DETERMINING HOW ANTHROPOGENIC CHANGE FILTERS BIOLOGICAL COMMUNITIES AND HOW SPECIES MODIFY THEIR TRAITS IN RESPONSE TO URBANIZATION

By

KRISTOPHER W ROW

Presented to the Faculty of the Graduate School of
The University of Texas at Arlington in Partial Fulfillment
of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON
AUGUST 2023

ACKNOWLEDGEMENTS

Completing this dissertation and the accompanying work would not have been possible without the help and support of so many people. First, I want to thank my friends and colleagues at UTA that I have made during this process and who have helped and been a source of encouragement to me through this process, particularly Douja Chamseddine, Aly Blanton, Maddison Emery, Kaitlyn Howel, Daren Card, Drew Shield, Zackary Lange, Edita Folfas, Alexander Murray, Heather Arterburn and Kim Bowles. Additionally, I owe so much to TJ Firneno and Corey Roelke: I cannot fully express how much your support, inspiration, and mentorship has meant to me. Also, thank you to my undergraduate research mentees Taylor Wright, Adriana Bailey, Monica Miles, Grace Urbina, Oscar Villasana-Espinosa, and Jaele Perez for allowing me to mentor you throughout your time as undergraduates. To my committee members, Drs. Todd Castoe, Matt Walsh, Matt Fujita, and Alison Ravenscraft – I am tremendously thankful for sharing your passion, insight, and support over the past five years – your contributions of time and knowledge have helped shaped me into a better researcher and scientist. Furthermore, to Dr. Todd Castoe, you gave me my first opportunity to conduct research in your lab and I am eternally grateful for you taking that chance on me and everything that you have done for me. Also, To Dr. Jean Maines, one of my first biology teachers, without you I would have never known this path was available to someone with my background and without you pushing me to pursue this who knows where I would be now. To my partner, Jennifer Row, I know that the past five years have been difficult, but I honestly would not have been able to do this without your love and support and thank you for not letting me quit when I thought I was letting everyone down. Finally, a heartfelt thank you

to my advisor, Luke Frishkoff. You were generous with your time, patience, knowledge, support, and encouragement throughout my career with you. I honestly could not have asked for a better mentor, and I can only hope to be half the advisor, mentor, and scientist you were to me.

ABSTRACT

DETERMINING HOW ANTHROPOGENIC CHANGE FILTERS BIOLOGICAL COMMUNITIES AND HOW SPECIES MODIFY THEIR TRAITS IN RESPONSE TO URBANIZATION

Kristopher Warren Row

The University of Texas at Arlington, 2023

Supervising Professor: Luke O. Frishkoff, PhD

Humans are significantly altering the environment at an alarming pace, leading to a reduction in species diversity. This transformation has created modified habitats where some species struggle to survive, while a few others not only manage to survive but thrive. One of the most drastic forms of habitat modification is urbanization, which is spreading worldwide and contributing to the decline of biodiversity. Urbanization has given rise to urban-tolerant species that differ ecologically from species that avoid urban areas across various niche dimensions. These urban-tolerant species must adapt to changes in food sources, microhabitat conditions, and alterations in physical habitat structures. The focus of my dissertation is to investigate the impact of urbanization on morphological and dietary changes in lizard populations. First, I aim to identify and characterize specific morphological traits associated with urbanization by determining how ecological filters are shaping urban lizard communities. Secondly, I will determine if lizard species inhabiting urban environments are undergoing phenotypic changes and categorize these changes as convergent, divergent, or idiosyncratic. Lastly, I will assess whether dietary niche breadth is expanding or contracting in urban environments compared to natural ones.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2:

Chapter 3:

Chapter 4:

LIST OF FIGURES

Chapter 2:

Chapter 3:

Chapter 4:

CHAPTER 1:

INTRODUCTION

The transformation of natural habitats into urban areas due to human activities represents a significant driver of biodiversity loss (Brooks *et al.*, 2002; Pereira *et al.*, 2012; Newbold *et al.*, 2015). Urbanization, a particularly drastic form of habitat modification, is expanding globally and is becoming an increasingly significant factor contributing to declines in biodiversity. The human population living in urban areas has increased significantly, from 751 million in 1950 to 4.2 billion today, representing 55% of the world's population. It is projected to grow by an additional 2.5 billion by 2050 (United Nations, 2018). As urban areas replace natural habitats, substantial biodiversity losses occur, often resulting in a decrease in species richness by more than 50% (Newbold *et al.*, 2015). The environmental changes triggered by human-mediated habitat conversion led to significant alterations in ecological conditions, favoring the loss of locally endemic species and promoting the proliferation of a few species that thrive in human-altered environments (McKinney & Lockwood, 1999).

Urban-tolerant species likely differ ecologically from species that avoid urban areas along multiple niche dimensions. Urban species must adapt to changes in food sources (Reznick & Ghalambor, 2001), microhabitat conditions (including light pollution and temperature increase; (Shochat *et al.*, 2006)), and physical habitat structure (McKinney, 2002). It has been suggested that these environmental changes act as an environmental filter, limiting the presence of species based on their biological traits (Clergeau *et al.*, 2005). Environmental filtering is considered a preliminary step in understanding community assembly before the processes addressed by coexistence theory (Kraft *et al.*, 2015).

Morphology plays a crucial role in establishing the connection between an organism's physical traits and its ability to thrive in natural environments. In human-altered ecosystems, understanding how morphology contributes to ecological success is vital for comprehending the structure of flourishing urban communities. Existing research has primarily focused on examining the morphological changes within individual species as they adapt to urban settings, thereby showcasing adaptive evolution in response to novel habitats (Marnocha *et al.*, 2011; Winchell *et al.*, 2016; Putman *et al.*, 2019). However, there remains a significant gap in our understanding of how morphology influences entire communities in urban environments. Drawing inspiration from similar approaches used in other systems, such as Mediterranean fish, where morphology helps predict successful species invasions in communities (Azzurro *et al.*, 2014), we aim to investigate which morphological traits are associated with urban success. Our strategy involves categorizing species morphospaces, representing all possible organism morphologies, with each axis corresponding to distinct shape or morphological characteristics (Mitteroecker & Huttegger, 2009). By comparing these morphospaces between species that persist in urban environments and those that do not, we seek to unravel the role of morphology in shaping urban communities.

Previous research on convergent evolution has revealed that over long periods, evolution can surprisingly exhibit predictability (Mahler *et al.*, 2013; Hart *et al.*, 2018). However, it remains uncertain whether rapid environmental changes will trigger convergent physiological evolution across species as they strive to adapt. This question poses an intriguing area for exploration, as the impact of swift environmental transformations on convergent evolution is yet to be fully understood.

A crucial determinant of how successful a species will be in a novel environment lies in its dietary interactions with existing resources (Baiser *et al.*, 2010). Notably, insects serve as a primary dietary component for many other species, but unfortunately, their populations are declining worldwide, primarily due to habitat loss (Sánchez-Bayo & Wyckhuys, 2019). The dynamics of a species' dietary niche space are intricately tied to the availability of prey resources (Macarthur & Pianka, 1966; Schoener, 1971). Consequently, when the abundance of food changes, predators are expected to also alter their dietary niche (Stephens *et al.*, 2019). The ongoing decline in insect populations could potentially reshape the dietary preferences and interactions of various predators, leading to significant shifts in the structure and functioning of ecological communities. Understanding these cascading effects is essential for predicting the broader implications of insect declines on the stability and dynamics of ecosystems.

My dissertation focuses on exploring how urbanization impacts community assembly, specifically in relation to multiple lizard species distributed across Texas. Lizards are chosen as the primary subjects of investigation for three key reasons. Firstly, considerable prior research has extensively studied the correlation between lizards, their adaptive traits, and the environment (Losos, 2009; Luxbacher & Knouft, 2009; Kaliontzopoulou *et al.*, 2010). Secondly, lizards have demonstrated a remarkable ability to swiftly adapt in response to sudden environmental changes (Kolbe *et al.*, 2012; Eloy De Amorim *et al.*, 2017). Lastly, numerous lizard species are frequently found thriving in urban settings, where they often occupy man-made structures in significant numbers (Perry *et al.*, 2008; Meshaka Jr, 2011; Winchell *et al.*, 2018). In Chapter 2, I asses the morphological constraints imposed by urban environments on lizard communities to understand how urbanization filters species based on their physical traits. In Chapter 3, I determine if urban lizards undergo phenotypic changes, and if so, examining whether these changes lead to (a) species approaching a global optimum and becoming more similar, (b) species showing phenotypic changes towards multiple local optima, forming groups of more similar species, or (c) species undergoing idiosyncratic phenotypic changes. In Chapter 4, I Identify the impact of urbanization on dietary niche breadth by comparing the insect family consumption of lizards in urban areas to those in natural habitats.

LITERATURE CITED

Azzurro, E., Tuset, V.M., Lombarte, A., Maynou, F., Simberloff, D., Rodríguez-Pérez, A. & Solé, R. V. (2014) External morphology explains the success of biological invasions. *Ecology Letters*, **17**, 1455–1463.

Baiser, B., Russell, G.J. & Lockwood, J.L. (2010) Connectance determines invasion success via trophic interactions in model food webs. *Oikos*, **119**, 1970–1976.

Brooks, T.M., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B., Rylands, A.B., Konstant, W.R., Flick, P., Pilgrim, J., Oldfield, S., Magin, G. & Hilton-Taylor, C. (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology*, **16**, 909–923.

Clergeau, P., Croci, S., Jokimä Ki, J., Kaisanlahti-Jokimä Ki, M.-L. & Dinetti, M. (2005) Avifauna homogenisation by urbanisation: Analysis at different European latitudes.

Eloy De Amorim, M., Schoener, T.W., Santoro, G.R.C.C., Lins, A.C.R., Piovia-Scott, J. & Brandão, R.A. (2017) Lizards on newly created islands independently and rapidly adapt in morphology and diet. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 8812–8816.

Hart, J.C., Ellis, N.A., Eisen, M.B. & Miller, C.T. (2018) Convergent evolution of gene expression in two high-toothed stickleback populations. *PLoS Genetics*, **14**, 1–22.

Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A. (2010) Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat-related patterns within Podarcis bocagei wall lizards. *Journal of Evolutionary Biology*, **23**, 1234–1244.

Kolbe, J.J., VanMiddlesworth, P.S., Losin, N., Dappen, N. & Losos, J.B. (2012) Climatic niche shift predicts thermal trait response in one but not both introductions of the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, USA. *Ecology and Evolution*, **2**, 1503–1516.

Kraft, N.J.B., Adler, P.B., Godoy, O., James, E.C., Fuller, S. & Levine, J.M. (2015) Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology*, **29**, 592–599.

Losos, J.B. (2009) *Lizards in an Evolutionary Tree*, 1st edn. University of California Press.

Luxbacher, A.M. & Knouft, J.H. (2009) Assessing concurrent patterns of environmental niche and morphological evolution among species of horned lizards (Phrynosoma). *Journal of Evolutionary Biology*, **22**, 1669–1678.

Macarthur, R.H. & Pianka, E.R. (1966) ON OPTIMAL USE OF A PATCHY ENVIRONMENT. *The American Naturalist*, **100**.

Mahler, D.L., Ingram, T., Revell, L.J. & Losos, J.B. (2013) Exceptional Convergence on the Macroevolutionary Landscape in Island Lizard Radiations. *Science*, **341**, 292–295.

Marnocha, E., Pollinger, J. & Smith, T.B. (2011) Human-induced morphological shifts in an island lizard. *Evolutionary Applications*, **4**, 388–396.

McKinney, M.L. (2002) Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, **52**, 883–890.

McKinney, M.L. & Lockwood, J.L. (1999) Biotic homogenization: A few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution*, **14**, 450–453.

Meshaka Jr, W.E. (2011) A runaway train in the making: the exotic amphibians, reptiles, turtles, and crocodilians of Florida. *Herpetological Conservation and Biology*, **6**, 1–101.

Mitteroecker, P. & Huttegger, S.M. (2009) The Concept of Morphospaces in Evolutionary and Developmental Biology: Mathematics and Metaphors. *Biological Theory*, **4**, 54–67.

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger, L., Bennett, D.J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M.J., Feldman, A., Garon, M., Harrison, M.L.K., Alhusseini, T., Ingram, D.J., Itescu, Y., Kattge, J., Kemp, V., Kirkpatrick, L., Kleyer, M., Correia, D.L.P., Martin, C.D., Meiri, S., Novosolov, M., Pan, Y., Phillips, H.R.P., Purves, D.W., Robinson, A., Simpson, J., Tuck, S.L., Weiher, E., White, H.J., Ewers, R.M., MacE, G.M., Scharlemann, J.P.W. & Purvis, A. (2015) Global effects of land use on local terrestrial biodiversity. *Nature*, **520**, 45–50.

Pereira, H.M., Navarro, L.M. & Martins, I.S. (2012) Global Biodiversity Change: The Bad, the Good, and the Unknown. *Annual Review of Environment and Resources*, **37**, 25–50.

Perry, G., Buchanan, B.W., Fisher, R.N., Salmon, M. & Wise, S.E. (2008) *Effects of artificial night* *lighting on amphibians and reptiles in urban environments*,.

Putman, B.J., Gasca, M., Blumstein, D.T. & Pauly, G.B. (2019) Downsizing for downtown: limb lengths, toe lengths, and scale counts decrease with urbanization in western fence lizards (Sceloporus occidentalis). *Urban Ecosystems*, **22**, 1071–1081.

Reznick, D.N. & Ghalambor, C.K. (2001) The population ecology of contemporary adaptations: What empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112**–**113**, 183–198.

Sánchez-Bayo, F. & Wyckhuys, K.A.G. (2019) Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, **232**, 8–27.

Schoener, T.W. (1971) Theory of Feeding Strategies. *Annual Review of Ecology and Systematics*, **2**, 369–404.

Shochat, E., Warren, P.S., Faeth, S.H., McIntyre, N.E. & Hope, D. (2006) From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology and Evolution*, **21**, 186–191.

Stephens, R.B., Hobbie, E.A., Lee, T.D. & Rowe, R.J. (2019) Pulsed resource availability changes dietary niche breadth and partitioning between generalist rodent consumers. *Ecology and Evolution*, **9**, 10681–10693.

United Nations (2018) *World Urbanization Prospects, 2018 revision.*, New York, NY.

Winchell, K.M., Carlen, E.J., Puente-Rolón, A.R. & Revell, L.J. (2018) Divergent habitat use of two urban lizard species. *Ecology and Evolution*, **8**, 25–35.

Winchell, K.M., Reynolds, R.G., Prado-Irwin, S.R., Puente-Rolón, A.R. & Revell, L.J. (2016) Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution*, **70**,

1009–1022.

Chapter 2:

Morphological limitations imposed on lizards facing urbanization

Kristopher W. Row (1,2), Oscar Villasana-Espinosa (1), Jaele Perez (1), Grace Urbina (1), Luke O. Frishkoff (1,2).

(1) Department of Biology, University of Texas Arlington, 501 S. Nedderman Dr., Arlington TX, 76019, USA

(2) Amphibian and Reptile Diversity Research Center, University of Texas Arlington, 501 S. Nedderman Dr., Arlington TX, 76019, USA

Note: in review at Global Ecology and Biogeography

ABSTRACT

Habitat conversion in general, and urbanization in particular, are thought to create ecological filters that eliminate some species while simultaneously replacing them with others that thrive under novel conditions. The specific nature of these filters is unclear, but morphology may play an important role. Here, we seek to assess which lizard morphologies are favored in urban habitats. We quantified 17 linear measurements of morphology from museum specimens from 37 lizard species from across the continental United States. We then correlate these morphological measurements with the relative incidence of observations in urban versus non-urban environments from the citizen science database iNaturalist to determine whether particular morphologies predispose species to tolerate urban environments. We then use functional diversity and morphospace volume metrics to quantify both the total amount of unique morphological diversity represented by urban associated species, versus those restricted to natural areas. Based on our results morphology appears to be filtering lizard species from urban environments. Specifically, species with intermediate body sizes and relative tails lengths, as well as larger heads and shorter hind-limbs were more likely to occupy urban zones. As a result of this filtering, there was substantially diminished morphological diversity among urban tolerant species. While natural restricted species had a high amount of morphological diversity that was unrepresented in urban tolerant species, most urban species' morphologies were shared by natural restricted ones. Only a small subset of morphologies found in natural environments persist in urban ones, but urban lizards do possess a small number of unique morphological features that may facilitate their success. Strong selection pressures in evolutionarily novel environments are not only diminishing species diversity but

pruning phenotypic diversity to favor a much smaller subset of functional possibilities. Due to the connection between phenotype and function, such diminished morphological diversity is likely to impact ecosystem functioning in impoverished human-modified systems.

INTRODUCTION

Habitat conversion is a primary driver of biodiversity loss (Brooks *et al.*, 2002; Pereira *et al.*, 2012; Newbold *et al.*, 2015). The environmental impacts unleashed by such conversion result in drastically altered ecological conditions that promote the loss of locally endemic species and replace them with a few expanding species that thrive in human-altered environments (McKinney & Lockwood, 1999). Urbanization is an especially drastic form of habitat modification (McDonald *et al.*, 2013), and is expanding rapidly across the globe (Seto *et al.*, 2013). While we have a broad understanding of how urbanization effects biodiversity and species richness, we are only beginning to understand the impact that urbanization is having on the phenotypic and morphological diversity of biological communities (Sol *et al.*, 2020; Winchell *et al.*, 2020).

Urban areas create massive changes in the environment, such as increases in impervious surfaces and pollution, elimination of natural vegetation, and elevated local temperatures (Shochat *et al.*, 2006; Grimm *et al.*, 2008). As a result of these changes, urbanization negatively affects the abundance and diversity of many native species (McKinney, 2008) while also disrupting the availability of resources that animals need to survive (Raupp *et al.*, 2009). As such, the replacement of natural habitats by urban areas can precipitate large biodiversity losses—often diminishing species richness by >50% (Newbold *et al.*, 2015). For example, in Southern Chile, the total number of bird species declined in urban environments due to the severe reduction in green space availability (Silva *et al.*, 2016). The combination of the extreme environmental difference from natural environments and their burgeoning prevalence makes urban areas an increasingly relevant force in biodiversity declines.

However, some species buck the trend and tolerate urban areas quite well. These urban-tolerant species are likely ecologically different from urban-avoiding species along multiple niche axes. Urban species must simultaneously withstand changing food sources (Reznick & Ghalambor, 2001) altered temperatures regimes (Shochat *et al.*, 2006), and broad changes in physical habitat structure (McKinney, 2002). Because morphology provides one of the key links between phenotype and fitness, many of the required shifts in niche necessary for persistence in urban environments will likely be reflected in species' morphologies. As a result, determining whether and how morphology equates to ecological success in human-modified environments constitutes a core requirement for explaining community structure in proliferating urban ecosystems.

To date, most morphology-based research into urban-associated phenotypes has focused on quantifying the change within single species from natural to urban environments—building a case for contemporary adaptive evolution within species to novel urban habitats (Marnocha *et al.*, 2011; Winchell *et al.*, 2016; Putman *et al.*, 2019; Putman & Tippie, 2020). For example, populations of *Anolis cristatellus* lizards in Puerto Rico possess longer limbs and more sub-digital lamallae than those in natural areas, adaptations that facilitate grasping broad surfaces such as buildings. Similarly, urban dark-eyed juncos (*Junco hyemalis),* located in southern California, have developed shorter wings and tails than their neighboring natural populations (Rasner *et al.*, 2004). While evidence of such adaptation illustrates the power of strong selective pressures in individual species, the ways in which entire communities or faunas are filtered by urbanization based on their morphology remains largely unexplored (but see Sol *et al.*, 2020; Winchell *et al.*, 2020).

A community-wide morphological perspective has been illuminating in understanding the mechanisms behind other global change drivers: For example, in the Mediterranean, fish species with unique morphologies are most successful in invading communities (Azzurro *et al.*, 2014). Elucidating community-level morphological filters determining community membership in urban areas will help contextualize species-specific results, pointing towards the generality of adaptation and pre-adaptation for survival in the city.

In this study, we assess the morphological limits imposed on lizard communities by urban environments. To do so we compare the morphological traits of lizard species that are commonly found within urban areas to lizards that rarely associate with urban zones and ask what morphologies correlate with urban success. We address three interrelated questions: First, do urban tolerant species on average come from distinct zones of morphospace in comparison to their natural counterparts? Second, do urban species take up less morphospace — indicative of only a subset of possible morphologies being viable? Finally, are morphologies that succeed in urban zones a nested subset of those in natural environments, or are urban lizards morphologically unique possessing traits that rarely occur in species restricted to natural zones? To answer these questions, we use observational data from the citizen science initiative iNaturalist, which has been shown to be a valuable resource in determining specie sensitivity to modified land use (Todd *et al.*, 2016, 2017), along with the National Land Cover Database to establish the frequency with which species use urban areas. We then gather morphological data for species that span the continuum of urbanization use. By comparing morphological data to species' frequency of occurrence in urban areas, we test the hypothesis that urbanization imposes morphological limits on lizards.

METHODS

Occurrence data and urban tolerance

Observational occurrence data were obtained for lizards in the continental United States from the iNaturalist database in March 2019. These data were filtered to only include research-grade observations, meaning each observation is georeferenced, has a photo, is not captive, have been reviewed and agreed upon by the iNaturalist community, had a positional uncertainty of less than 20 meters, and were observed after 2010 to ensure the observations were in line with the National Land Cover Database map used (see below). We included only species that had at least 100 post-filtering observations. The initial search yielded 67 species, of which 43 remained after filtering. Of these 43 species, on average ~52% of the original observations remained post-filtering.

