

THERMAL ECOLOGY OF THE FEDERALLY ENDANGERED BLUNT-NOSED  
LEOPARD LIZARD (*GAMBELIA SILA*)

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Master of Science in Biology

by

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Blunt-nosed Leopard Lizard (*Gambelia sila*)

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## ABSTRACT

### Thermal Ecology of the Federally Endangered Blunt-nosed Leopard Lizard (*Gambelia sila*)

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Recognizing how climate change will impact populations can aid in making decisions about approaches for conservation of endangered species. The Blunt-nosed Leopard Lizard (*Gambelia sila*) is a federally endangered species that, despite protection, remains in extremely arid, hot areas and may be at risk of extirpation due to climate change. We collected data on the field-active body temperatures, preferred body temperatures, and upper thermal tolerance of *G. sila*. We then described available thermal habitat using biophysical models, which allowed us to (1) describe patterns in lizard body temperatures, microhabitat temperatures, and lizard microhabitat use, (2) quantify the lizards' thermoregulatory accuracy, (3) calculate the number of hours they are currently thermally restricted in microhabitat use, (4) project how the number of restricted hours will change in the future as ambient temperatures rise, and (5) assess the importance of Giant Kangaroo Rat burrows and shade-providing shrubs in the current and projected future thermal ecology of *G. sila*. Lizards maintained fairly consistent daytime body temperatures over the course of the active season, and use of burrows and shrubs increased as the season progressed and ambient temperatures rose. During the hottest part of the year, lizards shuttled among kangaroo rat burrows, shrubs, and open habitat to maintain body temperatures below their upper thermal tolerance, but occasionally, higher than their preferred body temperature range. Lizards are restricted from staying in the open habitat for 75% of daylight hours and are forced to seek refuge under shrubs or burrows to avoid surpassing their upper thermal threshold. After applying climatic projections of 1 and 2°C increases to 2018 ambient temperatures, *G. sila* will lose additional hours of activity time that could compound stressors faced by this population, potentially leading to extirpation.

Finally, temperature-based activity estimation (TBAE) is an automated method for predicting surface activity and microhabitat use based on the temperature of an organism and its habitat. We assessed continuously logged field active body temperatures as a tool to predict the surface activity and microhabitat use of *Gambelia sila*. We found that TBAE accurately predicts whether a lizard is above or below ground 75.7% of the time when calculated using air temperature, and 60.5% of the time when calculated using biophysical models. While surface activity was correctly predicted about 93% of the time using either method, accuracy in predicting below ground (burrow) occupancy was 62% for air temperature and 51% for biophysical models. Using biophysical model data, TBAE accurately predicts microhabitat use in 79% of observations in which lizards are in the sun, 47% in the shade, and 51% in burrows. Heliotherms bask in the sun, and thus body temperatures can shift rapidly when the animal moves to a new microhabitat. This sensitivity, makes TBAE a promising means of remotely monitoring animal activity, particularly for specific variables like emergence time and surface activity.

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# **1. Thermal Ecology of the Federally Endangered Blunt-nosed Leopard Lizard (*Gambelia sila*)**

## **1.1 Introduction**

As anthropogenic climate change accelerates, so has the urgency for studies examining how rising temperatures will impact sensitive species. By 2050, up to 18% of species worldwide will be extinct (Thomas *et al.*, 2004; Urban, 2015), with human-caused land use changes having the potential to further increased extinction risk (Powers & Jetz, 2019). The actions we take now in terms of habitat management may mitigate the potential impacts of climate change on endangered species. Because ectotherms depend on ambient temperatures for their normal physiological processes (Stevenson, 1985; Walther *et al.*, 2002), they are very sensitive to thermal changes in their habitats (Sinervo *et al.*, 2010; Buckley *et al.*, 2015). This is especially true for ectotherms living in extremely hot environments where they are thermally constrained (Grant & Dunham, 1988; Bashey & Dunham 1997; Zamora-Camacho *et al.*, 2016) and have limited plasticity that would allow them to survive with further warming (Gunderson & Stillman, 2015). Because of their abundance, ease of study, and diversity in extremely hot environments worldwide, lizards have become model organisms for studies of thermal tolerance, with important implications for conservation biology. As many as 30% of all lizard populations could be extinct by 2080 (Sinervo *et al.*, 2010), and it is unclear whether thermoconforming lizards or heliothermic (sun-basking) lizards will fare worse (Sinervo *et al.*, 2010).

Habitat heterogeneity is important to lizards and other ectotherms as it allows them to exploit behavioral thermoregulation to maintain a body temperature close to their

preferred body temperature (Sears *et al.*, 2011, Carroll *et al.*, 2016). Temperatures on the surface of the ground exposed to the sun often exceed the thermal tolerance of lizard species (Sunday *et al.*, 2014), and so the availability of shade is important for thermoregulation (Kearney *et al.*, 2009). Notably, refugia not only provide refuge from predators, but also from thermally unsuitable conditions (Bradshaw & Main, 1968; Souter *et al.*, 2007; Pike & Mitchell, 2013; Lortie *et al.*, 2015; Moore *et al.*, 2018; Suggitt *et al.*, 2018). Vegetation may assist animals with thermoregulation by providing them with a complex mosaic of thermal and radiative properties on the surface of the ground (Carroll *et al.*, 2016; Milling *et al.*, 2018). Plants are important to the thermoregulation of lizards inhabiting extremely hot environments because they allow lizards to be surface active while protecting the lizards from intense solar radiation. (e.g., Porter *et al.*, 1973; Bauwens *et al.*, 1996). This includes, but is not limited to, essential activities like territory defense, mate guarding, and feeding.

