11-14-2022

Reproduction in Relation to Heavy-Metal Contamination Along an Urbanization Gradient in anInvasive Florida Lizard Species (*Anolis sagrei*)

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Reproduction in Relation to Heavy-Metal Contamination Along an Urbanization Gradient in an Invasive Florida Lizard Species (*Anolis sagrei*).

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
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Date of Approval:
November 14th, 2022

Keywords: ecotoxicology, heavy metals, urban level, reproductive parameters, lizards

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Abstract

Environmental contamination is accumulating rapidly worldwide due to increasing urbanization rates. Heavy metals are known to be particularly toxic to organisms, to accumulate in biological systems, and to be found in higher concentrations in animals located in areas of high urban levels. However, the physiological impacts of these toxic heavy metals in wildlife remain largely unknown. Lizards are excellent bioindicator species for environmental contaminants due to their trophic level as mesopredators and their susceptibility of exposure via air, water, diet, and soil through egg deposition. To assess the impacts heavy-metal contamination has on reproductive parameters in lizards and how these effects vary across an urbanization gradient, a total of 36 brown anoles (*Anolis sagrei*) were taken from 9 different field sites with urbanization levels ranging from low to moderate to high. Breeding pairs from each urban level were bred in a laboratory and reproductive parameters (hatchling survivorship, initial body size , and growth rates) were monitored. I found with statistical significance that hatchlings born from adults from areas of high urban levels have higher mortality, smaller initial body size and slower growth rates than those from adults captured from areas of low and moderate urban levels.

Additionally, soil samples from all 9 field sites along with tissue samples from tail clippings were taken from the adult females to test for contamination levels of heavy metals (Arsenic, cadmium, chromium, copper, lead, mercury, and zinc). Interestingly, soil contamination increased with urban level, but there was no significant relationship of heavy-metal contamination levels in tissue samples across different urban levels. These results suggest that high urban levels are negatively impacting *A. sagrei* reproduction, but factors other than heavy-metal contamination may be contributing to these effects. Future experimental studies are required to further understand the adverse physiological impact urbanization has on lizards, and specifically test various factors that may be contributing to decreased reproductive parameters.

Chapter One: Introduction

Environmental contamination is pervasive and accumulating rapidly worldwide due to anthropogenic activities (Azam et al., 2015).While there are various harmful contaminants present in many ecosystems, heavy metals are known to be particularly toxic to organisms (Wu et al., 2016), are known to accumulate in biological systems, and tend to be found in higher concentrations in animals when they are located in areas of heavy human activity (Salvador et al., 2017; Campbell and Campbell, 2002; Nasri et al., 2017). The most common heavy metal contaminants in urban areas include lead (Pb), mercury (Hg), arsenic (As), chromium (Cr) and cadmium (Cd). These heavy metals are dense, toxic in low concentrations, known endocrine-disrupting contaminants, non-biodegradable in nature, and commonly used in urban areas for multiple applications (Tchounwou et al., 2012). Vehicular traffic and urban runoff are main sources of these contaminants (Prestes et al., 2006). Heavy metals are acutely present and toxic in low concentrations (Wu et al., 2016) with the ability to impact entire ecosystems via bio-accumulation and bio-magnification through air particulates, water, soil, and food webs (Nasri et al., 2017; Salvador et al., 2017; Fletcher et al., 2006; Wu et al., 2017). Additionally, heavy metals are classified as endocrine disrupting contaminants (EDCs). EDCs are substances in the environment that are capable of affecting the endocrine system across various vertebrate taxa (Salvador et al., 2017). These contaminants interfere with the natural hormones (estrogen, androgen, testosterone and corticosterone) in wild organisms that work to maintain normal reproduction and development functions (Crain and Guillette, 1998; Guillette et al., 2000; World

Health Organization, 2002; World Health Organization, 2013). Population declines can be caused directly by lethal contamination levels (Ciliberti et al., 2012) as well as indirectly with persistent sublethal levels affecting reproductive success, such as embryo/offspring mortality, health, and development. (Guillette & Iguchi, 2003). Cadmium has been found to affect many aspects of reproductive success and endocrine function in mammals and birds, including decreased fertility rates, prenatal death, sexual dysfunction, and abnormal embryo development (Marettová et al., 2015). Within reptiles, Crain and Guillette (1998) demonstrated exposing alligator embryos to these endocrine-disrupting contaminants caused permanent damage to the endocrine and reproductive systems of the hatchlings. Additionally, EDCs can have been shown to significantly increase the proportion of females in the clutches (Bergeron et al., 1994; Crain and Guillette, 1998). Thus, the presence of heavy-metal contamination in urban environments may represent an alarming threat to biodiversity conservation. As urbanization rates are expected to increase (Ritchie, 2018), it is vital to assess the impact of heavy-metal contamination on the reproductive success of urban vertebrates for biodiversity conservation (Silvia et al., 2020).

Vertebrate species across several taxa living in urban areas have higher levels of heavy-metal contamination in their blood and tissue than species found in less urbanized areas (Campbell & Campbell, 2001). However, discernable trends in vertebrate taxa across urbanization gradients, particularly in areas of moderate urbanization, are unclear (Riem et al., 2012; McKinney, 2008). Additionally, most studies on the impacts of environmental contaminants on wild vertebrate taxa focus on birds and mammals, while leaving our understanding of reptile ecotoxicology comparatively unknown (Weir et al., 2014). In fact, avian species have frequently been used as surrogate species for reptiles in studies examining exposure and effects of environmental contaminants, leading to a significant knowledge gap concerning how these pollutants actually affect reptile species (Weir et al., 2010; Weir et al., 2014).

Of the few studies testing the impacts of heavy metal contaminants on lizards across urbanization gradients, several cite increased levels of heavy-metal contamination in areas of high urbanization levels. Nasri et al. (2017) studied Bosk's fringe-toed lizard (*Acanthodactylus boskianus*) to measure metal contamination near a phosphate treatment factory complex in southeastern Tunisia. Lizards were sampled on a gradient according to distance from the factory. The lizards with the highest concentrations of Cd, Pb, and zinc (Zn) were located in the areas closest to the factory, with Pb and Cd concentrations (in ppm) found in the kidney tissues of the lizards twice that of those taken from sites the greatest distance from the factory. This supports previous findings that heavy-metal contamination in lizards tends to increase with proximity to pollution sources and high levels of urbanization (Campbell and Campbell 2002; Fletcher et al., 2006). Endocrine disruption is one of the few physiological effects of pollutant contamination that has been examined in lizards. Simoniello et al. (2011) demonstrated lizard embryos are affected by heavy metal exposure from contaminated soil. Italian wall lizard (*Podarcis siculus*) eggs were incubated in Cd-contaminated soil, and embryos exhibited several adverse developmental effects, including malformations in the brain, deformations in in the skull and jaw, and abnormal retinal development. Verderame and Scudiero (2019) were able to conclude that accumulation of EDCs in soil may affect the spermatogenic cycle of male Italian wall lizards, which may lead to lower rates of their reproductive output and survival. Non-lethal doses of Cd have been shown to negatively affect the reproductive system of female Italian wall lizards, and exposure Pb to both decreased clutch sizes and increased mortality in embryos (Simoniello et al., 2013). Increased levels of heavy-metal contamination have also been shown to skew sex ratios of sungazer lizards (*Smaug giganteus*), favoring more females than males (71% to 29%, respectively) in highly polluted areas (McIntyre et al., 2012). Additionally, Burger et al. (2004) compared heavy-metal contamination of the invasive brown anole (*Anolis sagrei*) in Florida between rural study sites and high-urban metropolitan areas. They did not find consistent patterns of heavy-metal contamination between study sites within a metropolitan area (ex. Campground vs. Industrialized ports); however, they were able to determine differences in contamination levels by gender, with significantly higher levels of heavy metals found within female lizards compared to the males, despite females being smaller. The impact of these heavy metal contaminants on the lizards' reproductive output was not tested in this study and warrants further investigation.