To estimate each species' affinity to urban environments we first obtained landcover data of the United States at 30-meter resolution from the National Land Cover Database for the year 2011 (Homer *et al.*, 2015). The NLCD classified urban land-covers into four categories based on the inferred amount of urban land-surface within the cell (1-19%, 20-49%, 50-79%, and 80-100% urban cover). We assigned urban values to each raster cell based on the upper level of urban cover that it contained: i.e. 80-100% received a value of 1, 50-79% received 0.8, 20-49% received 0.50, less than 20% received 0.2, and all other land-use types received a 0. We then extracted the urbanization values around the coordinates of each iNaturalist observation locality, averaging over a 100m, 500m, and 1000m radius. This buffer accommodates the 20m uncertainty tolerated around observations, and represents the degree of urbanization within the general vicinity where the observation was made and therefore the likely habitats that a lizard would encounter. The mean value for all individuals within a species constituted that species "urbanization score", and roughly corresponds to the expected percentage of urban area within the selected radius. Comparing the averages between the different radii showed minor changes in urbanization score while not affecting the species classification as being urban or natural associated allowing the use of a 100m radius as a standard metric to determine the urbanization score of the average individual. Urbanization scores ranged from 0.0130 to 0.371, with a mean of 0.101 ± 0.084 (**Figure S1**).

Some analyses are facilitated by categorizing species into discrete groups of urban tolerant vs. non-urban tolerant. For the purpose of categorization, we applied a cutoff value of 0.1: species with urbanization scores above this cut-off value were deemed urban tolerant, and those below it were deemed natural habitat affiliated. We additionally conducted a sensitivity analysis considering alternative cut-off values (**Figure S2)**, but the overall biological conclusions are identical to those reported in the main text for a broad range of potential cut-offs.

Morphological analysis

We assessed species' morphology using linear morphometrics of museum specimens. Due to limitations on specimen availability, of the 43 species with urbanization scores, we collected morphology data for 37 species, and the remaining 6 species were dropped from the analysis. After identifying the five largest males for each of the 37 species (N = 185 individual lizards), we used digital calipers to make 17 individual measurements which together characterized fore- and hind-limb length, head shape, body, and tail length and shape (**Figure**

1). Not all specimens measured had complete tails, due either to (presumed) predation/competition events prior to collection, or from damage to fragile tails after preservation. Because the tail length is an important ecological feature for many lizards, we sought to estimate the total tail length of individuals with incomplete tails based on information from individuals with tails. To do so we built a linear mixed effect model to predict log total tail length as a function of log SVL, with a random effect of identity species. The conditional R^2 of the model revealed 93% total variance of the log tail length is explained. The model allowed us to predict the total tail length of individuals with broken tails based on SVL and species identity. The predicted tail length was used as data for all individuals with broken tails (N = 23 out of 185), while real tail length was retained for individuals with complete tails.

To assess whether urban tolerant species occupy distinct zones of morphospace from their natural counterparts, we first ran a principal component analysis (PCA) based on the covariance matrix of all 17 morphological variables for all individuals (N = 185). All morphology measurements were log-transformed to account for body size variation being log-normally distributed. PCA reduced the dimensionality of the data by creating synthetic axes for multiple morphological traits that are highly correlated with one another. The first three principal component axes, which accounted for 95% of the total variation, were extracted and used for all subsequent analyses **(Table 1)**. To understand whether specific morphologies predispose species to tolerate urban environments we ran linear models predicting each species' (log) urban score based on the species' averages of the three PC axes, as well as the corresponding quadratic terms. We conducted the analysis in a multi-model framework, assessing the full model (as described previously), along with all combinations of the six predictor variables (3 linear and 3 quadratics). This multi-model framework was followed by an assessment of AIC to determine the best model. For individual models we used Wald estimates to determine the significance of model terms.

To test whether urban species take up less morphospace, we used the FD package (Legendre & Laliberté, 2010; Laliberté *et al.*, 2014) to calculate two functional diversity indices: functional richness (FRic) and functional dispersion (FDis). Functional richness represents the volume resulting from the convex hull whose vertices are defined by the species in morphological space (Villéger *et al.*, 2008). Functional dispersion in contrast describes how morphologically variable species in a community are, by calculating the average distance of each species from the morphological center of all species (Legendre & Laliberté, 2010). Together, FRic and FDis provide both the total size of occupied morphospace, as well as a holistic measure of morphological variation. We use both measures because functional richness can be susceptible to outlier species. These functional diversity indices were calculated using the PC axes for both urban classified species and natural classified species (with an urban score of 0.1 applied as cutoff). To control for the differences in sample number between urban and natural species we implemented bootstrap resampling by randomly selecting between 3 and 14 species for each classification, which was repeated 100 times to create a distribution of possible functional diversity values.

Finally, we determined the level of redundancy and uniqueness between morphologies that succeed in urban zones and those in natural environments. To do so we estimated the total amount of shared morphospace between urban and natural species groups using the 'hypervolume' package (Blonder, 2019). This package estimates the volume and shape of the morphospace occupied and calculates the overlap and uniqueness between urban and natural morphospaces. The volume of morphospace was calculated for both urban classified species and natural classified species. The volume of unique urban morphospace was then compared to the volume of unique natural morphospace to ascertain the level at which these two species groups overlapped morphologically. Again, to control for the differences in sample size between the number of urban versus non-urban species we implemented bootstrap resampling by randomly selecting between 3 and 14 species for each classification, which was repeated 100 times to create a distribution of possible morphospace overlaps.

RESULTS

The final dataset included a total of 185 individual specimens across 37 species. Urbanization scores ranged from 0.0130 to 0.371, with a mean of 0.101 ± 0.084 (**Figure S1**). This mean urbanization score, corresponding to the average individual of the average species occurring in an area with roughly 10% urban land cover within a 100m radius, was used as the cut-off value between classifying a species as either urban or natural. Doing so led to 14 species classified as "urban affiliated" and 23 classified as "natural affiliated". The average body length between urban and natural lizards was broadly similar (84.2mm vs 87.5mm; t = -0.845, P = 0.34). The range of body lengths was however smaller among urban species, ranging from 50.02mm to 138.5mm, whereas natural species spanned from 49.87mm to 207.4mm.

To determine if urban tolerant species occupy distinct zones of morphospace we examined the correlation between a species' morphology and its tolerance to urbanization. The first three principal component (PC) axes accounted for 95% of the total variation **(Figure 2a, 2b)**. The first axis (78% of morphological variation) was positively correlated with all measured morphological variables and represents overall lizard body size. Positive values of the second PC axis (11%) corresponded to species with long tails, narrow heads, and short forelimbs, while negative values indicated species with short tails, relatively wide heads, and long forelimbs. Finally, the third axis (7%) pertained to body length, head size, and hindlimb length relative to SVL, with positive values, linked to long, slender bodies, larger heads, and shorter hindlimbs. Our full model including both linear and quadratic effects of the three major morphological axes suggested that morphology strongly predicted tolerance to urbanization (F = 2.985, R2 = 0.25, P = 0.0209). Urban environments favored species of an intermediate size, with both large and small species filtered out (multiple linear regression, quadratic PC1 effect: beta=-0.083, t = -2.111, P = 0.043; linear PC1 effect: beta = -0.145, t = -1.817, P=0.079; **Fig 3a**). Similarly, species were more likely to occur in urban areas if they had longer body lengths, larger heads and shorter hindlimbs (quadratic PC3 effect: beta = -0.360, t=-0.664, P=0.512; linear PC3 effect: beta = 0.866, t=3.129, P=0.004; **Fig 3c**).

To verify the patterns from the full model are robust, we assessed all combinations of parameters using AIC in a multimodel framework (**Table 2**). The full model was 3.0 AIC units worse than the best-supported model. Across all models, the AIC weight of the linear component of PC3 (correlated to body length, head size, and hindlimb length) was most important (importance value: 0.92). While neither the quadratic nor the linear effect of PC2 (correlated to head size, tail length, and fore-limb length) is statistically significant in the full model (quadratic PC2 effect: beta=-0.573, t=-1.560, P=0.129; linear PC2 effect: beta=0.150, t=0.538, P=0.595), the quadratic effect of PC2 is significant in best-supported models based on AIC, and multimodel inference flags it as being relatively important (quadratic PC2 effect importance value: 0.58; **Fig 3b**). Finally, the quadratic effect of PC1 is also important (quadratic PC1 effect importance value: 0.56) which reaffirms the idea that intermediate body size is correlated to urban success.

To assess the volume of morphospace occupied by urban species we used functional richness (to determine the total volume of morphospace) and functional dispersion (to determine variation in morphospace). Non-urban species as a whole take up a larger amount of morphospace (FRic: 21.32) and have greater morphological variation (FDis: 1.67) than do their urban counterparts (FRic: 7.93, FDis: 1.33; **Figure 4a, 4c**). However, because 23 species were classified as non-urban while only 14 were urban these differences could simply arise from differences in sampling depth. But even when numbers of species in each category are equalized through bootstrap resampling, the morphospace occupied by urban species is still more limited than non-urban species (**Figure 4b,4d**).

Finally, to understand whether urban species contained unique morphologies, or simply represented a nested subset of morphologies contained within natural habitats, we examined the total amount of unique morphospace occupied by urban and natural species groups. When examining all species, the amount of morphospace shared by urban and natural species was relatively small (Sorensen similarity: 0.36; total overlap volume: 4.90, **Figure 5a**). Natural species possessed numerous unique morphologies—on average, 74% of morphospace occupied by 'natural' species did not overlap with urban morphospace (Natural unique volume: 13.97). In contrast, only 44% of morphospace occupied by urban species was unique from that of natural species (Urban unique volume: 3.83). These results were robust to resampling, such that when equal numbers of urban and natural species were analyzed, natural morphospace occupancy was 74% unique, while urban morphologies were 45% unique (**Figure 5b**).

DISCUSSION

We provide three key pieces of evidence that suggest that urban environments impose morphological limitations on lizard communities. First, species most prevalent in urban environments are predictable based on their morphology alone: urban areas favor species with intermediate body sizes, large heads, and short hind-limbs. Second, the morphological variation and the total amount of morphospace occupied by urban species is much smaller than that of their natural counterparts—a signal of ecological filtering, since filtering removes non-viable variation from a system. Finally, urban and natural species share only 35% of their morphospace, while >70% of morphospace occupied by natural habitat species is unrepresented among urban tolerant ones. As such, only a small subset of morphologies available in natural communities actually persist in urban environments.

While the exact reasons linking specific morphologies to tolerance of urbanization are not entirely clear, our findings suggest some likely mechanisms. Typically, large-bodied organisms are thought to be disfavored by anthropogenic impacts and are most threatened by extinction (Gaston & Blackburn, 1995; Cardillo *et al.*, 2005), since body size tends to correlate with a host of life-history strategies including small clutch sizes, long times to maturity, and large home range requirements. Together these slower life-history strategies and greater requirements are thought to be poorly adapted to resource-limited environments with high potential mortality. While we do show that large-bodied lizards are absent from urban areas, their small-bodied counterparts are also excluded. The propensity for intermediate body sizes being favored could have a variety of causes. The existence of an “optimal body size” has long been postulated (Stanley, 1973; Brown *et al.*, 1993), such that in the absence of competition, a given clade with a given diet and general ecology is best equipped to be a specific size due to energetic tradeoffs (Brown *et al.*, 1993). A clade's optimal body size is thought to be approximately the observed mean, as stabilizing selection pulls most species towards this mean, while competition between species for resources pushes a few species far from the mean. If species near the mean size are energetically more efficient than either small or large-bodied species, such intermediate-sized organisms may be best able to handle the stresses associated with urban life. Indeed, more recent and taxonomically expansive assessments of overall extinction risk back up the findings presented here—extinction risk is highest for both the largest and smallest species and is lower for those of intermediate size (Ripple *et al.*, 2017). Other organisms experience similar reductions in body size variability in urban environments, as birds from either end of the body size distribution are also typically absent from cities (La Sorte *et al.*, 2018).

Other explanations related to resource competition or physiological tolerance may however exist that specifically filter out small-bodied lizards. Thermotolerance may be essential for ectotherm survival in hotter and more variable anthropogenic environments (Frishkoff *et al.*, 2015; Nowakowski *et al.*, 2018b), but small-sized lizards retain less heat, directly affecting their ability to thermoregulate (Michael *et al.*, 2014). Alternatively, resource limitation and both interference and exploitative competition may play a role in explaining the failure of smaller species. Most lizards are generalist insectivores and select prey based on their size. Insect abundance is severely reduced in urban environments (Merckx *et al.*, 2018). Due to gape limitation, intermediate-bodied species have access to a larger range of prey sizes (both small prey and large prey), while smaller lizards are forced to compete for small prey both amongst themselves and with larger lizards (Herrel *et al.*, 1995; Lima *et al.*, 2000; Vitt, 2000). This mechanism would also explain why species with large head sizes are preferentially abundant in urban areas. Interference competition between species in urban environments may similarly play a role, as larger heads are also paramount in aggressive displays, both within and between species (Donihue *et al.*, 2016; Wegener *et al.*, 2019). More behaviorally aggressive species and individuals are often more common in human-modified environments, with the best cases coming from studies of birds (Shochat *et al.*, 2010; Scales *et al.*, 2011; Hernández-Brito *et al.*, 2014). For example in agricultural landscapes, birds feedings at isolated trees are primarily aggressive dominant species with large bill sizes, while subordinate species are restricted to trees near forest-agriculture ecotones (Daily & Ehrlich, 1994).

Limb length reflects a trade-off between agility and speed on broad surfaces versus narrow surfaces. In general, longer hind-limbs are associated with faster-running speeds and ecologies in which an individual needs to flee from predators and run-down prey. Shorter limbs in contrast grant individuals the ability to navigate narrow and irregular surfaces, greater climbing ability, and are often associated with sit-and-wait predator strategies (Losos, 2009). Urban environments are dominated by buildings and older trees, and possess a denuded understory—features that in some ways mimic natural environments where the need to climb benefits lizards with shorter limbs (Herrel *et al.*, 2001). This trend was also directly observed in western fence lizards where females in urbanized environments had shorter limbs than their non-urban counterparts (Sparkman *et al.*, 2018). However, urban populations of *Anolis cristatellus* possess longer limbs than natural populations (Winchell *et al.*, 2016), indicating that the benefits of short limbs are not ubiquitous and that multiple eco-morphological strategies might exist to maximize fitness in urban environments. Alternatively, the preference for short limbs may come about due to its association with sit-and-wait foraging, a strategy that in contrast to active foraging, may avoid risks from enhanced predation from mesopredators, like feral cats, or pseudo-predation from automobiles. Indeed our findings are consistent with some emerging patterns of urban success found in other lizard faunas. In the Caribbean urban tolerance is negatively associated with relative hind limb length across the genus *Anolis* (Winchell *et al.*, 2020)

At the level of the "urban lizard fauna", we find that the total morphological diversity represented in these urban systems is much smaller than that of the primarily natural fauna. Such a reduction of morphological variation among the urban assemblages mirrors general losses of functional diversity (of which morphological diversity is a subset) that are frequently observed in human-modified environments. For example, urban bird assemblages show an average decrease of 20% in functional diversity when compared to their surrounding natural habitats (Sol *et al.*, 2020), and for birds and mammals, functional diversity declines sharply as agricultural land use intensifies (Flynn *et al.*, 2009). Among these North American lizards, the reduction in morphological variability is partially attributable to large and small-bodied lizards being preferentially excluded from urban areas. While overall the urban tolerant species group contained less overall morphological diversity, the remaining diversity was not a simple subset of the diversity among the natural habitat affiliated species. Instead, roughly 40% of the morphological diversity of urban species was unique. This finding does not necessarily mean that there are truly unique morphotypes in urban environments, as even the most urban affiliated species in this dataset still occur frequently in natural environments as well.

Addressing whether urban environments typically contain morphologically unique species that are absent or rare in nearby natural environments would require standardized surveys of individual lizard communities, a task that presence-only citizen science occurrence data is not suited for. Regardless, the overall reduction in morphospace occupation, along with the shift towards some unique morphologies among the most urban tolerant lizards, suggest that urban environments represent a strong selection pressure at the community level.

The ability of anthropogenic pressures to reduce diversity beyond the taxonomic level has become more widely recognized as a conservation challenge (Devictor *et al.*, 2010). In particular, phylogenetic and functional diversity a multiple scales are eliminated by intense forms of anthropogenic change (Sol *et al.*, 2020). often above and beyond that expected from species loss alone (Flynn et al., 2009; Frishkoff et al., 2014; Hagen et al., 2017). Such diversity declines are especially worrisome because functional diversity is more tightly linked with ecological functioning (Tilman *et al.*, 1997), and its decline therefore likely portends loss of the services that ecosystems provide to humans (Karp *et al.*, 2013; Monagan *et al.*, 2017; Echeverri *et al.*, 2021). Whether reductions in lizard diversity observed here result in meaningful declines in the services lizards provide, such as pest control, remain unknown. However, lizard abundance has been linked with agricultural insect pest consumption (Monagan *et al.*, 2017), suggesting that their role as service providers may be relevant in urban areas.

Together our data show that the urban lizard fauna is a morphologically restricted set of species, implicating the urban environment as imposing ecological filters on urban community assembly. Importantly we go beyond simply demonstrating morphological diversity losses, but show specifically how changes in morphological composition results in this diversity loss—

namely through reduction of large and small body sizes and elimination of lizards with smaller heads and longer limbs. While our analysis shows that morphology predicts roughly 25% of the variation in relative affiliation with urban versus natural environments, other unconsidered features likely also play a role. For example, species with higher trophic positions, or those that depend on aquatic habitats may be especially vulnerable to changing land-use (Todd *et al.*, 2016, 2017). Critically however, many of these ecologies will be reflected in species morphologies. However, other traits will not be reflected in morphology. Of these, physiological traits, especially heat tolerance, are likely to be paramount, as thermal tolerance has been repeatedly linked with success in human-modified systems (Frishkoff *et al.*, 2015; Nowakowski *et al.*, 2018a).

This study highlights an analytical framework to assess the prevalence of morphological filters in human-dominated landscapes using widely available, and ever-increasing citizen science data. Whether the patterns documented here are general features of urban systems across taxa or across geographic space remains to be seen. The traits that predict tolerance to other forms of anthropogenic change are sometimes inconsistent between regions (Hatfield *et al.*, 2018) or types of habitat conversion (Bartomeus *et al.*, 2018). Indeed the same species traits may increase tolerance to land-use change in some climate zones while decreasing tolerance in others (Murray *et al.*, 2021). As more of the filters determining community composition in anthropogenic systems are understood, the ecological rules that define the prevailing biological communities of the Anthropocene will come into focus. Hopefully, with an appreciation of these rules, targeted interventions to make anthropogenic systems more wildlife-friendly can be implemented, which will support species with traits that were previously filtered out.

Acknowledgements: We would like to thank the museums and biodiversity collections that made collecting the lizard morphological data possible. Specifically, we thank Carol Spencer, Michelle Koo, as well as the staff of the UC Berkeley Museum of Vertebrate Zoology for allowing access to the herpetology collection. Additionally, we thank the faculty and staff that support the Amphibian and Reptile Diversity Research Center at the University of Texas at Arlington for their instructions on proper museum etiquette and procedures.

Literature Cited

Azzurro, E., Tuset, V.M., Lombarte, A., Maynou, F., Simberloff, D., Rodríguez-Pérez, A. & Solé, R. V. (2014) External morphology explains the success of biological invasions. *Ecology Letters*, **17**, 1455–1463.

Bartomeus, I., Cariveau, D.P., Harrison, T. & Winfree, R. (2018) On the inconsistency of pollinator species traits for predicting either response to land-use change or functional contribution. *Oikos*, **127**, 306–315.

Blonder, B. (2019) Package “hypervolume” Type Package Title High Dimensional Geometry and Set Operations Using Kernel Density Estimation, Support Vector Machines, and Convex Hulls.

Brooks, T.M., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B., Rylands, A.B., Konstant, W.R., Flick, P., Pilgrim, J., Oldfield, S., Magin, G. & Hilton-Taylor, C. (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology*, **16**, 909–923.

Brown, J.H., Marquet, P.A. & Taper, M.L. (1993) Evolution of Body Size : Consequences of an Energetic Definition of Fitness. *American Naturalist*, **142**, 573–584.

Cardillo, M., Mace, G.M., Jones, K.E., Bielby, J., Bininda-Emonds, O.R.P., Sechrest, W., Orme, C.D.L. & Purvis, A. (2005) Evolution: Multiple causes of high extinction risk in large mammal species. *Science*, **309**, 1239–1241.

Daily, G.C. & Ehrlich, P.R. (1994) Influence of social status on individual foraging and community structure in a bird guild. *Oecologia 1994 100:1*, **100**, 153–165.

Devictor, V., Mouillot, D., Meynard, C., Jiguet, F., Thuiller, W. & Mouquet, N. (2010) Spatial mismatch and congruence between taxonomic, phylogenetic and functional diversity: the need for integrative conservation strategies in a changing world. *Ecology Letters*, **13**, 1030–1040.

Donihue, C.M., Brock, K.M., Foufopoulos, J. & Herrel, A. (2016) Feed or fight: testing the impact of food availability and intraspecific aggression on the functional ecology of an island lizard. *Functional Ecology*, **30**, 566–575.

Echeverri, A., Karp, D.S., Frishkoff, L.O., Krishnan, J., Naidoo, R., Zhao, J., Zook, J. & Chan, K.M.A. (2021) Avian cultural services peak in tropical wet forests. *Conservation Letters*, **14**.

Flynn, D.F.B.B., Gogol-Prokurat, M., Nogeire, T., Molinari, N., Richers, B.T., Lin, B.B., Simpson, N., Mayfield, M.M. & DeClerck, F. (2009) Loss of functional diversity under land use intensification across multiple taxa. *Ecology Letters*, **12**, 22–33.

Frishkoff, L.O., Hadly, E.A. & Daily, G.C. (2015) Thermal niche predicts tolerance to habitat conversion in tropical amphibians and reptiles. *Global Change Biology*, **21**, 3901–3916.

Frishkoff, L.O., Karp, D.S., M'Gonigle, L.K., Mendenhall, C.D., Zook, J., Kremen, C., Hadly, E.A. & Daily, G.C. (2014) Loss of avian phylogenetic diversity in neotropical agricultural systems. *Science*, **345**, 1343–1346.

