Behavioral Responses to Thermal Variation in a Color Polymorphic Lizard

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Abstract

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The Thermal Biology of Behavior: Implications for a Changing Climate

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Climate change is creating novel thermal environments worldwide. The alteration of thermal niches is especially challenging for ectotherms due to their reliance on body temperature for nearly all aspects of organismal performance. Characterizing the relationships between performance and temperature is therefore critical in predicting how ectotherm species and populations will persist in rapidly changing and challenging environments. To do so, scientists often characterize thermal traits with respect to aspects of physiological performance. In challenging environments, organisms will use behavioral adjustments to either enhance heating or avoid overheating. Behavior also influences many critical aspects of ecology and ultimately fitness. However, despite its ecological relevance and underlying physiological basis, behavior has largely been ignored in analyses pertaining to altered thermal niches. This dissertation explores multiple relationships between temperature and behavior in the ornate tree lizard, *Urosaurus ornatus*. As different species cope with novel thermal environments, elucidating the nature of these relationships will provide insight into how species may navigate altered thermal niches from both behavioral and physiological perspectives. First, I investigate the thermal sensitivity of push-up display rate, a social communication behavior utilized by *U. ornatus*. Display rate is influenced by an interaction between body temperature and microhabitat use, where body temperature influences display rate

in different directions and magnitudes depending on the microhabitat from which the lizard displays from. Second, I quantify how thermoregulatory behavior is influenced by the presence of conspecifics. Social interactions with individuals of multiple sexes and color morphs result in individual lizards adjusting their selected body temperatures to varying degrees. Third, I assess covariation of behavioral and thermal preference traits between color morphs. Dominant male morphs are bolder, more exploratory, and prefer higher body temperatures than subordinate morphs, which helps explain habitat use variation observed between the two groups. Last, I quantify the influence of heat stress on boldness behavior. When lizards undergo a heat hardening response, a cellular mechanism that temporarily increases thermal tolerance in response to unfavorable environmental conditions, they also increase boldness expression, which could influence the adaptive potential of heat hardening as a response to heat stress. Together, this dissertation documents a suite of behavioral traits that mediate how a model species interacts with its environment. Assessing relationships between these phenotypes and temperature provides novel insight into how rising temperatures and altered thermal niches attributed to climate change may influence behavioral expression, social structure, and survival.

Dedication

To Mom and Dad,

for making me go outside

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Chapter 1: Introduction

Background Information

Anthropogenic climate change is resulting in rising average temperatures worldwide as well as increased frequency and severity of extreme climate events, such as heat waves and droughts. These changes are resulting in altered thermal niches that threaten biodiversity worldwide but are particularly challenging for ectotherms due to their reliance on external sources to maintain body temperature (Deutsch *et al*., 2008; Huey *et al*., 2009; Huey *et al*., 2012). Further, body temperature influences nearly all aspects of ectotherm function and performance, including processes and traits such as metabolic rate, activity, locomotion, and growth rate (Huey & Stevenson, 1979; Bennett, 1990; Angilletta *et al*., 2004). Scientists often characterize the relationships between body temperature and performance using thermal performance curves (TPCs). These describe the range of body temperatures in which performance can occur (thermal tolerance breadth) as well as the upper and lower thermal limits of activity (CT_{max} and CT_{min}, respectively). Performance exhibits a gradual increase with body temperature up to a thermal optimum (T_{opt}), after which performance drastically declines with small temperature increases (Huey & Stevenson, 1979). Many ectotherms are adapted to their local thermal conditions, maximizing performance by maintaining body temperatures at or near T_{opt} via evolutionary processes and behavioral adjustments. However, the rapid pace of climate change and associated temperature increases threaten performance reduction and mortality as field active body temperatures exceed T_{opt}. Characterizing the thermal sensitivities of performance traits is critical when assessing potential species responses to rapid alterations of thermal environments.

The majority of these studies have documented the thermal sensitivity of locomotor performance, either measured as sprint speed or endurance capacity (e.g., Hertz *et al*., 1983; Huey *et al*., 2009; Artacho *et al*., 2013; Gilbert & Miles, 2017). Locomotion is a whole-organism performance that is often used as a proxy for survival due to relevant ecological ramifications such as predator escape ability and dominance (Robson & Miles, 2000; Miles, 2004). Suites of studies on the thermal sensitivity of locomotor performance have concluded that tropical, subtropical, and desert ectotherms are particularly vulnerable to climate warming because they are already living in environments with temperatures near performance optima (Miles, 1994; Deutsch *et al*., 2008; Huey *et al*., 2009; Gunderson & Leal, 2012; Gilbert & Miles, 2017). Thus, even moderate temperature increases in these environments are expected to approach CT_{max} of resident ectotherms, threatening drastic performance reduction and restricted available hours of activity. However, estimates of species persistence in altered climates based on the thermal sensitivity of locomotor performance are likely incomplete. Gunderson and Leal (2015) documented that patterns of activity in an anole lizard (*Anolis cristatellus*) were temperature dependent in a manner that was uncoupled with the thermal sensitivity of sprint speed. Specifically, activity rate is more sensitive to temperature than sprinting as indicated by a narrower thermal performance breadth. Therefore, rising temperatures will likely influence some aspects of behavior more severely than locomotor performance, highlighting the need for documenting the relationships between temperature and other aspects of organismal performance when considering species responses to climate change (Gunderson & Leal., 2015).

Behavior plays an important role in mediating an organism's interaction with its environment. Behaviors such as thermoregulation, habitat use, social interactions, territory maintenance, and courtship carry important ecological ramifications and influence survival and reproduction. Behaviors are rooted in motor patterns that have a physiological basis (Hasselmo, 1995) and are therefore expected to be temperature dependent. Despite this, the relationships between behavior and temperature are rarely considered when contemplating how species will navigate altered thermal environments. Here, I assess the relationships between behavior and temperature in a model lizard species, the tree lizard (*Urosaurus ornatus*). *Urosaurus ornatus* is a territorial color polymorphic lizard that occupies thermally challenging desert environments ranging from the southwest United States to northern Mexico. Throat color morphs in *U. ornatus* males correspond to alternative reproductive strategies and are associated with dominance and presumable behavioral variation among morphs. This assessment of behavioral responses to thermal variation in a model species provides insight into how ectotherms may navigate changing environments from an understudied behavioral perspective.

Chapter Two

Many lizard species utilize push-up displays to communicate information regarding territory and consequent access to resources. The rate of push-display can vary based on factors such as the sex of the displayer, social context and structure, and exposure to predators facilitated through perch selection. Temperature may also influence display rate due to the physiological basis of displays. I quantified push-up display rate in *U. ornatus* and modeled potential determinants of display rate. Display rate exhibited thermal sensitivity and lizards were able to maximize display rate at a wide range of body temperatures. Display rate was influenced by an interaction between body temperature and microhabitat usage. Lizards displayed at different rates on exposed dead trees (snags), tree trunks, and the inner branches of trees. Display rate increased with body temperature on inner branches, decreased with body temperature on trunks, and remained constant on snags. I propose that display rate variation with body temperature in distinct microhabitats is driven by shifts in microhabitat temperature over the course of the day as well as varying levels of exposure to conspecifics and potential predators in distinct microhabitats. This study highlights the importance of the interactions between temperature, habitat structure, and social context when considering how changing thermal environments may influence behavior.

Chapter Three

Preferred body temperature (T_{pref}) refers to the body temperatures that ectotherms select given the absence of ecological cost or constraint. T_{pref} often reflects optimization of performance and can be used to model thermoregulation accuracy and effectiveness as well as extinction risk due to rising temperatures. However, the assumption of no ecological constraints is unrealistic in the field, as thermoregulation is associated with multiple costs including time, energy, and increased predation risk. Introducing constraints when collecting T_{pref} data may provide more realistic representation of thermoregulation in the field. I introduced the presence of conspecifics as a realistic thermoregulatory constraint for *U. ornatus* individuals. I assessed the influence of sex and color morph on thermoregulatory behavior by pairing conspecifics in a thermal gradient. Lizards were significantly displaced from optimal T_{pref} values by conspecifics with considerable influence of the sex and morph of the challenger. Male thermoregulatory interactions were influenced by a dominance hierarchy relating to the amount of blue pigmentation in the throat. Females also competed for basking space and were most affected by individuals of the same color morph. Males and females paired together also competed for basking space. These data provide insight into how social structure may affect thermoregulatory behavior in the field and highlights the influence of ecological constraints on thermoregulation.

Chapter Four

Distinct color morphs within a population often exhibit ecological divergence highlighted by variation in morphology, physiology, whole-organismal performance, and behavior. Variation between morphs often facilitates the long-term maintenance of color polymorphism via negative frequency-dependent selection acting on alternative reproductive strategies. Behavioral variation in traits such as boldness and exploration often underlie dominance status and mating success in lizards. Further, morphs exhibiting alternative reproductive strategies are assumed to occupy distinct ecological niches that may coincide with dissimilar habitat usage and thermal properties. Therefore, while assessing behavioral and thermal trait variation among morphs can provide insight into polymorphism maintenance and ecological niche segregation, behavioral and thermal trait covariation in polymorphic species is understudied. I quantified covariation among boldness, exploration, and thermal preference with relation to male *U. ornatus* dominance status. Dominant males were bolder, more exploratory, preferred higher body temperatures, and selected a narrower range of body temperatures than subordinate males. Dominant males have been shown to occupy larger territories in high-quality habitat compared to subordinate males. This study highlights the behavioral and thermal mechanisms that underly this ecological segregation, reinforcing the importance of documenting trait variation in polymorphic species and how behavior influences the ways in which ectotherms interact with their thermal environments.

Chapter Five

Species can utilize phenotypic shifts (plasticity) to navigate changing and challenging environments. Some ectotherms can undergo a rapid plastic response to increase thermal tolerance when exposed to heat stress in a process called heat hardening. This short-term increase in CT_{max} can confer immediate increases in survival during events such as heat waves. However, stress responses are energetically costly and often require trade-offs with other traits; for example, in *U. ornatus*, a heat hardening response is associated with a reduction in locomotor performance and thermal preference. Quantifying the nature of these trade-offs provides insight into the adaptive potential of heat hardening as a response to warming environments. I assessed the influence of heat hardening on boldness behavior in *U. ornatus* due to the ecological importance of boldness behavior in the species and pilot data suggesting that boldness is sensitive to temperature. I quantified and compared boldness behavior before and after inducing a heat hardening response in individual lizards. Boldness behavior increased with heat hardening in *U. ornatus,* specifically in males. I propose that this response is associated with the previously documented reduction in thermal preference, which is presumed to increase competition for limited shaded microhabitats in the system. This study documents a behavioral shift associated with rising temperatures and highlights the need for further assessment of how behavior expression may be influenced by altered thermal niches attributed to climate change.

Chapter 2: Habitat Use and Body Temperature Influence Push-Up Display Rate in the Tree Lizard, *Urosaurus ornatus*

Introduction

Visual communication plays a major role in conveying information across multiple contexts in vertebrate species. The information may pertain to mating status, coordination of parental care, predator detection and response, and territorial status (Hartmann *et al*., 2005; Osorio & Vorobyev, 2008). Many lizard species engage in physical displays that involve postures, exaggerated movements, and coloration as a method of communication between conspecifics (Carpenter & Grubitz, 1960; Carpenter & Ferguson, 1997; Martins, 1994; LeBas & Marshall, 2000; Radder *et al*., 2006). Displays by male and female lizards include push-ups, head bobs, extension of a dewlap or neck frills, tail movements, and lateral compressions of the body to signal dominance or aggressive intent; these displays may be critical for securing key resources, such as territory and access to potential mating opportunities (Martins, 1991; Martins, 1994). Males will display when engaged in direct competition with other males in agonistic territorial encounters, towards females in courtship, and, most often, as a broadcast without an obvious, direct recipient for territory maintenance and courtship advertising (Martins, 1993a).

Among lizard species that engage in displays, substantial variation exists in the patterns and rates of push-up displays, both between species and among populations (Martins, 1993b). Elucidating contextual causes of such variation is of interest due to the ecological role that displays play and because push-up displays are thought to be honest signals that communicate reliable information about the sender (Brandt, 2003). In lizards, males have been found to display more frequently, using different patterns than females (Martins, 1991). The context of a display can also influence display patterns and intensity. For example, the displays in *Sceloporus graciosus* differ depending on whether the sender is engaged with a conspecific (courtship behavior or agonistic contests) or general broadcasting (Martins, 1993a). Other ecological factors have been shown to influence display rate: brown anoles (*Anolis sagrei*) decrease display rate after a simulated predator attack, possibly to reduce the conspicuousness of an individual to a predator (Simon, 2007).

Whereas substantial attention has focused on biotic factors that influence display patterns, abiotic factors such as temperature and microhabitat have received scant investigation. Heterogeneity in microhabitats influence the variability of the thermal landscape as well as the detectability of the signaler (Baird *et al*., 2020). Just as variation in the thermal environment modulates physiological performance in ectotherms, it has been proposed that temperature should also limit other traits that have a physiological underpinning, such as behavior (Gunderson & Leal, 2015). As a consequence, the perch site selected by an individual may promote or constrain the vigor of displays, because of the thermal properties of the substrate. In addition, differences in visibility among microhabitats may influence display rate.

Push-up displays are influenced by endurance capacity in lizards (Brandt, 2003). Endurance capacity is sensitive to temperature, indicating that the intensity and duration of push-up displays may also covary with temperature in a manner similar to thermal performance curves. The rate at which physiological functions occur accelerates as temperature increases up to a peak, known as the thermal optimum, after which performance declines (Huey & Stevenson, 1979). Thermal optima are often at or close to the preferred temperature, that is the body temperatures that lizards select in the absence of ecological costs (T_{pref}, Hertz *et al*., 1993). In addition, T_{pref} has been demonstrated to influence social interactions in lizards, including direct correlations between T_{pref}, aggression, and courtship in mountain log skinks (*Pseudemoia entrecasteauxii*; Stapley, 2006; Baird, 2013). Individuals with higher T_{pref} values may also display at higher rates. To maximize display rates, individuals may display most effectively at body temperatures that match their T_{pref}.

In one of the few studies that has investigated the thermal sensitivity of display performance, Ord & Stamps (2017) tested three factors that could influence variation in push-up display rates in male *Anolis* lizards. They found that push-up display rate covaried with ambient temperature, with display rates increasing with temperature up to a maximum followed by a rate decrease. Male *Anolis* push-up display rates did not vary based on metabolic rate or the number of potential recipients of the display (Ord & Stamps, 2017).

I investigated both abiotic and biotic factors as possible determinates of display rate in a model species, the tree lizard (*Urosaurus ornatus*). *Urosaurus ornatus* occurs in a diversity of habitats and can be found on rocks and trees. In populations that are arboreal, lizards may be found on a range of substrates, including tree trunks, branches, and the canopy on both live and dead trees. In addition, both males and females are characterized by a throat color polymorphism that varies among populations (Hews *et al.*, 1997; Zucker, 1989). Males are characterized by a blue, orange, or yellow throat. In addition, there are mosaic morphs that include an orange or yellow background with a central blue spot. Female *U. ornatus* may have orange, yellow, or white throats (Carpenter, 1995; personal observation). Prior studies of the species have shown that the throat morphs are fixed and have divergent social roles, including variation in dominance status (Hover, 1985; Thompson & Moore, 1991a; Hews *et al.*, 1997; Moore *et al.*, 1998, Miles unpublished). The mating system of tree lizards is polygynous, with males having a despotic hierarchy (Zucker, 1989; Deslippe *et al.*, 1990). Males are territorial and perform push-up displays in dominance and courtship interactions. The displays consist of series of push-ups in which all four limbs are extended and the entire body, head, and tail of the lizard are off the perch (Carpenter & Grubitz, 1961). Push-up displays can be accompanied by lateral compression of the body and an extension of the dewlap. In addition, males have bright blue ventral patches that are exposed during displays; by broadcasting their bright ventral and throat badges during push-up displays, male tree lizards become very conspicuous. The pattern of these displays is consistent in *U. ornatus* (Carpenter & Grubitz, 1961).

I investigated whether *U. ornatus* exhibits thermal sensitivity in display rates. I measured preferred body temperatures of each lizard to determine whether individuals selected body temperatures that maximized display rates. I also examined additional factors that are relevant to the natural history of *U. ornatus* that are known to alter display behavior in other species (Baird, 2013). These include sex, throat color, body size, microhabitat use, and social context (presence/absence of conspecifics within the visual field of the displaying lizard). I tested the following predictions: 1) Lizards with higher T_{pref} values display at higher rates, 2) Lizards select for and display at body temperatures that match their T_{pref}, and 3) Lizards that display at temperatures near their T_{pref} values display at higher rates.

Materials and Methods

Study Population

I studied adult tree lizards during the reproductive season, from 5 June–23 July 2018, at the Appleton-Whittell Research Ranch (AWRR) of the National Audubon Society in southeastern Arizona (31.365° N, –110.303° W). The focal population is located within a 2-hectare site in a semi-arid grassland. The abundance of the population during the study was estimated at 100 individuals (direct count). Adult female body sizes range from 45–55 mm and males range from 46–57 mm (personal observation). At this study site adult lizards are arboreal, spending the majority of their time on live oak (*Quercus emoryi, Q. arizonica*), mesquite (*Prosopsis velutina)*, and standing dead trees (snags). Microhabitats available to lizards differ in frequency: there are ~150 live trees but fewer than 10 snags. Although there are fewer snags than live trees, their large size and exposure to the sun offer ample basking opportunities. At this site, males and females exhibit a clustered distribution whereby multiple males and females occupy the same tree. At AWRR, male throats are yellow, orange, yellow/orange, blue, or yellow/blue. Females had yellow or orange throats.

Operative Environmental Temperature

I quantified the operative thermal environment (T_e) using Thermochron iButtons (Thermochrons^{tm}, Maxim Integrated Products, Sunnyvale, CA, USA). T_e data characterizes the range of thermal microhabitats available to *U. ornatus* individuals throughout the day over the course of the study, which is important when considering how microhabitat use influences display rate. I placed iButtons in operative temperature models constructed using PVC pipe. Models matched the length and mass of adult *U. ornatus* and were painted to match the reflectivity of the lizards (Gilbert & Miles, 2017; Gilbert & Miles, 2019b). I placed seven models each on tree trunks, inner branches of trees, outer branches of trees, sun-exposed snags (dead, woody substrates), and snags in shade environments. These microhabitats are used by *U. ornatus* at the study site with thermal properties among microhabitats were expected to differ based on sun exposure throughout the day. Each iButton sampled temperature once per hour for the duration of the study, 5 June–23 July 2018.

Quantifying Display Rate

I recorded push-up display rates by visually scanning trees for lizards between 07:00 –12:00 and using focal animal sampling when individuals were visually located. Most activity ceased by 12:00 when ambient temperatures exceeded the voluntary body temperatures for lizard activity (personal observation). All observations were made by the same individual (TMG), who wore grey and brown clothes to avoid any potential influence of brightly colored clothing on lizard behavior (Putman *et al.*, 2017). Observations were made at a distance of at least 3m to avoid influencing the behavior of the focal individual. Past experiences have shown that *U. ornatus* will cease displays and flee when approached to within 1–2m (Miles, unpublished data). I found no evidence that the presence of an observer at 3m altered the display behavior of the focal lizard. I observed lizards either until they performed push-up displays or until 15 minutes elapsed without display behaviors. Displays by *U. ornatus* consist of sequences of 3-8 push-ups, which may be repeated multiple times. I counted the number of push-ups and recorded the time elapsed during the display sequence. I continued to observe a lizard until it moved to a new substrate. I quantified display rate as the number of push-ups performed over the duration of the display bout.

Field Measurements, Lizard Capture, and Husbandry

I captured lizards using a noose as soon as it changed perches to obtain a measurement of its T_b for the display. Lizards that evaded capture for over one minute after displaying were excluded from analysis due to the possibility of T_b changing from the value during display. Because different perch types (e.g., trunk, branch, or twig) vary in thermal properties, I also measured the substrate temperature (T_s) of the lizard perch. I measured T_b and T_s where the lizard displayed using an infrared digital thermometer (Amprobe IR-750), that was validated against a quick-reading cloacal thermometer (Gilbert & Miles, 2019b). I recorded the sex of lizards based on the presence/absence of enlarged post-anal scales (present in males) and the color morph of individuals as described in “study population”. Color was assessed using visual inspection. Previous studies of tree lizards at this population used spectrometry to verify color scores of males and females (Lattanzio & Miles, unpublished data). I also recorded time of capture, microhabitat type at the display site (trunk, inner branch, outer branch, snags in exposed sun, snags within the shade), and social context. To quantify social context, I surveyed the environment surrounding a displaying lizard for the presence/absence of male or female conspecifics within the presumptive visual field of the displaying lizard. In arboreal lizards, horizontal and vertical visual exposure must be considered by accounting for tree trunks and branches that obscure the body of potential recipients (Baird *et al*., 2020). I considered conspecifics present if they were in the visual field of a displaying lizard and within 3m of the individual (Martins, 1993a; Ord & Stamps, 2017). Conspecific individuals were often much closer to the signaler (within 0.5m, personal observation). It was clear when the focal individual was displaying towards a conspecific because the individual would orient towards the receiver to enhance ventral and throat coloration during display and move towards the receiver between display sequences. I considered the social context as a challenge display when the receiver matched the sex of the displayer. I considered the social context to be courtship when the receiver was the opposite sex of the displayer. I did not observe any instances of multiple recipient conspecifics. In the absence of conspecifics, I designated the social context of the displaying lizard as general broadcasting (Martins, 1993a). If the recipient conspecific was unmarked, it was captured to determine its sex. Behavior of recipient conspecifics was not recorded.

I transported lizards to a laboratory at AWRR. I measured snout-vent length (SVL) and tail length (to the nearest mm) and body mass (to the nearest 0.1 g). I considered males and females larger than SVL > 42 mm as adults (Dunham, 1982;

Zucker, 1989). During captivity lizards were housed in individual terraria and provided a thermal gradient with an upper limit at their field active body temperature (36°C) to allow for thermoregulation. Lizards were maintained on a 13hr/11hr light/dark cycle to mimic local photoperiod. Nocturnal temperatures were ambient. Lizards were offered mealworms daily and provided water *ad libitum.*

Thermal Preference

To measure T_{pref} of lizard subjects, I constructed a linear photothermal gradient using aluminum flashing on a plywood base (120 x 16 x 20 cm, L x W x H) covered with sand. I had four lanes in the experimental setup. I suspended a 100W incandescent bulb at one end of the track and a second 60W bulb in the middle to generate a thermal gradient of 27°C–45°C. Lizards were placed individually at the gradient center and allowed to acclimate for 10 minutes. I then used an infrared digital thermometer to record body temperature every 10 minutes for 90 minutes (Gilbert & Miles, 2017). I calculated T_{pref} as the average T_b from the 9 measurements and the interquartile range, T_{set}, as the central 50% of selected T_bs. Following laboratory experiments, lizards were given unique toe clips for future identification. Toe clipping has been shown to not influence performance or increase individual stress levels in lizards, nor affect survivorship (Borges-Landáez & Shine, 2003; Langkilde & Shine, 2006). Lizards were then released back to their location of capture as determined by GPS coordinates. Individuals were in captivity for no longer than one week.

Statistical Analysis

Each operative temperature model provided hourly T_e data for the duration of the study. I generated mean hourly T_e values for each of the seven models in the five microhabitats (trunks, inner branches, outer branches, sunny snags, shady snags). I used these values to obtain mean T_e values for each microhabitat over a 24 hour period.