As reptile populations and abundance are declining across the globe (Gibbons et al., 2000; Doherty et al., 2020) and approximately 34% of known species are threatened with extinction (IUCN, 2020), their conservation is extremely important to preserving global biodiversity. Most reptiles are mesopredators, and therefore provide important services to their ecosystems by both controlling their prey species' populations and providing food sources for their predators (Gibbons and Buhlmann, 2001; Jaksić et al., 1982). They are also excellent bioindicator species for ecotoxicological studies , as they are particularly sensitive to environmental contaminants and are at risk of dietary and dermal exposure (Weir et al., 2010; Silvia et al., 2020). Dermal exposure in particular may be a large contributing factor for contamination in reptiles compared to other vertebrate taxa (Weir et al., 2010). As reptiles are ectotherms, and therefore have slower metabolisms, they have a lower recovery efficiency than endothermic vertebrate species, making them more sensitive to toxic contaminants (Schaumburg et al., 2012). They might also be exposed through several other routes, including bioaccumulation through the food chain, ingesting contaminated soil/water, contact with contaminated substrates, and gas inhalation (Hopkins, 2000; Burger et al., 2004; Márquez-Ferrando et al., 2009; Zocche, et al., 2013). Despite these traits, reptiles remain the most understudied vertebrate group in ecotoxicology research (Hopkins, 2000). Lizards in particular, are ideal terrestrial bioindicators the ecosystems they inhabit due to their predominately insectivorous diets (Gainsbury and Meiri, 2017) and relatively small home ranges (Indest et al., 2018). Lizards may absorb heavy metals through contaminated soil during egg deposition, making them innately susceptible to any adverse effects caused by an increase of these contaminants in their bodies (Salvador et al., 2017). As urbanization continues to increase globally, it’s imperative to that we better understand how reptiles are affected by these contaminants and high urban levels to implement successful conservation strategies and work to mitigate any adverse reproductive affects.

Objectives and hypotheses

The primary objective of this study is to better understand terrestrial reptile ecotoxicology, by testing the impacts of heavy-metal contamination (arsenic, cadmium, chromium, copper, lead, mercury, and zinc) on reproductive parameters (hatchling survivorship, initial body size, and growth rates) of an invasive anole species: the brown anole (*Anolis sagrei*) across an urbanization gradient across Florida.

Specific hypotheses are as follows:

1. The reproductive parameters will differ across urban levels. The individuals from areas high urban levels will show decreased hatchling survivorship, initial body size, and growth rates compared to lower urban levels.
2. Heavy-metal contamination concentrations in soil samples will increase with urban level.
3. Increased heavy contamination concentrations in soil and urban level will be negatively associated with initial hatchling body size.
4. Heavy-metal contamination concentrations in adult female tissue samples from low urban sites will be lower than in females from high urban sites.

Chapter Two: Materials and Methods

Study species: Anolis sagrei

The brown anole (*Anolis sagrei)* is the most abundant vertebrate in all of Florida (Burger et al., 2004). In areas that are heavily urbanized (such as South Florida) non-native species, such as the brown anole, sometimes fill the niches left by decreasing native species populations (McKinney, 2008). Therefore, understanding the effects of human activities on this species in wild habitats will provide a more comprehensive understanding of an ecosystem's health. Brown anoles are easily accessible, allowing for a large sample size across all study sites. As mesopredators, anoles may experience the effects of bioaccumulation, absorbing heavy metals from their prey items (Nasri et al., 2017). Aside from their diet, anoles may also absorb heavy metals through contaminated soil during egg deposition, making them innately susceptible to any adverse effects caused by an increase of these pollutants in their bodies (Salvador et al., 2017). This makes them useful indicator species, for both the ecosystems they inhabit, and other oviparous species.

Study location: The Florida scrub

The state of Florida has been heavily impacted by urbanization (Frances et al., 2019). Accumulation of Hg in natural ecosystems in coastal regions of Florida has been found to be among the highest in the country (Frances et al., 2019). Many known toxic heavy metals like As, Cd, and Pb are also elevated in the area (Frances, 2019). Thus, Florida an ideal location to study heavy metal accumulation.

The Florida scrub is an imperiled habitat (Ricketts, et al., 1999). However, it hosts a wide variety of plant and animal species, many endemic to the region (Gainsbury 2020; Noss, et al., 2015). Widely considered to be one of Florida's most distinctive ecosystems, this biodiversity hotspot has been negatively affected by habitat destruction and pollution caused by increasing rates of urbanization and agricultural activity (Mushinsky and McCoy, 2017). Unfortunately, many native reptile species in the ecosystem are experiencing sharp declines in population as a direct consequence of habitat loss from human development (Mushinsky and McCoy, 2017). Additionally,. It is expected that the human population will continue to increase within the state of Florida (Smith, 2005). This will exacerbate the rate of habitat destruction and pollution wreaking havoc on the region, and thus it is imperative for local conservation efforts to mitigate the impacts of environmental contamination on native wildlife reproductive success caused by increasing human activities.

Study sites

Brown anoles were sampled from nine different locations, with varying urban levels (Figure 1). Three sites were located in high urban areas, three were located in areas of moderate levels, and three were located in low urban areas. This urbanization gradient was based on the Human Footprint Index (HFI) of each field site. The nine study sites were categorized to each urban level based on their HFI rating. The HFI assigns a numerical value to each study site based on the level of impact that past and current human activity has on the location (Venter et al., 2016). To create this index, multiple factors and human environmental stressors and pressures (built-up environments, population density, electric infrastructure, crop lands, pasture lands, roads/highways, railways, and human-navigable waterways) were overlaid to create standardized maps. Each site is assigned a number ranging from 0-50, with 0 indicating low urban levels, and 50 indicating high urban levels (Venter et al., 2016). Study sites with the 3 highest HFI ratings were assigned as high urban areas, the next 3 highest were assigned as moderate urban areas, and the study sites with the 3 lowest HFI ratings were assigned as low urban areas (Table 1). The only exception was Jay B. Starkey Wilderness Area, which had the 4th highest Venter's Index rating (27) and was categorized as a high urban area instead of a moderate urban area due to high heavy metal soil contamination levels (Appendix A for field site legacy information).

1.) The high urban areas sampled from were the University of South Florida St. Petersburg (USFSP) campus, Weedon Island Preserve, and Jay B. Starkey Wilderness Area. The USFSP campus is located in a highly urbanized area, with high human activity/traffic levels and minimum natural habitat. It is located in Pinellas country, the most densely populated county in Florida (Rayer and Wang, 2014), with a population of approximately 960,000 people as of the 2020 census. Weedon Island Preserve is also located in Pinellas county, and hosts various native plant and animal species, and consists mainly of wetland, upland, and aquatic ecosystems ("Weedon Island Preserve…," 2020). Frequent human activity occurs within the site, and the preserve is used recreationally for birding and fishing ("Weedon Island Preserve…," 2020). This site has a long history of human impact, and was also previously used as an airport in the mid-20th century. Starkey Wilderness State Park is open to the public, and allows for moderate human activity, such as camping, hiking, fishing, and equestrian trails. It consists mainly of pine flatwood, scrub, freshwater marsh, and sandhill natural ecosystems (“Starkey Wilderness Preserve,” 2018). It was previously used as a cattle ranch in the early-mid 20th century. It is located in Pasco County, with a population of approximately 539,885 people as of the 2020 census.

2.) The moderate urban locations sampled from were Lower Hillsborough Wilderness Preserve, Highlands Hammock State Park, and Econ River Wilderness Area. Lower Hillsborough Wilderness Preserve, while located in a natural floodplain, is also subject to moderate amounts of human activity, and borders roads with high levels of vehicular traffic. It is located in Hillsborough County, with a population of approximately 1.5 million people as of the 2020 census. The majority of the park consists of riverine forest, wetland, and pine flatwood communities (Lower Hillsborough Wilderness Preserve,” 2018). Highlands Hammock State Park is located in Highlands County, and consists mainly of scrub, scrubby flatwood, and bayhead ecosystems (“Highlands Hammock State Park,” 2021) and hosts a wide range of native plant and animal species (“Highlands Hammock State Park.” 2021). Econ River Wilderness Area allows moderate human activity and hosts many native wildlife species. Its main ecosystems include pine flatwoods, sandhill habitats, and river swamp habitats (“Econ River Wilderness Area: Seminole County,” 2020). It is located in Seminole Country, with a population of approximately 471,826 people as of the 2020 census.

