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Impacts of Anthropogenic Disturbances and Urbanization on the Behavior and Morphology of Two Free-Living Lizard Species (*Uta stansburiana* and *Aspidoscelis neotesselatus*)

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IMPACTS OF ANTHROPOGENIC DISTURBANCES AND URBANIZATION ON THE BEHAVIOR AND MORPHOLOGY OF TWO FREE-LIVING LIZARD SPECIES (*UTA STANSBURIANA* AND *ASPIDOSCELIS NEOTESSELATUS*)

by

Layne O. Sermersheim

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

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Approved:

Susannah S. French, Ph.D. Major Professor	Julie K. Young, Ph.D. Committee Member
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ABSTRACT

Impacts of Anthropogenic Disturbances and Urbanization on the Behavior and Morphology of Two Free-Living Lizard Species (*Uta stansburiana* and *Aspidoscelis neotesselatus*)

by

Layne O. Sermersheim, Master of Science

Utah State University, 2024

Major Professor: Dr. Susannah French
Department: Biology

Urbanization can alter wildlife, requiring species to adjust to anthropogenic changes via life history strategies. Reptiles are particularly vulnerable to these changes, as ectotherms directly rely on their environment to maintain their optimal homeostatic state. As urbanization changes the landscape, reptiles will have to adapt to anthropogenic change, but it is unclear what behaviors may be aiding in this adaptation and whether there are morphological tradeoffs existing to support this change. To analyze the impact of urbanization and anthropogenic disturbances on reptile behavior and morphology, I conducted three research studies on two different species – the common side-blotched lizard and the Colorado checkered whiptail (*Aspidoscelis neotesselatus*).

In my first chapter, I characterized differences in side-blotched lizard body condition, aggressive behaviors, and thermoregulatory behaviors between urban sites experiencing an urban heat island effect, and rural field sites in St. George, UT, USA.

Although temperature differences were recorded, there were minimal behavioral differences between sites, yet a strong trade-off between thermoregulatory and aggressive behaviors, regardless of site type.

In my second chapter, I analyzed the impacts of military aircraft noise at Fort Carson Military Base in Colorado Springs, CO, USA on the behavior, stress, and metabolism of the Colorado checkered whiptail. During the flyovers, corticosterone and ketone bodies were upregulated, while lizards spent a greater proportion of their time eating, not moving, to help buffer the effect of the flyovers.

Because I detected considerable variation in behavior within sites studied in my first chapter, I investigated morphological and behavioral differences between side-blotched lizards within an urban site for my third chapter. At one of my urban sites, Interstate 15 bisects lizard habitat and introduces anthropogenic disturbances via noise pollution and an increased predator abundance. I observed poor body condition closer to the interstate but detected no difference in exploratory or anti-predator behavior by proximity to the interstate, though lizards fled faster and farther from a simulated aerial predator than the terrestrial model. A higher proportion of lizards fled from the terrestrial model, highlighting the potential for urban lizards to adjust their behavior based on predator type.

(179 pages)

PUBLIC ABSTRACT

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To my younger self - you never stopped being curious.

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Layne Sermersheim

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Chapters 2 and 4

I acknowledge the Paiute (Nung'wu) Nation, the Paiute Indian Tribe of Utah (Cedar Band, Kanosh Band, Koosharem Band, Indian Peaks Band, and Shivwits Band), and the Kaibab Paiute Tribe, on whose ancestral homelands the work in St. George was conducted.

Chapter 3

I acknowledge the Ndé Kónitsąąíí Gokíyaa (Lipan Apache), Núu-agha-tʉvʉ-pʉ̱ (Ute) and Tséstho'e (Cheyenne) Nations, on whose ancestral homelands the work in Colorado Springs and Fort Carson was conducted. The founding and development of Colorado Springs was in direct violation of the Fort Laramie (1851 & 1868).

Unless required in a citation, the side-blotched lizard scientific name is not used in this thesis. The individual in which this species was named after supported Brigham Young's 1848 "extermination order" of the Timpanogos Nation, starting the Black Hawk War, which spanned 25 years and left thousands dead. This individual also played a key role in the Battle Creek Massacre of three innocent Timpanogos people on 05 March 1849 in present day Pleasant Grove, Utah, USA (Gottfredson, 2003).

Layne Sermersheim

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CHAPTER 1

INTRODUCTION

Urbanization-based impacts on wildlife

Life. Death. ~~Taxes.~~ **Urbanization**, or the emergence and growth of cities into natural landscapes, stands as one of the few future certainties in life (Berry, 2008; Vlahov & Galea, 2002). Urban environments are characterized by concrete sidewalks, tall buildings, and the urban heat island (UHI) effect (Chapman et al., 2017; Luber & McGeehin, 2008; Piracha & Chaudhary, 2022; Tam et al., 2015), which is detrimental to humans (Aghamohammadi et al., 2022) and wildlife alike.

Urbanization impacts wildlife at individual (Liker et al., 2008; Putman et al., 2019; Villaseñor et al., 2014), population (Harveson et al., 2007; Rivera-Pérez et al., 2023; Vardi et al., 2023), ecosystem (Bohus et al., 2023; Ramachandra et al., 2019; Zang et al., 2011), and biome (de Barros Ruas et al., 2022; Filloy et al., 2019) levels, making it a huge problem for pretty much any species on Earth. Even if a species is not directly affected by urbanization via habitat degradation or the UHI effect, they could experience indirect effects, such as losing a necessary resource, prey item, or migration route. Plants (McKinney, 2008) and fungi communities (Abrego et al., 2020) are also impacted by urbanization, not just the vertebrates and invertebrates (Clark et al., 2007). One of the most detrimental aspects of urbanization is its homogenizing effects (McKinney, 2008). There is extensive evidence that demonstrates marked declines in biodiversity (Bohus et al., 2023; Gomes et al., 2011; Xu et al., 2018) and abundance (Fortel et al., 2014; Thaweepworadej & Evans, 2023), yet urbanization also impacts the life history strategies and patterns of animals via changes in their physiology (Aulsebrook et al., 2020; Giraudeau et al., 2014; Malisch et al., 2020), morphology (Jones et al., 2023; Liker et al., 2008), and behavior (Moiron et al., 2015; Murray et al., 2019). As urbanization persists, available habitat and resources for wildlife will continue to decline, the effects exacerbated by anthropogenic disturbances, like highways and noise pollution (Lehtinen et al., 1999; Santos & Tabarelli, 2002).

Species have three main choices when dealing with a changing environment. They can either **(1)** disperse to find a new, more suitable location **(2)** adapt to the changing environment, or **(3)** perish. One of the main ways animals acclimatize to a new environment is via behavioral shifts.

Behavioral adjustments to anthropogenic disturbances

Phenotypic plasticity, animal temperament, boldness, and behavioral modifications are just a few of the mechanisms used by wildlife in order to adjust to urbanizing environments (Lowry et al., 2013). Changes in behavior are linked to many different life history strategy traits that are affected by anthropogenic disturbances, such as diet (Prange et al., 2004) and reproduction (Robbins, 1993; Slabbekoorn & Peet, 2003). Behavior flexibility or plasticity, the ability of animals to adjust their behaviors based on experienced stimuli, may be one way animals can live in urban environments (Gross et al., 2010; Miranda, 2017). Behavioral plasticity has even been shown to facilitate evolution in urban areas, as shifts in behavior could shift population dynamics (Caspi et al., 2022). Most research examining the relationship between urban environments and plasticity has focused on avian groups (Bressler et al., 2020; Slabbekoorn, 2013; Slabbekoorn & Peet, 2003), yet reptiles are extremely susceptible to the UHI effect due to their direct relationship with their thermal environment. Because reptiles cannot produce their own heat, like endotherms, behavior is an extremely important mechanism in which reptiles such as snakes (Mukherjee, 2018) and lizards (Huey, 1974) maintain a healthy body condition. is also important to reptiles because it allows them to flee from predators (Moule et al., 2016; Trompeter & Langkilde, 2011) and unsuitable environments.

Side-blotched lizards and Colorado checkered whiptail models

Side-blotched lizards and Colorado checkered whiptails are ideal models to investigate the impacts of urbanization on behavior and morphology. They are both free-living lizard that are both relatively easy to catch and observe to quantify behavior.

Side-blotched lizards are small lizards (<5in) in the Phrynosomatid family that feed on small invertebrates (Tinkle, 1967) and are well known for their “rock, paper, scissors” mating strategies (Sinervo & Lively, 1996). Being a polymorphic species, each morphotype has a different mating strategy that outcompetes another, hence the “rock, paper, scissors”-esque evolutionary pattern. The morphotype distribution is maintained by female choice across the population, never allowing one morphotype to remain the top choice each year. They have a wide distribution, spanning from the southern tip of

Washington to tip of the Baja region in California, USA. Side-blotched lizards in urban areas of St. George, UT, USA, have shorter lifespans and a higher reproductive input than those found in rural areas (Lucas & French, 2012). They also have lower immunity and higher corticosterone levels, signaling an impact of urbanization on body condition. The lizards found in St. George did not migrate into the city, rather, the city grew out to encompass the populations.

The Colorado checkered whiptail (*Aspidoscelis neotesselatus)* is a triploid, all-female species of lizard in the Teiid family that reproduces via parthenogenesis (LIVO et al., 2019; Manning, 2008; Walker et al., 1997). The species was born at a single hybridization event between a female, diploid common checkered whiptail (*A. neotesselatus*) and a male six-lined racerunner (*A. sexlineatus*). Scientists believe it occurred in either Otero or Las Animas County, CO, because all three species are syntopic there (Walker et al., 1997; Walker et al., 2012).

Objectives

In my three thesis research chapters, I aim to further investigate the relationships between urbanization, morphology, and behavior. More specifically, I aim to uncover evidence of behavioral plasticity aiding the side-blotched lizards' and Colorado checkered whiptails' responses to anthropogenic disturbances. My research consisted entirely of *in-situ* behavioral observations to **(1)** observe differences thermoregulatory and aggressive behaviors by site type (urban versus rural); **(2)** investigate the impact of anthropogenic noise pollution on stress and behavior; and **(3)** gain a better understanding on how behavioral plasticity affects anti-predator and exploratory behaviors near a roadway disturbance.

Chapter 2 - Implications of urbanization on the morphology and behavior of a free-living lizard

In my second chapter, I tested the effects of urbanization on behavior, morphology, and body condition in different populations of side-blotched lizards inhabiting urban and rural areas around St. George, Utah, USA. I hypothesized that significant behavioral and morphological differences exist between rural and urban lizards; within the site types, perch environment and proximity to a disturbance affect behavior and morphology; and behavior and morphology are linked. More specifically, I predicted that urban lizards will show more aggressive behaviors; lizards closer to disturbances, regardless of site type, will perform more aggressive and fewer thermoregulatory behaviors; body condition will be directly linked to aggressive behaviors; and lizards with actively regrowing tails will perform fewer aggressive behaviors.

Chapter 3 - Behavior, stress and metabolism of a parthenogenic lizard in response to flyover noise

For my third chapter, I worked with co-authors to sample 82 female Colorado checkered whiptails at the Fort Carson Army Base in Colorado Springs, CO, USA, during the 2021 reproductive season to assess whether scheduled flyovers would impact the behavior, stress, and metabolism of *A. neotesselatus,* while controlling for size and reproductive stage differences. We measured corticosterone (CORT) as a marker of anthropogenically induced stress during flyovers compared to a control. We further tested for the downstream effects of flyovers on plasma glucose (free energy available to tissues), elevated metabolism with oxidative stress (ROMs), and ketone bodies (alternative cerebral energy substrates to glucose).

Chapter 4 - The effects of an interstate and recreational walking trail on lizard body condition and antipredator behavior

In chapter 4, I utilized field and experimental techniques to examine the effects of an interstate and walking path on the behavior, morphology, and body condition of side-blotched lizards found at an urban park. I hypothesized that significant morphological differences exist depending on proximity to the interstate; thermoregulatory, aggressive, exploratory, and anti-predator behaviors differ based on distance to the disturbance; and morphological and environmental factors are linked to behavior. I predicted that lizards found on perches closer to I-15 will have worse body condition, as distance from the disturbance shortens, individuals will be less likely to explore a novel environment and flee more quickly from aerial and terrestrial predators, individuals perched near the disturbances will spend less time thermoregulating and perform more push-ups, and lizards in better body condition will flee more quickly than those who do not. Alternatively, if lizards can habituate to the disturbance, I would see the reverse relationship in terms of behavior and distance from the disturbance.

Chapter 5 - Conclusions

In my last chapter, I summarize the findings of my projects and discuss realms of research I am interested in exploring in the future.

CHAPTER 2

IMPLICATIONS OF URBANIZATION ON THE MORPHOLOGY AND BEHAVIOR OF A FREE-LIVING LIZARD

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Abstract

Rapid human migration into urban areas creates unique challenges for wildlife, including habitat fragmentation and altered resource availability. Moreover, the addition of infrastructure creates a higher thermal capacity than rural landscapes, which can generate an urban heat island (UHI) effect. Behavioral and morphological responses to urbanization have been shown repeatedly in avian and mammalian groups, yet little work has focused on ectotherms. Here, we explore behavioral and morphological effects of urbanization on side-blotched lizard populations in St. George, Utah, USA. We tested the hypothesis that lizards in urban sites exhibit significant morphological and behavioral differences compared to their rural counterparts. In each landscape, we performed individual focal follows, recording specific perch characteristics and aggressive, thermoregulatory, and solitary behaviors. Urban sites were hotter than rural sites, supporting the presence of an UHI effect. Although temperature differences were recorded, there were minimal behavioral differences between sites. Instead, we found a trade-off existed between thermoregulatory and aggressive behaviors regardless of site

type. Tail status was an important factor impacting aggressive behaviors and linking behavior to morphology. Because further urban development is unavoidable, it is essential that the mechanisms of ectotherm adaptation continue to be studied, with particular focus on perch conditions across the broader urban environment.

Introduction

In 2018, 55% the world's population resided in cities, a trend expected to persist with continued population growth (Nations, 2018). Urban environments, characterized by increased infrastructure, creates a higher thermal capacity than the original rural environment, a phenomenon known as the urban heat island (UHI) effect (Chapman et al., 2017b; Luber & McGeehin, 2008; Piracha & Chaudhary, 2022). The UHI effect poses major health risks to humans (Aghamohammadi et al., 2022; Heidari et al., 2020; Piracha & Chaudhary, 2022), and can be just as detrimental to urban wildlife.

Like humans, heat-stress (Chapman et al., 2017a; Pipoly et al., 2022) and air quality (Aulsebrook et al., 2020; Bauerová et al., 2017; de Oliveira et al., 2023) impact urban wildlife, in addition to the negative impacts of habitat alteration and increased disease risk. Invertebrates (Bohus et al., 2023; White & Walsh, 2020), vertebrate (Thaweepworadej & Evans, 2023; Tscharntke & Batáry, 2023), and even fungi (Abrego et al., 2020; Li et al., 2023) have been negatively affected by urbanization. Increased disease risk has been seen across taxa as well, with much of the research focused on avian species (Berlow et al., 2021; Giraudeau et al., 2014; Mourkas et al., 2023). For example, the microbiome of wild bird populations from highly urbanized areas contained as much as four times more multi-host enteric pathogen genotypes and six times more antimicrobial resistant (AMR) strains of the pathogen relative to populations located farther from urban areas (Mourkas et al., 2023).

Urbanization also impacts the physiology, morphology, and behavior of nearby populations. A phylogenetic meta-analysis of over 516 physiological comparisons found a small, negative relationship between urbanization and corresponding wildlife health, with invertebrates and amphibians particularly affected (Murray et al., 2019). Morphological changes have been seen across taxa, including birds (Jones et al., 2023; Liker et al., 2008) and reptiles. For example, urban morphology of the lizard *Aspidoscelis costatus costatus* differed in nearly every variable measured, including: smaller heads, longer limbs, and smaller length of longest finger (Gómez-Benitez et al., 2021).

As urbanization continues, wildlife faces a reduction in available habitat and resources, as well as a heightened level of anthropogenic disturbance (Isaksson, 2015; Kowarik, 2011; Lowry et al., 2013). Past studies have described changes in behavior linked to life history strategies in diet (Prange et al., 2004), reproduction (Robbins, 1993; Slabbekoorn & Peet, 2003), movement and activity (Riley et al., 2003), disease susceptibility (Chandler et al., 2004; Hyngstrom et al., 1994), and mortality (Forman & Alexander, 1998; Lepczyk et al., 2004) in urban environments (Ditchkoff et al., 2006; Sol et al., 2013). A literature review conducted in 2020 described adaptive mammalian responses to urbanization, finding that 93% of the studied behaviors of mammals in urban environments differed from those in rural environments (Ritzel & Gallo).

Species can either adapt to the changing environment or disperse to find a new habitat altogether. There are several reasons why some species seemingly thrive in urban areas, one being behavioral flexibility or plasticity (Caspi et al., 2022; Gross et al., 2010; Sol et al., 2013). Plasticity in response to an urban setting has been observed mainly in avian groups (Slabbekoorn & Peet, 2003). However, reptiles may be particularly at risk to UHI effect and urbanization, given that they are primarily ectotherms. Behavioral studies between populations of reptiles are essential because behavior is an important mechanism by which urban animals can escape an unsuitable environment. Past studies on reptile behavior in urban areas have yielded mixed results, with some species acclimating to urban conditions, such as garden skinks (*Lampropholis guichenoti)* in Sydney, Australia (Prosser et al., 2006), whereas others, like Puerto Rican crested anoles (*Anolis cristatellus*), have higher embryonic mortality rates due to UHI effects (Hall & Warner, 2018). However, because one urban environment is different from the next, urban effects can vary considerably. Urban reptiles tend to have larger body sizes, but show no consistent patterns in boldness, exploratory behaviors, or immunity (Putman & Tippie, 2020). This variability in responses (French et al. 2018), underscores the need for comprehensive research that considers behavioral and morphological responses to the multi-faceted challenges of urban environments.

Side-blotched lizards are an ideal model in which to investigate the impacts of urbanization on morphology, condition, and behavior because there are established populations living in both urban and rural environments. By comparing body condition, we may be able to determine whether urban lizards are in better or worse physiological condition than their rural counterparts. Body condition is determined by the residuals of the linear regression between SVL (mm) and mass (g), with lizards in “better” condition falling above the line of isometry. Observing each population’s behaviors may reveal changes in response to urbanization, among other factors. Ultimately, these factors have the capacity to alter population fitness.

Physiological differences have been reported in this species (Hudson, 2023), with females having higher ectoparasite loads and a higher proportion of unfertilized eggs in urban environments (Virgin et al., 2023). Additionally, side-blotched lizards exhibited lower immunity and elevated corticosterone and oxidative stress levels in response to a stressor (Lucas & French, 2012). Behavioral changes have also been recorded, with side-blotched lizards inhabiting wind turbine farms being less wary of predators than those at corresponding reference sites (Keehn & Feldman, 2018), although the links between behavior and morphology of these lizards are relatively unstudied.

Here, we tested the effects of urbanization on behavior, morphology, and body condition in different populations of side-blotched lizards inhabiting urban and rural areas around St. George, Utah, USA. We hypothesized that **(1)** significant behavioral and morphological differences exist between rural and urban lizards; **(2)** within the site types, perch environment and proximity to a disturbance affect behavior and morphology; and **(3)** behavior and morphology are linked. More specifically, we predicted that **(1)** urban lizards will show more aggressive behaviors; **(2)** lizards closer to disturbances, regardless of site type, will perform more aggressive and fewer thermoregulatory behaviors; **(3)** body condition will be directly linked to aggressive behaviors; and **(4)** lizards with actively regrowing tails will perform fewer aggressive behaviors.

Because aggressive behaviors are linked to reproduction (Ferguson, 1971), analyzing the relationship between aggressive and thermoregulatory behaviors may provide an explanation for why populations in urban areas are stable. We also recorded tail status, mass, and snout-vent length (SVL), which may later inform whether urban lizards are in better or worse condition than their rural counterparts. By collecting metrics such as air temperature, substrate type, and average basking time per minute, we can make inferences not only across different site types but also according to circadian and temperature changes within those environments. Comparing the metrics of each population will indicate whether animals are responding behaviorally to urbanization and whether site differences are linked to habitat features, temperature, or some other factor.

Methods

Species and study area

In April 2021, 40 free-living male side-blotched lizards (snout-vent length (SVL) = 47.29 mm ± 0.40) were sampled from four different sites across the eastern half of Washington County, Utah (IACUC 2529). All sites are located along drainages of the Virgin River. Two sites, Man-O-War (MOW) and Sullivan Park (SP), are characterized as urban due to multiple anthropogenic stressors being present at each site (MOW: Average temperature = 25.6°C, Location = 37.05232°N, 113.60265°W; SP: Average temperature = 25.8 °C, Location = 37.12028°N, 113.49908°W) (Durso et al., 2020). Both sites are close to each other and within X km to downtown St. George, where urban development is ongoing. The two rural sites, Browse (BROW) and Leeds (LEEDS), are located around X km from St. George and experience minimal anthropogenic disturbance (BROW: Average temperature = 24.5°C, Location = 37.33097°N, 113.28053°W; LEEDS: Average temperature = 20.0°C, Location = 37.26009°N, 113.36965°W).

MOW, named after the road which intersects the site, is directly adjacent to a walking and biking path, the driving range of a golf course, an apartment complex, and a sports complex. Traffic noise is consistent throughout the day but minimal after sunset. MOW extends further into an area with more natural terrain, which includes the Virgin River and red-rock formations. The same walking path intersects side-blotched lizard habitat, and therefore both areas are considered the same site. Additionally, lizards located on this edge of MOW are exposed to Interstate 15, which is an added disturbance. At MOW, lizards commonly hid under retaining rocks and small shrubs that lined the walking path. SP is surrounded by multiple soccer fields, apartment complexes, and biking paths. Soccer tournaments are very common during the spring and summer months, attracting hundreds of spectators, which lead to multiple, direct human interactions. A soccer tournament was held on each day of observation and collection at SP. At SP, lizards are found among artificial landscaping boulders around the soccer fields and on free-standing rocks and dirt expanses along the walking path. Data was collected as far along the path as lizards were found.

BROW and LEEDS experience minimal anthropogenic disturbances. BROW is adjacent to a small, paved road with scarce traffic, only used by utility and farming vehicles. An ephemeral riverbed containing large boulders and overhanging trees creates a secluded habitat for lizards, only accessible by scrambling and hiking in and around the riverbed. LEEDS is intersected by a single gravel road that only sees ATV and occasional RV traffic. Unlike BROW, LEEDS has more open dirt expanses and some rocky outcrops. There are more cacti and scattered desert shrubs at LEEDS than at BROW, which hosts more trees and grasses.

In-situ behavioral observation

Side-blotched lizards were characterized by color pattern and known sexually dimorphic traits. Only males were included in this study, due to the possibility that reproductive hormone shifts might affect female behaviors (Adkins & Schlesinger, 1979; Sinervo & Miles, 2011). Once identified, the observer voice recorded in-situ behaviors (**Table 1**) and any disturbances for five minutes using the “Voice Memos” application on an Apple iPhone 12. Only voice recordings were conducted, no video data was collected. Solitary, aggressive, and thermoregulatory behaviors were captured in the recordings, all of which were either categorized as states or events. States, like basking or null, described stints of behavioral conditions. Events, such as a push-up display or pursuit, described behaviors that occur at a single point in time. Pursuit was considered an event due to difficulties determining precisely when a pursuit concluded. Head bobs were included under push-ups, due to difficulty differentiating between the two behaviors. The main component of the aggressive behavior category were push-ups, as very few chases and pursuits were observed. Human disturbances that may have affected lizard behavior during the recording were also noted, such as the number of pedestrians who ran or biked past the observation area, but those disturbances were not included in the analyses.

The same observer (LOS) recorded all individuals in the study over the 10 days of sampling, to maintain consistent descriptions of behavior. LOS maintained a minimum distance of two meters (Zhao et al., 2022) from the individual to ensure behaviors recorded were naturally occurring, and not due to observer disturbance. If the focal individual appeared to become alarmed by the observer’s presence at any time before or during the recording, the session was concluded. If visibility of the individual was lost during the observation period, the observer changed locations to continue the analysis.