The Blunt-nosed Leopard Lizard (*Gambelia sila*) is a federally endangered species with isolated populations in the San Joaquin Valley and the southeastern Carrizo Plain in California (U.S. Fish & Wildlife, 1998; Germano & Rathbun, 2016; IUCN, 2017), an area with extremely hot and arid conditions. Since the 1960's, the species' range has decreased by 85% due to agriculture, oil exploration, and invasive grasses (Germano *et al.*, 2001; Filazzola *et al.*, 2017). The active season for adults is limited to as little as 2.5 months in the spring and early summer (Germano *et al.*, 1994), after which time they estivate and then transition directly into hibernation. It is therefore likely that *G. sila* is already clinging to existence in a thermally stressful environment, and evaluation of the thermal ecology of this species is likely to provide managers and

researchers with valuable information about climate change mitigation efforts for this species (Germano, 2019). A significant amount of the remaining habitat occupied by *G. sila* is dominated by the Mormon Tea shrub, *Ephedra californica* (Stout *et al.*, 2013), which creates spotty microhabitats that are cooler and more humid than the open ground (Filazzola *et al.*, 2017). Lizards are regularly found in the shade of these shrubs, especially in the afternoon when temperatures are highest (Westphal *et al.*, 2018; Germano, 2019). Given that the habitats occupied by *G. sila* tend to be structurally simple (i.e. lizard microhabitat choices are limited to the open desert floor, rodent burrows, and *Ephedra* shrubs), modeling the thermal ecology of *G. sila* provides an excellent opportunity to quantify the importance of these microhabitats, both currently and as the climate warms.

In this study, we describe the thermal ecology of a population of *G. sila* in the southeastern Carrizo Plain over the course of one active season with the goals of (1) quantifying the daily and seasonal body temperatures of lizards, (2) describing how lizards behaviorally use available microhabitats, (3) determining their thermoregulatory accuracy, (4) calculating the number of hours they are currently restricted to shade and/or burrows due to extreme heat, and (5) projecting how these values are likely to be impacted by climate change during this century. Given that the only above ground shade available to lizards at this site is provided by sparsely distributed *Ephedra* shrubs, we also explicitly test the hypothesis that shrubs currently act as thermal buffers that allow the lizards to remain active above ground longer than they would if there were no shrubs.

## 1.2 Materials and Methods

### 1.2.1 Field Site

Our study site is located within the Elkhorn Plain (35.117998° -119.629063°) in the Carrizo Plain National Monument, California, USA. This area is characterized by extremely harsh, arid summers (average high 30 - 40°C) and cool winters (average low 5 - 9°C, Germano & Williams, 2005; Raws USA Climate Archive, 2019). This site is part of the San Joaquin Desert (Germano *et al.*, 2011), which in modern times has been frequently misclassified as a grassland prairie, despite early European explorers describing the landscape as lacking dominant annual or perennial grasses (D'Antonio *et al.*, 2007; Schiffman, 2007; Minnich, 2008). When temperatures rise in this area, the vegetation dies off in early May, leaving the ground barren and resembling that of an arid desert with occasional small saltbush plants (Minnich, 2008) and in some areas, including our site, sparsely distributed *Ephedra* shrubs. The area is dominated by Giant Kangaroo Rat (*Dipodomys ingens*) precincts with extensive burrow networks. Our study spanned one active season of *G. sila* (May - July 2018). We obtained ambient temperature data from a weather station (Cochora Ranch, station ID: CXXC1) 3.7 km due east of the field site.

### 1.2.2 Study Species and Field Monitoring

Adult *G. sila* (N = 30) were captured by hand-held lasso in early May 2018. Snout-vent length (SVL,  $\pm 0.1$  cm), mass (Pesola® 50 -100g precision scale,  $\pm 0.5$ g), and sex were recorded upon capture (Table S1). Females were palpated for follicles and recorded as gravid or not. Lizards were fitted with VHF temperature-sensitive radio-

transmitter collars (Holohil model BD-2T, Holohil Systems Ltd., Carp, ON, Canada) following the methods of Germano & Rathbun (2016). The transmitters were epoxied to nickel-plated ball chain collars, which were fitted around the lizards' necks, with whip antennas (16cm) extending dorsally from the collars. Lizards were released the same day of capture. Following release, lizards were tracked 1-3 times per day using a VHF receiver and Yagi antenna (R-1000 Telemetry Receiver, Communications Specialists, Inc., Orange, CA, USA), resulting in an average of 55 observations on each lizard over the active season. Behavioral observations, microhabitat (open desert floor, under shrub, or in burrow), GPS location, and time of day were recorded at each tracking event. At the end of the study, lizards were recaptured by lasso or excavated from burrows and collected for measurement of preferred body temperature and thermal tolerance (see below). Collars were then removed, SVL and mass data were recorded again, and lizards were released at their sites of capture, at which time they entered estivation for the remainder of the summer.

### *1.2.3 Field Active Body Temperature ( $T_b$ ) and Microhabitat Use*

We continually recorded the temperatures of the radio-transmitters as the field active lizard body temperature ( $T_b$ ) using a Telonics TR-5 receiver with data acquisition system (Telonics Option 320) and 10ft tall omni antenna (Telonics model RA-6B). We programmed the system to log the interpulse intervals of the transmitters about every ten minutes and used manufacturer-provided calibration equations to convert interpulse interval to temperature. This resulted in a total of ~90,000  $T_b$  points for the 30 lizards spanning their active season from May-July. Because radio-transmitters were external



(collars), it is possible that they could heat more rapidly than the lizard's core actual  $T_b$ , especially when lizards are in the sun. This may lead to a slight overestimate of lizard  $T_b$  than if core  $T_b$  had been collected, which is not possible with external radio-transmitters. Data were checked manually for aberrant points, which were removed. We used an ANCOVA to test whether SVL, mass, sex, or gravidity affected mean  $T_b$ , and a repeated measures ANOVA with time of day (daytime or nighttime), month, the interaction between time of day and month, and lizard ID as a random effect, to analyze how  $T_b$  changed over the active season (May-July), and Tukey posthoc tests to compare monthly nighttime temperatures or monthly daytime temperatures. We also used field-active  $T_b$  data to calculate the field-active voluntary maximum  $T_b$  ( $VT_{max}$ ), or the average maximum daily  $T_b$ , which presumably occurred when the lizard was active above ground exposed to solar radiation (Brattstrom, 1965), to use in the activity restriction analysis (see below). To test the hypothesis that lizard microhabitat differed by month, we calculated an initial Pearson's chi-square statistic from the observed data. We then ran a permutation test by shuffling the observations across months and computing a chi-square statistic for each permutation. This analysis was performed in R (R Core Team, 2017), and all other analyses were performed in JMP® (v. Pro 14).