3.) The low urban locations sampled from were Archbold Biological Station, Duette Preserve and Cedar Key Scrub State Reserve. Archbold Biological station is a research institute located in Highlands County (population approximately 106, 221 people as of the 2020 census), in south central Florida, in the headwaters of the Florida Everglades (“Archbold Biological Station…”, 2018). It is located in the Florida scrub, and consists primarily of scrub, wetland, sand dune, and ranchland ecosystems (“Archbold Biological Station…”, 2018). It hosts several endemic species, and human activity is largely restricted apart from scientific research or educational purposes. Duette Preserve also allows for limited human activity, such as hiking and camping, and hosts many native ecosystem types. This includes oak scrub, dry prairie, pine flatwoods, swamps, and depression marsh (“Duette Preserve,” 2018). The land is managed to protect native plant and animal species. Additionally, the land in Duette Preserve’s has previously been used for agricultural purposes and is still in the process of being restored to its natural condition (“Duette Preserve,” 2018). It is located within Manatee County, with a population of approximately 403,250 people as of the 2020 census. Cedar Key Scrub State Reserve is located in Levy County, with a population of approximately 41,503 people as of the 2020 census. The reserve allows for limited human activity, and hosts natural scrub, flatwood, and salt marsh communities (“Cedar Key Scrub State Reserve,” 2021).

Table 1. Table showing all 9 field sites, their location, their associated HFI rating, and their assigned level.

Study Site	Longitude	Latitude	HFI	Urban Level
USFSP Campus	-82.6374	27.76275	38	High
Weedon Island Preserve	-82.607	27.85	35	High
Jay B. Starkey Wilderness Preserve	-82.6401	28.25267	27	High
Econ River Wilderness Area	-81.1741	28.61378	30	Moderate
Lower Hillsborough Wilderness Preserve	-82.2781	28.14847	26	Moderate
Highlands Hammock State Park	-81.5328	27.47011	12	Moderate
Cedar Key Scrub State Park	-82.9883	29.20583	11	Low
Archbold Biological Station	-81.3385	27.18561	8	Low
Duette Preserve	-82.11	27.53683	5	Low

All nine sample sites were located in West-Central Florida, where brown anoles are known to be abundant. Additionally, many areas of Florida are highly industrialized, and have been shown to have high levels of heavy metals in the soil, making it an ideal place to study terrestrial heavy-metal contamination (Burger et al., 2004).

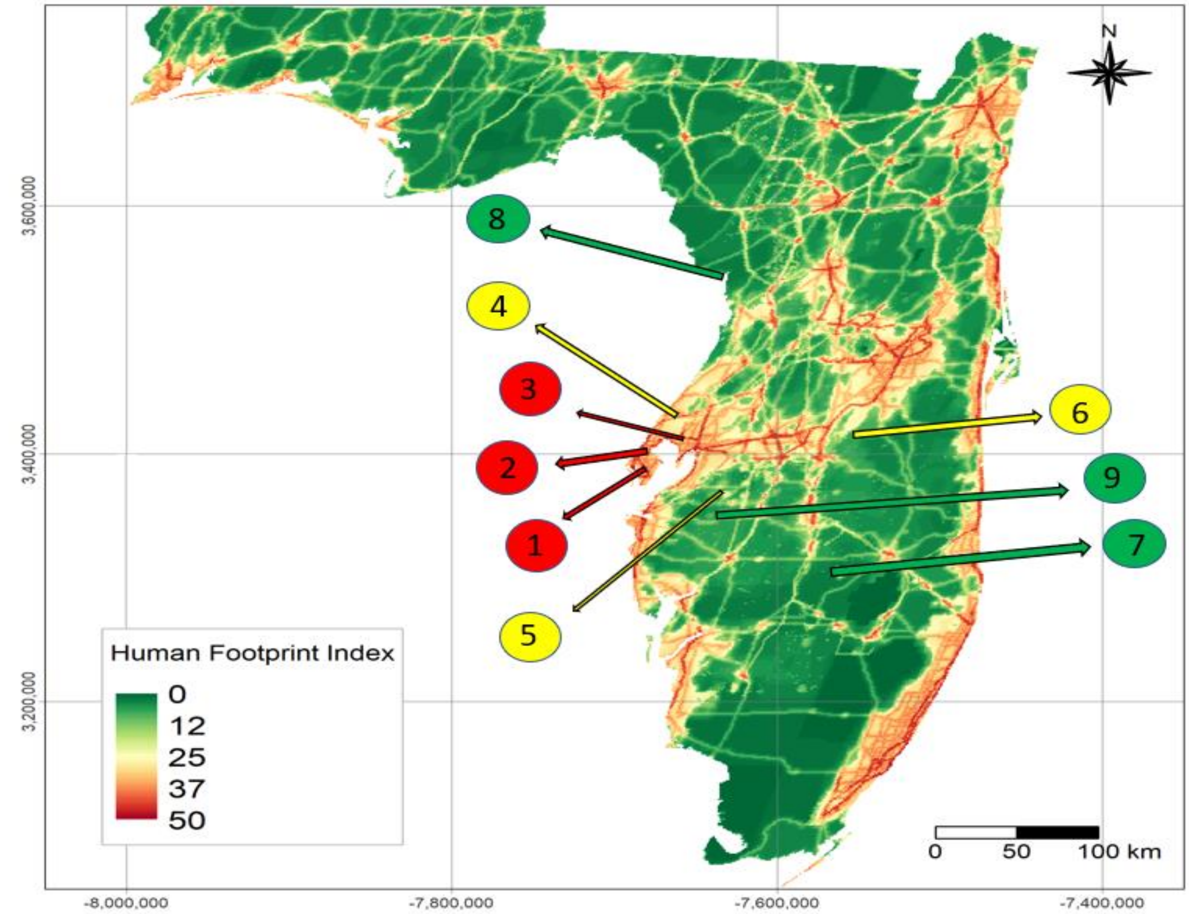

Fig 1. Field site map showing the locations and urban levels of the nine field sites used in this study overlaid with the Human Footprint Index (Venter et al., 2016). High urban (red) sites: USFSP Campus [1] Weedon Island State Preserve [2], Lower Hillsborough Wilderness Preserve [3]. Moderate urban (yellow) sites: Jay B Starkey Wilderness Park [4], Duette Preserve[5], Econ River Wilderness Area[6]. Low urban (green) sites: Archbold Biological Station [7], Cedar Key Scrub State [8] Reserve, Highlands Hammock State Park [9.]

Capture and handling

Field work began in summer, 2021, with eight brown anoles captured from each field site (4 female and 4 male). Lizards were caught via the “noosing” technique. This involved tying a piece of string with a loop at one end and slipping the loop over the lizards’ head to capture them. Each lizard was weighed with Pesola scale (g), sexed, and measured for total length and snout-to-vent length (SVL) with a digital caliper (mm). All brown anoles were be taken back to University of South Florida St. Petersburg (USFSP) for further laboratory experiments. Brown anoles are known to be sexually mature when their snout-vent length is between 34-39 mm (Burger et al., 2004), and only individuals in this size range were taken for laboratory experiments. Soil from each location (approximately 250 grams) was collected, and brown anoles used in laboratory experiments were kept in terrariums containing soil from their wild locations.

Heavy metal analyses

Tissue samples from the tail clippings and soil samples were sent to Michigan State University Diagnostic Veterinary Laboratory (MSU VDL) for analysis. MSU VDL is a fully accredited veterinary diagnostic laboratory through the American Association of Veterinary Laboratory Diagnosticians (AAVLD). It offers full-service diagnostic tests for all species, both domestic and exotic. The quality standard used for accreditation of this laboratory incorporates the principles of the World Organization for Animal Health document "Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases" along with the requirements of the AAVLD.