Physiological and environmental data collection

Once the behavioral observation was complete, each focal lizard was caught using a snaring pole. Internal body temperature was then taken via cloacal insertion of a Type K Thermocouple (REED Instruments TP-01). Each lizard was then placed in a separate, opaque, breathable bag for safe transportation to the laboratory.

After capture, broad environmental and perch-specific measurements were recorded, including site type, air temperature, and presence or absence of nearby disturbances. The three possible disturbance types found at the sites were walkways, highways, or roads. Perch substrate-related variables included surface temperature, type (rock, dirt, or gravel), and size (width, height, and length, all in cm). Distance from the nearest disturbance, noted earlier, was recorded either by measuring wheel (CST/berger 200ft. Pro Series Nylon-Clad Steel Tape) or rangefinder (Nikon PROSTAFF S). The rangefinder was used when the distance to the nearest disturbance exceeded the range of the measuring wheel or if the distance could not be accurately taken with the wheel. Finally, a count of other side-blotched lizards (male or female) and other lizard species (*Sceloporus occidentalis, S. magister,* etc.*)* was recorded, if such lizards were present during the behavioral observation.

Morphological data collection

Once in the lab, individuals were removed from their bags to collect morphometric data. Mass (g) to the nearest 0.01 g was taken by placing the lizard in a small cup and weighing on a scale (OHAUS HH320). Snout-vent-length (SVL) and side-blotch length and width was measured to the nearest 0.01mm with a pair of calipers (SPI Dial Caliper). Recorded indicators of body condition measurements included: **(1)** tail status - categorized as intact (never autotomized or lost due to unknown reasons), re-growing (still re-growing after tail loss), or regrown (fully regrown after tail loss); **(2)** injuries - presence or absence of bites or open wounds; **(3)** scarring – type, presence or absence, and location; and **(4)** ectoparasite load – count, location on body, and type of mites or ticks. Finally, throat color was recorded and categorized as yy, yo, yb, oo, oy, ob, bb, by, or bo, where y = yellow, o = orange, and b = blue (Sinervo & Lively, 1996). All body condition and throat color data were determined via visual inspection. Once processed, lizards were held overnight in individual plastic containers with a paper towel at the bottom and water in a shallow dish. They were then released to their initially spotted location the subsequent day.

In-situ behavioral analysis

Audio recordings of behavior were played back and converted to numerical data via the program JWatcher, Version 1.0 (Blumstein et al., 2006). By using key-binds to link a specific keyboard key to a single behavior, JWatcher calculates total time spent in each state or the number of occurrences for each event. The sums and counts calculated by JWatcher were then used in later statistical analyses.

Statistical analyses

All analyses and plots were performed in RStudio version 4.3.0 (R Core Team, 2023) with use of installed packages 'tidyverse' (Wickham et al., 2019), 'pwr' (Champely, 2020), 'rstatix' (Kassambara, 2023b), 'ggpubr' (Kassambara, 2023a), and 'rcompanion' (Mangiafico, 2023). The statistical test used in each comparison was chosen by interpreting both diagnostic plots and the results of a Shapiro-Wilks Test. In cases where data were not normally distributed, we used nonparametric tests. Each two-sample, unpaired T-test used Cohen's d to calculate effect size. All analyses used a significance level of p = 0.05.

Broad environmental comparisons

A two-sample, unpaired T-test was run to determine site type (urban versus rural) differences in air temperature. Cloacal temperature was related to site type using a Wilcoxen Rank Sum Test. Pearsons' correlations were used to analyze the relationships between different temperature types (site, substrate, and cloacal).

Behavior, morphology, perch, and site type comparisons

Three behavioral categories were created from the many behaviors recorded: solitary, aggressive, and thermoregulatory. The solitary behavior unit included the number of movements a lizard made during the five-minute audio recording. The aggressive unit combined the number of chases, pursuits, and push-ups. The thermoregulatory unit included seconds spent basking.

Body condition was determined by the residuals of the linear regression between SVL (mm) and mass (g), a calculation frequently used in many herpetological studies (Peig & Green, 2009). Positive values represent good condition, where residual values are above the line of isometry. Negative values represent bad condition, where residual values are below the line of isometry. A priori testing revealed body condition did not differ by site type. Therefore, it was not included as a random effect in further analyses.

Tail status was considered a feature of morphology due to the nature of tail autotomy or loss to a predator. The external morphology of the regenerated tail may not differ from the original tail, but the internal morphology has calcified cartilaginous tubes instead of vertebrae (Bateman & Fleming, 2009; Bellairs & Bryant, 1985). Tail status types - intact, regrowing, and regrown - were condensed into two specific groups: “Actively regrowing” (regrowing) and “not actively regrowing” (intact and regrown). Splitting the status types in this way distinguishes which lizards that may have been prioritizing behaviors or choosing perches that promote tail growth. Tests run with an ANOVA of the three distinct tail status categories yielded similar results as those conducted using Wilcoxen Rank Sum Tests.

Throat color types were categorized into groups based on the color most lateral on the throat: “Yellow” (yy, yo, yb), “Orange” (oo, oy, ob), or “Blue” (bb, by, or bo). After grouping by lateral color, there were 20 yellow individuals, 19 orange individuals, and one blue individual. Because there was only one blue individual in the dataset, it was removed and a Wilcoxen Rank Sum Test was used.

Comparisons between **(1)** behavioral categories, **(2)** body condition, or **(3)** temperature variables by **(1)** site type, **(2)** tail status, or **(3)** throat color were conducted, either by Wilcoxen Rank Sum Tests or two-sample, unpaired T-tests. Analyses between the categorical variables - site type, throat color, and tail status - were conducted either by Chi-square or Fisher's Exact tests.

Statistical analyses between **(1)** behavior categories, **(2)** temperature variables, **(3)** body condition, or **(4)** overall behavior unit by **(1)** distance to nearest disturbance (m), **(2)** perch height (m), **(3)** temperature variables, or **(4)** body condition was conducted using Spearman or Pearson correlations. Distance to disturbance was not separated by disturbance type (walkway, roadway, or no nearby disturbance), due to the unequal number of observations of each type.

Certain behaviors were looked at individually to make specific comparisons. For example, number of chases and pursuits were removed from aggressive behaviors to isolate the number of push-ups. These unique cases were also calculated by Spearman or Pearson correlations, or by simple linear regressions.

Results

Environment

Urban sites (mean = 25.2 °C ± 3.58) were significantly hotter than rural sites (mean = 22.3 °C ± 4.54; **Figure 1**; t(38) = -2.27, effect: -0.717, p = 0.029). Cloacal temperature was positively correlated with both air (**Figure 2a**; r(38) = 0.56, p = <0.001) and substrate (**Figure 2b**; r(38) = 0.35, p = 0.026) temperature, but there was no evidence to support a correlation between site and substrate temperature (r(38) = 0.27, p = 0.096).

Behavioral and morphological differences between sites

Neither behavior (Wilcoxen Rank Sum Test: **solitary**: z = -0.851, p = 0.41; **aggressive**: z = 1.01, p = 0.321; **thermoregulatory**: z = 0.216, p = 0.841), body condition (z = 0.667, p = 0.517), nor throat color (x2 (1, N = 39) = 0.235, p = 0.628) were significantly different between site types. The only difference between site types was in tail status (**Figure 3**; p = 0.020). At rural sites, 5% of lizards sampled had actively regrowing tails, compared to 40% at urban sites.

Within-site differences in behavior and morphology associated with perch and disturbance

At both site types, both lizard behavior (Spearman's r: **solitary**: r(38) = 0.001, p = 1; **aggressive**: r(38) = -0.041, p = 0.801), and morphology (Pearson's r: **body condition**: r(38) = 0.0035, p = 0.830); **throat color:** (Mann-Whitney: z = -0.347, p = 0.739) were not significantly correlated with distance from the nearest disturbance. However, there was a trend toward animals spending less time basking when closer to a disturbance (Spearman's r: thermoregulatory: r(38) = 0.3, p = 0.060). No behavior or morphometric variable was significantly correlated with perch height (all p-values > 0.2). However, there was a marginally significant relationship between perch height and both tail status (Mann-Whitney: z = -1.8, p = 0.0748) and thermoregulatory behavior (Spearman's r: r(38) = 0.28, p = 0.077). Lizards that spent more time basking were more likely to be found on taller perches, and those not actively regrowing tails may have preferred taller perches as well. Regardless of site type, there was a strong negative correlation between time spent basking and the number of push-ups performed (**Figure 4**;

Spearman's r: r(38) = -0.75, $p < 0.001$). Lizards that spent more time basking performed fewer push-ups in the five-minute focal behavior recording.

Linking behavior and morphology

Only one statistic significantly supported a link between morphology and behavior: individuals actively regrowing their tails performed more aggressive behaviors (Wilcoxen Rank Sum Test: $z = 2.13$, $p = 0.034$). The same result was seen when using the number of push-ups as compared to all aggressive behaviors (**Figure 5**: Wilcoxen Rank Sum Test: $z = 2.12$, $p = 0.036$). Nearly all other behavioral and morphological comparisons (**Table 2**) did not provide sufficient evidence to support this relationship (all p-values > 0.1). However, there was marginal support for a positive correlation between body condition and aggressive behaviors performed (Spearman's r: r(37) = 0.3, $p = 0.066$).

Discussion

Summary

We observed differences between urban and rural sites, and marginal relationships among perch and disturbance, related to morphology, condition, and behavior. As predicted, urban sites were hotter than rural sites, but there was no difference in body temperature by site type. Instead, body temperature was correlated with substrate and air temperatures. As site and substrate temperature increased, so did body temperature, suggesting lizards track closely with their perch. Urban and rural lizards only differed in tail status, where urban sites had more animals actively regrowing their tails compared to rural lizards.

We also found marginal support for a relationship between basking and distance to the nearest disturbance. Animals closer to a disturbance tended to spend less time basking. Additionally, there was a trend for lizards that were not actively regrowing tails to prefer taller perches, and those spending more time basking were also found more frequently on taller perches. Regardless of site type, perch environment, and distance to a disturbance, we saw a significant relationship between thermoregulatory behavior and push-ups; as time spent basking increased, the number of push-ups performed decreased.

Finally, lizards that were actively regrowing tails performed more aggressive behaviors, specifically push-ups, and body condition was marginally related to aggressive behaviors, which may suggest that performing more aggressive behaviors, including push-ups, is related to greater body condition.

Environment

As predicted, urban sites were hotter than rural sites, a result that corroborates extensive research on the UHI effect (Baker et al., 2002; Chow et al., 2012; Kim, 1992; Li et al., 2022; Luber & McGeehin, 2008; Tam et al., 2015). UHIs may have even more profound implications for ectotherms, as the environment is directly linked to body temperature (Johnson et al., 2019; Lettoof et al., 2020; Putman et al., 2019). As expected, lizard body temperature was correlated with air temperature.

However, urban lizards did not have significantly higher body temperatures than rural lizards. One might assume that urban lizards thermoregulated more efficiently than urban lizards, but we found no evidence that urban lizards basked for longer periods of time than their rural counterparts. It is plausible that urban habitats have been significantly altered, offering more shade structures or artificially created burrows. For example, MOW and SP had large boulders and trees artificially added to create an appealing landscape. This may allow urban lizards to remain active during the hottest days, whereas activity of rural lizards may be restricted to only a few hours of the day, as seen in Ackley et al. (2015) . Our results align with that study, as we found body temperature was correlated with both site and substrate temperature, yet substrate and air temperature were not correlated. This discrepancy could be due to the diverse composition of human-modified urban habitats, indicating that perch substrate temperature may play a more important role in determining lizard body temperature than air temperature, as seen in multiple studies on reptiles (Bauwens et al., 1999; Gaudenti et al., 2021; Gifford et al., 2012; Grover, 1996). Side-blotched lizards on Antelope Island, near Salt Lake City, Utah, were found to select sites that significantly differed from the average air temperature, using movement behaviors to thermoregulate (Goller et al., 2014). Variation in perch availability may also influence push-up display rate. Temperature and perch type were the best indicators of display rate in tree lizards (*Urosaurus ornatus*), further emphasizing the importance of different niche dimensions within a broader habitat (Goerge & Miles, 2022).

Behavioral and morphological site differences

Despite temperature and habitat differences among site types, we found no behavioral differences, contrary to our prediction. Our results contrast with many other studies that have documented anywhere from mild to severe changes in behavior in response to urbanization. For example, *Anolis cristatellus* had altered wariness behavior and locomotor constraints due to artificial surfaces commonly found in urban habitat (Avilés-Rodríguez & Kolbe, 2019). The absence of behavioral differences in our study could, again, be attributed to the diverse range of habitats present in urban sites. It is possible that perch use and availability were no more variable across sites than within, allowing urban side-blotched lizards to acclimate to their environment through more frequent perch shifts.

The wide variety of microhabitats within urban areas of St. George may offer survival advantages by providing more distinct “zones” in which the lizards can occur, further contributing to their behavior and body condition. For example, injured lizards may establish their territory in hotter microhabitats to prioritize healing, which has been shown to benefit from warmer temperatures in lizards (Hudson et al., 2021) and garter snakes (*Thamnophis sirtalis*) (Smith et al., 1988). This may require a greater variety of ecological niches that can be used for a variety of purposes within urban sites, as in *Zootoca vivipara*, in which perch selection varied with sex, parity mode, and coloration (Recknagel et al., 2023). This highlights the importance of diverse perch conditions within a single species.

Interestingly, the only difference between sites in morphology or condition was tail status. At rural sites, there were fewer lizards actively regrowing tails. This may be due to fewer encounters with predators or with con-/hetero- specifics in an individual's immediate perch. Although urban lizards are more likely to have actively regrowing tails, the warmer environment may aid in healing, counteracting some of the costs of tail loss and regrowth (Fox et al., 1990; Hudson, 2023; Wilson, 1992). As noted, previous research shows that warm environments aid in immune performance, and therefore we predicted that animals in worse condition would prefer warmer perches. Although perch substrate temperature was positively correlated with cloacal temperature, it was not significantly related to tail regrowth, body condition, nor any other variable recorded.

Finally, it is important to consider an alternative hypothesis regarding length of time since development. Because the lizards have short generation times and high replacement rates (Tinkle, 1965), especially in urban areas (Lucas & French, 2012), we may have caught and observed lizards who had potentially evolved, or adapted, to the environment already. We saw little variation in behavior within urban sites, which may point to an adapted population, considering the above-state average population growth of St. George for nearly the past 50 years (Pacific Northwest Regional Economic Analysis Project (PNREAP), 2023). If the population had not adapted, we may have seen more variation within urban sites.

Within-site perch and disturbance differences in behavior and morphology

Perch height did not have a significant effect on any variable measured, but two marginally significant ($p < 0.077$) trends appeared: lizards that spent more time basking were more likely to be found on taller perches, and those not actively regrowing tails may have preferred taller perches. Basking on taller rocks may aid in defense of home ranges, whereas lizards not actively regrowing their tails may prefer taller perches for their better vantage points. These trends may seem to contradict our earlier claim that lizards regrowing their tails would prefer warmer perches. However, because resting on the top of a rock may make a lizard more visible and susceptible to predator attacks (Vanhooydonck & Van Damme, 2003), lizards actively regrowing their tails may avoid these spots. There is currently mixed support for whether tail loss negatively impacts locomotor functions, such as sprint speed (McElroy & Bergmann, 2013), but it has been shown that in female side-blotched lizards at least, sprint speed and stride length are decreased by tail loss (Anderson et al., 2012). A reduction in sprint speed may make fleeing from the tops of rocks more difficult for lizards with no tail, leading them to remain in lower spaces.

We anticipated that perch features and disturbance would influence lizard behavior. While we initially predicted that lizards closer to a disturbance, regardless of site type, would behave more aggressively, we found no significant differences between aggressive and solitary behaviors, nor in throat color, related to distance from the nearest disturbance. Past research investigating the relationship between behavior and disturbances in urban environments has yielded mixed results, where most studies found that behavior was altered by disturbances (Pittfield & Burger, 2017; Rytwinski & Fahrig, 2012; Smit et al., 2022; Williams et al., 2020), whereas in others, such as ours, it was not. The varied results could shed light on behavioral plasticity in urban environments, or they may simply different demeanors of animals that thrive in urban environments. Lowry et al. (2013) note that both plasticity and demeanor change in response to urban environments the animal kingdom, especially in birds (Da Silva et al., 2014; Di Giovanni et al., 2022; Miranda et al., 2013). Side-blotched lizards have already been shown to adapt physiologically to urban environments and to show population-level genetic differences between urban and rural populations (Hudson, 2023), so this species' adaptative capability may be due, in part, to behavioral plasticity.

We predicted that animals closer to disturbances would spend less time thermoregulating, and we found marginal evidence to support this prediction. The observed reduction in thermoregulatory behaviors near disturbances may be related to an increase in various environmental pressures near disturbances. First, perceived threats near the disturbances, such as bikers on walking paths and noise near the interstate, may prompt lizards to bask less to prioritize awareness. By reducing basking behavior, lizards may prioritize awareness of the environment. Past research indicates that lizards adjust their push-up display rate in accordance to perceived threats in the area (Simon, 2007), supporting their ability to assess the surrounding environment and adjust their behaviors accordingly. Second, there is less protection from predators near the disturbances. For example, there are no rock formations on or immediately beside the walking path, offering no overhead or rock crevice protection. No lizard that was initially spotted on the walking path remained in the same spot throughout the behavioral recording, and most of the lizards found close to the walking path (within 3-5 feet) were either hiding under or perched atop a single, small rock (unpublished observation). Lastly, unlike natural rock formations, black asphalt walking paths offer no camouflage from predators. Increased conspicuousness has increased costs, as observed in collared lizards (*Crotaphytus collaris*) where model individuals with high contrast against the substrate were most frequently attacked by predators (Husak et al., 2006). It may be beneficial to spend less time performing thermoregulatory behaviors, and any other conspicuous behavior, near or on substrates that contrast with body color and pattern. This change in behavior could also help reduce tail loss, which was more prevalent in urban habitats.

Regardless of site type, lizards that spent more time basking performed fewer push-ups. This relationship could present a trade-off between metabolism and reproduction, as body temperature directly relates to metabolism in ectotherms, and pushups are key to defending territory and attracting mates (Ferguson, 1971; Martins, 1991; Ventura et al., 2023). This relationship was also observed in a separate study by Sermersheim et al. (in preparation). This idea complements the findings of other physiological studies conducted on the same population of lizards in St. George. Side-blotched lizards presented with an immune challenge altered their energetic strategy by downregulating their metabolism (Smith et al., 2017). Spending less time basking, as suggested in our study, could facilitate this reduction in metabolism and correlate with more push-ups performed, potentially increasing investment in reproduction. Indeed, Lucas and French (2012) found that urban side-blotched lizards had a higher reproductive investment than their rural counterparts, in addition to lower immunity and elevated corticosterone and oxidative stress levels in response to a stressor. These results could point to a life history shift in the urban populations of side-blotched lizards in St. George (Smith et al., 2017). Prioritizing reproduction over self-maintenance could be one way the urban populations maintain a stable population, despite having shorter lifespans than rural populations (Lucas & French, 2012).

Behavior and morphology are linked

We found marginal support for relationships among morphology and behavior, particularly tail status. Contrary to our prediction, lizards actively regrowing their tails performed more push-ups, regardless of site type. This result was unexpected, as we predicted that there would be a trade-off between aggressive and thermoregulatory behaviors. There are several possible explanations for why we observed the opposite relationship. First, the behavioral phenotype of some lizards could simply be more aggressive than others, and those individuals may be more likely to encounter and engage with con- and hetero-specifics, as well as being bolder. Flight initiation distance (FID) is a common metric used to measure wariness of individuals between populations, and past research on differences in FID among lizards has provided mixed results. In some species, greater FIDs were recorded in modified habitats (Prosser et al., 2006), whereas in others, shorter FIDs were recorded (Batabyal et al., 2017; Keehn & Feldman, 2018). Second, lizards regrowing their tails may perform more push-ups to compensate for their lack of a tail, both to decrease the chance of predation (make the predator assume its tail is intact), and to increase the chance of mating. For example, male side-blotched lizards without tails face multiple negative consequences, such as increased mortality (Hudson, 2023; Wilson, 1992) and lowered social status (Fox et al., 1990). This suggests that an increase in aggressive behaviors, regardless of environment, may help promote survival and ultimately fitness.

Finally, there was a trend for individuals in better body condition to perform more aggressive behaviors. This result may seem contradictory when considering the earlier finding that lizards regrowing tails performed more aggressive behaviors, and the disadvantage that such lizards face in predatory and reproductive situations (Fox et al., 1990; Wilson, 1992). We found no difference in body condition among urban and rural lizards, and therefore tail loss may not necessarily be detrimental to condition if behavioral adaptation can counteract its negative effects. Regardless of tail status, if an individual is performing more aggressive behaviors, they are more likely to maintain an appropriate metabolic rate and have success in foraging. More research is needed to determine whether tail loss necessarily implies "worse" body condition in these populations.

Conclusion

Our study had three main results. First, we found evidence for the presence of the UHI effect in St. George, UT, as urban sites were hotter than rural sites. Second, although temperature differences were recorded, there were minimal behavioral differences found between sites, yet a strong trade-off between thermoregulatory behaviors and aggressive behaviors regardless of site type arose. Third, tail status repeatedly surfaced as an important factor impacting aggressive behaviors, linking behavior and morphology. These results shed light on our understanding of the responses of reptiles to urbanization, by emphasizing the adaptability of lizards to diverse habitats.

Our results highlight the importance of including perch conditions in behavioral and morphological assessments of responses to urban environments. Perch use varied by tail status and thermoregulatory duration in our study, but it has been found also to vary by age, sex, parity mode, body condition, and coloration. Picking apart a species' perch, rather than their broad environment, may lead to better predictions of how their life history traits may shift in future, hotter climates. Different perches may elicit behavioral shifts, which may have the potential to counteract the impacts of UHI effect and urbanization. This perspective is especially important when creating effective conservation strategies, as considering the perch may be just as important as considering the overall environment.

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Tables and Figures

Table 1

Description of Side-Blotched Lizard Behaviors Recorded During the Five-Minute Observation Period

Type of Behavior	Behavior	Code	Description of Behavior
Solitary	Move	M	SBL moves to a different location – either shade, rock, or dirt (event)
Aggressive	Chase	CH	SBL rapidly follows or darts at conspecifics or heterospecifics (state)
	Pursuit	P	SBL actively pursued or followed another conspecific or heterospecifics (event)
	Push-up	PU	SBL raises upper-body up and down (event)
Thermoregulatory	Basking	BA	SBL flattens body and remains in sunlight (state)

Note. Code represents the key-bind nomenclature later used in JWatcher for audio analysis.

Table 2

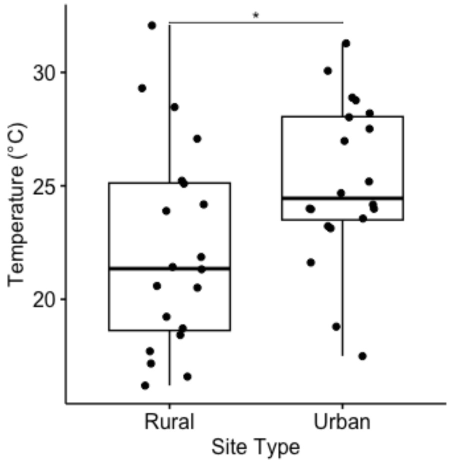
Statistical Analyses of Behavior and Morphology

Behavior	Throat color (Orange vs. yellow)	Condition (mass~SVL)	Tail status (Actively regrowing vs. not)
	(Wilcoxen Rank Sum Test – Mann-Whitney)	(Spearman’s r)	(Wilcoxen Rank Sum Test – Mann-Whitney)
Aggressive (push-ups, pursuits, and chases)	p = 1 z = 0	p = 0.0663 r(37) = 0.3	**p = 0.034** z = 2.13
Solitary (movements)	p = 0.474 z = 0.735	p = 0.304 r(38) = 0.17	p = 0.317 z = 1.02
Thermoregulatory (time spent basking)	p = 0.749 z = 0.337	p = 0.593 r(38) = -0.087	p = 0.114 z = -1.6

Note. Only one statistic supports the link between the two: individuals actively regrowing their tails performed more aggressive behaviors. (z = 2.13, p = 0.034).