Gaston, K.J. & Blackburn, T.M. (1995) Birds, body size and the threat of extinction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **347**, 205–212.

Grimm, N.B., Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X. & Briggs, J.M. (2008) Global change and the ecology of cities. *Science*, **319**, 756–760.

Hagen, E.O., Hagen, O., Ibáñez-álamo, J.D., Petchey, O.L. & Evans, K.L. (2017) Impacts of urban areas and their characteristics on avian functional diversity. *Frontiers in Ecology and Evolution*, **5**, 1–15.

Hatfield, J.H., Orme, C.D.L., Tobias, J.A. & Banks-Leite, C. (2018) Trait-based indicators of bird species sensitivity to habitat loss are effective within but not across data sets. *Ecological Applications*, **28**, 28–34.

Hernández-Brito, D., Carrete, M., Popa-Lisseanu, A.G., Ibáñez, C. & Tella, J.L. (2014) Crowding in the city: Losing and winning competitors of an invasive bird. *PLoS ONE*, **9**, e100593.

Herrel, A., Van Damme, R. & De Vree, F. (1995) Sexual Dimorphism of Head Size in Podarcis Hispanica Atrata’. Testing the Dietary Divergence Hypothesis by Bite Force Analysis. *Netherlands Journal of Zoology*, **46**, 253–262.

Herrel, A., Meyers, J.J. & Vanhooydonck, B. (2001) Correlations between habitat use and body shape in a phrynosomatid lizard (Urosaurus ornatus): A population-level analysis. *Biological Journal of the Linnean Society*, **74**, 305–314.

Homer, C., Dewitz, J., Fry, J., Coan, M., Hossain, N., Larson, C., Herold, N., McKerrow, A., VanDrie, J.N. & Wickham, J. (2015) Completion of the 2011 National Land Cover Database for the Conterminous United States. *Photogrammetric Engineering and Remote Sensing*, **81**, 345–353.

Karp, D.S., Mendenhall, C.D., Sandí, R.F., Chaumont, N., Ehrlich, P.R., Hadly, E.A. & Daily, G.C. (2013) Forest bolsters bird abundance, pest control and coffee yield. *Ecology Letters*, **16**, 1339–1347.

Laliberté, E., Legendre, P. & Shipley, B. (2014) FD: measuring functional diversity from multiple traits,and other tools for functional ecology. R package version 1.0-12.

Legendre & Laliberté (2010) A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, **91**, 299–305.

Lima, A.P., Magnusson, W.E., Williams, D.G., Journal, S. & Mar, N. (2000) Differences in Diet among Frogs and Lizards Coexisting in Subtropical Forests of Australia. *Society for the Study of Amphibians and Reptiles*, **34**, 40–46.

Losos, J.B. (2009) *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*, First. University of California Press, Berkeley, CA.

Marnocha, E., Pollinger, J. & Smith, T.B. (2011) Human-induced morphological shifts in an island lizard. *Evolutionary Applications*, **4**, 388–396.

McDonald, R.I., Marcotullio, P.J. & Güneralp, B. (2013) *Urbanization and Global Trends in Biodiversity and Ecosystem Services*. *Urbanization, Biodiversity and Ecosystem Services: Challenges and Opportunities*, pp. 31–52. Springer Netherlands, Dordrecht.

McKinney, M.L. (2008) Effects of urbanization on species richness: A review of plants and animals. *Urban Ecosystems*, **11**, 161–176.

McKinney, M.L. (2002) Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, **52**, 883–890.

McKinney, M.L. & Lockwood, J.L. (1999) Biotic homogenization: A few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution*, **14**, 450–453.

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F., Backeljau, T., Bonte, D., Brans, K.I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J.M.T., Fontaneto, D., Gianuca, A.T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Van Doninck, K., De Meester, L. & Van Dyck, H. (2018) Body-size shifts in aquatic and terrestrial urban communities. *Nature*, **558**, 113–116.

Michael, D.R., Banks, S.C., Piggott, M.P., Cunningham, R.B., Crane, M., MacGregor, C., McBurney, L. & Lindenmayer, D.B. (2014) Geographical variation in body size and sexual size dimorphism in an Australian lizard, Boulenger's Skink (Morethia boulengeri). *PLoS ONE*, **9**, e109830.

Monagan, I. V., Morris, J.R., Rabosky, A.R.D., Perfecto, I. & Vandermeer, J. (2017) Anolis lizards as biocontrol agents in mainland and island agroecosystems. *Ecology and Evolution*, **7**, 2193–2203.

Murray, A.H., Nowakowski, A.J. & Frishkoff, L.O. (2021) Climate and land-use change severity alter trait-based responses to habitat conversion. *Global Ecology and Biogeography*, **30**, 598–610.

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger, L., Bennett, D.J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M.J., Feldman, A., Garon, M., Harrison, M.L.K., Alhusseini, T., Ingram, D.J., Itescu, Y., Kattge, J., Kemp, V., Kirkpatrick, L., Kleyer, M., Correia, D.L.P., Martin, C.D., Meiri, S., Novosolov, M., Pan, Y., Phillips, H.R.P., Purves, D.W., Robinson, A., Simpson, J., Tuck, S.L., Weiher, E., White, H.J., Ewers, R.M., MacE, G.M., Scharlemann, J.P.W. & Purvis, A. (2015) Global effects of land use on local terrestrial biodiversity. *Nature*, **520**, 45–50.

Nowakowski, A.J., Frishkoff, L.O., Thompson, M.E., Smith, T.M. & Todd, B.D. (2018a) Phylogenetic homogenization of amphibian assemblages in human-altered habitats across the globe. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, E3454–E3462.

Nowakowski, A.J., Watling, J.I., Thompson, M.E., Brusch, G.A., Catenazzi, A., Whitfield, S.M., Kurz, D.J., Suárez-Mayorga, Á., Aponte-Gutiérrez, A., Donnelly, M.A. & Todd, B.D. (2018b) Thermal biology mediates responses of amphibians and reptiles to habitat modification. *Ecology Letters*, **21**, 345–355.

Pereira, H.M., Navarro, L.M. & Martins, I.S. (2012) Global Biodiversity Change: The Bad, the Good, and the Unknown. *Annual Review of Environment and Resources*, **37**, 25–50.

Putman, B.J., Gasca, M., Blumstein, D.T. & Pauly, G.B. (2019) Downsizing for downtown: limb lengths, toe lengths, and scale counts decrease with urbanization in western fence lizards (Sceloporus occidentalis). *Urban Ecosystems*, **22**, 1071–1081.

Putman, B.J. & Tippie, Z.A. (2020) Big City Living: A Global Meta-Analysis Reveals Positive Impact of Urbanization on Body Size in Lizards. *Frontiers in Ecology and Evolution*, **8**, 1–13.

Rasner, C.A., Yeh, P., Eggert, L.S., Hunt, K.E., Woodruff, D.S. & Price, T.D. (2004) Genetic and morphological evolution following a founder event in the dark-eyed junco, Junco hyemalis thurberi. *Molecular Ecology*, **13**, 671–681.

Raupp, M.J., Shrewsbury, P.M. & Herms, D.A. (2009) Ecology of Herbivorous Arthropods in Urban Landscapes. *Annual Review of Entomology*, **55**, 19–38.

Reznick, D.N. & Ghalambor, C.K. (2001) The population ecology of contemporary adaptations: What empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112**–**113**, 183–198.

Ripple, W.J.J., Wolf, C., Newsome, T.M.M., Hoffmann, M., Wirsing, A.J.J. & McCauley, D.J.J. (2017) Extinction risk is most acute for the world’s largest and smallest vertebrates. *Proceedings of the National Academy of Sciences*, **114**, 10678–10683.

Scales, J., Hyman, J. & Hughes, M. (2011) Behavioral syndromes break down in urban song sparrow populations. *Ethology*, **117**, 887–895.

Seto, K.C., Parnell, S. & Elmqvist, T. (2013) *A Global Outlook on Urbanization*. *Urbanization, Biodiversity and Ecosystem Services: Challenges and Opportunities*, pp. 1–12. Springer Netherlands, Dordrecht.

Shochat, E., Lerman, S.B., Anderies, J.M., Warren, P.S., Faeth, S.H. & Nilon, C.H. (2010) Invasion, Competition, and Biodiversity Loss in Urban Ecosystems. *BioScience*, **60**, 199–208.

Shochat, E., Warren, P.S., Faeth, S.H., McIntyre, N.E. & Hope, D. (2006) From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology and Evolution*, **21**, 186–191.

Silva, C.P., Sepúlveda, R.D. & Barbosa, O. (2016) Nonrandom filtering effect on birds: species and guilds response to urbanization. *Ecology and Evolution*, **6**, 3711–3720.

Sol, D., Trisos, C., Múrria, C., Jeliazkov, A., González-Lagos, C., Pigot, A.L., Ricotta, C., Swan, C.M., Tobias, J.A. & Pavoine, S. (2020) The worldwide impact of urbanisation on avian functional diversity. *Ecology Letters*, **23**, 962–972.

La Sorte, F.A., Lepczyk, C.A., Aronson, M.F.J., Goddard, M.A., Hedblom, M., Katti, M., MacGregor-Fors, I., Mörtberg, U., Nilon, C.H., Warren, P.S., Williams, N.S.G. & Yang, J. (2018) The phylogenetic and functional diversity of regional breeding bird assemblages is reduced and constricted through urbanization. *Diversity and Distributions*, **24**, 928–938.

Sparkman, A., Howe, S., Hynes, S., Hobbs, B. & Handal, K. (2018) Parallel behavioral and morphological divergence in fence lizards on two college campuses. *PLOS ONE*, **13**, e0191800.

Stanley, S.M. (1973) An Explanation For Cope's Rule. *Evolution*, **27**, 1–26.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. & Siemann, E. (1997) The influence of functional diversity and composition on ecosystem processes. *Science*, **277**, 1300–1302.

Todd, B.D., Nowakowski, A.J., Rose, J.P. & Price, S.J. (2017) Species traits explaining sensitivity of snakes to human land use estimated from citizen science data. *Biological Conservation*, **206**, 31–36.

Todd, B.D., Rose, J.P., Price, S.J. & Dorcas, M.E. (2016) Using citizen science data to identify the sensitivity of species to human land use. *Conservation Biology*, **30**, 1266–1276.

Villéger, S., Mason, N.W.H. & Mouillot, D. (2008) New Multidimensional Functional Diversity Indices for a Multifaceted Framework in Functional Ecology Published by : Ecological Society of America content in a trusted digital archive. *Ecology*, **89**, 2290–2301.

Vitt, L.J. (2000) Ecological Consequences of Body Size in Neonatal and Small-Bodied Lizards in the Neotropics. *Herpetological Monographs*, **14**, 388–400.

Wegener, J.E., Mulder, K.P., Pringle, R.M., Losos, J.B. & Kolbe, J.J. (2019) HEAD SIZE OF MALE AND FEMALE LIZARDS INCREASES WITH POPULATION DENSITY ACROSS ISLAND POPULATIONS IN THE BAHAMAS. *https://doi.org/10.3099/0006-9698-566.1.1*, **566**, 1–9.

Winchell, K.M., Reynolds, R.G., Prado-Irwin, S.R., Puente-Rolón, A.R. & Revell, L.J. (2016) Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution*, **70**, 1009–1022.

Winchell, K.M., Schliep, K.P., Mahler, D.L. & Revell, L.J. (2020) Phylogenetic signal and evolutionary correlates of urban tolerance in a widespread neotropical lizard clade*. *Evolution*, **74**, 1274–1288.

Figures and tables

Table 1: Principal component axis loadings of the 17 morphological measurements across the 185 individuals and 37 species included in the study. All loadings above 0.15 and below -0.15 are color-coded to indicate the strength of positive (red) and negative (blue) correlation with each axis.

Measurement	PC1	PC2	PC3
Snout Vent Length	**0.18**	0.04	**0.25**
Head Length	**0.21**	0.02	**0.27**
Head Width	**0.24**	**-0.24**	**0.27**
Head Height	**0.23**	**-0.15**	**0.19**
Lower Jaw Length	**0.21**	0.12	**0.24**
Outlever	**0.21**	0.10	**0.26**
Jugal to Symphasis	**0.20**	0.01	**0.19**
Femur	**0.27**	-0.08	-0.12
Tibia	**0.30**	**-0.10**	**-0.33**
Metatarsal	**0.32**	-0.03	**-0.45**
Longest Toe (4th)	**0.27**	**0.26**	**-0.40**
Humerus	**0.24**	**-0.24**	-0.04
Radius	**0.25**	**-0.19**	-0.06
Longest Finger (4th)	**0.25**	-0.01	**-0.18**
Pelvis Height	**0.23**	-0.01	**0.17**
Pelvis Width	**0.24**	-0.23	**0.18**
Tail Length	**0.24**	**0.81**	0.10
Prop. Variation	0.78	0.11	0.07

Table 2: Significance and parameter values of key models evaluated in the manuscript regressing species' (log) urbanization index score against the three major morphospace axes and their quadratic terms. 'Rank' displays the most likely models (all models within 2 AIC of best model) as well as the AIC ranks of the full model, and the null model against all 65 possible combinations of the six predictor variables. 'Weight' indicates the AIC weight, and ΔAIC indicates the difference in AIC between the focal model and the best model. Intercept and PC terms denote parameter estimates. Significance of parameter estimates is indicated by adjacent symbols (+ $p<0.1$, * $p<0.05$, ** $p<0.01$). Finally, the 'importance values' indicate the sum of model weights over all 65 models that include each parameter.

Rank	AIC	ΔAIC	Weight	Intercept	PC1	$PC1^2$	PC2	$PC2^2$	PC3	$PC3^2$
1	83.0	0.0	0.16	-2.25	-0.14 +	-0.08 *		-0.63 *	0.84 **	
2	84.4	1.4	0.08	-2.14	-0.15 +	-0.09 *		-0.70 *	0.88 **	-0.38
3	84.5	1.5	0.08	-2.28		-0.07 +		-0.61 *	0.84 **	
4	84.6	1.6	0.07	-2.3	-0.14 +	-0.08 +	0.16	-0.49	0.83 **	
7 Full	86.0	3.0	0.04	-2.19	-0.15 +	-0.08 *	0.15	-0.57	0.87 **	-0.36
64 Null	180.96	98.0	0.00							
		Importance Values:			0.50	0.56	0.40	0.58	0.92	0.21

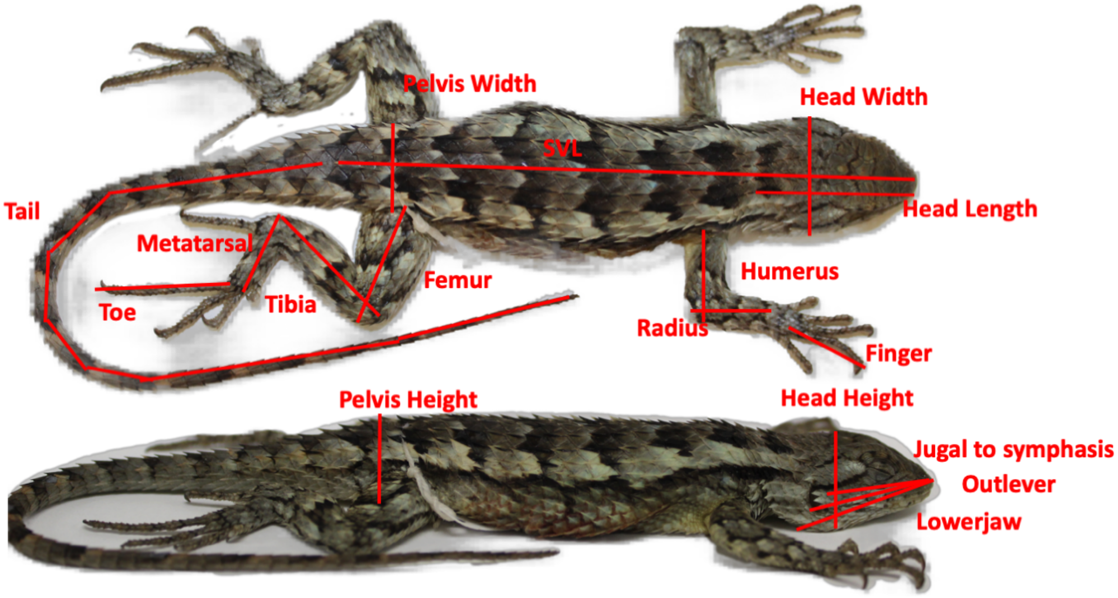


Figure 1: A representation of the 17 morphological traits measured, depicted against a *Sceloporus olivaceus* specimen.

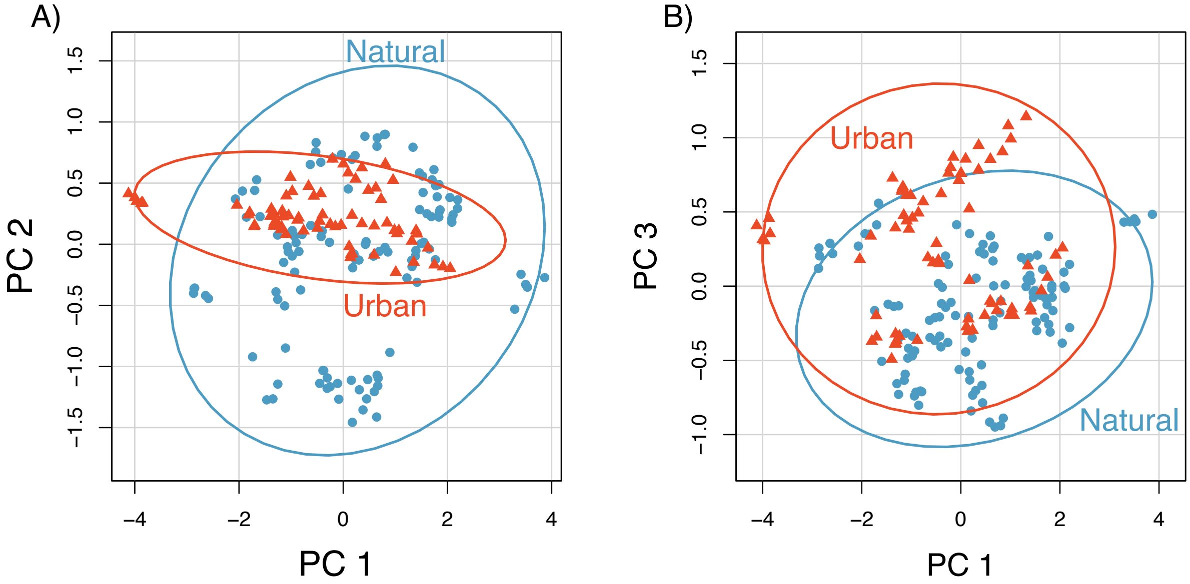


Figure 2: Morphological associations with urban environments. (A) Scatterplot of all individuals of all species PC1 (body size) and PC2 values (tail length, head width, fore-limb length). (B) Corresponding PC3 (body length, head size, and hind-limb length) versus PC1 (body size) values. In A and B each point represents an individual of either an urban (red) or natural (blue) affiliated species. Ellipses represent the 95% confidence intervals.

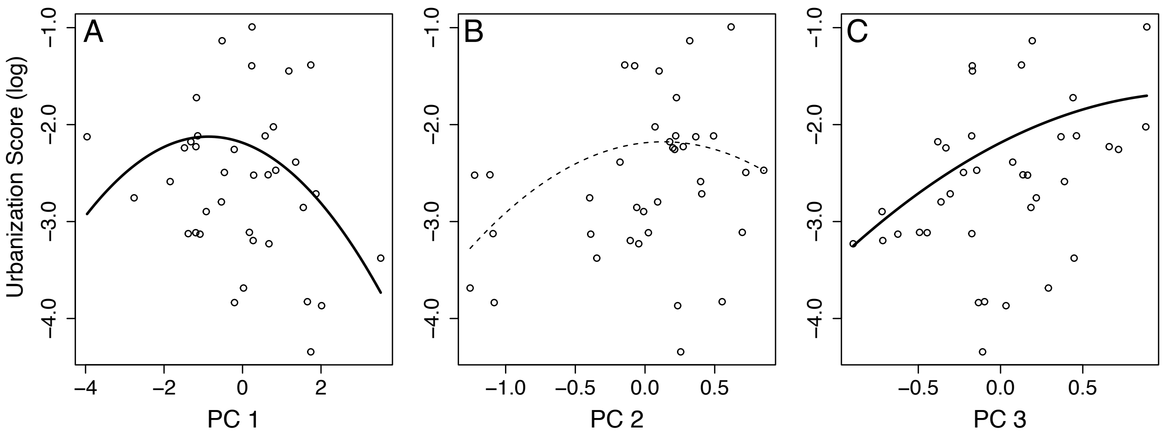


Figure 3: Relationships between morphology and urbanization based on the full model containing linear and quadratic effects of three major morphological PC axes. (A) Relationship between PC1 and the average amount of urban area where species is encountered (log urbanization score), depicting that intermediate-sized species are most likely to occur in urban environments. (B) Relationship between PC2 and log-transformed urbanization score. While not significant, the multimodel framework highlighted a high amount of importance in the quadratic PC2 effect. (C) Relationship between PC3 and (log) urbanization score, highlighting that urban environments favor long bodies, bigger heads, and short hind-limbs. Each point represents a species' mean PC value. Lines depict best-fit relationship from multiple linear regression models (after taking into account the effects of other PC axes).