#### *1.2.4 Preferred Body Temperature ( $T_{set}$ ) and Thermoregulatory Accuracy ( $d_b$ )*

At the end of the study (mid-July), lizards were collected from the field site and brought to a field station to collect data on their preferred body temperature range ( $T_{set}$ ) in a thermal gradient. The gradient consisted of sand substrate divided into three lanes (250 x 20 x 25 cm each) separated by wood dividers so lizards could not see lizards in

neighboring lanes. One end of the gradient was heated to 47 °C with a closed circuit 4 gallon water heater (Stiebel Eltron model no. SHC4, Germany), and the other end was cooled to 10 °C with a closed circuit 400L water cooler (ActiveAQUA Refrigerateur model no. AACH10, Petaluma, CA, USA). Water circulated under the gradient in insulated pipes from the heated side to the cold side to create the thermal gradient. Thermocouples (model 5SRTC-TT-K-40-72, Omega Engineering, UK) were inserted into the lizard's cloacae and held in place by medical tape wrapped around the base of the tail. The thermocouples recorded  $T_b$  every ten minutes on a data logger (model RDXL4SD, Omega Engineering, Egham, Surrey, UK). Lizards were placed in the center of the gradient and left undisturbed for three hours (the first two hours were used as an acclimation period, and the final hour was used to determine  $T_{set}$ ). We designated  $T_{set}$  as the 25-75% interquartile range of the final hour  $T_b$ . Data collection for the 30 lizards ran continually day and night over several days to minimize the amount of time the lizards were kept in captivity before release. We excluded  $T_{set}$  data for three lizards from the analysis (10.6, 14.3, 18.2°C) because they were  $> 2$  SD away from the median and were likely from lizards that failed to actively thermoregulate within the gradient in the time allotted. We used an ANCOVA to test the effects of sex, SVL, mass, capture method (lasso or excavation), and time of day on median  $T_{set}$ . We calculated lizard thermoregulatory accuracy ( $d_b$ ) by subtracting the mean  $T_{set}$  IQR from each instance of  $T_b$  (Hertz *et al.*, 1993), then averaged all  $d_b$  values for a single lizard within each 1-hour period per day from 0700-1900, then averaged all  $d_b$  by hour of day to create average hourly  $d_b$  values. Either very high positive or very low negative values of  $d_b$  represent poor accuracy and zero represents perfect accuracy.

### 1.2.5 Upper Thermal Tolerance ( $T_{pant}$ )

The upper thermal tolerance of lizards is typically measured as a loss of righting response or the onset of muscular spasms in response to high temperature, which represents the critical thermal maximum ( $CT_{max}$ ), or the high temperature at which a lizard loses muscular coordination and will die if heated further (Cowles & Bogert, 1944; Larson, 1961; Prieto & Whitford, 1971; Shea *et al.*, 2016). At  $T_b$  slightly below the  $CT_{max}$ , lizards begin gaping and panting, presumably to increase evaporative cooling rates (Dawson & Templeton, 1963; Heatwole *et al.*, 1973; Tattersall *et al.*, 2006). Given that *G. sila* is a federally endangered species, we chose to use their panting threshold ( $T_{pant}$ ) as a conservative measure of their upper thermal tolerance so that we did not expose lizards to excessively stressful or potentially fatal high temperatures. To measure  $T_{pant}$ , we used a Cal Poly-engineered device, the Gas Analysis Temperature Oxygen Regulation System (GATORS). Lizards were fitted with cloacal resistance thermometers, heated at  $1^\circ\text{C}$  ambient temperature per minute in individual temperature-controlled chambers (18cm length, 4cm diameter), observed for panting behavior (open mouth and rapid thoracic compression), then promptly removed and cooled.  $T_{pant}$  was recorded immediately following collection of  $T_{set}$  data. We used an ANCOVA to test the effects of sex, SVL, mass, capture method (lasso or excavation), and time of day on  $T_{pant}$ .

### 1.2.6 Biophysical Models and Microhabitat Temperatures

We used biophysical models to model the ranges of temperatures within microhabitats throughout the course of a day a lizard would experience if it were behaviorally neutral to, or non-thermoregulating within, the environment. Models ( $N =$

18) consisted of 1” (2.5cm) diameter copper pipes, welded with a copper female end on one side and a male end on the other. A Thermochron iButton (DS1921G-F5) programmed to record temperature every 10 minutes and coated in PlastiDip was suspended in the center of each pipe by a 3D-printed plastic insert to avoid contact with the pipe walls, then pipes were filled with water (Dzialowski, 2005), and PVC caps were screwed onto the male copper ends. Models were fitted with two 3.8cm “legs” made from copper wiring to prop models above ground on one end, mimicking a lizard propped up on its front legs. Biophysical model temperatures were validated by comparing internal temperatures to those of a preserved lizard over the course of 120 minutes of heating in the sun (models were continually within  $\pm 1$  °C of the lizard). Models were deployed from July 1-19 (a very hot period) in three different microhabitats: on the desert floor exposed to the sun (open, N = 6), in the shade under *Ephedra* shrubs (shrub, N = 6), and approximately 1 meter inside Giant Kangaroo Rat burrows (burrow, N = 6). Models in burrows did not have legs to mimic lizards lying prone on the burrow floor. We compared the mean hourly temperatures of the three microhabitats during *G. sila* activity hours (0700-1900) using a two-way ANOVA followed by a Tukey-Kramer post hoc test.