Figure 1

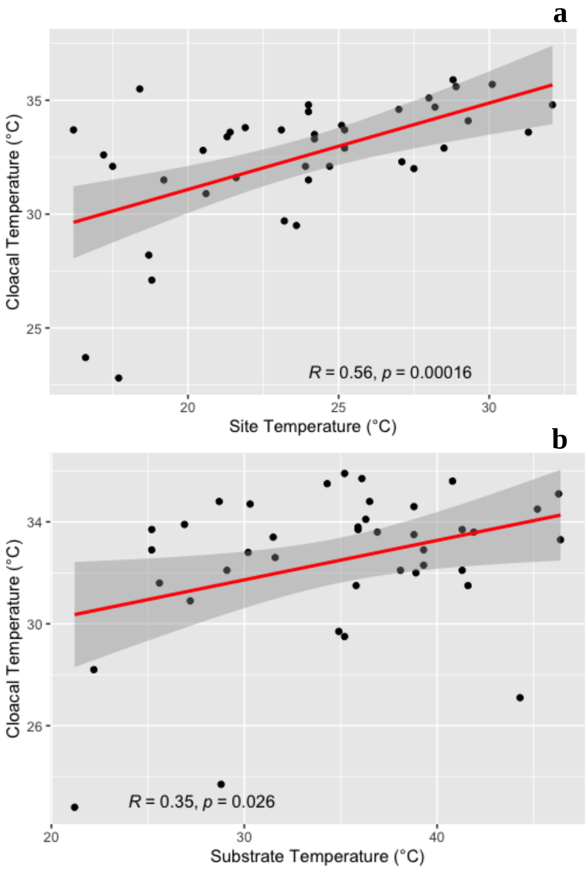
Urban and Rural Sites Have Significantly Different Air Temperatures



Note. (t(38) = -2.27, effect: -0.717, p = 0.029).

Figure 2

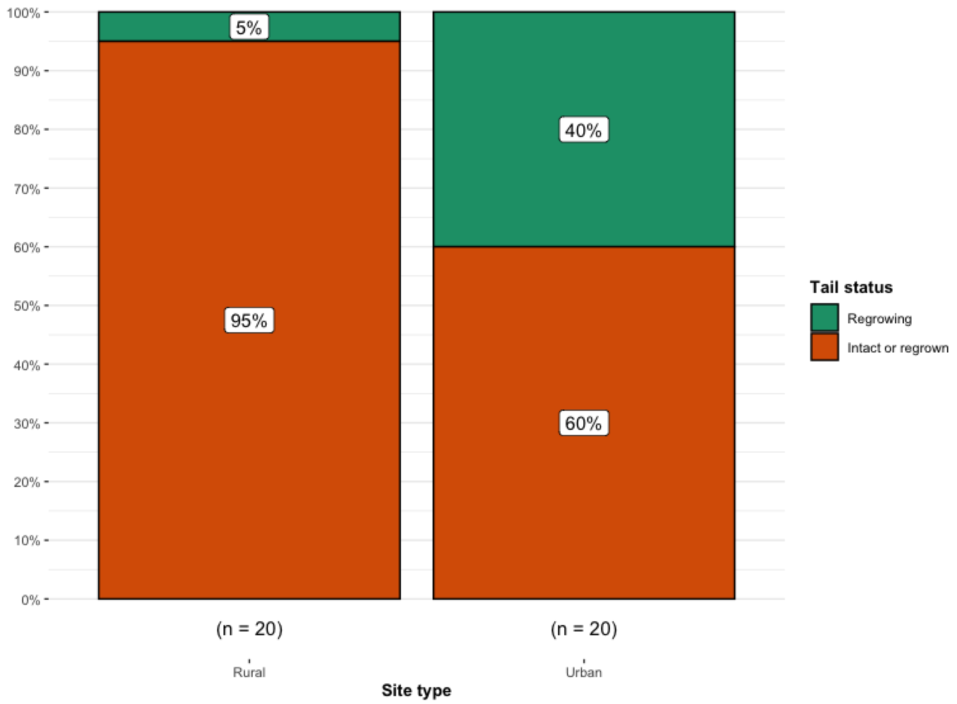
Cloacal Temperature Comparisons



Note. 2a) Cloacal temperature is positively correlated with air (site) temperature (r(38) = 0.56, p = <0.001). 2b) Cloacal temperature is positively correlated with substrate temperature r(38) = 0.35, p = 0.026).

Figure 3

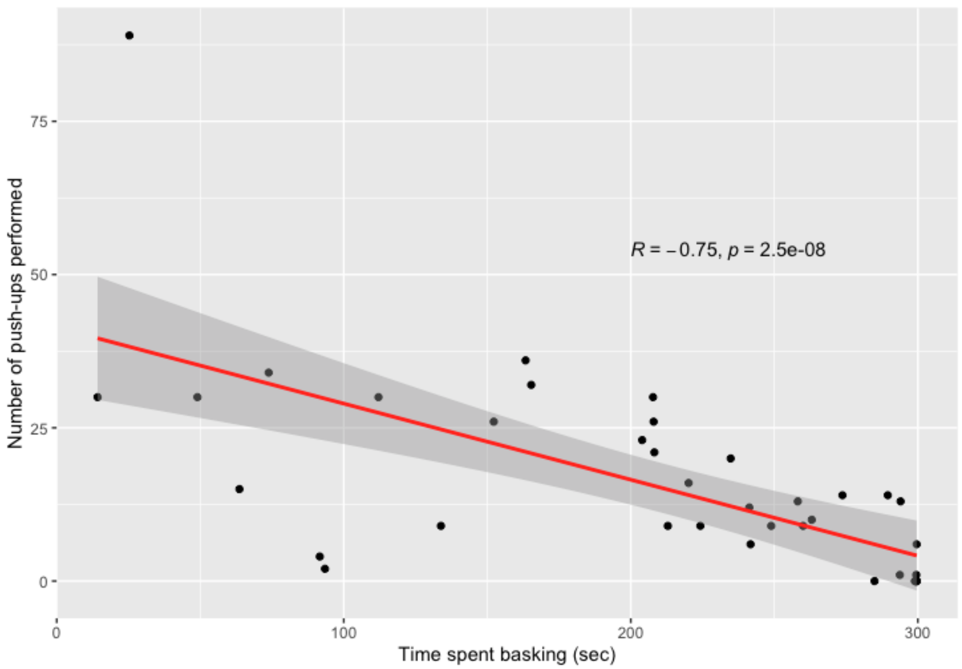
Site type by Tail Status



Note. Fisher's exact test (p = 0.02).

Figure 4

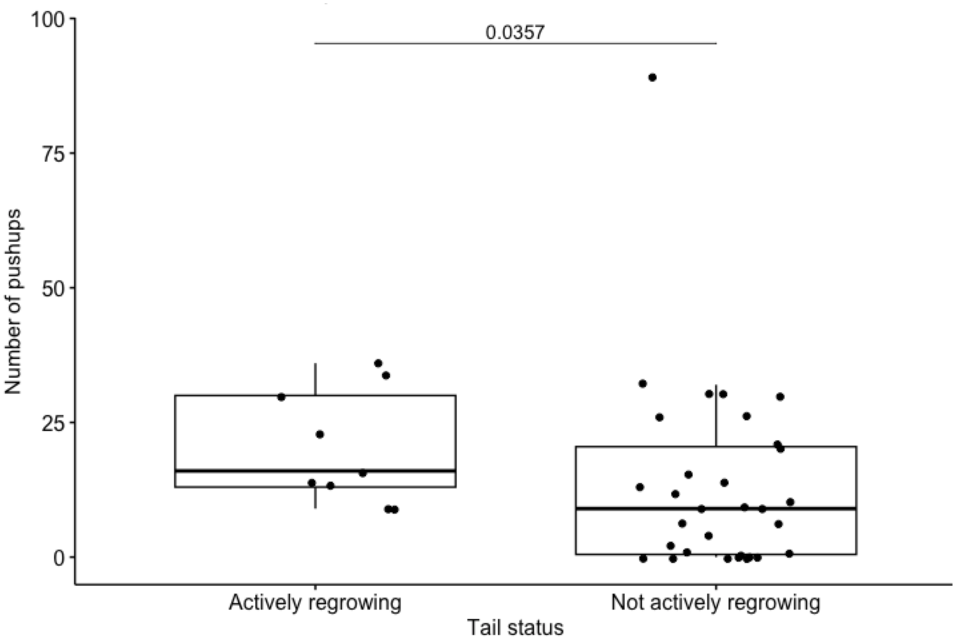
Time Spent Basking by Number of Pushups Performed



Note. Statistic was run with Spearman's r correlation due to the outlier and the variables not being normally distributed. When the outlier was removed, the same results were present (r(38) = -0.75, p = 2.50e-08).

Figure 5

Tail Status by Number of Pushups Performed



Note. (z = 2.12, p = 0.036). The same result is seen when comparing tail status and all aggressive behaviors (push-ups, pursuits, and chases). The results are presented with the outlier included to show natural variation, but a similar result was achieved when it was removed (p < 0.05).

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CHAPTER 3

BEHAVIOR, STRESS AND METABOLISM OF A PARTHENOGENIC LIZARD IN RESPONSE TO FLYOVER NOISE

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Abstract

The Colorado checkered whiptail (*Aspidoscelis neotesselatus*) is a parthenogenetic lizard that is listed as a “species of special concern” in the state of Colorado. *A. neotesselatus* occupies a small range that includes the US Army Fort Carson Military Base in Colorado Springs, Colorado. The species is exposed to a variety of military disturbances, including aircraft flyover noise. We sampled 82 females during the 2021 reproductive season to assess whether scheduled flyovers would impact the behavior, stress, and metabolism of *A. neotesselatus*, while controlling for size and reproductive stage differences. We measured corticosterone (CORT) as a marker of anthropogenically induced stress during flyovers compared to a control. We further tested for the downstream effects of flyovers on plasma glucose (free energy available to tissues), elevated metabolism with oxidative stress (ROMs), and ketone bodies (alternative cerebral energy substrates to glucose). When disturbed by flyovers, these lizards spent less time moving but more time eating. Aircraft noise also increased CORT when controlling for clutch size, indicating a stress response driven by flyovers, as well as an independent effect of reproductive investment on CORT. CORT did not affect plasma glucose. Flyovers led to a marginally decrease in circulating ROMs, with gravid females experiencing lower plasma ROMs than non-gravid females, but that later effect was independent of flyovers. Flyovers significantly increased ketone bodies, with smaller animals experiencing higher ketone concentrations than larger individuals, yet the effect of size on ketone bodies was independent of the flyover treatment. Although *A. neotesselatus* seem to adjust their behavior and eat more to buffer the potentially negative effect of flyovers on energetic pathways, they still appear to suffer a metabolic cost driven by the stress response via ketone accumulation, as well as a reproductive cost driven by clutch size investment that is independent of flyover disturbance. We suggest that military aircraft operators attempt to avoid dense populations of *A. neotesselatus* during the reproductive season or fly at altitudes that lead to decibel reads that fall below 50 dB at ground level, as a cautious management step that ensures the resilience and local abundance of *A. neotesselatus* at Fort Carson. **Keywords:** conservation, hormones, flyovers, glucose, metabolism, noise pollution, physiology, reproduction, whiptail.

Introduction

Reptiles are on the decline worldwide and vulnerable to many anthropogenic threats (Gibbons et al., 2000). Habitat loss and degradation introduced invasive species, environmental pollution, disease and parasitism, unsustainable land use, and global climate change all have profound, detrimental impacts on reptile populations (Gibbons et al., 2000). Human factors have greatly increased the need for long-term monitoring and conservation efforts aimed at improving the trajectories of native reptile populations (Tan et al., 2023; Todd et al., 2010). Most of these threats are obvious in their impacts on reptile population viability; habitat loss, including fragmentation and land use conversion, is thought to be the leading cause of decline for reptile populations (Amburgey et al., 2021; Sala et al., 2000). Habitat loss can impact reptiles directly through mortality events, or indirectly by limiting their ability to meet survival and reproductive ends (e.g. Todd & Andrews, 2008). Shifts in niche space driven by climate change are predicted to be a further detriment to habitat suitability by shifting physiology, life history traits, and population distribution. This may ultimately lead to extinctions directly related to increasing temperature and decreasing precipitation (e.g. Dunham & Overall, 1994).

There are additional threats that have received less attention, likely due to how overlooked anthropogenic disturbance may be to human perception. However, “minor” disturbances may have dramatic impacts on wildlife. Light and noise pollution are amongst the fastest growing, but least understood, anthropogenic stressors on the planet (Dominoni et al., 2020; Buxton et al., 2017; Kyba et al., 2017), altering sensory environments at a global scale. While anthropogenic light sources affect visual perception, noise reduces the ability to receive other acoustic signals and may cause distress. These sensory disturbances can, in turn, impact communication (e.g. Francis & Barber, 2013), movement (e.g.

Cabrera-Cruz et al., 2018), foraging (e.g. Bennie et al., 2015), energetic budgets (e.g. Touzot et al., 2019), predation risk (Francis & Barberm, 2013), and stress (e.g. Ouyang et al., 2018) within wild animal populations. Anthropogenic changes such as these are often telling of the pathways by which an individual organism can be negatively influenced. As sound and light pollution continues to expand over space and time, there is an urgent need to understand how such sensory pollutants affect physiology, life history traits, and fitness (Kernbach et al., 2021).

Anthropogenic noise is often louder and more frequent than natural sounds (Patricelli & Blickley, 2006; Popper & Hastings, 2009). Sound or noise, especially those that are intense and unpredictable, can negatively affect animals (e.g. Warren et al., 2006; Slabbekoorn and Ripmeester, 2008; Popper and Hastings, 2009; Barber et al., 2010; Slabbekoorn et al., 2010; Shannon et al., 2015), and lead to a stress response (Jakob-Hoff et al., 2019). The release of adrenal corticosteroids in response to a stressor is well-documented across vertebrates. Stimulation of the hypothalamus during a stressful event leads to the release of CORT from the adrenal gland. While the primary function of CORT is to mobilize energy for the central nervous system, it has also been found to be an accurate indicator of a systemic stress response. Anthropogenic noise can initiate a hormonal stress response by elevating circulating glucocorticoids, “GCs”, in birds (Hayward et al., 2011; Blickley et al., 2012b), mammals (Creel et al., 2002), amphibians (Tennessen et al., 2014; Kaiser et al., 2015) and fishes (Wysocki et al., 2006; Nichols et al., 2015; Mills et al., 2020), but not systematically (Crino et al., 2013; Angelier et al., 2016).

Anthropogenic noise may also lead to shifts in behaviors that trigger a stress response or prove maladaptive in other ways. In a study of the eastern blue-tongued lizard

(*Tiliqua scincoides*), individuals spent more time in a “freezing” behavior when exposed to high frequency mining machinery noise, a sign of chronic stress (Mancera et al., 2017). With more unconventional types of noise pollution, green, flatback and hawksbill turtles showed disturbance behaviors in response to drones flying at low elevations (20-30m) (Bevan et al., 2018). Traffic noise increased levels of a stress-relevant glucocorticoid in female wood frogs (*Lithobates sylvaticus*) and impaired female travel towards a male breeding chorus in the field (Tennessen et al., 2014). Behavioral responses to noise pollution often translate into stress responses, as loud noise increase cortisol levels in several species (Hall, 2010; for a review see Kight & Swaddle, 2011).

Noise-related stress can shift behaviors and activity budgets by increasing vigilance (i.e. more time spent retreating or hiding), which can ultimately result in less time spent foraging. Reduction in foraging activity and the potential for associated weight loss are predicted to increase metabolic processing and mobilization of cholesterol to fuel the production of stress hormones; when stressed, individuals rapidly use stored resources to regulate neuroendocrine responses to said stress (Kight and Swaddle, 2011). Anthropogenic noise disturbance increased serum glucose, cortisol, and lactate in European sea bass (Santulli et al., 1999) and oxygen consumption in shore crabs (Wale et al., 2013), suggesting that anthropogenic noise my increase metabolic rate by increasing both physical activity and energy consumption.

Noise-induced stress also appears to affect females disproportionately, most likely because of sex differences in size, hormone expression, and reproductive investment (Kight and Swaddle, 2011). In this study, we propose to quantify the impact of flyover noise pollution on the behavior, stress physiology and metabolism of the Colorado checkered whiptail, *Aspidoscelis neotesselatus*, a parthenogenic, all-female, whiptail species whose range mostly overlaps the Fort Carson military installation near Colorado Springs, Colorado, USA. The reduced genetic variation of this parthenogenetic species, made up entirely of female congeners, could increase susceptibility to environmental disturbances (Aubry et al., 2019; Aubry et al., 2020; Caracalas et al., 2021). To-date, the species has had multiple conservation listings, likely due to its small natural range. It is designated as near threatened by the International Union for Conservation of Nature (IUCN, 2007), deemed a species of special concern by Colorado Parks and Wildlife, and listed by the U.S. Army as a species at risk.

Fort Carson Military Base houses both military and air force units and their aircraft. This includes but is not limited to Apache, Chinook, and Blackhawk helicopters, transport aircraft, and F-16 fighter jets. *A. neotesselatus* on Fort Carson are exposed to regular aircraft flyovers within 20,000 feet above ground with a reported max decibel capability of 161dB produced during operation. This decibel range has been reported as harmful and/or agitating to humans, even at a substantial distance from the source (Nykaza et al., 2009). Mexican Spotted owls have also shown to initiate a significant response to noise disturbance as low as 68 decibels (Pater et al., 2010). We measured the behavior, stress, and metabolic response of *A. neotesselatus* by comparing a control versus flyover treatment, each a week in length, during a flyover experiment. We specifically measured: **(1)** activity budgets to document behavioral responses to flyovers; **(2)** the stress response in activating corticosterone release (‘CORT’); **(3)** the downstream effects of stress on glucose; **(4)** oxidative reactive oxygen metabolites (‘ROMs’) as an index of oxidative stress level (oxidant capacity); and **(5)** ketone bodies, which can increase when the brain demands extra glucose from the body in times of stress. We investigated the effect of flyovers on **(1-5)** while controlling for differences in size, weight, and reproductive state (**Figure 6**). We hypothesized that anthropogenic disturbance in the form of military aircraft flyover events would have significant behavioral and physiological impacts on the Colorado checkered whiptail population via behavioral responses and shifts in energetic expenditures related to the sensory stress driven by noise pollution.

Methods

Field collection

Data were collected from *A. neotesselatus* lizards living on the Fort Carson (FC) U.S. Army Installation located in unincorporated El Paso County, Colorado, near the city of Colorado Springs, USA (**Figure 7**). FC covers 55,000 hectares and extends into Pueblo and Fremont Counties. *A. neotesselatus* was sampled and surveyed in the northern edge of its range in FC. Lizards were surveyed and sampled in 2021 for the purpose of this specific study, which is part of a longer-term study that was initiated in 2018. The species is known to occur in several different training areas (TAs) (**Figure 7**), but we specifically surveyed TA 55 in coordination with military activities where our liaison biologist at FC scheduled aircraft flyovers on specific days over the study period, so that we could time our surveys with respect to flyovers.

In TA 55, the majority of *A. neotesselatus* were found in and along the banks of a dry creek bed. The dominating vegetation type was one-seeded Juniper (*Juniperus monosperma*) and mixed grassland, located around the edges of the sample area. The shrubs found included shadscale (*Atriplex confertifolia*), four-wing saltbush (*Atriplex canescens*), James' seaheath (*Frankenia jamesii*), and rubber rabbitbrush (*Chrysothamnus nauseosus*).

Flyover experimental design

Military aircraft flown over the field site included UH-60 Black Hawks, AH-64 Apaches, CH-47 Chinooks, and F-16 fighter jets. Given that helicopter range from the ground during flight is highly variable (up to 20,000 ft maximum), readings were taken on the ground using a data logging sound reader (ExTech, EN300 5-in-1 Environmental Meter) at the time that each lizard was captured. Ground noise was measured in decibels; the decibel scale is logarithmic with value increases representing significant increases in the perceived noise intensity. Sampling dates on which no flyovers occurred were 06/14/2021-06/20/2021. Sampling dates on which flyovers occurred were 06/23/2021-06/25/2021. On the non-flyover dates, ground decibel readings ranged from 30.1 to 55.8 decibels. During the flyover period, ground decibel readings ranged from 33.9 to 112.2 decibels. Because the experiment took place on a military base where each training area is access-restricted, there were no anthropogenic disturbances at the field location else than the presence of 3 to 4 field workers during the control, and the presence of field workers in addition to aircraft noise during the treatment.

Note that in order to minimize the influence of external environmental factors on our flyover experiment: **(1)** all observations were taken from the same location (**Figure 7**), which limits potential differences in habitat, predation rates, and resource availability; **(2)** the treatment was applied right after the control, which limits any potential life history differences since the study took place over a two-week period; **(3)** clutch size (i.e. ranges from 0 to 4) and reproductive stage (i.e. gravid versus not) differences were present across individuals, which is why we controlled for these effects in our models; and **(4)** since the same animal was never resampled either within or across treatments, all observations were independent from one another (i.e. no pseudoreplication). Microclimatic conditions averaged over sampling days were similar between the control (mean = 30.4 ºC, sd = 5.3 ºC) and the treatment (mean = 30.5ºC, sd = 5.1 ºC). We were limited in our ability to replicate our experiment, with only one control and one treatment.

Behavioral data collection

Behavior was recorded by observing individuals for three minutes prior to initiating capture (reference **Table 3a** for behavior-based sample size data). The observers were quiet across the sampling period and remained at minimum two meters away from the individual to ensure the behaviors recorded were naturally occurring, and not displayed in response to human presence. We ended the session if the individual was alarmed by the observers' presence at any time before or during the recording. We recorded audio descriptions of the ongoing behaviors on the "Voice Memos" app of an Apple iPhone 12 to instantly save the completed memo to a local database. We categorized In-situ behaviors into one of nine behaviors: basking, searching, eating, basking while eating, eating while searching, moving, moving while searching, null, and out of sight (reference **Table 4** for an explicit description of each behavior). We summarized behavior by playing back video recordings and noting the proportion of time each lizard spent in a specific behavior category. The out of sight behavior was not used in final analyses due to the lizard being out of the observer's sightline, therefore a behavior was not observed.

Animal captures and blood sample collection

We conducted transect surveys during the morning and early afternoon hours (0900-1300) to seek out and capture individuals. After collecting behavioral data, lizards were caught using a snare pole and kept in mesh fabric bags in the shade to later be processed. Coordinates were recorded for the exact location of each individual capture. Toeclip ID has been used at all FC sites and across all study years to track long term data for individual animals. For recaptures, the unique preexisting toeclip ID was recorded. Newly caught individuals were given a unique toeclip ID. Individuals from the 2021 season were marked with a unique number using a non-toxic paint pen; these highly visible, temporary cohort markings allowed us to avoid recapturing individuals and exposing them to repeated capture stress over the course of the study. Weight, tail length, tail diameter, and Snout vent length (SVL) were measured on the day of capture. Ultrasounds were performed to assess gravidity, clutch size, and follicular/egg size of adult females. Follicle is henceforth used as an inclusive term to span the range of reproductive development from the smallest measurable follicle, to the largest clearly defined egg, which ranged from 0.17 to 1.05 cm. Follicle size measurement, i.e. the length along the longest axis, was conducted with a SonoSite Turbo ultrasound unit with an external linear probe (SonoSite Turbo ultrasound, FUJIFILM SonoSite Inc., Bothell, Washington, USA), and each follicle in a clutch was our sampling unit. Once processed, lizards were released in the exact coordinate location of capture.