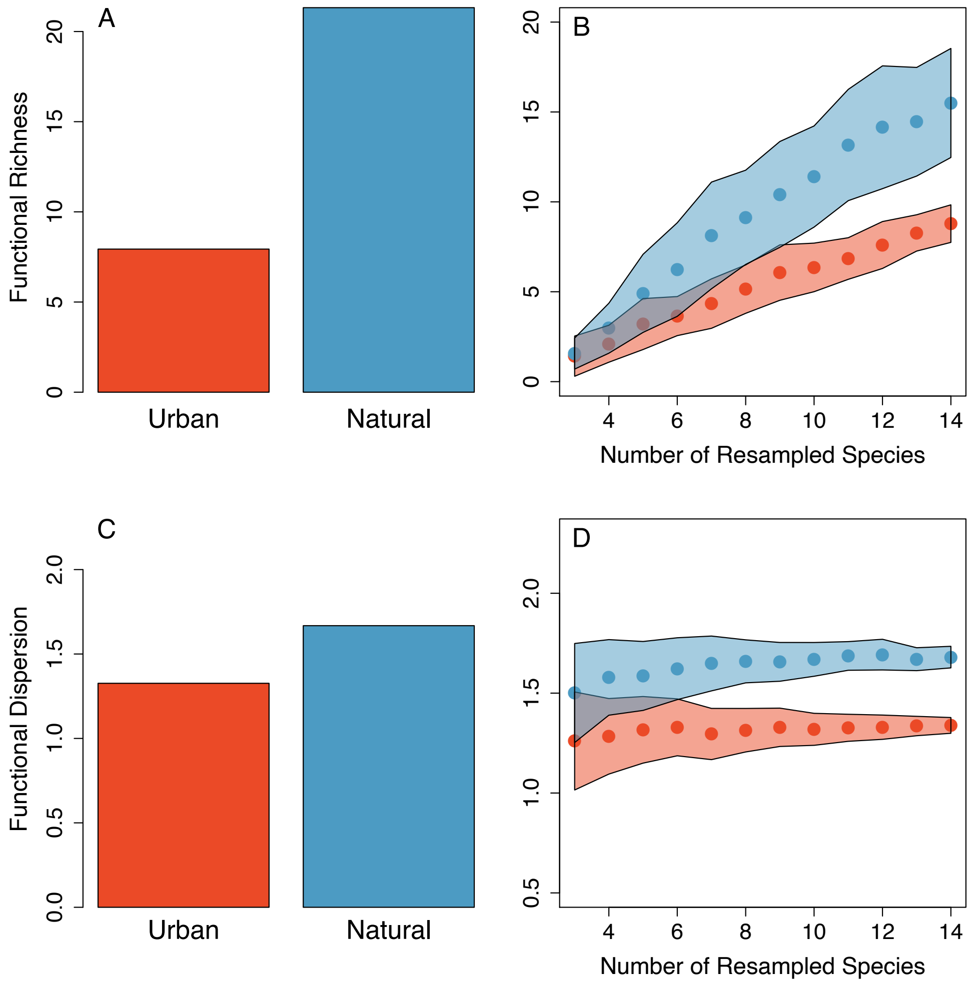


Figure 4: Functional diversity contained within the urban-associated and natural-restricted lizard faunas. (A) Barplots showing the total functional richness contained in urban (N = 14 species), and natural restricted (N = 23 species) faunas, with (B) bootstrap resampling used to account for a difference in sample size between faunas. Panels (C) and (D) show corresponding differences between urban and natural species for functional dispersion. In B and D points represent the bootstrapped means, and the shaded regions represent standard deviation.

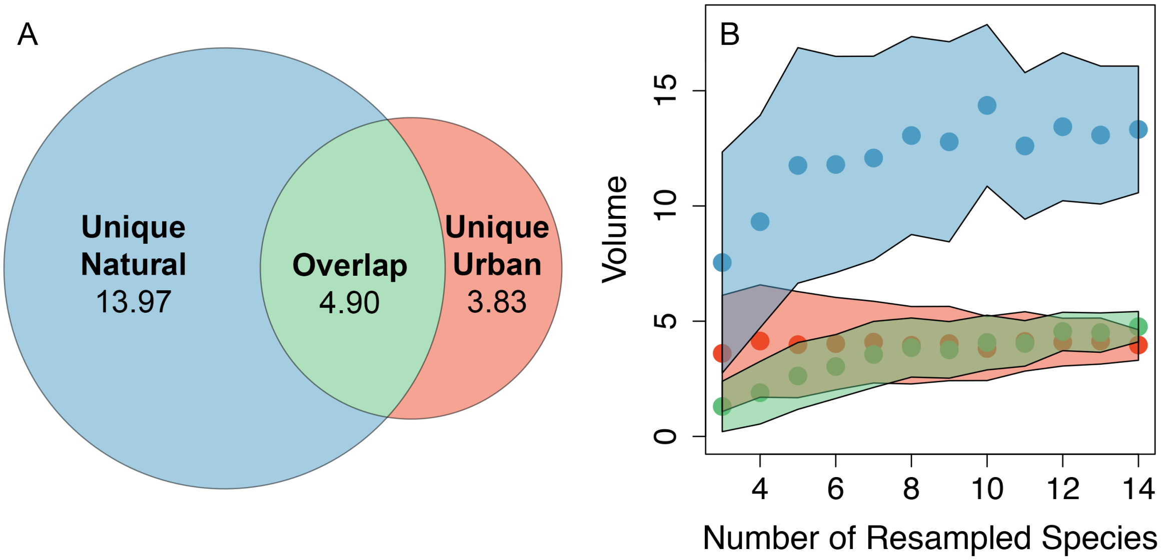


Figure 5: Unique and overlapping morphospace volumes occupied by urban-associated and natural-restricted lizard faunas. (A) Venn diagram showing the volume of unique and overlapping morphospace occupied by urban-associated (N = 14 species), natural-restricted (N = 23 species) faunas, with (B) bootstrap resampling used to account for a difference in sample size between faunas. In B points represent the respective the bootstrapped means, and the shaded regions around B represent standard deviations.

Supplemental Figures

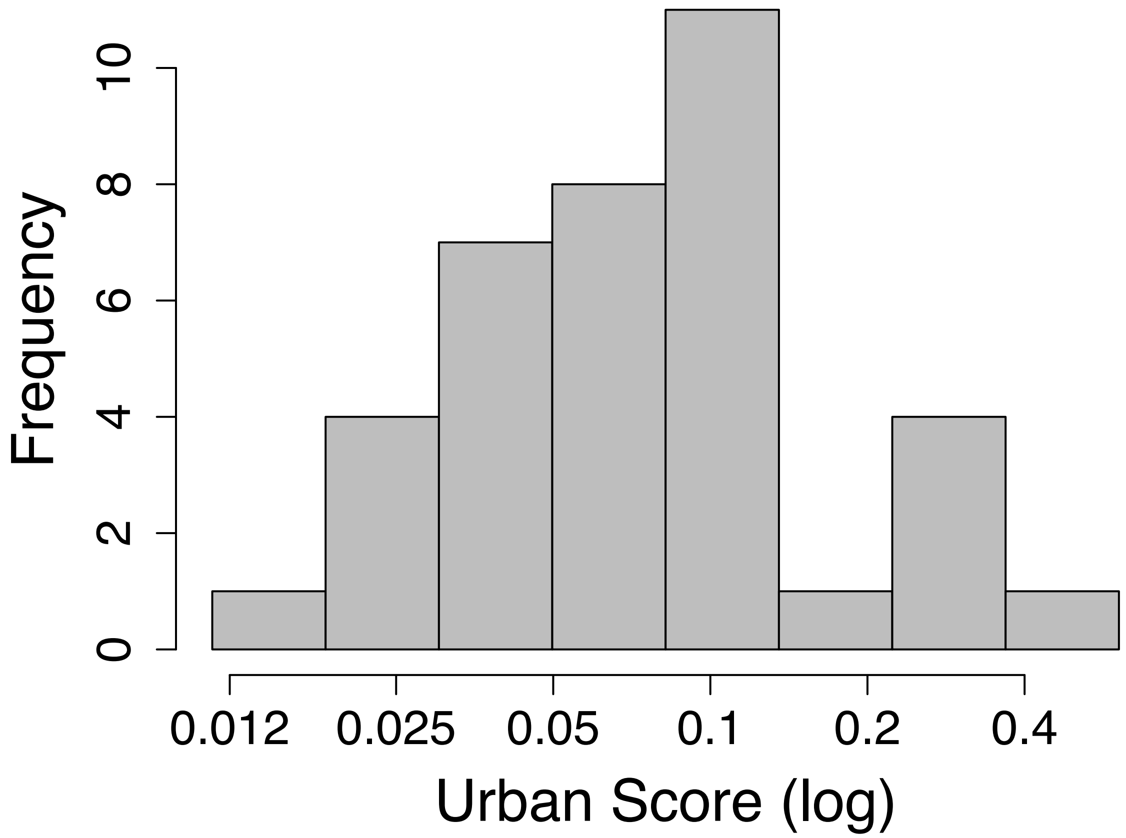


Figure S1: Histogram showing the distribution of urbanization score across all 37 species used in the analysis. Note that x-axis is on log-scale.

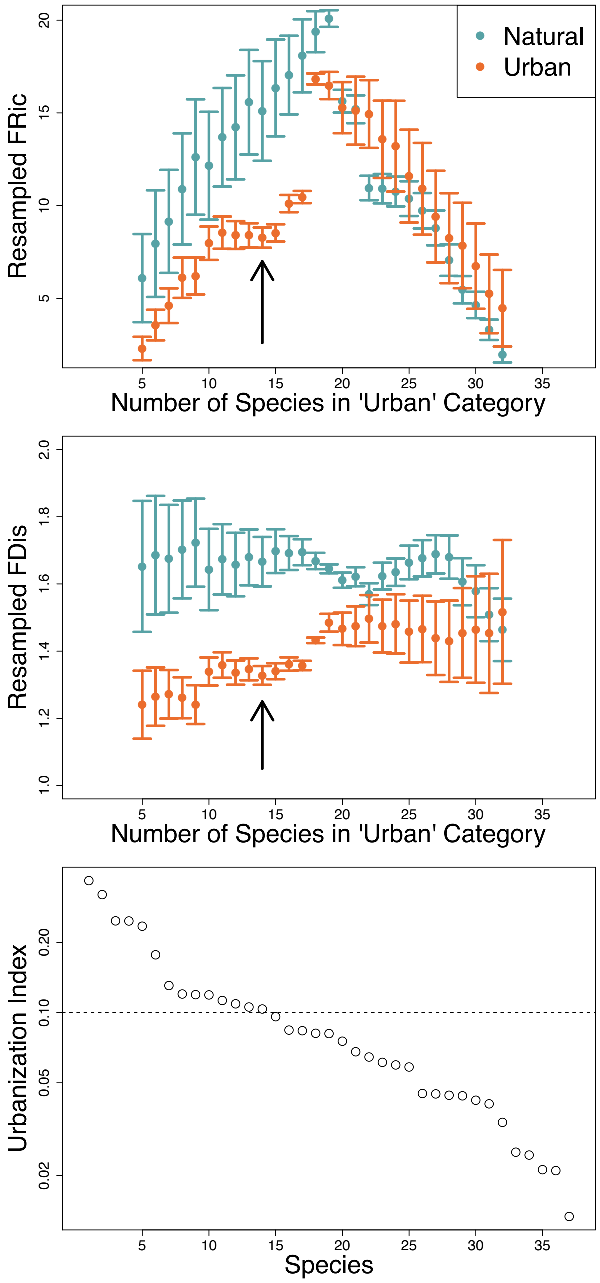


Figure S2: Sensitivity analysis evaluating the consequences of alternative cut-off values to determine “urban” species. In the top two panels functional richness (FRic) and functional dispersion (FDis) are evaluated under alternative cut-off values by considering the X most “urban” species, based on their urbanization index value, and then resampling (without replacement) the remaining (more natural affiliated) species down to X, and calculating FRic and FDis on this equal-sized subset. We evaluated all values of urbanization cut-offs, starting with the five most urbanized species up to 32 when there were only 5 species in the “natural” category. In top and middle panel points depict means and lines show the standard deviations over 1000 resamplings for each value of X. Black arrow shows the number of species considered “urban” in the main text. The lower panel shows the corresponding urbanization values for all species in the dataset, ordered from most to least urban affiliated, such that placement along the x-axis corresponds to the resampled values of species in the upper two panels. The dashed line shows the urbanization cut-off (0.1) used in the main text. Note that the y-axis is logarithmic.

Chapter 3:

Classifying morphological change across multiple genera of lizard in response to urbanization.

Kristopher Row (1), Alexander Murray (1), Monica Miles (1), Luke Frishkoff (1)

(1) Department of Biology, University of Texas Arlington, 501 S. Nedderman Dr., Arlington TX,

76019, USA

Abstract:

Urbanization represents a crucial aspect of global transformation that is exerting substantial selection pressures on species inhabiting these areas. While it is widely known that numerous plant and animal species manage to survive in these urban environments, there is still a limited understanding of whether these urban inhabiting populations are undergoing adaptations to prove successful and even less information is known regarding this effect on a global level. Here we seek to look at the local effects of urbanization on the morphological traits of two lizard species found in the state of Texas, *Anolis carolinensis* and *Sceloporus olivaceus*. Furthermore, we then compare our findings to two other species shown to alter their morphology in response to urbanization, *Anolis cristatellus* and *Sceloporus occidentalis*. In Texas, we found that both *A. carolinensis* and *S. olivaceus* show alterations in their morphology in urban environments presenting with longer fore limb and hind limb lengths while urban *A. carolinensis* are also increasing their head length, *S. olivaceus* are increasing their head height. Finally, when comparing our findings to other known accounts of phenotypic change brought on by urbanization, we find a similar pattern in *A. cristatellus* but an opposite trend in *S. occidentals* where limb length is reportedly getting shorter. Our findings suggest that while most species found in urban environments are altering their morphologies in predictable ways, there remains a subset of species that will have unique experiences resulting in unpredictable phenotypic change.

Introduction

Human-mediated habitat conversion is one of the primary drivers of biodiversity loss (Brooks *et al.*, 2002; Pereira *et al.*, 2012; Newbold *et al.*, 2015). Urbanization is an increasingly relevant form of habitat modifications due to its rapid expansion and drastic change on the landscape. This rapid expansion can be seen in the massive increase in human populations concentrated in urban areas. In 1950, the human population in urban areas was roughly 750 million, today, that number is 4.4 billion (56% of the world's population) and is expected to grow by another 2.5 billion by the year 2050 (United Nations, 2018). Natural habitats are being replaced by urban ones to accommodate the rapid increase in population, which is precipitating large biodiversity loses, often reducing species richness by great than 50% (Newbold *et al.*, 2015).

The expedited nature of urban growth leads to almost instantaneous selection pressures that alter the topography and dimensionality of the adaptive landscape (Svensson & Calsbeek, 2013). Species found in urban environments are forced to not only adapt to altered microhabitat conditions (Shochat *et al.*, 2006) and changing food sources (Reznick & Ghalambor, 2001) but they must also adapt to novel physical structures (McKinney, 2002). Determining the link between morphology and ecological success in these urban environments constitutes a core requirement in understanding how species adapt and thrive there.

To date, most morphology-based research into urban phenotypes has been focused on studying its effect on single species (Marnocha *et al.*, 2011; Winchell *et al.*, 2016; Putman *et al.*, 2019). While this invaluably builds a case for contemporary adaptive evolution within species to novel urban habitats, the field is lacking in how combined communities adapt and if they exhibit similar patterns. Based on the single species studies we have a general idea on how the adaptive landscape of urban environments effects phenotypic traits, but it remains unknown if rapid environmental change will precipitate predictable convergent evolution (Mahler *et al.*, 2013; Hart *et al.*, 2018) across species or if the landscape will drive multiple species in a more idiosyncratic fashion (Obolski *et al.*, 2018). For example, convergent evolution would be seen where multiple related species alter their morphology along similar vectors in response to urbanization. We also see evidence of morphological similarities in lizards with species that are found in high abundance in urban areas, specifically, we see that urban associated species tend to favor more intermediate body sizes (filtering out species to large or small), larger heads, and shorter hind limbs than naturally associated species (Row *et al.*, 2023).

Lizards offer an excellent opportunity for studying the impact of urbanization on morphological traits. Extensive research has focused on lizards and their adaptive traits in relation to the environment. Studies have solidly established the correlation between morphology and habitat preferences (Losos, 2009; Kaliontzopoulou *et al.*, 2010), which suggests that lizards may undergo morphological adaptation in response to urbanization. Moreover, lizards have demonstrated their ability to rapidly adapt to sudden changes (Kolbe *et al.*, 2012; Eloy De Amorim *et al.*, 2017). Furthermore, a notable characteristic of many lizard species is their frequent presence in urban environments, where they utilize man-made structures for basking, foraging, nesting, and social interactions (Perry *et al.*, 2008; Meshaka Jr, 2011). This unique behavior makes them particularly well-suited for investigating how urbanization influences morphological traits. Together, due to their well-documented relationship with environmental factors, rapid adaptability, and propensity for inhabiting urban areas, lizards represent an ideal model system for studying the effects of urbanization on morphological traits.

Here we seek to understand and characterize the phenotypic change of the two major urban-associated lizards in the state of Texas: *Anolis carolinensis* and *Sceloporus olivaceus*. Based on previous studies that strongly associate structural habitat performance to morphology (Macrini & Irschick, 1998; Calsbeek & Irschick, 2007) and our knowledge on how Anole species have shown increases in limb length (Winchell *et al.*, 2016) we predict a similar pattern to present in main-land *A. carolinensis* while the opposite can be predicted in *S. olivaceus* based on the findings of previous studies (Sparkman *et al.*, 2018; Putman *et al.*, 2019). Furthermore, due to our predictions, we suspect that when comparing our findings to previous studies we will find morphological convergence within species on how they respond to urbanization.

Methods

Species and Study Locations

Data collection was conducted between the months of May and July 2019-2022 in four regions centered around urban centers in Texas: Austin, Dallas – Fort Worth (DFW), Houston, and San Antonio. Each region consisted of a single urban site, with natural sites placed nearby in habitat representative of minimally modified forest habitat in the given ecoregion. All urban sites selected were university campuses, which are primarily comprised of buildings, impervious surfaces, with limited tree cover and manicured greenery. *A. carolinensis* was sampled at all cities where *S. olivaceus* was only sampled in San Antonio (the only city where a substantial urban population seemed to be present).

Each site was visited for 2-4 days and adult individuals were captured indiscriminately using a pan fish pole with a lariat made of fishing line. The capture of lizards indiscriminately was done to prevent a bias in data due to targeted collection of, say, larger individuals. Both males and females were collected in an effort to ascertain phenotypic differences for an entire population.

Once captured, local habitat data were recorded including GPS coordinates, substrate/perch description (i.e. was is natural or man-made), ambient and lizard temperatures (using a handheld thermometer gently inserted into the cloaca), and canopy cover (using a convex spherical densiometer). After capture the lizards were first sedated using orally administered 20% benzocaine (Oragel) or a 0.7% dose of calcium carbonate buffered Tricaine Methanesulfonate (MS-222), then weighed using a standard field scale. Lizards were then euthanized with an unbuffered 50% MS-222 and preserved in 70% ethanol.

Morphological Analysis

Using Mitutoyo digital calipers, 15 individual measurements were made which, collectively, characterized fore- and hind-limb lengths, head shape, body length, tail length, and hip shape. While the right limb was usually used for measurements, the left limb was used instead if the right limb was damaged or was otherwise in better condition.

To determine the effect of urbanization on morphology we compared all measured traits from urban populations to that of their natural counterparts. For the comparison, we used the R programing language (R Core Team, 2021) to conduct multiple ANCOVAs, all of which included SVL as a covariate in order to account for the overall size of the lizards. First, a “global” model was run which included “habitat type” (hereafter referred to as simply “type”), which tested the effect of urban vs natural environments, locality (i.e. which of the four urban associated regions they came from) to determine if different cities had an effect on the trait, sex (male vs female), to test if differences in traits are not due to sexual dimorphisms, a locality-by-type interaction, to see if each cities environments are having the same effect on morphology, and finally a type-by-sex interaction (to test whether urbanization affects the sexes differently). In summary, the global model accounts for how large an individual is (SVL), if the specimen was from an urban or natural environment (type), which city the specimen was found in (locality), the sex of the individual (sex), how environment type interacts with different cities, and how sex interacts with environment type.

To facilitate comparing regions independently, a separate set of ancova models were used, one per region. This model accounted for body size (SVL), which environment the individuals came from (type), and their sex. After determining that urbanization was having an effect on morphological traits, we used emmeans (Lenth *et al.*, 2020) to quantify the effect across populations.

Consistency of morphological change in urban environments

Previously, there has been notable studies which have also looked at the effects of urbanization of morphology (Marnocha *et al.*, 2011; Winchell *et al.*, 2016; Sparkman *et al.*, 2018; Putman *et al.*, 2019; Putman & Tippie, 2020). We selected two of those studies that contained species similar to the two species we targeted here and made their morphological data available. First, Winchell et al. (2016) was selected to compare *Anolis cristatellus* to *A. carolinensis* while Sparkman et al. (2018) was selected to compare *Sceloporus occidentalis* to *S. olivaceus*. We incorporated the raw data from these two studies on two lizard species, with ours to ascertain whether changes in morphology between species were (i) consistently converging towards some specific value, (ii) were consistently moving in the same direction, or (iii) were idiosyncratic, with each species moving in unique directions. Using relative trait data (obtained by dividing all trait values by SVL) we standardized the effect of change and eliminate the comparisons of larger lizards to smaller ones and just focus on the traits of interest. We then reanalyzed the data from the Winchell et al (2016) and Sparkman et al (2018) data on all shared traits that we measured, through our global models (for the Winchell data all terms involving sex were removed due to the data only containing males). Emmeans were then used to quantify the magnitude of change for the morphological traits that showed a significant difference.