Tissue samples

Previous studies utilized several different methods to test contaminant concentrations in reptiles. Reliable, non-destructive indices of heavy-metal contamination in reptiles have been developed (particularly blood samples, skin samples, and tail clippings) and proven to be reliable methods for detecting contaminants within the bodies of reptiles (Clark et al., 2000). Tail clippings and skin samples are recommended indices when measuring contaminants in lizards, as they contain blood, tissue, and bone (Nasri et al., 2017; Ciliberti et al., 2012; Schneider et al., 2013). These non-destructive sampling techniques in lizards are largely preferable to previous methods (typically dissections and/or organ/tissue sampling after euthanasia), as they are easily applicable when evaluating contaminants in tissue and skin samples and are far less destructive to both the reptiles and their habitats (Hopkins et al., 2001; Nasri et al., 2017).

Tissue samples were taken in the form of tail clips, between 20-50 mg from each individual. Tail clippings were taken from 15 adult female brown anoles; 5 from each urban level. As brown anoles are able to drop and regenerate their tails as a defense mechanism. To limit the possibility of tail regeneration impacting contamination level, all tail clippings were taken from females with no visible signs of tail loss/regeneration. All 15 adult females were tested for heavy metals (As, Cd, Cr, Cu, Hg, Pb, and Zn) present within their tissue samples.

Once the tail clippings were sent to MSU VDL, the samples were digested in nitric acid in a 95°C oven for four hours. The digested samples were diluted with water to 100x the dried tissue mass. Tissue from tail clippings were analyzed following the protocol in Wahlen et al. (2005) using an Agilent 7900 Inductively Couples Plasma Mass Spectrometer (ICP/MS). Elemental concentrations were calibrated using a 6-point linear curve of the analyte-internal standard response ratio. Standards were from Inorganic Ventures and NIST Bovine Liver and Mussel standards were used as controls. A second source calibration check from “High Purity Standards” was also used. The detection limits for the tissue analyses was 0.01 ug/g of heavy metals.

Soil analysis

Soil samples of approximately 250 g were taken from each study site. Sevel soils samples using a sterile spade were taken at each study site where lizards were captured to get a representative soil sample from each site. The samples were sent to MSU VDL to be analyzed for heavy-metal contamination. Soil samples were completely dried out in an oven and only dry matter was used. Aqua regia was used to leach out heavy metals from each sample for analysis, and results were reported on a dry matter basis. The detection limits for the soils analyses was 0.03 ug/g of heavy metals. This analysis tested for the relationship between heavy metals across different urban levels.

Laboratory experiments

Captured brown anoles were taken to the laboratory at USFSP for further tests. The potential effects of heavy-metal contamination on hatchling mortality, initial size, and growth rates were examined by housing breeding pairs in separate terraria, categorized by urban level. Each terrarium housed one male and one female brown anole and lined with soil from each of their study sites. A total of 36 adult male and 36 adult female brown anoles were collected for laboratory work, with four breeding pairs from each study site (12 pairs for each urban level). Therefore, a total of 72 brown anoles were used in laboratory experiments. Each breeding pair was closely monitored, and an egg check was conducted daily for the presence of newly-laid eggs. A pot with soil from each breeding pairs’ field site was provided for egg deposition. The total number of eggs from each brood was counted, and hatchling survival rate was recorded. Once an egg was found, it was weighed on a digital scale, and placed in a 0.3 gram plastic container, with a 1:1 ratio by mass of vermiculite and deionized water and covered loosely with soil (~0.2 grams) from each correlated field site. Each container was then placed in an incubator at approx. 75% humidity and 27 degrees Celsius.

Reproductive parameters: Hatchling survivorship, initial body size, and growth rates

Hatchling survivorship, initial body size, and growth rates were measured for each hatchling. Hatchling survivorship was determined by documenting any hatchlings mortality observed during daily laboratory work. Initial body size was measured by taking hatchling snout-vent length (SVL (mm)) within 24 hours of hatching, and growth rates of hatchlings were monitored by taking weekly SVL measurements.

Data analysis

All statistical analyses were performed in *R* 4.0.3. (R Core Team, 2022). Data met assumptions of normality and homogeneity of variance and were tested with Shapiro-Wilk and Bartlett tests. Linearity of data was tested with diagnostic plots in the ggfortify package in *R* and met assumptions (Tang et al., 2016). Significance was determined at alpha 0.05.

To test my first hypothesis, a chi-square test of independence was performed to examine the relation between urban level and hatchling survivorship. An analysis of variance test (ANOVA) was used test for a significant difference in hatchling initial body size across urban levels. Tukey Post-Hoc tests were used to determine which pair-wise differences were significant. The lme4 package (Bates et al., 2015) was used to perform a linear mixed effect model to test the effect of urban level (fixed effect; categorical variable) on hatchling growth rates, using hatchling ID as the random variable.

To test my second hypothesis, ANOVAs were used test for significant differences in heavy metal soil contamination levels across urban levels. Tukey Post-Hoc tests were used to determine which pair-wise differences were significant.

To test my third hypothesis, a linear model was used to examine the influence of soil contamination levels of four heavy metals that varied significantly across urban level (Cr, Cu, Pb, and Zn: continuous variables), urban level (categorical variable), mother SVL (continuous variable; covariate) and their interactions associated to initial hatchling body size (SVL: dependent variable). A variance inflation factor (VIF) test was used with the “car” package in *R* to test for high correlation among the predictor variables (Fox & Weisburg, 2019). VIF values above 10 were removed from the analysis. This removed arsenic, cadmium, and mercury. The linear model was conducted using the “visreg” package (Breheny & Burchett, 2017).

To test my fourth hypothesis, a linear model was used to investigate the influence of urban level, adult female SVL (used as a covariate) and their interaction on heavy metal (Copper, Lead, and Zinc) concentration in adult female tissue samples. Arsenic, cadmium, chromium, and mercury test results all registered as > 0.5 ug/g and had no variation between the 15 samples; thus, were not included in the analysis.

Chapter Three: Results

Reproductive parameters across urban levels

A total of 58 hatchlings were included in this study: 30 from high urban areas, 14 from moderate urban areas and 14 from low urban areas. It is worth noting that the adults from high urban areas were kept in the laboratory longer than those from moderate/low urban areas, and therefore had ~3-4 weeks longer to reproduce. Each breeding pair produced 1-6 hatchlings, with the exception of one breeding pair from Duette Preserve (a low urban site). All incubated eggs hatched successfully; There were no embryo/hatchling mortalities pre-hatching. Hatchling reproductive parameters (hatchling survivorship, initial body size, and growth rates) were significantly affected by urban level, as hypothesized. Hatchlings with parents from low and moderate urban sites were significantly more likely to survive than those with parents from high urban sites (X^2 (3, N = 58) = 14.12, p<0.01). Hatchling survivorship in the 3 low urban sites and in the 3 moderate urban sites were 100%, while the survivorship rates in the 3 high urban sites reduced significantly to 60%. Hatchling mortality was only seen in hatchlings born from adults taken from high urban field sites (USF St. Petersburg Campus, Weedon Island Preserve, and J.B. Starkey Wilderness Area) (Figure 2). All hatchlings born from parents from low and moderate urban areas survived until the end of the study on November 21st, 2021, with total lifespans ranging from 8-13 weeks in the laboratory .Hatchling initial SVL length varied significantly across urban level(F(2, 55)=11.59, p<0.01) (Figure 3). Hatchlings from low ($\bar{x}$=17.77, *SD*=0.91) and moderate ($\bar{x}$ =17.27, *SD*=1.01) study sites had greater initial SVL measurements than those from high ($\bar{x}$ =16.40, *SD*=0.75) urban areas. The mean hatchling initial length was significantly different between high urban levels and low urban levels (p <0.01) and high urban levels and moderate urban levels (p=0.017). However, there was no statistically significant difference in mean initial hatchling lengths between low and moderate urban levels (p=0.30). Finally, mean hatchling growth rates (taken by weekly SVL measurements) were slower when hatchlings were from high urban areas ($\bar{x}$ =0.87, *SD*= 0.15) than those from moderate ($\bar{x}$ =0.99, *SD*= 0.16) and low urban areas ($\bar{x}$ =01.00, *SD*= 0.13). (F(2, 55)=4.95, p=0.01) (Figure 4).