All statistical analyses were conducted using the R statistical computing environment (v3.5.2, R Core Team, 2019). I calculated summary statistics for T_b, T_{pref}, and T_{set}. I used t-tests to test for differences in T_{pref} and T_{set} between sexes. I used mixed effects models to test for differences in T_b between sexes, using lizard ID as a random effect to account for multiple T_b recordings on individuals. All future mixed effects models (function *lme* in the package *nlme* (Pinheiro *et al*., 2019)) included lizard ID as a random effect to take multiple observations of individual lizards into account. I measured the repeatability of display behavior using an intraclass correlation coefficient using the function *ICC* in the package *ICC* (Wolak *et al*., 2012). I analyzed the relationship between T_b and T_{pref} using a mixed effects model to determine if lizards displayed at temperatures matching T_{pref}. I also calculated the difference between T_b and T_{pref} and used this variable in a mixed effects model to investigate whether lizards that were closer to T_{pref} values in the field displayed at higher rates. I used linear mixed-effects models to assess factors that explain variation in display rate. I built models including sex, throat color, microhabitat, and social context (challenge, courtship, or broadcasting) as fixed effects. I included T_b, T_s, T_{pref}, time of day, and SVL as covariates. I checked for multicollinearity between variables using the *vif* function in the package *car* (Fox &

Weisberg, 2019) and found no evidence for multicollinearity (VIF for each variable < 5). To compare models I used maximum likelihood and the *model.sel* function in the package *MuMIn* (Bartón, 2022). I used the Akaike information criterion corrected for small sample size (AICc) to determine the best model. I first built and compared models with each variable isolated as a determinate of display rate. Based on this comparison, I constructed more complex models that combined multiple variables, starting with the two most significant variables. When building these models, I included interaction terms based on predicted biological relevance (e.g., microhabitat and T_b). If these interactions were nonsignificant, I built an additional model without the interaction terms. I continued this process by adding one variable at a time to the current best model. This process generated my candidate models. I refit the best model with restricted maximum likelihood and checked the residuals for departures from the main assumptions of GLMM. I used Type II sum-of-square to determine significant terms in the model. Because past studies have demonstrated that T_e influences temporal and spatial variation in the thermal properties of microhabitats, I investigated the influence of the time of day on display rate and T_b in separate microhabitats using mixed effects models. I expected the thermal sensitivity of display rate to be nonlinear (Huey & Stevenson, 1979), so I analyzed the relationship between display rate and T_b using a generalized additive mixed model (GAMM, function *gamm,* package *mgcv* v. 1.8-40; Wood, 2017). I chose to use a GAMM approach because I had multiple observations for each lizard and other nonlinear methods do not allow random effects (see Zajitschek *et al.*, 2012). I anchored the display curve using the critical thermal minimum (CT_{min}) and maximum (CT_{max}) values for *U.*

ornatus from Gilbert & Miles (2019). These two traits were measured on lizards from the same population as the study population. The critical thermal limits are defined as the lower and upper endpoints for physiological activity. In my analysis the display rate would be 0 for CT_{min} and CT_{max}. I calculated the core statistics of the performance curve, including thermal optima (T_{opt}), and the 90% performance breadth from the display data. I estimated the performance curve with an autoregressive correlation structure to account for within individual variation. Lizard ID was included in the model as a random term.

Results

Microhabitat Variation in Operative Environmental Temperatures

The operative temperature models indicated a dynamic thermal profile across microhabitats over the course of the day. Early in the morning, trunks were the warmest microhabitat, though all microhabitats were below the preferred temperature range (T_{set}) of *U. ornatus* (Figure 1). All microhabitats began warming at a rapid rate around 08:00. Near midday, T_e values began showing spatial heterogeneity, with sunny snags being the most exposed and warmest microhabitat. Less exposed microhabitats, such as trunks and inner branches, offered the coolest temperatures (Figure 1).

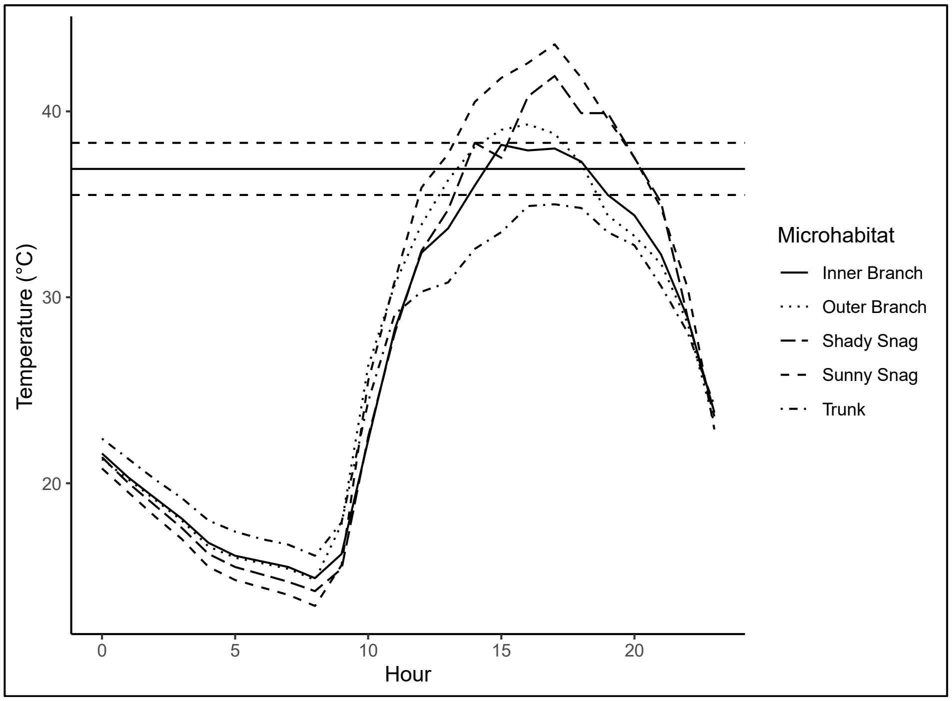

Figure 1
Mean operative temperatures available in five microhabitats used by *Urosaurus ornatus*. Although I provide values for a 24 hour period, the activity period of *U. ornatus* is from about 07:00 - 19:00 over the course of an average day in June. T_{pref} is shown as the solid horizontal line; T_{set} is bracketed within the horizontal dotted lines.

Field Active T_b and Thermal Preference

I recorded 88 field active T_bs from 50 individuals that displayed (75 T_bs from 39 males and 13 T_bs from 11 females). The field active T_bs of the lizards when displaying ranged from 29.2°C–40.1°C with an average of 35.2°C. There was no difference in T_b between males (35.2 ± 0.23°C (mean ± SE), N = 75) and females (34.7 ± 0.74°C, N = 13; $\chi^2_1 = 0.34$, $P = 0.56$). I measured T_{pref} and T_{set} of the 50 individuals with measurements of display behavior. Individual T_{pref} values ranged from 34.4°C–39.3°C with an average of 37.1°C. There was no difference in T_{pref} between males (37.0 ± 0.20°C, N = 39) and females (37.5 ± 0.40°C, N = 11; $t_{48} = -1.01$, $P = 0.32$). T_{set} values ranged from 0.7°C–

6.4°C with an average of 2.5°C. The T_{set} of males was narrower ($T_{set} = 2.24 \pm 0.18$°C, N = 39) than females ($T_{set} = 3.35 \pm 0.53$°C; $t_{48} = -2.48$, $P = 0.02$).

Display Rates

I recorded 88 distinct displays from 50 different *U. ornatus* adults: 75 displays from 39 males and 13 displays from 11 females. Because lizards were captured after displaying, repeated observations on the same individual were separated by at least 24 hours (mean number of days between repeated observations = 6.7). On average, I measured 1.76 display observations per individual. The number of push-ups performed by a lizard ranged from three over 5.6 seconds to 57 over 81 seconds. Lizards averaged 0.66 displays/second. There was no difference in display rate between males (0.65 ± 0.007 displays/second, N = 75) and females (0.71 ± 0.03 displays/second, N = 13; $\chi^2_1 = 2.44$, $P = 0.12$). Display rates among individuals with multiple measurements had low repeatability ($r_{intra} = 0.02$, N = 14).

There was no detectable influence of T_{pref} on display rate ($\chi^2_1 = 1.28$, $P = 0.26$), and individuals did not display at temperatures that matched their preferred body temperatures ($\chi^2_1 = 0.53$, $P = 0.47$; Figure 2). How close a lizard's body temperature was during display to its preferred body temperature (the difference between T_b and T_{pref}) also had no discernable impact on display rate ($\chi^2_1 = 0.74$, $P = 0.39$). I found no significant influence of sex, body size, color morph, time of day, social context, or substrate temperature on display rate ($P > 0.05$; see Table 1 for summary statistics). The mixed effects model best describing push-up display rate included microhabitat, T_b, and the interaction between T_b and microhabitat. This model had the lowest AICc (-233.6), the highest Akaike weight (0.39), and a delta AICc of over 2 when compared to the next-best model (2.47). Display rate was influenced by an interaction between T_b and microhabitat ($\chi^2_4 = 18.82$, $P < 0.001$; Figure 3). Lizards utilized microhabitats nonrandomly as determined by a chi-square test ($\chi^2_4 = 76.71$, $P < 0.001$), using sunny snags the most (N = 50) and outer branches the least (N = 3), even though snags were less available than trunks, inner branches, and outer branches (see "study population"). I compared the slopes of the different microhabitats (that represent the interaction between microhabitat and T_b) using the Tukey method via *lstrends* in *lsmeans* (Lenth, 2016) and found significant differences between the slopes of sunny snags and trunks ($P = 0.02$) and between inner branches and trunks ($P = 0.006$). There was a positive relationship between T_b and display rate on inner branches ($\chi^2_1 = 12.3$, $P < 0.001$, N = 10), a negative relationship between T_b and display rate on trunks ($\chi^2_1 = 8.7$, $P = 0.003$, N = 15), and no relationship between T_b and display rate on sunny snags ($\chi^2_1 = 0.18$, $P = 0.67$, N = 50) (Figure 3; Figure 4).

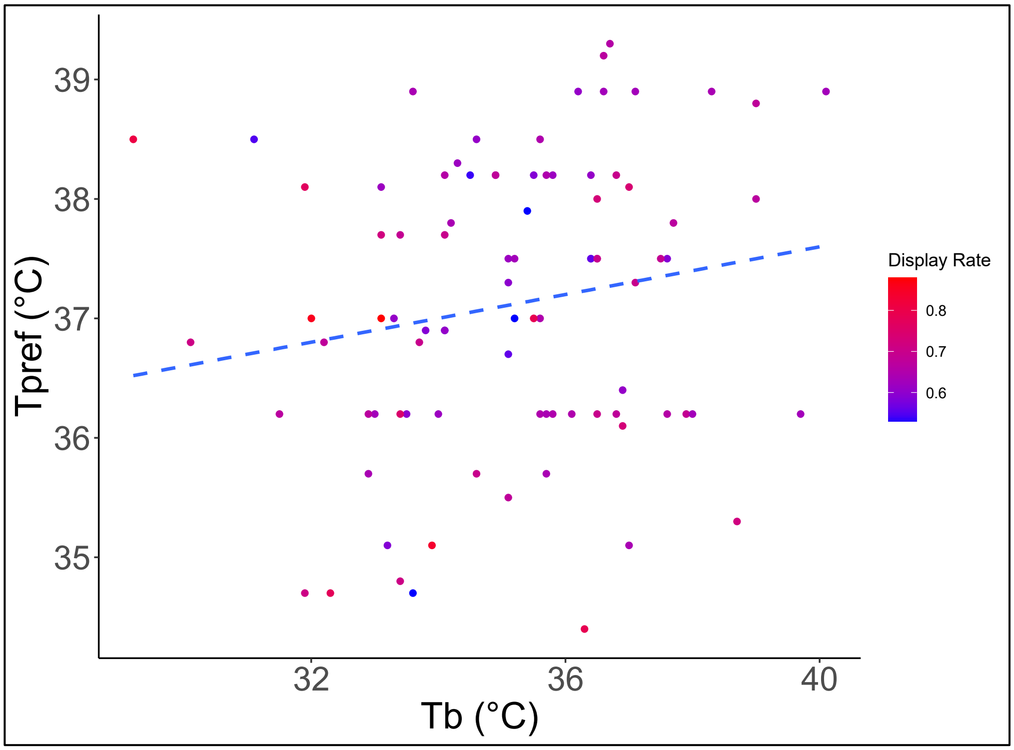

Figure 2
The relationship between a lizard's preferred body temperature (T_{pref}) and its body temperature during the time of display (T_b). Lizards did not display at temperatures that matched T_{pref} ($\chi^2_1 = 0.53$, $P = 0.47$). Display rates are represented with a color gradient, with high rates of display represented with red and low rates represented with blue.

Table 1
Display rate ± SE of all considered fixed effects: sex, throat color, microhabitat, and social context.

		Display Rate (push-ups/second) ± SE
Sex	Male (N = 75)	0.65 ± 0.007
	Female (N = 13)	0.71 ± 0.03
Throat Color (Male)	Blue (N = 19)	0.65 ± 0.02
	Blue/Yellow (N = 2)	0.66 ± 0.01
	Yellow (N = 40)	0.64 ± 0.009
	Orange (N = 3)	0.66 ± 0.02
	Yellow/Orange (N = 11)	0.68 ± 0.009
Throat Color (Female)	Yellow (N = 4)	0.72 ± 0.04
	Orange (N = 9)	0.70 ± 0.04
Microhabitat	Sunny Snags (N = 50)	0.65 ± 0.008
	Shady Snags (N = 10)	0.65 ± 0.02
	Trunks (N = 15)	0.73 ± 0.03
	Inner Branches (N = 10)	0.63 ± 0.02
	Outer Branches (N = 3)	0.69 ± 0.05
Social Context	Broadcast (N = 75)	0.66 ± 0.008
	Challenge (N = 3)	0.63 ± 0.01
	Courtship (N = 10)	0.64 ± 0.02

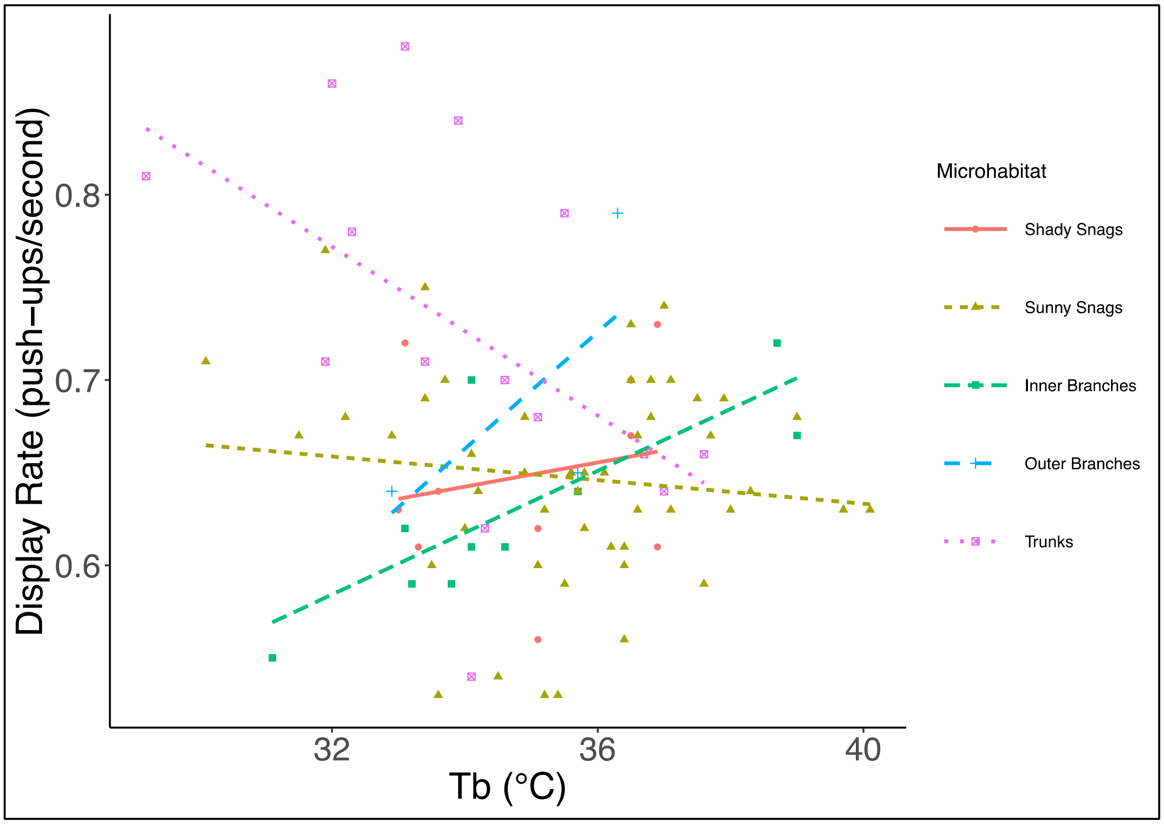

Figure 3
The influence of body temperature on display rate in five different microhabitats. Slopes between sunny dead trees (snags) and trunks (*P* = 0.02) and between inner branches and trunks (*P* = 0.006) are significantly different.

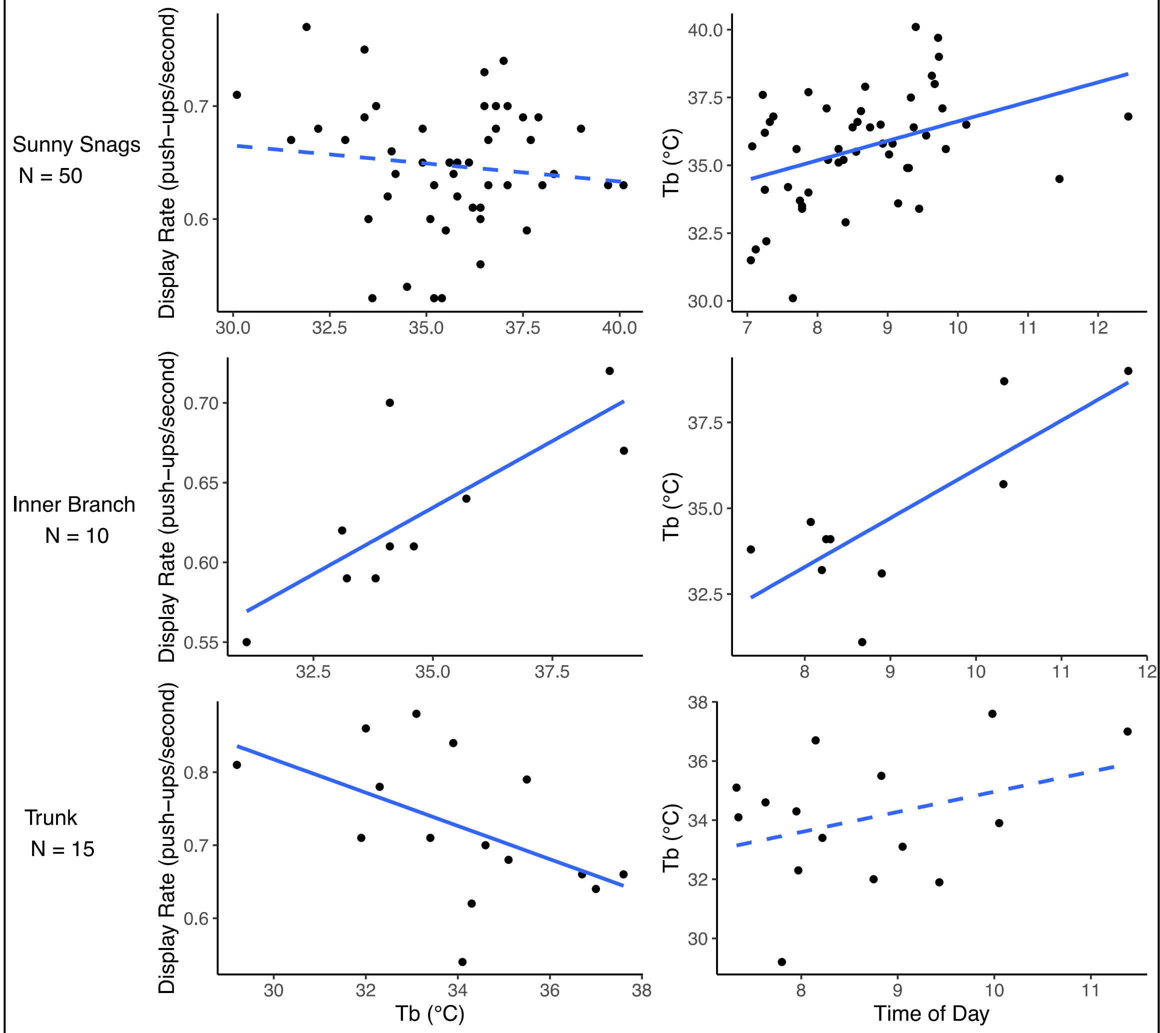

Figure 4

Relationships between display rate, T_b, and time of day on sunny dead trees (snags), inner branches, and trunks. Significant relationships are designated by solid trendlines; insignificant relationships are designated by dotted trendlines. In sunny snags: T_b ~ time of day $\chi^2_1 = 10.5$, $P = 0.001$. In inner branches: display rate ~ T_b $\chi^2_1 = 12.3$, $P < 0.001$; T_b ~ time of day $\chi^2_1 = 12.6$, $P < 0.001$. In trunks: display rate ~ T_b $\chi^2_1 = 8.7$, $P = 0.003$.

Lizards occupying sunny snags showed a significant positive relationship between time of day and T_b ($\chi^2_1 = 10.5$, $P = 0.001$, N = 50; Figure 4). On inner branches, I found a significant positive relationship between time of day and T_b ($\chi^2_1 = 12.6$, $P < 0.001$, N = 10; Figure 4). There was no observed relationship between time of day and T_b on trunks ($\chi^2_1 = 1.86$, $P = 0.17$, N = 15; Figure 4). I also tracked lizard activity, measured as the number of observations of individuals on each microhabitat, over the course of the morning (Figure 5). Among the three microhabitats shown to be significant in my model, activity was highest on sunny snags throughout the day. Activity in the early morning was concentrated on sunny snags and on trunks. Lizard use of inner branches was lower throughout the day (Figure 5).

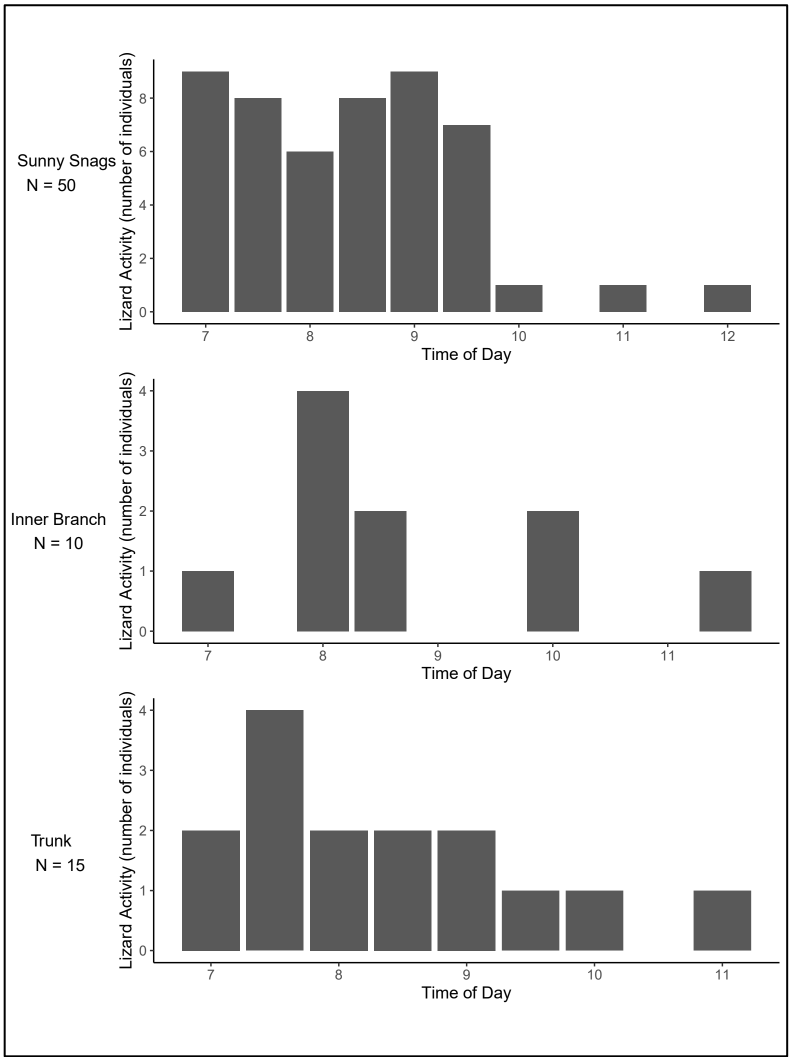

Figure 5
Lizard activity on sunny dead trees (snags), inner branches, and trunks over the course of the morning.

