

# Thermal refugia and persistence of Texas horned lizards (*Phrynosoma cornutum*) in small towns

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## Abstract

Texas horned lizards (*Phrynosoma cornutum*) have disappeared from many areas in Texas, especially from urbanized areas, probably in large part due to loss of suitable habitat. Our previous studies have found that horned lizards persist and occur at high densities in some small towns in southern Texas. Nevertheless, this species has continued to decline and disappear from these towns. Long-term data from Kenedy and Karnes City indicate that when study sites experienced significant shrub and vegetation removal horned lizards declined by 79%. We hypothesize this may in part be due to the degradation of the thermal landscape for these lizards. We determined the preferred temperature range ( $T_{set25}$  -  $T_{set75}$ ) of lizards at our study sites and took field measurements of body temperature ( $T_b$ ). Temperature loggers were also placed in three microhabitats across our study sites. Shrubs and vegetation provided the highest quality thermal environment, especially for about 5 hours midday when temperatures in the open and buried under the surface exceeded the lizards' critical maximum temperature ( $CT_{max}$ ) or were above their preferred temperature range. Horned lizard density was positively related to the thermal quality of the habitat across our sites. Texas horned lizards in these towns require a heterogenous mix of closely spaced microhabitats and especially thermal refugia, such as shrubs and vegetation along fence lines and in open fields. Maintaining thermal refugia is one of the most important and practical conservation actions that can be taken to help small ectotherms persist in human modified landscapes and cope with increasing temperatures due to climate change.

## INTRODUCTION

Vegetation loss is one of the primary drivers of habitat degradation and the reduced abundance of many reptile species (Fleischner 1994; Smith et al. 1996; Attum and Eason 2006). Several reasons have been proposed to explain why vegetation loss results in decreased reptile numbers including: decreased diversity and number of prey, exposure to more predation due to loss of cover, and reduction of important microhabitats required for thermoregulation (Jones 1981; Norbury 2001). Loss of vegetation can reduce prey availability causing reptiles to move further and forage longer, which can leave them more susceptible to predation (MacArthur and Pianka 1966; Hinsley 2000). Vegetation loss adversely effects lizards by reducing available microhabitats to thermoregulate and hinders their ability to escape lethal substrate temperatures (Adolph 1990; Carrascal et al. 1992). Declines often occur when vegetation complexity decreases, resulting in reduced thermoregulatory patches that serve as thermal refugia, which is one of the primary mechanisms that allow species to persist in harsh and arid habitats (Attum et al. 2013).

Organisms occupy different habitats based on trade-offs between maintaining proper physiological processes within certain limits and the need to avoid predators, forage, and find mating opportunities (Huey 1991; Angilletta 2009). Lizards are ectothermic and entirely dependent on their surroundings to maintain proper body temperatures, which are potentially strong determinates of habitat choice (Cowles and Bogert 1944; Vitt et al. 2008). Lizards utilize a variety of behaviors, physiological adaptations, and microhabitats to maintain ideal body temperatures throughout the day, even as daytime temperatures fluctuate by as much

17°C in some areas like the American southwest (Angilletta 2009). When lizards are exposed to environmental temperatures ( $T_e$ ) that are greater than their preferred body temperature ( $T_{set}$ ) their activity is restricted (Sinervo et al. 2010). Because of this, lizards are particularly vulnerable to extinction from climate change because at higher temperatures females must spend more time thermoregulating in the shade and less time foraging out in the open (Sinervo et al. 2010). This lower foraging efficiency decreases the number and quality of offspring they can produce, leading to declining populations and eventual extinction (Sinervo et al. 2010). At least 20% of all lizard species are predicted to be extinct by 2080 under current climate models (Sinervo et al. 2010).

Suitable thermal habitat is fundamental for lizards and other squamates (Heatwole 1977; Dunham et al. 1989; Huey 1991). The availability of microhabitats that offer temperatures suitable for lizards determine how far lizards must move and how much energy they expend in finding an ideal thermal environment (Grbac and Bauwens 2001; Sears et al. 2016). While preferred body temperature may be achieved by behavior, physiology, and morphology; reptile activity patterns are constrained by the distribution of microhabitats across space and time (Grbac and Bauwens 2001). Heterogenous landscapes are characterized by a diversity of environmental gradients and land cover types (August 1983). These landscapes support microhabitats with thermoregulatory patches that are variable in temperature and spatially closer together (Sears et al. 2016). This microhabitat configuration allows lizards to expend less energy moving to a favorable thermal patch to regulate body temperature and allows more time for foraging and reproductive opportunities (Sears et al. 2016). Homogeneous landscapes (i.e., pasture or dense forest) decrease available microhabitats and increase the distance lizards must travel between sun and shade, increasing their exposure to predators. An understanding of the thermal regimes in different microhabitats is important to understand thermoregulatory behavior, habitat quality, and cost of living in different types of environments.

Lizards living in urban environments face additional challenges when it comes to thermal environments. Urban areas are often warmer and warm faster than natural areas because roads, buildings, and other concrete surfaces lower albedo rates, thus increasing surface temperatures (Taha 1997; Ackley et al. 2015; Kolbe et al. 2016). Research has shown that different types of landscaping in urban areas can have a large effect on whether temperatures are within preferred temperature ranges for lizards (Ackley et al. 2015). It has been shown that mesic landscaping, with spray irrigation and increased heterogeneity of plants, can keep areas significantly cooler and within lizards' preferred temperature range during 100% of their active period compared to xeric and native landscaping in some areas (Ackley et al. 2015). The importance of thermal microhabitats in urban areas for determining the densities of lizards living in urban areas is currently unknown.

Texas horned lizards inhabit arid and semi-arid open habitat with some vegetation. Horned lizards are often active over longer periods of time than sympatric lizard species and display variable body temperatures which is attributed to relaxed thermoregulation (Pianka and Parker 1975). Texas horned lizards have a high preferred body temperature ( $T_{set}$ ), ranging from 34.2 - 38.5 degC (Prieto and Whitford 1971; Russell 2001; Lara-Resendiz et al. 2015; Table 1). Their critical maximum temperature ( $CT_{max}$ ), the temperature at which they lose the ability to move, is also very high (45.9 - 48.1 °C; Ballinger and Shrank 1970; Kour and Hutchison 1970; Prieto and Whitford 1971). These temperature tolerances are higher and more variable than other sympatric species of desert lizards (Pianka and Parker 1975). Due to their ecology of being an ant specialist, it is likely that relaxed thermoregulation allows horned lizards to withstand direct sunlight for longer periods of time while foraging for ants in the open. Their cryptic camouflage and ability to withstand higher temperatures for longer aids in reducing predation risk since they do not have to move as frequently between sun and shade (Pianka and Parker 1975; Guyer and Linder 1985).