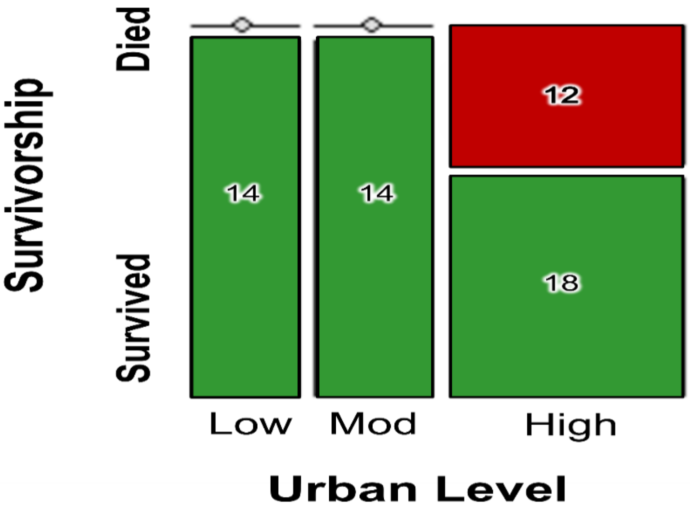

Fig. 2. A contingency analysis showing the relationship between urban level and hatchling survivorship. Hatchlings with parents from low and moderate urban sites were significantly more likely to survive than those with parents from high urban sites.

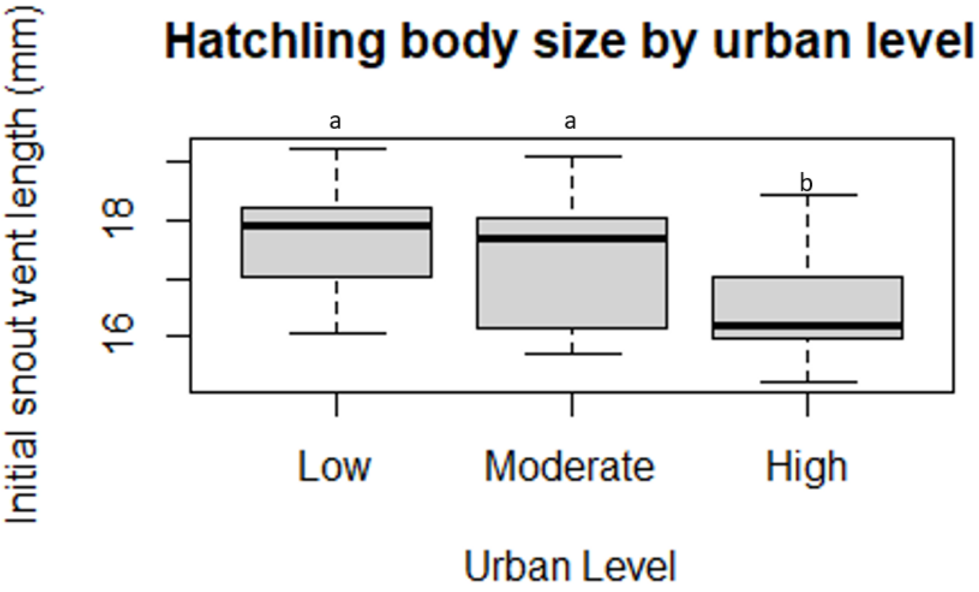

Fig. 3. Initial hatchling body sizes across urban level. Lowercase letters indicate significant difference between mean initial snout-vent length across urban levels.

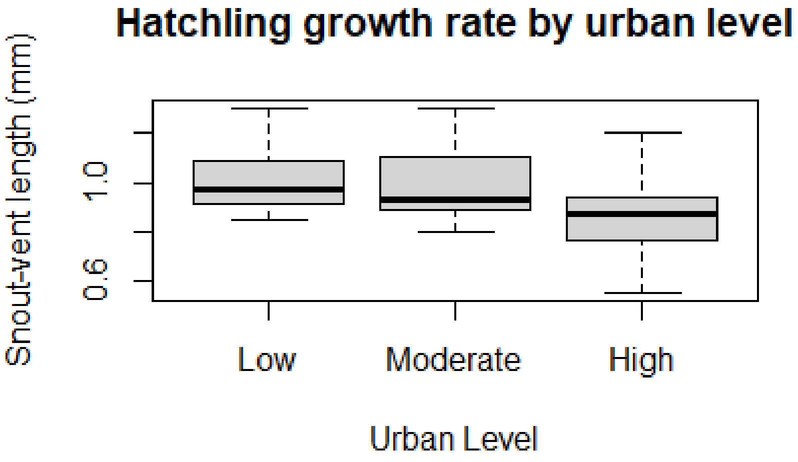

Fig. 4. Effects of urban level on hatching growth (measured in repeated, weekly SVL measurements over 5 weeks).

Soil heavy-metal contamination across urban levels

As hypothesized, heavy-metal contamination in the soil samples from the nine different field sites were significantly related to urban level, with the highest levels of heavy-metal contamination in high urban field sites (Table 2). However, only four metals (Cr, Cu, Pb, and Zn) showed contamination levels a greater than 1 ppm, and therefore these are the metals we report on. Chromium (F(2,6)=90.07, p<0.01), copper (F(2,6)=23.1, p<0.01, lead (F(2,6)=11.58, p<0.01) and zinc (F(2,6)=18.2, p<0.01) soil contamination levels differed significantly among urban levels. Chromium (p<0.01), copper (p=0.01), lead (p<0.01) and zinc (p<0.01) were all significantly higher in high urban areas (chromium: $\bar{x}$ = 5.46, *SD*= 0.83; copper: $\bar{x}$ = 50.0, *SD*= 22.1; lead: $\bar{x}$ =25.1, *SD*= 23.9; zinc: $\bar{x}$ = 107.35, *SD*= 90.3) than in low urban areas (chromium: $\bar{x}$ = 1.65, *SD*= 0.92; copper: $\bar{x}$ = 4.6, *SD*= 3.7.1; lead: $\bar{x}$ =1.78, *SD*= 0.42; zinc: $\bar{x}$ = 3.46, *SD*=1.85) (Figure 5). Soil contamination levels of chromium, copper, lead, and zinc were also significantly greater in high urban areas compared to moderate urban areas (chromium: $\bar{x}$ = 1.89, *SD*= 1.71; copper: $\bar{x}$ = 1.89, *SD*= 1.71; lead: $\bar{x}$ =4.7, *SD*= 0.99; zinc: $\bar{x}$ = 3.74, *SD*=3.05). There was no significant variation in soil contamination levels between low and moderate urban areas in chromium (p=0.96), copper (p= 0.90), lead (p=0.89), or zinc (p=.99). It is worth noting that none of the soil samples analyzed exceeded regulatory levels for heavy-metal contamination in soil as set by the United States Environmental Protection Agency (US EPA) ("Regional Screening Levels …", 2022).

Table 2. Soil sample results from MSU VDL for heavy-metal contamination from 9 different field sites (concentrations in parts per million (ppm).