There was a significant nonlinear relationship between display rate and T_b ($F_{2.57,87.4} = 6.82$; $P < 0.001$; $R^2 = 0.27$, N = 90). The GAMM showed a relatively high rate of display across a broad range of T_bs. The optimal temperature for display was 33.1°C with a maximum display rate of 0.66 displays/second (Figure 6). The 90% thermal performance breadth spanned 16°C, from 23°C to 39°C. Although the optimal temperature for display was below T_{pref} the performance breadth overlapped the interquartile range.

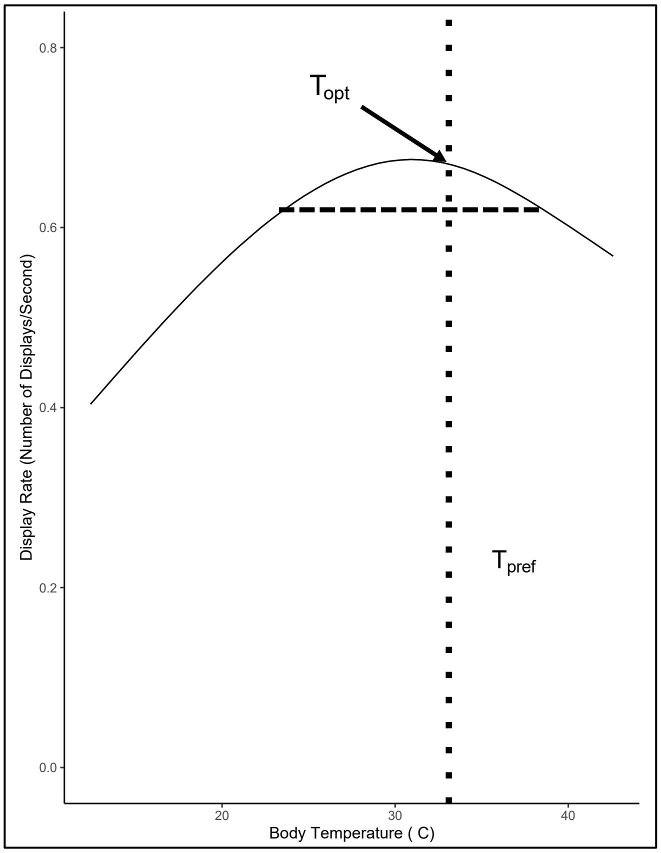

Figure 6
Thermal sensitivity of push-up display rate for *Urosaurus ornatus* based on a GAMM. Vertical dotted line portrays the optimal temperature for display rate (T_{opt}), horizontal dashed line is the thermal performance breadth (B_{90}), and shaded rectangle is thermal preference interquartile range. B_{90} represents temperatures at which performance is 90% of maximum.

Discussion

The goal of this analysis was to identify factors influencing display rate in *U. ornatus.* I did not detect an influence of sex, body size, color morph, time of day, substrate temperature, or social context (challenge, courtship, or broadcasting) on display rate. However, I found a significant influence of the microhabitat used by an individual for displaying and its T_b. Lizards used microhabitats nonrandomly but evenly among morphs, and the relationship between display rate and T_b was significantly different among three microhabitats: sunny snags, inner branches, and tree trunks. On sunny snags, display rate was unaffected by T_b and remained at relatively low rates across differing T_bs. On inner branches, display rate increased with T_b. On tree trunks, display rate decreased with T_b. An explicit test of the thermal sensitivity of display rate showed a nonlinear association with body temperature. Two patterns emerged from the display performance curve. First, lizards can maintain a high display rate over a broad range of temperatures. Second, the optimal temperature for display rate was below both mean active body temperature and mean thermal preference. I predicted that individuals with higher T_{pref} values would display at higher rates, that lizards would select for and display at body temperatures that match their T_{pref}, and that lizards that displayed at temperatures near their T_{pref} values would display at higher rates. I found no support for these hypotheses.

I propose that the variation in display rate is driven by shifts in microhabitat temperature over the course of the day as described by the interaction between microhabitat and T_b. In addition, the cost of displaying in more open environments may influence display rate. As in many other mating or territorial behaviors, push-up displays are performed in locations to increase the detection by receiver individuals. The combination of color signals with variation in dewlap coloration and brilliant blue belly patches combined with overt and exaggerated patterns of movement enhance the conspicuousness of a displaying individual. One cost of being conspicuous is the potential to attract the attention of predators (Husak *et al.*, 2006). One of the main predators of *U. ornatus* is a visual hunting snake, the coachwhip (*Masticophis flagellum*) (Goerge & Miles, unpublished observations). When sexually selected traits such as bright coloration and intense bouts of displays attract predators, the force of sexual selection on the traits is often counteracted by reduced survivorship (Kotiaho *et al.*, 2002), and occupancy of microhabitats that carry a high risk of predation can affect courtship behavior (Candolin, 1997). Lizards have been shown to be capable of adjusting display rate as a response to perceived predation threat (Simon, 2007). Rather than displaying at maximum capacity, tree lizards might adjust display rates depending on the probabilities of being detected by both conspecifics and predators. This could explain the lack of relationship between display rate and T_b observed on sunny snags and the inverse association of display rate and T_b on tree trunks. Sunny snags had the highest rate of lizard activity across all microhabitats, largely driven by the early hours of the morning during which lizards were basking to increase T_b. As the most exposed microhabitat, the T_e of sunny snags favors basking behavior in the morning but becomes too warm for individuals by 10:00 (Figure 1). Despite lizard T_b increasing over the course of the day on sunny snags, display rate remained constant and at a lower rate than other microhabitats. The pattern of displays on open microhabitats (snags) may reflect a strategy of lizards to transmit information to conspecifics without attracting the attention of visual hunting predators. As opposed to displaying towards conspecifics within a close visual field, *U.*

ornatus in this study engaged in general broadcasting displays in 74/88 (84%) instances, a rate comparable to similar studies (e.g., 88%, Martins 1993a). Although social context was not significant when modeling display rate, overall lizard movement across microhabitats over the course of the day may have influenced display behavior of broadcasting individuals.

I observed an inverse relationship between display rate and T_b on tree trunks. During early mornings many individuals of *U. ornatus* will use trunks as a basking site. The high display rate on trunks at low T_b may be a consequence of the ability to signal to multiple conspecifics. I therefore propose that display rate is highest at low temperatures on trunks because this is when more conspecifics are present, making social broadcasting more beneficial, as later in the afternoon trunk occupancy decreases. Ord & Stamps (2017) did not find evidence of *Anolis* lizards adjusting display rate based on the number of nearby conspecifics; however, they also found that display rates were dependent on ambient temperature. My thermal performance curve showed that lizards could maximize display rates over a broader range of T_bs, indicating that the drivers of display behavior may differ between species or systems. Indeed, other taxa have been shown to alter the rate of advertisement signal production based on potential receivers (Aiken, 1982; Wellendorf *et al*., 2004; How *et al*., 2008). For example, *Sceloporus* species alter display patterns based on the presence (or absence) of males or females in the immediate vicinity (Martins, 1993a). Display rates on tree trunks at low temperatures is high compared to other microhabitats. Tree trunks are also exposed to conspecifics and predators, but unlike sunny snags, offer easy and accessible refuge via individuals running up into dense tree branches when approached (personal observation). This access to shelter could provide higher degrees of predator safety to vigorous displayers, and this combination of high visibility with relative safety could result in the high observed display rates relative to sunny snags and other microhabitats.

During the warmer hours of the day lizards spend more time in cooler (but within their T_{set}) microhabitats such as inner tree branches. As with trunks, the interplay between T_b, time of day, and high density of conspecifics on a common microhabitat is a potential driver of the positive relationship between display rate and T_b on inner branches. Display rate is likely low at low temperatures on inner branches because the probability of interaction with conspecifics is also low. As conspecifics shift their perch selection into inner branches during the warmer hours of the day, display rate increases with T_b.

Unlike other studies on display patterns and rates, I was surprised to find no evidence that sex, throat morph, or social context (territoriality vs. courtship vs. broadcasting) influenced display rate (Martins, 1991; Martins, 1993a; Martins, 1994). These and other studies (e.g., Partan *et al*., 2011) report differences in the relative frequencies of full push-ups, head bobs, dewlap extensions, and lateral compressions depending on sex and social context. I observed limited variation among the sexes in display pattern. Males, regardless of during broadcasting, territory disputes, or courtship, displayed using similar patterns consisting of 3–8 distinct push-ups accompanied by simultaneous lateral compressions and raised tails. Females exhibit similar display behaviors but with far less lateral compression, likely due to the absence of ventral coloration (or when present pale).

I found display rate to be driven by ecological contexts, similar to the findings by Simon *et al*. (2007), who showed that individuals altered display rate based on perceived predation threat. Gunderson & Leal (2015) proposed a model under which physiological constraints would limit display rate production, and Ord & Stamps (2017) found that *Anolis* lizards displayed at rates predicted by the influence of temperature on physiological performance. My data also showed thermal sensitivity in display rate. However, the optimal temperature for displays was 4°C below T_{pref} (37.1 °C–33.1°C). The thermal performance breadth for display rates exhibited a broad temperature range (23–39 °C), which overlapped the range of preferred T_bs. I note that the majority of displays were at temperatures outside the T_{set} of *U. ornatus* (60/88 T_bs below the mean T_{set} range of 35.9–38.4°C). My results are concordant with the patterns observed in Ord and Stamps (2017). Whereas Ord and Stamps found that T_{opt} for display rate was within the range of peak performance in *Anolis*, my results showed that *U. ornatus* could display at high levels below T_{pref}. These differences may be due to differences in the thermal ecology of *Anolis* and *U. ornatus*. *Anolis* occupying forest environments tend to be thermoconformers (Hertz *et al*., 1993); hence, Ord and Stamps (2017) used air temperature as a proxy for T_b rather than the actual T_b of lizard the lizard. In contrast, *U. ornatus* are thermoregulators (Gadsden *et al*., 2020). I therefore used body temperature rather than air temperature, and to ensure that my recorded display rate matched the lizard's body temperature at the time of display, stopped recording when lizards moved to thermally distinct locations. This presumably resulted in shorter recording times than those used in Ord & Stamps (2017) which could play a role in the differing results. My findings could also be a result of differences in the thermal environment. For instance, in arid climates where ambient temperatures are often above T_{pref} and CT_{max}, the cost of thermoregulation may be low. Hence, lizards may have the capacity of displaying early in the morning at temperatures below T_{pref} because the risk of predation is low and the information being broadcasted may involve resource holding potential rather than seeking mates. Thus, lizards avoid the need to use perches that may exceed T_{pref} and CT_{max} to use visual displays to convey dominance. Given my findings, I suggest that future studies would benefit by considering the influence of microhabitat and season when dissecting how display rates may be affected by temperature.

Chapter 3: The Influence of Intraspecific Competition on Thermal Preference Depends on Sex and Color Morph

Introduction

In ectothermic animals, temperature influences physiological processes and impacts organismal performance and ultimately survival and reproduction (Huey & Stevenson, 1979; Kingsolver & Huey, 2008; Angilletta, 2009). Ectotherms use behavior to maintain body temperatures (T_b) at or near temperatures that maximize performance (Huey & Stevenson, 1979; Seebacher, 2005). Such behaviors may entail bouts of basking to increase body temperature or retreating to cooler microhabitats to avoid overheating. Because ectotherms tend to maintain their T_bs within a narrow temperature range, thermal ecologists have suggested that organisms may have an intrinsic set point that coincides with temperatures optimizing performance (Dawson, 1975).

Preferred body temperature, or the body temperature that an organism selects in the absence of ecological costs or constraints, is measured in the laboratory using a thermal gradient, providing a range of temperatures that can be used for thermoregulation. Researchers often measure preferred body temperature as T_{pref}, the average body temperature that an individual selects over a given duration of time, or as T_{set}, a range of values (often the middle 50%) that an individual selects.

Thermal preference data have been identified as critical for assessing several important ecological applications, frequently tested in lizards. Preferred body temperatures often reflect optimization of physiological performance (Angilletta *et al.*, 2002), which in turn can confer fitness advantage (Miles, 2004). Preferred body temperature data can be combined with body temperature data from the field to calculate thermoregulation accuracy and can be further analyzed with habitat thermal quality data to calculate thermoregulatory effectiveness (sensu Hertz *et al*., 1993). Thermal preference data are also frequently applied to model local extinction risks of populations, which become heightened when projected altered thermal niches increase to temperatures exceeding measured T_{pref} and T_{set} values (Kearney *et al*., 2009; Sinervo *et al*., 2010; Sears & Angilletta, 2015; Kirchhof *et al*., 2017).

Although thermal preference captures the temperature a lizard selects under ideal conditions, i.e., without cost or constraint, these circumstances are rarely, if ever, realized in the field. Costs and constraints of thermoregulation are numerous (Huey & Slatkin, 1976) and include time and energy that cannot be devoted to other activities (e.g., foraging or courtship; Grant & Dunham, 1988; Adolph & Porter, 1993) and increased predation risk (Herczeg *et al*., 2008). The costs of thermoregulation, both energetic and non-energetic, are usually factored in when modeling optimal thermoregulation strategies and behaviors (Huey & Slatkin, 1976; Sears & Angilletta, 2015; Basson *et al*., 2017; Lymburner & Blouin-Demers, 2019). However, constraints on thermoregulatory behavior are almost never considered when measuring thermal preference data. Although such a measure of thermal preference may not represent preference *per se*, individuals are rarely allowed to select body temperatures in the field without consideration of constraints. Therefore, by introducing realistic constraints into thermal preference measurements, researchers should be able to derive a clearer, more realistic understanding of thermoregulatory behavior in the field for a given study system.

The ornate tree lizard, *Urosaurus ornatus*, is a small-bodied, territorial lizard that primarily lives on tree and rock substrates. *U. ornatus* tends to be densely populated, with significant amounts of home range overlap among males and females; it is not uncommon to observe multiple males and females inhabiting the same tree over the course of an active season (Zucker, 1995; Goerge, personal observation). In ectotherms, the thermal quality of the environment is a driving factor in habitat selection (Huey, 1991; Halliday & Blouin-Demers, 2014), leading to high levels of competition for basking sites in dense populations (Cadi & Joly, 2003; Polo-Cavia *et al*., 2010). Therefore, considering how the presence of conspecifics influences social dynamics and thermoregulation could result in a more realistic understanding of field thermoregulatory behavior in densely populated species. Here, I investigated how the presence of conspecifics influenced T_{pref} in *U. ornatus,* a species that exhibits throat color polymorphisms in both males and females. Previous work has detailed relationships between lizard color morph, T_{pref}, and social behavior (e.g., Stapley, 2006). I made several predictions regarding how conspecifics would alter thermoregulatory behavior in *U. ornatus*: (i) male conspecifics should compete for basking territory, resulting in one or both competing males being displaced from an optimal thermal position in the gradient leading to a lower body temperature; (ii) a male's color morph should influence the magnitude of thermal displacement, with less dominant color morphs being displaced from an optimal temperature more frequently by more dominant color morphs; (iii) female conspecifics should compete for basking territory less aggressively than males, with thermoregulation less impacted than in males; and (iv) males and females would have limited interactions because females were gravid during the time of study (Ruiz *et al.*, 2008). By considering how interactions among different male and female color morphs influence T_{pref}, I aim to provide data on what body temperatures *U. ornatus* may select in the field.

Materials and Methods

Study Site and Capture

I sampled adult lizards from a population at the Appleton-Whittell Research Ranch (AWRR), a National Audubon Society site in southeastern Arizona (31.365° N, –110.303° W) from 6 May-26 June 2019. The site sits in an arid desert grassland. The tree lizards at this site are almost entirely arboreal, spending their time on live oak trees (*Quercus* sp.), mesquite trees (*Prosopsis* sp.), and dead tree snags. *U. ornatus* is found in dense populations at this site; multiple males and females simultaneously inhabit the trees throughout the active season, resulting in frequent territorial and courtship interactions (personal observation). I walked transects through the study site during mornings from about 7:00–12:00 and captured lizards using standard noosing techniques or by hand. Captured lizards were transported to a laboratory at AWRR. Adult males in this population exhibit a diversity of throat colors; males can have solid blue, yellow, or orange throats, but many individuals preserve varying combinations of these colors. Females exhibit less diversity, having solid orange, yellow, or white throats. Based on previous studies that found that blue *U. ornatus* males tend to be more dominant than other color morphs (Thompson & Moore, 1991a; Carpenter, 1995; Taylor & Lattanzio, 2016), I grouped males into three throat color categories: blue males, partially blue males (blue throat cores surrounded by orange or yellow), and non-blue males (yellow or orange males). Females were categorized as having orange, yellow, or white throats. Snout-vent length (SVL) was recorded and lizards were housed in individual terraria in the lab. The terraria were maintained around the field active *U. ornatus* body temperature of 36°C and on a 13hr/11hr light/dark cycle to mimic field conditions. Lizards were offered mealworms daily, provided water *ad libitum,* and allowed to acclimate in the laboratory for at least 24 hours before initiating thermal preference trials.

Thermal Preference Trials

To investigate the influence of conspecifics on thermoregulatory behavior in *U. ornatus,* I measured thermal preference in a 120 cm L x 16 cm W x 20 cm H thermal gradient constructed with aluminum sheet metal on a plywood base. I layered the gradient with a sandy substrate and used 60W and 100W heating bulbs to create a linear temperature gradient ranging from about 27°C–45°C. This gradient provided the lizards with a range of temperatures comfortably encompassing *U. ornatus*' average active field body temperature of 36°C (personal observation). Each lizard completed a solo trial in the thermal gradient; individuals were placed in the center of the gradient and allowed to acclimate for 10 minutes, after which body temperature was measured with an infrared digital thermometer every 10 minutes for 60 minutes total. T_{pref} was calculated as the average body temperature selected over the duration of the trial. In addition to a solo trial, individuals completed up to three more paired trials in which two lizards shared a gradient simultaneously: a trial with an individual of the opposite sex, a trial with an individual of the same sex and same color morph group, and a trial with an individual of the same sex and different color morph group. For example, a blue male might have completed a trial with a female (any color morph), another blue male (same sex same morph group), and with either a partially blue male or a male with no blue (same sex different morph group). T_{pref} was calculated in the same way in paired trials as in solo trials. I attempted to ensure that each individual completed all three of the paired trials, but some individuals were unable to do so depending on which individuals were in the lab at the time and because some color morphs were more common in the population than others (e.g., there were many more partially blue and non-blue males than blue males in the population). After the solo trials, the order of the three paired trials was randomized for each lizard, and although SVL was recorded and accounted for in analyses, lizards were paired solely based on color morph groups and not size. Trials were separated by at least 24 hours and lizards were fed immediately after completing trials so that they could fast 24 hours before the next trial. The sandy substrate in the thermal gradient was changed after trials to avoid confounding influences from chemical cues. After completing all trials, individuals were released into the field at the location from which they were captured.

Statistical Analysis

All statistical analyses were carried out in R version 3.6.3 (R Core Team 2020). To investigate the influence of conspecifics on thermal preference, I compared solo T_{pref} values to values from paired trials. I first separated males and females and for each sex used mixed effects models to determine if trial type (solo, opposite sex, same sex same morph, and same sex different morph) had an influence on T_{pref}. For all mixed effects models, I included SVL to account for any influence of body size and included lizard ID as a random effect to account for repeated measures across trial type. I also used a model selection process to determine whether including an interaction term between trial type and SVL was necessary. I ran a Tukey post-hoc analysis if trial type was found to have a significant impact on T_{pref}. I then split up males and females into their color morph groups and again investigated for the influence of trial type on T_{pref} using mixed effects models (i.e., does trial type influence T_{pref} in blue, partially blue, and non-blue males and in orange, yellow, and white females). For each morph group, I then examined how an opponent's color morph in a paired trial influenced T_{pref} using mixed effects models. For example, after investigating how T_{pref} in blue males changed from solo to paired trials with females and with other male morphs, I examined whether orange, yellow, or white females were associated with different T_{pref} values among blue males (opposite sex trials) and whether partially blue or non-blue males were associated with different T_{pref} values among blue males (same sex trials).

Results

The dataset exploring the influence of *Urosaurus ornatus* conspecifics on thermal preference comprised a total of 96 individual tree lizards: 61 males and 35 females completed solo T_{pref} trials. For all models tested, those without an interaction term between trial type and SVL were superior (lower AIC) to models with the interaction term. In both females and males, T_{pref} during solo trials were higher than in any paired trials (Figure 7). For females, trial type had a significant effect on T_{pref} ($\chi^2_3 = 13.41$, $P = 0.004$). A Tukey post-hoc analysis found that in females, T_{pref} during solo trials was significantly higher than T_{pref} during paired trials with males (P = 0.006) and with females of the same color morph (P = 0.02; Figure 7). For males, trial type also had a significant effect on T_{pref} ($\chi^2_3 = 19.55$, $P < 0.001$) wheras the influence of SVL was not significant ($\chi^2_1 = 0.11$, $P = 0.74$). A Tukey post-hoc analysis on trial type found that in males, T_{pref} during solo trials was significantly higher than T_{pref} during paired trials with males of the same color morph group ($P < 0.001$) and with males of different color morph groups ($P = 0.002$; Figure 7).

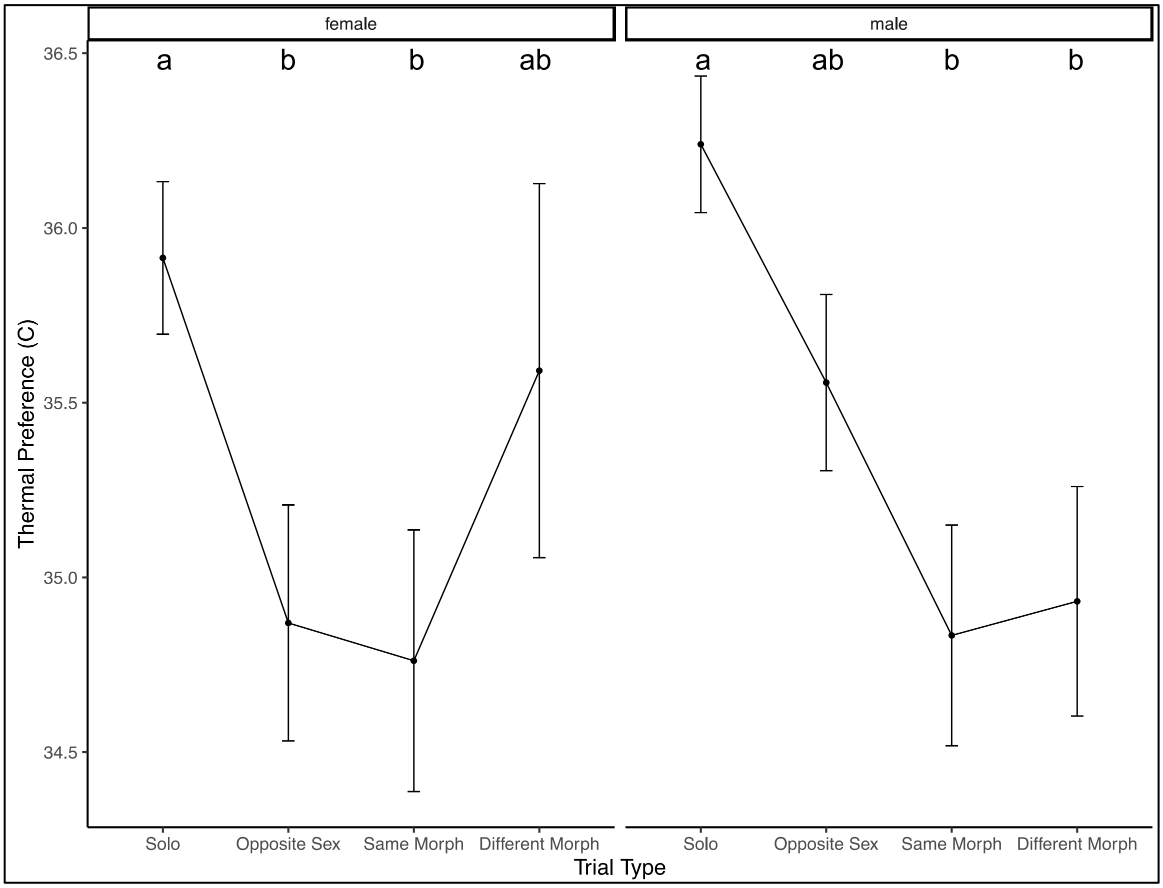

Figure 7
Differences in thermal preference by trial type (Solo = solo trial; Opposite Sex = trial with a conspecific of opposite sex; Same Morph = trial with a conspecific of same sex and same color morph group; Different Morph = trial with a conspecific of same sex and

different color morph group) in females (left) and males (right). Points represent the average T_{pref} value for each sex and trial type combination and error bars represent standard error. Significant differences are noted with letters above each plot.