We collected blood from the retroorbital sinus of each captured lizard using a heparinized microhematocrit capillary tube (Fisherbrand, #22-362566). Blood samples were collected within the first 3 minutes of being pursued for capture, a validated window of time to measure baseline physiological activity in lizards (Romero and Reed, 2005). If capture attempts exceeded 3 minutes in duration, we did not collect blood samples from that individual lizard to limit the effect of a stress response elicited by the actual pursuit of the animal. After collection, blood samples were immediately stored on ice after centrifugation at 6000 RPM for 10 minutes to isolate plasma. We separated plasma from red blood cells by drawing it from the top of the sample with a Hamilton syringe (SigmaAldrich, #20888). Samples were frozen, transported to the laboratory on dry ice, and stored at −20 °C for assays. Samples were only taken during a standardized timeframe to avoid daily circadian differences in CORT released by stimuli (Maclean et al., 1973; Dallman et al., 1987; Jones & Gillham, 1988; Romero & Wingfield, 2001). We recorded site temperature at each capture, which averaged 30.48°C with a standard deviation of 4.05, thus quite consistently hot across the sampling period. The sampling period was between 0700-1200 hours and was found to have no relationship between circulating CORT and time of day (Hudson et al., 2020). Body temperature at the time of capture does not impact baseline plasma CORT levels either in field caught lizards (Racic et al., 2020). Plasma samples were analyzed to measure baseline levels of physiological activity as described below (Romero & Reed, 2005; Sheriff et al., 2011). Reference **Table 3b** for blood collection-based sample data.

Blood plasma analysis

To determine CORT concentrations, enzyme-linked immunosorbent assay

(ELISA) kits were validated and used with blood plasma (10 uL), following Hudson et al. (2020). This colorimetric ELISA is based on competitive binding between sheep polyclonal antibodies and plasma hormone that takes place on a donkey anti-sheep immunoglobulin (IgG) microtiter plate. We assayed each sample in duplicate across one 96-well plate according to manufacturer guidelines (Product #ADI-901-097; Enzo Life Sciences, Farmingdale, NY). Due to sample volume constraints, some samples were not run in duplicate. The intra-assay coefficient of variation (CV) was 9.2%.

Blood samples were collected within 3 min of capture, and blood glucose was immediately measured from the whole blood using a handheld glucometer (Accu-Chek Aviva Plus glucose monitoring system; Roche 182 Diagnostics, Indiana, USA). If there was an initial error in the glucose reading, then we re-tested a blood subsample to obtain a valid measure.

Circulating ROMs were quantified using an assay kit (MC435, Diacron International, Italy), which detects levels of hydroperoxides that oxidize an alkyl-substituted aromatic amine (A-NH2). Plasma was diluted in the assay buffer solution (5 µl : 100 µl). Samples were run in duplicate in a 96-well microplate following the manufacturer's instructions for “endpoint” mode with modifications for use (French et al., 2017). This reaction resulted in a color change that was measured with a spectrophotometer at 505 nm (xMark; Bio-Rad). Values were calculated as absorbance change relative to the standard. The inter-assay coefficient of variation (CV) was 5.38% and the intra-assay CV was 6.88 %.

Whole blood analysis

We tested whole blood concentrations of β-ketones by pipetting blood samples (1 uL) into test strips designed for a STAT-Site dual analyte measurement system (#7000-001, EKF Diagnostics, Boerne, Texas, USA). Results were automatically acquired within 10 seconds of use if concentrations ranged 0.1 to 8.0 mmol/L. If the meter yielded a calculation error, samples were re-pipetted into a new strip and tested for a valid measure unless out of range.

Statistical analyses

All analyses and visual representation of data were performed using default packages in RStudio (version 1.2.5001; R Core Team, 2019) and additional packages: ‘dplyr’ (Wickham, 2020) and ‘ggplot2’ (Wickham, 2016).

Impact of flyovers on behavior statistical analyses

We quantified the impact of flyover treatment on three behavioral categorizations: moving vs. not moving (i.e. “moving”, “moving while searching” vs. all other behaviors), eating vs. not eating (i.e. “eating”, “eating and searching”, "eating and basking” vs. all other behaviors), and active vs. inactive (i.e. “searching”, “eating while searching”, “moving”, “moving while searching” vs. all other behaviors). We then calculated the proportion of time each individual spent in any given behavior and averaged these proportions across individuals to test for significant differences in the proportion of time spent moving vs. not moving. We used a two-way ANOVA model to test for these differences using the ‘aov’ function in program R (Chambers et al., 1992).

Impact of flyovers on physiology statistical analyses

We first tested for preliminary effects of flyovers treatments on each measure of interest (i.e. CORT, glucose, ROMs, and ketones) using t-tests. We then used generalized linear models with a gaussian distribution and an identity link to model the relationship between flyover treatments (control vs. flyover) on dependent variables of interest (Bolker et al., 2009) while controlling for the effect of size 'svl', body mass, reproductive state (gravid versus not), and clutch size (which varied from 0 to 4 follicles), which are known predictors of physiology in this species (Aubry et al., 2019, 2020; Hudson et al., 2020; Caracalas et al., 2021). A set of a priori defined models competed within a model selection framework (Burnham & Anderson, 2002) where models were compared based on their respective AIC values (Akaike, 1973). The top-performing model within each set is further discussed in the result section.

For all models, we tested for normality of model residuals using Shapiro's test (Royston, 1982) and for homogeneity of variance using Levene's test (i.e., homoscedasticity, Hines & Hines, 2000). Note that ROMs were log-transformed, and ketone concentrations were square-rooted, to un-skew their distribution and normalize residuals. Model assumptions of normality of residuals and homoscedasticity were tested using appropriate statistics and are reported in the result section below. We adopted a significance level alpha of 0.05 for statistical significance in all analyses.

Results

Impact of flyovers on behavior

Although **Figure 8** indicated that animals, on average, spent more time moving when there were no flyovers, ANOVA test results indicated that there was no significant difference between the average time spent moving vs. remaining stationary (**Table 5**). The flyover treatment did not have a significant effect on the time spent in either group of behaviors (**Table 5**). Further, the interaction between behavior and treatment did not significantly impact the average proportion of time spent in either moving or stationary behaviors (**Table 5**).

Figure 9 indicated that animals spent more time basking (B), moving (M) or searching (S) for food than actively eating (E) when undisturbed. However, the pattern was reversed when flyovers occurred. While animals spent less time basking, moving or searching when flyovers occurred, focusing more on eating and eating while searching instead (**Figure 9**). There was a significant difference between the average time spent eating vs. all other behaviors (**Table 5**). Yet, the interaction between behavior and treatment did not significantly impact the average proportion of time spent eating vs. all other behaviors (**Table 5**).

Animals disturbed by flyovers spent less time moving or searching (**Figure 10**). They spent significantly less time in assessing vs. passive behaviors such as basking and eating (**Table 5**). The flyover treatment had a marginally significant effect on the time spent in either group of behaviors, with animals spending significantly less time assessing surroundings when flyovers occurred (**Table 5**). Yet, the interaction between behavior and treatment did not significantly impact the average proportion of time spent assessing vs. all other behaviors (**Table 5**).

Impact of flyovers on physiology

There was no significant relationship between noise disturbance and circulating CORT (*t-value* = -1.3476, *df* = 36.238, *p-value* = 0.1861). Mean concentrations of plasma CORT averaged 0.3452 ng/mL in the control, and 0.4479 ng/mL within the flyover treatment (**Figure 11**). Model selection results (**Table 6**) indicated that the best fitting model supported an effect of the flyover treatment on plasma CORT while correcting for clutch size. The effect of flyover treatment had significant and positive effect on CORT ($\beta_{treatment}$ = 0.3644, *std. error* = 0.1655, *p-value* = 0.0312), and so did clutch size, whereby an increase in clutch size was associated with increased CORT as well ($\beta_{clutchsize}$ = 0.1060, *std. error* = 0.0418, *p-value* = 0.0136), whilst the interaction between clutch size and treatment did no significantly affect CORT ($\beta_{clutchsize*treatment}$ = -0.0897, *std. error* = 0.0597, *p-value* = 0.1375).

There was no significant relationship between plasma glucose and noise disturbance during flyovers (Welch Two Sample t-test: *t-value* = -0.2664, *df* = 25.259, *p-value* = 0.7921), where mean concentrations of plasma glucose averaged 325.30770 mg/dL for the control and 328.1579 mg/dL for the flyover treatment (**Figure 12**). Further, model selection results (**Table 6**) indicated that the best fitting model did not support a relationship between flyovers and plasma glucose while correcting for size, mass, or reproductive investment ($\beta_{treatment}$ = 2.850, *std. error* = 9.228, *p-value* = 0.758).

Noise disturbance marginally impacted circulating ROMs (Welch Two Sample t-test: *t-value* = 1.8963, *df* = 45.975, *p-value* = 0.06422) where mean concentrations of plasma ROMs averaged 16.9616 mg/H2O2/dL within the control, and 11.6889 mg/H2O2/dL within the flyover treatment (**Figure 13**). Model selection results (**Table 6**) indicated that the best fitting model supported an effect of the flyover treatment on plasma ROMs while correcting for reproductive stage. The effects of flyover treatment had a significant effect on ROMs ($\beta_{treatment}$ = 2.8305, *std. error* = 0.4328, *p-value* < 0.001), while gravidity had a negative effect on ROMs when compared to non-gravid females ($\beta_{rep.stage}$ = -1.0622, *std. error* = 0.4007, *p-value* = 0.0098), but the interaction between reproductive stage and treatment was not significant in affecting ROMs ($\beta_{rep.stagel*treatment}$ = -0.4648, *std. error* = 0.6692, *p-value* = 0.4895).

Noise disturbance did correspond significantly with plasma ketones (Welch Two Sample t-test: *t-value* = -2.9504, *df* = 29.367, *p-value* = 0.0062), where mean concentrations of ketones averaged 4.6895 within the control, and 10.13043 within the flyover treatment (**Figure 14**). Model selection results (**Table 6**) indicated that the best fitting model supported a positive effect of the flyover treatment on ketones ($\beta_{treatment}$ = 6.9416, *std. error* = 2.5692, *p-value* = 0.0085) while correcting for size mattered as well, with smaller animals experiencing higher ketone concentrations (β_{slv} = -0.0645, *std. error* = 0.0256, *p-value* = 0.0138); yet, the interaction between size and treatment was not significant ($\beta_{svl*treatment}$ = 0.0142, *std. error* = 0.0411, *p-value* = 0.7313).

Discussion

Our results suggest that *A. neotesselatus* spent less time assessing their environment and more time eating when disturbed by flyovers. This was determined when lizards were observed feeding in a stationary position or traveling while carrying prey items. Specifically, our results suggest that animals, on average, spent significantly more time in passive behaviors such as basking (B), stationary eating (E), and eating while searching (ES) when disturbed. Aircraft noise increased CORT when clutch size was controlled for; but did not impact plasma glucose during the flyover treatment. Contrary to our predictions, noise disturbance marginally decreased circulating ROMs and was negatively correlated with CORT (t=-3.007, df=67, p-value=0.0037, Pearson's product-moment correlation cor=-0.344). Reproductive stage was important in explaining these differences as well, with gravid females experiencing lower plasma ROMs than non-gravid females; there were no significant interactions between noise and reproductive stage in affecting ROMs. Noise disturbance did significantly increase ketone production, and smaller animals experienced higher ketone concentrations, but the latter was independent of noise exposure despite model selection indicating that the interaction between size and noise was important in explaining variability in ketones.

Behavioral activity shifted in response to noise pollution, which has been observed in a variety of taxa, including reptiles (for review, see for e.g., Warren et al., 2006; Slabbekoorn & Ripmeester, 2008; Popper & Hastings, 2009; Barber et al., 2010; Slabbekoorn et al., 2010). The shift towards more elusive behavior and a lack of movement corroborates other studies which observed “freezing” behavior when exposed to noise disturbance (e.g., Warwick et al., 2013; Mancera et al., 2017). However, we did find animals did spend significantly more time eating while flyovers took place, suggesting an adjustment in their behavior that could potentially mitigate the negative impact of flyovers on their metabolism.

Both flyover disturbance and clutch size influenced plasma CORT, though their effects were independent of one another. Positive correlations between reproductive mass and glucocorticoid levels have been reported in common side-blotched lizards (Byron & Wingfield, 1992), yet some studies of reptiles do not show elevated CORT in the presence of chronic anthropogenic stress (Injaian et al., 2020; Malisch et al., 2020). Given that most of the studied anthropogenic sources of stress are fixed (e.g., roadways, light and noise pollution from cities), our results may be indicative of an acute stress response whereby the transient and sporadic nature of flyovers may act as an acute rather than chronic stressor.

We hypothesized that glucose mobilization by CORT may reduce plasma glucose levels in the presence of acute flyover stress, leading to the production or utilization of the alternate energy sources (Romero & Beattie, 2002). Specifically, we expected that lower plasma glucose during flyover noise disturbance would reflect a rapid uptake of energy to cope with an acute stressor. However, we found plasma glucose was unaffected by the flyover treatment, which refutes the idea of energetic reallocation because of sensory disturbance, at least in the context of glucose levels. Most birds, amphibians, and reptiles secrete CORT to maximize short-term fitness through the modulation of lipid metabolism, but there is considerable variation in these responses (Gangloff et al., 2017; Neuman-Lee et al., 2020; Kelley et al., 2021). Butler et al. (2020) compared free-living house sparrow (*Passer domesticus*) nestlings exposed to a one-hour stressor to control individuals, and quantified circulating CORT, triglyceride, and glycerol levels. Nestlings exposed to a stressor had reduced circulating triglycerides consistent with an increased rate of glucogenesis during an acute stressor. Although the timeframe of our sampling may not have detected stress-induced glucose changes (e.g. transient or slow responding), our results do not support links between CORT and aspects of glucogenesis in response to an acute stressor, a finding supported by other reptile studies (Neuman-Lee et al., 2020). The molecular pathways connecting these metrics still need to be elucidated via controlled experiments in the lab.

Our results suggest behavioral variation in *A. neotesselatus* during flyover and non-flyover conditions. *A. neotesselatus* spent a longer duration of time eating during the flyovers. This behavioral change may serve to buffer the potentially negative effect of flyovers on energetic pathways. This result is quite novel in that most studies report on the negative impacts of noise pollution on body mass and condition via shifts in glucogenesis driven by decreased feeding time when exposed to noise (reviewed in Kight & Swaddle, 2011). It has been hypothesized that noise-driven declines in foraging activity, and potential weight loss, may lead to increased metabolic processing and mobilization of cholesterol to fuel the production of stress hormones, because when stressed, individuals rapidly use stored resources to regulate neuroendocrine responses to stress (Kight & Swaddle, 2011). Although we did detect a stress response (increased CORT) during flyovers, we did not detect the expected decline in plasma glucose levels, most likely because of the behavioral adjustment *A. neotesselatus* made in eating more and moving less, which may have fueled plasma glucose levels. This result would need to be confirmed by additional controlled noise pollution experiments in the field.

Reproductive investment did play a role in modulating some of these responses, as suggested by the interaction between clutch size and noise in elevating CORT, which was retained by the model selection process. In rock iguanas, free glycerol levels are highest during the beginning of the breeding season and decrease with gravidity (Webb et al., 2019). Likely due to fat catabolism for vitellogenesis at the beginning of the season and uptake during gravidity. In the context of our study, sampling occurred one month into the breeding season and reproductive stages ranged from mid to post reproduction as determined by ultrasound. Although our findings are consistent with the seasonal timeframe in which glycerol trends were observed by Webb et al. (2019), we did not observe a relationship between reproductive stage or investment, glucogenesis and noise exposure. The behavioral adjustment which we qualify here as “compensatory feeding” in *A. neotesselatus* is a plausible explanation for the lack of impact of noise exposure on glucose metabolism. We did, however, observe an increase in CORT with reproductive investment, which we had observed before in the same population (Aubry et al., 2020; Caracalas et al., 2021).

ROMs values were marginally impacted by noise, but not in the expected direction, in that they were higher under control conditions and reduced during the flyover treatment. The ROMs test measures blood levels of hydroperoxides, which are produced when biomolecules (fatty acids, nucleic acids, proteins, etc.) are oxidized (Constantini, 2016). ROMs may exceed the capacity of antioxidant defense and repair mechanisms (i.e., oxidative stress), leading to oxidative damage of biomolecules (Metcalfe & Alonso-Alvarez, 2010). Lower ROMs during flyovers may be indicative of a lack of oxidative damage. During flyover events, lizards were observed eating and eating while searching their environment for a significantly higher proportion of time than was spent in other behaviors (**Table 5, Figure 4**), and were most likely not in a digestive state which sometimes carried an oxidative cost. Results suggest that compensatory eating in *A. neotesselatus* may keep metabolic processing at bay, which would explain the lower ROMs values observed during flyovers.

Although *A. neotesselatus* seem to adjust their behavior during flyovers by increasing the time they spend eating to buffer the potentially negative effect of flyovers on energetic pathways, they still suffered a metabolic cost driven by the stress response via ketone accumulation, especially when considering smaller-sized animals. Indeed, plasma ketones were significantly higher during flyover noise disturbance when we accounted for body size. An increase in ketones in response to acute and prolonged stress has been documented before (Neuman-Lee et al., 2015; Ricart-Jane, 2002). When blood glucose levels are significantly altered due to stress or physical activity, fatty acid metabolism may be initiated (Rojas-Morales et al., 2020). Ketones are products of fat metabolism that can be utilized as alternative sources of energy when glucose is low, but plasma glucose was unaffected by the flyover treatment in our study, suggesting individuals may preferentially mobilize ketones, even when glucose may be maintained via observed "compensatory eating" behavior we observed in the field.

Conclusion

Colorado checkered whiptails at Fort Carson responded to acute anthropogenic stress in a variety of ways that included: a compensatory behavioral response (less time spent moving, but more time spent eating), a stress response (increased CORT) and partial energy mobilization (increase in ketones). In an oviparous lizard actively allocating yolk, we found that results must be analyzed in the context of reproductive timing and maternal investment by controlling for reproductive status and clutch size, as conducted here. Anthropogenic disturbance may have a higher impact on parthenogenetic lizards during the breeding season when maternal sources of energy must be invested into developing offspring. However, *A. neotesselatus* seem to adjust their behavior to minimize the negative impacts of noise disturbance on some energetic pathways and may maintain energy levels through compensatory eating during flyovers. In summary, although anthropogenic disturbance in the form of military aircraft flyover events have significant physiological impacts on this Colorado checkered whiptail population, behavioral adjustments and compensatory feeding appear to limit energetic expenditures to some extent in relation to noise pollution. Although behavioral plasticity may help mitigate some of the potentially negative impacts of noise exposure on *A. neotesselatus* metabolism, the impacts may also be mitigated if aircraft operators maintain an elevation that is sufficiently high enough to lead to decibel reads on the ground that fall below 50 dB.

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Declarations

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Authors' contributions

LMA & SSF conceived the study; MK, LS, & SH collected the data; MK, LS, & SH conducted the lab work; LS analyzed the behavior audio recordings; LMA analyzed the data; AJL facilitated field activities; LMA wrote the first draft of the manuscript; MK, LS, SH, AJL & SFF participated in the writing of subsequent versions of the manuscript.

Tables and Figures

Table 3

Sample Sizes for Behavioral and Dependent Variables of Interest

a

Treatment	Control	Flyover
Basking	10	6
Eating while Basking	1	0
Eating	17	4
Eating while Searching	1	1
Moving	43	15
Moving while Searching	2	9
Null	51	15
Searching	12	9

b

Treatment	Control	Flyover
CORT	49	24
Glucose	53	21
ROMs	55	24
Ketones	58	24

Note. 3a) Sample size for behavioral data (Null; Eat; Basking; Basking while eating; Move; Searching; Eating while searching; Moving while searching; Out of sight) collected on *A. neotesselatus*, with respect to treatment (control versus flyovers). 3b) Sample size for data collected on *A. neotesselatus*, with respect to treatment (control versus flyovers) and the dependent variables of interest (i.e. CORT, Glucose, ROMs, and Ketones).

Table 4

Description of Behaviors Recorded for A. neotesselatus

Behavior	Code	Description of Behavior
Null	N	Lizard remains in one location and remains still
Eating	E	Lizard actively consumes a food item while stationary
Basking	B	Lizard flattens body and remains in sunlight
Basking while eating	BE	Lizard consumes a food item while basking
Moving	M	Lizard moves to a different location
Searching/scanning	S	Lizard actively searches for food or other unknown objects – characterized by prodding snout into the substrate or raising head to assess surroundings

Eating while searching	ES	Lizard actively consumes food while searching/scanning
Moving while searching	MS	Lizard rapidly moves from one location to the next, stopping only to prod substrate or scan environment
Out of sight	O	Lizard was not visible to observer - behavior not used in analysis

Note. Code represents the nomenclature later used in analyses.

Table 5

ANOVA Test Results

a.

	df	SS	MSS	F-value	P-value
Behavior: Moving vs. Other	1	0.025	0.0248	0.152	0.697
Treatment: Flyovers yes/no	1	0.351	0.351	2.148	0.144
Behavior x Treatment	1	0.265	0.2651	1.623	0.204
Residuals	192	31.364	0.1634		

b.

	df	SS	MSS	F-value	P-value
Behavior: Eating vs. Other	1	1.658	1.658	12.947	<0.001***
Treatment: Flyovers yes/no	1	0.774	0.7745	6.048	0.015*
Behavior x Treatment	1	0.307	0.3075	2.401	0.123
Residuals	192	24.588	0.1281		

c.

	df	SS	MSS	F-value	P-value
Behavior: Active vs. Other	1	1.941	1.9407	14.889	<0.001***
Treatment: Flyovers yes/no	1	0.349	0.3486	2.675	0.104
Behavior x Treatment	1	0.014	0.0139	0.107	0.744
Residuals	192	25.025	0.1303		

Significance codes: ‘***’ p-value < 0.001; ‘**’ p-value < 0.01; ‘*’ p-value < 0.05

Note. a) movement, flyover treatment, and their interaction; b) eating, flyover treatment, and their interaction; and c) active, flyover treatment, and their interaction, on the proportion of time spent in the behavior

Table 6

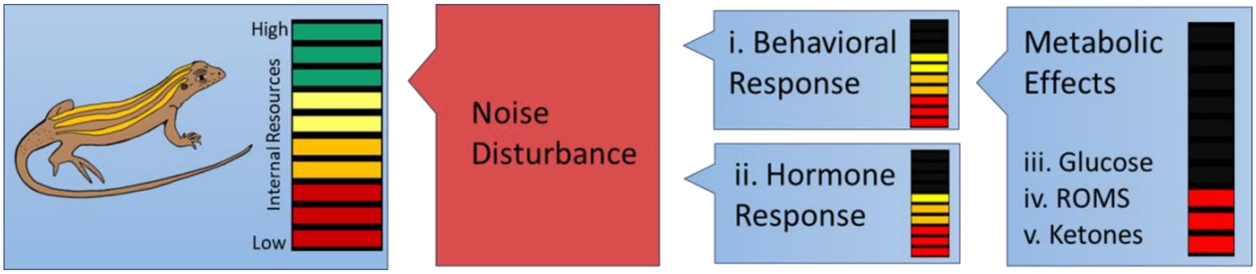
Model Selection Results

Dep. Variable	Model covariates	K	AICc	Delta_AICc	AICc Wt	Cum. Wt	LL
Plasma Cort	**Treatment*Clutch size**	**5**	**21.643**	**0**	**0.362**	**0.362**	**-5.353**
	Treatment*svl	5	22.958	1.315	0.188	0.550	-6.010
	Null	2	23.554	1.910	0.139	0.689	-9.687
	Treatment	3	23.592	1.949	0.137	0.826	-8.614
	Treatment*mass	5	24.453	2.810	0.089	0.915	-6.758
	Treatment*Reprod. Stage	5	24.541	2.897	0.085	1	-6.802
Plasma Glucose	**Null**	**2**	**706.242**	**0**	**0.608**	**0.608**	**-351.033**
	Treatment	3	708.325	2.084	0.214	0.822	-350.984
	Treatment*Clutch size	5	710.668	4.427	0.066	0.889	-349.873
	Treatment*svl	5	710.965	4.723	0.057	0.946	-350.021
	Treatment*mass	5	712.178	5.937	0.031	0.977	-350.627
	Treatment*Reprod. Stage	5	712.800	6.559	0.023	1	-350.939
Plasma dROMs	**Treatment*Reprod. Stage**	**5**	**256.416**	**0**	**0.908**	**0.908**	**-122.785**
	Treatment*Clutch size	4	262.030	5.614	0.055	0.962	-126.737
	Treatment*mass	5	264.868	8.452	0.013	0.976	-127.011
	Treatment*svl	5	265.264	8.848	0.011	0.987	-127.209
	Null	2	266.048	9.632	0.007	0.994	-130.943
	Treatment	3	266.413	9.997	0.006	1	-130.042
Ketone	**Treatment*svl**	**5**	**246.433**	**0**	**0.783**	**0.783**	**-117.811**
	Treatment	3	250.710	4.277	0.092	0.876	-122.197
	Treatment*mass	5	251.128	4.695	0.075	0.951	-120.158
	Treatment*Clutch size	5	252.553	6.120	0.037	0.987	-120.871
	Treatment*Reprod. Stage	5	254.750	8.318	0.012	1.000	-121.970
	Null	2	261.719	15.287	0.000	1	-128.782

Note. Comparison of generalized linear models testing for the effect of flyover treatments (control vs. flyover) on circulating Cort (in ng/mL), Glucose (mg/dL), ROMs (mg/H202/dL), and Ketones, while controlling for the effect of size (svl in mm), mass (in gr), reproductive state (gravid versus not), and clutch size (0-4 follicles). K: the number of parameters. AICc-related metrics measures the fit of the model to the data, with the lowest AICc value, smallest Delta AICc, and highest Cumulative Weight indicating best fit.