Field Site	Urban Level	Arsenic	Cadmium	Chromium	Lead	Mercury	Copper	Zinc
USFSP	High	0.7	0.28	5.87	52	0.09	46	208.9

Table 2 (Continued)

Weedon Island Preserve	High	0.37	0.1	4.5	6	0.04	23.8	27.2
Jay B. Starkey Wilderness Area	High	1.08	0.05	6	3	0.02	85.8	32.5
Lower Hillsborough Wilderness Preserve	Moderate	0.6	0.01	0.2	2	0.02	0.6	1.2
Econ River Wilderness Area	Moderate	0.29	0.04	2.82	6	0.03	4.1	7.6
Highlands Hammock State Park	Moderate	0.28	0.04	4.59	4	0.11	0.9	3
Duette Preserve	Low	0.18	0.05	2.57	2	0.02	8.7	4.9
Archbold Biological Station	Low	0.18	0.02	1.45	2	0.02	2.5	3.7
Cedar Key Scrub State Reserve	Low	0.04	0.01	0.16	1	0.02	0.2	0.2

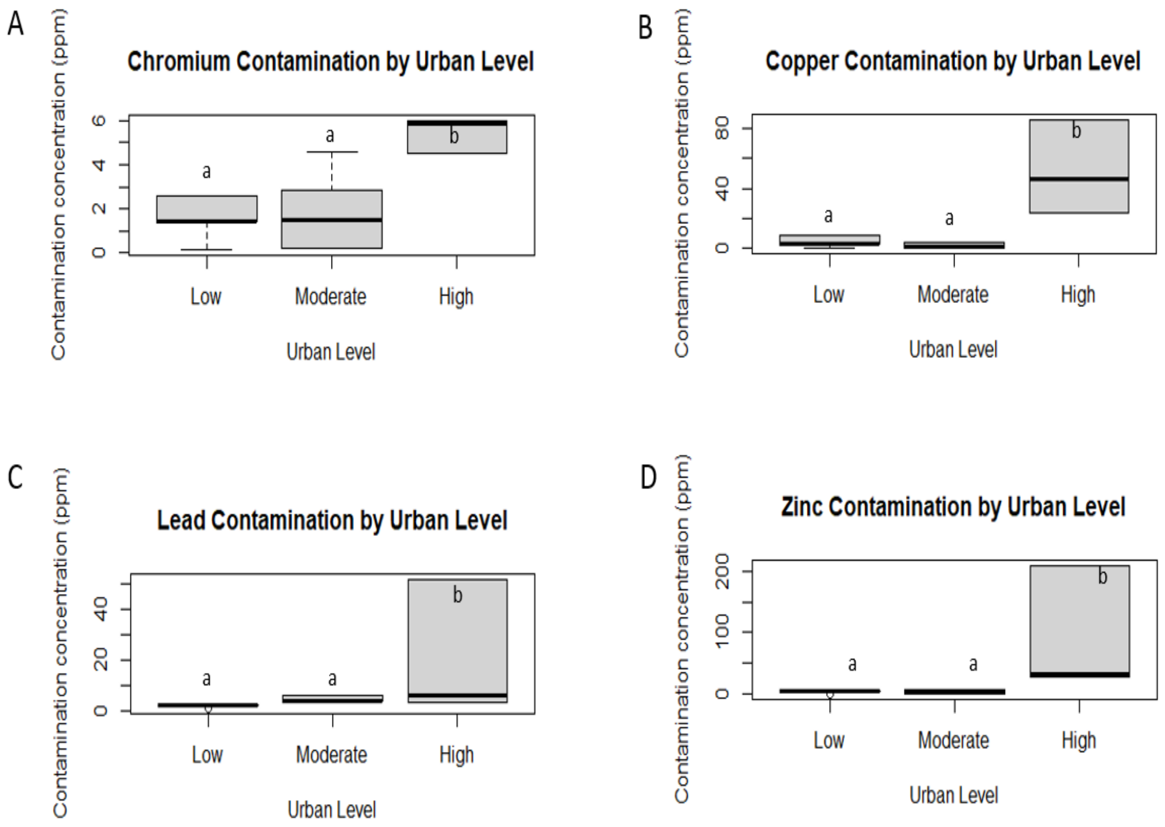

Fig. 5. Relationship of four heavy metals. (A. chromium, B. copper, C. lead, D. zinc) across urban level.

The effects of soil heavy metals and urban level on hatchling initial body size

Hatchling initial body size (SVL) interacted significantly with soil heavy-metal contamination and urban level (Table 3). Thus, the effect of soil heavy metals on hatching initial body size depended on urban level.

More specifically, Cr soil concentrations (LM, F2,56 = 90.07, p <0.01), Cu soil concentrations (LM, F2,56 = , p = 51.56, p< 0.01), Pb soil concentrations (LM, F2,56 = 11.58, p <0.01), and Zn soil concentrations (LM, F2,56 = 18.1, p <0.01) were positively and significantly related to urban level.

Table 3. Linear models investigating the relationships between selected heavy-metal contamination levels in soil (Cr, Cu, Pb, and Zn), urban level and mother SVL along with their interactions on and hatchling initial body size (initial SVL). Significant variables (p<0.05) are shown in bold.

Dependent Variable	Independent Variable/Factor	df	F	p-value
Hatchling Initial size (SVL)	**Cr**	**2, 56**	**8.51**	**>0.01**
	Urban Level	**2, 56**	**10.36**	**>0.01**
	Mother SVL	2, 56	7.28	0.47
	Cr x Urban Level	**2, 56**	**90.7**	**>0.01**
	Cr x Urban Level x Mother SVL	2, 56	3.24	0.74
	Cu	2, 56	2.73	0.5
	Urban Level	**2, 56**	**8.01**	**>0.01**

Table 3 (Continued)

	Mother SVL	2, 56	2.24	0.77
	Cu x Urban Level	**2, 56**	**51.5**	**>0.01**
	Cu x Urban Level x Mother SVL	2, 56	2.61	0.61
	Pb	2, 56	2.78	0.49
	Urban Level	**2, 56**	**10.5**	**>0.01**
	Mother SVL	2, 56	3.61	0.21
	Pb x Urban Level	**2, 56**	**11.6**	**>0.01**
	Pb x Urban Level x Mother SVL	2, 56	3.57	0.32
	Zn	**2, 56**	**8.19**	**>0.01**
	Urban Level	**2, 56**	**7.61**	**>0.01**
	Mother SVL	2, 56	4.21	0.07
	Zn x Urban Level	**2, 56**	**18.1**	**>0.01**
	Zn x Urban Level x Mother SVL	56	2.21	0.69

Adult female tissue heavy-metal contamination across urban levels

Neither urban level nor adult female SVL was significantly related to heavy-metal contamination levels in the adult female tissue samples (Table 4). The interactions were also not significant (Table 5). Thus, heavy-metal contamination in adult female tissue samples did not differ across urban level nor with female size. It is worth noting that none of the soil samples analyzed exceeded regulatory levels for heavy-metal contamination in body tissue as set by the US EPA ("Framework for Metals Risk Assessment," 2007).

Table 4. MSU VDL tissue analysis results from 15 adult female *A. sagrei* tail clipping tissue samples (metal concentrations in ug/g)

Field Site	Urban Level	ID Number	Copper	Lead	Zinc
USFSP	High	HF1	2	1.55	150.23
USFSP	High	HF3	2.47	1.47	60.3
Weedon	High	HF9	3.32	0.93	92.93
Weedon	High	HF12	2.55	0.83	113.06
Starkey	High	MF6	2.67	0.98	148.05
Lower Hillsborough	Moderate	HF5	2.9	0.79	104.33
Econ River	Moderate	MF1	2.81	1.13	133.8
Econ River	Moderate	MF4	2.4	0.45	60.01
Highlands Hammock	Moderate	LF6	2.5	1.64	114.58
Highlands Hammock	Moderate	LF7	2.56	0.23	106.26
Duette	Low	MF9	2.34	2.86	109.46
Duette	Low	MF10	2.79	1.36	106.05
ABS	Low	LF3	3.02	1.02	104.45
ABS	Low	LF4	2.36	1.64	90.91
Cedar Key	Low	LF11	2	0.75	107.1

Table 5. Linear models investigating the influence of urban level, adult female SVL (used as a covariate) and their interaction on heavy metal (Copper, Lead, and Zinc) concentration in adult female tissue samples. Significant variables (p<0.05) are shown in bold

