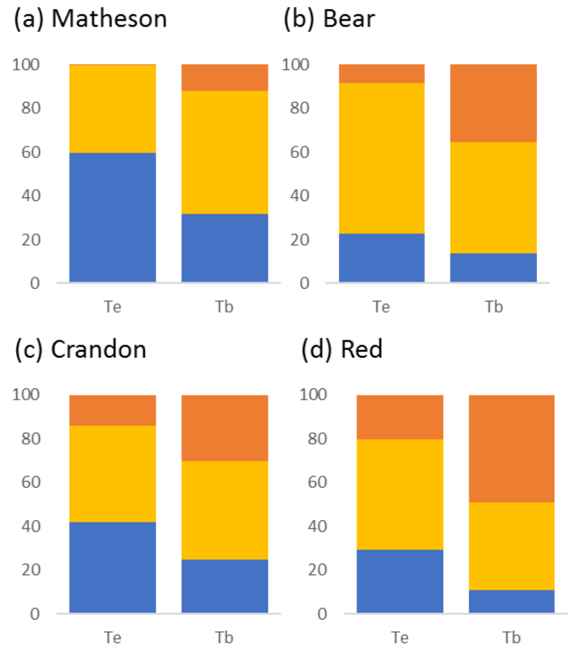

Figure 5 Percentage of operative (T_e) and body (T_b) temperatures below (blue), within (yellow), and above (orange) the preferred temperature range (28.2°C – 31.7°C) for *A. crisatellus*.

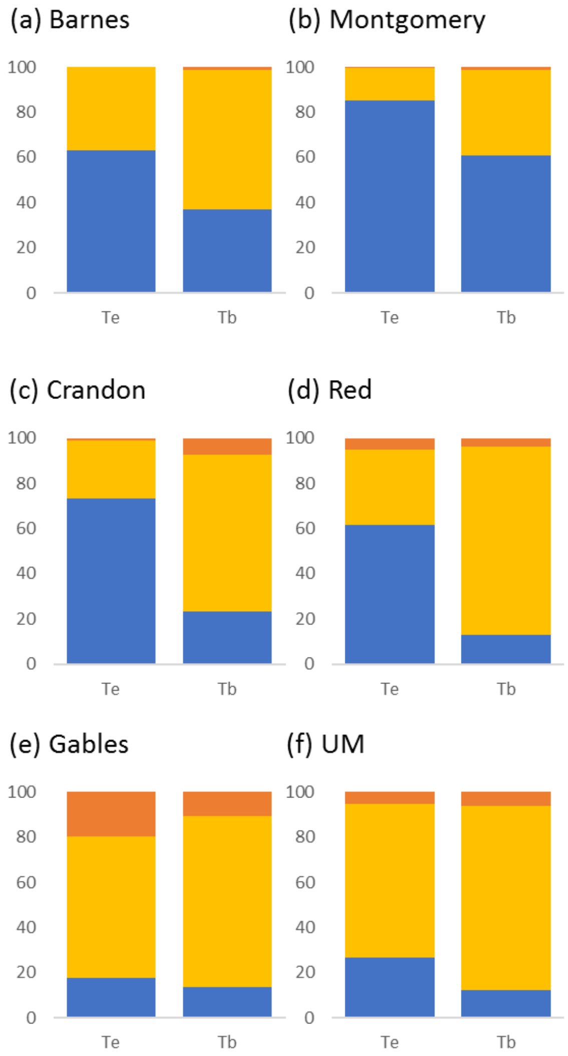

Figure 6 Percentage of operative (T_e) and body (T_b) temperatures below (blue), within (yellow), and above (orange) the preferred temperature range (30.2°C – 35.7°C) for *A. sagrei*.

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APPENDIX 1

STUDY LOCATION INFORMATION AND MAP

Table 1. Site type, species present, and GPS coordinates for each site used in chapters 1-3.