### 1.2.7 Activity Restriction

We used data from the biophysical models along with  $T_{pant}$ ,  $V_{T_{max}}$ , and  $T_{set}$  data to calculate the activity constraint or hours of restriction ( $h_r$ ), or the number of hours that a lizard could not be active in a given microhabitat because its  $T_b$  would be too high, in several ways:

1. Basking restriction: the average number of hours per day that lizards are currently restricted from continually basking in the open and are confined to burrows or shade because temperatures of biophysical models in the open exceed  $T_{\text{pant}}$ ,  $VT_{\text{max}}$ , or  $T_{\text{set}}$  (we calculated hours of restriction separately for each variable).
2. Above ground restriction: the average number of hours per day that lizards are currently restricted from remaining active above ground and are confined to burrows because temperatures of biophysical models in the open or in the shade exceed  $T_{\text{pant}}$ ,  $VT_{\text{max}}$ , or  $T_{\text{set}}$ .
3. Total restriction: the average number of hours per day that temperatures of biophysical models in all microhabitats exceed  $T_{\text{pant}}$ ,  $VT_{\text{max}}$ , or  $T_{\text{set}}$ .

### *1.2.8 Climatic Projections*

To assess how  $h_r$  might change in the future due to consequences of anthropogenic climate change, we used Cal-Adapt's representative concentration pathway (RCP) climate scenario 4.5 and 8.5 (Cal-Adapt, 2019). RCP 4.5 is a conservative scenario which predicts a steady decline following peak carbon emissions in 2040. RCP 8.5 is a worst-case scenario in which carbon emissions continue throughout the 21st century, peaking in 2050 and plateauing around 2100. Using the "modeled projected annual mean" tool, we identified the years where the annual average temperatures in the Elkhorn Plain are projected to increase 1 and 2 °C from the 2018 average. To make our predictions, we added a 1 °C increase unilaterally across the 2018 biophysical model data. We projected how each  $h_r$  variable would be affected by climate change by adding 1 and 2 °C to current biophysical model temperatures (+1 °C  $h_r$  and +2

°C h<sub>r</sub>). Note that temperatures inside burrows, under shrubs, and out in the open are unlikely to actually increase at the same rates, but this method provides us with a coarse estimate as to how h<sub>r</sub> might change with warming climates (Brusch *et al.*, 2016).

## 1.3 Results

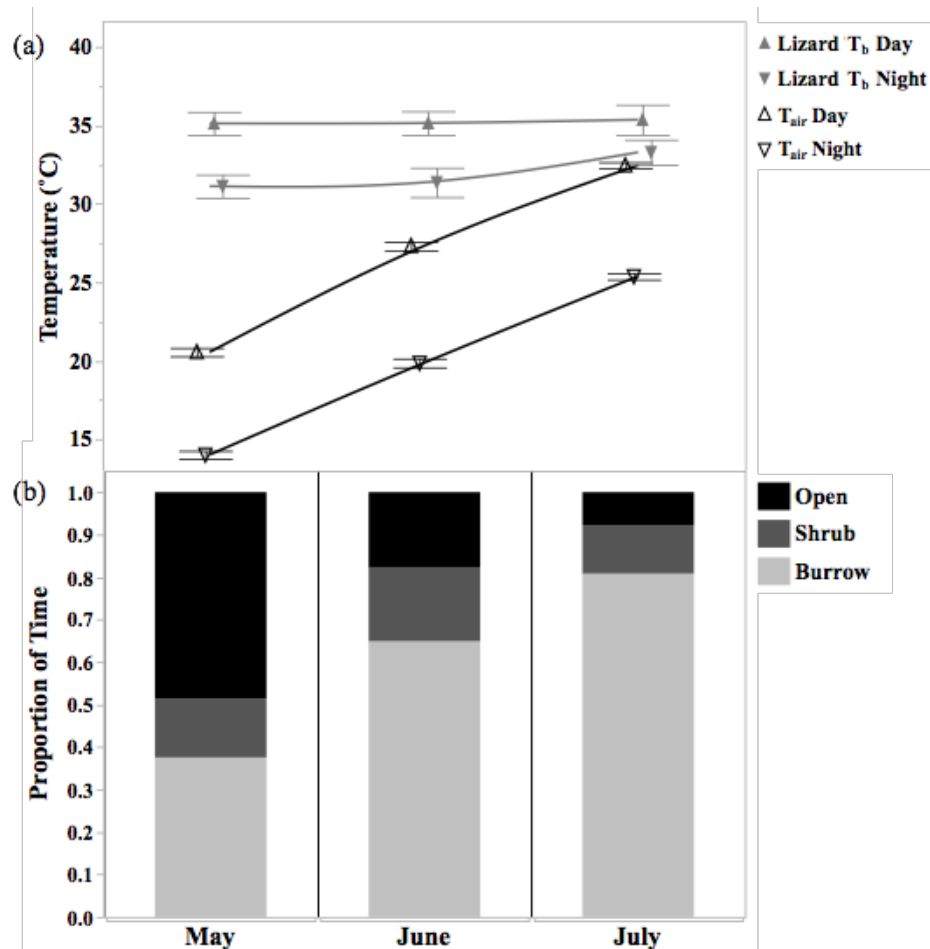
### 1.3.1 Field Active Body Temperature ( $T_b$ ) and Microhabitat Use