Figure 6

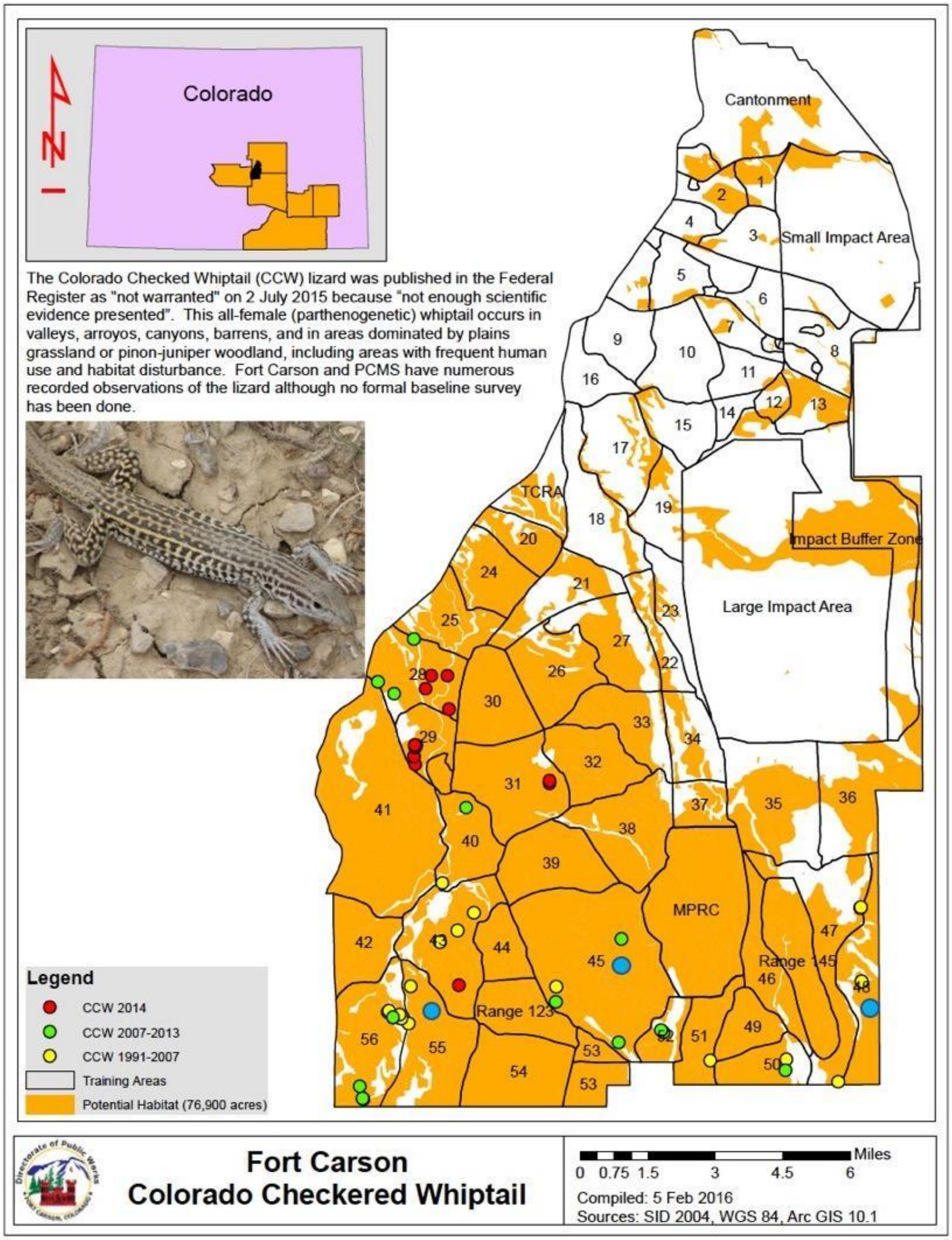
Conceptual Energetic Budget Allocation of A. neotesselatus



Note. Conceptual energetic budget allocation of *A. neotesselatus* following noise disturbance (i.e. flyover treatment) when resources are partitioned into multiple competing processes involving a behavioral response, energy mobilizing hormones, and shifts in metabolic allocation. Internal resources are represented in the right column across a gradient with high internal resource indicated in green and low internal resources indicated in red. Resources are expected to diminish with the initiation of multiple physiological processes in the setting of noise induced physiological and behavioral changes.

Figure 7

Surveys of A. neotesselatus Conducted in Previous Years

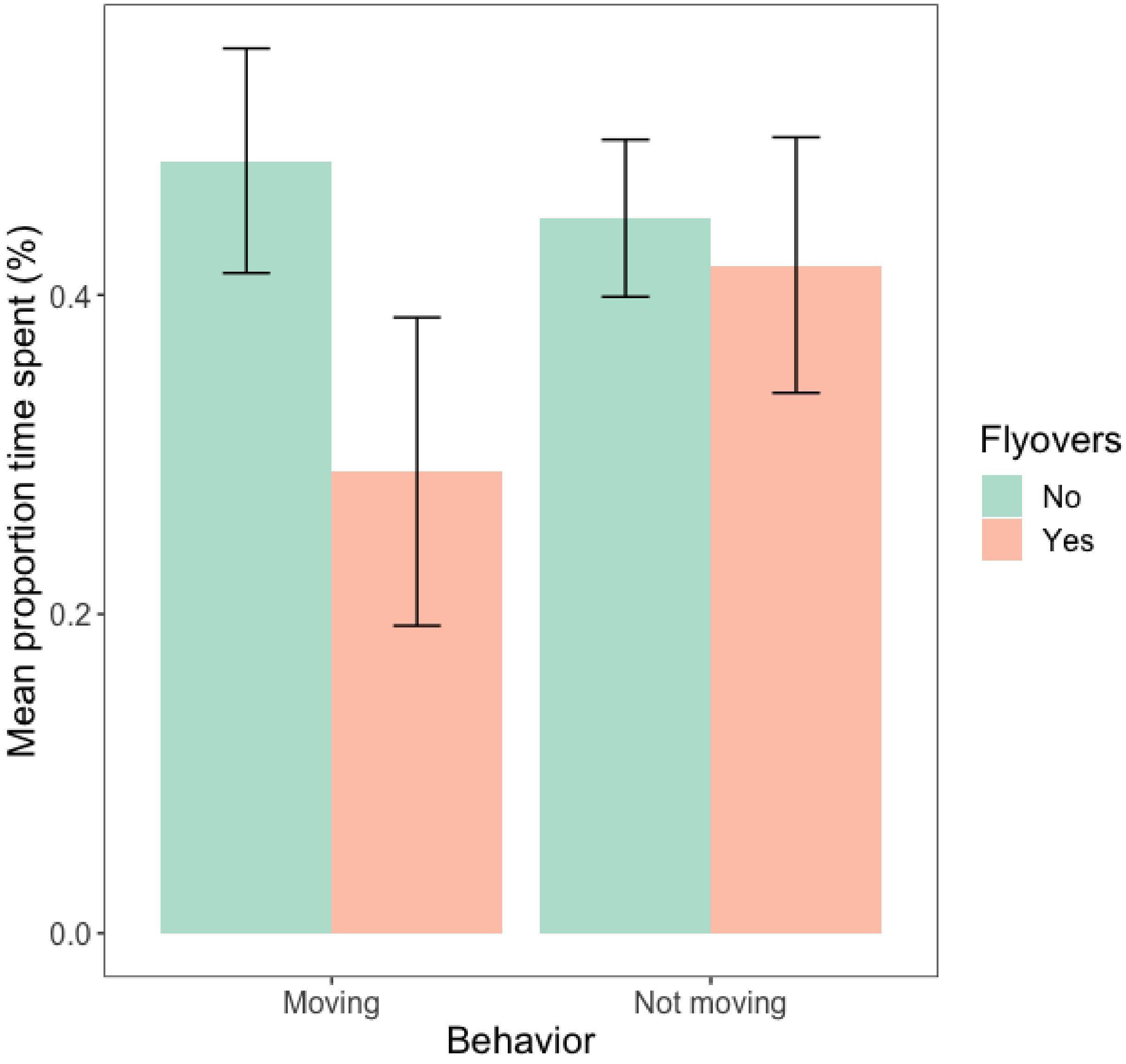


The Colorado Checked Whiptail (CCW) lizard was published in the Federal Register as "not warranted" on 2 July 2015 because "not enough scientific evidence presented". This all-female (parthenogenetic) whiptail occurs in valleys, arroyos, canyons, barrens, and in areas dominated by plains grassland or pinon-juniper woodland, including areas with frequent human use and habitat disturbance. Fort Carson and PCMS have numerous recorded observations of the lizard although no formal baseline survey has been done.

Note. Surveys of *A. neotesselatus* conducted in 1991-2007 (yellow dots), 2007-2013 (green dots), and 2014 (red dots) at Fort Carson, CO. Surveys used in this study (2018-2019) are represented by blue dots, with intensive sampling focused on TA45, TA48, and TA55.

Figure 8

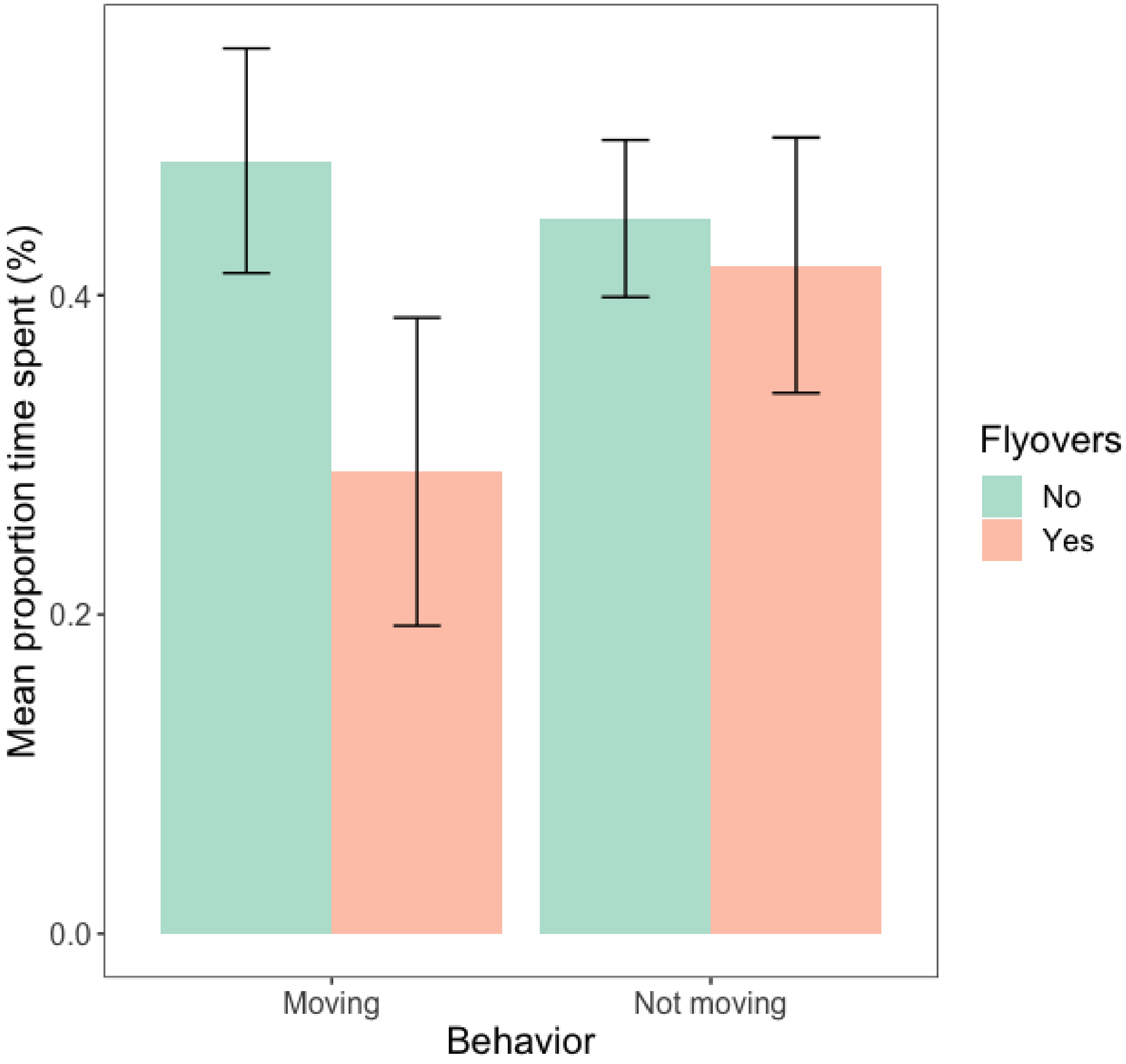
Average Proportion of Time (%) Spent by A. neotesselatus Moving Vs. All Other Behaviors Between the Treatment (Flyovers) and the Control (No Flyovers)



Note. Moving: ‘M’ Moving, ‘MS’ Moving While Searching; All Other Behaviors: (‘B’ Basking, ‘BE’ Basking While Eating, ‘E’ Eating, ‘ES’ Eating While Searching, ‘N’ Null, ‘S’ Searching/Scanning/Foraging)

Figure 9

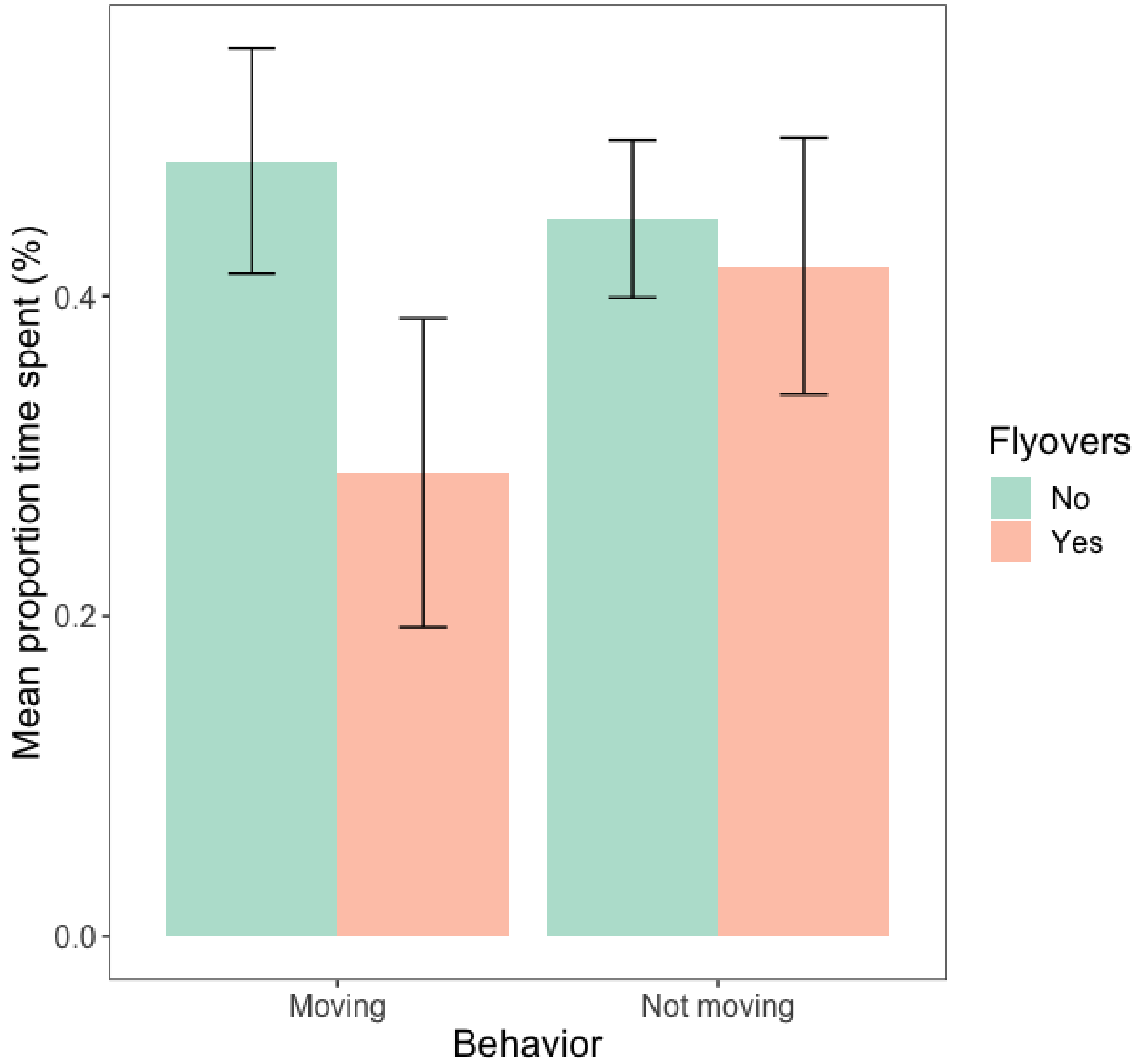
Average Proportion of Time (%) Spent by A. neotesselatus Eating) Vs. All Other Behaviors) Between the Treatment (Flyovers) and the Control (No Flyovers)



Note. Eating: ‘BE’ Basking While Eating, ‘E’ Eating, ‘ES’ Eating While Searching; All Other Behaviors: ‘B’ Basking, ‘M’ Moving, ‘MS’ Moving While Searching, ‘N’ Null, ‘S’ Searching/Scanning/Foraging

Figure 10

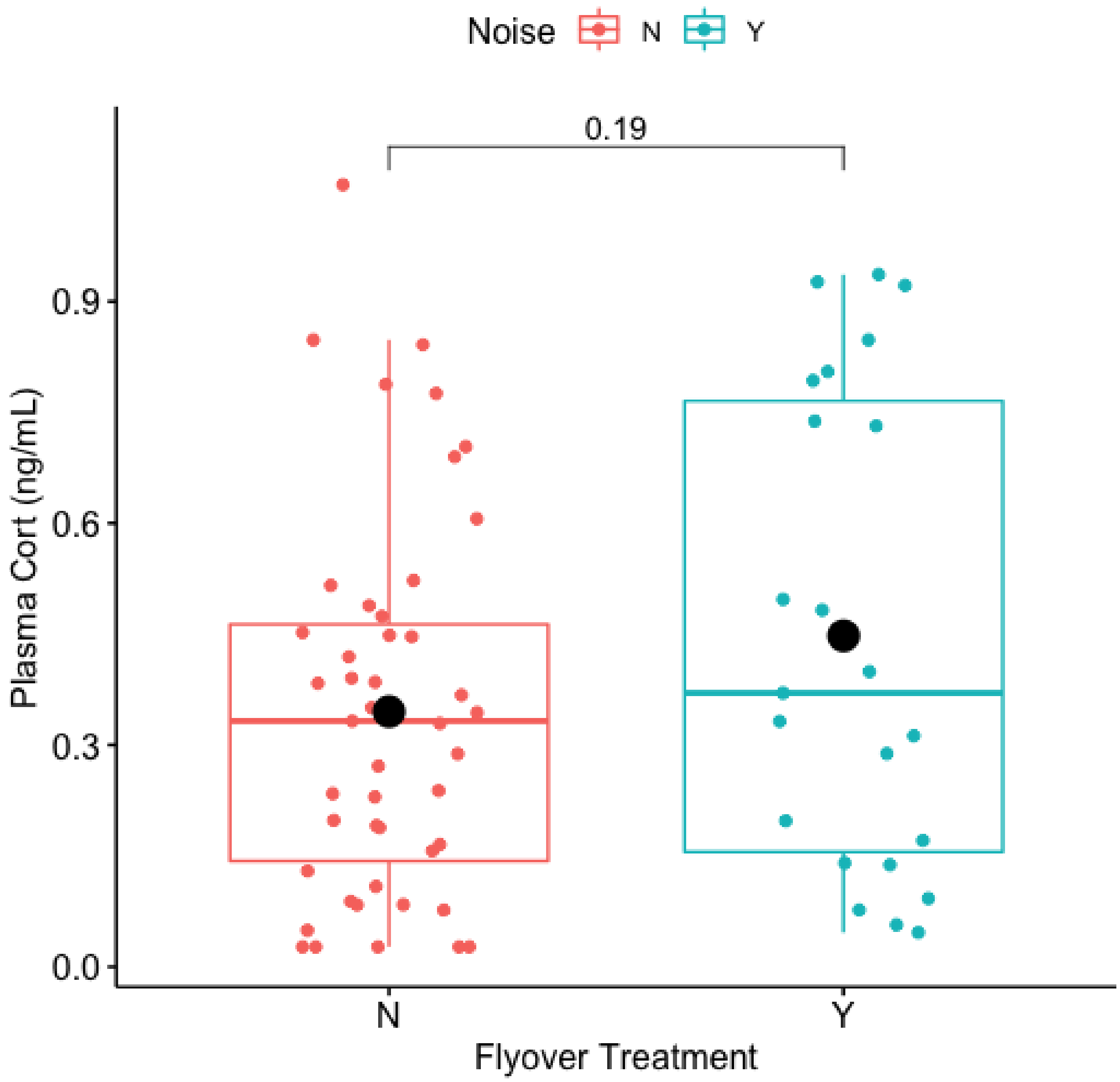
Average Proportion of Time (%) Spent Assessing the Environment () Vs. Any Other Behavior by A. neotesselatus In Key Behaviors Between the Treatment (Flyovers) and the Control (No Flyovers).