The Texas horned lizard (*Phrynosoma cornutum*) is a threatened species in the state of Texas (Donaldson et al. 1994) due to widespread population declines. These declines are attributed to a variety of factors including habitat loss, introduction of the red imported fire ant (*Solenopsis invicta*), loss of their preferred prey harvester ants (*Pogonomyrmex* spp.), and over-collecting for the pet trade (Donaldson et al. 1994; Dixon 2000; Henke 2003). However, they are found in two small towns in south Texas, Kenedy and Karnes

City, at higher densities (52 lizards/hectare; Ackel 2015) than are observed in more natural areas (3 - 10 lizards/hectare; Whitford and Bryant 1979; Whiting et al. 1993). Research has shown that the high density of lizards observed in these towns may be due to a variety of factors including isolation due to roads and buildings (which could increase horned lizard densities due to limited dispersal; Wall 2014), a dietary shift to consuming smaller more abundant prey items (Alenius 2018), and reduced predation pressure compared to natural areas by some types of predators (Mirkin et al. 2021). Nevertheless, Texas horned lizard populations in these towns have been declining since the 1990s (Wade Phelps pers. comm.).

We hypothesize that vegetation is important for thermoregulation in Texas horned lizards, and their decline in these towns could in part be due to degradation of the thermal landscape caused by the removal of vegetation they utilize for thermoregulation. In this study, we determined the preferred body temperature ( $T_{set}$ ) in a laboratory gradient to better understand temperature preferences at our field sites. We determined body temperatures ( $T_b$ ) of horned lizards in the field and placed models with temperature loggers in different microhabitats to better understand the range of environmental temperatures ( $T_e$ ) available to horned lizards. Using data collected since 2013, we ask if horned lizard densities decline at a site after major vegetation removal, and if thermal quality ( $d_e$ ) at a site is associated with lizard presence and density. We also asked if vegetation provides a better thermal environment for Texas horned lizards compared to open areas or being buried under the soil, which are two other microhabitats the lizards often utilize. We also evaluated the accuracy ( $d_b$ ) and effectiveness ( $d_e - d_b$  and  $E$ ) of thermoregulation of horned lizards in an urban environment, and we compare our results with data available for other *Phrynosoma* species.

## METHODS

### Study sites and fieldwork

Texas horned lizards have been studied since 2013 in two small towns (~3,042 - 3,296 people) in south Texas; Kenedy (28.8191degN, 97.8486degW; elevation = 81 m) and Karnes City (28.8850degN, 97.9008degW; elevation = 131 m). Between 2013 and 2021, we censused 16 sites (3 in Kenedy and 13 in Karnes City). Each site was censused between 5 and 9 years (average 7.6 years). The sites are in school yards, alleyways, and abandoned lots that vary in size from 0.07 - 1.11 hectares (Alenius 2018). These sites are classified into two groups based on spatial structure: alleyways, which have a dirt road bordered by houses, fences, and vegetation (i.e., tree canopy cover, ornamental shrubs, and native vegetation) and fields, which have short vegetative cover interspersed with clumps of trees and shrubs (Fig. 1).

Sites are censused by walking transects with 2 - 4 people between 8:00 - 12:00 and 17:00 - 20:00, which corresponds to active periods for Texas horned lizards (Moeller et al. 2005; Ackel 2015). One of the authors (Williams) was present at all transects over the course of this study. Transects are conducted at each site 8 times each summer, divided up into three sampling periods: 2 weeks in late May/early June (each site is censused 4 times), 1 week in early July (each site is censused 2 times), and 1 week in late July and early August (each site is censused 2 times). Upon capture, we record time, sex, weight (g), length (mm), and location using ArcGIS Collector.<sup>?</sup> We photograph belly spots for identification and obtain a DNA sample by swabbing the cloaca with a small Puritan<sup>?</sup> cotton-tipped applicator (Williams et al. 2012).

We noted when the sites experienced major changes to their vegetation, such as removal of the vegetation along fence rows, removal of isolated bushes and bushes around the bases of trees in parks, and removal of large brush piles. Mowing and trimming of grasses and forbs, which usually occurred several times during the summer months at all sites, was not counted as significant vegetation removal. We calculated the percent change in horned lizard density (lizards/hectare) between years for each site. For each site, we averaged the percent change across the years when there was no vegetation removal and compared it to the average percent change the year after vegetation removal using a t-test after checking test assumptions.

Average annual temperature for both towns is 18degC. Although air temperature is higher in the summer, reaching an average of 36degC and a maximum temperature of 40degC in the shade. Average annual precipitation is 790 mm and monthly averages vary from 33 - 76.2 mm, with May receiving the most rainfall and December receiving the least. Rainfall patterns are typically higher during the beginning of the field

season in late May and continue to decrease through August. The habitat is dominated by honey mesquite (*Prosopis glandulosa*), anacua (*Ehretia anacua*), and sugarberry (*Celtis laevigata*) along with native grasses, forbs, and ornamental plants.

### Body temperature in the field ( $T_b$ )

In 2019 – 2021, field cloacal temperature ( $T_b$ ) was recorded within 30 seconds of capture by inserting a small temperature probe connected to a digital thermometer (GDEALER Model DT8; accuracy  $\pm 1^\circ\text{C}$ ; resolution  $\pm 0.1^\circ\text{C}$ ) one centimeter into the cloaca. The microhabitat where the lizard was found was also recorded and classified as open sunny, open overcast, or shade/vegetation.

### Operative environmental temperatures ( $T_e$ ) and model calibration

Simultaneously, during the 2019 – 2021 field season, we determined environmental temperatures ( $T_e$ ) at sites that currently have horned lizards and sites where they have been extirpated within the last 10 years.  $T_e$  has historically been determined using copper or polyvinylchloride (PVC) models to estimate available temperatures for small ectotherms, but we used 3D printed models of adult Texas horned lizards for morphological accuracy (Watson and Francis 2015; Mirkin et al. 2021). Models were printed with acrylonitrile butadiene styrene (ABS) and painted with 33% reflective paint (Rustoleum™ gray primer) that corresponds to the reflectivity of horned lizards (Adolph 1990; Lara-Resendiz et al. 2015a). The underside of the model had a recessed opening that held a DS1922L Thermochron™ temperature logger that records temperature at a resolution of  $\pm 0.2^\circ\text{C}$  (Fig. 2). Self-fusing repair tape was used to seal the temperature logger in the model.

In 2019, 15 models were placed at 5 sites from June 2 – 8<sup>th</sup>. After we were able to purchase more temperature loggers, 30 models were placed at 10 sites from June 30 – July 6<sup>th</sup> and August 1 – 8<sup>th</sup>. In 2020 and 2021, 45 models were placed at 15 sites from June 1 – June 14<sup>th</sup>, July 1 – 6<sup>th</sup>, and August 1 – 6<sup>th</sup>. At each site, one model was placed in the open, one under vegetation (shrubs at 12 sites and thick grass under tree canopies at 3 sites), and one buried ~2 cm under the soil surface in open areas to mimic the three common microhabitats Texas horned lizards utilize (Burrow et al. 2001; Wall 2014). Models were placed in areas horned lizards had been previously observed for all the sites by using prior GPS locations from ArcGIS Collector.<sup>2</sup> Models were placed in the same sites and the same microhabitat locations each year. Temperature loggers were programmed to record temperature every 10 minutes from 7:00 – 20:00 to estimate environmental temperatures ( $T_e$ ) throughout the horned lizard’s activity period (Lara-Resendiz et al. 2015a). We calculated mean  $T_e$  values for each time point (every 10 minutes during the lizard’s active period) for each month and year for open, dirt, and vegetation microhabitats. The models were calibrated against live lizards by first capturing a lizard and measuring the cloacal temperature ( $T_b$ ) using a small temperature probe connected to a digital thermometer (GDEALER Model DT8; accuracy  $\pm 1^\circ\text{C}$ ; resolution  $\pm 0.1^\circ\text{C}$ ) within 30 seconds of capture and then placing a model in the same spot as the captured lizard and recording the temperature on the logger after 10 minutes (Dzialowski 2005; Kolbe et al. 2016). Hourly ambient air temperatures were determined by using publicly available historic weather data from the KBEA weather station in Beeville, TX (28.4008degN, 97.7483degW; altitude = 64 m), which is located approximately 50 km away.