Site Name	Site Type	Species	Coordinates (decimal degrees)
Matheson	Natural	*A. cristatellus*	25.681132, -80.273902
Bear	Natural	*A. cristatellus*	25.716863, -80.150807
Barnes	Natural	*A. sagrei*	25.737188, -80.310536
Montgomery	Natural	*A. sagrei*	25.659544, -80.282323
Crandon	Urban	*A. cristatellus* and *A. sagrei*	25.688333, -80.163691
Red	Urban	*A. cristatellus* and *A. sagrei*	25.683461, -80.284701
Gables	Urban	*A. sagrei*	25.745724, -80.258362
UM	Urban	*A. sagrei*	25.719079, -80.281732

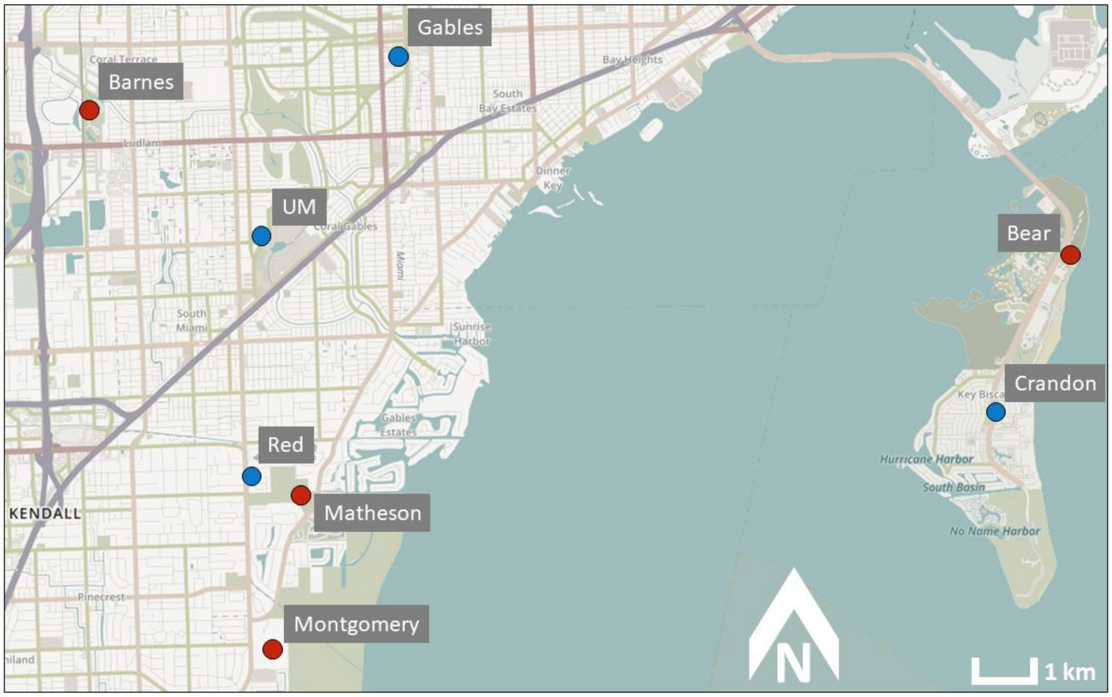

Figure 1. Map of southern Miami, with all field sites indicated in circles. Red represents natural sites and blue represents urban sites. See table 1 for species present and exact coordinates.

APPENDIX 2

PHENOTYPIC VARIATION IN URBAN AND NATURAL LIZARD POPULATIONS

This section presents a survey of phenotypic variation in one urban and one natural population of *A. sagrei* in and around two of the study sites used in chapters 1-3.

METHODS

We collected lizards in the urban population (Male: N = 119; Female: N = 114) within an area of Coral Gables ~ 3 x 10 blocks. One of the urban sites in chapters 1-3 was a subset of this larger area, and our collection included lizards within that site. We collected lizards in the natural population (Male: N = 116; Female: N = 113) from another of the sites used in chapters 1-3, a remnant oak forest patch in Montgomery Botanical Center. See chapters 1 and 3 for detailed site descriptions, and results for how these sites differ in their structural and thermal microhabitats.