Mean  $T_b$  was not impacted by sex ( $F_1 = 0.91$ ,  $p = 0.35$ ) or by initial SVL ( $F_1 = 0.10$ ,  $p = 0.75$ ), mass ( $F_1 = 0.29$ ,  $p = 0.59$ ). Within female lizards,  $T_b$  did not differ between gravid and non-gravid lizards ( $F_1 = 0.16$ ,  $p = 0.70$ ). Monthly mean daytime and nighttime ambient temperatures increased from May to June to July, as expected, with daytime average temperatures consistently about 6-8 °C higher than nighttime temperatures (Figure 1.1a). Despite the dramatic increase in ambient temperatures over the course of the active season, lizard  $T_b$  did not vary across each month ( $F_{123.6} = 2.0$ ,  $p = 0.14$ ), and there was no interaction between month and time of day ( $F_{122.9} = 1.26$ ,  $p = 0.29$ ). As expected, average monthly mean daytime  $T_b$  of lizards was significantly higher than nighttime  $T_b$  ( $F_{122.9} = 38.6$ ,  $p < 0.001$ ). Tukey post-hoc tests showed that nighttime  $T_b$  increased significantly in July compared to May and June, presumably because burrow temperatures increased. The calculated  $VT_{\max}$  of *G. sila* was  $40.4 \pm 0.8^\circ\text{C}$ .

As ambient temperatures increased, we observed a concomitant increase in burrow use and decrease in time spent in the open microhabitat during daylight hours (Figure 1.1b). In the permutation independence test, the chi-square test statistic computed from the original data was 250. Of the 5,000 independent permutations performed, our

initial statistic was only exceeded 3 times, resulting in a permutation p-value of 0.0006, showing that microhabitat selection significantly differed by month.

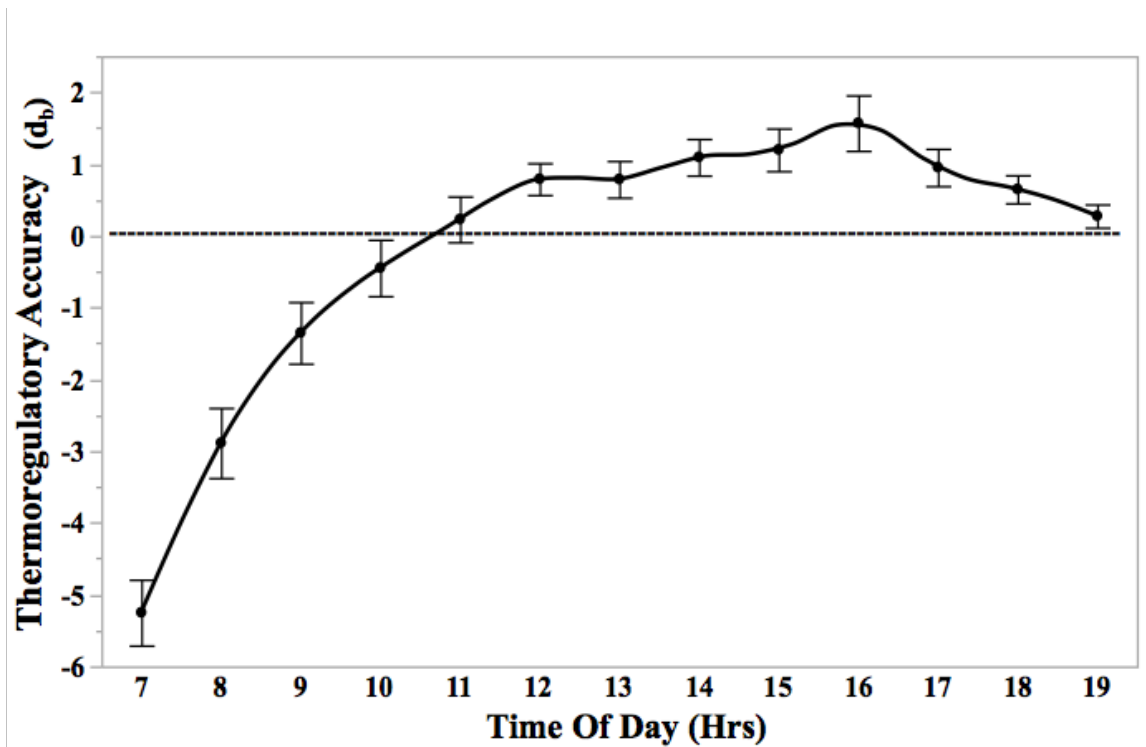
Average daily  $T_b$  of *G. sila* during an extremely hot part of their active season (1-19 July 2019) is shown in Figure 1.3, along with  $T_{set}$  range,  $T_{pant}$ , and  $T_e$  in the three microhabitats (results below).



**Figure 1.1: Monthly body and ambient temperatures and microhabitat selection by *Gambelia sila* (N=30) over the course of their active season (May - July 2018).** (a) Both daytime and nighttime ambient temperatures ( $T_{air}$ ) increased as the season progressed, but lizard  $T_b$  remained constant during daytime hours as they thermoregulated. Nighttime  $T_b$  increased in July. Values shown are means  $\pm$  1 SEM. (b) As ambient temperatures increased, lizards increased the proportion of time spent in burrows and decreased the proportion of time spent in the open during daylight hours. Shrubs therefore represented an increasing proportion of the above-ground microhabitat use as temperatures increased over the season.