I performed similar analyses within each sex and color morph group combination. In orange females (*n* = 15), trial type had a significant effect on T_{pref} ($\chi^2_3 = 11.41$, $P = 0.01$), as did SVL ($\chi^2_1 = 5.84$, $P = 0.016$). Specifically, solo orange females had higher T_{pref} values compared to when they were with males ($P = 0.006$; Figure 8). Orange females (SVL = 44.98 ± 4.67 mm) were also larger than either yellow females (43.62 ± 3.93 mm) or white females (41.93 ± 4.13 mm) and larger orange females preferred warmer temperatures than smaller orange females ($r = 0.41$, $P = 0.002$). To further investigate the influence of males on orange females, I compared orange female solo T_{pref} with orange female T_{pref} when in the gradient with each of the male color morph groups (Figure 9). The color morph of the male conspecific had a significant influence on orange female T_{pref} ($\chi^2_3 = 10.68$, $P = 0.014$); specifically, solo T_{pref} was significantly higher than T_{pref} when in the gradient with non-blue males ($P = 0.03$).

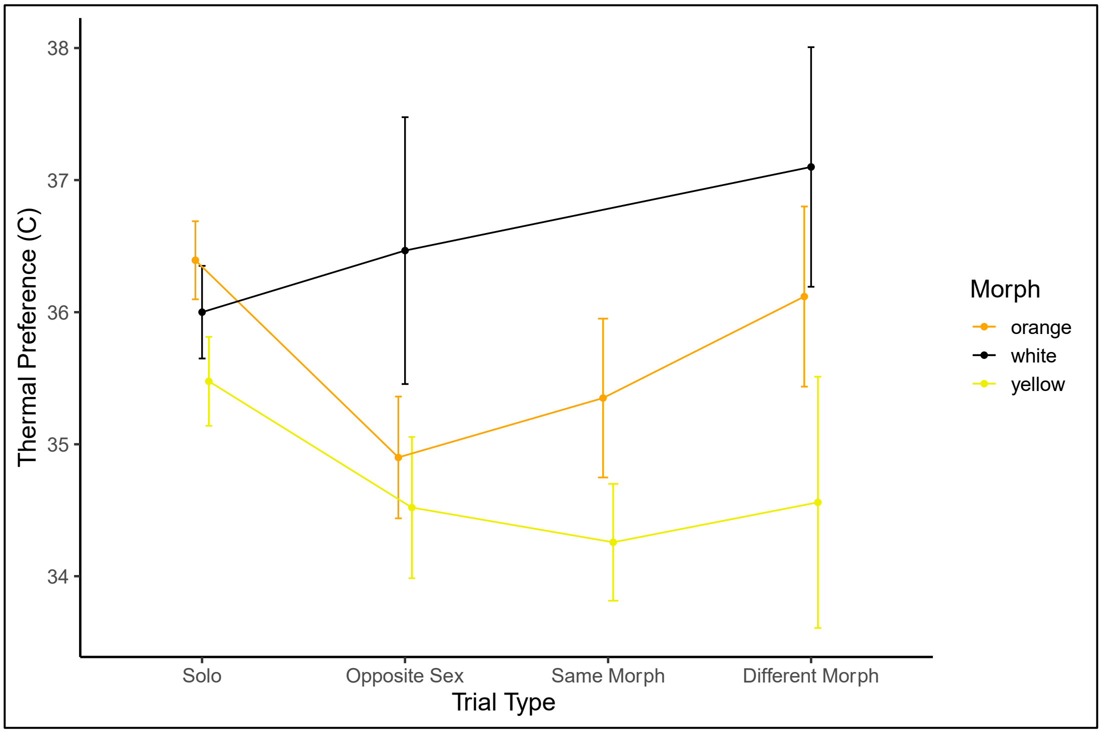

Figure 8
Differences in thermal preference by trial type (Solo = solo trial; Opposite Sex = trial with a conspecific of opposite sex; Same Morph = trial with a conspecific of same sex and same color morph group; Different Morph = trial with a conspecific of same sex and different color morph group) in three female color morphs. Points represent the average T_{pref} value and error bars represent standard error. The sole significant difference between trial type within each morph is that in orange females, solo was higher than opposite sex (*P* =0.006).

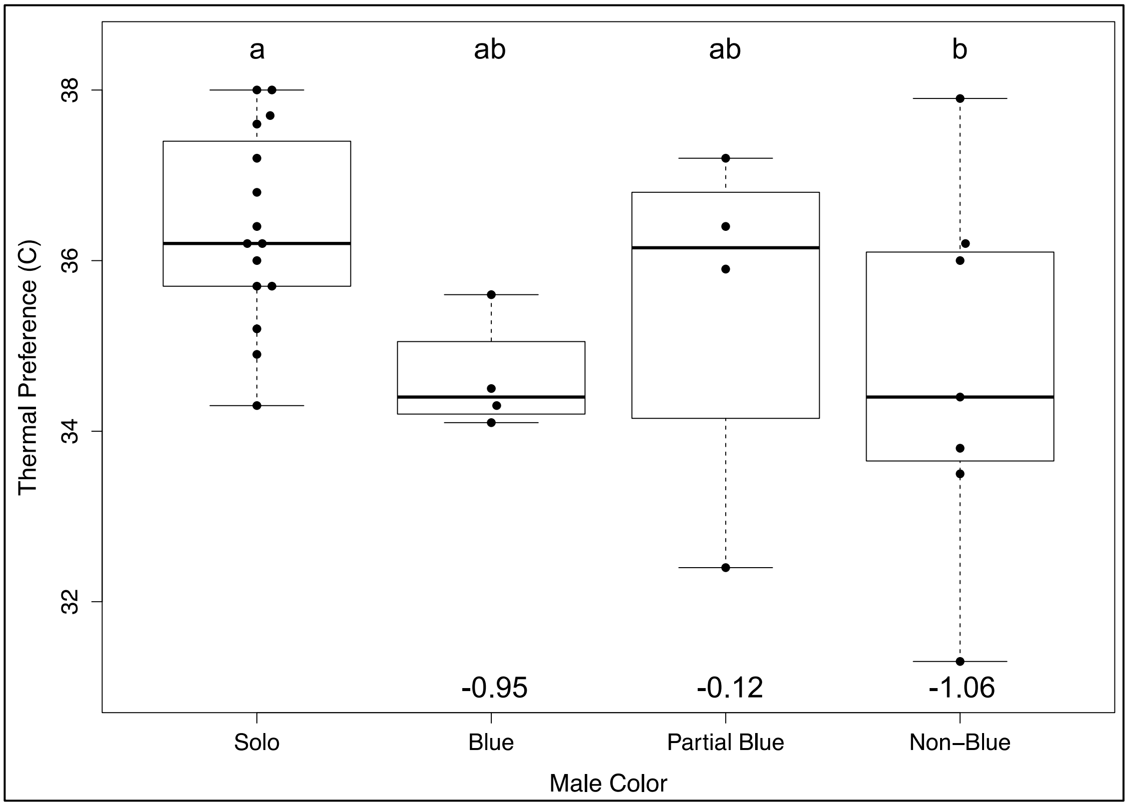

Figure 9
Comparison in orange female T_{pref} between solo trials and paired trials with different male color morph groups. Horizontal bar in each box represents the median and each point is a T_{pref} value from an individual orange female lizard for her respective trial. Significant differences are noted with letters above the boxplots. Values below each male morph group boxplot represent that male color group's T_{pref} difference between their solo trials and trials with orange females (i.e., blue males reduced their T_{pref} by 0.95°C when in the gradient with orange females compared to their solo trials).

In yellow females (*n* = 17), trial type did not have an effect on T_{pref} ($\chi^2_3 = 4.46$, $P = 0.22$; Figure 8). However, when I isolated the relationships between yellow solo and paired trials with males, I found that male color morph influenced yellow female T_{pref} ($\chi^2_3 = 17.88$, $P < 0.001$) and specifically that solo yellow females and yellow females paired with partially blue males both had higher T_{pref} values than when with non-blue males ($P = 0.002$; $P = 0.02$, respectively; Figure 10). White females ($n = 3$) had insufficient sample size for significance testing.

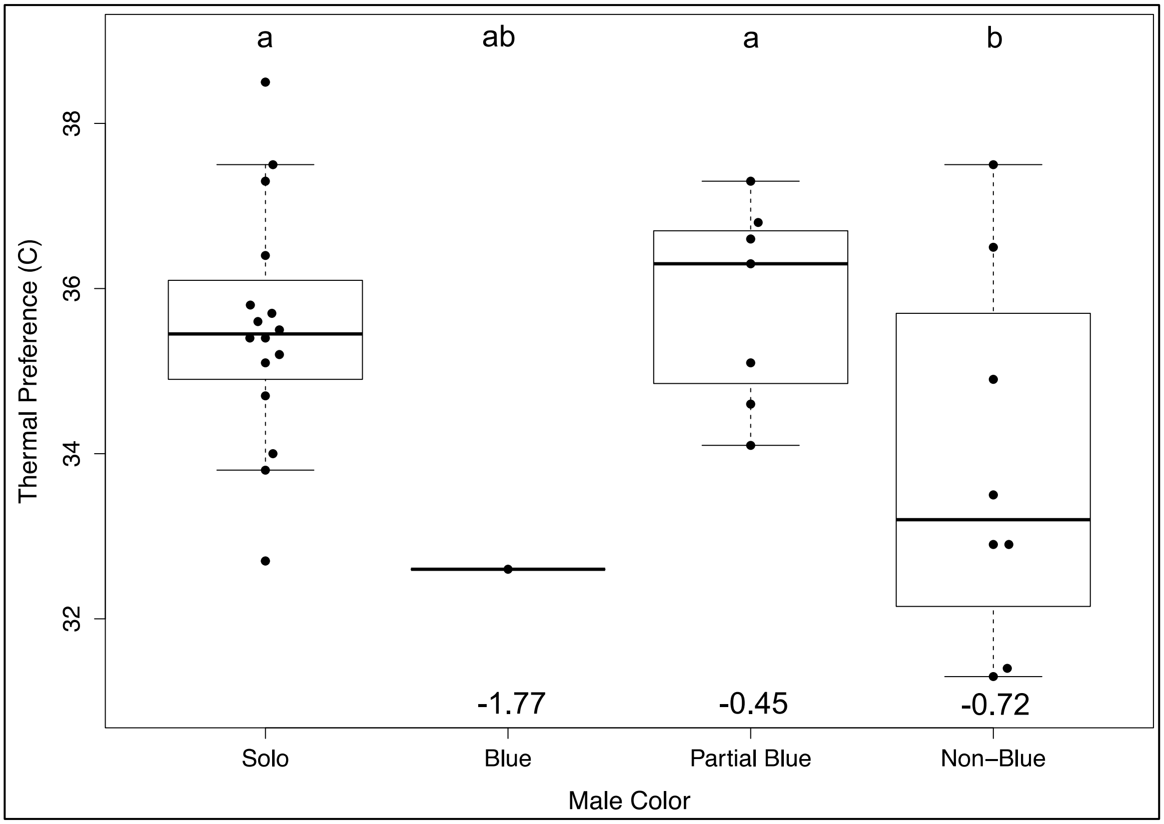

Figure 10
Comparison in yellow female T_{pref} between solo trials and paired trials with different male color morph groups. Horizontal bar in each box represents the median while each point is a T_{pref} value from an individual yellow female lizard for her respective trial. Significant differences are noted with letters above the boxplots. Values below each male morph group boxplot represent that male color group's T_{pref} difference between solo trials and trials with yellow females (i.e., partial males reduced their T_{pref} by 0.45°C when in the gradient with yellow females compared to their solo trials).

Among male lizards, the three morph groups followed a similar pattern across trial types, with partial blue males consistently having the highest T_{pref} values and non-blue males having the lowest (Figure 11). In blue males ($n = 13$), trial type did not have any influence on T_{pref} ($\chi^2_3 = 3.19$, $P = 0.36$; Figure 11). In partial blue males ($n = 22$), trial type and SVL both had a significant influence on T_{pref} ($\chi^2_3 = 8.03$, $P = 0.045$; $\chi^2_1 = 4.66$, $P = 0.031$, respectively). The sole significant combination in partial blue males was between solo and same morph trials ($P = 0.028$; Figure 11). In these same morph trials in which two partial blue males share a gradient, almost every individual exhibited a lower T_{pref} value compared to solo trials, yet the larger male had a higher T_{pref} value than the smaller male that he shared a gradient with in eight out of ten trials. In non-blue males ($n = 26$), trial type influenced T_{pref} ($\chi^2_3 = 10.43$, $P = 0.017$) such that solo non-blue males exhibited higher T_{pref} values than when in the gradient with different male morphs ($P = 0.017$; Figure 11). The influence of neither blue males nor partial blue males on non-blue males was significant ($P = 0.081$; $P = 0.063$, respectively), although non-blue males did decrease their T_{pref} values slightly more when in the presence of partial blue males (Figure 12).

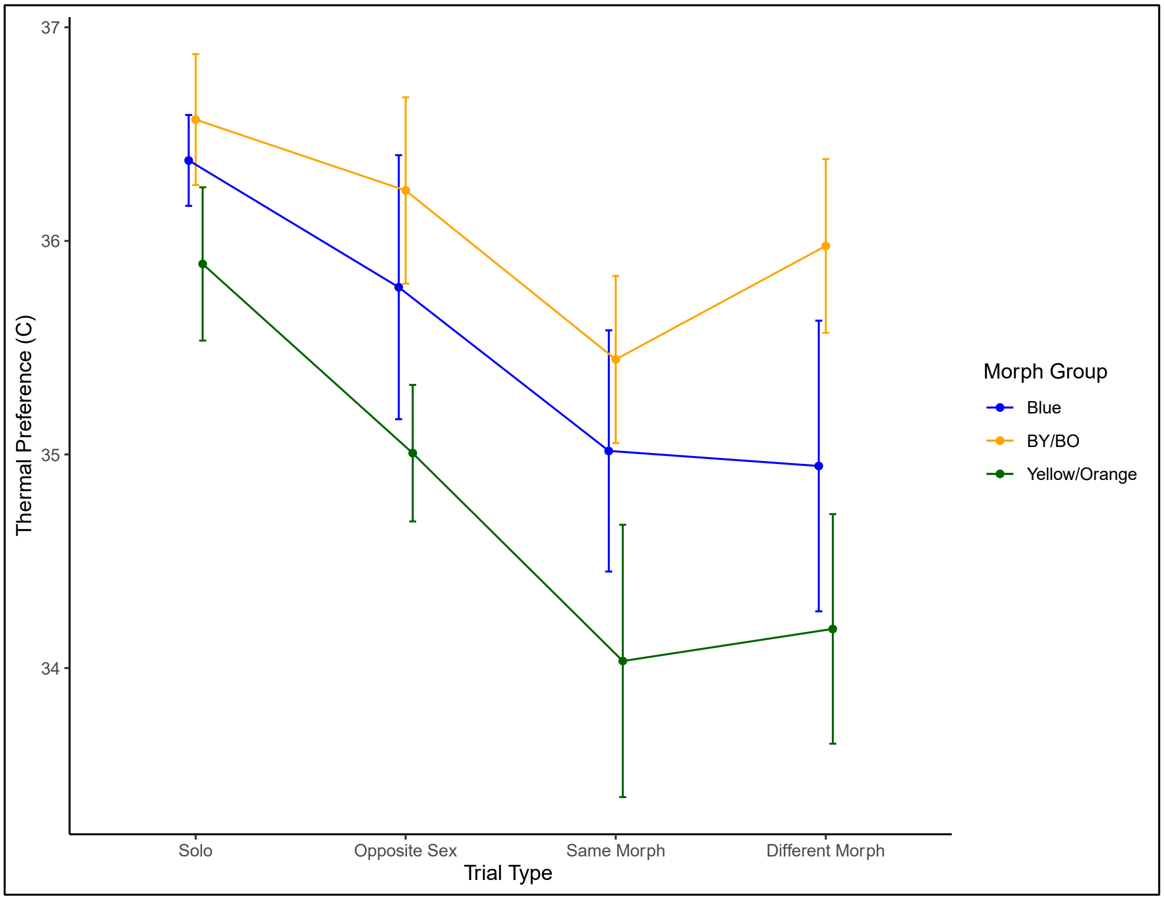

Figure 11
Differences in thermal preference by trial type (Solo = solo trial; Opposite Sex = trial with a conspecific of opposite sex; Same Morph = trial with a conspecific of same sex and same color morph group; Different Morph = trial with a conspecific of same sex and different color morph group) in three male color morph groups. Points represent average T_{pref} value and error bars represent standard error. The following trial type differences within each morph group were significant: solo and same morph in partial blue males (*P* =0.028) and solo and different morph in non-blue males (*P* = 0.017).

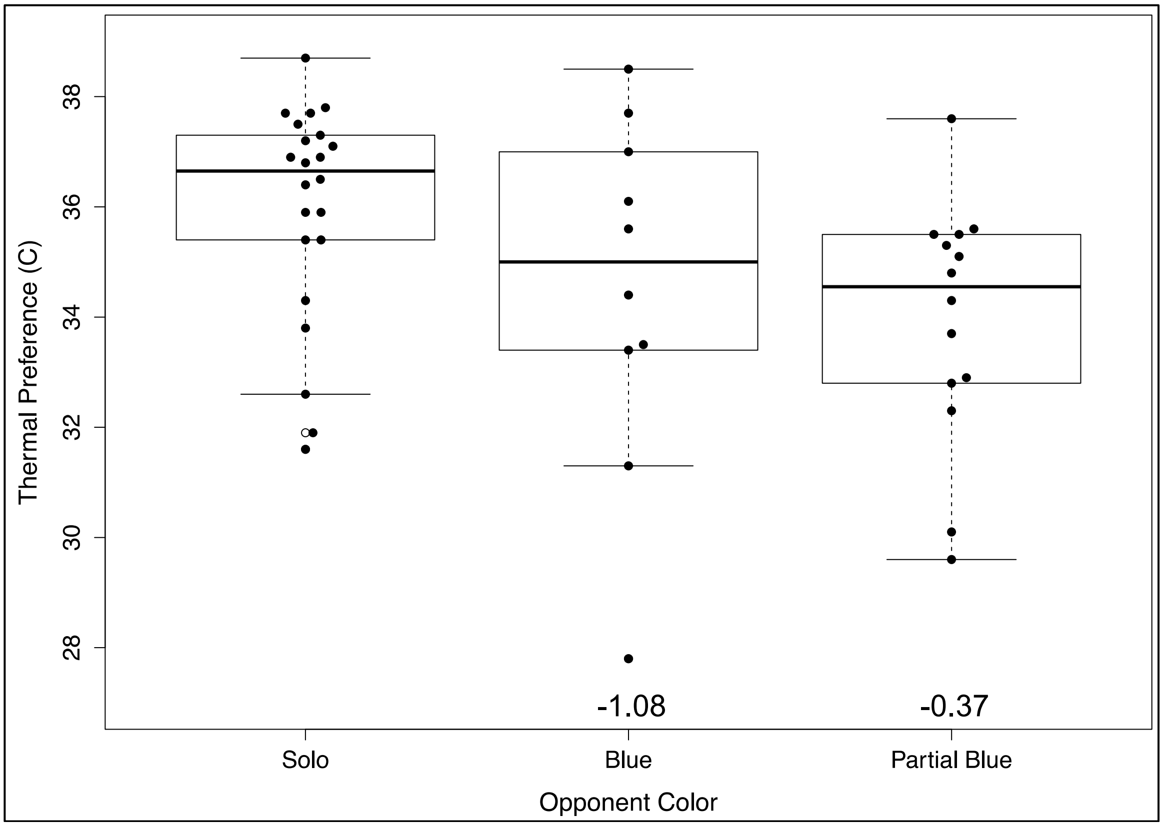

Figure 12
Comparison in non-blue male T_{pref} between solo trials and paired trials with different male color morph groups. Horizontal bar in each box represents median and each point is a T_{pref} value from an individual non-blue male lizard in his respective trial. Values below blue and partial blue boxplots represent that male color group's T_{pref} difference between solo trials and trials with non-blue males (i.e., partial blue males reduced their T_{pref} by 0.37°C when in the gradient with non-blue males compared to their solo trials).

Discussion

Male Interactions

Across sex and color morph, I found clear evidence that the presence of conspecifics influences thermoregulatory behavior in *U. ornatus.* In paired trials involving two males, I predicted that males would displace one another from solo T_{pref} values. I did indeed find that among males, high degrees of displacement occurred when males were paired in the gradient together; average T_{pref} values decreased across all males when two males were paired together, regardless of whether the paired males were of the same or different color morphs. When alone in the gradient, male T_{pref} values averaged at 36.2°C, consistent with previous studies that report *U. ornatus'* T_{pref} values around 36°C (Miles, 1994; unpublished data). When males competed for basking territory, T_{pref} values were displaced to 34.8°C and 34.9°C when males were paired with the same and different color morphs, respectively. Although there was ample space provided in the thermal gradient in temperature zones both above and below 36°C, thermally displaced lizards almost always selected for body temperatures below 36°C rather than above. This is likely a reflection of the general relationship between ectotherm body temperature and physiological performance. As ectotherm body temperatures rise, so does physiological performance, up to a performance peak at an optimal body temperature (T_{opt}). As body temperatures increase above T_{opt}, performance declines at a rapid rate, giving these curves an asymmetrical shape (Huey & Stevenson, 1979). If individuals are unable to select for T_{opt} — about 36°C in *U. ornatus* (Gilbert & Miles, 2016) — body temperatures below T_{opt} are likely to confer greater physiological performance than body temperatures equally above T_{opt}, likely explaining why I observed displaced individuals overwhelmingly selecting for body temperatures below 36°C.

Male Color Morph Dynamics

I further predicted that throat color of competing males would influence the degree of T_{pref} displacement; blue males, having previously shown to be a dominant morph (Thompson & Moore, 1991a; Carpenter, 1995; Taylor & Lattanzio, 2016), were predicted to be least displaced, non-blue males were predicted to be most displaced, and partial blue males were predicted to fall somewhere in between. Although I did observe significant influence of throat color on male T_{pref}, the relationships between color morph groups were somewhat surprising. When in paired trials, non-blue males selected for the lowest temperatures as predicted, but partial blue males were less displaced than blue males, indicating dominance in thermoregulatory interactions. Indeed, partial blue males were only significantly displaced from their average solo T_{pref} value of 36.6°C when in the gradient with other partial blue males; in these trials, partial blue males had an average T_{pref} value of 35.4°C and the larger partial blue male maintained a higher T_{pref} value than the smaller partial blue male in eight out of 10 trials. In comparison, when in the gradient with different morphs, partial blue males were only displaced to 36.0°C.