We captured roughly 50 individuals at a time in nine different groups (first group captured 29 May 2016, final group captured 11 July 2016), and each of these groups was held for four days while we measured aspects of their morphology, thermal biology, and behavior.

Morphology

We measured snout-vent length (SVL) with a handheld ruler and body mass with a small scale. We used a field-portable x-ray system (X-Ray Associates East, LLC, Nutley, NJ, USA) to capture images of lizards to precisely measure bone lengths. Lizards were chilled to limit their movement and affixed to a clear plastic card to capture images. Using the ObjectJ plugin in ImageJ, we took the following measurements from x-rays (in mm): skull length; skull width at the widest point; shoulder width, the length of the clavicle; humerus length; ulna length; hand length, from 3rd finger tip to base of hand; hip width, the widest points on the pelvis; femur length; tibia length; metatarsal length; 4th toe length, from the tip of the 4th toe to the end of the metatarsal. We measured the right side of the lizard except in instances of damage or missing limbs or digits in which case we measured the left side. In addition to individual bones, we measured the total limb length of the hind and forelimb. For later analyses, we calculated relative lengths of all body measurements as the residuals of a linear regression against SVL, separate for each sex.

In addition to x-rays, we used a flatbed scanner (Epson V500 Photo, Suwa, Nagano, Japan) to capture images of the toepads on the 3rd toe on the forefoot and 4th toe on the hindfoot. In ImageJ, we counted the number of toepads on each of these digits and measured the area.

Thermal Traits

We determined the maximum temperature at which lizards lost their righting response, or critical thermal maximum (CT_{max}), by warming lizards at a rate of about 1°C per minute, starting from 37°C. After being placed on their backs, the temperature at which they could not flip over was recorded as the CT_{max}.

We generated thermal performance curves by recording maximum sprint velocity on a standard racetrack, a 8.6 cm-wide board covered in window screen at a

37° angle to encourage quadrupedal movement, at five target temperatures: 16°C, 21°C, 26°C, 32°C, and 37°C. We randomized the trial order for each group. We kept lizards in a small, field incubator for at least 30 minutes to reach each target temperature. Lizards were placed at the base of each track and allowed to run upwards. We encouraged movement with gentle taps near their tails when needed. We filmed all lizard runs at 240 frames-per-second with a digital camera (Casio Exilim Ex-zr1000), and used ImageJ (Schneider et al., 2012) to analyze maximum velocity. To estimate thermal performance curves, we fitted data with 21 asymmetrical peak curves using TableCurve 2D (SysStat Software Inc, San Jose, CA). Data points for the curve were the five sprints at increasing temperatures and the CT_{max}. For each individual, we calculated AIC scores of the generated models and chose the best fit. When AIC values were too close to identify a single model, we chose the model with fewer parameters, and when models with the fewest parameters were indistinguishable, we chose the model with the highest R^2 value.

Behavior

We assessed exploratory behavior in an arena (87.9 cm by 47.6 cm plastic Sterilite® box) with two horizontal perches placed 10 cm and 30 cm from one side of the arena. We placed a lizard in the opposite end of the arena, under a refuge for 2 minutes. After this acclimation period, we raised the refuge and started the trial, measuring the time it took for the lizard to 1) move at least one body length from their original position and 2) climb onto a perch (either the near or far one). After 15 minutes, we simulated a predator attack using a small, yellow and black hexagonal-patterned ball slowly advanced toward the lizard. We measured the distance between the ball and the lizard when the lizard fled (flight initiation distance, FID) and the distance the lizard fled (flight distance).

Statistical Analyses

All statistical analyses were performed for each sex separately. We compared morphology between urban and natural populations using t-tests. Following estimation of thermal performance curves (see above), we used t-tests to compare moments of the TPCs: optimal performance temperature (T_{opt}), maximal sprint speed (P_{max}), and performance breadth (95% T_{Br} and 80% T_{Br}) between urban and natural populations. We performed an ANCOVA to test for differences in CT_{max} between the two populations using capture date as a covariate. We compared the behavior variables (time to first movement and time to first perch) and flight variables using t-tests between urban and natural populations.