### 1.3.2 Preferred Body Temperature ( $T_{set}$ ) and Thermoregulatory Accuracy ( $d_b$ )

The median preferred body temperature of *G. sila* is  $34.1 \pm 1.2^\circ\text{C}$ , with a  $T_{set}$  range of  $32.3 \pm 1.2^\circ\text{C} - 37.5 \pm 1.1^\circ\text{C}$  (Figure 1.3). There was no significant effect of sex ( $F_1 = 3.93$ ,  $p = 0.08$ ), SVL ( $F_1 = 0.02$ ,  $p = 0.90$ ), mass ( $F_1 = 0.26$ ,  $p = 0.62$ ), capture method ( $F_1 = 0.55$ ,  $p = 0.47$ ), or time of day ( $F_4 = 1.10$ ,  $p = 0.41$ ) on  $T_{set}$ . Before 9am,  $d_b$  values were negative because lizard  $T_b$  was lower than  $T_{set}$ , as even burrows are too cool for lizards to achieve  $T_{set}$  at night and early morning (Figure 1.2). After about 1100,  $d_b$  values become positive as lizard  $T_b$  often exceeded  $T_{set}$ , especially from about 1400 to 1900 (see Figure 1.2).



**Figure 1.2: Average thermoregulatory accuracy ( $d_b$ ) of *Gambelia sila* (N=30) over the course of the active season (May - July 2018) plotted against time of day from 0700-1900. Error bars represent  $\pm 1$  SEM. Values at zero (dashed line) indicate that the lizard is thermoregulating within the  $T_{set}$  range. As values move away from zero in either direction, the accuracy of thermoregulation decreases.**

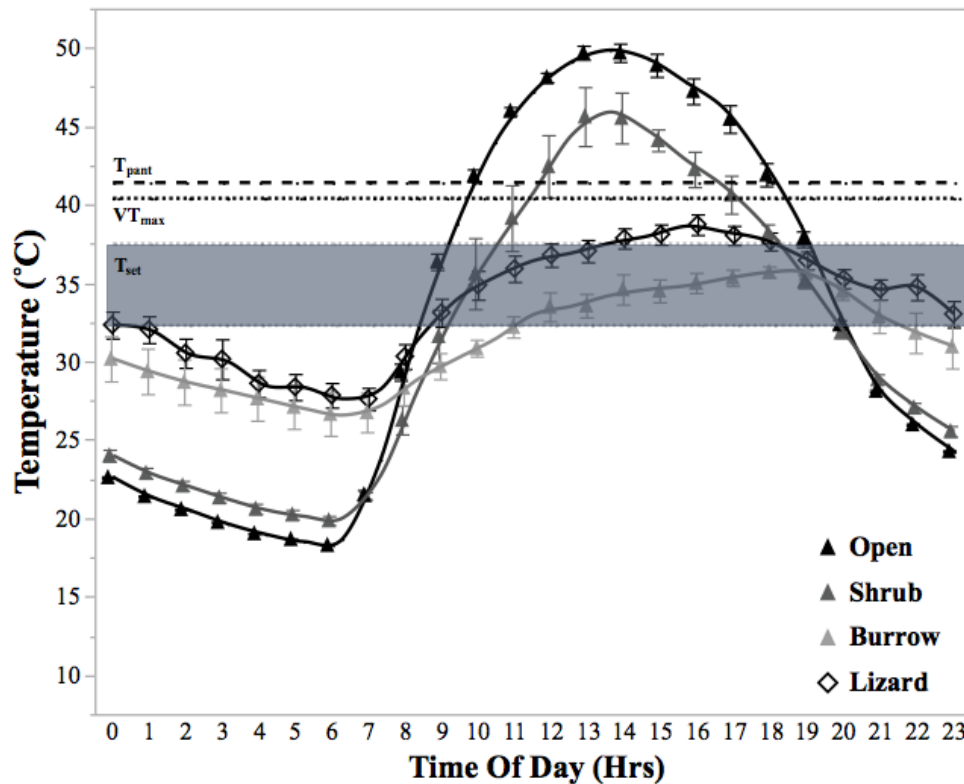


### 1.3.3 Upper Thermal Tolerance ( $T_{pant}$ )

There was no effect of sex ( $F_1 = 2.81$ ,  $p = 0.11$ ), SVL ( $F_1 = 0.01$ ,  $p = 0.92$ ), mass ( $F_1 = 2.27$ ,  $p = 0.15$ ), or capture method ( $F_1 = 1.39$ ,  $p = 0.26$ ) on  $T_{pant}$ . Mean  $T_{pant}$  was  $41.4 \pm 0.2^\circ\text{C}$  (Figure 1.3). Given that the true upper thermal threshold ( $CT_{max}$ ) is usually several degrees higher than  $T_{pant}$  (e.g., Heatwole *et al.*, 1973; Shea *et al.*, 2016), the  $CT_{max}$  of *G. sila* is probably in the mid 40 °C range.

### 1.3.4 Biophysical Models

Based on data from biophysical models, hourly daytime (0700-1900) temperature from 1-19 July 2019 varied significantly among microhabitats ( $F_{14,38} = 11.07$ ,  $p < 0.0001$ ), with temperatures in the open highest, under shrubs intermediate, and in burrows lowest (Figure 1.3).