### Preferred body temperature in the laboratory ( $T_{set}$ )

In 2021,  $T_{set}$  was recorded using a laboratory thermal gradient that consisted of a plastic box 88.6 cm x 42.2 cm x 15.6 cm (length, width, and height) that was filled with 2 - 3 cm of sand (Hertz et al. 1993; Angilletta 2009; Sinervo et al. 2010). At one end of the box, a Phillips™ 250 W heat light bulb was placed 33.7 cm above the substrate to create a thermal gradient ranging from 25 - 60oC. The gradient was housed in a climate-controlled room at a constant temperature of 20oC. We captured adult lizards (> 69 mm) in the field and allowed them to acclimate in the thermal gradient overnight (between 12 - 23 hours).

Lizards were exposed to natural lighting through windows and artificial lighting, which mimicked the natural daylight cycles at our field location. No food or water was offered during the experiments given the short captive period and their natural history (i.e., ant specialist and do not drink frequently), but enclosures

were kept humid with a damp towel during the acclimation period. Lizards were placed in the middle of the gradient when the trial started. Preferred body temperature ( $T_{set}$ ) was measured every two hours from 8:00 – 20:00 with the same temperature probe used for  $T_b$ . After the experiment, all lizards were released at their capture site. The 25 and 75 percent quartiles for preferred body temperature ( $T_{set25} - T_{set75}$ ) were used as the upper and lower  $T_{set}$  (Hertz et al. 1993; Lara-Resendiz et al. 2015a).

### Habitat thermal quality and thermoregulatory indices

Data from  $T_b$ ,  $T_{set}$ , and  $T_e$  were used to calculate the accuracy of thermoregulation ( $d_b$ ) and habitat thermal quality ( $d_e$ ) as follows: if  $T_b$  or  $T_e < T_{set}$ , then  $d_b = T_b - T_{set25}$  and  $d_e = T_e - T_{set25}$ , and if  $T_b$  or  $T_e > T_{set}$  then  $d_b = T_b - T_{set75}$  and  $d_e = T_e - T_{set75}$ , respectively. When  $T_b$  or  $T_e$  values were within  $T_{set}$  range,  $d_b$  and  $d_e$  were considered equal to zero. High values of  $d_b$  and  $d_e$  indicate low accuracy and low thermal quality, while values equal to or near zero indicate high accuracy of thermoregulation and represent thermally ideal environments. Thermoregulatory effectiveness ( $E$ ) was then calculated using  $d_b$  and  $d_e$ , where the overbars represent mean values of the deviations, using the following equation:  $E = 1 - (\overline{d_b} / \overline{d_e})$ . When calculating  $E$ , we calculated  $d_e$  using  $T_e$  temperatures from 8:00 – 12:00 and 17:00 – 20:00 since field cloacal  $T_b$  temperatures (and therefore  $d_b$ ) were only measured during those time periods. When calculating  $E$ , we used the mean value of  $d_e$  for all microhabitats across each time point. Given that  $d_e$  did not vary between years (One-way ANOVA,  $F_{2,223} = 2.2$ ,  $P = 0.11$ ), we averaged  $d_e$  across years to obtain  $\overline{d_e}$ . An  $E$  value near to one indicates an organism that actively thermoregulates because environmental temperature is far from its preferred temperature. These lizards are under thermal stress and must increase or decrease their  $T_b$  with respect to  $T_e$ . An  $E$  value equal or near to zero indicates a thermoconformer, which is not regulating temperatures actively since the environmental temperature is within its preferred temperature range (Hertz et al. 1993). However, an  $E$  value can come from a variety of combinations of  $d_b$  and  $d_e$  (Hertz et al. 1993). For example, a species could occupy a difficult thermal environment (i.e., high  $d_e$  values) and utilize a different thermoregulatory strategy compared to a species that occupies a more benign thermal environment, but these species could still have the same  $E$  value if the ratios between  $d_b$  and  $d_e$  are the same (Blouin-Demers and Weatherhead 2001). Thus, it is also important to consider the difference in magnitude between  $d_b$  and  $d_e$  when interpreting  $E$  (Blouin-Demers and Nadeau 2005). Another way to calculate thermoregulatory effectiveness is by using the following equation:  $d_e - d_b$ . This method avoids the limitations associated with ratios and can quantify the extent of departure from perfect thermoconformity with values of zero representing thermoconformity and positive values indicating thermoregulation (Blouin-Demers and Nadeau 2001). We calculated  $d_e - d_b$  using  $T_e$  (and therefore  $d_e$ ) data from 8:00 – 12:00 and 17:00 – 20:00 as we did for  $E$ .

When calculating  $d_e$  for each site ( $n = 15$ ), we calculated  $d_e$  using  $T_e$  temperatures from 8:00 – 20:00 across all microhabitats for each month and year for that site. We calculated  $d_e$  for each microhabitat (open, buried in dirt, and underneath vegetation) for all months and years to measure the average thermal quality of the microhabitats available to horned lizards during their active period. We also calculated the percent time each microhabitat  $T_e$  (open, buried in dirt, or under vegetation) fell within their preferred temperature range ( $T_{set25} - T_{set75}$ ) and exceeded their critical thermal maximum ( $CT_{max}$ ). We then used Kruskal-Wallis and Dunn’s post hoc tests to examine differences between microhabitats. Hours of restriction ( $h_r$ ) were expressed as the hours in each day that  $T_e$  exceeds  $CT_{max}$  (Ivey et al. 2020; Taylor et al. 2021).

### Statistical analysis

*Body temperature in the field ( $T_b$ ) and model calibration* — We performed a generalized linear model (Minitab(r) Version 19) to explore the variability in field cloacal temperatures ( $T_b$ ) among lizards. Recaptured lizards within the same year were identified by belly spots and only the first  $T_b$  measurement was included in analysis to avoid pseudoreplication.  $T_b$  temperature was added as the response variable and time of capture (grouped into two-hour blocks), month, year, body condition (weight/SVL), age, sex, and

microhabitat classification (open sunny, open overcast, shade/vegetation) were added as factors. We started with the full model with all relevant interaction terms and proceeded with stepwise selection. The model with the lowest Akaike information criterion (AIC) score was selected. Tukey post hoc tests were then performed on significant factors to see what groups were different from one another. Microsoft Excel<sup>(r)</sup> and Minitab<sup>(r)</sup> Version 19 were used for regression analysis to examine calibration between cloacal body temperature ( $T_b$ ) with environmental operative temperatures measured by models ( $T_e$ ). We used the student's  $t$  distribution ( $t = (\text{slope} - 1) / \text{SE}$  with  $df = n - 2$ ) to test if the slope was significantly different than one.