Non-blue males were significantly displaced from their average solo T_{pref} value of 35.9°C when in the gradient with different male morphs. Non-blue males decreased their T_{pref} values when in the gradient with both partial blue and blue males, but slightly more so when in the gradient with partial blue males compared to blue males. In these same trials, the opposing blue males were much more influenced by the presence of non-blue males than the opposing partial blue males were; while they still maintained higher T_{pref} values than the non-blue males, blue males were displaced by 1.08°C in these trials compared to only a 0.37°C displacement of partial blue males. In addition, blue males were displaced an intermediate amount when in paired trials and neither same morph nor different morph trials resulted in a significant displacement from solo T_{pref} values in blue males. Together, these data depict a thermoregulatory behavior social hierarchy in male

U. ornatus that places partial blue males as most dominant, blue males as intermediate, and non-blue males as least dominant.

Female Interactions

I predicted that females would compete amongst each other for basking territory but be less displaced than males; although female tree lizards can be territorial, males tend to be more so (Mahrt, 1998; unpublished data). This prediction was largely upheld. Females were significantly displaced when in the gradient with females of the same color morph. Although this relationship did not remain statistically significant when breaking females down into color morphs, the trend was still observed in orange and yellow females. In partial blue males that showed significant displacement in same-morph trials, SVL seemed to serve as a tiebreaker, with the larger male maintaining a higher T_{pref} than the smaller male in eight out of ten trials. The role of size in female same morph trials was less clear. Orange females were larger than yellow females and also had higher average T_{pref} values. Although larger orange females preferred higher T_{pref} values than smaller orange females in solo trials, the larger orange female only maintained a higher T_{pref} in paired trials three of six times. Across all females, it is interesting that territorial disputes were greater within color morphs than among them. In other polymorphic lizards, female morphs have been shown to utilize differential reproductive strategies (Sinervo, 2001). In *Uta stansburiana,* which also has orange and yellow female throat color morphs, Svensson *et al*. (2001) found that orange females in the field tend to space themselves out such that they rarely interact with other orange females and that orange females became stressed when exposed to other orange females. Orange *U. stansburiana* females also have lower lifetime fitness when surrounded by other orange females (Sinervo *et al*., 2000). Although not explicitly tested for in *U. ornatus*, a similar mechanism may explain the higher levels of same morph aggression observed in females: similar reproductive strategies within morphs leading to higher levels of same morph aggression and thus spatial and thermal displacement in same morph pairings.

Male-Female Interactions

In the presence of females, males did not alter their T_{pref}. In contrast, females and in particular orange females, were significantly displaced when sharing the gradient with males. The behavior of females in the gradient may be a consequence of reproductive activity. The study took place during the reproductive season and most if not all females were gravid during T_{pref} trials. Male lizards have been shown to decrease courtship efforts when nearby females are gravid (Ruiz *et al*., 2008). The pattern of male behavior in the presence of females is consistent with prior results. Males did not follow females in the gradient nor engage in courtship displays. Rather, males may have competed for basking territory in the gradient similar to interactions with other males. An alternative explanation is that gravid females actively avoiding courtship interactions with males. It would be of interest to repeat the study prior to females carrying eggs to observe how males and nongravid females interact under similar conditions. Both orange and yellow females were most significantly displaced by non-blue males rather than blue and partially blue males. Orange *U. ornatus* male morphs utilize sneaker reproductive tactics, often behaving like females to gain close proximity to potential mates (Lattanzio *et al*., 2014). Perhaps females paired with these males received cues that they perceived as other females, resulting in similar thermoregulatory interactions as described in "Female Interactions."

Conclusions

I demonstrate clear influence of conspecifics in the thermoregulatory behavior of *U. ornatus* that following fairly predictable patterns based on polymorphic social dominance hierarchies. The influence of conspecifics in thermoregulatory behavior may carry important ramifications especially in high density populations. For instance, recent attempts to build on Huey & Slatkin's (1976) cost-benefit model of optimal thermoregulation have investigated how lizards thermoregulate in habitats of varying thermal quality and found surprising results indicating that lizards thermoregulate heavily and accurately in low thermal quality habitat compared to higher quality habitat (Basson *et al*., 2017; Lymburner & Blouin-Demers, 2019). Perhaps increased population density in high thermal quality habitat (Paterson & Blouin-Demers, 2017) contributes to increased thermoregulatory displacement as shown in this study, resulting in increased comparative thermoregulatory accuracy in lower quality habitat. Additionally, population density could have an influence on the thermal quality of a habitat. In this system, multiple lizards share a tree and I have documented as many as 24 individuals utilizing the same tree. High densities of individuals could make accurate thermoregulation more difficult due to competitive displacement for high quality perches. Conspecific density is rarely considered in studies that assess thermoregulatory accuracy and efficiency in ectotherm populations or species (e.g., Sartorius *et al*., 2002; Lara-Reséndiz *et al*., 2015) but perhaps should be considered given these results. In high density territorial species, it may be beneficial to compare field body temperatures to conspecific-influenced T_{pref} measurements in order to determine a more realistic depiction of thermoregulatory accuracy and efficiency. More realistic representations of thermoregulation in dense populations could in turn be used to inform more accurate models that predict ectotherm responses to climate change based on thermal preference data.

Chapter 4: Dominance Status is Explained by Covariation Between Boldness, Exploration, and Thermal Preference in a Color Polymorphic Lizard

Introduction

Color polymorphism, or the presence of at least two distinct, genetically determined color morphs within an interbreeding population (Huxley, 1955) has been documented in a wide range of taxa including insects (Tanaka, 2001; Willink *et al.*, 2020), fish (Maan *et al.*, 2008; Hurtado-Gonzales *et al.*, 2014), amphibians (Harkey & Semlitsch, 1988), and reptiles (Roulin, 2004; Olsson *et al.*, 2013). Distinct color morphs often exhibit substantial ecological divergence that can incorporate variation in morphology, physiology, whole-organismal performance, and behavior (Moreno, 1989; Huyghe *et al.*, 2007; Korzan & Fernald, 2007; Kusche *et al.*, 2015; Brock, *et al.*, 2020). How distinct color morphs persist within a population is a fascinating question in evolutionary biology that is achieved by some degree of balancing selection, where morphs exhibit situational advantages and disadvantages compared to others in the population (Fisher, 1930; Gray & McKinnon, 2007; Stuart-Fox *et al.*, 2020). Within populations, disruptive selection and negative frequency-dependent selection, both via predation (apostatic selection) and sexual selection through variation in mate choice preferences or intrasexual mechanisms, can all contribute to specific color morph situational advantages that lead to color polymorphism maintenance (Olendorf *et al.*, 2006; Bond, 2007; Gray & McKinnon, 2007; Takahashi *et al.*, 2010). The contributions of these mechanisms to polymorphism maintenance vary widely among populations and species (reviewed in Gray & McKinnon, 2007), thus quantifying trait variation among color morphs remains an important step in improving our understanding of situational processes that facilitate phenotypic variation and maintenance.

Frequency-dependent selection facilitated through alternative reproductive strategies among morphs has received recent attention, especially in species with three or more morphs whose frequencies oscillate through time (Sinervo & Lively, 1996; Olendorf *et al*., 2006; Sánchez-Guillén *et al*, 2017). Male color morphs utilizing alternative reproductive strategies is perhaps best documented in the side-blotched lizard (*Uta stansburiana*); in that species, males with orange throats are hyper-aggressive and defend large territories, males with blue throats defend smaller territories and are less aggressive, and males with yellow throats are not territorial and utilize 'sneaker' mating tactics. These alternative reproductive strategies are maintained through a rock-paper-scissors negative frequency-dependent selection model, where blue males perform well against orange males but are more susceptible to yellow male sneaker tactics than orange males (Sinervo & Lively, 1996; Sinervo *et al*., 2007). In systems with multiple reproductive strategies, substantial behavioral and performance variation tends to exist among morphs (Sinervo & Lively, 1996; Dijkstra *et al*., 2008; Huyghe *et al*., 2009). Variation in phenotypes that influence predation and access to food, mating opportunities, and territory, such as locomotor performance, fighting ability, and immune response, can confer survival and reproductive advantages to particular morphs depending on environmental or situational context (Sinervo *et al*., 2000; Coladonato *et al*., 2020).

Behavioral variation in traits such as boldness, exploration, and aggression are thought to be major contributors to variation in dominance observed among color morphs that exhibit alternative reproductive strategies (Kingston *et al*., 2003). The Aegean wall lizard (*Podarcis erhardii*), which consists of three color morphs, exhibits consistent boldness variation (defined as willingness to take risks) where the orange morphs are the least bold, as measured by predator escape and avoidance behavior. Orange morphs are also the least aggressive morph and perform worst in staged contests for space (Brock & Madden, 2022; Brock *et al*., 2022). Boldness behavior can influence habitat use, predation, and reproduction; for example, in yellow-spotted monitor lizards (*Varanus panoptes*), bolder lizards have larger home ranges and spend increased time in areas with more predators than do shyer lizards and also enjoy higher mating success (Ward-Fear *et al*., 2018). Bolder individuals also tend to exhibit higher locomotor capacity (Le Galliard *et al*., 2012; Goulet *et al*., 2017; Chen *et al*., 2019), influencing survivorship (Miles, 2004), but they also often suffer higher rates of predation (Carter *et al*., 2010). Variation in exploration and activity rates can also influence survivorship; in Iberian wall lizards (*Podarcis hispanicus*), more exploratory lizards habituate faster to predators than do less exploratory individuals (Rodríguez-Prieto *et al*., 2011). Prior studies have demonstrated links between behavior and fitness. Despite this, data on differing behavioral strategies among sympatric color morphs remains scarce outside of a few model systems (but see Yewers *et al*, 2016; Sreelatha *et al*., 2021; Brock & Madden, 2022).

In ectotherms, body temperature influences biochemical processes, whole-organism performance, and life-history traits (Huey, 1982). Because of the thermal sensitivities of these processes, ectotherms that utilize behavioral thermoregulation should prefer and select for body temperatures that optimize traits to maximize fitness (Huey & Bennett, 1987). In polymorphic species that have multiple behavioral and reproductive strategies, morphs are assumed to occupy different environmental niches that may coincide with dissimilar microhabitat usage (Forsman *et al*., 2008; Lattanzio & Miles, 2014; Lattanzio & Miles, 2016; i de Lanuza & Carretero, 2018). In habitats characterized by thermal heterogeneity, variation in thermal preference among morphs is expected. Although thermal preference has been shown to covary with lizard behavior (Goulet *et al*., 2016; Goulet *et al*., 2017), very few studies have evaluated thermal preference variation among distinct color morphs (but see Paranjpe *et al*., 2013; Thompson *et al*., 2023). Assessing thermal preference variation in color polymorphic species, as well as covariation with behavior, offers the potential to provide valuable insight into niche specialization and the maintenance of color polymorphism.

The tree lizard (*Urosaurus ornatus*) is a small-bodied tree and rock dweller that occupies the southwestern United States and northern Mexico. Male tree lizards are characterized by a throat color polymorphism that correlates with alternative reproductive strategies. Blue males are dominant territory holders. Yellow males are satellites, defined by occupying home ranges on the periphery of dominant male territory. Orange males are sneakers, exhibiting nomadic behavior and often behaving like females to gain close proximity to potential mates. However, some populations also include individuals of mixed color morphs, and because some populations are monomorphic and others can contain up to six morphs, mating systems may vary geographically (M’Closkey *et al*., 1990; Thompson & Moore, 1991b; Moore *et al*., 1998). Despite geographic variation in morph frequency, a consistent pattern in *U. ornatus* morph dominance hierarchies is that males with blue throats are dominant over males that are yellow, orange, or orange/yellow (Thompson & Moore, 1991a; Hews & Moore, 1995; Taylor & Lattanzio, 2016). Females are also polymorphic and have orange, yellow, or white throats. Female *U. ornatus* morphs differ in mate preference where orange females prefer dominant males and yellow females may avoid dominant males (Lattanzio *et al*., 2014). Female morphs also vary in reproductive traits (Zucker & Boecklen, 1990); however, it is unknown if they exhibit alternative behavioral strategies.

In this study, I assessed covariation between behavior and thermal preference among *U. ornatus* morphs. Specifically, I investigated boldness behavior, which I define as the willingness to take risks, and exploratory behavior. I selected these behaviors because they are often correlate with dominance status (Sundström *et al*., 2004) but are less studied than aggression and dominance. Further, these behaviors provide insight into ecological niche differentiation among morphs that can result in covariation among thermal preference, boldness and exploration. The latter two traits often correlate with territorial defense, whereas the former trait affects habitat use and exploitation of variable thermal opportunities. I hypothesize that dominant males will be bolder and more exploratory than are satellite and sneaker males. Previous results demonstrate that male morphs exhibit differences in habitat occupancy where dominant males occupy higher quality habitat than subordinate males (Lattanzio & Miles, 2014). Therefore, I also hypothesize that thermal preference in different morphs should covary with boldness and exploratory behavior. I also investigate trait covariation among female morphs.

Materials and Methods

Study Site and Lizard Capture

Adult lizards were observed and captured during 6 May-31 June 2019 from a population at the Appleton-Whittell Research Ranch (AWRR) of the National Audubon Society in southeastern Arizona. (31.365° N, –110.303° W). The population is located in a semi-arid grassland and adult lizards are arboreal, spending the majority of their time on live oak (*Quercus* sp.), mesquite (*Prosopsis* sp.), and dead tree snags. I searched for lizards during mornings between 07:00–12:00. By around 12:00, rising air temperatures at the study site resulted in a reduction in lizard activity (personal observation). I captured lizards using a pole and lasso.

Morphological Measurements and Husbandry

I transported lizards to a laboratory at AWRR. I measured snout-vent length (SVL) using calipers (mm) and body mass (in grams) using a digital Pesola$^{®}$ scale. I accounted for these traits because body size may influence behavior in lizards (de Barros *et al*., 2010). I determined sex for each individual based on the presence of enlarged post-anal scales in males and recorded color morph. In the sample population, males are characterized by blue, orange/blue, yellow/blue, orange, yellow, and orange/yellow throats. Data on behavioral variation in mosaic morphs is less common in *U. ornatus*, but in this population, yellow/blue males are abundant and similar in aggressive and territorial behavior to blue males (personal observation). I categorized males into two groups, dominant and subordinate, to investigate differences in behavior and thermal preference. Blue, orange/blue, and yellow/blue males were classified as dominant and orange, yellow and orange/yellow males were considered subordinate. Females in the sample population have yellow, orange, or white throats. During captivity, lizards were housed in individual terraria and provided a thermal gradient including their field active body temperature (36°C) to allow for thermoregulation. I maintained lizards on a 13hr/11hr light/dark cycle to mimic local photoperiod. Nocturnal temperatures were ambient. Lizards were offered mealworms daily and provided water *ad libitum.*

Thermal Preference

In a thermal gradient in the laboratory setting, lizards are able to select preferred temperatures in the absence of ecological costs. Thermal preference (T_{pref}) is expected to reflect available thermal niches to individuals in the field. I constructed a 120 cm L x 16 cm W x 20 cm H thermal gradient with aluminum sheet metal on a plywood base to assess individual lizards' thermal preferences. The gradient base was layered with a sand and spanned 27°C–45°C with temperatures maintained by 60W and 100W heating bulbs. I placed lizards in the center of the gradient and allowed them a 10-minute acclimation period, after which I measured T_b with an infrared digital thermometer every 10 minutes for 60 minutes total. For each individual, I calculated T_{pref} (the mean T_b selected over the 60-minute trial) and the coefficient of variation of T_{pref} (T_{pref}CV) to quantify the variability of individuals' T_{pref} in relation to the population average. I used T_{pref}CV rather than the interquartile range (T_{set}) because I used mean T_b as the estimate for T_{pref}. I also included a shuttling variable, calculated as the summed absolute values of the change in

T_b at 10-minute intervals. Information on shuttling behavior was included because it describes behavior of individuals in the thermal gradient. Individuals with low shuttling values selected and basked at a single location for the duration of the trial, whereas individuals with a high shuttling value moved frequently between extreme temperature values to attain a given T_{pref}.

Behavioral Measurements

The exploratory behavior of each lizard was assessed in a novel environment: a 60 cm L x 30 cm W x 25 cm H enclosure made from insulation material. The enclosure had a plywood base upon which a grid of 18 separate 10 cm x 10 cm squares was drawn. The enclosure was maintained at about 36°C to match the *U. ornatus* active field body temperature. I placed a lizard in the center of the grid under a translucent container for 5 minutes, then removed the container and gave the lizard 30 minutes to explore the novel enclosure. I recorded activity of the lizard on a camera during trials to reduce observer disturbance on subjects. I defined exploratory behavior of an individual as the number of square transitions the lizard made in the grid over the course of a 30-minute trial. I wiped the grid with isopropyl alcohol after each trial to mitigate effects from chemical cues. Boldness was measured as the response of a lizard to a simulated predation attack. I placed individuals in a 60 cm L x 30 cm W x 25 cm H enclosure with a sandy substrate and a perch (a branch heated to 36°C) on one end. Lizards were given 15 minutes to initiate basking on the perch, after which I chased them to the other end of the enclosure into a refuge box, simulating an act of predation. The refuge box covered cool sand and had a small opening on each side. I recorded lizards for 45 minutes after being chased into the refuge box and calculated boldness in two ways: amount of time lizards spent perching on the branch and amount of time they spent basking in the general heated area, but not necessarily on the branch. I replaced the sandy substrate after each trial to mitigate chemical cues. Thermal preference and behavioral trials were separated by 24 hours to reduce stress on study individuals. After the completion of trials, I gave lizards a unique toe clip for future identification and released them back to the capture site.

Statistical Analysis

Statistical analyses were conducted using R version 3.5.2 (R Core Team 2019). I assessed repeatability of thermal preference traits, exploratory behavior (number of transitions), and boldness behavior (both time spent basking and perching) using an intraclass correlation coefficient. I used type ICC3 for single fixed raters using the “ICC” function in the package “psych” (Revelle, 2020). Repeatability analyses entailed measuring thermal preference and behavior on individuals twice: quantifying the suite of traits soon after their first capture, releasing lizards at the capture site following the conclusion of the first measurements, and waiting at least one week before recapturing the individuals to conduct a second set of measurements. A one-week period before retesting was selected to avoid potential confounding effects arising from acclimation to the laboratory settling. I assessed trait repeatability in males as a recapture of females would have occurred during the initiation period of egg-laying in females.

I investigated whether behavioral or thermal preference traits varied between male status groups with a MANCOVA, using Pillai’s trace as it is robust and can accommodate unequal group sizes and Type III sum of squares. I included exploration (number of transitions), boldness (time spent perching and time spent basking), T_{pref}, T_{pref}CV, and shuttling as dependent variables. I included SVL as a covariate and excluded body mass due to its high correlation with SVL ($r = 0.8$). I also assessed whether these traits varied by morph (orange and yellow) in females. I excluded two white females from the analysis because I had fewer observations than dependent variables. I used univariate one-way ANOVAs for post-hoc analyses on significant terms to determine which variables contributed to the separation of groups in the MANCOVA.

I used the function "princomp" in the stats package to conduct a principal component analysis (PCA) for males and females to describe the patterns of covariation among thermal traits ($T_{pref,}$ T_{pref}CV, shuttling) and behavioral traits (exploration, perching, basking) in relation to male status and female morph. I also included SVL and mass. The PCAs were calculated using a correlation matrix because the traits had different scales. Three principal components (PCs) were retained for males and four PCs were retained for females based on the broken-stick criterion (Jackson, 1993). To determine whether the set of behavioral and thermal traits differed between status groups or color morphs, I compared the groups using marginal PERMANOVAs via the function "adonis2" in the package "vegan" (Oksanen *et al*., 2020). I assessed which axes contributed to significant differences between groups using ANOVAs with the retained PCs as response variables.

Results

Repeatability

Repeatability estimates were based on 27 male lizards. All behavioral traits were repeatable (transitions: ICC = 0.43, $P = 0.01$; basking: ICC = 0.69, $P < 0.001$; perching: ICC = 0.56, $P < 0.001$). Among thermal preference variables, T_{pref}CV (ICC = 0.56, $P < 0.001$) was repeatable. T_{pref} (ICC = 0.25, $P = 0.096$) and shuttling were not repeatable (ICC = 0.03, $P = 0.43$).

Thermal and Behavioral Traits

Sample Size. Thermal preference and behavioral traits were measured in 91 individuals: 62 males and 29 females. Of the 62 males, I sampled 37 dominant individuals and 25 subordinate males. Of the 29 females, I sampled 14 orange, 13 yellow, and 2 white (excluded from analysis due to low sample size) individuals.

Trait Variation Between Male Status Groups. In males, behavioral and thermal trait expression differed between status groups (Pillai's Trace = 0.22, $F_{1,54} = 2.6$, $P = 0.03$). Dominant male morphs were bolder and more exploratory than subordinate males (basking: dominant = 1,187.5 ± 60.1 seconds, subordinate = 793.9 ± 76.3 seconds; $F_{1,60} = 5.1$, $P = 0.03$, Figure 13A; perching: dominant = 909.1 ± 57.2 seconds, subordinate = 577.8 ± 69.3 seconds; $F_{1,60} = 4.1$, $P = 0.046$, Figure 13B; transitions: dominant = 77.1 ± 7.5, subordinate = 32.3 ± 4.6; $F_{1,60} = 6.7$, $P = 0.01$, Figure 13C). Dominant males also exhibited higher values for preferred body temperature than subordinate males (dominant = 36.6 ± 0.12°C, subordinate = 35.8 ± 0.19°C; $F_{1,60} = 3.9$, $P = 0.05$, Figure 13D) and had lower T_{pref}CV and shuttling values than subordinate males (T_{pref}CV: dominant = 4.4 ±

0.30°C, subordinate = 6.6 ± 0.40°C; $F_{1,60} = 6.0$, $P = 0.02$, Figure 13E; shuttling: dominant = 9.7 ± 0.49°C, subordinate = 13.7 ± 0.50°C; $F_{1,60} = 9.1$, $P = 0.004$, Figure 13F). Due to the significant differences between groups, all traits were considered for further analysis.