Results

In total the morphologies of 308 *Anolis carolensis* were measured across the four urbanized regions studied, of them, 125 from urban environments, and 183 from natural settings. Similarly, 57 *Sceloporus olivaceus* were analyzed from a single region, 39 from urban habitats, and 18 from natural ones. When assessing all regions together, but allowing for the potential for regions to have different effects of urbanization on morphology (i.e. the “global” model), multiple aspects of morphology were consistently different between urban and natural populations. Furthermore, in almost every model run, the sex term shows up as significant indicating that there is a significant difference in all traits between males and females which is congruent with sexual dimorphism found in both *A. Carolinensis* and *S. Olivaceus.*

In *A. carolinensis*, measurements related to overall head length (head length, lower jaw, outlever, and jugal), hind limb length (tibia, metatarsals, and full hind limb [femur + tibia + metatarsal], but not femur on its own), and radius, were all significantly larger for a given overall body size (SVL) in individuals from urban environments (Table 1, Figure 1). For example, tibia length was significantly longer in urban environments in our global model ($p < 0.001$) while also being significantly longer in all individual regions with the exception of Austin (Austin $P = 0.059$, DFW $p = 0.039$, Houston $p = 0.004$, and San Antonio $p < 0.001$, Figure 2).

In general, region-by-urbanization interaction effects were non-significant (all $P > 0.05$), suggesting that urbanization operated in similar ways across all cities. The exceptions were pelvic width, where DFW deviated from all other regions by urban individuals having smaller pelvic widths versus minimal differences elsewhere.

We further examined evidence for consistency in morphological shifts between regions by running individual models in each of the four regions where *A. carolinensis* occurred. Most of the individual regions showed the same patterns of morphological change displayed in the global models with one region just barely falling outside the range of significance (Table 2). Specifically, for the outlever measurement, urban associated lizards had larger traits in all 4 regions while the head length, lower jaw, jugal to symphasis, and tibia measurements showed all but one region following the same pattern as the global model. For the other traits identified as significant by the global model, at least 2 cities were identified as having non-significant trends, though in all cases the trend was in the same direction in the non-significant and significant regions.

Many similar features were also enlarged in urban *S. olivaceus*. These lizards possessed increased head heights (instead of lengths as in *A. carolinensis*), hind limb lengths (femur, tibia, and full hind limb) and fore limb length (specifically the radius) in urban affiliated individuals (Table 1, due to *S. olivaceus* only being collected in one region, their global model represents the entirety of their samples).

Consistency of morphological change in urban environments across species

To evaluate the potential for either parallelism or the convergence of a morphological evolutionary response to urbanization we combined our data with previous data for the crested anole (*A. cristatellus*; (Winchell *et al.*, 2016)) from Puerto Rico, and the western fence lizard (*S. occidentalis*; (Sparkman *et al.*, 2018)), which is distributed along the west coast of North America. While not all traits were measured in these two species, all limb traits that varied between urban and natural populations for *A. carolinensis* were also varied in the same direction for *Anolis cristatellus*, however urban *A. cristatellus* also had greater femur and humerus lengths. When observing relative tibia length, we see this difference across all sites and studies where *A. carolinensis*, *A. cristatellus*, and *S. olivaceus* show relative tibia length getting significantly longer in urban environments but *S. occidentalis* showing an opposite trajectory (Table 1). Additionally, while urban *A. carolinensis* had longer jaws (and longer heads generally), urban *A. cristatellus* did not differ from their natural counterparts in jawlength (head length was not measured). Instead, natural *A. cristatellus* had taller heads than urban populations, unlike *A. carolinensis*, and the opposite direction as displayed in *S. olivaceus*.

Only a subset of limb measurements exists for *Sceloporus occidentalis*, and of these only femur and upper hind limb length were different between urban and natural populations. In contrast to the other species, urban associated individuals had shorter hindlimbs than their natural counterparts.

Comparing the SVL-standardized combined limb lengths (i.e. femur + tibia; humerus + radius) of all populations of all species shows that *S. olivaceus*, *A. carolinensis*, and *A. cristatellus* all exist on the same plane of relative hind to fore limb lengths, with *A. carolinensis* having the shortest limbs, and *A. cristatellus* having the longest. Rather than converging on some “optimal” cross species value for limb length, urban populations all simply possess longer limbs, making the urban *A. carolinensis* shift in the direction of the limb lengths of *S. olivaceous*, while urban *S. olivaceous* limbs lengths shift towards *A. cristatellus*. *Sceloporus occidentallis* in contrast has hindlimbs that are comparatively longer than their forelimbs, and urban populations shift to have shorter hindlimbs, which has the effect of shifting them towards the limb length ratio exhibited by the other species (Figure 3).

Discussion

In *A. carolinensis* populations we found consistent morphological change where urban populations presented with significantly longer head length traits (head length, lower jaw, outlever, and jugal to symphasis), longer radius, and longer hind-limbs (tibia, combined tibia + femur, metatarsal, and combined tibia + femur + metatarsal). These findings are congruent with what we expected to find based on previous research, specifically in regard to limb morphology (Winchell *et al.*, 2016, 2018).

In Puerto Rican anoles, head shape has a tendency to correlate with substrate usage in that species found to mostly use broad perches are found to have taller heads where species occupying narrower perches present with flatter head shapes (Harmon *et al.*, 2005; Losos, 2009). With that information we would expect to see a similar pattern to present itself in urban environments being that urban substrates tend to be much wider, but we did not. Interestingly, we did find evidence that urban *A. carolinensis* have longer heads in all related head morphologies which is commonly associated with dietary changes. In San Antonio we also collected *S. olivaceus* where urban individuals presented with taller heads rather than the longer heads found in urban *A. carolinensis*. Taller heads have been known to correlate to with increased bite forces (De Meyer *et al.*, 2019) which could indicate a similar adjustment in diet we predict in urban anoles. Furthermore, it has been documented that insect abundance in urban areas is severely reduced (Merckx *et al.*, 2018) which in theory should force urban anoles to broaden their diets. This information, along with the evidence that most lizard species tend to be insectivores, selecting prey based on size (Costa *et al.*, 2008), leads us to the conclusion that urban lizards may be presenting with longer heads to allow them to eat a more size diverse diet.

In addition to the differences in head morphology, the differences in limb length observed between urban and natural areas this study are captivating. Previously conducted research which has extensively compared ecomorphological studies shows that not only are longer limb lengths in anoles associated with increased locomotory performance on wider surfaces (Losos, 1990; Larson & Losos, 1996; Macrini & Irschick, 1998; Calsbeek & Irschick, 2007), but that these individuals with longer limbs also have a preference of using broader substrates (Losos, 1994). Our data suggests that Texas populations of urban *A. carolinensis* have longer limbs which we believe is due to the greater abundance of broad substrates that dominate urban habitats (e.g. building walls, and large shade trees). Furthermore, we detected a very similar pattern to that of A. carolinensis in urban limb morphologies where urban *S. olivaceus* presented with longer hind limbs (all measured traits except metatarsal) and longer radius. Interestingly, this is opposite to the trends detected in *S. occidentalis* individuals from Sparkman et al. (2018) as well as *S. occidentalis* samples reported from Putman et al. (2018).

Another factor which may drive longer limb lengths in urban populations is the openness (lack of tree cover and sparsely distributed perches) of the environment. Generally, we find that urban areas are much more open which leads to greater distances between perch-able substrates. Given these longer distances, longer limb length (which facilitate faster sprint speeds (Vanhooydonck *et al.*, 2006)) would be advantageous in aiding individuals swift transition between perches. Given the evidence, we suspect that the longer hind limbs detected in Texas urban anoles improves locomotory performance not only on the wider perches available but on the ground in urban areas which is the same conclusion found by Winchell et all. (2016) and Marnocha et al. (2011).

Community morphological predictions

While the number of urban phenotypic effects in lizard studies are increasing, we are still trying to piece together the global effects of urbanization on lizard populations. Here we combine our findings on *A. carolinensis* and *S. olivaceus* with published data looking at the effects of urbanization on *A. cristatellus* (Winchell et al. 2016) and *S. occidentalis* (Sparkman et al. 2018). While we are unable to draw conclusions on all traits we measured in Texas, we can directly compare the effects of urbanization on limb morphology between on all four species groups. While *A. carolinensis*, *A. cristatellus*, and *S. olivaceus* all showed similar significant trends of limb length being longer in urban environments is this interestingly opposite of what was detected in *S. occidentalis*. Sparkman et al. (2018) concludes that these reductions in limb length are most likely correlated with a decline in predation, but this may be specific to their study sites. While three out of four of our studies show similar trends which also tend to be in line with a recent meta-analysis (Putman & Tippie, 2020), the deviation seen in *S. occidentalis* is most informative and emphasizes the captivating notion that diverse lizards could be experiencing morphological transformations due to urbanization at a global level with the acknowledgement that these reactions might vary based on urban characteristics and/or specific species. Together, *Anoles* appear to be altering their limb morphology in a parallel pattern (both *A.* carolinensis and *A. cristatellus* are developing longer limbs) but we are unable to determine if they are convergent due to the size differences while *Sceloporus* species appear to modify their limb lengths in a more idiosyncratic fashion due to their morphological changes being in opposite directions.

Conclusion

In recent years we have started seeing an increased interest into the effects of urbanization on lizard morphology not just on a local scale (Winchell *et al.*, 2016; Sparkman *et al.*, 2018; Putman *et al.*, 2019), but globally (Putman & Tippie, 2020). Here, we show that locally, Texas *A. carolinensis* and *S. olivaceus* urban populations present with longer forelimbs compared to their natural counterparts. Furthermore, by comparing our findings to that of Winchell et al. (2016) and Sparkman et al. (2018) we attempted to determine if globally, lizard morphology changes in predictable ways across species. While it appears, most species are modifying morphology in predictable patterns, the presence of one or more species behaving differently highlights a need for further analysis. Specifically, *Anoles* appear to modify morphology predictably due to similar directions of morphological change, but *Sceloporus* present with opposite directions.

Literature Cited

Brooks, T.M., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B., Rylands, A.B., Konstant, W.R., Flick, P., Pilgrim, J., Oldfield, S., Magin, G. & Hilton-Taylor, C. (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology*, **16**, 909–923.

Calsbeek, R. & Irschick, D.J. (2007) The quick and the dead: Correlational selection on morphology, performance, and habitat use in island lizards. *Evolution*, **61**.

Costa, G.C., Vitt, L.J., Pianka, E.R., Mesquita, D.O. & Colli, G.R. (2008) Optimal foraging constrains macroecological patterns: Body size and dietary niche breadth in lizards. *Global Ecology and Biogeography*, **17**, 670–677.

Eloy De Amorim, M., Schoener, T.W., Santoro, G.R.C.C., Lins, A.C.R., Piovia-Scott, J. & Brandão, R.A. (2017) Lizards on newly created islands independently and rapidly adapt in morphology and diet. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 8812–8816.

Harmon, L.J., Kolbe, J.J., Cheverud, J.M. & Losos, J.B. (2005) Convergence and the multidimensional niche. *Evolution*, **59**.

Hart, J.C., Ellis, N.A., Eisen, M.B. & Miller, C.T. (2018) Convergent evolution of gene expression in two high-toothed stickleback populations. *PLoS Genetics*, **14**, 1–22.

Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A. (2010) Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat-related patterns within Podarcis bocagei wall lizards. *Journal of Evolutionary Biology*, **23**, 1234–1244.

Kolbe, J.J., VanMiddlesworth, P.S., Losin, N., Dappen, N. & Losos, J.B. (2012) Climatic niche shift predicts thermal trait response in one but not both introductions of the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, USA. *Ecology and Evolution*, **2**, 1503–1516.

Larson, A. & Losos, J.B. (1996) Phylogenetic systematics of adaptation. *Adaptation*.

Lenth, R., Singmann, H., Love, J., Buerkner, P. & Herve, M. (2020) Package ‘ emmeans ’ R topics documented : *R package version 1.15-15*, **34**.

Losos, J.B. (1990) Ecomorphology, Performance Capability, and Scaling of West Indian Anolis Lizards : An Evolutionary Analysis. *Ecological Monographs*, **60**, 369–388.

Losos, J.B. (1994) Integrative approaches to evolutionary ecology: Anolis lizards as model systems. *Annual Review of Ecology and Systematics*, **25**.

Losos, J.B. (2009) *Lizards in an Evolutionary Tree*, 1st edn. University of California Press.

Macrini, T.E. & Irschick, D.J. (1998) An intraspecific analysis of trade-offs in sprinting performance in a West Indian lizard species (Anolis lineatopus). *Biological Journal of the Linnean Society*, **63**.

Mahler, D.L., Ingram, T., Revell, L.J. & Losos, J.B. (2013) Exceptional Convergence on the Macroevolutionary Landscape in Island Lizard Radiations. *Science*, **341**, 292–295.

Marnocha, E., Pollinger, J. & Smith, T.B. (2011) Human-induced morphological shifts in an island lizard. *Evolutionary Applications*, **4**, 388–396.

McKinney, M.L. (2002) Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, **52**, 883–890.

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F.F., Backeljau, T., Bonte, D., Brans, K.I.I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J.M.T.M.T., Fontaneto, D., Gianuca, A.T.T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Van Doninck, K., De Meester, L. & Van Dyck, H. (2018) Body-size shifts in aquatic and terrestrial urban communities. *Nature*, **558**, 113–116.

Meshaka Jr, W.E. (2011) A runaway train in the making: the exotic amphibians, reptiles, turtles, and crocodilians of Florida. *Herpetological Conservation and Biology*, **6**, 1–101.

De Meyer, J., Irschick, D.J., Vanhooydonck, B., Losos, J.B., Adriaens, D. & Herrel, A. (2019) The role of bite force in the evolution of head shape and head shape dimorphism in Anolis lizards. *Functional Ecology*, **33**.

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger, L., Bennett, D.J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M.J., Feldman, A., Garon, M., Harrison, M.L.K., Alhusseini, T., Ingram, D.J., Itescu, Y., Kattge, J., Kemp, V., Kirkpatrick, L., Kleyer, M., Correia, D.L.P., Martin, C.D., Meiri, S., Novosolov, M., Pan, Y., Phillips, H.R.P., Purves, D.W., Robinson, A., Simpson, J., Tuck, S.L., Weiher, E., White, H.J., Ewers, R.M., MacE, G.M., Scharlemann, J.P.W. & Purvis, A. (2015) Global effects of land use on local terrestrial biodiversity. *Nature*, **520**, 45–50.

Obolski, U., Ram, Y. & Hadany, L. (2018) Key issues review: Evolution on rugged adaptive landscapes. *Reports on Progress in Physics*, **81**.

Pereira, H.M., Navarro, L.M. & Martins, I.S. (2012) Global Biodiversity Change: The Bad, the Good, and the Unknown. *Annual Review of Environment and Resources*, **37**, 25–50.

Perry, G., Buchanan, B.W., Fisher, R.N., Salmon, M. & Wise, S.E. (2008) *Effects of artificial night lighting on amphibians and reptiles in urban environments*,.

Putman, B.J., Gasca, M., Blumstein, D.T. & Pauly, G.B. (2019) Downsizing for downtown: limb lengths, toe lengths, and scale counts decrease with urbanization in western fence lizards (Sceloporus occidentalis). *Urban Ecosystems*, **22**, 1071–1081.

Putman, B.J. & Tippie, Z.A. (2020) Big City Living: A Global Meta-Analysis Reveals Positive Impact of Urbanization on Body Size in Lizards. *Frontiers in Ecology and Evolution*, **8**.

R Core Team (2021) R core team (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www. R-project. org*.

Reznick, D.N. & Ghalambor, C.K. (2001) The population ecology of contemporary adaptations: What empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112**–**113**, 183–198.

Row, K.W., Villasana-Espinosa, O., Perez, J., Urbina, G. & Frishkoff, L.O. (2023) Morphological limitations imposed on lizards facing urbanization. *bioRxiv*, 2023.05.09.540039.

Shochat, E., Warren, P.S., Faeth, S.H., McIntyre, N.E. & Hope, D. (2006) From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology and Evolution*, **21**, 186–191.

Sparkman, A., Howe, S., Hynes, S., Hobbs, B. & Handal, K. (2018) Parallel behavioral and morphological divergence in fence lizards on two college campuses. *PLOS ONE*, **13**, e0191800.

Svensson, E.I. & Calsbeek, R. (2013) *The Adaptive Landscape in Evolutionary Biology*, (ed. by E. Svensson) and R. Calsbeek) Oxford University Press.

United Nations (2018) *World Urbanization Prospects, 2018 revision.*, New York, NY.

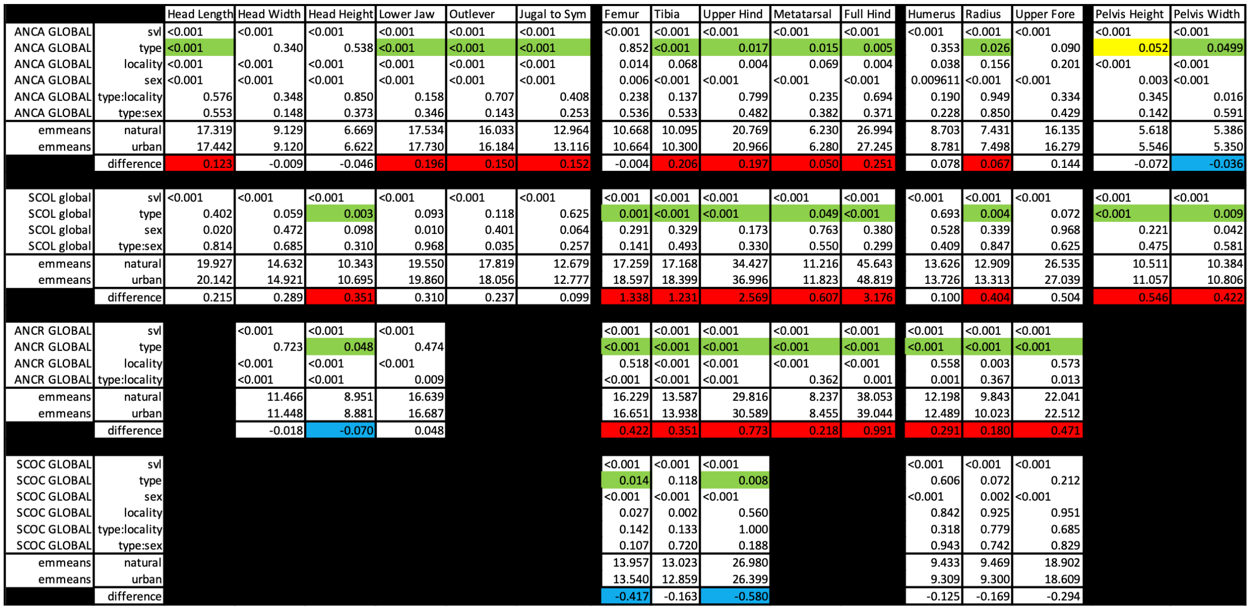
Vanhooydonck, B., Herrel, A., Van Damme, R. & Irschick, D.J. (2006) The quick and the fast: the evolution of acceleration capacity in anolis lizards. *Evolution*, **60**, 2137.

Winchell, K.M., Carlen, E.J., Puente-Rolón, A.R. & Revell, L.J. (2018) Divergent habitat use of two urban lizard species. *Ecology and Evolution*, **8**, 25–35.

Winchell, K.M., Reynolds, R.G., Prado-Irwin, S.R., Puente-Rolón, A.R. & Revell, L.J. (2016) Phenotypic shifts in urban areas in the tropical lizard *Anolis cristatellus*. *Evolution*, **70**, 1009–1022.

TABLES

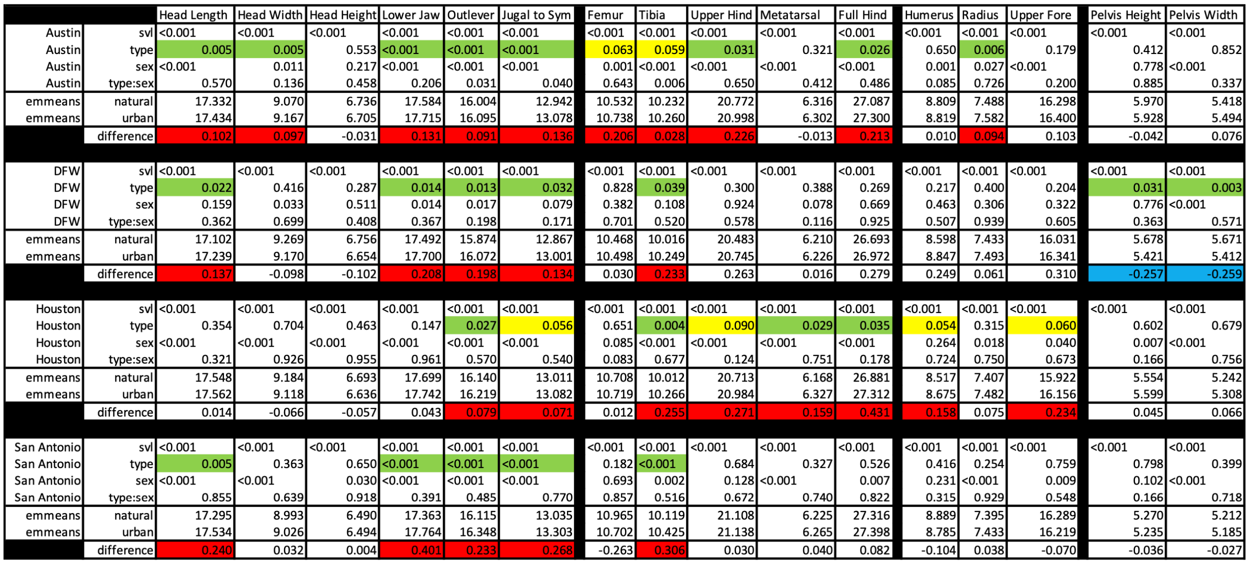
Table 1: The raw output of the global ancova models which accounts for how large an individual is (SVL), if the specimen was from an urban or natural environment (type), which city the specimen was found in (locality = region), the sex of the individual (sex), how environment type interacts with different cities, and how sex interacts with environment type. *A. carolinensis* and *S. occidentalis* Included all terms, *S. olivaceus* is missing the locality (region) term due to only being captured in San Antonio, *A. cristatellus* is missing the sex term since only males were collected. A green box in the type row signifies a significant difference between urban and natural associated lizards of that species for a giving trait where yellow is close to significant. In difference row a red box signifies that lizards associated with the urban habitat type were larger/longer while a blue box signifies it was smaller. The difference row is only highlighted for traits that were deemed significant in the type row.