*Preferred body temperature ( $T_{set}$ )* — We performed a mixed effects model (Minitab<sup>(r)</sup> Version 19) with lizard ID as a random effect and month and time of day as fixed effects. We used a mixed effects model to account for repeated measurements of the same lizard in the thermal gradient. We then found the model predicted means and compared them to the observed means to find our mean preferred body temperature (Camacho and Rusch 2017). We performed a student's  $t$ -test to see if there was a significant difference between model predicted means and the observed mean preferred temperature.

*Habitat thermal quality ( $d_e$ )* — Assumptions of normality were analyzed visually and by using Kolmogorov-Smirnov test. Homogeneity of variances were analyzed using Levene's test. To explore differences in thermal quality ( $d_e$ ) between years and microhabitats, we used one-way ANOVA and Tukey post hoc test and Kruskal-Wallis and Dunn's post hoc test, respectively. Student's  $t$ -test was used to explore differences in  $d_e$  between alleyways and fields. Microsoft Excel<sup>(r)</sup> and Minitab<sup>(r)</sup> Version 19 were used for spearman rank correlation to see if lizard density (lizards/hectare) correlated to site thermal quality ( $d_e$ ). We accepted significance of statistical tests at  $\alpha = 0.05$ . Mean  $\pm$  standard error is presented in the results, including figures and tables unless otherwise stated.

## RESULTS

### Change in density

There were only four sites which did not experience significant vegetation removal between 2013 and 2021, and horned lizards declined an average of  $-0.04 \pm 0.08\%$  at these four sites. Twelve sites experienced significant vegetation and brush removal during this period. At five of these sites, all horned lizards disappeared for the remainder of the study and at one site all horned lizards disappeared for three years then recolonized once vegetation along the fence row grew back. Average percent change in density (lizards/hectare) for the 12 sites in years before vegetation removal was  $+19.7 \pm 12.1\%$  with horned lizard density declining on average  $-78.9 \pm 6.2\%$  the year after vegetation clearing (Fig. 3;  $t_{0.05(2), 16} = 7.27$ ,  $P = 1.9 \times 10^{-6}$ ).

### Body temperature in the field ( $T_b$ )

One hundred and fifty-three *P. cornutum* were captured (66 in 2019, 47 in 2020, and 40 in 2021) and their body temperature ( $T_b$ ) recorded. Of the 102 individuals for which we had microhabitat data, 37 were in the open under sunny conditions, 17 were in the open under overcast conditions, and 48 were in the shade of vegetation. The overall mean  $T_b$  was  $33.6 \pm 0.30^\circ\text{C}$  ( $n = 153$ , range =  $23.6 - 41.2^\circ\text{C}$ ; Fig. 4). The distribution of  $T_b$  and  $T_e$  indicates that horned lizards avoided higher temperatures and used microhabitats that kept their mean  $T_b$  slightly lower than mean environmental temperatures (Fig. 4). Body temperature ( $T_b$ ) was different between years ( $F_{2, 141} = 6.11$ ,  $P = 0.003$ ) and was significantly lower in 2021 ( $n = 40$ ,  $32.3 \pm 0.60^\circ\text{C}$ ) than in 2019 ( $n = 57$ ,  $33.7 \pm 0.48^\circ\text{C}$ ; Tukey,  $P = 0.034$ ), and 2020 ( $n = 46$ ,  $34.2 \pm 0.53^\circ\text{C}$ ; Tukey,  $P = 0.002$ ). Body temperature ( $T_b$ ) fluctuated through time of day ( $F_{3, 140} = 17.34$ ,  $P < 0.00001$ ) with early morning temperatures ("8:00 & 9:00" category) being significantly cooler than all later temperature categories ("10:00 & 11:00" difference in means =  $-3.10 \pm 0.64^\circ\text{C}$ , Tukey,  $P < 0.0001$ ; "17:00 & 18:00" difference in means =  $-5.10 \pm 0.76^\circ\text{C}$ , Tukey,  $P < 0.001$ ; "19:00 & 20:00" difference in means =  $-3.67 \pm 0.98^\circ\text{C}$ , Tukey,  $P = 0.002$ ). Body temperature ( $T_b$ ) also differed by microhabitat ( $F_{2, 97} = 8.92$ ,  $P = 0.0003$ ) with lizards found in the shade of vegetation being on average  $3 \pm 0.70^\circ\text{C}$  cooler than ones found in open sunny microhabitat conditions (Tukey,  $P < 0.001$ ). There was no significant difference in  $T_b$  between months ( $F_{2, 141} = 0.26$ ,  $P = 0.77$ ), age ( $F_{1, 142} = 2.18$ ,  $P = 0.14$ ), sex ( $F_{1, 142} = 1.18$ ,  $P = 0.28$ ), or body condition ( $F_{1, 142} = 0.39$ ,  $P = 0.53$ ).

## Preferred body temperature in the laboratory ( $T_{set}$ )

Individual as a random variable did not explain variability in  $T_{set}$  temperatures ( $P = 0.06$ ). Time of day ( $F_{5, 90} = 1.75$ ,  $P = 0.13$ ) and month ( $F_{2, 16} = 1.99$ ,  $P = 0.17$ ) also had no significant effect on  $T_{set}$ . Model predicted means averaged to  $36 \pm 0.47^\circ\text{C}$ . We decided to use the observed mean of  $35.7 \pm 0.33^\circ\text{C}$  since it was not statistically different than the model predicted mean ( $t_{0.05(2), 26} = 0.45$ ,  $P = 0.65$ ). Therefore, preferred body temperature in the thermal gradient was  $35.7 \pm 0.33^\circ\text{C}$  ( $n = 19$ , range =  $27.2 - 41.5^\circ\text{C}$ ). The  $T_{set}$  interquartile range ( $T_{set25} - T_{set75}$ ) was  $33.5 - 38.5^\circ\text{C}$ .

## Operative environmental temperatures ( $T_e$ )

There was a highly significant linear relationship between field  $T_b$  and model estimated  $T_e$  ( $y = 0.80x + 6.57$ ,  $R^2 = 0.89$ ,  $P = 0.02$ ) and the slope was not significantly different than 1.0 ( $n = 71$ ,  $t_{0.05(2), 69} = -0.159$ ,  $P = 0.87$ ), suggesting that models accurately measured  $T_e$  available to horned lizards during their active hours.

Environmental temperatures ( $T_e$ ) for open microhabitats averaged to  $40.8 \pm 0.83^\circ\text{C}$  in 2019 ( $n = 88$ , range =  $26.7 - 51.8^\circ\text{C}$ );  $41.3 \pm 0.91^\circ\text{C}$  in 2020 ( $n = 79$ , range =  $28.7 - 51.2^\circ\text{C}$ ); and  $36.1 \pm 0.64^\circ\text{C}$  in 2021 ( $n = 78$ , range =  $26.4 - 42.9^\circ\text{C}$ ). Average open temperatures exceeded the critical maximum temperature ( $CT_{max}$ ) for 5 hours in the middle of the day in 2019 and 2020 and were considered hours of restricted activity ( $h_r$ ). Open temperatures never reached  $CT_{max}$  in 2021 but exceeded the upper preferred temperature ( $T_{set75}$ ) for 5 hours during the middle of the day (Fig. 5). Open microhabitats fall in preferred temperatures in the morning (9:00 - 10:00) and are probably important for increasing body temperature during the beginning of their activity period (Fig. 5).