RESULTS

Morphology

In females, the following morphological variables were significantly greater in the urban population: toepad area on both the fore and hind feet, lamellae number on the forefinger, head length, head width, 4th toe length (hindfoot), hand and finger length (forelimb) (Table 1). Female relative humerus length was significantly greater in natural lizards. In males, urban lizards were larger (SVL and mass) and had more lamellae on the 3rd finger of the forelimb (Table 2).

Thermal Traits

We did not find any differences between the urban and natural populations for optimal performance temperature, maximum sprint speed, or performance breadth

(Table 3). Interestingly, in both males and females, we found evidence for acclimation in CT_{max}. CT_{max} increased with capture date, such that lizards captured near the end of the summer (29 May 2016) had a CT_{max} roughly 2°C higher than those captured in the beginning of the summer (11 July 2016; Fig. 1). In males, these values did not differ by population source (capture date: $F_{1,231} = 59.9$, $P < 0.001$). Urban females had an overall higher CT_{max} ($F_{1,222} = 24.8$, $P < 0.001$), and there was a significant interaction between capture date and population source ($F_{1,222} = 6.5$, $P < 0.02$), such that the increase from the beginning of the summer to the end was stronger in natural lizards (Fig. 1).

Behavior

In both males and females, lizards from the natural site spent more time exploring visually before moving than did lizards from urban areas (females: $t = 5.0$, $df = 213.9$, $P < 0.001$; males: $t = 3.1$, $df = 220.7$, $P < 0.01$; Fig. 2). We also found that for both males and females, urban lizards moved to a perch faster than natural lizards (females: $t = 5.2$, $df = 213.6$, $P < 0.001$; males: $t = 2.9$, $df = 220.5$, $P < 0.01$; Fig. 3). While flight initiation distance did not differ between populations for either sex, males from natural areas fled nearly twice as far after a simulated attack as did males from natural areas ($t = 3.7$, $df = 139.1$, $P < 0.001$; Fig. 4). We did not find a difference in flight distance in females between natural and urban populations.

Table 1. Female morphology means (± S.E.) of natural and urban populations. SVL, mass, and lamellae number means are calculated from absolute values. Bone lengths and toepad area means are calculated from relative (residuals from regression on SVL) values.

Trait	**Natural**	**Urban**
SVL	43.8 ± 0.3	44.0 ± 0.2
Mass	2.06 ± 0.04	2.13 ± 0.03
Lamellae number, 3rd finger	**8.31** ± 0.08	**8.66** ± 0.07
Lamellae number, 4th toe	11.54 ± 0.11	11.62 ± 0.10
Humerus length	**0.030** ± 0.013	**-0.029** ± 0.004
Ulna length	-0.002 ± 0.005	-0.002 ± 0.004
Hand and 3rd finger length	**-0.009** ± 0.006	**0.008** ± 0.006
Forelimb length	-0.002 ± 0.004	0.002 ± 0.003
Shoulder Width	0.002 ± 0.006	-0.002 ± 0.006
Hip Width	0.001 ± 0.007	-0.001 ± 0.007
Femur length	0.002 ± 0.004	-0.002 ± 0.004
Tibia length	-0.004 ± 0.004	0.004 ± 0.004
Metatarsal length	-0.004 ± 0.006	0.004 ± 0.006
4th toe length	**-0.012** ± 0.009	**0.012** ± 0.008
Hindlimb length	-0.001 ± 0.003	0.001 ± 0.003
Toepad area, 3rd finger	**-0.037** ± 0.008	**0.037** ± 0.007
Toepad area, 4th toe	**-0.028** ± 0.006	**0.028** ± 0.007
Head length	**-0.006** ± 0.005	**0.006** ± 0.004
Head width	**-0.007** ± 0.005	**0.007** ± 0.004

Table 2. Male morphology means (± S.E.) of natural and urban populations. SVL, mass, and lamellae number means are calculated from absolute values. Bone lengths and toepad area means are calculated from relative (residuals from regression on SVL) values.