		Head Length	Head Width	Head Height	Lower Jaw	Outlever	Jugal to Sym	Femur	Tibia	Upper Hind	Metatarsal	Full Hind	Humerus	Radius	Upper Fore	Pelvis Height	Pelvis Width
ANCA GLOBAL	svl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ANCA GLOBAL	type	<0.001	0.340	0.538	<0.001	<0.001	<0.001	0.852	<0.001	0.017	0.015	0.005	0.353	0.026	0.090	0.052	0.0499
ANCA GLOBAL	locality	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	0.068	0.004	0.069	0.004	0.038	0.156	0.201	<0.001	<0.001
ANCA GLOBAL	sex	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001	<0.001	<0.001	0.009611	<0.001	<0.001	0.003	<0.001
ANCA GLOBAL	type:locality	0.576	0.348	0.850	0.158	0.707	0.408	0.238	0.137	0.799	0.235	0.694	0.190	0.949	0.334	0.345	0.016
ANCA GLOBAL	type:sex	0.553	0.148	0.373	0.346	0.143	0.253	0.536	0.533	0.482	0.382	0.371	0.228	0.850	0.429	0.142	0.591
emmeans	natural	17.319	9.129	6.669	17.534	16.033	12.964	10.668	10.095	20.769	6.230	26.994	8.703	7.431	16.135	5.618	5.386
emmeans	urban	17.442	9.120	6.622	17.730	16.184	13.116	10.664	10.300	20.966	6.280	27.245	8.781	7.498	16.279	5.546	5.350
	difference	0.123	-0.009	-0.046	0.196	0.150	0.152	-0.004	0.206	0.197	0.050	0.251	0.078	0.067	0.144	-0.072	-0.036
SCOL global	svl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SCOL global	type	0.402	0.059	0.003	0.093	0.118	0.625	0.001	<0.001	<0.001	0.049	<0.001	0.693	0.004	0.072	<0.001	0.009
SCOL global	sex	0.020	0.472	0.098	0.010	0.401	0.064	0.291	0.329	0.173	0.763	0.380	0.528	0.339	0.968	0.221	0.042
SCOL global	type:sex	0.814	0.685	0.310	0.968	0.035	0.257	0.141	0.493	0.330	0.550	0.299	0.409	0.847	0.625	0.475	0.581
emmeans	natural	19.927	14.632	10.343	19.550	17.819	12.679	17.259	17.168	34.427	11.216	45.643	13.626	12.909	26.535	10.511	10.384
emmeans	urban	20.142	14.921	10.695	19.860	18.056	12.777	18.597	18.399	36.996	11.823	48.819	13.726	13.313	27.039	11.057	10.806
	difference	0.215	0.289	0.351	0.310	0.237	0.099	1.338	1.231	2.569	0.607	3.176	0.100	0.404	0.504	0.546	0.422
ANCR GLOBAL	svl		<0.001	<0.001	<0.001			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
ANCR GLOBAL	type		0.723	0.048	0.474			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
ANCR GLOBAL	locality		<0.001	<0.001	<0.001			0.518	<0.001	<0.001	<0.001	<0.001	0.558	0.003	0.573		
ANCR GLOBAL	type:locality		<0.001	<0.001	0.009			<0.001	<0.001	<0.001	0.362	0.001	0.001	0.367	0.013		
emmeans	natural		11.466	8.951	16.639			16.229	13.587	29.816	8.237	38.053	12.198	9.843	22.041		
emmeans	urban		11.448	8.881	16.687			16.651	13.938	30.589	8.455	39.044	12.489	10.023	22.512		
	difference		-0.018	-0.070	0.048			0.422	0.351	0.773	0.218	0.991	0.291	0.180	0.471		
SCOC GLOBAL	svl							<0.001	<0.001	<0.001			<0.001	<0.001	<0.001		
SCOC GLOBAL	type							0.014	0.118	0.008			0.606	0.072	0.212		
SCOC GLOBAL	sex							<0.001	<0.001	<0.001			<0.001	0.002	<0.001		
SCOC GLOBAL	locality							0.027	0.002	0.560			0.842	0.925	0.951		
SCOC GLOBAL	type:locality							0.142	0.133	1.000			0.318	0.779	0.685		
SCOC GLOBAL	type:sex							0.107	0.720	0.188			0.943	0.742	0.829		
emmeans	natural							13.957	13.023	26.980			9.433	9.469	18.902		
emmeans	urban							13.540	12.859	26.399			9.309	9.300	18.609		
	difference							-0.417	-0.163	-0.580			-0.125	-0.169	-0.294		

Table 2:

The raw output of the regional ancova models for *A. carolinensis* after being subset to each region, which accounts for how large an individual is (SVL), if the specimen was from an urban or natural environment (type), the sex of the individual (sex), how environment type interacts with different cities, and how sex interacts with environment type. A green box in the type row signifies a significant difference between urban and natural associated lizards of that species for a giving trait where yellow is close to significant. In difference row a red box signifies that lizards associated with the urban habitat type were larger/longer while a blue box signifies it was smaller. The difference row is only highlighted for traits that were deemed significant in the type row.



		Head Length	Head Width	Head Height	Lower Jaw	Outlever	Jugal to Sym	Femur	Tibia	Upper Hind	Metatarsal	Full Hind	Humerus	Radius	Upper Fore	Pelvis Height	Pelvis Width
Austin	svl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Austin	type	0.005	0.005	0.553	<0.001	<0.001	<0.001	0.063	0.059	0.031	0.321	0.026	0.650	0.006	0.179	0.412	0.852
Austin	sex	<0.001	0.011	0.217	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.027	<0.001	0.778	<0.001
Austin	type:sex	0.570	0.136	0.458	0.206	0.031	0.040	0.643	0.006	0.650	0.412	0.486	0.085	0.726	0.200	0.885	0.337
emmeans	natural	17.332	9.070	6.736	17.584	16.004	12.942	10.532	10.232	20.772	6.316	27.087	8.809	7.488	16.298	5.970	5.418
emmeans	urban	17.434	9.167	6.705	17.715	16.095	13.078	10.738	10.260	20.998	6.302	27.300	8.819	7.582	16.400	5.928	5.494
	difference	0.102	0.097	-0.031	0.131	0.091	0.136	0.206	0.028	0.226	-0.013	0.213	0.010	0.094	0.103	-0.042	0.076
DFW	svl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
DFW	type	0.022	0.416	0.287	0.014	0.013	0.032	0.828	0.039	0.300	0.388	0.269	0.217	0.400	0.204	0.031	0.003
DFW	sex	0.159	0.033	0.511	0.014	0.017	0.079	0.382	0.108	0.924	0.078	0.669	0.463	0.306	0.322	0.776	<0.001
DFW	type:sex	0.362	0.699	0.408	0.367	0.198	0.171	0.701	0.520	0.578	0.116	0.925	0.507	0.939	0.605	0.363	0.571
emmeans	natural	17.102	9.269	6.756	17.492	15.874	12.867	10.468	10.016	20.483	6.210	26.693	8.598	7.433	16.031	5.678	5.671
emmeans	urban	17.239	9.170	6.654	17.700	16.072	13.001	10.498	10.249	20.745	6.226	26.972	8.847	7.493	16.341	5.421	5.412
	difference	0.137	-0.098	-0.102	0.208	0.198	0.134	0.030	0.233	0.263	0.016	0.279	0.249	0.061	0.310	-0.257	-0.259
Houston	svl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Houston	type	0.354	0.704	0.463	0.147	0.027	0.056	0.651	0.004	0.090	0.029	0.035	0.054	0.315	0.060	0.602	0.679
Houston	sex	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.085	<0.001	<0.001	<0.001	<0.001	0.264	0.018	0.040	0.007	<0.001
Houston	type:sex	0.321	0.926	0.955	0.961	0.570	0.540	0.083	0.677	0.124	0.751	0.178	0.724	0.750	0.673	0.166	0.756
emmeans	natural	17.548	9.184	6.693	17.699	16.140	13.011	10.708	10.012	20.713	6.168	26.881	8.517	7.407	15.922	5.554	5.242
emmeans	urban	17.562	9.118	6.636	17.742	16.219	13.082	10.719	10.266	20.984	6.327	27.312	8.675	7.482	16.156	5.599	5.308
	difference	0.014	-0.066	-0.057	0.043	0.079	0.071	0.012	0.255	0.271	0.159	0.431	0.158	0.075	0.234	0.045	0.066
San Antonio	svl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
San Antonio	type	0.005	0.363	0.650	<0.001	<0.001	<0.001	0.182	<0.001	0.684	0.327	0.526	0.416	0.254	0.759	0.798	0.399
San Antonio	sex	<0.001	<0.001	0.030	<0.001	<0.001	<0.001	0.693	0.002	0.128	<0.001	0.007	0.231	<0.001	0.009	0.102	<0.001
San Antonio	type:sex	0.855	0.639	0.918	0.391	0.485	0.770	0.857	0.516	0.672	0.740	0.822	0.315	0.929	0.548	0.166	0.718
emmeans	natural	17.295	8.993	6.490	17.363	16.115	13.035	10.965	10.119	21.108	6.225	27.316	8.889	7.395	16.289	5.270	5.212
emmeans	urban	17.534	9.026	6.494	17.764	16.348	13.303	10.702	10.425	21.138	6.265	27.398	8.785	7.433	16.219	5.235	5.185
	difference	0.240	0.032	0.004	0.401	0.233	0.268	-0.263	0.306	0.030	0.040	0.082	-0.104	0.038	-0.070	-0.036	-0.027

FIGURES

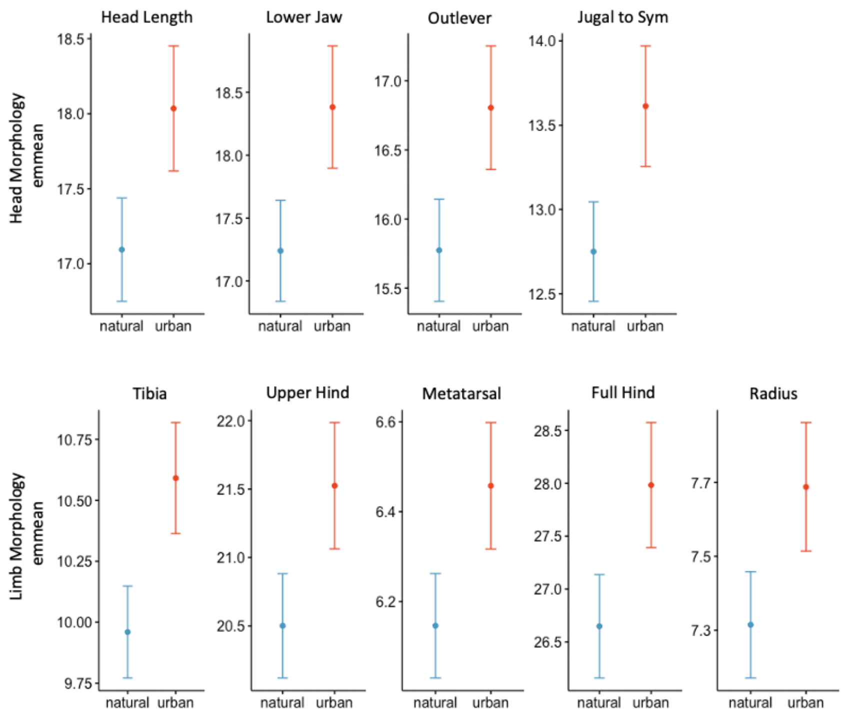


Figure 1

Visual representation of the global trait emmeans associated with *A. carolinensis*. The top row of graphs represents head morphology and the bottom row represents limb morphology. Only morphological traits that were deemed significant are highlighted here. Blue lines represent natural values where red lines represent urban ones.

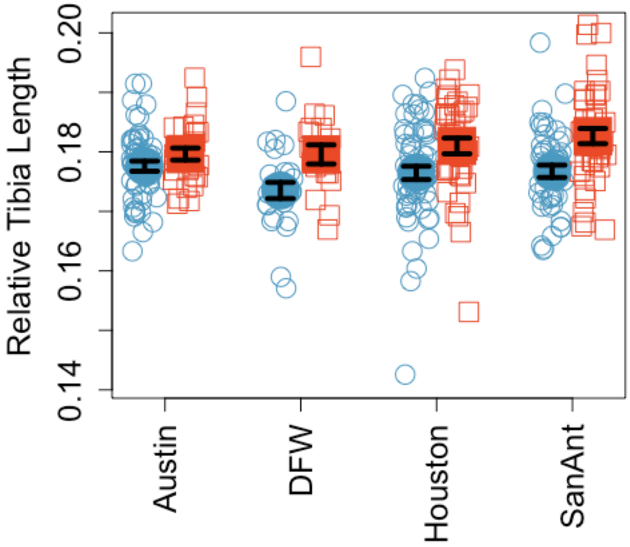


Figure 2

A. Carlinensis was collected in four regions across Texas: Austin, DFW, Houston, and San Antonio. For all traits, ancova models were ran subsetting to each region to determine if the significant signals detected in the global model held for each region. Here is an example of one of those traits, relative tibia length. Blue hollow circles represent the individual naturally associated lizards where the red hollow squared represent the individual urban ones. The solid blue circle and red square symbolize their means along with standard error bars. Here, DFW, Houston, and San Antonio show evidence of the urban and natural means being distinct from one another with Austin being closer together. This is representative of the regional models for the tibia trait where all but Austin show a significant signal of urban lizards having longer tibias.

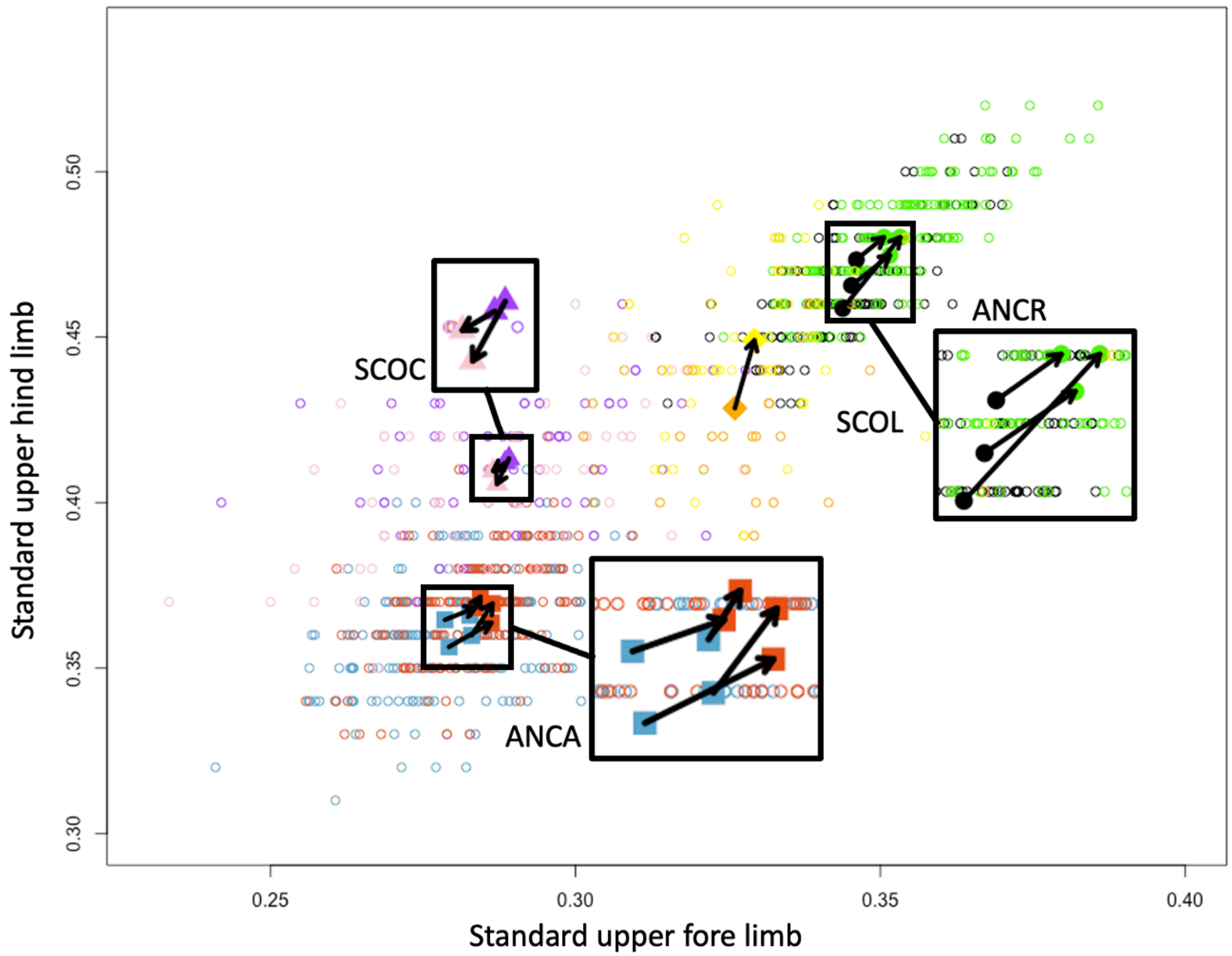


Figure 3

Comparing the standardized combined limb lengths of all individuals represented in this study. All arrows are drawn from natural habitat means to urban habitat means to show the direction of change. All replications in means and arrows signify multiple regions present for that species. For *A. carolinensis* (ANCA) solid blue squares represent the mean value for each region where red squares are urban ones. For *A. cristatellus* (ANCR) solid black circles are natural means and solid green circles are urban ones. For *S. occidentalis* solid purple triangles represent natural means and solid pink triangles are urban ones. Finally, for *S. olivaceus*, the orange diamond represents the natural mean where the yellow diamond represents the urban mean.

Chapter 4:

Classifying how dietary niche space is changing in response to urbanization

Kristopher Row (1), Adriana Bailey (1), Alison Ravenscraft (1), Luke Frishkoff (1)

(1) Department of Biology, University of Texas Arlington, 501 S. Nedderman Dr., Arlington TX, 76019, USA

Abstract

Due to massive environmental change caused by humans, there is a reported expected decline in insect communities over the next few decades. Predictably, as insect communities decline, we can expect cascading effects throughout the food web. We predict that if insect abundance is decreasing in urban environments, then lizard species will be forced to broaden their dietary niche space in order to maintain their required energy intake. We collected fecal samples in four distinct regions across the state of Texas, in two lizard species (*Sceloporus olivaceus* and *Anolis carolinensis*). For these samples we leveraged DNA barcoding techniques to determine the diets of lizards in paired urban and natural sites and quantify their dietary niche breadth. Here, we show that lizards found in urban environments are in fact consuming significantly different insect families compared to natural populations. Furthermore, we also provide evidence that urban lizards are expanding their dietary niche breadth when compared to natural ones. Our observed shifts in dietary patterns among urban lizards shed light on the potential resilience of these reptiles in the face of human modified environmental transformations.

Introduction

Humans are causing massive environmental change which in turn is leading to a major reduction in species diversity. These environmental changes brought about primarily through habitat conversion is driving biodiversity loss (Brooks *et al.*, 2002; Newbold *et al.*, 2015). One of the more pervasive and drastic forms of habitat modification is urbanization which is rapidly expanding across the globe. Urbanization creates a series of environmental pressures which in turn alters ecological conditions and leads to the loss of endemic species at multiple trophic levels, while also creating space for a few species, which are able to tolerate these modified landscapes, to expand and thrive (McKinney & Lockwood, 1999).

One factor that is instrumental to the determination if a new species will be successful in a novel environment are the dietary interactions with existing resources (Baiser *et al.*, 2010). Predictably, insects, which provide the primary dietary component for many other species is declining all over the world and the primary driver of this decline is habitat loss (Sánchez-Bayo & Wyckhuys, 2019). As insect communities continue to decline, we can expect cascading effects throughout the food web. The dynamics of a species dietary niche space are driven by prey resources (Macarthur & Pianka, 1966; Schoener, 1971) so when the availability of food changes, the dietary niche of the predator is liable to change as well (Stephens et al., 2019).

How a species responds to resource modification can directly influence its ability inhabit a new environment. To determine how species respond to modifications in food availability we seek to characterize the difference between urban and natural populations dietary niche breadth. This goal requires the identification of and breadth of insects consumed by lizards. We predict that if insect abundance is decreasing in urban environments, then lizard species will be forced to broaden their dietary niche space in order to maintain their required amount of energy intake. This hypothesis is supported by the evidence that when high-quality food resources are in abundance, diets tend to specialize leading to the decrease in dietary niche space but when those resources become scarce, diets tend to generalize as alternative food sources are consumed, which results in the broadening of the dietary niche space (Schoener, 1971; Stephens & Krebs, 1986). An example of this can be seen in hummingbird-plant interactions where they looked at high-quality forest habitat foraging versus coffee plantations and found that in coffee plantations with reduced resources, hummingbirds became more opportunistic in their foraging becoming far less specialized (Morrison & Mendenhall, 2020).

Many lizard species are commonly found through urban environments in high abundance (Winchell *et al.*, 2018). Not only do lizard species persist in urban environments, but they are prevalent across multiple regions, habitat types, and temperate zones (iNaturalist, 2018). Furthermore, there are also a subset of lizards that are generalists (Rose, 1976; Losos, 2009) that make them ideal for leveraging the intricacies of diet modification due to the expectation of large diets.

Here, we target two lizard species, *Anolis carolinensis* and *Sceloporus olivaceus* to answer two primary questions, (1) are lizards from urban habitats eating the same, or different insect families than those found in natural habitats and is there a significant difference in that insect consumption and (2) are urban lizards modifying their niche breadth in urban habitats. Together, this will allow us to determine how urban lizard diets are changing in response to urbanization.