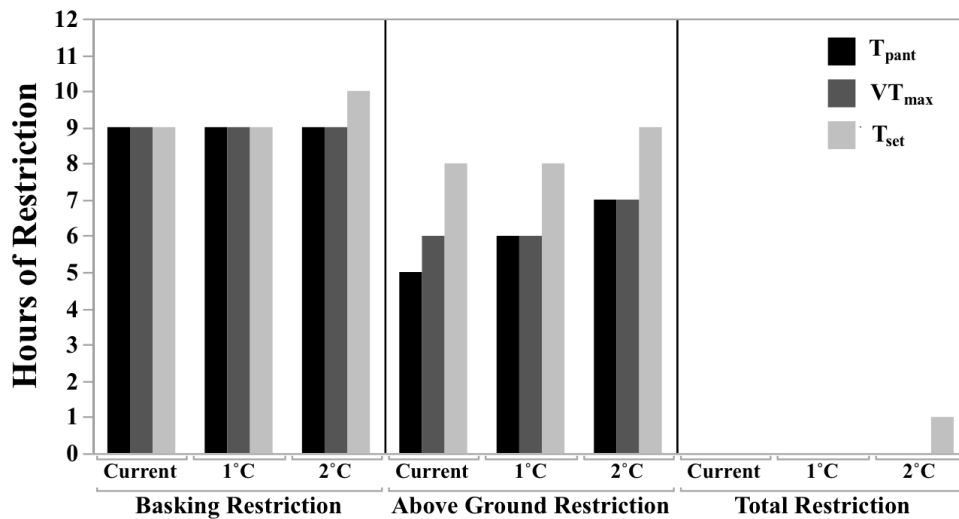
**Figure 1.3: Average daily temperatures of biophysical models in three microhabitats (N=6 each) and *Gambelia sila* body ( $T_b$ ) temperatures (N=30) during an extremely hot part of their active season (1-19 July 2019).** Temperatures above ground (open and shrub) regularly exceeded the upper thermal tolerance ( $T_{pant}$ ), whereas temperatures in burrows were most often within lizard preferred body temperature ( $T_{set}$ ) range. Average maximum voluntary body temperature ( $VT_{max}$ ) did not exceed  $T_{pant}$ . Lizards maintained  $T_b$  within  $T_{set}$  for most of the daylight hours, and lizard  $T_b$  never exceeded  $T_{pant}$ . Error bars represent  $\pm 1$  SEM.

### 1.3.5 Activity Restriction Time

Currently, during the hottest time of the active season, *G. sila* are restricted from continually basking in the sun for 8-9 hours a day (Figure 1.4), forcing them into burrows or under shrubs because temperatures of biophysical models in the direct sun exceed all three thermal variables ( $T_{pant}$ ,  $VT_{max}$ , and  $T_{set}$ ). Even the ground beneath shrubs is above  $T_{pant}$  for 5 hours a day, where lizards are restricted to using burrows only. Currently, mean burrow temperatures do not exceed lizard  $T_{set}$  even in the hottest part of the summer.

### 1.3.6 Climatic Projections

In the RCP 4.5 scenario, our field site will have increased from its 23.5 °C 2018 annual average to 24.5 °C by 2079, and to 25.5 °C at some point beyond 2099. For the RCP 8.5 scenario, our field site will have increased 1 °C to 24.5 °C by 2059 and to 25.5 °C by 2097. Assuming that equal warming occurs across all microhabitats, the hours restricted to shade or burrows will not be impacted with a 1 °C increase, but there will be an additional hour above  $T_{set}$  with a 2 °C increase (Figure 1.4). The number of hours restricted to burrows because  $T_b$  would exceed thermal variables will increase by 1-2 hours. Currently, burrow temperatures do not exceed  $T_{pant}$ ,  $VT_{max}$ , or  $T_{set}$ , and a 1 °C increase in temperatures will not change this. However, with a 2 °C increase, burrows will exceed  $T_{set}$  for 1 hour per day.



**Figure 1.4: The number of daytime hours (0700 to 1900) that *Gambelia sila* are restricted from being in the open (basking restriction), from being in the open or shade (above ground restriction), or from being inside burrows (total restriction) calculated as hours above  $T_{pant}$ ,  $VT_{max}$ , and  $T_{set}$ , at the current climate and with 1 and 2 °C increase in temperature. These data encompass a very hot portion of the active season (1-19 July), so there will be fewer restricted hours earlier in the season when daytime ambient temperatures are lower.**

## 1.4 Discussion

In this study on the thermal ecology of *G. sila*, we have shown that these lizards exist in a very hot environment by taking refuge from extreme midday heat under *Ephedra* shrubs and inside *Dipodomys* burrows. Our analysis of monthly changes in lizard  $T_b$  reveals that daytime  $T_b$  does not significantly change over the course of their active season (Figure 1.1a), indicating that despite mean monthly increases in ambient temperatures in this extremely hot environment, lizards are thermoregulating to keep their  $T_b$  consistent. This finding is consistent with other studies on diurnal lizards; for example, the skink *Tiliqua rugosa* thermoregulates at a relatively consistent 33-35 °C from spring through autumn by changing their thermoregulatory behaviors (Firth & Belan, 1998). In addition to shuttling among various microhabitats, thermoregulatory behaviors include changes in posture (Cowles & Bogert, 1944; Muth, 1977), lying flat on the ground when temperatures are low, and raising limbs and tail off the ground when temperatures are high (Losos, 1987). In *G. sila*, nighttime  $T_b$  was lower than daytime  $T_b$  and nighttime  $T_b$  increased in July, most likely because the temperatures of the burrows they inhabit at night also increased. Over the course of the active season from May to July, lizards increased burrow use and decreased time spent in the open. At the beginning of the season, milder ambient temperatures allowed the lizards to stay above ground longer and utilized the open to defend territories, forage, and mate (Buckley *et al.*, 2015; Grimm-Seyfarth *et al.*, 2017; Germano, 2019). As temperatures in each of these microhabitats increased, we observed an increased reliance on burrows and, to a lesser extent, shade plants when temperatures in the open are too high for these lizards to stay active for extended periods of time because they exceed the lizards'  $T_{set}$  and  $T_{pant}$ .