Note. Assessing the Environment: Stationary: 'ES' Eating While Searching, 'M' Moving, 'MS' Moving While Searching, 'S' Searching/Scanning/Foraging; Any Other Behavior: ('B' Basking, 'BE' Basking While Eating, 'E' Eating, 'N' Null)

Figure 11

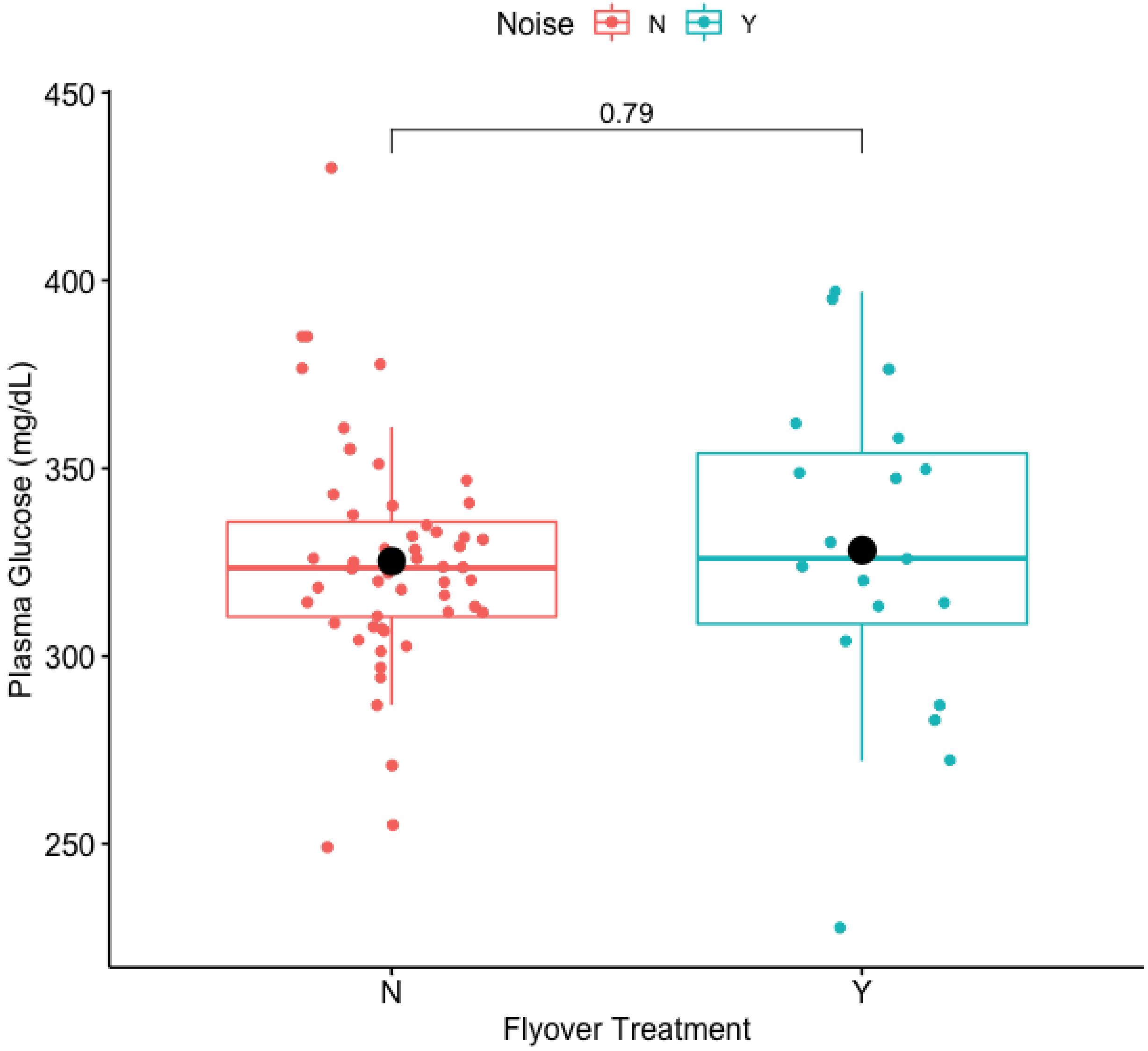
Impact of Flyover Treatments on Circulating Plasma Corticosterone (ng/mL)



Note. Noise: ‘N’ = control, ‘Y’ = flyover

Figure 12

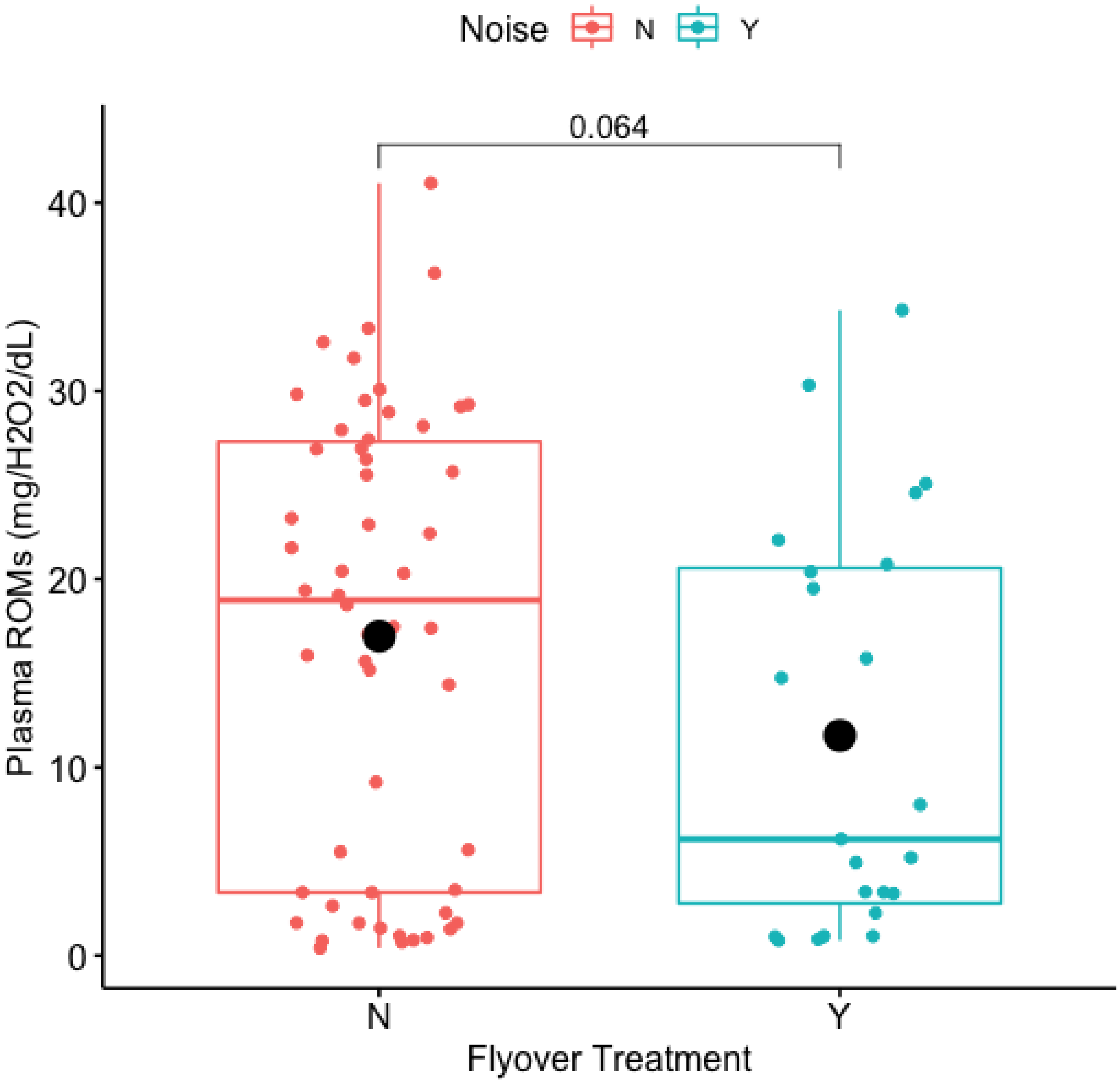
Impact of Flyover Treatments on Plasma Glucose (mg/dL)



Note. Noise: ‘N’ = control, ‘Y’ = flyover

Figure 13

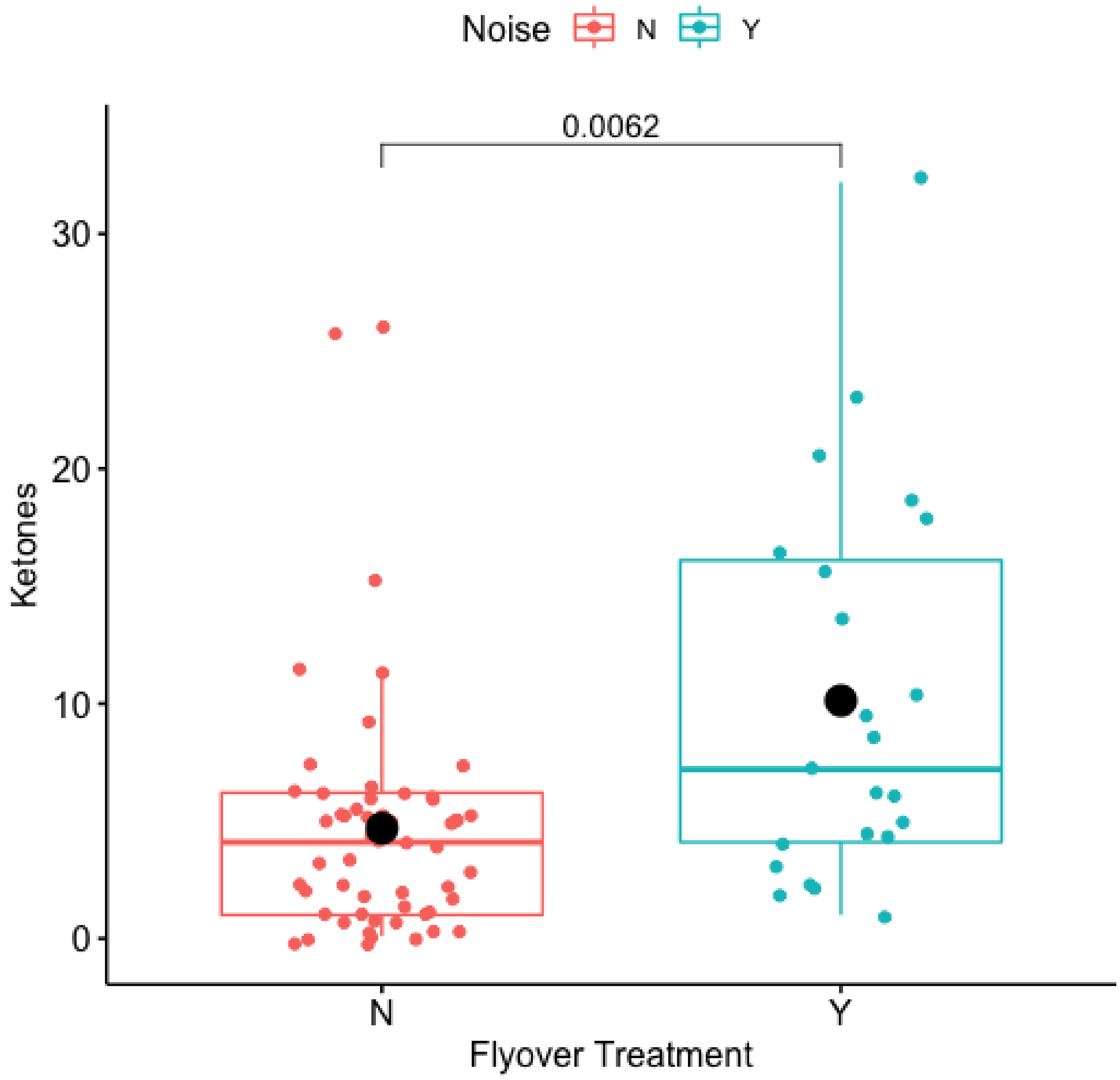
Impact of Flyover Treatments on Plasma ROMs (mg/H202/dL)



Note. Noise: ‘N’ = control, ‘Y’ = flyover

Figure 14

Impact of Flyover Treatments on Ketones



Note. Noise: ‘N’ = control, ‘Y’ = flyover

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CHAPTER 4

THE EFFECTS OF AN INTERSTATE AND RECREATIONAL WALKING TRAIL ON LIZARD BODY CONDITION AND ANTIPREDATOR BEHAVIOR

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Abstract

Human population growth leads to greater urban sprawl, increasing humans' reliance on roads, highways, and interstates. Roadway infrastructure fragments preexisting habitats into smaller areas that may support less biodiversity. Predator abundance has also been shown to increase near roadways, as the wide-open areas offer better visibility for hunting and easier access to roadkill. One way species may adjust to the increased predator abundance is via behavioral adjustments. Lizards are ideal organisms because of the wide variety of behaviors they exhibit and the relative ease in categorizing them. Past research indicates roads are linked to elevated stress hormone levels and poor body condition in lizards, yet the impacts of roadways on lizard behavior and morphology are unclear. US Interstate-15 bisects Confluence Park, a long-term field site for research on established, urban side-blotched lizard in St. George, UT, USA. The park disturbances create an ideal experiment to address whether the distance of a lizard's perch from I-15 and the walking path affect morphology or behavior, including aggressive, thermoregulatory, exploratory, and anti-predator behaviors. We observed lizard behavior in-situ for two minutes, then introduced each into an arena where exploratory and anti-predator behaviors were assessed via aerial and terrestrial models. Body condition, determined by the residuals of the linear regression between SVL (mm) and mass (g), was worse near the highway, while body temperature was higher closer to the walking trail. There was a strong, negative correlation between time spent basking and the number of pushups performed. A higher proportion of lizards fled from the terrestrial model, but latency to flee was shorter and distance fled was higher for the aerial model. Lastly, there was no relationship between any exploratory behavioral measures by lizard morphology nor urban site characteristics. Our results suggest that behavioral plasticity may allow lizards to respond optimally to different predator types existing in Confluence Park, yet the presence of Interstate-15 may compound the negative impacts of urbanization already present in urban sites.

Introduction

The impacts of urbanization on surrounding wildlife diversity, abundance, and health have been repeatedly documented in taxa across the animal kingdom. Habitat loss and fragmentation are some of the most detrimental effects of urbanization, causing declines in species richness and abundance (Hanski, 2005, 2015; Pimm et al., 2014; Rytwinski & Fahrig, 2012). For example, urbanization negatively impacted the species richness and abundance of the wood mouse (*Apodemus sylvaticus*), greater white-toothed shrew (*Crocidura russula*), Algerian mouse (*Mus spretus)* and house mouse (*Mus* *musculus)* in the Porto Metropolitan Area in Portugal (Gomes et al., 2011). Additionally, invertebrate assemblages instreams flowing from moderately urban landscapes had reduced richness and Shannon diversity compared to assemblages sampled downstream from forested areas (Bohus et al., 2023), while squirrel species abundance and diversity was reduced in urban areas near Bangkok, Thailand (Thaweepworadej & Evans, 2023). A small, albeit negative relationship between urbanization and wildlife health was found in a phylogenetic meta-analysis of over 516 physiological comparisons, with invertebrates and amphibians most noticeably affected (Murray et al., 2019). In at least one instance, the relative distance of nearby roads and cities has effectively predicted the degree of habitat loss and fragmentation of vegetation (Santos & Tabarelli, 2002).

The presence of roads and highways, necessary biproducts of urbanization, create unique problems for urban wildlife. Collison-based mortality (Klöcker et al., 2006), noise pollution (McClure et al., 2013), and artificial light (Jones et al., 2023) sources are all prevalent near roadways (Dean et al., 2019). Additionally, greater wetland isolation and road density have been linked to decreased amphibian species richness (Lehtinen et al., 1999). Roads are not only pathways for humans but also wildlife. Predators may use roadways for improved visibility and accessibility during hunting (Doherty et al., 2015; James, 1999; Raiter et al., 2018) impacting when and where prey can be active. The many problems caused by roadways may require animals to adjust their life-history strategies to cope with their presence. One way species may adjust to the challenges presented by roadways is via behavioral adjustments.

Changes in behavior linked to life-history strategies in reproduction (Robbins, 1993; Slabbekoorn & Peet, 2003), movement and activity (Riley et al., 2003), and mortality (Forman & Alexander, 1998; Lepczyk et al., 2004) may exacerbate effects of road-based disturbances (Ditchkoff et al., 2006). For example, the activity patterns of black-tailed prairie dogs (*Cynomys ludovicianus*) and white-tailed deer (*Odocoileus virginianus*) shifted during a motorcycle rally in Devils Tower National Monument (Buxton et al., 2020). Male reed buntings (*Emberiza schoeniclus*) adjusted their song frequency and rate to high, man-made noise levels (Gross et al., 2010), similar to how túngara frog (*Engstomops pustulosus*) calls in urban areas had a higher complexity and amplitude (Smit et al., 2022).

Taxonomic groups are affected by roads differently, with small mammals showing positive to no effects and reptiles and amphibians showing predominately negative effects (Rytwinski & Fahrig, 2012). Reptiles are at particular risk to the negative impacts of roadways directly and indirectly. Ectotherms may be drawn to roads or pathways to thermoregulate, as black asphalt is typically hotter than the surrounding environment (Ashley & Robinson, 1996). Reptiles are then at an increased chance of being struck by vehicles - snake mortality was correlated to the number of vehicles traveling through the Everglades each month (Bernardino & Dalrymple, 1992). Reptiles have also been observed using sections of roadways underneath lights to hunt for insects (Brehme et al., 2013; Neill, 1950). If smaller prey use roads more frequently, it may attract the attention of larger predators, like feral cats and birds. Indirectly, the road fragments the landscape and alters reptile habitats.

In some cases, reptiles seem to acclimatize to human developments, changing their behavior and reproductive strategies to fit the environment. Although lizards became less conscientious of predators due to composition and abundance changes at wind farms, minimally developed farms have been able to sustain healthy populations (Keehn & Feldman, 2018). Conversely, lizards in highly developed farms, those with a higher abundance of highways and interstates, had poor body condition and decreased survival rates (Keehn et al., 2019).

While it is known that lizards avoid highways with loud noise and vibrations (Brehme et al., 2013), it is unclear how highway disturbances may affect lizard behavior and morphology (Andrews et al., 2015; Jochimsen et al., 2004). Increased road usage has been linked to elevated stress hormone levels (Kechnebbou, 2019) and suppressed immunity (Lucas & French, 2012), both of which may impact lizard behavior. Additionally, multiple studies have examined how reptile basking behavior is affected by anthropogenic disturbances - time spent basking was lower in areas with more human disturbance and recreational use (Heppard & Buchholz, 2019; Mukherjee, 2018).

Because of a widespread species distribution and a locally abundant population, side-blotched lizards are the ideal study species to examine behavioral and morphological effects of anthropogenic disturbances. In a previous study conducted on the same population of side- blotched lizards, we found no differences in body condition between our urban and rural field sites (Sermersheim et al., in preparation). This could imply that lizards are able to adapt and live seemingly well in disturbed urban areas, a result that was also seen in aforementioned studies (Keehn et al., 2019; Lucas & French, 2012).

Our previous study did not support the claim that behavior differed between urban and rural sites, but there was considerable variation within sites (Sermersheim et al., in preparation). This conclusion suggests that other factors may be influencing behavior on a finer scale, and we found marginal support for a relationship between thermoregulatory behavior and distance to the nearest disturbance, a roadway - lizards perched closer to the roadway spent less time basking (Sermersheim et al., in preparation). This relationship could be due to many factors, one of which might be the perceived risks associated with the roadway (Jochimsen et al., 2004). Not only is the noise level higher, but there are more aerial predators near the highway (LOS and ELL personal observation). Lizards may be less likely to explore novel areas near the roadway due to the increased predation risk.

Similarly, because of the high predator abundance, lizards may have quicker anti-predator responses closer to the roadway. Lizards living on high feral cat density islands in Greece displayed similar exploratory and anti-predator behaviors: *Podarcis erhardii,* remained closer to their refuge when approached by a person in the field, had a higher FID (flight initiation distance) when approached by a person or cat model, and was more likely to autotomize their tails compared to lizards found on low feral cat density islands (Li et al., 2014). Alternatively, it is possible that lizards could habituate and adapt to the increased predation risk and disturbance presence. In this case, habituating via plastic exploratory and anti-predator responses may offer an explanation as to how populations can adapt to anthropogenic disturbances, like roadways.

In the present study, we utilized field and experimental techniques to examine the effects of an interstate and walking path on the behavior, morphology, and body condition of side-blotched lizards found at an urban park. We hypothesized that **(1)** significant morphological differences exist depending on proximity to the interstate; **(2)** thermoregulatory, aggressive, exploratory, and anti-predator behaviors differ based on distance to the disturbance; and **(3)** morphological and environmental factors are linked to behavior. We predicted that **(1)** lizards found on perches closer to I-15 will have worse body condition, **(2)** as distance from the disturbance shortens, individuals will be less likely to explore a novel environment and flee more quickly from aerial and terrestrial predators, **(3)** individuals perched near the disturbances will spend less time thermoregulating and perform more push-ups, and **(4)** lizards in better body condition will flee more quickly than those who do not. Alternatively, if lizards can habituate to the disturbance, we could potentially see the reverse relationship in terms of behavior and distance from the disturbance.

Methods

Species and study area

In April 2022, 31 free-living male *U. stansburiana* lizards (snout-vent length (SVL) = 51.1 mm ± 2.5; mass = 4.46 g ± 0.4) were sampled along the Crosby Family Confluence Park Virgin River Trail (Average temperature = 28.4°C, Location = 37.07444°N, -113.58326°W) in St. George, UT, USA (IACUC 2529). I-15 is a major and busy thoroughfare, with its northern terminus in Sweet Grass, Montana, USA at the Canada-United States border, while the southern terminus is in San Diego, California, USA just north of the Mexico-United States border (Google Maps) . I-15 spans 2,306 km (1,433 miles) and passes directly over the Confluence Park walking trail (**Figure 15a**). Traffic noise is consistent throughout the day, dying down after sunset. Lizards are typically found beneath retaining rocks that line the Virgin River, perched throughout red-rock formations that line the northern edge of the walking path, and underneath small shrubs and trees that are interspersed along the eastern edge of the walking path. Data was collected as far along the path as lizards were found. All but one lizard was collected from the east side of Interstate 15; the individual collected on the west side was not an outlier in any statistical test, therefore included in the results (**Figure 15b**).

The river trail walking path is an added disturbance, as pedestrians are running, biking, and using small, motorized scooters constantly throughout the day. Lizards may have become habituated to this constant human presence; therefore, non-human predators were utilized in this study.

Bird species present in the area include cliff swallows (*Petrochelidon pyrrhonota)*, greater roadrunners (*Geococcyx californianus*), Cooper's hawks (*Accipiter cooperii*), great blue herons (*Ardea herodias*), verdins (*Auriparus flaviceps*), white-crowned sparrows (*Zonotrichia leucophrys*), green-winged teals (*Anas crecca*), and red-tailed hawks (*Buteo jamaicensis*). *P. pyrrhonota* are commonly seen nesting and flying underneath the I-15 overpass, swooping down into the Virgin River to collect insects. Other reptile species present include western fence lizards (*Sceloporus occidentalis*), yellow-back spiny lizards (*Sceloporus uniformis*), western whiptails (*Aspidoscelis tigris*), Mojave Desert tortoises (*Gopherus agassizii*), and ground snakes (*Sonora semiannulata*). *S. occidentalis* are often seen nearby the side-blotched lizards.

In-situ behavioral observation

Side-blotched lizards were characterized by color pattern and known sexually dimorphic traits. Only males were included in this study, due to the possibility that reproductive differences might affect female behaviors (Adkins & Schlesinger, 1979;

Sinervo & Miles, 2011). All in-situ behavior recordings were conducted using the same methods as outlined in Sermersheim et al. (in preparation). Solitary (movements), aggressive (push-ups and head bobs), and thermoregulatory (basking) behaviors were captured as either states, stints of behaviors, or events, behaviors that occur at a single point in time. Head bobs were included within the push-up count, due to difficulty differentiating between the two behaviors. The only changes in methodology from the previous study was in the duration of audio recordings and the researchers who performed the in-situ recordings. The recording time was reduced in the current study after analyzing behavior data from the previous study. Most behaviors occurred in the first two minutes of the five-minute audio duration; therefore, the recording time was reduced to two minutes. Audio recordings were completed by LOS, RTT, and ELL after proper training on identifying behaviors. Each researcher used the “Voice Memos” application on an Apple iPhone to complete audio recordings.

Novel environment (wagon arena) and prop creation

The mobile arena (**Figure 16a**) was created by cutting 4-inch flaps in each side of a collapsible, all-terrain wagon (TIMBER RIDGE Double Decker Wagon, Dimensions = 32" x 18.5" x 34.5", Weight capacity = 204.12 kg). On the outside of the arena, each flap had a piece of tape securing the flap down to ensure the lizard could not escape. Clear acrylic sheets (Make Market® Clear Acrylic Craft Sheet) were measured and cut according to the interior wagon-wall dimensions, then covered with vinyl sheets (ORACAL® 651 Intermediate Cal Vinyl – Color: Beige) to mimic a dirt or sand habitat and to prevent lizards from climbing up the fabric walls of the wagon. The wagon was then filled with a combination of dirt, sand, and small sticks/plant material found at the park to mimic a “natural” habitat. Two larger rocks were placed in the center of the wagon and stacked to create a “hide” that lizards could seek cover beneath. Space was left on both sides of the hide to ensure lizards could flee parallel to the hide without needing to run underneath it. The substrate and hide were re-adjusted before each trial, ensuring the environment was consistent between individual focal lizards. To record the behavior within the wagon, a phone clip (Slopehill Flexible Long Arm Mount Stand, Dimensions = 70 cm (27.5in) overall length with 55 cm (21.5in) flexible arm) was attached to the bottom lefthand side of the wagon. An Apple iPhone 12 was used to video record the behavioral trials within the wagon. On the opposite side of the wagon from the phone clip was the wagon handle. A 2.7 m (9ft) transparent fishing line was tied to the handle to be later used in the aerial predator trials.