Methods

Four regions in the state of Texas were identified as target areas for lizard fecal collection. The four regions are Austin, Dallas – Fort Worth (DFW), Houston, and San Antonio. Due to them being present at high abundance and being known to feed on arthropods, *Anolis carolinensis* was targeted in all regions and *Sceloporus olivaceus* was targeted only in San Antonio. In each region one urban site was identified (a major university in the target region) along with a paired natural site which was required to be undisturbed and disconnected from large human modified environments. In two regions (San Antonio and Austin) multiple natural sites were identified due to limited detectable abundance of target lizard species at the initial natural site selected. These sites were sampled once per year (between May and July) for 4 years where lizard fecal samples were collected.

Fecal collection and processing

Lizards were identified in the target area and captured using lassos attached to fly fishing poles. Following capture, the lizards were held in individual collected bags and observed for no more than 48 hours. During that time all excrement was collected and preserved in RNA later (Malmstrom, 2015). Some lizards were also collected as specimens as part of another study. For these lizards, we collected fecal matter from the lower intestine to further add to our sample size while they were being preserved. A total of 667 fecal samples were collected for this project, with 580 from *A. carolinensis* and 87 from *S. olivaceus*. For each region samples were collected in urban and natural environments across 14 unique sites (for a full breakdown of samples by field season and site see Table 1(base)).

Once back in the lab, all samples were stored in a -20C freezer pending DNA extraction. Once ready for extraction, all fecal samples were removed from the RNA later and placed on kim-wipes for no more than one minute to allow the RNA to run off but not too long to allow the sample to dry out. The samples were then immediately put through a DNA extraction protocol using the ZYMO Quick-DNA Fecal/Soil Microbe Miniprep Kit.

DNA amplification and sequencing

Arthropod DNA in fecal samples were amplified by PCR with a 16s mitochondrial primer set (IN16STK-1F: TGAACTCAGATCATGAA and IN16STK-1R: TTAGGGATAACAGCGTAA; designed by (Kartzinel & Pringle, 2015)). The arthropod amplicons were indexed with barcoded with forward and reverse primers (Caporaso *et al.*, 2012; Kozich *et al.*, 2013). The PCR reaction contained 0.5 umol/L forward and 0.5 umol/L reverse primer, 12.5 ul of Q5 master mix, 8 ul of nuclease free water, and 2 ul of sample DNA in a total volume of 25ul. The thermocycler program began with denaturation at 98C for one minute, followed by 35 cycles of denaturation at 98C for 10 seconds, annealing at 55C for 30 seconds, and extension at 72C for 30 seconds, with a final extension of 72C for 2 minutes (adapted from (Kartzinel & Pringle, 2015) and New England Biology recommended annealing temperatures for Q5 master mix).

Arthropod PCR products were cleaned using AMPure XP magnetic beads followed by sample quantification using a fluorometric 96-well plate reader (Qubit dsDNA HS kit). To create the arthropod library an equal mass of DNA was added for each quantified sample. Finally, the fecal arthropod library was sequenced on an illumina MiSeq platform at the University of Texas in Arlington, with a v3 (1 x 150-cycle) reagent kit.

Sequence data processing and cleaning

The program cutadadapt (Martin, 2018) was used to remove priming sites along with poor quality bases at the 5’ and 3’ ends of the sequences. The resulting sequences were then run through the R package “DADA2” (Benjamin Callahan *et al.*, 2020) which applies run-specific quality scores and error rates along with quantifying the number of times each sequence was observed to ascertain the true biological sequences that were present allowing for the analysis to be ran at the arthropod family level. Furthermore, as a conservative measure, based on expected read length of approximately 108 bp, and to eliminate any possible false reads, we eliminated sequences that were shorter than 106 bp or longer than 110bp. Finally, using the DADA2 function “removeBimeraDenovo” we checked and removed any chimera sequences. The remaining sequence variants were exported as a fasta file and ran through a BLAST (Madden, 2013) pipeline to determine the accession IDs associated with each sequence from GenBank (Sayers *et al.*, 2022). Then, those accession IDs were run through the “taxonomizr” R package (Sherrill-Mix, 2019) to assign taxonomic ranks to all sequence variants. Finally, the data was then combined in R using the “phyloseq” (McMurdie & Holmes, 2013) package which generated the data for downstream analysis.

Analysis

To account for variations in sequencing depth and ascertain the total number of reads associated with each sequence variant, we employed a rarefaction technique on the complete dataset, standardizing it to 5070 reads per sample. This chosen cutoff of 5070 reads allowed us to maintain a maximum number of samples in the dataset while still effectively profiling the majority of arthropod reads present. First, to test for population-level differences in the insect families consumed by lizard populations we calculated the Bray-Curtis distances between samples using unrarefied, but proportion normalized data (McMurdie & Holmes, 2014). Next, we used the R package "vegan" (Oksanen *et al.*, 2020) to run a PERMANOVA test to determine if for each region, the insect families being consumed in urban environments are statistically different than those eaten in natural environments. Then, to further investigate the difference between urban and natural diets we ran Principal Coordinates Analysis (PCoA) to visualize these Bray-Curtis distances.

After determining if urban and natural lizard populations are consuming different insect families, we ran differential abundance tests (R package "DESeq2" (Love *et al.*, 2014)) of individual insect families. This was done to determine specifically which insect families were being targeted by either urban populations more commonly or natural ones.

Finally, to determine if the dietary niche space is changing in urban habitats compared to natural ones, we first standardized all our samples to a depth of ten lizards. This was done to account for some sites only having ten fecal samples and to ensure our results were not influenced by other sites having much greater sampling. By merging all lizard samples within each site, we use the alpha diversity mean as a proxy to dietary niche breadth, indicative of the number of types of insects a standardized number of lizards are expected to consume. To do this we ran extracted alpha diversity values at the maximum depth to include all samples and replicated that extraction ten times sampling from random lizards. We then extracted the alpha diversity means of each site and ran an ancova test looking to see if there are significant difference when comparing urban habitats from natural ones while accounting for the region the lizards' fecal samples were collected from.

Results

After sequence processing and quality filtering, 39,558,051 total reads were generated from 680 samples, from across four MiSeq runs. Together, this led to 11,581 sequence variants of which 9,469 blasted to known genbank samples. Furthermore, 7,947 of those were arthropod samples (the remainder were a combination of bacteria, squamates, and other random assortments of organisms that probably represent DNA that was extracted from the lizards themselves and possible parasites that were occasionally observed in the samples rather than actual components of the lizards' diets). After sub-setting to arthropod only reads, rarefaction curves were generated to determine at which point all the samples were adequately sampled while minimizing the amount of samples lost. The samples were standardized to 5070 reads resulting in the loss of 86 samples (leaving 594 in the dataset). This rarefaction cut off allowed us to retain a minimum of 10 fecal samples per site (Table 1(rare)). Once the data set was rarefied, all sequence variants were further pooled at the family level for analysis with a total of 356 insect families found in the fecal samples. Finally, the data was broken into five groups: the first four were made up of *A. carolinensis* samples in the regions Austin, DFW, Houston, and San Antonio (to be referred to as San Antonio (A)) and the last was for San Antonio *S. olivaceus* (San Antonio (S)) samples.

Looking at which insect families were found most commonly in fecal samples we determined that prey populations of *A. carolinensis* and *S. olivaceus* most commonly consisted of *Orthopterans* (grasshoppers and crickets), *Coleoptera* (beetles), *Diptera* (flys) and *Hemiptera* (true bugs) (Supplemental Table 2). We extracted insect families that were found in at least 5 lizard samples to ensure our comparison's represented true dietary samples rather than one off consumptions (we refer to these as "high abundance" insect families). Of the 116 high abundance insect families, every insect was detected at least once in every region. Then, we generated Bray-Curtis dissimilarity distances for each group (Figure 1) which showed that in all regions except for San Antonio, urban lizards are eating more dissimilar insect families than one another then their natural counterparts. Furthermore, In San Antonio, both *S. olivaceus* and *A. carolinensis* show that both urban and natural habitat lizards are eating equally dissimilar insect families. PERMANOVA tests revealed that in every region when comparing the insect families between urban and natural habitat types, there is a significantly difference in their insect family consumption (Austin: p = 0.001, r^2 = 0.046. DFW: p = 0.001, r^2 = 0.033. Houston: p = 0.001, r^2 = 0.021. San Antonio (A): p = 0.001, r^2 = 0.018. San Antonio (S): p = 0.001, 0.05). Finally, PCoA plots highlight that while urban lizards are consuming a significantly different group of insect families, there is still a large amount of overlap signifying that the difference in insect family consumption is not only in the families but most likely in the amount consumed of insect families found in both habitats (Figure 2).

To further investigate the difference in insect family consumption, differential abundance testing was done in each region to determine which insect families are consumed at significantly different rates (Supplemental Table 1). For some families, urban lizards are always consuming insects in greater abundances in natural environments (ex. Figure 3a) while other families show the opposite trend (ex. Figure 3b). Finally, there also exists variation across regions where it can be seen that in one region you see urban affiliated lizards eating more of a given insect family while in another region natural affiliated lizards are eating more of that insect family (ex. Figure 3c).

Using alpha diversity means of each site (Supplemental Table 3) the ancova showed that the estimated dietary niche breadth of urban associated *A. carolinensis* is significantly broader than those associated with natural environments while also showing that which region an anole was sampled from did not have a significant effect on their niche breadth (habitat type p value = 0.012, region p value = 0.087, Figure 4). We were unable to measure the dietary niche breadth of *S. olivaceus* due to only collecting samples from two sites.

Discussion

It has been documented that insect communities all over the world are declining primarily due to habitat loss (Sánchez-Bayo & Wyckhuys, 2019). Given that the interwoven dynamics of a species dietary niche space are driven by prey resources (Stephens et al., 2019), our findings support our hypothesis that urban associated lizards are in fact eating a significantly different group on insect families while also broadening their niche space signifying, they are becoming less specialized. While most anoles tend to be generalists in nature (Losos, 2009) the evidence that they are being even less selective of prey items further illustrates that urban associated species need to be able to accommodate a wider range of prey item to be successful. This is also evidenced by the hummingbird-plant system that in reduced resource environments, they must become less selective (Stephens et al., 2019). Furthermore, these finding of increased dietary niche space in modified environments make sense if insect communities are diminished in urban environments like expected (Merckx *et al.*, 2018) then our findings of urban lizards eating a wider selection of insect families are even more severe due to the limited resources. Again, this is further evidenced by the bray-curtis dissimilarity showing that urban lizards are eating more unique lizard families when compared to other urban lizards in the same region.

While looking at the different families found in each habitat, we can discern that while urban and natural lizards share a great deal of consumed insect families, they each have their own subset of uniquely consumed insect families. This was determined by looking at the unique and overlapping insect families the amount of overlapping insect families was between 75 (DFW) families and 98 (San Antonio (A)) or at a minimum, 65% of total insect families available were shared between habitat types and a maximum of 84% (Supplemental Table 4). Along with PERMANOVA testing we can conclude that in every region (and for both species), urban and natural lizard populations are eating significantly different groups of insect families and can also be visualized in the PCoA figures (Figure 3) which highlight the difference. While this allows us to determine that they are consuming significantly different groups of insect families we used differential abundance testing to determine specifically which of those insect families are most different between urban and natural population.

Without quantifiable evidence describing the differences in insect communities between urban and natural habitats, our conclusions on the severity of our findings are lacking. While this doesn't mean our interpretations are baseless, it just highlights a much-needed addition to the overall project.

Our study utilized high-throughput sequencing techniques to analyze the insect prey consumed by urban and natural habitat-associated *Anolis carolinensis* (green anoles) and *Sceloporus olivaceus* (Texas spiny lizards). We identified a total of 356 insect families in the fecal samples, with *Orthopterans*, *Coleoptera*, *Diptera*, and *Hemiptera* being the most commonly consumed prey. By comparing the diets of urban and natural habitat lizards, we found that urban lizards consumed more dissimilar insect families than their natural counterparts, except for San Antonio, where both urban and natural lizards showed similar dietary patterns while also confirming significant differences in insect family consumption between these groups. Additionally, our results indicated that urban-associated *A. carolinensis* had a significantly broader dietary niche breadth compared to those associated with natural environments showing that urban associated lizards are generalizing their diets consuming more unique prey items than natural lizards. Overall, this study provides valuable insights into the dietary ecology of urban reptile populations and highlights the influence of urbanization on their insect prey consumption patterns.

Literature Cited

Baiser, B., Russell, G.J. & Lockwood, J.L. (2010) Connectance determines invasion success via trophic interactions in model food webs. *Oikos*, **119**, 1970–1976.

Benjamin Callahan, A., McMurdie, P. & Holmes, S. (2020) *Package "dada2,."*

Brooks, T.M., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B., Rylands, A.B., Konstant, W.R., Flick, P., Pilgrim, J., Oldfield, S., Magin, G. & Hilton-Taylor, C. (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology*, **16**, 909–923.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G. & Knight, R. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal*, **6**.

iNaturalist (2018).

Kartzinel, T.R. & Pringle, R.M. (2015) Molecular detection of invertebrate prey in vertebrate diets: Trophic ecology of Caribbean island lizards. *Molecular Ecology Resources*, **15**, 903–914.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. & Schloss, P.D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, **79**.

Losos, J.B. (2009) *Lizards in an Evolutionary Tree*, 1st edn. University of California Press.

Love, M.I., Huber, W. & Anders, S. (2014) DESeq2 Manual. *bioRxiv*.

Macarthur, R.H. & Pianka, E.R. (1966) ON OPTIMAL USE OF A PATCHY ENVIRONMENT.

The American Naturalist, **100**.

Madden, T. (2013) *The BLAST Sequence Analysis Tool*,.

Malmstrom, R. (2015) RNAlater Recipe. *protocols.io*.

Martin, M. (2018) *cutadapt Documentation Release 1.17*,.

McKinney, M.L. & Lockwood, J.L. (1999) Biotic homogenization: A few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution*, **14**, 450–453.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, **8**, e61217.

McMurdie, P.J. & Holmes, S. (2014) Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLoS Computational Biology*, **10**.

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F.F., Backeljau, T., Bonte, D., Brans, K.I.I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J.M.T.M.T., Fontaneto, D., Gianuca, A.T.T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Van Doninck, K., De Meester, L. & Van Dyck, H. (2018) Body-size shifts in aquatic and terrestrial urban communities. *Nature*, **558**, 113–116.

Morrison, B.M.L. & Mendenhall, C.D. (2020) Hummingbird-plant interactions are more specialized in forest compared to coffee plantations. *Diversity*, **12**.

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger, L., Bennett, D.J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M.J., Feldman, A., Garon, M., Harrison, M.L.K., Alhusseini, T., Ingram, D.J., Itescu, Y., Kattge, J., Kemp, V., Kirkpatrick, L., Kleyer, M., Correia, D.L.P., Martin, C.D., Meiri, S., Novosolov, M., Pan, Y., Phillips, H.R.P., Purves, D.W., Robinson, A., Simpson, J., Tuck, S.L., Weiher, E., White, H.J., Ewers, R.M., MacE, G.M., Scharlemann, J.P.W. & Purvis, A. (2015) Global effects of land use on local terrestrial biodiversity. *Nature*, **520**, 45–50.

Oksanen, J., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J. & Suggests, M. (2020) Vegan: Community Ecology Package. R package version 2.5-7. *Community ecology package*, **10**.

Rose, B.R. (1976) Dietary Overlap of Sceloporus occidentalis and S. graciosus. *Copeia*, **1976**.

Sánchez-Bayo, F. & Wyckhuys, K.A.G. (2019) Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, **232**, 8–27.

Sayers, E.W., Cavanaugh, M., Clark, K., Pruitt, K.D., Schoch, C.L., Sherry, S.T. & Karsch-Mizrachi, I. (2022) GenBank. *Nucleic Acids Research*, **50**.

Schoener, T.W. (1971) Theory of Feeding Strategies. *Annual Review of Ecology and Systematics*, **2**, 369–404.

Sherrill-Mix, S. (2019) *Package "taxonomizr" Title Functions to Work with NCBI Accessions and Taxonomy*,.

Stephens, D.W. & Krebs, J.R. (1986) *Foraging Theory*, Univeristy Press.

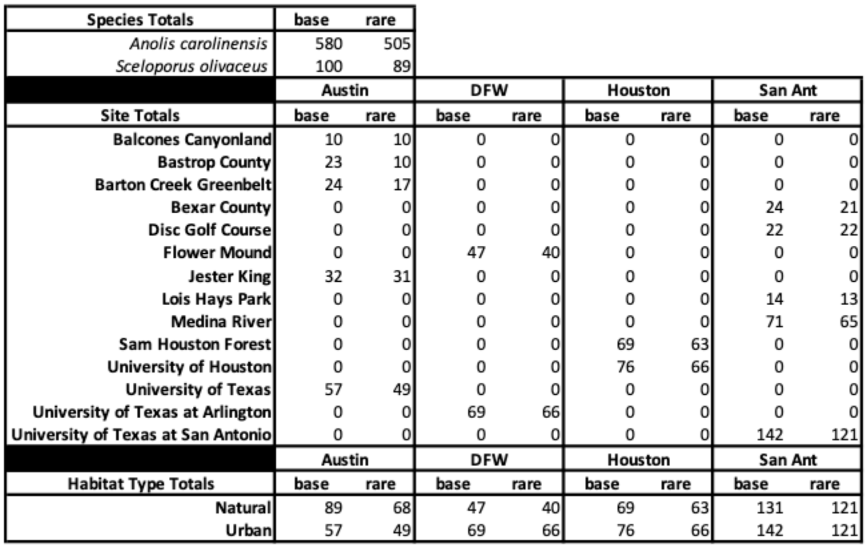
Stephens, R.B., Hobbie, E.A., Lee, T.D. & Rowe, R.J. (2019) Pulsed resource availability changes dietary niche breadth and partitioning between generalist rodent consumers. *Ecology and Evolution*, **9**, 10681–10693.

Winchell, K.M., Carlen, E.J., Puente-Rolón, A.R. & Revell, L.J. (2018) Divergent habitat use of two urban lizard species. *Ecology and Evolution*, **8**, 25–35.

TABLES

Table 1

Summary of sample totals. First, of the species totals before (base) and after rarefaction (rare) followed by the specimen totals of all the lizards captured and at which site they were from before and after rarefaction. Finally, a summary of how each region is broken down by habitat type and sample size before and after rarefaction.



Species Totals	base	rare						
Anolis carolinensis	580	505						
Sceloporus olivaceus	100	89						
	Austin		DFW		Houston		San Ant	
Site Totals	base	rare	base	rare	base	rare	base	rare
Balcones Canyonland	10	10	0	0	0	0	0	0
Bastrop County	23	10	0	0	0	0	0	0
Barton Creek Greenbelt	24	17	0	0	0	0	0	0
Bexar County	0	0	0	0	0	0	24	21
Disc Golf Course	0	0	0	0	0	0	22	22
Flower Mound	0	0	47	40	0	0	0	0
Jester King	32	31	0	0	0	0	0	0
Lois Hays Park	0	0	0	0	0	0	14	13
Medina River	0	0	0	0	0	0	71	65
Sam Houston Forest	0	0	0	0	69	63	0	0
University of Houston	0	0	0	0	76	66	0	0
University of Texas	57	49	0	0	0	0	0	0
University of Texas at Arlington	0	0	69	66	0	0	0	0
University of Texas at San Antonio	0	0	0	0	0	0	142	121
	Austin		DFW		Houston		San Ant	
Habitat Type Totals	base	rare	base	rare	base	rare	base	rare
Natural	89	68	47	40	69	63	131	121
Urban	57	49	69	66	76	66	142	121

FIGURES

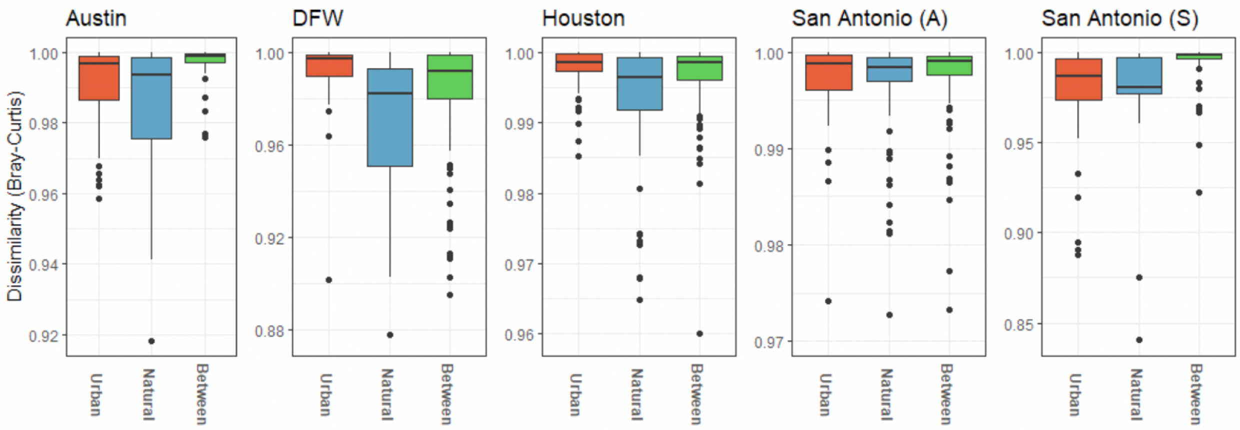


Figure 1

Bray-Curtis dissimilarity plots show how dissimilar all the samples are within each group. The closer to 1, the more dissimilar any two samples are within that group. For example, in Austin, if you sampled any two urban associated their insect consumption would be more dissimilar than if you compared two natural associated lizard. Furthermore, if you randomly sampled a lizard from an urban site and compared it to one in an natural one, they would be even more dissimilar than either two group independently.