Trait	Natural	Urban
SVL	**54.4** ± 0.4	**57.4** ± 0.4
Mass	**4.19** ± 0.10	**4.98** ± 0.11
Lamellae number, 3rd finger	**10.03** ± 0.08	**10.53** ± 0.08
Lamellae number, 4th toe	13.49 ± 0.10	13.59 ± 0.09
Humerus length	**0.030** ± 0.013	**-0.030** ± 0.004
Ulna length	-0.001 ± 0.004	0.001 ± 0.004
Hand and 3rd finger length	0.008 ± 0.005	-0.008 ± 0.009
Forelimb length	0.003 ± 0.003	-0.003 ± 0.003
Shoulder Width	0.002 ± 0.006	-0.001 ± 0.005
Hip Width	0.001 ± 0.006	-0.001 ± 0.005
Femur length	-0.001 ± 0.003	0.001 ± 0.003
Tibia length	-0.003 ± 0.004	0.002 ± 0.003
Metatarsal length	0.000 ± 0.006	0.000 ± 0.004
4th toe length	-0.001 ± 0.007	0.001 ± 0.005
Hindlimb length	0.000 ± 0.003	0.000 ± 0.003
Toepad area, 3rd finger	-0.003 ± 0.006	0.003 ± 0.007
Toepad area, 4th toe	-0.006 ± 0.007	0.006 ± 0.007
Head length	-0.002 ± 0.005	0.002 ± 0.005
Head width	-0.007 ± 0.006	0.006 ± 0.004

Table 3. Mean values on thermal performance curves for urban and natural females and males, ± S.E. No thermal performance curve measurements were significantly different.

	Females		**Males**	
Thermal Trait	**Natural**	**Urban**	**Natural**	**Urban**
T_{opt} (°C)	36.0 ± 0.2	35.8 ± 0.2	36.1 ± 0.2	35.7 ± 0.2
P_{max} (m/s)	1.2 ± 0.02	1.2 ± 0.03	1.4 ± 0.03	1.4 ± 0.03
80% Breadth	9.4 ± 0.4	9.3 ± 0.3	10.1 ± 0.3	9.8 ± 0.3