Environmental temperatures for dirt microhabitats averaged to  $36.1 \pm 0.50^\circ\text{C}$  in 2019 ( $n = 88$ , range =  $27.2 - 42.5^\circ\text{C}$ );  $36.3 \pm 0.58^\circ\text{C}$  in 2020 ( $n = 79$ , range =  $27.8 - 42.8^\circ\text{C}$ ); and  $33.4 \pm 0.47^\circ\text{C}$  in 2021 ( $n = 78$ , range =  $26.5 - 38.8^\circ\text{C}$ ). Dirt microhabitat temperatures never reached  $CT_{max}$  in all three years, but temperatures exceeded the upper preferred temperature ( $T_{set75}$ ) for 6 hours in the middle of the day in 2019 and 2020, which would require lizards to seek refuge elsewhere to stay within their preferred temperature range (Fig. 5). Dirt microhabitat temperatures stayed within the  $T_{set}$  range for most of the day in 2021 (Fig. 5).

Environmental temperatures for vegetation microhabitats averaged to  $32.1 \pm 0.28^\circ\text{C}$  in 2019 ( $n = 88$ , range =  $26 - 34.9^\circ\text{C}$ );  $31.6 \pm 0.32^\circ\text{C}$  in 2020 ( $n = 79$ , range =  $26.7 - 35.1^\circ\text{C}$ ); and  $28.6 \pm 0.29^\circ\text{C}$  in 2021 ( $n = 78$ , range =  $25.6 - 30.7^\circ\text{C}$ ). Vegetation microhabitats provided temperatures within the  $T_{set}$  range during the hottest parts of the day in 2019 and 2020, when open and dirt microhabitats were above preferred temperatures or sometimes above  $CT_{max}$  (Fig. 5). Vegetation temperatures never reached  $T_{set}$  in 2021 and stayed below their preferred temperature range the whole day (Fig. 5). Ambient air temperatures were closest to temperatures found under vegetation, which is expected since temperature data is measured in the shade (Fig. 5).

There was a significant difference in percent time that  $T_e$  was at critical maximum temperature ( $F_{2, 323} = 13.09$ ,  $P < 0.0001$ ) and percent time that  $T_e$  was at preferred temperatures ( $F_{2, 323} = 2.95$ ,  $P = 0.05$ ) between years. This difference was due to 2021 being on average cooler than 2019 and 2020 (Tukey,  $P < 0.05$  both cases). After looking at temperature abnormalities at our field sites, 2021 was the only year that temperatures were cooler on average since monitoring this population starting in 2013 (NOAA Climate at a Glance: Global Time Series). We therefore decided to remove 2021  $T_e$  from the percent time at critical temperature and percent time at preferred temperature analyses below to give a more representative view of the temperatures commonly experienced by lizards at our sites.

Dirt microhabitat was above their critical temperature ( $CT_{max}$ ) for 15.6% of the day and within their preferred temperature range ( $T_{set25} - T_{set75}$ ) for 20.7% of the day. Open microhabitat was above their critical temperature for 39.1% of the day and within their preferred temperature range 13.5% of the day. Vegetation microhabitat was above their critical temperature for 0.3% of the day and within their preferred temperature range 25.1% of the day (Fig. 6 and 7). All microhabitats were significantly different from each other for percent time at critical temperature (Fig. 6; Dunn,  $P < 0.001$ ). Percent time at preferred

temperature was only significantly different between dirt and open and vegetation and open microhabitats (Fig. 7; Dunn,  $P < 0.001$ ).

### Habitat thermal quality ( $d_e$ )

Habitat thermal quality ( $d_e$ ) did not differ between years (2019 – 2021; One-way ANOVA,  $F_{2,223} = 2.2$ ,  $P = 0.11$ ) and therefore data was pooled and  $d_e = 2.30 \pm 0.19$  (Table 2). There was a negative correlation between the average density of horned lizards (lizards/hectare) and average thermal quality ( $d_e$ ) across sites ( $r_s = -0.68$ ,  $P = 0.01$ ; Fig. 8), meaning as thermal quality degraded (i.e., higher  $d_e$  values) horned lizard density decreased. Thermal quality was higher (i.e., lower  $d_e$  value) for Karnes City, which still has a good population of horned lizards ( $d_e = 4.5 \pm 0.22$ ), compared to Kenedy, which has experienced steep declines and had no horned lizards present in 2019 - 2021 ( $d_e = 6.0 \pm 0.32$ ;  $t_{0.05(2), 8} = -3.85$ ,  $P = 0.005$ ). Average thermal quality ( $d_e$ ) was highest (i.e., lower value) for vegetation followed by dirt then open microhabitats (Dunn,  $P < 0.001$  in both cases; Fig. 9). Thermal quality was higher for sites that contained alleyways ( $d_e = 4.4 \pm 0.70$ ) rather than fields ( $d_e = 5.5 \pm 0.90$ ;  $t_{0.05(2), 12} = -2.78$ ,  $P = 0.017$ ).

### Thermoregulatory indices

The average deviations of  $T_b$  from  $T_{set}$  range (i.e.,  $d_b$ ) was low (1.59oC), indicating the lizards were active close to their preferred temperature range and suggests that *P. cornutum* exhibits accurate thermoregulation ( $d_b = 1.59$ ; Table 2). Effectiveness of thermoregulation ( $E$ ) was 0.31, indicating *P. cornutum* is a moderate thermoregulator. Effectiveness of thermoregulation for  $d_e - d_b$  was 0.71, also indicating some thermoregulatory behavior and a thermally benign environment (Table 2).

## DISCUSSION

In tropical and desert areas the major challenge for lizards is to lower their body temperature and vegetation plays a key role in providing shade and cooling temperatures (Kearney et al. 2009; Grimm-Seyfarth et al. 2017). In fact, maintaining thermal refugia like shrubs is probably key to buffering increasing temperatures due to climate change (Kearney et al. 2009; Attum et al. 2013; Grimm-Seyfarth et al. 2017; Suggitt et al. 2018; Ivey et al. 2020; Gaudenti et al. 2021). Studies of microhabitat utilization have all found that Texas horned lizards need a mosaic of bare ground, sparse grass and herbaceous plants, and woody vegetation for unimpeded movement, foraging for ants, and thermoregulation (Fair and Henke 1998; Burrow et al. 2001; Eifler et al. 2012; Anderson et al. 2017). Texas horned lizard spatial distribution appears to be governed more by habitat than prey availability with lizards occupying habitats with numerous patches of heterogeneous microhabitats (Munger 1984; Whiting et al. 1993). Our data reveals that shrubs and thick vegetation provide a critical refuge for thermoregulation and represented the highest quality microhabitat ( $d_e$ ) that is closest to their preferred temperature range. Environmental temperatures ( $T_e$ ) underneath vegetation never exceeded the lizards' upper  $T_{set75}$  or  $CT_{max}$ , whereas temperatures in the open often exceeded  $CT_{max}$  or were above their upper preferred temperature ( $T_{set75}$ ) for 5 hours in the middle of the day during their active period. We found when shrubs and brush piles had been removed from fields, fence rows, and the base of trees, the number of lizards at a site declined by almost 80%. It is unknown to what extent the vegetation removal may have caused direct mortality, however, in all cases there were nearby areas with horned lizards that could have recolonized the site and yet the site either lost all lizards or stayed at a much lower density. In only one case was a site recolonized after vegetation grew back within the time frame of the study. We suggest that these declines have occurred in large part because the lizards lost a critical thermal refuge from temperatures at ground level that often exceed their  $CT_{max}$  during the summer months.