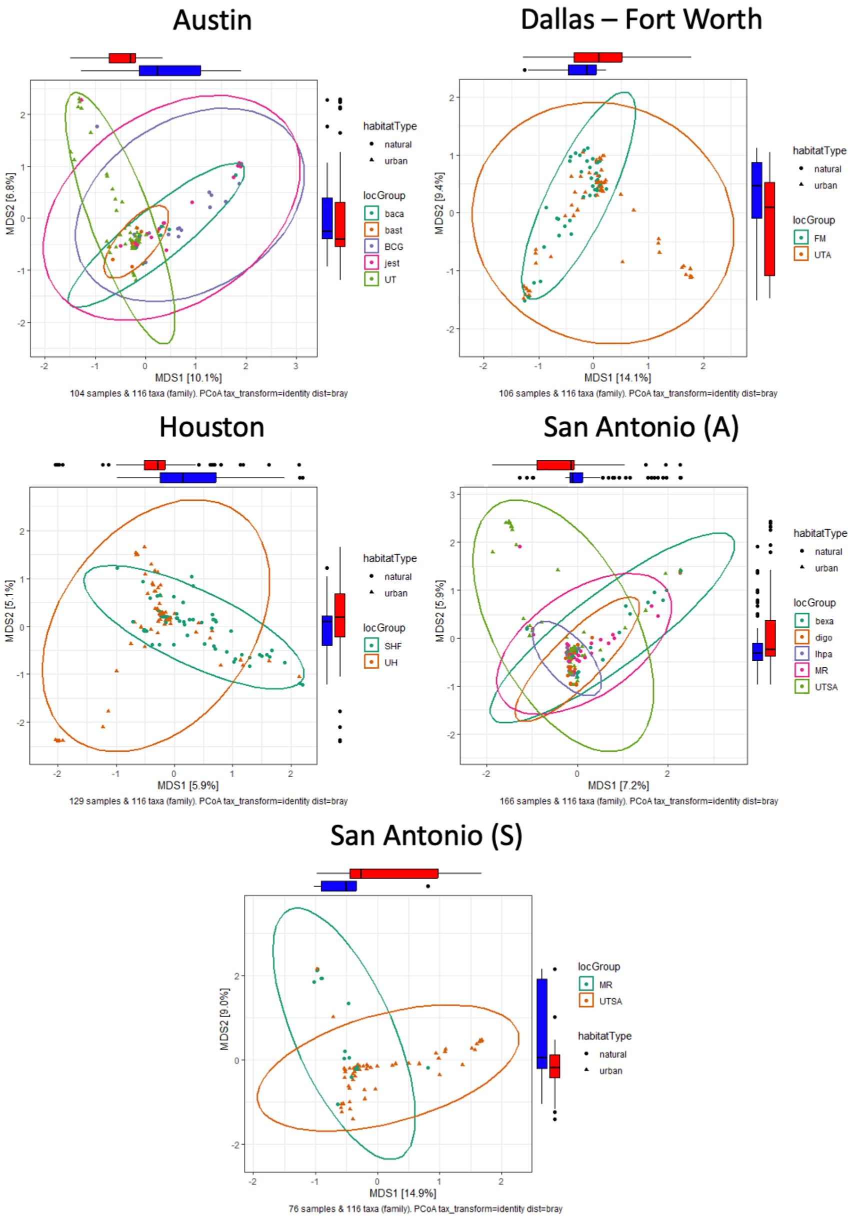


Figure 2

Principal coordinate analysis for each region broken up by target species. Within each plot, all sites where lizards were captured are present with a 95% confidence ellipse. Furthermore, all urban associated samples are denoted by triangles and natural ones are circles while the colors are based on the site (locGroup). On the top and right of every plot is a combined box plot showing the merged urban versus natural comparison for each region to highlight how different each group is.

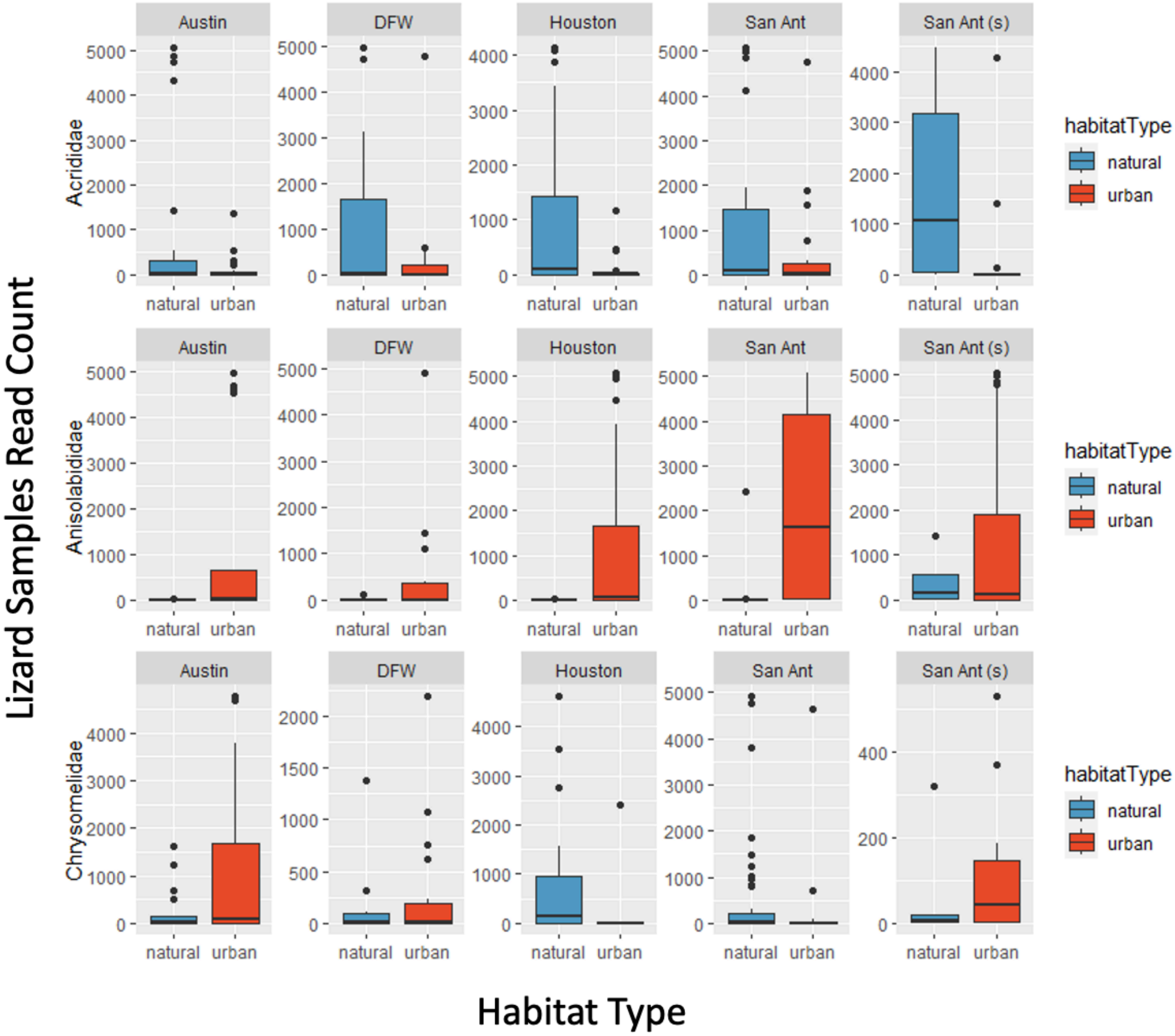


Figure 3

Differential abundance testing highlighted specific insect families which were consumed in both urban and natural environments but were significantly consumed at different rates. Here, we provide examples of the possible combinations of results. First, with the family *Acrididae*, in every region they were always consumed more in natural habitats. Second, with the family *Anisolabididae*, In every region they were always consumed more in urban habitats. Finally, with the family *Chrysomelidae* we see an example where in some regions it is consumed more in urban environments and in other regions it is consumed more in natural ones.

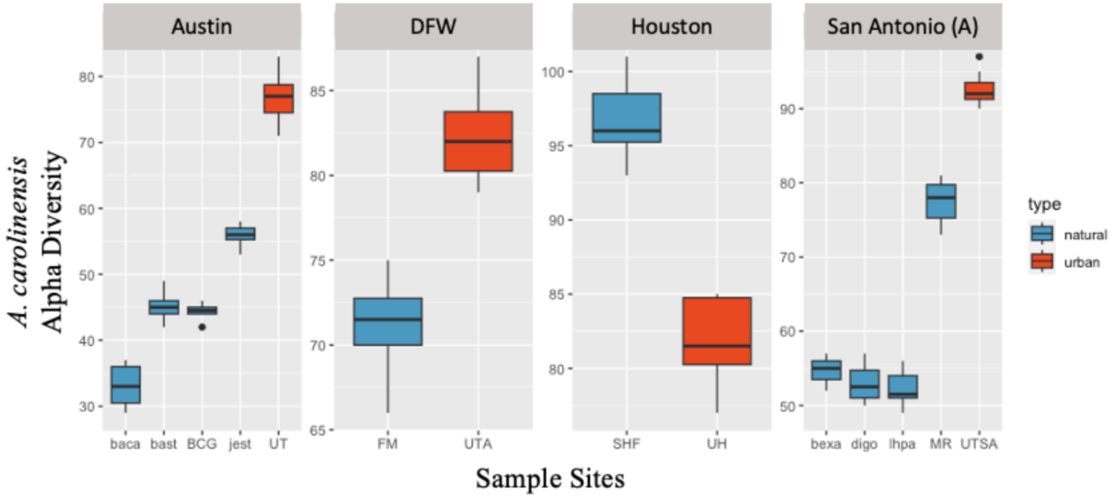


Figure 4

Visual representation of the dietary niche space (alpha diversity values) compared between urban (red) and natural (blue) sites across each region. Only *A. carolinensis* was able to be viewed due to the lack of sample sites where *S. olivaceus* was collected. In every region except Houston there is a clear difference in dietary niche space showing that on average urban associated lizard have a broader dietary niche space than natural lizards.

Supplemental Tables

Table 1:

List of all the insect families that were flagged as being significantly differentially consumed when comparing them between urban and natural lizards organized by region. A “stat” value with a positive number means that it was consumed more in urban environments while a “stat” value with a negative number means it was consumed more by natural lizards. Note: SAN = *A. carolinensis* San Antonio samples and SANs = *S. olivaceus* San Antonio samples.

stat	pvalue	class	order	family	region
4.17	0.00	Insecta	Dermaptera	Anisolabididae	AUS
-3.32	0.00	Insecta	Orthoptera	Acrididae	AUS
4.16	0.00	Insecta	Diptera	Dolichopodidae	AUS
10.14	0.00	Insecta	Diptera	Sarcophagidae	AUS
2.65	0.01	Insecta	Coleoptera	Scarabaeidae	AUS
-1.92	0.05	Insecta	Coleoptera	Elateridae	AUS
4.81	0.00	Insecta	Hemiptera	Pentatomidae	AUS
-5.66	0.00	Insecta	Orthoptera	Gryllidae	AUS

-3.16	0.00	Insecta	Orthoptera	Tettigoniidae	AUS
3.95	0.00	Insecta	Lepidoptera	Uraniidae	AUS
-11.14	0.00	Insecta	Hemiptera	Issidae	AUS
2.27	0.02	Insecta	Hymenoptera	Formicidae	AUS
-5.65	0.00	Insecta	Hemiptera	Fulgoridae	AUS
2.49	0.01	Insecta	Diptera	Chironomidae	AUS
3.49	0.00	Insecta	Coleoptera	Chrysomelidae	AUS
2.14	0.03	Insecta	Hemiptera	Rhyparochromidae	AUS
10.33	0.00	Insecta	Diptera	Mycetophilidae	AUS
9.90	0.00	Insecta	Trichoptera	Hydropsychidae	AUS
2.40	0.02	Insecta	Coleoptera	Hydraenidae	AUS
-9.95	0.00	Insecta	Coleoptera	Anthribidae	AUS
-1.94	0.05	Arachnida	Araneae	Salticidae	AUS
2.13	0.03	Insecta	Dermaptera	Anisolabididae	DFW
-5.38	0.00	Insecta	Orthoptera	Acrididae	DFW
11.51	0.00	Insecta	Diptera	Tipulidae	DFW
-2.71	0.01	Insecta	Hemiptera	Pentatomidae	DFW

-3.96	0.00	Insecta	Orthoptera	Tettigoniidae	DFW
2.25	0.02	Insecta	Coleoptera	Cerambycidae	DFW
4.01	0.00	Insecta	Coleoptera	Curculionidae	DFW
2.70	0.01	Insecta	Hymenoptera	Formicidae	DFW
-2.51	0.01	Insecta	Diptera	Asilidae	DFW
9.52	0.00	Insecta	Hymenoptera	Cimbicidae	DFW
-2.41	0.02	Insecta	Lepidoptera	Hesperiidae	DFW
-3.21	0.00	Arachnida	Araneae	Salticidae	DFW
2.04	0.04	Insecta	Hemiptera	Aphididae	DFW
-8.69	0.00	Insecta	Diptera	Bibionidae	DFW
7.39	0.00	Insecta	Dermaptera	Anisolabididae	HOU
-4.75	0.00	Insecta	Orthoptera	Acrididae	HOU
2.35	0.02	Insecta	Coleoptera	Carabidae	HOU
4.07	0.00	Insecta	Coleoptera	Scarabaeidae	HOU
-3.13	0.00	Insecta	Hemiptera	Pentatomidae	HOU
-3.37	0.00	Insecta	Orthoptera	Gryllidae	HOU
3.22	0.00	Insecta	Hemiptera	Miridae	HOU

2.30	0.02	Insecta	Coleoptera	Cerambycidae	HOU
2.95	0.00	Insecta	Coleoptera	Curculionidae	HOU
2.75	0.01	Insecta	Blattodea	Blaberidae	HOU
-2.68	0.01	Insecta	Diptera	Chironomidae	HOU
-3.84	0.00	Insecta	Coleoptera	Chrysomelidae	HOU
-3.34	0.00	Insecta	Hemiptera	Cicadellidae	HOU
2.94	0.00	Insecta	Diptera	Asilidae	HOU
-3.05	0.00	Insecta	Hemiptera	Coreidae	HOU
-9.15	0.00	Insecta	Hemiptera	Acanthosomatidae	HOU
-2.55	0.01	Insecta	Lepidoptera	Noctuidae	HOU
9.05	0.00	Insecta	Blattodea	Ectobiidae	HOU
-2.25	0.02	Insecta	Lepidoptera	Saturniidae	HOU
-1.94	0.05	Insecta	Hemiptera	Reduviidae	HOU
2.17	0.03	Insecta	Hemiptera	Scutelleridae	HOU
-10.36	0.00	Insecta	Blattodea	Rhinotermitidae	HOU
4.35	0.00	Insecta	Lepidoptera	Erebidae	SAN
7.76	0.00	Insecta	Dermaptera	Anisolabididae	SAN

-6.37	0.00	Insecta	Orthoptera	Acrididae	SAN
4.16	0.00	Insecta	Coleoptera	Elateridae	SAN
-3.18	0.00	Insecta	Orthoptera	Tettigoniidae	SAN
-3.98	0.00	Insecta	Hemiptera	Miridae	SAN
2.28	0.02	Insecta	Blattodea	Blaberidae	SAN
-2.55	0.01	Insecta	Lepidoptera	Nymphalidae	SAN
-10.97	0.00	Insecta	Hemiptera	Fulgoridae	SAN
-4.80	0.00	Insecta	Coleoptera	Chrysomelidae	SAN
3.09	0.00	Insecta	Lepidoptera	Geometridae	SAN
1.97	0.05	Insecta	Hemiptera	Rhyparochromidae	SAN
-2.56	0.01	Arachnida	Araneae	Lycosidae	SAN
-2.07	0.04	Insecta	Hemiptera	Alydidae	SAN
-2.45	0.01	Insecta	Hemiptera	Cicadidae	SAN
-4.29	0.00	Insecta	Hemiptera	Coreidae	SAN
-1.95	0.05	Insecta	Lepidoptera	Noctuidae	SAN
-12.02	0.00	Insecta	Trichoptera	Hydropsychidae	SAN
-3.19	0.00	Insecta	Lepidoptera	Sphingidae	SAN

2.04	0.04	Insecta	Hemiptera	Rhopalidae	SAN
-1.96	0.05	Insecta	Coleoptera	Coccinellidae	SAN
-2.54	0.01	Insecta	Lepidoptera	Papilionidae	SAN
-2.71	0.01	Insecta	Hemiptera	Flatidae	SAN
-9.69	0.00	Insecta	Ephemeroptera	Baetidae	SAN
-9.17	0.00	Insecta	Hemiptera	Membracidae	SAN
5.42	0.00	Insecta	Dermaptera	Anisolabididae	SANs
-4.84	0.00	Insecta	Orthoptera	Acrididae	SANs
2.70	0.01	Insecta	Coleoptera	Scarabaeidae	SANs
2.57	0.01	Insecta	Coleoptera	Elateridae	SANs
3.69	0.00	Insecta	Hemiptera	Pentatomidae	SANs
-2.92	0.00	Insecta	Orthoptera	Tettigoniidae	SANs
-2.99	0.00	Insecta	Lepidoptera	Uraniidae	SANs
3.00	0.00	Insecta	Coleoptera	Cerambycidae	SANs
2.18	0.03	Insecta	Coleoptera	Curculionidae	SANs
9.67	0.00	Insecta	Hemiptera	Issidae	SANs
4.21	0.00	Insecta	Hymenoptera	Formicidae	SANs

-2.14	0.03	Insecta	Coleoptera	Buprestidae	SANs
7.50	0.00	Insecta	Lepidoptera	Pieridae	SANs
7.34	0.00	Insecta	Hemiptera	Cicadidae	SANs
2.97	0.00	Insecta	Lepidoptera	Noctuidae	SANs
7.65	0.00	Diplopoda	Polydesmida	Paradoxosomatidae	SANs
9.12	0.00	Insecta	Blattodea	Blattidae	SANs

Supplemental Table 2:

Table showing the top 20 most consumed insect families by region. “nsamps” represents the number of lizard samples in that region that consumes said insect family where “rank” informs you on how much that insect family was consumed compared to the other families (rank 1 is the most consumed insect family where rank 20 is least consumed out of the top 20).

		Austin		Dallas-Fort Worth		Houston		San Antonio (Anole)		San Antonio (scelop)	
Insect Order	Insect Family	nsamps	Rank	nsamps	Rank	nsamps	Rank	nsamps	Rank	nsamps	Rank
Orthoptera	Acrididae	54	1	54	2	48	2	68	1	30	4
Dermaptera	Anisolabididae	27	7	29	11	39	6	43	8	53	1
Coleoptera	Anobiidae			30	10						
Blattodea	Blaberidae									12	18
Blattodea	Blattidae									13	17
Coleoptera	Carabidae					19	19			29	5
Coleoptera	Cerambycidae	15	20	20	17	24	16			19	11
Diptera	Chironomidae			44	4	21	18				
Coleoptera	Chrysomelidae	40	3	31	8	36	10	61	2	17	13
Hemiptera	Cicadellidae	21	12	30	9	38	8	30	13		
Hemiptera	Clastopteridae	15	19								
Coleoptera	Curculionidae	20	14	19	18	29	14	42	9	18	12
Diptera	Dolichopodidae	16	17	61	1	47	3	52	4	16	16
Coleoptera	Elateridae	19	15			33	11	35	10	28	6
Lepidoptera	Erebidae	28	6	24	14	38	7	44	7	26	7
Hymenoptera	Formicidae			17	20	37	9	27	16	23	10
Orthoptera	Gryllidae	24	9	43	5	54	1	59	3	25	8
Lepidoptera	Hesperiidae	18	16							12	19
Hemiptera	Issidae	35	5	28	12			29	15	10	20
Lepidoptera	Lycaenidae			28	13	24	17				
Hemiptera	Miridae	16	18			19	20	30	12		
Lepidoptera	Noctuidae	25	8	18	19					17	14
Hemiptera	Pentatomidae	45	2	37	7	40	5	47	6	33	2
Diptera	Phoridae			52	3	31	12	25	18		
Lepidoptera	Pieridae							30	14		
Hemiptera	Rhyparochromidae							22	20		
Araneae	Salticidae					26	15				
Coleoptera	Scarabaeidae	20	13	24	15	29	13	33	11	24	9
Diptera	Tachinidae							26	17		
Coleoptera	Tenebrionidae									17	15
Orthoptera	Tettigoniidae	36	4	41	6	41	4	49	5	31	3
Diptera	Tipulidae	21	11	24	16			22	19		
Lepidoptera	Uraniidae	24	10								

Supplemental Table 3:

List alpha diversity means with their standard deviations of every site lizards were captured at organized by genus. Region reflects where each site was located (A) is for *A. carolinensis* and (S) is for *S. olivaceus*.

Site	Genus	Region	Type	Mean	SD
baca	Anole	AUS	natural	33.2	2.94
bast	Anole	AUS	natural	45.2	2.20
BCG	Anole	AUS	natural	44.4	1.07
bexa	Anole	SAN (A)	natural	54.9	1.73
digo	Anole	SAN (A)	natural	52.8	2.39
FM	Anole	DFW	natural	71.1	2.73
jest	Anole	AUS	natural	55.9	1.52
lhpa	Anole	SAN (A)	natural	52.2	2.20
MR	Anole	SAN (A)	natural	77.5	2.92
SHF	Anole	HOU	natural	96.8	2.74
UH	Anole	HOU	urban	81.8	2.94

UT	Anole	AUS	urban	76.8	3.77
UTA	Anole	DFW	urban	82.2	2.66
UTSA	Anole	SAN (A)	urban	92.5	2.22
MR	Sceloporus	SAN (S)	natural	48.7	1.95
UTSA	Sceloporus	SAN (S)	urban	71.6	1.84

Supplementary Table 4:

Summary table reflecting the number of insect families that were found only in either urban or natural habitats along with how many insect families were consumed on both habitat types in every region.

	Number of high abundance insect families		
Region	Urban Only	Natural Only	Overlap
Austin	13	17	86
DFW	34	7	75
Houston	15	13	88
San (A)	5	13	98
San (S)	28	9	79