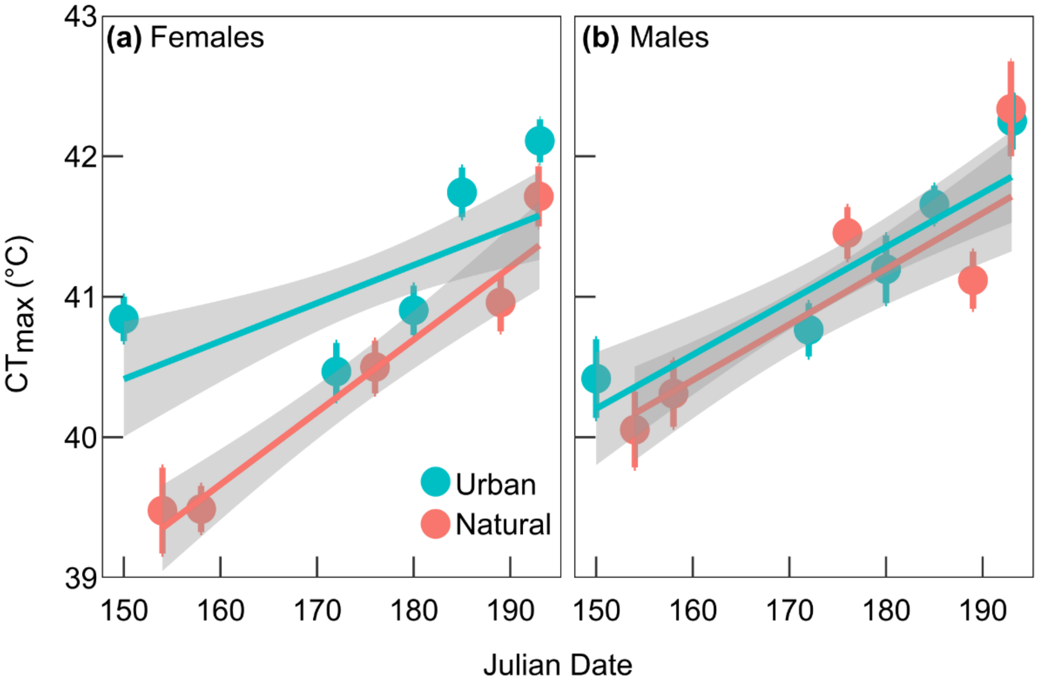

Figure 1. Mean CT_{max} values (± S.E.) for each capture group during the summer, separate for (a) females and (b) males. Urban populations are represented and blue and natural in red. Linear regression overlaid (line) with shaded area representing 95% confidence interval.

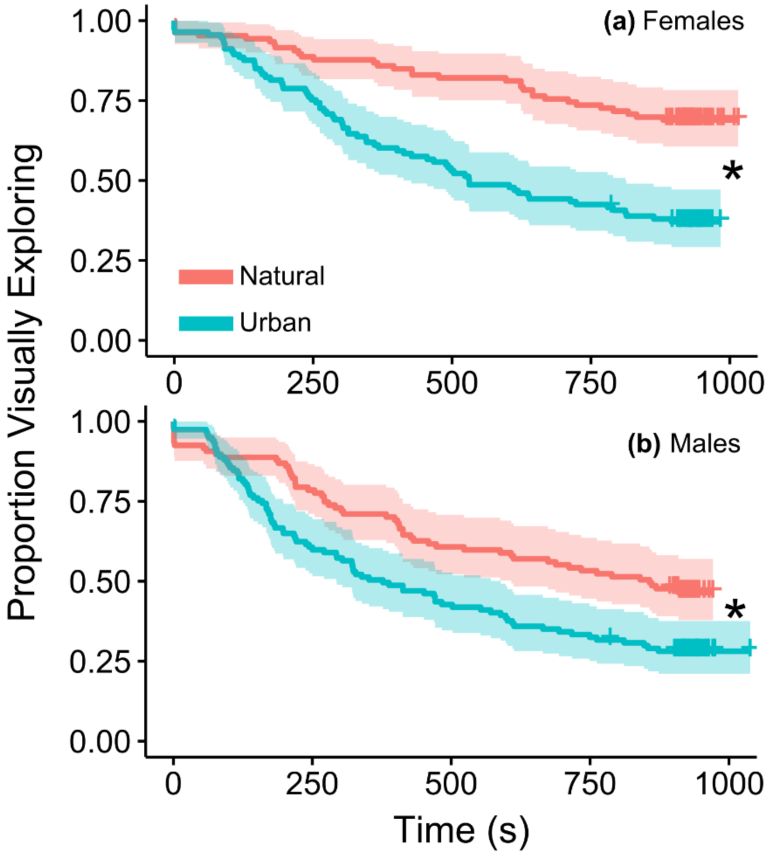

Figure 2. Survival curves for time spent visually exploring for (a) females and (b) males. Natural population represented in red and urban population represented in blue. Shaded area shows 95% confidence interval for survival analysis. Asterisk indicates significant difference between populations.