The shade provided by vegetation may also increase foraging opportunities for lizards at our field sites. In Kenedy and Karnes City, Texas horned lizards eat mostly smaller ants (*Pheidole* spp., 40% of diet) and harvester termites (*Tenuirostritermes cinereus*, 34% of diet), while harvester ants only make up 8% of their diet (Alenius 2018). *Tenuirostritermes cinereus* exhibits diurnal open-air foraging that is constrained by high temperatures and low humidity levels and are usually found foraging in overcast and humid conditions,

such as under vegetation or during the early morning hours (Nutting et al. 1974; Scheffrahn and Rust 1983; Alenius 2018). Vegetation provides shade and a humid microenvironment for termites, and therefore could increase foraging time for horned lizards.

Open microhabitats (i.e., bare ground) are still important for thermoregulation during the morning and evening hours to reach adequate  $T_b$  and to forage for ants that also have a bimodal pattern of activity (Whitford and Bryant 1979; Whitford et al. 1980). Burrowing in the middle of the day can potentially reduce temperatures below  $CT_{max}$ , although burrowing in more open areas such as the places we placed the models would result in a  $T_e$  that was often above their upper  $T_{set75}$  for about 6 hours of the day. It is not clear how often burrowing behavior may be related to thermoregulation since they only bury themselves a few centimeters under the surface of loose dirt or sand and they often burrow while under vegetation (Whitford and Bryant 1979; Burrow et al. 2001). Burrowing is very effective at making the lizards virtually invisible, and so it may more often function as a predator avoidance strategy when they are inactive. Texas horned lizards will also climb onto the trunk or lower branches of shrubs during the hottest times of the day presumably for thermoregulation (Whitford and Bryant 1979; Burrow et al. 2001). We have never observed this behavior in Kenedy or Karnes City, so we did not place models in those areas, however studies of this species in other areas should include models in shrubs to evaluate their daily temperature profiles.

Preferred body temperature at our study sites ( $T_{set} = 35.7 \pm 0.33^\circ\text{C}$ ) was in between reported  $T_{set}$  from other studies of Texas horned lizards (Table 1), but close to the average  $T_{set}$  of 20 species of *Phrynosomatids* ( $35.1 \pm 2.2^\circ\text{C}$ ; Clusella-Trullas and Chown 2014). Field body temperature ( $T_b = 33.6 \pm 0.30^\circ\text{C}$ ) was similar to one reported value ( $33.4 \pm 0.45^\circ\text{C}$ ; Lara-Resendiz et al. 2015b) and lower than three other reported body temperatures for Texas horned lizards ( $35.7 \pm \text{ND}^\circ\text{C}$ , Brattstrom 1965;  $37.3 \pm 0.30^\circ\text{C}$ , Pianka and Parker 1975;  $35.2 \pm 3.44 \text{ SD}^\circ\text{C}$ , Russell 2001). Nonetheless,  $T_b$  is lower than the mean environmental temperatures available to them ( $T_e = 35.2 \pm 1.1^\circ\text{C}$ ), but falls within the lower  $T_{set25}$  range for lizards at our study sites. Horned lizards at our field sites thermoregulated with lower effectiveness ( $E = 0.31$ ), which is consistent with less precision and relaxed thermoregulation found across *Phrynosoma* spp. (Pianka and Parker 1975). Thermoregulatory effectiveness, as measured by  $d_e - d_b$ , was 0.71, which also indicates some thermoconforming behavior and a thermally benign environment. Horned lizards at our site could be keeping their  $T_b$  lower because precise thermoregulation is less important than other activities. For example, foraging could potentially be prolonged in shaded areas with lower temperatures.

There was a negative correlation between the average thermal quality ( $d_e$ ) at a site and average density (lizards/hectare) of horned lizards. Kenedy had sites with lower thermal quality (i.e., higher  $d_e$  values) and a lower density of horned lizards compared to Karnes City. This was in part related to the configuration of sites in Kenedy, which were all open fields, while many of the sites in Karnes City were alleyways or had alleyways associated with an open field. Alleyways had significantly higher thermal quality than fields, and past studies have also found higher densities of lizards in the alleyways compared to the fields (Ackel 2015). Alleyways in these towns consist of dense vegetation (e.g., shrubs and grasses) along the fence lines and a variable canopy cover with a dirt road in the middle. This configuration allows lizards to sun and forage, then retreat into the nearby vegetation when temperatures increase (Ackel 2015). Fields have isolated bushes, trees with bushes, or brush piles that are relatively separated from each other. Alleyways probably represent a configuration of thermal refugia that are more dispersed with a gradient of temperatures near each other, whereas fields have a more clumped distribution of thermal refugia, and so are less favorable because of the energetic costs of moving between relatively distant clusters of favorable microhabitats to maintain body temperature (Huey and Slatkin 1976; Sears et al. 2016).

Texas horned lizards may be well suited to living in some types of human modified habitats that result in a heterogenous mix of microhabitats. A recent meta-analysis of reptile responses to anthropogenic habitat modification found that the family *Phrynosomatidae* had a less negative response to human habitat modification than other groups of lizards, suggesting they may be adapted to more disturbed habitats or habitats that have features of disturbance such as arid lands with sparse vegetation (Doherty et al. 2019). Within town, Texas horned lizards utilize areas that contain native grasses, have some bare ground, and are mowed

or trimmed regularly (Wall 2014). The lizards use both non-native and native shrubbery, large prickly pear cacti, brush piles, the inside of old sheds, and under pier and beam houses as thermal refugia and hiding places. In the past, pier and beam houses were much more common in the area and residents report anecdotally that it was common to have horned lizards living under the house. Maintaining Texas horned lizards in these towns will require maintaining this heterogenous mix of closely spaced microhabitats and especially maintaining thermal refugia. There should be minimal clearing of brush in alleyways and parks, or possibly conducting removal in incremental stages, and replacing it with appropriate shrubbery. Fence rows should maintain shrubbery and more shrubbery should be planted in open yards and parks. Landscaping style (e.g., types of vegetation planted, fencing and borders, extent of tree canopy cover) can result in maximum daily air temperature differences up to 10oC between two adjacent habitats (Todd and Andrews 2008; Robinson et al. 2013) and reduce surface temperatures over 10oC during the day (Brazel et al. 2007). Landscaping can also create habitat that can increase the diversity and abundance of reptiles in human modified areas (Ackley et al. 2015; Pulsford et al. 2017; Nopper et al. 2017). Maintaining and creating thermal refugia is probably one of the most important and practical conservation actions that can be taken to help small ectotherms persist in human modified landscapes and cope with increasing temperatures due to climate change (Kearney et al. 2009; Kearney 2013; Grimm-Seyfarth et al. 2017; Suggitt et al. 2018).

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## TABLES

**Table 1:** Preferred body temperatures ( $T_{set}$ ) of Texas horned lizards and  $T_{set}$  range ( $T_{set25} - T_{set75}$ ) in degC. Mean +- SE (standard error) or SD (standard deviation) reported for  $T_{set}$ . ND is standard error that was not presented.