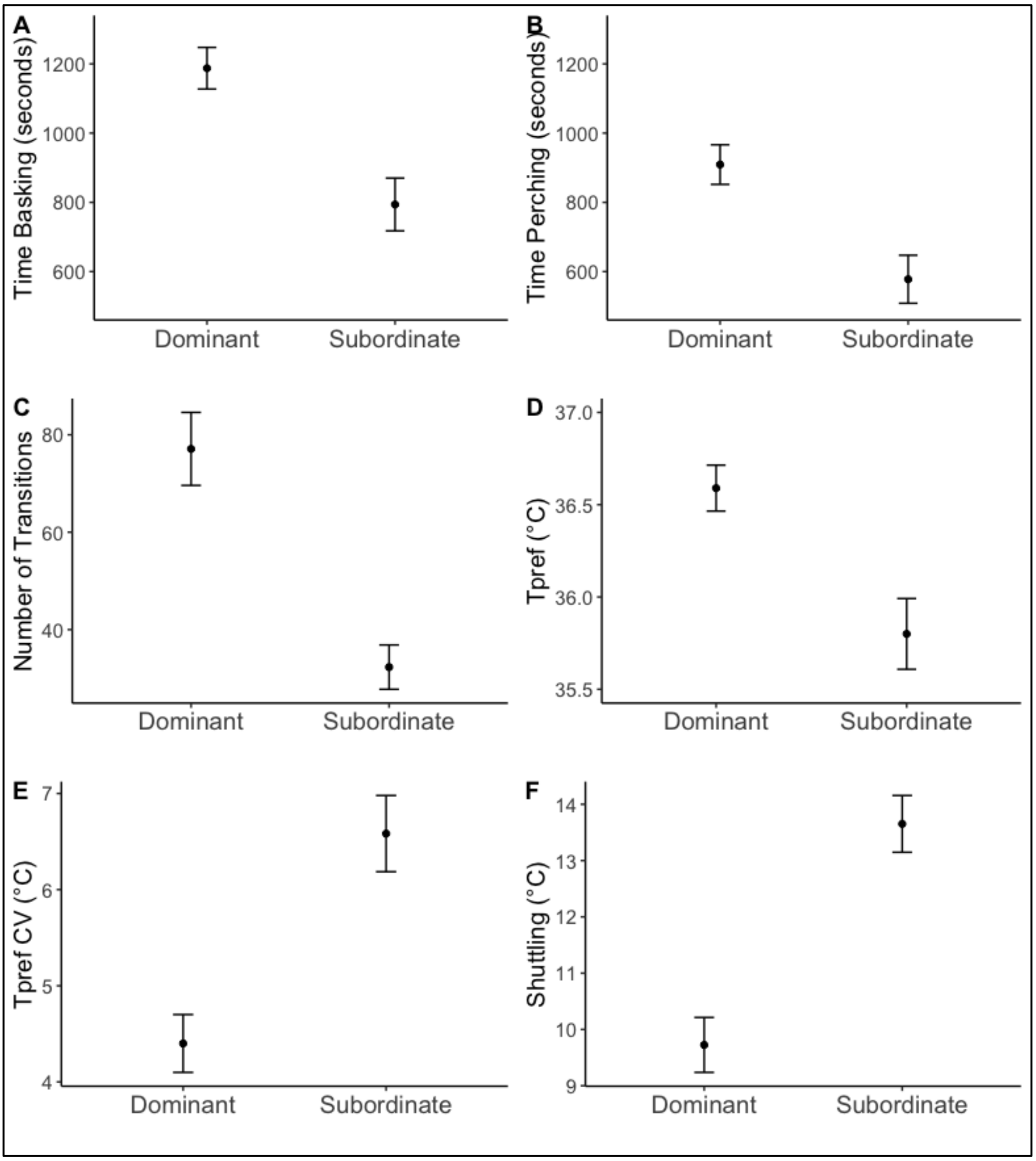

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Figure 13
Variation in thermal and behavioral traits between dominant and subordinate males. All traits varied significantly between male status groups.

Trait Variation Between Female Morphs. In females, behavioral and thermal trait expression did not differ between orange and yellow morphs (basking: orange = 992.2 ± 75.3 seconds, yellow= 924.1 ± 79.9 seconds, Figure S2A; perching: orange =

740.4 ± 73.1 seconds, yellow = 689.8 ± 73.2 seconds, Figure S2B; transitions: orange = 74.4 ± 7.6, yellow = 70.9 ± 6.3, Figure S2C; T_{pref}: orange = 36.5 ± 0.11°C, yellow = 35.8 ± 0.14°C, Figure S2D; T_{pref}CV: orange = 5.3 ± 0.26°C, yellow = 5.0 ± 0.23°C, Figure S2E; shuttling: orange= 12.3 ± 0.58°C, yellow = 11.7 ± 0.61°C, Figure S2F; Pillai's Trace = 0.06, $F_{1,19} = 0.22$, $P = 0.97$). However, SVL was associated with trait expression in females (Pillai's Trace = 0.47, $F_{1,19} = 2.8$, $P = 0.04$). Specifically, larger females preferred higher body temperatures ($F_{1,25} = 16.6$, $P < 0.001$, Figure 14).

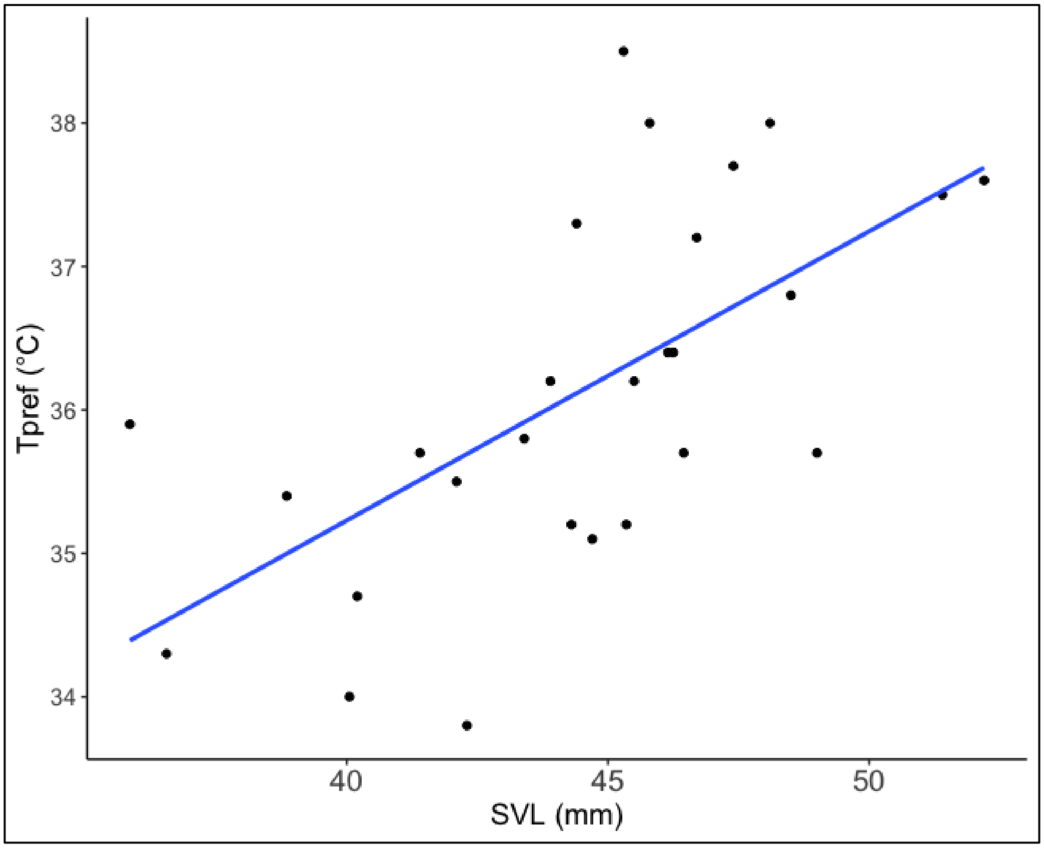

Figure 14
Larger female individuals prefer warmer body temperatures ($F_{1,25} = 16.6$, $P < 0.001$).

PCA. I assessed behavioral and thermal trait covariation in relation to male status and female morph using PCAs. In males (N = 62), I retained three PC axes based on the broken stick criterion, which explained 79.3% of the total variation. The first PC axis explained 33.9% of the total variation and described covariation between thermal and behavioral traits, showing that males with high boldness scores had lower T_{pref}CV and shuttling values. The second PC axis explained 27.3% of the total variance and had the greatest contribution from thermal traits such that males with higher T_{pref}CV and shuttling values preferred lower body temperatures. The third PC axis explained 18.1% of the total variation and showed that larger, heavier males were bolder and more exploratory (Table 2; Figure 15). Dominant and subordinate males differed significantly in PC space ($F_{1,60} = 4.6$, $R^2 = 0.07$, $P = 0.02$), and specifically along PC1 ($F_{1,60} = 17.6$, $P < 0.001$).

Table 2

Loadings (variable coordinates/square root of eigenvalue) of behavioral and thermal preference traits and body size to PC Axes 1, 2, and 3 in male lizards.

Variable	PC Axis		
	1	2	3
Transitions	0.26	0.13	0.36
Basking	0.43	0.29	0.37
Perching	0.42	0.30	0.34
T_{pref}	0.21	-0.56	
T_{pref}CV	-0.41	0.44	
Shuttle	-0.40	0.40	
SVL	-0.34	-0.27	0.55
Mass	-0.30	-0.29	0.56
Eigenvalue	2.72	2.19	1.45
Percent Variance Explained	33.9%	27.3%	18.1%
Cumulative Variance Explained	33.9%	61.2%	79.3%

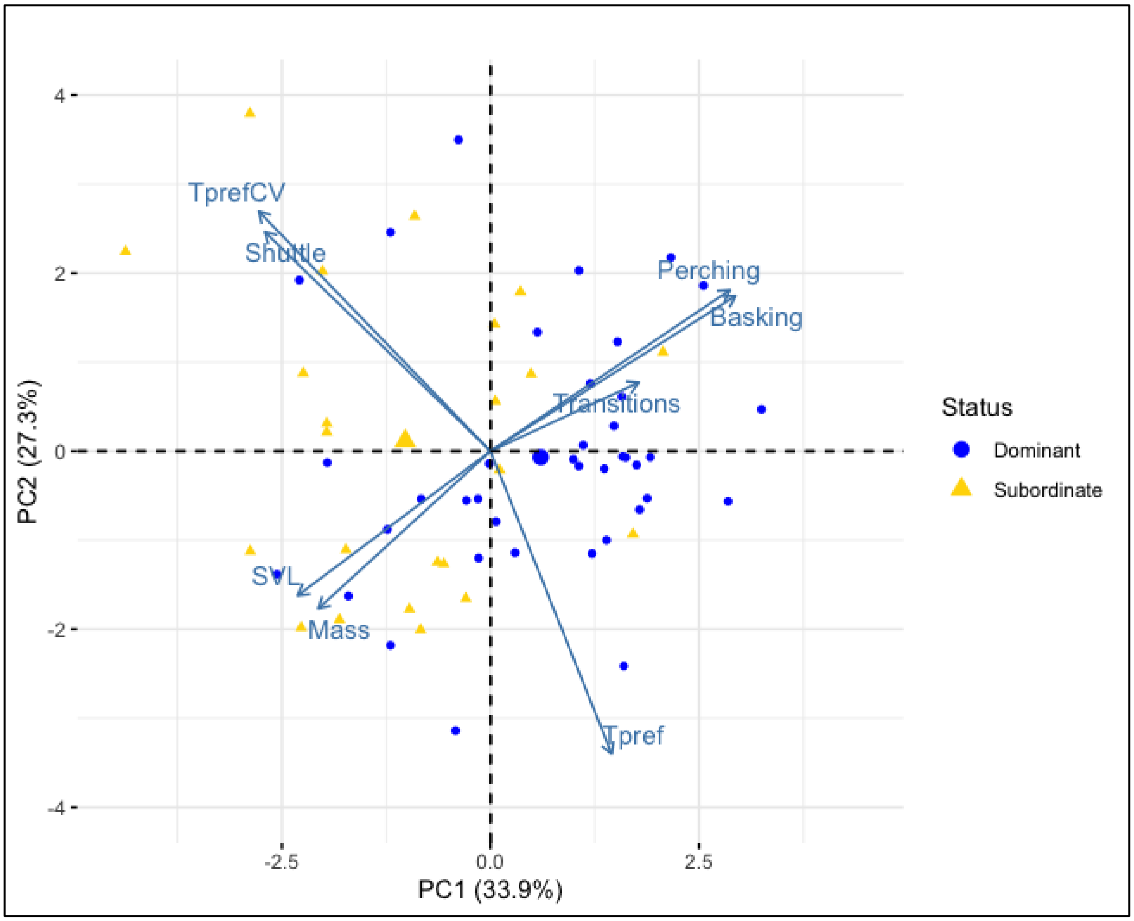

Figure 15
Patterns of covariation among behavioral and thermal traits and body size based on the first two axes from a principal component analysis in males. Dominant and subordinate males differed in PC space, specifically on PC1 ($F_{1,60} = 17.6$, $P < 0.001$). Mean position of each status group is designated with a larger shape in the plot.

In females (N = 29), four PC axes were retained based on the broken-stick criterion and accounted for 91.9% of the total variation. The first PC axis explained 39.1% of the total variation and had the greatest contributions from SVL and mass, which covaried positively with T_{pref} and negatively with boldness. The second PC axis explained 24% of the total variation and was dominated by negative loadings from T_{pref}CV and shuttling. The third PC axis explained 18% of the total variation and described larger females as bolder and preferring higher body temperatures. The fourth PC axis explained 10.8% of the total variation and was dominated by a positive loading from exploratory behavior (Table 3; Figure 16). There was no difference between female color morphs in PC space ($F_{1,25} = 0.04$, $R^2 = 0.002$, $P = 0.93$).

Table 3
Loadings (variable coordinates/square root of eigenvalue) of behavioral and thermal preference traits and body size to PC Axes 1, 2, 3, and 4 in female lizards.

Variable	PC Axis			
	1	2	3	4
Transitions	0.29		0.14	0.87
Basking	0.34	-0.24	0.55	-0.15
Perching	0.36	-0.19	0.54	-0.14
T_{pref}	-0.37	0.23	0.35	0.34
$T_{pref}CV$	-0.22	-0.61	-0.16	0.29
Shuttle	-0.11	-0.69		
SVL	-0.47		0.38	
Mass	-0.50		0.30	
Eigenvalue	3.13	1.92	1.44	0.87
Percent Variance Explained	39.1%	24.0%	18.0%	10.1%
Cumulative Variance Explained	39.1%	64.1%	81.1%	91.9%

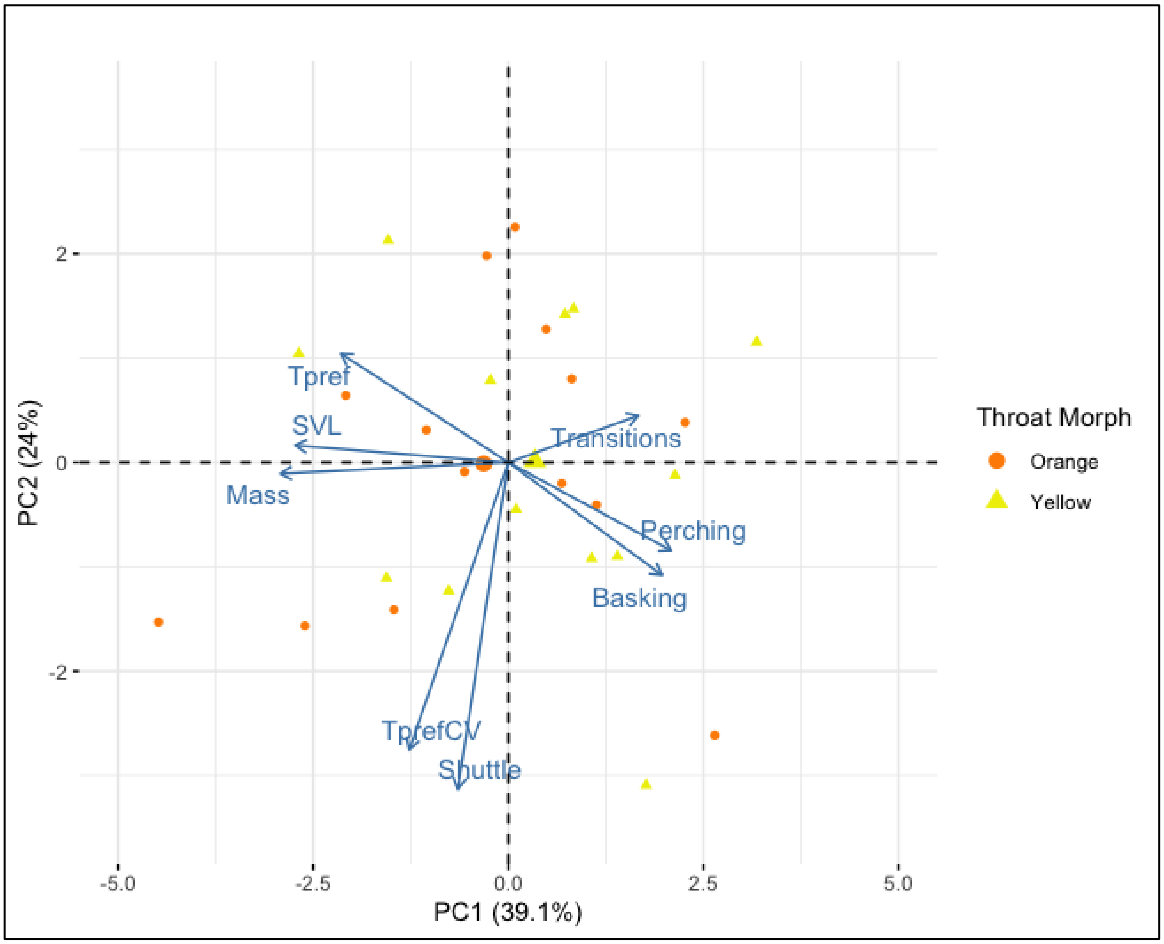

Figure 16
Patterns of covariation among behavioral and thermal traits and body size based on the first two axes from a principal component analysis in females. Mean position of each status group is designated with a larger shape in the plot. Orange and yellow females did not differ in PC space.

Discussion

Color polymorphic lizard species often exhibit behavioral variation among morphs that reflects alternative reproductive strategies and dominance hierarchies (Sinervo & Lively, 1996; Stuart-Fox *et al*., 2021). Morph variation in other behaviors and in thermal traits is less often studied. Here, I found that in *U. ornatus* males, behavioral and thermal trait expression covaried with morph associated dominance status. Dominant males were bolder and more exploratory than were subordinate males. In addition, subordinate males exhibited greater amounts of shuttling behavior and higher variation in preferred temperatures. In contrast, dominant males minimized movement while basking, resulting in higher preferred body temperatures and a lower CV. Male body size had no discernable influence on relationships between dominance status and trait expression. All behavioral and some thermal traits were repeatable in males. In females, color morph had no discernable influence on behavior or thermal traits and I found no significant covariation between any traits. However, larger females selected for higher body temperatures in the thermal gradient.

Color polymorphic species are known to occupy different environmental niches with varying thermal quality and access to resources such as food and mating opportunities (Forsman & Åberg, 2008; Forsman *et al*., 2008; i de Lanuza & Carretero, 2018; Thompson *et al*., 2023). In *U. ornatus*, blue and yellow/blue males are dominant over yellow and orange morphs and defend larger territories. There is also evidence that when overall environmental quality is low, dominant *U. ornatus* males utilize microhabitats that offer high-quality prey items at a higher trophic level and increased thermal quality at higher rates than the microhabitats occupied by subordinate males (Lattanzio & Miles, 2014; Lattanzio & Miles, 2016). My study provides insight into behavioral mechanisms that underly ecological segregation and variation in microhabitat use between status groups. In other lizard species, bolder and more exploratory individuals occupy larger territories than shyer, less exploratory individuals. Bolder individuals also enjoy higher mating success and habituate to predators more quickly

(Rodríguez-Prieto *et al*., 2011; Ward-Fear *et al*., 2018). Increased territory size can enable dominant males to exploit higher-quality habitat (Fox *et al*., 1981). In the population, individuals of *U. ornatus* occupy both high-quality thermal habitats, i.e., live trees and lower-quality dead snags. The latter habitat type exhibits lower habitat heterogeneity, canopy cover, and fewer prey items. Similar to the findings of Lattanzio & Miles (2014), dominant males in this study occupied high-quality live trees at greater rates than did subordinate males: 22/39 (56%) of dominant males were captured on live trees whereas only 9/23 (39%) of subordinate males were captured on live trees. This variation in habitat use between dominant and subordinate morphs provides evidence that ecological niche segregation between color morphs may be driven in part by variation in behavioral expression.

I documented significant thermal trait variation between dominant and subordinate morphs. In addition to being bolder and more exploratory, dominant male morphs preferred higher body temperatures and maintained body temperatures in a narrower range than did subordinate morphs. Despite the prevalence of color polymorphisms in lizards, limited data exist pertaining to morph variation in preferred body temperature. I am aware of two other studies that report differences in thermal preference among morphs. Paranjpe *et al*. (2013) found that in *U. stansburiana*, yellow female morphs preferred higher body temperatures than did orange and yellow/orange females. In addition, Thompson *et al*. (2023) demonstrated that orange *Podarcis erhardii* morphs preferred lower temperatures than did white and yellow morphs, coinciding with variation in microhabitat use. These findings are further evidence that variation in thermal preference should be considered in studies of polymorphic species. Importantly, variation in T_{pref} between morphs covaried with boldness and exploratory behavioral strategies, consistent with the ecological niche separation between morph groups documented by Lattanzio and Miles (2016). I expected a heterogeneous environment to favor divergence in thermal preference coinciding with differences in microhabitat exploitation (Lelièvre *et al.*, 2011). Indeed, behavioral differences observed between morphs could reflect the fact that they occupy microhabitats that vary in thermal quality. In addition to preferring higher body temperatures in the thermal gradient, dominant males selected for higher body temperatures in the field than did subordinate males (average field T_b of dominant males: 34.1°C; average field T_b of subordinate males: 32.9°C). I suggest a pattern of greater precision associated with a higher T_{pref} and field active T_b may reflect the fact that dominant males exclude subordinates from access to higher-quality thermal environments, as maintenance of preferred body temperatures is easier for individuals in high-quality habitat (Waldschmidt & Tracy, 1983; Calsbeek & Sinervo, 2002). In contrast, subordinate males demonstrate wider body temperature ranges in the thermal gradient, using shuttling to a greater extent during thermoregulation. Together with less frequent boldness and exploration behaviors, these data indicate ecological and thermal niche partitioning such that subordinate males occupy lower-quality habitat and rely on increased shuttling behavior throughout a wider range of thermal conditions to achieve preferred body temperatures.

Homogeneous patterns of trait variation in females suggest that color polymorphism in female *U. ornatus* is not related to boldness, exploration, or exploitation of the thermal environment. Although some studies have documented behavioral and thermal trait variation among female lizard morphs (Paranjpe *et al.*, 2013; Thompson *et al.*, 2023), my findings are consistent with previous work indicating that in many cases, trait variation between sympatric female morphs coincides more with mate choice and reproductive traits than with behavior. The few studies that have addressed variation between female morphs in *U. ornatus* retrieve similar relationships. Lattanzio *et al.* (2014) found that that yellow and orange females differ in mating preference, with orange females preferring dominant males and yellow females preferring subordinate males. Zucker & Boecklen (1990) described throat color changes when females became gravid, suggesting that female throat color was a good predictor of clutch size. Although my results indicate lack of behavioral variation between female morphs, future studies could investigate the influence of the interaction between female morph and environmental condition on *U. ornatus* reproductive behavior; for example, in *U. stansburiana*, female morphs alter clutch size and egg mass differentially depending on the presence of same- and different-morph neighbors (Svensson *et al.*, 2001).

In any case, larger females preferred higher body temperatures than did smaller females. Female *U. ornatus*, although less aggressive and territorial than males, do establish and maintain territories (Mahrt, 1998), indicating that variation in thermal environments may be available to individual females. It is unclear whether body size variation confers competitive advantage in microhabitat acquisition, as variation in female lizard thermal preference often does not correlate with body size (Beal *et al.*, 2014; Cecchetto & Zaretto, 2015). Instead, covariation between body size and T_{pref} in females may be reproductive in nature. I measured behavioral and thermal traits during the early part of vitellogenesis. Larger, gravid females may have preferred higher body temperatures to enhance the maturation of the eggs prior to oviposition (Webber *et al.*, 2015).

Overall, my results demonstrate that alternative reproductive strategies in male *U. ornatus* coincide with behavioral and thermal trait covariation that may underly exploitation of variable thermal environments. Future studies could investigate behavioral and thermal trait variation in color polymorphic species to bolster data on boldness, exploratory behavior, and thermal preference variation among color morphs given the ecological relevance of these traits (Carter *et al*. 2010; Rodríguiz-Prieto *et al*., 2011; Ward-Fear *et al*., 2018; Chen *et al*., 2019; Brock & Madden, 2022; Thompson *et al*. 2023). Elucidating trait variation in color polymorphic species is critical for exploring how natural and sexual selection promote and maintain color polymorphisms in natural populations (Gray & McKinnon, 2007; Chelini *et al*., 2021). Further, assessing distinct ecological roles among color morphs and the trait variation that underlies niche segregation can inform how different morphs may be influenced by and respond to rapidly changing environments.