DEPENDENT VARIABLE	**INDEPENDENT VARIABLE/FACTOR**	**DF**	**F**	***P*-VALUE**
CU	Urban Level	2, 13	1.35	0.72
	Adult Female SVL	2, 13	0.6	0.90
	Urban level x Adult Female SVL	2, 13	0.47	0.76
PB	Urban Level	2, 13	1.43	0.64
	Adult Female SVL	2, 13	1.45	0.31
	Urban level x Adult Female SVL	2, 13	1.5	0.60
ZN	Urban Level	2, 13	0.32	0.8
	Adult Female SVL	2, 13	0.58	0.9
	Urban level x Adult Female SVL	2, 13	2.14	0.26

Chapter Four: Discussion

Reproductive parameters across urban levels

In this study, I examined the influence of heavy-metal contamination on reproductive parameters in brown anole (*Anolis sagrei)* across an urbanization gradient in Florida. It is advantageous to use lizards for urban gradient studies, as they are typically not migratory or wide-ranging, and therefore are good indicators of environmental contamination for the areas they inhabit (Fletcher et al., 2006; Silvia et al., 2020). This means that these lizards likely do not stray far from the habitats they were found in and provide reliable data for each specific study site and urban level. We found that select reproductive parameters (hatchling survivorship, initial body size, and growth rates), did differ across urban levels, with brown anole hatchlings from high urban areas having significantly decreased hatchling survivorship, initial body size, and growth rates compared to hatchlings from moderate and low urban areas, showing that high urban levels have a negative relationship with *A. sagrei* reproductive parameters. These results support Seress et al. (2012) documenting bird species produce larger fledglings and in greater numbers when breeding pairs are found in rural areas rather than urban or suburban locations.

Both reptile species richness and population are documented to decrease in areas of high urban levels (Smolensky & Fitzgerald, 2011; Hunt et al., 2011; Kolbe et al., 2016). Hamer and McDonnell (2010) analyzed reptile and amphibian abundance in urban areas around Melbourne, Australia, and predicted the probability of persistence for these species. They found only 56% of the 39 included species of reptiles had greater than 95% probability of being extant if current urbanization trends continue. Urban areas have many factors resulting from increased human activity (including noise/light pollution, temperature changes, and high levels of environmental contamination) that can impact physiological functions and populations of reptiles (French et al., 2018). Lowe (1985) found that rapidly increasing urbanization rates in the American Southwest caused negatively impacted riparian habitat areas, and caused the extinction of several native reptile species. Hunt et al. (2013) found that damming in high urban areas lead to decreased in abundance in reptile species in South Carolina. Wolfe et al. (2013) found that housing development caused by increasing urbanization led to decreased survival rates in the Texas horned lizard (*Phyrnosoma cornutum*). Additionally, urban reptiles exhibit increased physiological stress and suppressed immunity compared to rural individuals (Lucas & French, 2012). There is precedent for high levels of urbanization impacting vertebrate reproduction, and many studies attribute these trends to increased environmental pollutants in high urban areas that are known to impact the endocrine system (Campbell and Campbell, 2002; Nasri et al., 2017; Salvador et al., 2017). Campbell and Campell (2009) found that toxins from insecticides and pesticides in urban areas bioaccumulate in snake and lizard species, causing mortality and lowered reproductive capability. However, while these results of this study point to a clear, negative relationship between urban level and *A. sagrei* reproductive parameters, it is mostly likely caused by multiple stressors associated to urbanization known to negatively impact wildlife (Fidino et al., 2021).

Soil heavy-metal contamination across urban levels

Heavy-metal contamination levels in soil samples taken from our study sites increased as urban level increased, as hypothesized. This is consistent with existing literature (Marambio-

Alfaro et al., 2020; Hanfi et al., 2019). Wei and Yang (2010) found that concentrations of chromium, copper, lead, and zinc were all significantly great in soil form urban and agricultural areas than soil taken from rural areas. Urban run-off and vehicular run-off, both main contributors to introducing high amounts of heavy metals and other EDCs into the environment, are major environmental stressors of high urban areas (Prestes et al., 2006). Heavy metals bio-accumulate in many natural substances, including soil (Wu et al., 2017), and concentrations of toxic elements do not lessen over time (Márquez-Ferrando et al., 2009). Nasri et al., (2017) found a correlation in cadmium, lead, and zinc contamination in lizards living near an industrialized phosphate plant, and their insect prey items. This shows bioaccumulation of these contaminants up food chains in contaminated areas. Fletcher et al. (2006) found that geckos inhabiting urban areas impacting by mining showed significantly higher levels of heavy metals in their tissue and blood than those from low urban areas and correlated with contamination levels in the soil. Lead has been found in high concentrations in the scales of snakes and lizards in high urban and heavy populated areas of India compared to species in rural settings (Kaur, 1988). Tissue samples from Atacamen Pacific Iguanas (*Microlophus atacamensis*) showed extremely high levels of cadmium, copper, lead, and zinc in industrialized areas in Chile (Marambio-Alfaro et al., 2020). Similar trends are also seen in other vertebrate taxa. Bauerová et al. (2017) were able to find a clear trend in avian species' health and heavy-metal contamination in urban areas. Birds with the highest levels of heavy-metal contamination in their blood came from sites with the greatest amount of air pollution, and had lower erythrocyte counts and decreased heterophil/lymphocyte (H/L) ratios, indicating hemolytic conditions of the birds' red blood cells.

These traits of heavy metals were reflected in our results, as four toxic heavy metals (Cr, Cu, Pb, and Zn) were found in significantly greater concentrations in all three high urban study sites than in the three low urban and three moderate urban sites. Interestingly, there was little variation of heavy-metal contamination in soil between the low and moderate urban sites. While there are many studies that document stark differences in environmental contaminants between high and low urban areas, trends in areas designated as moderately urban can be unclear (Riem et al., 2012; McKinney, 2008). For future ecotoxicology studies, increasing the number of sampling sites for soil and/or water heavy-metal contamination along a gradient will provide a better understanding of the relationship between heavy metals at varying urban levels.

The effects of soil heavy metals and urban level on hatchling initial body size

Heavy-metal contamination levels in soil samples taken from the study sites increased as urban level increased, as hypothesized. Chromium, copper, lead, and zinc negatively correlated with hatchling initial body size measurements, although it is worth noting that arsenic and cadmium were only present in trace amounts. All of these heavy metals are known EDCs and can disrupt reproduction and development in reptiles (Britta & Schiesari, 2010). Contaminated soil can impact lizards via dermal exposure, ingestion, and egg deposition (Nasri et al., 2017; Salvador et al., 2017; Fletcher et al., 2006). As the brown anole eggs were deposited and incubated in soil from their mothers' corresponding field sites, the increased levels of heavy-metal contamination found in the high urban field sites may have contributed to the decreased reproductive parameters found in the hatchlings from the high urban areas (Sahoo et al., 1996). Heavy metals have been known to cause death in reptile embryos and defects in newborn hatchlings (Simoniello et al., 2011; Simoniello et al., 2013). Bird and mammals species have been shown to have increased levels of toxic heavy-metal contamination in their blood and tissue in high urban areas (Marettová et al., 2015). Heavy-metal contamination has caused high rates of egg infertility and decreased embryo survivorship in snapping turtles (*Chelydra serpentina*) (Hopkins et al., 2013). Excess copper exposure in bird can cause decrease egg quality, hatchability, and embryonic growth (Gou et al., 2020). Chromium is a known reproductive toxicant and has been shown to accumulate in reptile cells (Wise et al., 2016). Chromium can be acutely toxic to vertebrates and impact vital organs such as the liver and can cause physiological stress that decreases reproductive output (Ni et al., 2020). Both zinc and lead can contribute to DNA damage in lizards and have adverse effects to overall health (Silva et al., 2020). Herkovits and Pérez-Coll (1991) exposed the larvae of a South American toad species (*Bufo arenarum*) to 32 mg/L of zinc for 3 days caused 65% mortality rates compared to 35% in the control group. McIntyre and Whiting (2012) found that heavy metals, including lead and copper, contributing to decreased body conditions in sungazer lizards (*Smaug ginganteus*). However, the physiological impacts of urbanization on reptile hatchlings is largely understudied, when compared to adults. The impacts, and possible transference, of adults to hatchlings are not well known, and the findings of this study warrant further investigation.