N	$T_{set}$	$T_{set25}$	$T_{set75}$	Location	References
10	$38.5 \pm$ ND	37.5	39	Dona Ana Co., New Mexico	Prieto and Whitford 1971
97	$34.2 \pm 0.1$ SE	32.5	36	Janos, Chihuahua, Mexico	Lara-Reséndiz et al. 2015a
9	$36.3 \pm 2.39$ SD	34.9	38.1	Dimmit/La Salle Co., Texas	Russell 2001
19	$35.7 \pm 0.33$ SE	33.5	38.5	Karnes Co., Texas	Present study

**Table 2 :** Field body temperature ( $T_b$ ), operative environmental temperature ( $T_e$ ), preferred temperature in laboratory ( $T_{set}$ ) and  $T_{set}$  range ( $T_{set25} - T_{set75}$ ) in °C, and accuracy of thermoregulation ( $d_b$ ), habitat thermal quality ( $d_e$ ), and thermoregulatory effectiveness ( $d_e - d_b$  and  $E$ ). Showing mean +- SE.

Species	$T_b$	$T_e$	$T_{set}$	$T_{set}$ range	$T_{set}$ range	$d_b$	$d_e$	$d_e - d_b$	$d_e - d_b$
<i>Phrynosoma cornutum</i>	$33.6 \pm 0.3$	$35.2 \pm 1.1$	$35.7 \pm 0.33$	33.5—38.5	$1.59 \pm 0.2$	$1.59 \pm 0.2$	$2.30 \pm 0.2$	$2.30 \pm 0.2$	0.71

## FIGURES

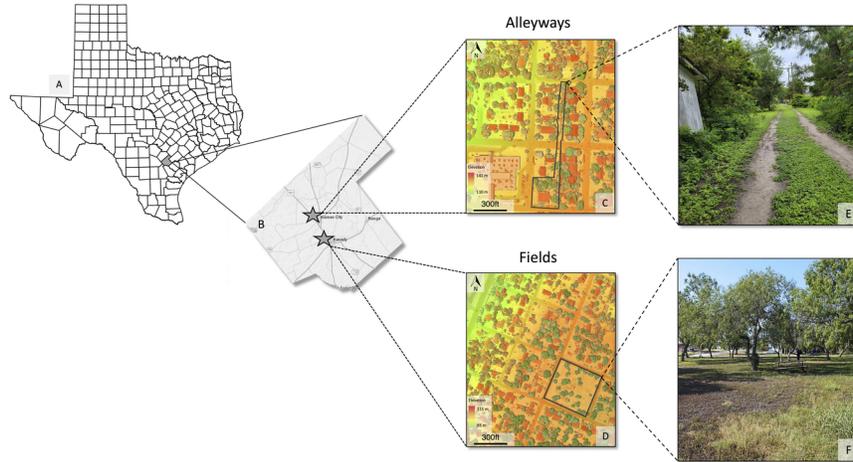


Figure 1: Figure 1: (A) Map of Texas counties, with Karnes County shown in grey. (B) Map of Karnes County, showing the locations of Kenedy and Karnes City. (C&D) Aerial map showing classification of trees and shrubs (dark green), houses (red), and ground (color ramp representing range of elevation in meters) from two of our study sites (outlined in black) within Karnes City and Kenedy, respectively. Maps generated by classifying LiDAR point cloud data (United States Geological Survey, TX Hurricane B4 2018, Date: 2019-01-12Z - 2019-02-21Z, Quality: QL 2) using Esri ArcGIS<sup>7</sup> Pro. Study sites are split up into two types of spatial structure: (C) alleyways, which are more heterogeneous in structure and thermal microhabitats, and (E) have dirt roads bordered by houses, fences, and vegetation (i.e., tree canopy cover, ornamental shrubs, and native vegetation), and (D) fields, which are less heterogenous and have thermal microhabitats spread apart since fields have (F) short vegetation cover interspersed with clumps of trees and shrubs.



Figure 2: Figure 2: Horned lizard model equipped with a temperature logger embedded in the belly area and secured with black self-fusing repair tape to approximate environmental temperatures ( $T_e$ ) available to lizards.

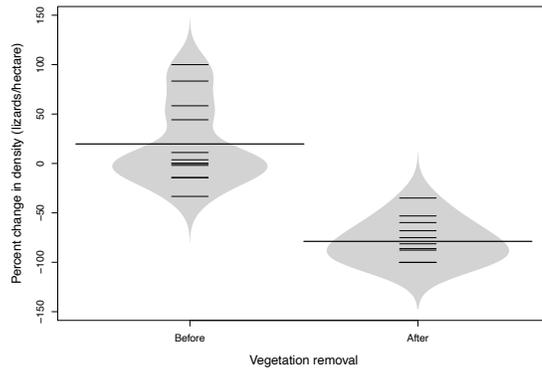


Figure 3: Figure 3: Bean plot showing percent change in horned lizard density (lizards/hectare) at sites that experienced extensive vegetation removal in Karnes Co., Texas from 2013 – 2021 ( $n = 12$  sites). Percent change in density is shown before vegetation removal and one year after vegetation removal between the same sites. Bold horizontal lines represent the average for change in density for before and after vegetation removal, respectively. Smaller lines corresponding to individual site values. Percent change in density was significantly different between a site before vegetation removal and one year after vegetation removal, with horned lizard density declining on average  $-78.9 \pm 6.2\%$  the year after vegetation clearing ( $t_{0.05(2), 16} = 7.27$ ,  $P = 1.9 \times 10^{-6}$ ).

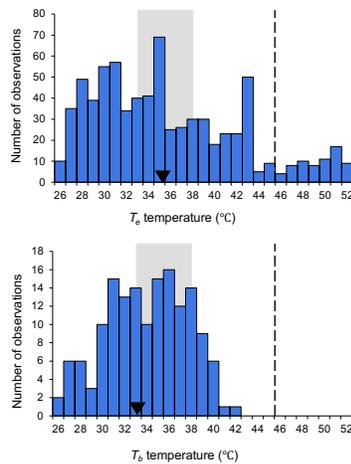
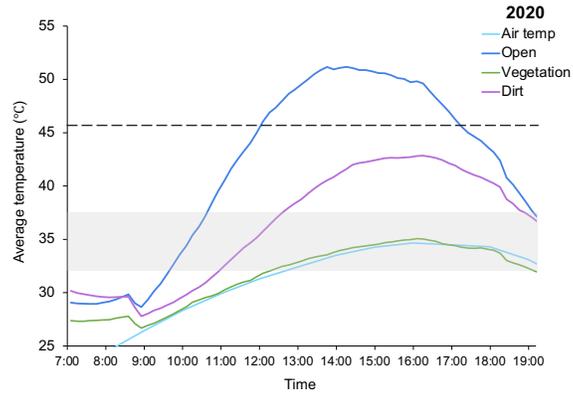
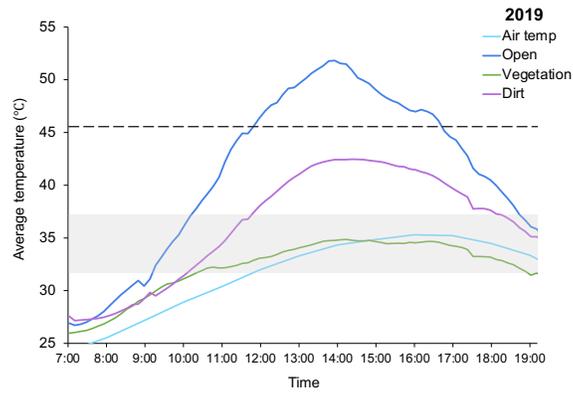