Chapter 5: Behavioral Plasticity During Acute Heat Stress: Heat Hardening Increases the Expression of Boldness Behavior

Introduction

Anthropogenic climate change is increasing average global temperatures and the frequency and severity of extreme weather events including heat waves and droughts (IPCC, 2022). Such changes threaten biodiversity worldwide (Garcia *et al*., 2014) but ectotherms, which depend on external sources to regulate body temperature, are particularly vulnerable to temperature fluctuations (Kingsolver *et al*., 2013; Paaijmans *et al*., 2013). Because of the underlying influence of body temperature on physiology (Huey & Stevenson, 1979), exposure to increased temperatures and climate extremes can alter ectotherm activity patterns, performance capacity, and ultimately fitness and survival (Folguera *et al*., 2009; Sinervo *et al*., 2010; Kingsolver *et al*., 2013; Gunderson & Leal, 2015).

If novel thermal environments threaten organismal performance and survival, species can respond in several ways. They can undergo range shifts to track favorable thermal conditions (Davis & Shaw, 2001; MacLean & Beissinger, 2017), but for many terrestrial vertebrates, dispersal capacity is limited. In these cases, organisms either adapt to novel thermal conditions via genetic changes, make behavioral or physiological adjustments via phenotypic plasticity, or face extirpation (Merilä & Hendry, 2014; Seebacher *et al*., 2015; Gunderson *et al*., 2017). Evolution of thermal traits such as optimal performance temperature (T_{opt}), preferred body temperature (T_{pref}), and thermal tolerance (critical thermal maximum CT_{max}) has received renewed attention as evolutionary biologists question whether thermal traits can evolve at a rate that matches the rapid pace of climate change (Bodensteiner *et al*., 2020). Many comparative studies have concluded that CT_{max} evolves more slowly than does cold tolerance (CT_{min}) as demonstrated by CT_{max} conservatism across evolutionary lineages and geographic locations (Araújo *et al*., 2013; Grigg & Buckley, 2013; Muñoz *et al*., 2014). Many ectotherms utilize behavioral thermoregulation to regulate body temperature by shuttling between thermally distinct microhabitats, adjusting activity time, or altering body position; these behavioral adjustments can buffer body temperature from environmental variation and thereby constrain selection pressure (Huey *et al*., 2003). However, a few recent studies have demonstrated selection operating on and evolutionary lability of thermal traits (Gilbert & Miles, 2017; Gilbert & Miles, 2019b; Herrando-Pérez *et al*., 2020), indicating that thermal traits, and especially CT_{max}, may have more evolutionary potential than presented in the literature (Bodensteiner *et al*., 2020).

Plastic responses, or phenotypic changes to environmental stimulus without genetic change, are thought to play a key role in buffering species from short-term environmental variation attributable to climate change (Keller & Seehausen, 2012; Urban *et al*., 2014). Well-documented adaptive plastic responses to warming environments include behavioral thermoregulation and in some species, developmental adjustments to T_{pref} such that individuals reared under warmer temperatures prefer warmer temperatures (Blouin-Demers *et al*., 2000; Paranjpe *et al*., 2013; Refsnider *et al*., 2019; Singh *et al*., 2020). Developmental plasticity has been shown to have little ability to increase heat tolerance (Dayananda *et al*., 2017; Llewelyn *et al*., 2018; Gunderson *et al*., 2020);

however, organisms can undergo a rapid plastic response to increase heat tolerance during periods of extreme physiological stress. When body temperatures approach CT_{max}, individuals can upregulate heat-shock proteins and prevent cellular damage in a process called heat hardening (Bowler, 2005). Increase in heat-shock proteins results in a temporary increase in CT_{max} that occurs in the span of minutes to hours (Loeschcke & Hoffman, 2007). Especially in hot climates, heat hardening may provide ectotherms with an effective first line of defense during extreme weather events such as heat waves and can increase short-term survivorship and fitness by increasing available activity time (Loeschcke & Hoffman, 2007; Seebacher *et al.*, 2015; Phillips *et al.*, 2016).

Plastic responses to environmental variation, and especially stress responses, are often energetically costly and involve trade-offs with life-history traits and tolerance plasticity (Kaitala, 1991; Jordan & Snell, 2002; Gervasi & Foufopoulos, 2008; Deery *et al.*, 2021). Likewise, recent evidence has demonstrated maladaptive phenotypic shifts associated with heat hardening. Tree lizards (*Urosaurus ornatus*) that underwent a heat hardening response manifested a decrease in both locomotor performance and preferred body temperature (Gilbert & Miles, 2019a). Both traits have been shown to be under directional selection in *U. ornatus* (Gilbert & Miles, 2017), and decreased locomotor performance is often coupled with lower survivorship and fitness in lizards (Le Galliard *et al.*, 2004; Miles, 2004; Calsbeek & Irschick, 2007). Not only does the study of Gilbert and Miles (2019a) demonstrate a physiological trade-off associated with heat hardening (reduced locomotor capacity), but a decrease in T_{pref} also indicates a behavioral response to heat stress. In a heterogeneous thermal environment, changes in thermoregulatory behavior likely lead to adjustments in microhabitat selection, mediating physiology and social interactions (Huey, 1991; Gilbert & Miles, 2019a). Given this link between heat hardening and behavior, and the physiological intensity of heat stress responses, it is plausible that other behavioral trade-offs accompany heat hardening (Gilbert & Miles, 2019a). Such phenotypic shifts could influence survival and reproduction. Elucidating the nature of these relationships is critical when considering the potential of heat hardening as an adaptive plastic response to warming temperatures and changing thermal environments.

Here, I investigated the influence of heat hardening on boldness behavior in the tree lizard (*U. ornatus*). The tree lizard is a desert species that inhabits a diversity of habitats and likely relies on heat hardening during its active season, as ambient temperatures often exceed the species' CT_{max} (Goerge & Miles, unpublished data). It is furthermore an excellent study species because duration and intensity of heat hardening responses, as well as associated phenotypic shifts, have been documented (Gilbert & Miles, 2019a). I investigated boldness, here defined as willingness or aversion to engage in risky behavior, as *U. ornatus* has demonstrated distinct, repeatable boldness behavior (Taylor & Lattanzio, 2016; Goerge & Miles, unpublished data). Males and females of *U. ornatus* are also characterized by a throat color polymorphism, with males having mosaics of blue, yellow, or orange throats and females exhibiting either orange, yellow or white throats. In males, throat color is associated with alternative reproductive strategies and behavioral differences, including boldness (Hover, 1985; Thompson & Moore, 1991a; Taylor & Lattanzio, 2016; Goerge & Miles, unpublished data). Boldness in lizards influences traits including territorial status, mating success, and survivorship (Kuo *et al.*, 2015; Ward-Fear *et al.*, 2018); therefore, changes in boldness, coupled with shifting social landscapes resulting from altered thermoregulatory behavior (Gilbert & Miles, 2019a), may prove maladaptive. My objectives in this study were to (i) assess the influence of heat hardening on boldness behavior in *U. ornatus,* (ii) investigate whether males and females exhibit concordant responses to heat hardening, and (iii) assess whether color morphs responded differently to heat hardening. Given previous results that indicate a reduction in locomotor performance and thermal preference associated with heat hardening (Gilbert & Miles, 2019a), I predicted that boldness behavior would decrease with a heat hardening response.

Materials and Methods

Study Site and Lizard Population Characteristics

I captured adult tree lizards from a site located at the Appleton-Whittell Research Ranch (AWRR) of the National Audubon Society in southeastern Arizona (31.365° N, –110.303° W) from 7 May – 2 June 2022. The study period falls within the reproductive season of *U. ornatus*, during which ambient and substrate temperatures often exceed 36°C, the average thermal preference and optimal performance temperature of the lizards. Both males and females establish and defend territory exclusively on trees (Mahrt, 1998). The focal population is located within a 2-hectare area situated in a semi-arid oak-grassland consisting of oak trees (*Quercus emoryi, Q. arizonica*), mesquite trees (*Prosopsis velutina*) and various grass species (*Bouteloua curtipendula, Eragrostis intermedia, Bothriochola barbinodis*). At this site, lizards also occupy large dead trees (snags) that offer direct sun exposure and ample basking opportunities. Territories of males and females are associated with oak and mesquite trees throughout the site and multiple males and females can occupy the same trees. At AWRR, relative frequencies of color morphs fluctuate yearly (Table 4), although some morphs remain rarer than others. Yellow/blue and blue males are common whereas orange/blue, yellow, orange, and yellow/orange males are rare. Most females are yellow or orange, and white females are less common.

Table 4
Frequencies of *U. ornatus* color morphs sampled by year from a single population in southeast Arizona. Values represent percentages that each morph makes up of its respective sex.

Sex	Morph	2018 ($N_f = 18$) ($N_m = 48$)	2019 ($N_f = 68$) ($N_m = 76$)	2021 ($N_f = 42$) ($N_m = 90$)	2022 ($N_f = 31$) ($N_m = 37$)
Female	Orange	38.9	52.9	42.9	45.2
	Yellow	55.6	42.6	52.4	35.5
	White	5.6	4.4	4.8	19.4
Male	Blue	20.8	22.4	18.9	21.6
	Orange/Blue	4.2	11.8	14.4	10.8
	Orange	2.1	3.9	4.4	0
	Orange/Yellow	16.7	10.5	2.2	10.8
	Yellow/Blue	47.9	39.5	55.6	54.1
	Yellow	8.3	11.8	4.4	2.7

Lizard Capture and Husbandry

I searched for lizards from 07:00–12:00. By noon, ambient temperatures at the study site exceed lizards' voluntary body temperature for activity, exacerbated by the exposed nature of the habitat (personal observation). I captured lizards using a noose and recorded body temperature (T_b) using an infrared digital thermometer (Amprobe IR-750).

The use of skin surface temperatures as a measure of T_b have been validated against cloacal thermometers in *U. ornatus* (Gilbert & Miles, 2019b). I recorded the sex of lizards (male *U. ornatus* have enlarged post-anal scales) and the color morph via visual inspection. I transported lizards to a laboratory at AWRR and measured snout-vent length (SVL) and tail length (to the nearest mm) and body mass (to the nearest 0.1 g). During captivity lizards were housed in individual terraria and provided with a thermal gradient via heat tape to allow for thermoregulation. Lizards were maintained on a 13hr/11hr light/dark cycle to mimic local photoperiod. Nocturnal temperatures were ambient. Lizards were offered mealworms daily and provided water *ad libitum.* I allowed lizards 24 hours to acclimate to the laboratory before further testing.

Thermal Preference

The T_b that ectotherms select in a thermal gradient (T_{pref}) is assumed to represent temperatures that optimize performance in the absence of ecological costs or constraints (Huey & Bennett, 1987). I quantified T_{pref} of individuals using a photothermal gradient constructed with cardboard on a plywood base (120 x 16 x 20 cm, L x W x H) covered with sand. I generated a linear thermal gradient of ~27°C–44°C by suspending a 100W incandescent lightbulb at one end of the tract and a second 60W bulb at the middle. I placed lizards in the middle of the gradient and allowed them to acclimate for 10 minutes, after which I recorded T_b every 10 minutes for a total of 60 minutes using an infrared digital thermometer (Gilbert & Miles, 2019b). I calculated T_{pref} as mean T_b over the duration of the trial.

Quantifying Boldness Behavior

I measured baseline boldness behavior in individuals 24 hours after completion of thermal preference trials to minimize stress on the lizards. I quantified boldness as latency to emerge from a refuge following a simulated predator attack. This approach follows other published methods for quantifying boldness in lizards (Carazo *et al*., 2014; Le Galliard, 2015). Lizards were tested in a 60 x 30 x 25 cm (L x W x H) enclosure with a sandy substrate. I placed a perch on one end of the enclosure and heated it to 36°C with an incandescent lightbulb suspended above the perch. A small refuge box (10 x 30 x 8 cm, L x W x H) with one low opening, meant to simulate a natural refuge that *U. ornatus* may use such as a crack in tree bark, was placed at the other end of the enclosure. Individuals were placed in the enclosure for 15 minutes for acclimation and to assess behavior before a simulated predator attack. All lizards initiated basking on or near the perch during this time. After 15 minutes, I simulated predation pressure by chasing lizards into the refuge box via gentle taps to the pelvic girdle with a paintbrush. I recorded the behavior of lizards for 30 minutes after the attack using a camera (Canon PowerShot SX30 IS) to avoid disturbing the lizard by presence of an observer. I quantified boldness as latency to emerge from the refuge box (in seconds). Lizards were considered out of the refuge box when their pelvic girdle had fully emerged. I replaced the sandy substrate after each trial to remove any potential chemical cues.

Heat Hardening

Twenty-four hours after lizards completed a baseline boldness test, I initiated a heat-hardening response by warming individuals until they reached CT_{max}. Lizards were first warmed to 36°C in a temperature-controlled chamber, after which they were moved to a separate enclosure heated to ~46°C using incandescent lightbulbs. This setup raises lizard T_b by about 1°C per minute (Gilbert & Miles, 2016). I considered lizards to have reached their CT_{max} when they lost their righting response (Huey & Stevenson, 1979). When lizards began to pant, indicating that the lizard was approaching CT_{max}, I checked for a righting response every 30 seconds. After righting response was lost, I immediately measured its T_b. I then placed the lizard into a cool water bath lower its body temperature. I had no mortality events from CT_{max} trials. In *U. ornatus* from this population, peak heat hardening response occurs six hours post CT_{max} and duration of the response lasts for about 24 hours (Gilbert & Miles, 2019a). Therefore, to measure influence of heat hardening on boldness behavior, individuals completed another identical boldness test six hours post CT_{max}. Because boldness behaviors may change due to familiarity of the refuge and not due to the hardening treatment, I measured boldness in a group of control lizards 24 hours after baseline measurement without inducing a heat hardening response. Post-recovery, individuals were released back at their site of capture using coordinates obtained with a Garmin GPSMAP device.

Statistical Analysis

Statistical analyses were performed in R version 4.0.3 (R Core Team 2020). I tested for differences between males and females in T_b, T_{pref}, baseline latency, and peak heat hardening latency using a MANOVA. I compared boldness behavior before and during peak heat hardening using mixed effects models with a log(x+1) transformation, eliminating extreme outliers and increasing normality of the residuals. I performed a mixed effects model on all lizards and included an interaction between treatment (baseline vs. heat hardening) and sex, SVL as a covariate, and lizard ID as a random effect. I also performed mixed effects models on males and females separately, including an interaction between treatment and color morph, SVL as a covariate, and lizard ID as a random effect, and on each morph if the sample size was large enough. I assessed influence of SVL, body mass, and T_{pref} on latency before and during heat hardening, as well as magnitude of change between the two trials, using linear models. I ran these models on all lizards, males, and females.

Results

I captured 68 *U. ornatus* individuals. The sample consisted of 37 males and 31 females. Distribution of morphs among males comprised: 20 yellow/blue, 8 blue, 4 orange/yellow, 4 orange/blue, and 1 yellow. The female sample had the following throat morphs: 14 orange, 11 yellow, and 6 white.

There were no differences between males and females in baseline latency to emerge from the refuge (males: 620.9 ± 119.5 seconds (mean ± standard error); females: 408.2 ± 109.0 seconds), peak heat hardening latency (males: 238.5 ± 52.0 seconds; females: 218.7 ± 48.0 seconds), T_{pref} (males: 35.9 ± 0.22°C; females: 35.7 ± 0.31°C), or field active T_b (males: 34.3 ± 0.24°C; females: 34.5 ± 0.38°C; Pillai's Trace = 0.04, $F_{1,65} = 0.59$, $P = 0.67$).

Among all lizards, heat hardening was significantly associated with increased boldness behavior (baseline latency: 523.9 ± 82.3 seconds; peak heat hardening latency: 229.5 ± 35.5 seconds; $t_{(67)} = -2.9$, $P = 0.005$; Figure 17A). This response was present in males (baseline latency: 620.9 ± 119.5 seconds; peak heat hardening latency: 238.5 ± 52.0 seconds; $t_{(36)} = -2.7$, $P = 0.01$; Figure 17B) and specifically within yellow/blue morphs (baseline latency: 662.9 ± 168.8 seconds; peak heat hardening latency: 241.5 ± 85.9 seconds; $t_{(19)} = 2.4$, $P = 0.03$). There was no significant difference among male morphs in the magnitude of change in latency between trials (all pairwise post-hoc comparisons $P > 0.25$; Figure 18). There was no significant difference in latency between trials in females (baseline latency: 408.2 ± 109.0 seconds; peak heat hardening latency: 218.7 ± 48.0 seconds; $t_{(30)} = -1.5$, $P = 0.14$) and no difference among morphs in the magnitude of change in latency between trials (all pairwise post-hoc comparisons $P > 0.25$; Fig. 19). The control group of lizards showed no difference in boldness between measurements (first trial: 580.5 ± 121.1 seconds; second trial: 490.4 ± 99.2 seconds; $t_{(26)} = -1.0$, $P = 0.31$; Fig. 20).

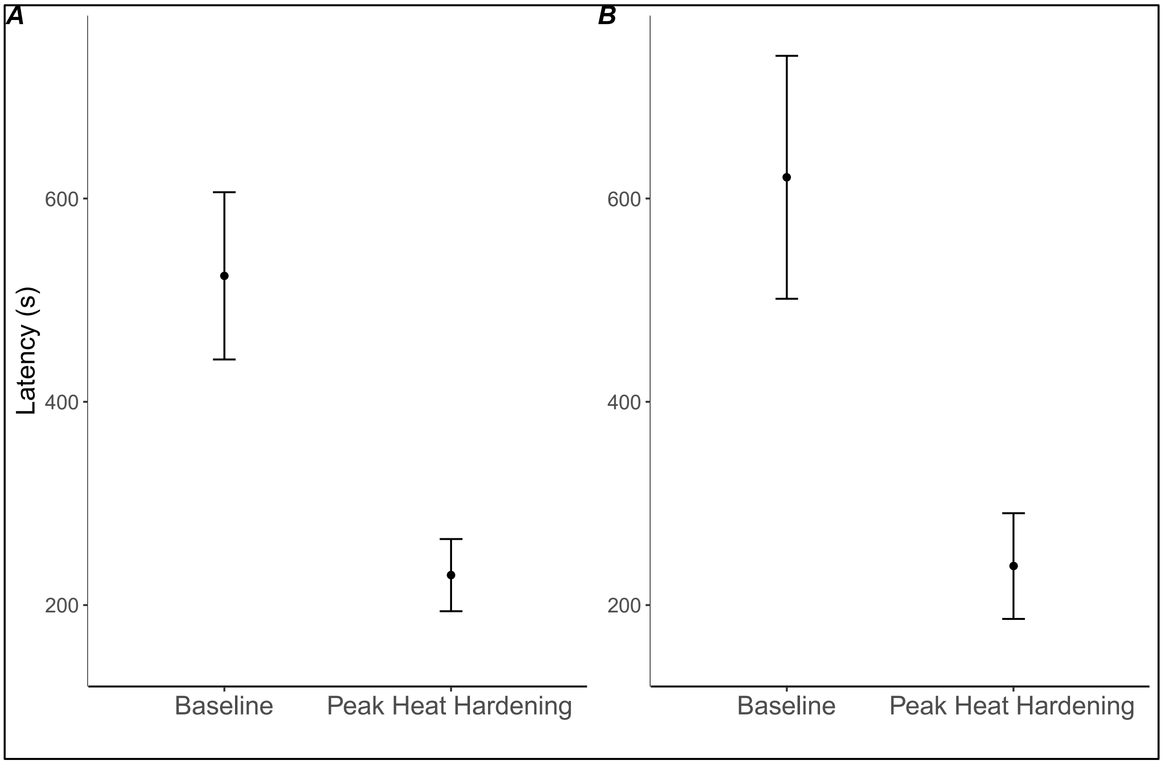

Figure 17
A, Influence of heat hardening on boldness behavior, measured as latency to emerge from a refuge after a simulated predator attack, among all measured *Urosaurus ornatus*. Lizards significantly increased boldness behavior during peak heat hardening ($P = 0.005$). Points represent means and error bars represent standard error. B, Influence of heat hardening on boldness behavior among males only. Males significantly increased boldness behavior during peak heat hardening ($P = 0.01$).

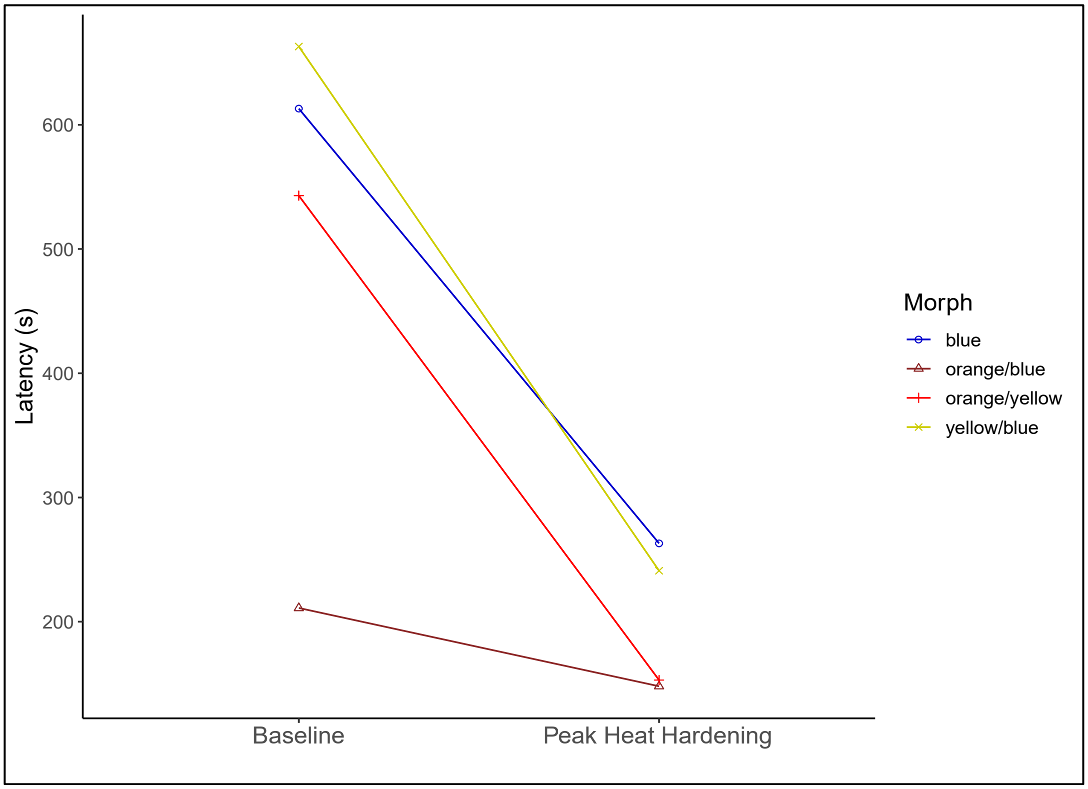

Figure 18
Mean change in boldness behavior (latency to emerge from a refuge after a simulated predator attack) before and during heat hardening among male morphs. No difference was observed in degree of change between morphs (all $P > 0.25$). Yellow/blue males (N = 20) significantly increased boldness behavior during heat hardening ($P = 0.03$). All other morphs (blue N = 8, orange/yellow N = 4, orange/blue N = 4) did not ($P > 0.05$). A single yellow male was excluded from the analysis.