Tissue contamination and reproductive parameters across urban levels

Interestingly, heavy-metal contamination levels in the lizards' tissue samples did not differ between urban levels as hypothesized, and in fact showed very little variation among samples. While some previous studies found heavy-metal contamination in lizard tissue is significantly greater in high urban areas (Márquez-Ferrando et al., 2006; Fletcher et al., 2009; Kaur, 1988) others found heavy-metal contamination levels in lizard tissue do not always follow urbanization gradients (Burger et al., 2004). However, high urban areas and urbanization are associated with not only increased levels of heavy-metal contamination, but several environmental contaminants that are known EDCs, such as organochlorides and phthalates (Green et al., 2021). In fact, heavy metals, along with pesticides, pharmaceuticals, and increased acidity are the main ecotoxicological threats facing reptiles in urban environments (Croteau et al., 2008). Therefore, while these results do suggest that high urban levels do in fact negatively affect reproductive parameters in brown anoles, further research is needed to better understand and disentangle the specific variables that cause these adverse impacts.

A potential reason for the lack of variation seen in the levels of heavy-metal contamination levels in the lizard tissue samples seen in this experiment may be the type of tissue used. While most studies include tail-clippings due to their non-lethal method of studying environmental contamination in reptiles (Clark et al., 2000; Hopkins et al., 2001; Nasri et al., 2017), tissue analyses in reptile have also been taken from liver samples or full body samples (Burger et al., 2004; Fletcher et al., 2006; Silva et al., 2020). It is possible the tissue derived from the tail clippings may not have been appropriate representations of heavy-metal contamination levels present within the lizards' whole bodies.

Adult female lizards are known to store toxic contaminants, including heavy metals, in high levels within their gonads, and therefore these high levels of contamination would not be present in tail clippings alone (Hopkins et al., 2004; Boman et al., 2001). Another possible explanation for the low levels of heavy metals in the female tissues is female lizards and other vertebrates may transfer these toxic contaminants to their offspring via embryos and eggshells in a process called maternal transference (Britta & Schiesari, 2010). In oviparous animals, such as the brown anole, contaminant transfer to embryos can occur 2 ways: internally from the mother, and externally from the environment (Britta & Schiesari, 2010). The first method of contaminant transfer is not well studied, but may explain why, in this study, adult anoles from the high urban sites in this experiment did not show increased heavy-metal contamination levels or decreased SVL measurements, but hatchlings born from them had increased mortality, were smaller, and grew slower. Rie et al. (2001) studied the accumulation of cadmium in painted turtles (*Chrysemys picta)* and found that the metal accumulates the highest in the reptiles' liver but is then transferred to the ovary and oviduct tissue, where it contaminates eggs in pregnant females. Stewart et al. (2011) found that contamination levels of PCBs in the blood of adult female leatherback sea turtles (*Dermochelys coriacea*) were significantly and positively correlated to contamination levels found their eggs, suggesting the transfer of these contaminants from mother to eggs. Another study on freshwater turtles (*Emydura macquarii macquarii*) found PFAS in egg shells, yolk, and embryos (Beale et al., 2022).

Other studies on sharks found that various environmental contaminants were detected in found in maternal plasma, uterine fluids, eggs/eggshells, and embryo stomach contents, with offloading rates from mother to embryos ranging from 2-13% (Lyons & Adams, 2015; Naidoo et al., 2017; Mariana et al., 2020). Additionally, concentration rates of environmental contaminants have been found to be up to two times higher in embryos than in ovulated eggs, suggesting that the offloading of toxic contaminants continues and accumulates throughout the developmental process (Naidoo et al., 2017). In areas of high environmental contamination and/or high urban levels, this may cause the effects of environmental contaminants to disproportionately impact embryos and hatchlings compared to adult vertebrates.

Chapter Five: Broader Implications

This study aims to better understand the effects of heavy-metal contamination on lizards' reproductive success along an urbanization gradient in Florida. The results of this study show that there is a negative relationship between high urban levels and reduced reproductive paraments in brown anoles in Florida. While heavy metals in adult lizard tissue samples were not found to increase with urban level, hatchling survivorship, SVL, and growth rates decreased in areas of high urban levels. These results suggest that hatchlings and juvenile lizards may be disproportionally impacted by environmental contamination when compared to adults (Guillette & Iguchi, 2003). The reproductive impacts of environmental contaminants in reptiles across urbanization gradients are poorly studied; the impacts on hatchings even more so. Reptiles as bioindicator species remain underutilized in the field of conservation biology, despite their trophic level and small home ranges making them ideal study specimens (Silvia et al., 2020). Additionally, the developmental systems of most lizard and other reptile species are evolutionarily conserved (Croteau et al., 2008). This means that the impacts of environmental contaminants seen in the endocrine systems of reptiles could help our understanding of how these contaminants impact development and reproduction in other vertebrate species, including humans, in urban environments. Furthermore, the effects of human activity are far-reaching, and extend outside of urbanized areas. Urban run-off leaches into soil and water systems, rural, and agricultural areas are polluted from pesticides and herbicides (Salvador et al., 2017). All of the selected field sites for this study, from all three urban levels, were impacted by human activity.

Even in protected parks and preserves, human activity is still prevalent, and a preserves' proximity to roads and other artificial structures are known to contaminate the ecosystem, and impact contamination levels (Salvador et al., 2017). Mining sites and similar industrial plants, even those that are no longer active, have shown long-lasting effects on their surrounding ecosystems, in the form of contamination in the soil, water, and wildlife (Fletcher et al., 2006). To implement effective conservation strategies, it is vital to fill-in our knowledge gaps on the impacts of urbanization and the associated heavy-metal contamination on the future generation.

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Appendix A. Field Site Legacy Table

Field Site Legacy Information

Field Site	**Background Information/History**
USFSP	The University of South Florida, St. Petersburg Campus has been in use since 1965. It is located in the heart of downtown St. Petersburg, and experiences both heavy human and vehicular activity daily.
Weedon Island State Preserve	The area of Weedon Island Preserve naturally consisted of oak, pine, flatwood and scrub habitats before human interference and activity. The land was used for an airport from for over 30 years in the 1930s-1950s. Although it is now used as a preserve, continued human activity and potential lasting contamination from past use may impact its wildlife.
Lower Hillsborough Wilderness Area	This site's primary purpose is water storage/ supply. The LHWP was designed for temporary impoundment of floodwaters by constructing a levee on its western boundary, a dam on the Hillsborough River and the Tampa Bypass Canal to reroute floodwaters.
Econ River State Park	The Econ River Wilderness Area was purchased by Seminole in 1994 through the county's Natural Lands Program and has been opened to the public since 1998. It attracts about 40,000 guests a year and is in close proximity to UCF.

Field Site Legacy Information (Continued)

Duette Preserve	Local timbering and turpentine production from slash and longleaf pine was the predominant use at Duette Preserve tract, followed later by cattle ranching and limited row crop production. In the 1960s large tracts of land were acquired by several phosphate mining companies who made plans for strip mining to obtain phosphate ore. Manatee County purchased a majority of the preserve from three phosphate companies in two major voter-approved acquisitions in 1984 and 1986 to protect the Lake Manatee reservoir and watershed.
Jay B. Starkey Wilderness Area	Over more than a century, there were turpentine camps and timber operations, a raised bed railway for logging and bridges built across the rivers.
Highlands Hammock State Park	The park opened in 1931 and was used for conservation efforts. Construction on a botanical gardens began soon after, but it was never completed. Since its opening, the park has been a popular camping/hiking area for the general public.
Archbold Biological Station	Founded in 1941, extensive research on the great diversity of natural habitats have been carried out since. The land was kept largely unchanged by human activity, and human/vehicle traffic has been limited since its founding.
Cedar Key Scrub State Reserve	Located of off Florida State Road 24, Cedar Key Scrub State Reserve is largely untouched by previous human activity, making it an ideal area to designate as a low urban field site.