To create the novel terrestrial predator, a wooden dowel rod (Creatology™ 12” Wood Dowels, ¼ in) was inserted and secured into a lizard model (Safari Ltd. Bearded Dragon Figurine, Dimensions = 8.2 x 5.7 x 2.8 in, Weight = 4.2 oz), which was painted solid black. The novel aerial predator was created with a fake, but realistic looking Halloween crow prop (Realistic Halloween Crow, Dimensions = 6.3 x 4.72 x 7.87 in, Weight = 1.2 oz). The wings were outspread to mimic a real aerial predator. A two-inch cutting of a straw was glued to the back of the crow to thread clear fishing line through to make the aerial predator “fly.”

Novel environment-based behavioral observation

After in-situ behavioral recordings were complete, each focal lizard was caught using a snaring pole. Once the video recording began, the lizard was immediately placed into the upper center of the arena (**Figure 16b**: AHC-above hide center) and a two-minute timer started. All observers were not visible from the arena, allowing only the video camera to capture the lizard behavior. At the two-minute mark, the anti-predator trials began. All trials were conducted by LOS, and predator types were alternated to ensure behavioral responses were not related to presentation order. At no point in time during the terrestrial predator trial did LOS stand above the wagon’s edge; the lizard model was inserted from a kneeling position to prevent behavioral responses to the observer. The terrestrial predator was pushed into the arena at a constant speed, by either the top or bottom flap, depending on the lizard’s location. The model was inserted on the same side as the lizard was located unless it was positioned directly in front of the flap (i.e., the flap could not be opened without touching the individual). In these scenarios, the model was inserted at the opposite end. Once the predator model’s snout touched the hide, it was removed at the same speed as its insertion. The flap was then taped shut again and a two-minute refractory period began. In the last 30 seconds of the refractory period, the next anti-predator trial preparations began. For the aerial predator trial LOS threaded the transparent fishing line though the straw cutting on the back of the bird model, ensuring the front (beak) of the bird model was facing the direction it would be “flying.” Once the two-minute refractory period ended, LOS moved the fishing line around and above the phone clip and centered it atop the arena. LOS then held the end of the fishing line at nose height for each trial (151cm), and released the bird model, sending it “flying” over the arena. The bird model hit the handle on the opposite side of the arena and immediate dropped. Once the lizard stopped moving (detected by listening for movement), the video recording was ended and immediately saved to a secure folder.

Morphological and environmental data collection

While the arena behavioral trials were ongoing, RTT recorded site and perch environmental measures. Air and perch temperature, noise level (ExTech, EN300 5-in-1 Environmental Meter, dB), and distance to disturbances was collected at each perch. Distance to each disturbance (path and highway) was recorded following methods outlined in Sermersheim et al. (in preparation). Perch location was pinned onto Google Maps to save latitude and longitude.

Once the behavioral trials were complete, body temperature (via cloacal probe insertion), mass (g) to the nearest 0.01, snout-vent length (SVL) to the nearest 0.01mm, and side-blotch length and width were taken following methods from Sermersheim et al. (in preparation). Body condition measurements were also replicated, and included tail status, presence or absence of injuries and scarring, and presence or absence of mites. Throat color was also recorded and categorized consistently with Sermersheim et al. (in preparation), using throat color descriptions from Sinervo and Lively (1996). Body condition measurements and throat color were determined via visual inspection. The individual was then released in the exact location it was first spotted.

In-situ and arena-based behavioral analysis

The audio recordings of the in-situ behavior were analyzed via JWatcher, Version 1.0 (Blumstein et al., 2006) in the same manner as outlined in Sermersheim et al. 2021 (unpublished). Data produced by JWatcher was later used in statistical analyses.

Each arena-based behavior video recordings were analyzed in two ways: **(1)** latency to movement and **(2)** quadrants moved during initial movement. Latency to movement was calculated slightly differently between the exploratory and anti-predator trials. The goal of the exploratory trail was to observe how long it took for a lizard to move within a novel environment, so latency to movement was the number of seconds it took for the lizard to move once it was placed in the arena. The goal of the anti-predator trials was different; we wanted to observe that if a lizard fled from a predator, how quickly it fled. Therefore, latency to movement was the number of seconds it took for the lizard to flee from the predator. A movement was considered a flee if it was a quick, haphazard burst. For predator types, the latency timer began the instant the model crossed the arena threshold. On average, it took the bird model one second to “fly” across the arena. The average amount of time the lizard model remained in the arena for a trial was 15.3 seconds. After analyzing the anti-predator behavior recordings, all flees from the bird model occurred within the first second of the aerial trial and within the first 15 seconds of the terrestrial trial. If a lizard did not flee from the aerial or terrestrial model, the time to latency was recorded as 2 seconds and 16 seconds respectively. If it was not obvious whether the lizard perceived the predator, latency to movement was marked as missing data (i.e. NA; only occurred in the terrestrial trials, n=5).

Distance moved during initial movement was calculated by placing a grid on top of the arena (**Figure16b**). The grid quadrants were named based off their distance and relation to the hide in the center. A lizard was considered “inside” a quadrant when its head and torso were within the confines. To describe distance from hide, quadrants moving away from the hide were RBH (right below hide), BH (below hide), and FBH (far below hide). To describe relation to the hide, quadrants to the left contained an L in their code, R for right, and C for center (in line with the hide). LH and RH describe a lizard’s quadrant directly to the left or right of the hide, while OTH and UH represent a lizard located over-the-hide and under-hide respectively. For example, if a lizard started the aerial predator trial in quadrant FAHR (far above hide right) and ended its flee in quadrant LH (left of hide), that individual moved five quadrants (**Figure 16c**). If a lizard did not move or flee, the distance was recorded as zero. If it was not obvious whether the lizard perceived the predator, quadrants moved was recorded as missing data (i.e., NA; only occurred in the terrestrial trials, n=5).

Statistical analysis

All plots and analyses were created in RStudio version 4.3.0 (R Core Team, 2023) with packages ‘tidyverse’ (Wickham et al., 2019), ‘rcompanion’ (Mangiafico, 2023), ‘ggpubr’ (Kassambara, 2023a), ‘pwr’ (Champely, 2020), and ‘rstatix’ (Kassambara, 2023b). Nonparametric tests were used when data was not normally distributed, determined via Shapiro-Wilks Tests and diagnostic plots. All relationships between environmental measures (interstate distance, noise level, air temperature, substrate temperature, and cloacal temperature) were conducted via Persons’ correlations. A priori testing revealed anti-predator behavior variables did not differ by order of anti-predator trial (all $p > 0.2$). Therefore, it was not included as a random effect in further analyses. All analyses used a significance level of $p = 0.05$.

Morphological comparisons

The residuals from a linear regression between SVL (mm) and mass (g) were used as a measure of body condition (Peig & Green, 2009). Residual values above the line of isometry represent lizards in good body condition, while those below represent bad body condition relative to the population (Pearson's correlation: $r(29) = -0.55$, $p = 0.001$). Tail status was condensed into two categories, "actively regrowing" (regrowing) and "not actively regrowing" (intact and regrown), following the same methodology from Sermersheim et al. (in preparation). There were 29 lizards not actively regrowing tails and 2 actively regrowing. All statistical analyses using the original three tail status (Kruskal-Wallis) group yielded similar results as those conducted with the condensed categories (Wilcoxen Rank Sum). Similarly, throat color types were condensed into groups based on the color most lateral on the throat: "Yellow", "Orange", and "Blue." After condensing, there were 23 orange individuals, 5 yellow, and 0 blue, therefore Wilcoxen Rank Sum Tests were used to make all throat color comparisons. 3 individuals had throat colors that were not recorded at time of collection and were not included in analyses. The consolidated tail status and throat color groupings were used in the current study to remain consistent with our previous research.

All but one comparison conducted between body condition or body temperature by **(1)** distance to the disturbances, **(2)** environmental measures, and **(3)** in-situ behaviors (aggressive – number of push-ups performed and thermoregulatory – time spent basking) were conducted via Spearmans' correlations. The relationship between body temperature and distance to the path was calculated with a Pearson's correlation. Analyses between **(1)** tail status, **(2)** throat color, **(3)** injury and scarring presence, and **(4)** mite presence were all performed via Wilcoxen Rank Sum Tests except one. The comparison between distance to the interstate and presence of injury or scarring utilized a two sample T-test (Cohen's d for effect size).

In-situ and arena-based behavioral comparisons

All but one analysis between in-situ behaviors by **(1)** environmental measures and **(2)** distance to the disturbances were conducted via Spearmans' correlations – the relationship between distance to the path and time spent basking required a Pearson's correlation. Comparisons between **(1)** exploratory and **(2)** anti-predator variables according to **(1)** environmental measures, **(2)** distances to the disturbances, **(3)** in-situ behaviors, were ran by Spearman's correlations. Exploratory and anti-predator variables comparisons by morphology were conducted via Spearmans' correlations and Wilcoxen Rank Sum Tests.

Comparisons between predator stimulus type (aerial versus terrestrial) by **(1)** latency to flee and **(2)** quadrants fled were conducted by paired Wilcoxen Rank Sum Tests. Latency to flee from an aerial predator was kept as a continuous variable to relate it to the latency to flee from the terrestrial predator. The relationship between predator stimulus type and proportion of lizards that fled was ran via McNemar's Chi-square test.

Results

Environment

Distance from the interstate was negatively correlated with sound level – as distance from I-15 increased, sound level declined (r(29) = -0.66, p = <0.001). Air temperature was positively correlated with both perch substrate temperature (r(29) = 0.42, p = 0.018) and body temperature (r(29) = 0.67, p = <0.001). Perch substrate temperature was also positively correlated with body temperature (r(29) = 0.37, p = 0.39).

Morphological differences based on distances to the disturbances

Body condition was strongly, positively correlated with distance from the interstate (**Figure 17**; Spearman's r: r (29) = 0.72, p = <0.001). As distance from I-15 increased, body condition improved. No other morphological differences were detected (Wilcoxen Rank Sum: **presence of mites** (z = -0.672, p = 0.52); **tail status** (z = -0.241, p = 0.84); **throat color** (z = -0.36, p = 0.74); Two-sample t-test: **presence of injury or scarring** (t(29) = 1.11, p = 0.28); Spearman's r: **body temperature** (r(29) = 0.062, p = 0.739).

Body temperature was negatively correlated with distance from the walking path (Pearson's r: r(29) = -0.5, p = 0.004). As distance from the walking path increased, body temperature declined. No other morphological differences were detected (Spearman's r: **body condition** (r (29) = 0.12, p = 0.0.51); Wilcoxen Rank Sum: **presence of mites** (z = 0.752, p = 0.47); **tail status** (z = 1.05, p = 0.32); throat color (z = -0.09, p = 0.95); **presence of injury or scarring** (z = 0.113, p = 0.93).

In-situ behaviors

There were no statistically significant relationships between in-situ behaviors and distance to the interstate or path (all $p > 0.2$). However, there is a negative correlation between time spent basking and number of push-ups performed, as previously found in Sermersheim et al. (in preparation) (Spearman's r: r(29) = -0.54, p = 0.002).

There was a marginal relationship between time spent basking and number of quadrants fled from an aerial predator – as time spent basking increased, lizards fled more quadrants (Spearman's r: r(29) = 0.35, p = 0.052). No other relationships were detected between in-situ behaviors and arena-based behaviors (all $p > 0.1$).

Arena-based behaviors

There was no significant relationship between exploratory and anti-predator latency to move by distance to the disturbances (see **Table 7** for all statistics). Only one statistic marginally supported the relationship between number of quadrants fled and distance to a disturbance: individuals perched farther from the path fled fewer quadrants from the terrestrial predator (Spearman's r: r(24) = -0.35, p = 0.0783). Otherwise, there was no evidence to suggest a connection between exploratory and anti-predator movements and distance to the disturbances.

There was a significant difference between predator stimulus type and latency to flee (**Figure 18a**). Lizards fled more quickly from the aerial predator than the terrestrial predator (Paired Wilcoxen Rank Sum: z: -4.21, p = <0.0001). Evidence suggested a marginal relationship between predator stimulus type and number of quadrants moved, where lizards fled more quadrants from the aerial predator than the terrestrial predator (**Figure 18b**; Paired Wilcoxen Rank Sum: $z = 1.74$, $p = 0.086$).

There was evidence to support a difference in the proportion of lizards that fled based on predator stimulus type (**Figure 19**). The proportion of lizards that fled from the terrestrial predator was higher than the proportion that fled from the aerial predator (McNemar's Chi-square: $X^2(1, n = 57) = 8.33$, $p = 0.004$).

Linking behavior, morphology, and environment

There were no statistically significant relationships between *in-situ* behaviors and morphology (all $p > 0.2$). Perch substrate temperature was negatively correlated with time spent basking – as perch temperature increased, time spent basking decreased (Pearson's r: $r(29) = -0.4$, $p = 0.026$). No other significant relationships were found between *in-situ* behaviors and environmental measures (all $p > 0.09$). Similarly, there were no significant relationships between arena-based behaviors and morphology (all $p > 0.09$), nor environmental measures (all $p > 0.2$).

Discussion

Summary

We observed morphological differences in body condition and body temperature based on distance to the disturbances. As predicted, lizards perched farther from the intestate had better body condition than those perched near I-15, but there was no effect of distance to the walking path on body condition. Body temperature, correlated with both perch substrate and air temperature, was lower in individuals that perched farther away from the walking path. There was no effect of distance to the disturbances on throat color, tail status, scarring and injury presence, and mite presence.

There was no evidence to support a relationship between time spent basking or number of push-ups performed by distance to both interstate and walking path disturbances, but there was a negative correlation between time spent basking and number of push-ups performed, similar to our other study (Sermersheim et al., in preparation). Only marginal support was found for a relationship between *in-situ* and arena-based behaviors - as time spent basking increased, lizards fled more quadrants from aerial predators. Contrary to our predictions, we did not observe differences in latency to move based on distance to both interstate and walking path disturbances. The only difference detected in number of quadrants moved by distances to both disturbances was a marginal - lizards perched farther from the path fled fewer quadrants from the terrestrial predator. Although distance to both disturbances did not appear to significantly affect behavior, lizards fled more quickly and moved marginally more quadrants from the aerial predator than the terrestrial predator, suggesting lizards may be able to distinguish predators by type and adjust their latency and movement accordingly. In a simulated in-situ predator attack, most wall lizards (*Podarcis muralis)* fled to nearby hides, yet some individuals fled without hiding, choosing to stop and tongue flick (Amo et al., 2005). This response may help lizards avoid entering refuges that contain predators. In the same study, lizards placed in an open terrarium, some with smooth snake (*Coronella austriaca)* chemical cues, some without, increased their number of tongue flicks, and number of movements in areas with the snake scent. This flexibility in behavior may help lizards effectively navigate situations in which multiple predator types are present. In the current study, a higher proportion of lizards fled from the terrestrial model than the aerial model, potentially describing a difference in perceived risk by predator type.

Finally, there were no links detected between aggressive and thermoregulatory behaviors by morphology, but as perch substrate temperature increased, time spent basking decreased. We did not observe any connections between exploratory and anti-predator behaviors by morphology or environmental factors, contrary to our predictions.

Morphological comparisons

As predicted, body condition was worse the closer a lizard's perch was to the interstate. There are few studies that directly compare body condition and distance to a roadway, but this result is consistent with other studies that found reduced body condition in urban environments (French et al., 2018; Lazić et al., 2017; Thawley et al., 2019; Winchell et al., 2019). A decline in body condition can have serious impacts on a wide range of physiological, immunological, and behavioral processes, and vice versa. For example, corticosterone has been shown to negatively impact body condition in red-sided garter snakes (*Thamnophis sirtalis parietalis*) (Lutterschmidt & Maine, 2014). Stressful situations induce corticosterone release, so if lizards that have withstanding poor body condition are continually exposed to stressful situations, body condition may only continue to decline (Kechnebbou, 2019). This feedback loop may have impacts on the lifespan of lizards with home ranges near the highway, a lifespan which is already inherently shortened by living in an urban area (Lucas & French, 2012). Not only are lizards perched near the highway more likely to encounter predators, but also, these lizards exist in an urban environment. Side-blotched lizards that live in urban areas have lower immunity and elevated corticosterone and oxidative stress levels compared to those in rural populations (Lucas & French, 2012). So, perching near the interstate may compound the effects of living in an urban environment.

Surprisingly, there was no relationship between body condition and distance to the walking path. This could be due to several factors. First, we observed a relationship between body temperature and distance to the path - lizards perched closer to the path had a higher body temperature than those farther away. It has been shown in numerous studies that warmer environments may aid in wound healing (Fox et al., 1990; Wilson, 1992), including within this same population of side-blotched lizards (Hudson, 2023). Therefore, it is possible that perching closer to the path may speed up healing. This may also suggest that lizards can detect their body condition and make active behavioral decisions to remedy their condition. Second, we may not have detected an effect of walking path distance to body condition because the individuals with worse body condition may not have approached the path. Although not observed in our study, ectoparasite load was higher in common-wall lizards (*Podarcis muralis*) that inhabited high-tourist level sites with many walking paths in the Guadarrama mountains in Spain (Amo et al., 2006). Additionally, the lizards had worse body condition at the high-tourist level sites. If a lizard is already in poor body condition, the cost of approaching the path and potentially being exposed to more mites or parasites may be too high. Third, anti-predator behaviors may depend on body condition (Beale & Monaghan, 2004). The trail at Confluence Park is used by humans and animals. Perching near the walkway may not be feasible if a lizard is already in bad body condition because it may not perform anti-predator behaviors as effectively as it should to remain alive (Lima, 1998; Lima & Dill, 1990; Ydenberg & Dill, 1986).

In our previous study, we found evidence to suggest that urban sites had more lizards actively regrowing their tails than rural sites (Sermersheim et al., in preparation). The higher abundance of predators in urban environments seemed like a potential explanation as to why we detected this difference, yet we saw no effect of distance to the disturbances on tail status in the current study. One explanation as to why we did not see a relationship could be linked to predator habituation. Although lizards near the highway were exposed to more predators, they may have decreased their response to predators because they are repeatedly exposed to them (Rankin et al., 2009). Therefore, lizards near the highway may be able to effectively escape predators without dropping their tail because they have habituated to the stimuli.

In-situ and arena-based behavior comparisons

It is extremely important to consider thermoregulatory behaviors when analyzing any data that relates to body condition, due to its direct link to survivorship and development (Adolph & Porter, 1993). In our previous study, we found marginal support for a negative correlation between time spent basking and distance to the disturbance (Sermersheim et al., in preparation). Spending a shorter amount of time spent basking near disturbances could be explained by the increased temperature of the interstate or walkway substrate – the black asphalt used to create the pathway has a higher heat capacity than the natural flora and fauna (Pan et al., 2017). Therefore, a lizard may bask for a shorter duration near the disturbance, a theory that would also support the results of our current study in which lizard body temperature is higher near the walking path.

Contrary to our prediction and past results, there was no evidence to support a relationship between time spent basking or number of push-ups performed by distance to the disturbances. Male push-up displays, or sexually selected signals used during courtship and territory defense, are key behaviors used to determine a lizard's allocation of energy into reproduction (Carpenter & Ferguson, 1978; Ferguson, 1971; Stamps, 1977; Ventura et al., 2023). Indicators of high intensity displays have higher conspicuousness: more push-ups are performed, more legs are used, and extreme lateral flattening is present (Albuquerque et al., 2023; Brandt, 2003; Carpenter & Ferguson, 1978; Martins, 1991). Because lizards near the disturbances were not performing fewer push-ups than those found farther away, their relative conspicuousness may be the same, despite a higher risk of predation. A study analyzing the geographic variation of side-blotched lizard behavior found that lizards generally perform fewer aggressive displays and have reduced conspicuousness in high predator density sites (Zani et al., 2013). The idea that lizards are able to rapidly adjust their conspicuousness in response to a predator via reduction in aggressive behavior, has been shown in male brown anole lizards (*Anolis sagrei*), which decreased their push-up display rate after an attack from a model kestrel (Simon, 2007). Our results may provide further evidence to support the side-blotched lizard's ability to habituate to an urban environment.

Consistent with our previous study (Sermersheim et al., in preparation), there was a strong, negative correlation between the amount of time spent basking and the number of push-ups performed. Because this result was found regardless of site type (urban versus rural) and regardless of distance to the disturbances, this tradeoff between body condition/metabolism with reproduction seems to be an important life history pattern across populations. Side-blotched lizards experiencing high levels of physiological stress, characterized by elevated CORT/oxidative stress hormone levels and suppressed immunity in urban areas of St. George, UT, USA, had higher reproductive output, yet decreased survival compared to those living in rural areas (Lucas & French, 2012). There is evidence to suggest that lizards living closer to roads have elevated CORT levels (Kechnebbou, 2019), which in turn, negatively impacts body condition in reptiles (Lutterschmidt & Maine, 2014), as discussed previously. This supports our result that body condition was lower for lizards perched near the highway.

We found a marginally significant relationship between *in-situ* and arena-based behaviors - as time spent basking increased, lizards fled more quadrants from aerial predators. Because lizards frequently bask at the apex of taller rocks to maximize radiative heat gain, birds may have an easier time spotting them. We found lizards perched on both dirt and rocks, but most (61%) preferred to perch specifically on top of the white retaining wall rocks. Perhaps the white rocks offer the perfect thermoregulatory conditions (Goller et al., 2014), but lizards are more conspicuous on white rocks compared to the red or even brown rocks found at Confluence Park. Balearic lizards (*Podarcis lilfordi*) approached by a human predator from above had a longer flight initiation distance (FID) and fled a farther distance compared to approaches from below (Cooper & Pérez-Mellado, 2011). This study suggests lizards may perceive differences in predator approach direction and speed and adjust their response accordingly (Cooper et al., 2010).

Our results suggest side-blotched lizards can adjust their anti-predator behaviors by predator approach type. Like the Balearic lizards mentioned above, side-blotched lizards fled significantly more quickly (higher latency to flee) and marginally more quadrants from the aerial model approaching from above, than the terrestrial model approaching at eye level. The opposite result was observed in a similar experiment analyzing escape behaviors of populations of side-blotched lizard in Washington, Nevada, and Oregon, USA, from using model predators. Researchers found that escape responses (FID, distance fled, and refuge entry) did not differ by model predator type, suggesting the lizards use more generalized escape responses (Wagner & Zani, 2017). A higher proportion of lizards in our study fled from the terrestrial model than the aerial model, potentially describing a difference in perceived risk by predator type. One reason why Wagner & Zani (2017) may not have detected differences is because they used two terrestrial based predators; it may be harder for a lizard to perceive the risk between two predators differently if they both use the ground (Stankowich & Blumstein, 2005). By including an aerial predator in our study, we were able to analyze perceived differences in risk, perhaps by locomotion type, approach direction, or speed.

The optimal escape theory model developed nearly 40 years ago by Ydenberg and Dill (1986) describes the relationship between predator approach and optimal time to flee (Cooper & Frederick, 2007; Martín et al., 2009). FID represents the intersection of the cost of remaining still, like potentially becoming the predator’s next meal, and the cost of fleeing, like upregulated CORT or oxidative stress hormones. Because the latency to flee was shorter for the aerial model, our results suggest that side-blotched lizards reach that intersection more quickly for aerial versus terrestrial predators. Phrynosomatid lizards fled more quickly from human predators as their speed and directness of approach increased (Cooper & Sherbrooke, 2013), a result that could explain why side-blotched lizards fled more intensely from the aerial model.