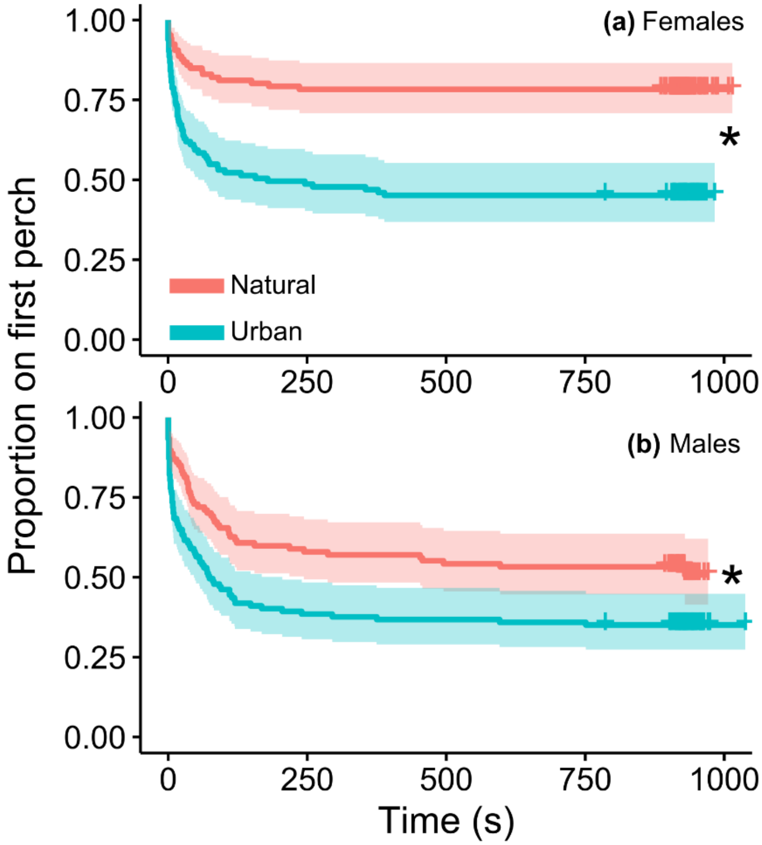

Figure 3. Survival curves for time until lizards reached the first perch for (a) females and (b) males. Natural population represented in red and urban population represented in blue. Shaded area shows 95% confidence interval for survival analysis. Asterisk indicates significant difference between populations.

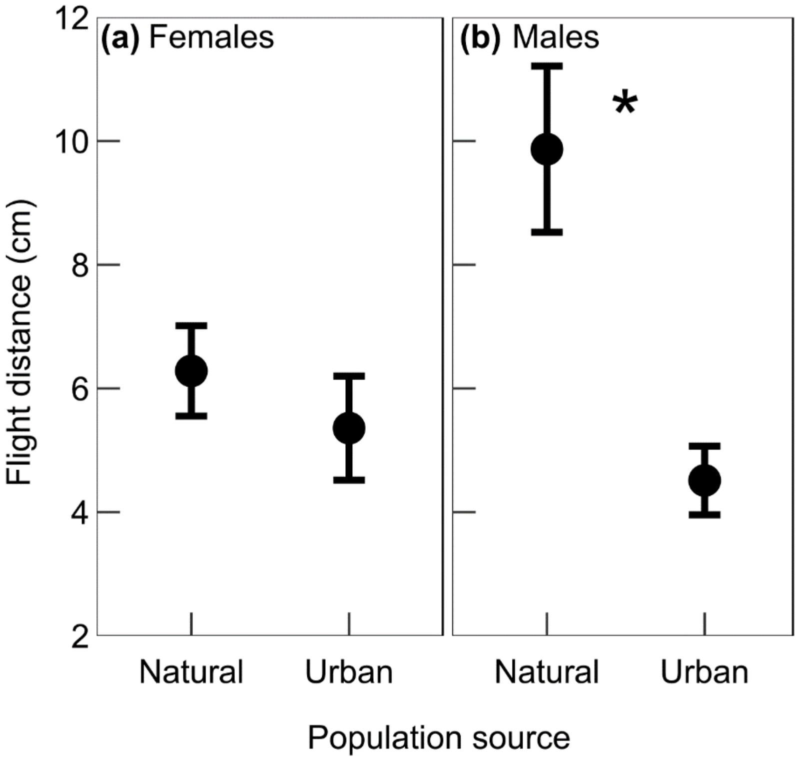

Figure 4. Mean flight distance (± S.E.) for natural and urban (a) females and (b) males after simulated attack in behavior trials. Asterisk indicates a significant difference in flight distance between urban and natural populations.