Figure 4: Figure 4: Frequency distribution of model temperatures  $T_e$  (top) and horned lizard  $T_b$  cloacal temperatures (bottom) from Karnes Co., Texas, 2019 - 2021. Mean  $\pm$  SE  $T_e$  was  $35.2 \pm 1.1^\circ\text{C}$  ( $n = 326$  models) and mean  $T_b$  was  $33.6 \pm 0.3^\circ\text{C}$  ( $n = 153$  lizards). Black arrows represent the mean, the grey box represents their preferred temperature ( $T_{set}$ ) interquartile range ( $33.5 - 38.5\text{degC}$ ), and the black dashed line represents their critical thermal maximum ( $CT_{max} = 45.9\text{degC}$ ; Prieto and Whitford 1971).



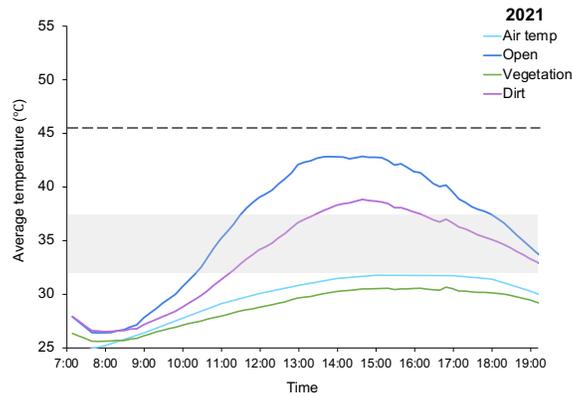


Figure 5: Figure 5: Average operative temperatures ( $T_e$ ) over Texas horned lizard’s active period measured by models in open, dirt, and vegetation microhabitats for 2019 - 2021(A - C). The dark blue line represents open, purple line represents dirt, green line represents vegetation, and the light blue line represents hourly ambient air temperatures from Karnes Co., Texas. The grey box represents their preferred temperature ( $T_{set}$ ) interquartile range (33.5 - 38.5°C). The black dashed line represents their critical thermal maximum ( $CT_{max} = 45.9^\circ\text{C}$ ; Prieto and Whitford 1971).

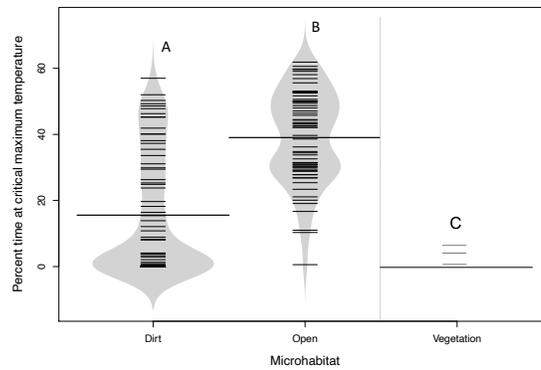


Figure 6: Figure 6: Bean plot showing percent time  $T_e$  was at critical temperature ( $CT_{max}$ ) for three different microhabitat classifications in Karnes Co., Texas from 2019 - 2020. Bold horizontal lines represent the average for each microhabitat, and smaller lines correspond to the average values of each model within that microhabitat. Groups sharing a letter are not significantly different from each other whereas different letters are significantly different from each other (dirt,  $n = 68$  models; open,  $n = 63$  models; and vegetation,  $n = 70$  models).

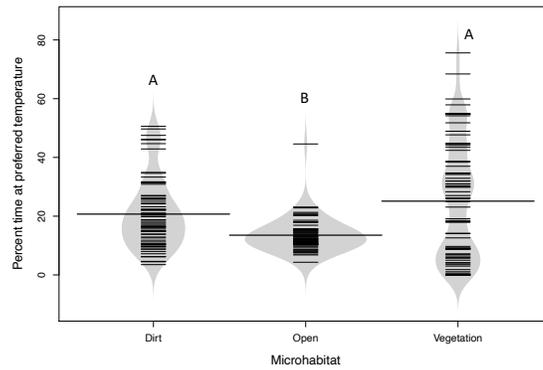


Figure 7: Figure 7: Bean plot showing percent time  $T_e$  was at preferred temperatures ( $T_{set25} - T_{set75}$ ) for three different microhabitat classifications in Karnes Co., Texas from 2019 - 2020. Bold horizontal lines represent the average for each microhabitat, and smaller lines correspond to the average values of each model within that microhabitat. Groups sharing a letter are not significantly different from each other whereas different letters are significantly different from each other (dirt,  $n = 68$  models; open,  $n = 63$  models; and vegetation,  $n = 70$  models).

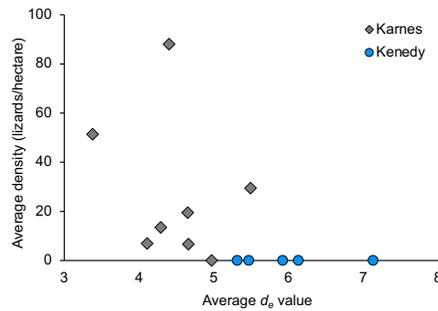


Figure 8: Figure 8: Scatterplot showing the relationship between average thermal quality score ( $d_e$ ) and average density of horned lizards (lizards/hectare) for 2019 - 2021 in two small towns Kenedy (blue circle) and Karnes City (grey diamond), Texas ( $n = 13$  sites).

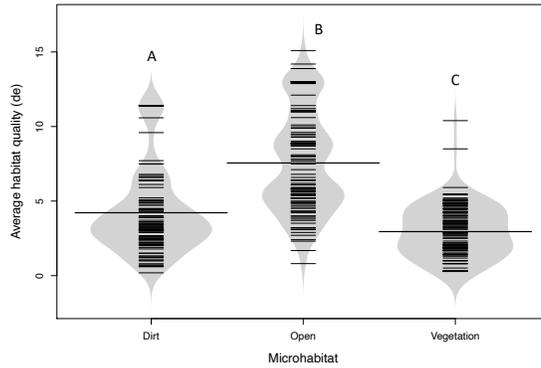


Figure 9: Figure 9: Bean plot showing the distribution of quality scores (*de*) for three different microhabitat classifications in Karnes Co., Texas from 2019 - 2021. Bold horizontal lines represent the average *de* for each microhabitat, and smaller lines correspond to the average *de* values of each model within that microhabitat. Groups sharing a letter are not significantly different from each other whereas different letters are significantly different from each other (dirt,  $n = 105$  models; open,  $n = 103$  models; and vegetation,  $n = 118$  models).

## ETHICAL APPROVAL

Our Texas horned lizard work was approved by the Institutional Animal Care and Use Committee at Texas Christian University and Scientific Research Permit No. SPR-0613-073 from Texas Parks and Wildlife.

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