Contrary to our predictions, we did not observe differences in any exploratory behavior measured, nor latency to move based on distance to the disturbances. The only difference detected in number of quadrants moved by distance to the disturbances was a marginal difference - lizards perched farther from the path fled fewer quadrants from the terrestrial predator. These results may highlight again the ability for side-blotched lizards to habituate to anthropogenic disturbances. Alternatively, because the lizards have short generation times and high replacement rates (Tinkle, 1965), we may have only collected data at a site where lizards have evolved and adapted their exploratory behaviors to best suit their environment. In urban areas, where lizard lifespan is even shorter compared to rural areas (Lucas & French, 2012), this may especially be true, considering the above-state average population growth of St. George since the 1970s (Pacific Northwest Regional Economic Analysis Project (PNREAP), 2023). Assuming lizards at Confluence Park live for one year on average, nearly 50 years have passed since I-15 was open to the public in St. George (Washington County Historical Society, 2022a), and 25 years since the original trailhead near the park was constructed (Washington County Historical Society, 2022b).

Finally, there were no links detected between aggressive and thermoregulatory behaviors by morphology, nor any connections between exploratory and anti-predator behaviors by morphology or environmental factors. But as perch substrate temperature increased, time spent basking decreased, highlighting the importance of analyzing behavior not only on a macro, site wide scale, but also at a microhabitat scale, a result taken away from our previous study as well (Sermersheim et. al, in preparation).

Conclusion

In summary, our study had four main takeaways. First, body condition was worse near the highway, and body temperature was higher closer to the recreational walking trail. Second, we observed a strong, negative correlation between time spent basking and number of pushups performed, the same result that we observed in our previous study. Third, a higher proportion of lizards fled from the terrestrial model, but latency to flee was shorter and number of quadrants fled was higher for the aerial model. Fourth, there was no relationship between any morphological or environmental variable measured by exploratory behavior.

Our results highlight the diversity of urban habitats and the responses lizards use to cope with disturbances. More particularly, behavioral plasticity may allow lizards to respond optimally to different predator types existing in Confluence Park. However, habituation has the potential to decrease short- and long-term fitness if a lizard interprets predator clues incorrectly, or if new predators migrate into the area with unknown risks. Lastly, our study suggests that the presence of Interstate-15 may compound the impacts of urbanization. The consistent upregulation of CORT due to anthropogenic stress has negative implications on survival, a result that has already been seen in urban populations of side-blotched lizards in St. George, UT, USA. Life history strategy differences between urban and rural environments may only be exacerbated as urbanization continues.

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Tables and Figures

Table 7

Statistical Analyses of Exploratory and Anti-Predator Behaviors by Distance to the Disturbances

	Exploratory		Anti-predator: Aerial		Anti-predator: Terrestrial	
	Latency	Quadrants moved	Latency	Quadrants moved	Latency	Quadrants moved
Distance to the interstate	r(28) = 0.23 p = 0.223	r(28) = -0.11 p = 0.553	r(29) = 0.14 p = 0.44	Spearman's r: r(29) = -0.2 p = 0.275	r(24) = 0.16 p = 0.445	r(24) = -0.11 p = 0.601
Distance to the walking path	r(28) = -0.19 p = 0.309	r(28) = 0.21 P = 0.276	r(29) = 0.13 p = 0.493	Pearson's r: r(29) = -0.12 p = 0.455	r(24) = 0.09 p = 0.662	r(24) = -0.35 p = 0.0783

Note. All analyses used Spearmans' correlations except quadrants moved from an aerial predator. Only one statistic marginally supports a potential link: individuals perched farther from the path fled fewer quadrants from the terrestrial predator (r(24) = -0.35, p = 0.0783).

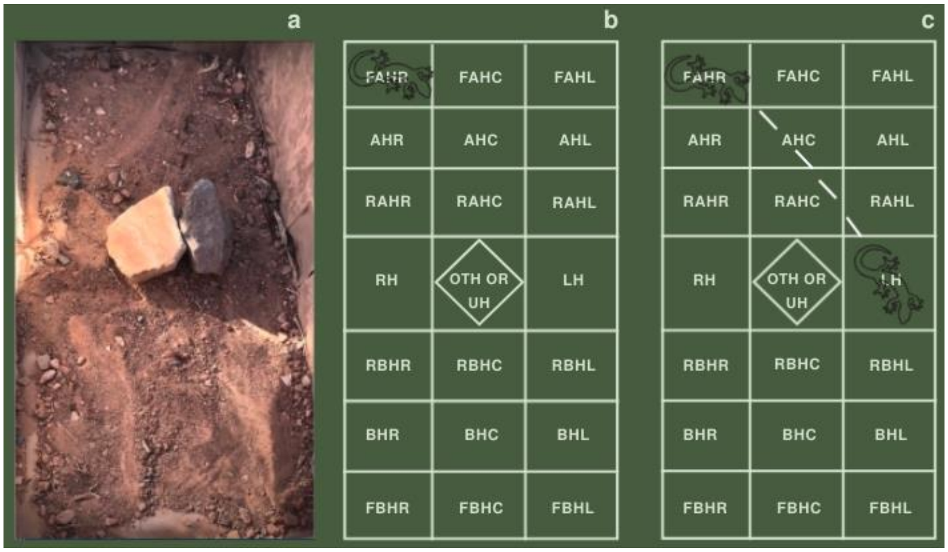
Figure 15

I-15 Overpass and Collection Distribution

Note. 15a) I-15 overpass along the Confluence Park Virgin River Trail. All lizards found close to the interstate were found in the rock bed seen in the image. 15b) Distribution of lizards found along the trail (n=31). Each point represents a unique lizard.

Figure 16

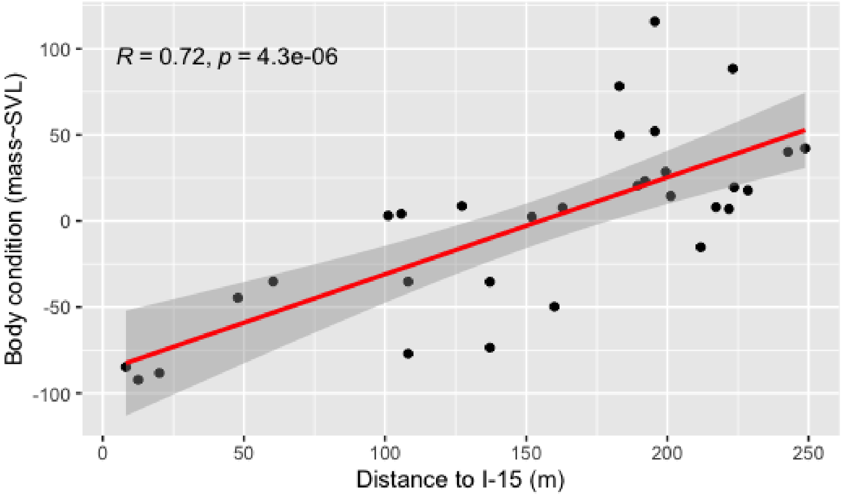
Arena Set-up



Note. 16a) Top-down photo of the arena. 16b) Arena grid with lizard beginning on FAHR (far above hide right). 16c) Lizard moving to quadrant LH (left of hide). Number of quadrants moved = 5.

Figure 17

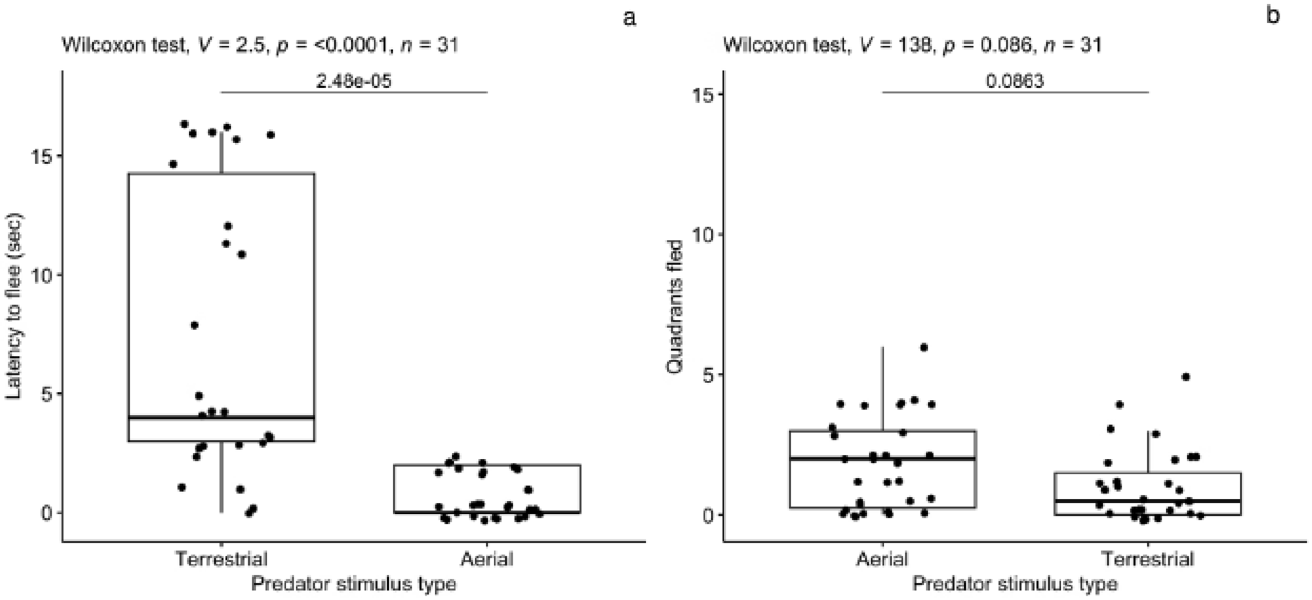
Distance to I-15 by Body Condition



Note. Spearman’s r: r(29) = 0.72, p = <0.001.

Figure 18

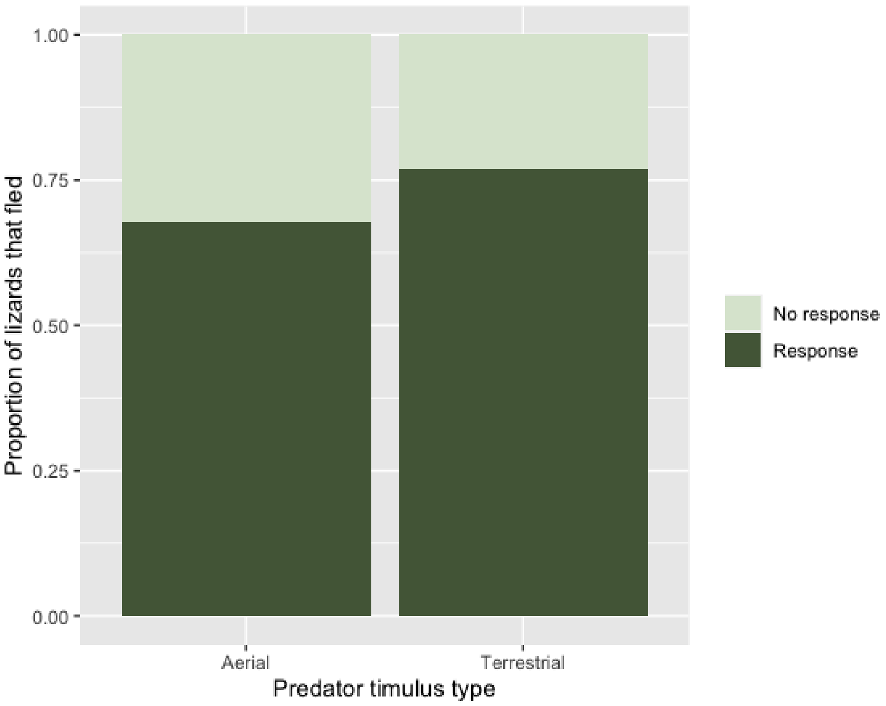
Predator Stimulus Type by Latency to Flee and Quadrants Fled



Note. 18a) A significant relationship exists between predator stimulus type and latency to flee. Lizards fled faster from the aerial predator than the terrestrial predator (Paired Wilcoxen Rank Sum: z: -4.21, p = <0.0001). 18b) A marginally significant relationship exists between predator stimulus type and quadrants fled. Lizards fled more quadrants from the aerial predator than the terrestrial predator (Paired Wilcoxen Rank Sum: z = 1.74, p = 0.086).

Figure 19

Predator Stimulus Type by Proportion of Lizards that Fled



Note. The proportion of lizards that fled from the terrestrial predator was higher than that of the aerial predator (McNemar's Chi-square: $X^2(1, n = 57) = 8.33$, $p = 0.004$).

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CHAPTER 5

CONCLUSIONS

Within my three data chapters, I aimed to evaluate the impacts of anthropogenic disturbances and urbanization on the behavior and morphology of two free-living lizard species: the common side-blotched lizard and the Colorado checkered whiptail. Although these species differ taxonomically, morphologically, reproductively, and behaviorally, both are doing seemingly well in their anthropogenic disturbance-ridden environments. I first compared the behavioral and morphological differences between side-blotched lizards that lived in urban versus rural areas of St. George, UT, USA. Then, to explore how particular disturbances affect within-site measures of behavior and morphology, I observed the Colorado checkered whiptail's responses to military flyover noise at Fort Carson Military Base in Colorado Springs, CO, USA. Lastly, I returned to St. George to explore the relationship between the distance of a lizard's perch to an on-site interstate, exploratory and anti-predator behaviors, and morphology.

In Chapter 2, I explored the behavioral and morphological effects of urbanization on side-blotched lizard populations across the urban gradient of St. George. By comparing environmental measures like air and perch substrate temperature, I was able to provide evidence of the urban heat island (UHI) effect in urban areas of St. George; on average, urban sites were 3°C warmer than rural sites. Although I observed temperature difference between sites, there was minimal evidence to support site types impacted lizard behavior. One of the most prominent results to come from this study was the tradeoff between thermoregulatory and aggressive behavior. Regardless of site type, lizards that spent more time basking performed fewer push-ups. This relationship may provide evidence for the life-history strategy divergence occurring between urban and rural populations of side-blotched lizards in St. George. Urban lizards have shorter lifespans, therefore invest more into reproduction than urban lizards - yet there is a cost. Because urban lizards prioritize reproduction, self-maintenance is neglected; reduced immunity and elevated corticosterone is commonly observed at urban sites. The tradeoff between basking and push-ups may support the diverging life history strategies. Push-up displays are related to reproductive success, and based off my data, lizards prioritizing and performing more push-ups spend less time basking, a behavior that is crucial for maintaining a healthy body condition. The only morphological difference found between sites was tail status – there were fewer lizards in rural areas with actively regrowing tails. This could be due to a higher predator abundance in urban sites, or other factors not measured. Ironically, lizards that were actively regrowing their tails performed more push-ups, regardless of site type. Perch use varied by tail status and time spent basking in my study, emphasizing the need for future research to dive deeper into ways lizards perches or home range microhabitats may support greater behavioral plasticity, which may counteract hotter temperatures in cities due to the UHI effect. Comparing rural and urban environments allowed me to ponder what disturbances within urban sites may have particularly detrimental effects to lizard populations living there.

In Chapter 3, I looked at physiological markers of anthropogenic induced stress responses. The checkered whiptails living in the US Army Fort Carson Military Base in Colorado are exposed to multiple disturbances, one of the most potent being aircraft flyover noise. My role in this project was to detect the behavioral modifications whiptails made during flyovers, but they also showed adjustments in corticosterone levels and energy mobilization via ketone bodies. Although a loud noise might seem to cause lizards to flee into refugia, there was no evidence to suggest lizards fled from the aircraft noise. In fact, I found the opposite. Whiptails spent less time moving during flyovers, signaling potential habituation to the anthropogenic disturbance. Habituation may suggest a reduced response and awareness of surroundings during the flyovers, which could relate to lizards spending less time assessing their environment. Indeed, this response was supported. I am extremely interested in conducting more research on this population. Not only are their behaviors unique due to their parthenogenetic nature, but they have also shown behavioral plasticity in response to an anthropogenic disturbance despite not existing in what I would consider an “urban” environment. If I were to conduct further research on this population of whiptails, I would be interested in testing whether we see other characteristics of habituation. I would design a lab experiment in which I removed whiptails from the training area, housed them in a controlled lab setting, and played a recorded audio of the flyovers at a continually decreasing intervals. If the whiptails are habituating to the noise, I would expect to see evidence of spontaneous recovery. I would also be interested in test their response to other loud noises. Perhaps there are other factors that are keeping the whiptail population stable at Fort Carson. In my second project, I again saw evidence suggesting behavioral plasticity to anthropogenic disturbances.

The goal of Chapter 4 was to determine whether there was support for within-site variation of anthropogenic disturbances and behavioral responses to the variation in side-blotched lizards. I did not observe any behavioral differences between individuals in urban and rural sites in chapter 2, suggesting there may be varying levels of disturbance within sites. The largest anthropogenic disturbance present in any of my urban sites was Interstate-15. I was interested in determining whether I could predict body condition by distance to the highway, as past literature suggests roadways significantly decrease amphibian and reptile species richness and abundance, while benefitting bird and small mammal species. At Confluence Park, there is also an extremely busy recreational walking and biking trail, adding more variation in disturbance to the environment. In addition to observing thermoregulatory and aggressive behaviors, I wanted to observe exploratory and antipredator behaviors. Because highways have been shown to attract small mammals, birds, and feral cats, I expected antipredator behaviors to vary by distances to the disturbance. By testing lizards with both an aerial and terrestrial predator, I was also able to detect whether lizards adjusted their anti-predatory behaviors according to predator type.

First, body condition was worse near the highway, but no other morphological measurements were detected. Corticosterone and oxidative stress hormones have already been shown to be upregulated in urban sites in St. George and are linked to a shorter lifespan and poor body condition. Lizards perched closer to the interstate also had poor body condition, suggesting the highway may only compound more stress on top of the urban environment. Lizard body temperature was higher near the walkway, potentially due to the black paved asphalt of the trail. The same strong, negative correlation between time spent basking and number of pushups performed was observed again, but within a site. Because I found this result regardless of site type, and now, regardless of distance to the disturbances, I believe this tradeoff between body condition/metabolism with reproduction seems to be an important life history pattern across populations.

I am interested in collecting data within rural sites near St. George to see if I find the same relationship. Although I did not see a relationship between latency to flee from a predator and distance to the disturbances, latency to flee was shorter and number of quadrants fled was higher for the aerial model. This result was more fun to find than my initially predicted relationship to the disturbances because there is now significant evidence to support that side-blotched lizards may adjust their behaviors in response to predator type. Behavioral plasticity is beneficial for wildlife living in urban environments due to rapidly changing predator composition and abundance; it may allow lizards to respond optimally to different predator types existing in Confluence Park, contributing to the narrative that although life history strategies are diverging between urban and rural areas, urban side-blotched lizards have maintained a steady population.

The results from my thesis research have contributed knowledge on the effects of urbanization, noise, and roadways on reptile behavior and morphology. Because the variability between and within urban environments is extremely wide, it is difficult to say whether my findings will ever accurately apply to another lizard species in an urban environment. However, one result that I consistently found throughout my research was the ability of behavioral modifications and responses to aid in lizard adaptation and acclimatization. Plastic behaviors, like habituation, may allow lizards to prioritize novel signals, benefitting their fitness in the long run.

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APPENDICES

Appendix A: Publisher Permission and Copyright Guidelines

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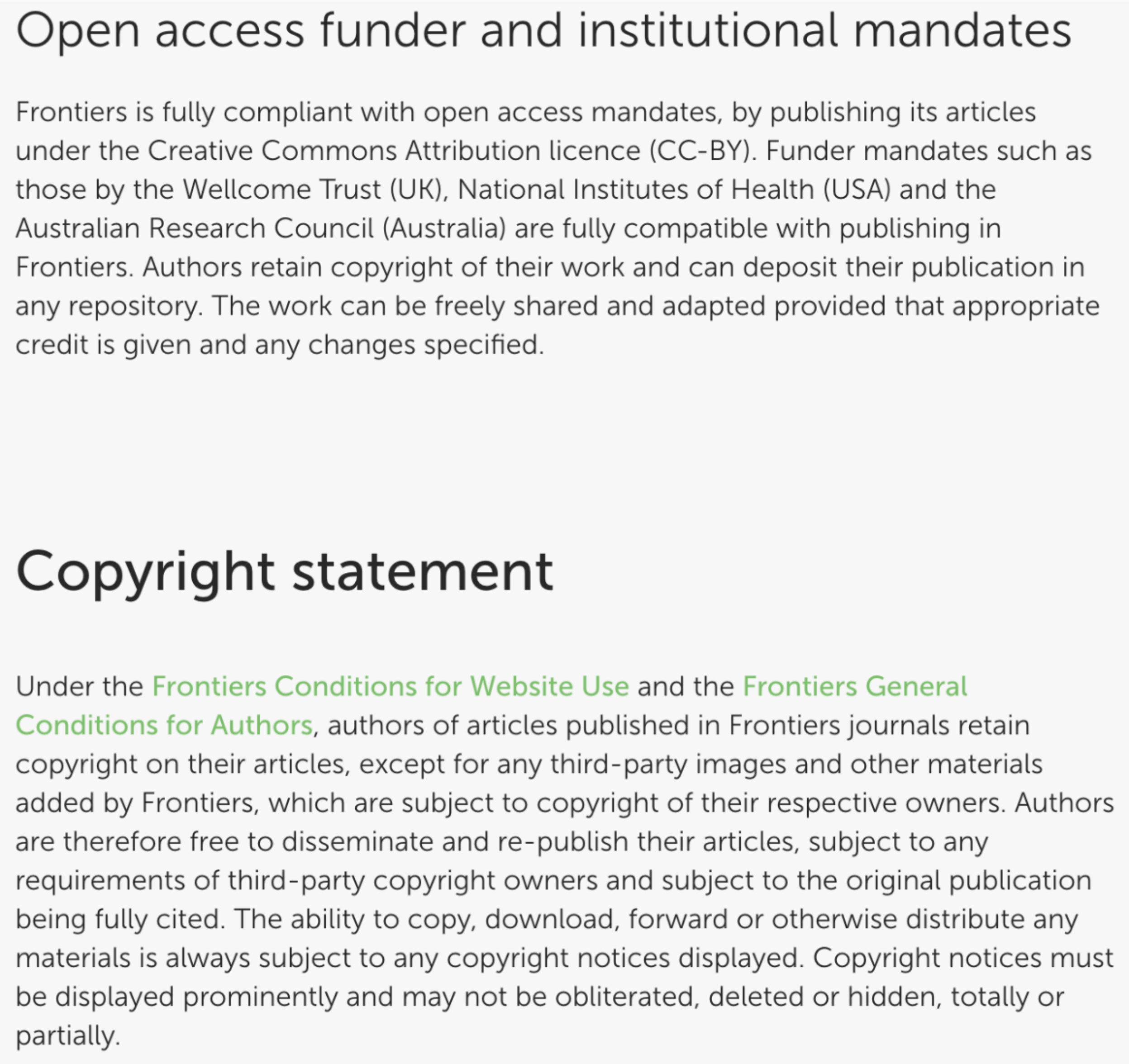

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Utah State University
Logan, UT, USA 84322

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Megan E. Kepas, PhD
Assistant Professor
Utah Tech University
St. George, UT, USA 84770

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Department of Biology
Utah State University
Logan, UT, USA 84322

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Sincerely,

Spencer B. Hudson, PhD
Wildlife Biologist
APHIS Wildlife Services (WS)
WS National Wildlife Research Center
Florida Field Station
2820 East University Avenue
Gainesville, FL, USA 32641

July 8th, 2024

Layne O. Sermersheim
Department of Biology
Utah State University
Logan, UT, USA 84322

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Anna Joy J. Lehmicke
Eagle Biologist – Migratory Birds Science Applications
U.S. Fish & Wildlife Service
134 Union Boulevard
Lakewood, CO, USA 80228

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Layne O. Sermersheim
Department of Biology
Utah State University
Logan, UT, USA 84322

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Susannah S. French, PhD
Associate Dean for Research and Faculty, College of Science
Professor of Biology
Utah State University
Logan, UT, USA 84322

July 8th, 2024

Layne O. Sermersheim
Department of Biology
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Assistant Professor
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Fort Collins, CO, USA 80523