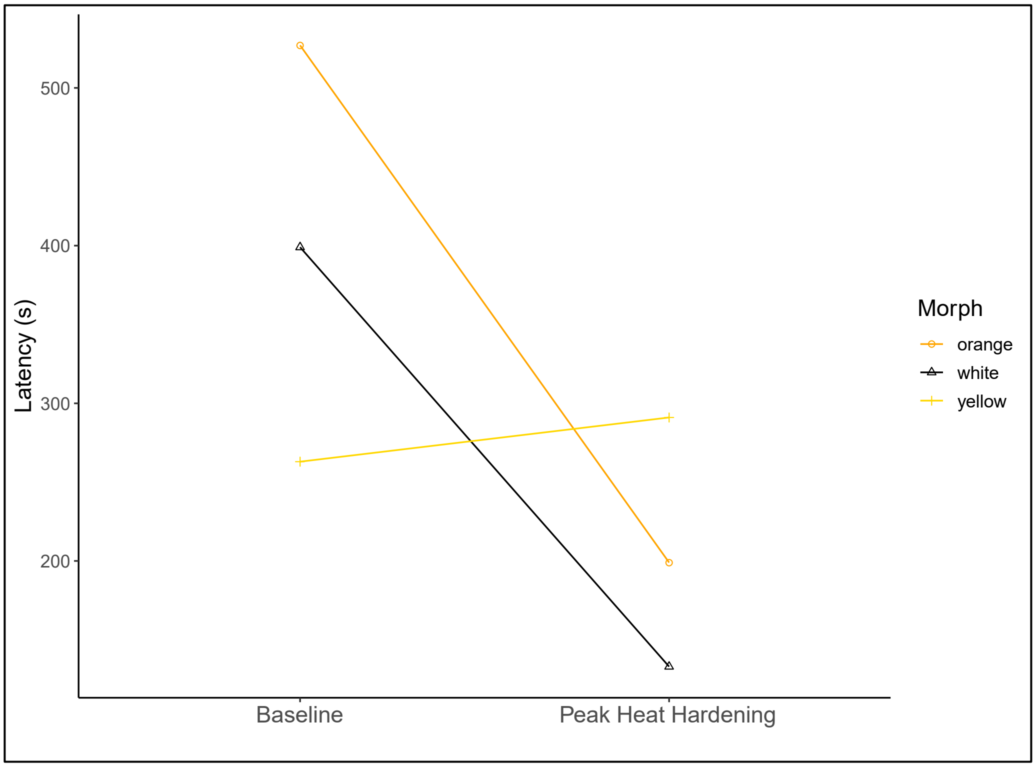

Figure 19
Mean change in boldness behavior (latency to emerge from a refuge after a simulated predator attack) before and during heat hardening among female morphs. There was no significant difference observed in degree of change between morphs (all $P > 0.25$). Boldness behavior before and during heat hardening was not significantly different for any female morph (orange N = 14, yellow N = 11, white N = 6; $P > 0.05$).

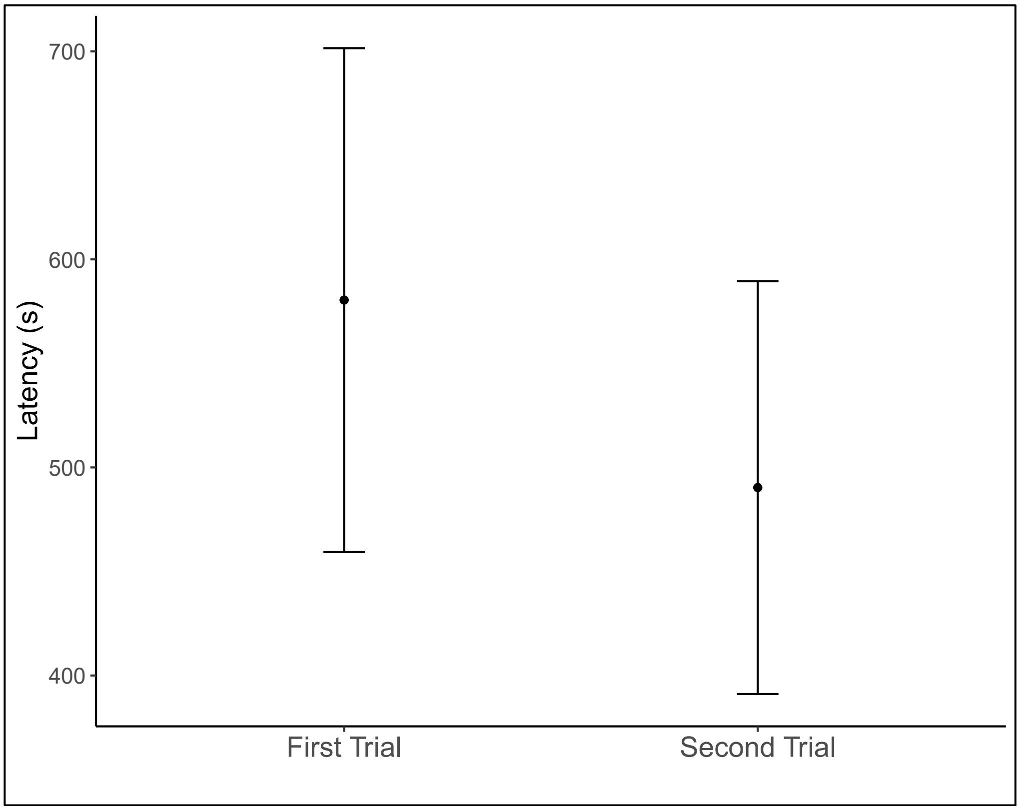

Figure 20
Latency to emerge from refuge in control group. Lizards did not differ in boldness behavior between trials ($P = 0.31$). Points represent means and error bars represent standard error.

Lizards with higher T_bs in the field were less bold prior to heat hardening ($F_{1,65} = 8.4$, $P = 0.005$), yet exhibited the largest increase in boldness after heat hardening ($F_{1,65} = 5.4$, $p = 0.02$; Fig. 21). Both males and females that selected higher T_bs were less bold before heat hardening (males: $R^2_{adj} = 0.09$, $F_{1,34} = 4.5$, $P = 0.04$; females: $R^2_{adj} = 0.12$, $F_{1,34} = 5.2$, $P = 0.03$). T_{pref}, SVL, and body mass had no measured influence on boldness in either trial, nor on degree of change between the trials, in all lizards, males, or females ($P > 0.05$).

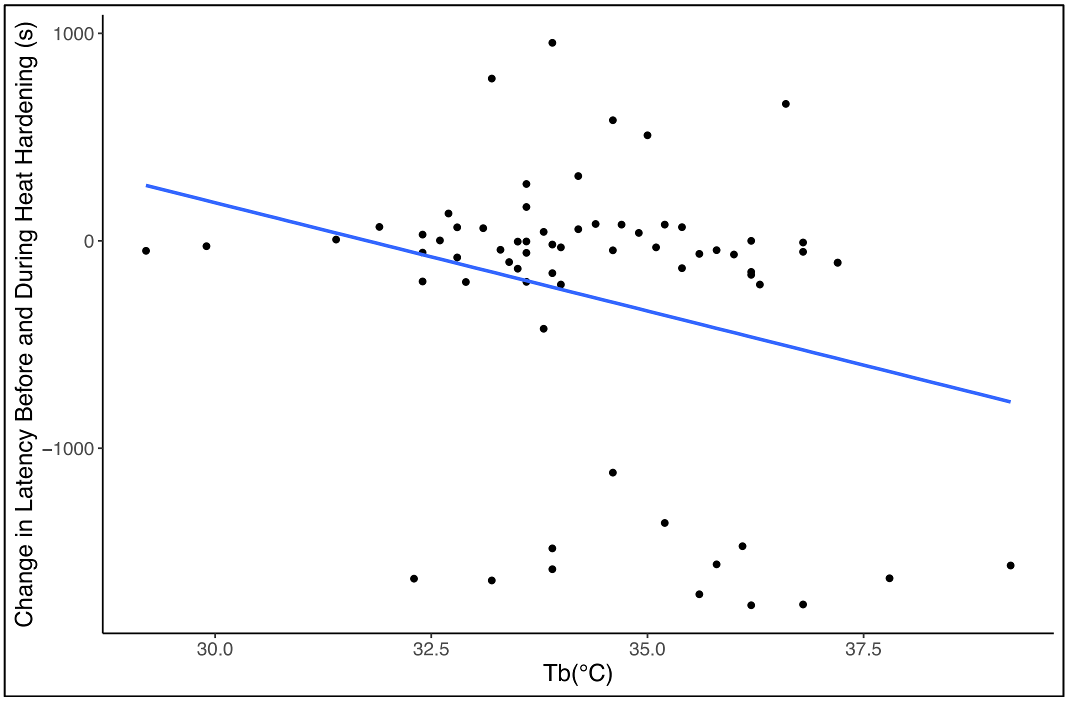

Figure 21
Lizards with higher body temperatures (T_b) in the field became significantly more bold during heat hardening than lizards that selected for moderate and low T_bs ($P = 0.02$). Negative change in latency values represent individuals that decreased latency to emerge during heat hardening, whereas positive change in latency values represent individuals that became less bold during heat hardening.

Discussion

Plasticity in thermal tolerance can increase short-term survival in ectotherms during periods of extreme heat by increasing available activity time (Loeschcke & Hoffman, 2007; Seebacher *et al*., 2015; Phillips *et al*., 2016). However, heat hardening responses can be accompanied by maladaptive phenotypic shifts in physiology and behavior (Gilbert & Miles, 2019a). Here, I found that heat hardening significantly enhances boldness behavior expression in *U. ornatus*, and specifically in males. The response occurred in yellow/blue males, a dominant, territorial morph that is common at the study site. Boldness behavior was also associated with T_b selection in the field. Shyer lizards selected for higher T_bs and demonstrated a larger increase in boldness behavior during heat hardening than individuals that selected for lower T_bs. There were no observed differences between baseline or post heat hardening boldness between males and females.

A key question pertaining to ectotherm responses to rising temperatures is what traits are altered as a consequence of the heat hardening response. Past research on *U. ornatus* found that heat hardened individuals experienced a decrease in T_{pref}. One potential outcome of a lower Tpref is a constraint on which microhabitats would be suitable to regulate at a lower body temperature (Gilbert & Miles, 2019a). I suggest that an increase in boldness following a heat hardening response in *U. ornatus* may reflect a consequence of this phenomenon. In desert landscapes, most available microhabitats are sun exposed and close to CT_{max}. During periods of heat stress, population-wide decreases in T_{pref} prompt lizards to seek out cooler microhabitats (Gilbert & Miles, 2019a), resulting in an increase in the local density of conspecifics in limited microhabitats. In lizards, boldness behavior is a key trait that influences territory size and maintenance (Ward-Fear *et al*., 2018). As competition for favorable space increases, increased boldness may enhance the ability to acquire and defend limited territory and resources. Indeed, lizards have been demonstrated to alter boldness expression in the presence of conspecifics (Brand *et al*., 2022), and *U. ornatus* becomes more likely to defend territory when resources are scarce (Taylor & Lattanzio, 2016). Territory defense in *U. ornatus* is more prevalent in males than in females (M'Closkey *et al*., 1987); this could explain why increases in boldness behavior are observed to be more pronounced in males than in females during heat hardening. In addition, females were gravid during the time of this study. Gravid females adjust their activity to reflect reduced locomotor capacity to decrease predation risk (Cooper *et al*., 1990); this may also influence why we did not observ a significant increase in boldness in females during the heat hardening response. Further, yellow/blue male morphs, which hold territory and are more dominant than many other morphs (Thompson & Moore, 1991a; personal observation), exhibited a significant increase in boldness during heat hardening. Other male morphs seemed to follow the same pattern, and it would be interesting to study the effects of heat hardening on behavioral expression in other desert populations with a more even distribution of morphs to determine whether this relationship is significant for subordinate morphs.

Lizards that maintained higher T_bs in the field were less bold before heat hardening occurred. However, although shyer lizards selected warmer temperatures in the field, no relationship was observed between T_{pref} and baseline boldness behavior. This indicates that the relationship between T_b and baseline boldness is not driven by variation in individual thermal preference and physiology but perhaps by environmental conditions and population social structure. Indeed, there is evidence that links between thermoregulatory strategy and behavior may be influenced in part by environmental conditions (Horváth *et al*., 2020). Most studies on the relationships between behavior and temperature found that bolder and more aggressive lizards prefer warmer temperatures (Stapley, 2006; Goulet *et al*., 2017; Michelangeli *et al*., 2018). However, these studies were conducted on cold-adapted, temperate species (e.g., *Iberolacerta cyreni, Lampropholis delicata, Pseudemoia entrecasteauxii*) where warm microhabitats that provide basking opportunities are more limited. For desert dwelling *U. ornatus*, cooler, shaded microhabitats are limited, and because substrate temperatures in exposed microhabitats often exceed CT_{max}, competition for cooler microhabitats is likely intense. Thus, bolder individuals may be more successful in securing shaded microhabitats, forcing shyer conspecifics to select for higher T_bs in the field that may exceed their T_{prefs}. When heat hardening occurred, these individuals exhibited a greater change in boldness behavior than individuals with lower field active T_bs. Although shyer individuals may avoid engaging in territorial disputes for more favorable microhabitats under typical conditions, the reduction in T_{pref} associated with heat hardening may make shyness more costly. Stress responses incur an energetic cost and individuals reduce energy by being active at lower body temperatures (Feder & Hofmann, 1999). An increase in boldness expression could be beneficial as the energetic incentive for competing for limited shady microhabitats rises.

Although heat hardening can act as an adaptive plastic response to extreme weather, associated increases in boldness behavior could represent a maladaptive phenotypic shift in environments characterized by repeated heat waves. Variation in expression of boldness behavior often provides survival and reproductive advantages or disadvantages depending on environmental context (Smith & Blumstein, 2008). For instance, bolder individuals are more likely to defend territory and enjoy greater foraging success, but they risk a greater chance of predation (Wilson *et al.*, 1993; Sih *et al.*, 2004; Ward-Fear *et al.*, 2018). Reduction in sprint speed was associated with heat hardening in *U. ornatus* (Gilbert & Miles, 2019a). Impairment in sprint capacity may have fitness consequences due to the importance of sprinting for predator avoidance (Miles, 2004; Gilbert & Miles, 2017). Higher rates of predator exposure coupled with reduced locomotor performance could result in higher mortality, diminishing adaptive potential of heat tolerance plasticity. Furthermore, increased boldness often leads to higher rates of tail loss in lizards, an injury resulting from predators or conspecifics (Carter *et al*., 2010; Talavera *et al*., 2021; unpublished data). Although tail loss increases immediate survivorship if it allows predator escape, it also incurs several costs. Individuals that drop tails lose access to high concentrations of lipids stored in the tail as well as the costs incurred for regenerating the tail. Tail loss also compromises dominance status, which can result in reduced home range size and mating success, and immunity can be impacted via trade-offs in resource allocation (Martin & Salvador, 1993; Salvador *et al*., 1995; Doughty *et al*., 2003; Kuo *et al*., 2013). If heat hardening promotes boldness that increases tail loss, it could therefore further impact survival.

As species encounter novel environments due to climate change, many will rely on phenotypic plasticity to buffer effects of altered thermal niches. Here, I demonstrate a phenotypic shift in boldness behavior associated with heat hardening that carries ecological implications with respect to social structure, survival, and reproduction. Although heat hardening provides short term increases in thermal tolerance, its efficacy for increasing survival may be compromised due to associated behavioral shifts in addition to decreased physiological capacity (Gilbert & Miles, 2019a). Such trade-offs may become even more pronounced in the face of extreme weather events as organisms are forced to rely on heat hardening responses with greater frequency. Quantifying how other behavioral traits tied to survival and fitness, such as aggression towards conspecifics, are impacted by heat hardening is critical for understanding how species will respond to changing thermal environments over time.

Chapter 6: Conclusions

The data in this dissertation demonstrate a variety of relationships between temperature and behavior in a model lizard species, *U. ornatus*. In Chapter 2, I document the thermal sensitivity of push-up display rate while considering other local environmental determinants. The display rate of *U. ornatus* was significantly influenced by body temperature. The thermal performance curve describing the relationship between body temperature and display rate was characterized by an optimal temperature for display rate that was below both mean active body temperature and mean thermal preference (T_{pref}). In addition, the thermal performance curve described a broad range of temperatures over which individuals were able to maintain high rates of display. Lizards displayed at 90% of maximum capacity over a 16°C range of body temperatures (23°C–39°C) that encompassed both T_{pref} and T_{set} ranges of the population.

The broad thermal sensitivity of display rate indicates additional influence of other factors on the rate of display. We found that display rate was best described by an interaction between body temperature (T_b) and microhabitat use. Lizards used microhabitats nonrandomly over the course of the day to take advantage of differing thermal properties and levels of exposure of the various microhabitats. Lizards displayed at significantly different rates on three microhabitats: sunny snags, inner branches of trees, and tree trunks. There was no relationship between T_b and display rate on sunny snags, whereas lizards increased display rate with T_b on inner branches and decreased display rate with T_b on trunks. I detected no influence of sex, body size, color morph, or social context on display rate.

In Chapter 3, I detected significant influence of conspecifics on T_{pref}. When males competed for basking space in the thermal gradient, I observed high levels of displacement from solo T_{pref} values. The throat color dynamics of the competing males had an influence on the magnitude of displacement. Yellow/blue and orange/blue males were displaced the least, while yellow and orange males were displaced the most, indicating a thermoregulatory dominance hierarchy of yellow/blue and orange/blue > blue > yellow and orange males. Female *U. ornatus* were also displaced from optimal T_{pref} values compared to solo T_{pref} when competing for basking space. In females, higher magnitudes of displacement were observed when females of the same color morph shared a gradient, perhaps due to female avoidance behavior when adjacent to individuals sharing a similar reproductive strategy. When males and females shared a gradient, females were significantly displaced while males were not. The gravid state of females during the study may have resulted either in male avoidance behavior or in a reduction of male courtship behavior.

In Chapter 4, I demonstrated covariation between boldness, exploration, and thermal preference traits between dominant (blue, yellow/blue, orange/blue) and subordinate (orange, yellow, orange/yellow) male *U. ornatus*, which represent alternative reproductive strategies. Dominant males were bolder and more exploratory than subordinate males and preferred higher body temperatures, while subordinate males selected for a wider range of body temperatures while engaging in more shuttling behavior in the thermal gradient. Dominant and subordinate males also occupied different combined behavioral and thermal trait space, with dominant males being most significantly described as bolder and selecting for a narrower range of preferred body temperatures.

In Chapter 5, I documented behavioral shifts associated with heat hardening, a rapid plastic response to heat stress. I found that boldness behavior in *U. ornatus* significantly increased as a consequence of heat hardening, most significantly in males. Yellow/blue male morphs most significantly contributed to this result. I also found that lizards that selected higher T_bs in the field were less bold prior to heat hardening but exhibited the largest increase in boldness after heat hardening.

Future climate change projections suggest widescale rapid alterations of thermal niches. Many environments, including deserts, are projected to become increasingly hotter and drier. As a result, resident ectotherms are anticipated to experience warmer body temperatures at higher rates. The data presented here offer a preliminary exploration as to how ectotherms may respond to these altered thermal niches. While the thermal sensitivity of display rate is broad in *U. ornatus*, the thermal performance curve describes a rapid decrease in display rate when body temperatures exceed T_{pref}. If lizards are increasingly exposed to environmental temperatures in this range, a reduction in display rate could alter conspecific communication pertaining to reproductive and territorial behaviors. Further, exposed microhabitats such as snags may become unhabitable for increased hours during the day, limiting access to a microhabitat that is heavily utilized for basking and communication purposes. In addition, a reduction in habitable microhabitats via increased temperatures may result in fewer high-quality basking sites available to ectotherm populations. The data on conspecific thermoregulatory interactions and covariation between behavioral and thermal traits between morph groups suggest that morphs interact with the environment differentially. In species that exhibit intraspecific variation in thermoregulatory behavior as well as behaviors such as boldness and exploration, changes in the thermal environment will not only influence how ectotherms interact with the environment but could be more detrimental to some individuals than others. These dynamics could influence the persistence of polymorphism maintenance as well as trait variation within populations. As ectotherms are exposed to hotter, potentially lethal temperatures, adaptive plastic responses offer a first line of defense. Our data indicate that heat stress responses may be associated with potentially maladaptive behavioral adjustments. These results and that of this dissertation as a whole highlight the need for further study on the relationships between behavior, physiology, and local environmental conditions when considering species responses to rising temperatures.

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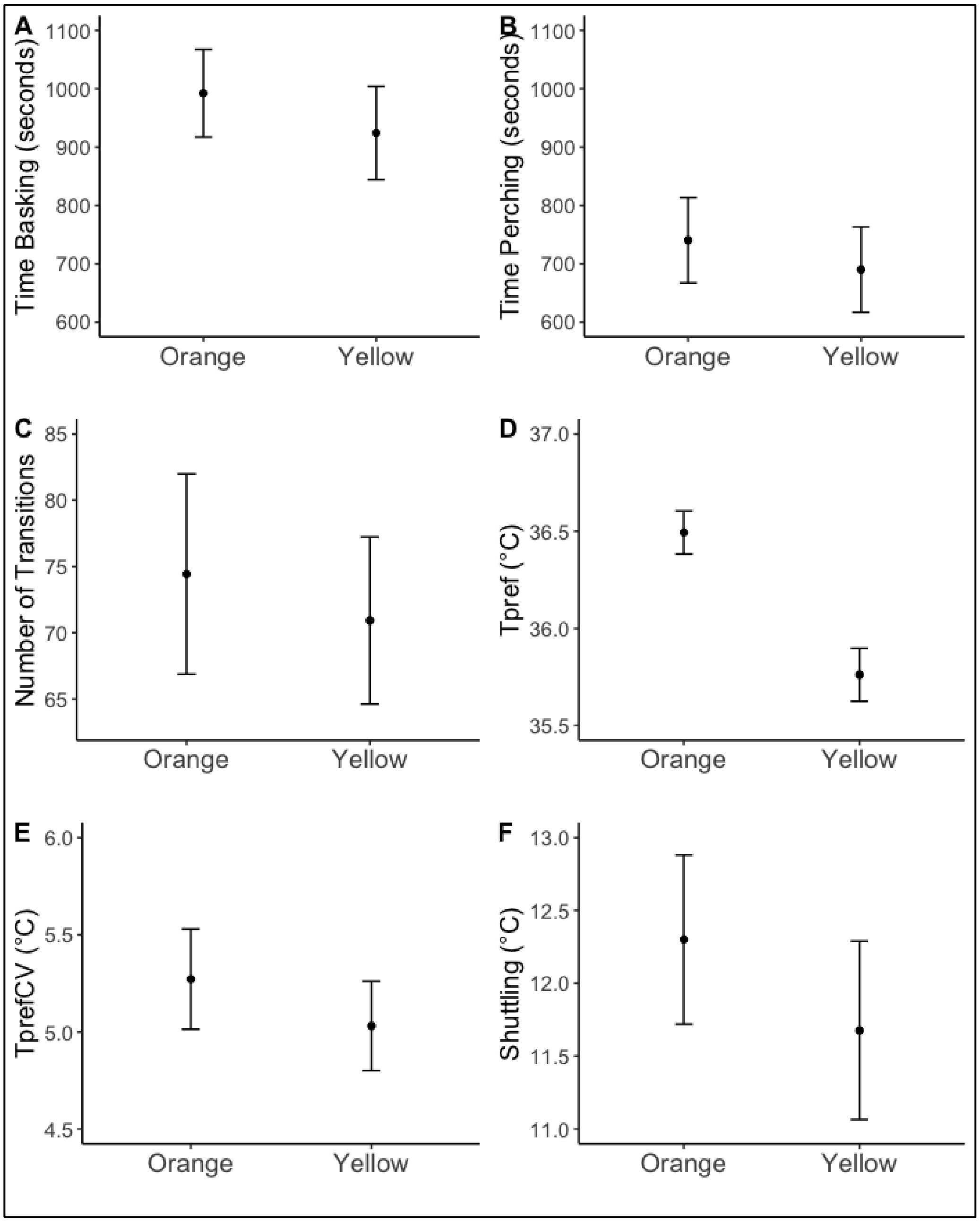
Appendix A: Supplementary Figures



Supplementary Figure 1
Variation in thermal and behavioral traits between orange and yellow females. No traits varied significantly between female morph groups.