Analysis of the biophysical models we placed out in the three major microhabitats available to lizards at our field site during an extremely hot window of their active season in July revealed the following patterns relevant to lizard thermoregulatory behavior: (1) temperatures in the open are highest during midday and lowest at night, with the greatest daily fluctuation, (2) temperatures in burrows are the most stable, providing the lowest temperatures available during midday and the highest at night across all three microhabitats, and (3) temperatures under shrubs tend to be intermediate between the open and burrows, suggesting that the shade from shrubs should provide a buffer from solar radiation to lizards during midday. Lizard  $T_b$  was lowest in the early morning hours when inside burrows, and increased rapidly upon emergence from burrows in the morning. In the morning,  $T_b$  is actually slightly elevated above  $T_e$  in the sun, which may be an artifact of the faster heating rates of the externally attached radio-transmitters than the biophysical models. However, this difference between lizard  $T_b$  and sun  $T_e$  is negligible until about 0900 hours, when  $T_e$  in the sun dramatically exceeds lizard  $T_b$ . As a result of shuttling thermoregulatory behavior, lizard  $T_b$  during the hottest part of the year tends to lie between that of the biophysical models in the shade of shrubs and those in burrows. Thermoregulation allowed lizards to maintain a fairly stable  $T_b$  during midday, and to remain within their  $T_{set}$  for a small portion of the day. Lizard  $T_b$  tended to exceed  $T_{set}$  during the hours of 1400-1900, probably because lizards traded off their  $T_{set}$  with the need to be above ground to defend territory and forage (Martín & Lopez, 1999; Polo *et al.*, 2005; Medina *et al.*, 2016). It is fairly common for diurnal lizards to allow their  $T_b$  to exceed their lab-measured  $T_{set}$  (e.g., Light *et al.*, 1966), sometimes even panting in order to evaporatively cool while active in extreme heat (reviewed in Tattersall *et al.*, 2006).

During the latter portion of the day, lizard  $T_b$  started to slowly decline as they entered burrows.

Collecting data on  $T_{set}$  and field active  $T_b$  allowed us to examine the lizards' thermoregulatory accuracy ( $d_b$ ) during daylight hours, when they can actively thermoregulate. Early in the morning,  $d_b$  is low because all three microhabitats are too cold for the lizards to achieve their preferred body temperatures. Interestingly, average  $T_b$  in the early morning is actually higher than all three microhabitats (Figure 1.1), which may be result in part from solar radiation heating up the external radio-transmitter more rapidly than the biophysical models. In addition, lizards may be thermoregulating by positioning their bodies perpendicular to the sun to absorb more solar radiation (Muth, 1977; Waldschmidt, 1980), standing on all four legs to avoid conductive heat loss to the ground (Cowles & Bogert, 1944), darkening their skin via melanophore dispersion to absorb more radiation (Sherbrooke *et al.*, 1994; Sherbrooke, 1997), and other mechanisms. Thermoregulatory accuracy is best at around 9am, when lizard  $T_b$  matches their  $T_{set}$ . As the day progresses,  $d_b$  becomes worse as available temperatures are higher and therefore further from  $T_{set}$ . They shuttle between burrows (where there is good thermal quality but no opportunity to forage, defend territories, etc.) and the open desert floor (poor thermal quality but facilitates the above behaviors). During the heat of the day, lizards can either seek refuge in burrows or continue above ground activity, at least for a time, by using *Ephedra* shrubs (Westphal *et al.*, 2018). Our data support the hypothesis that shrubs are valuable and aid in the thermoregulation of *G. sila* because on a hot day, they are currently able to spend four more hours above ground than if there were no shrubs and they were forced to enter burrows to avoid exceeding  $T_{pant}$ .

Furthermore, light can penetrate shrub canopies, resulting in a mosaic of thermal and radiative properties, so the thermal microhabitat under shrubs may be even more complex and variable than our biophysical models could measure. All biophysical models under shrubs were placed on the ground; in hindsight, after we realized that lizards sometimes climbed several inches off the ground into shrubs, we realized that placing models on shrub branches would have been an informative way of analyzing microhabitat heterogeneity underneath shrubs, as lizards could thermoregulate more accurately during the heat of the day by climbing in shrubs (Germano, 2019). Shrubs may therefore provide a valuable source of thermal heterogeneity in this relatively simple environment, a pattern that has been observed in many other studies (Bauwens *et al.*, 1999; Bauwens *et al.*, 1996; Stout *et al.*, 2013; Sears *et al.*, 2016; Filazzola *et al.*, 2017). For example, Egyptian tortoises (*Testudo kleinmanni*) in the deserts of Egypt depend on large shrubs to thermoregulate and survive; if loss of vegetation occurred, the species would not persist (Attum *et al.*, 2013). In general, thermal resources like shade may be important buffers for the effects of climate change, especially for organisms inhabiting areas experiencing rapid warming (Suggitt *et al.*, 2018). Given that *G. sila* do occur in sites without shrubs (Germano & Rathbun, 2016), we recommend future studies comparing the thermoregulatory accuracy and activity patterns of *G. sila* populations with and without shrubs would be informative.

Field-active lizards thermoregulate to achieve and maintain  $T_b$  within their  $T_{set}$  range, which is optimal for peak performance (e.g., sprint speed, reproduction, or digestion, Xiang *et al.*, 1996). While lizards by definition prefer to thermoregulate within their  $T_{set}$  range, they regularly exceed  $T_{set}$  to perform essential activities like feeding and

mating (Porter *et al.*, 1973; Adolph & Porter, 1993; Bauwens *et al.*, 1996), so examining their  $VT_{max}$  in the field is ecologically relevant. Similarly, measures of upper thermal tolerance like the  $CT_{max}$  are important because lizards *cannot* exceed these temperatures because they would lose motor function and die (Cowles & Bogert, 1944). If habitats become hot enough that lizards will exceed their  $CT_{max}$  for significant portions of the day, extirpation is likely to occur because the lizards will lack sufficient activity time, as *G. sila* is already restricted from basking in the open or even being above ground at all for large portions of the day. Clearly, shuttling behavior mitigates these restrictions; lizards can still move through hot, open areas in the middle of the day as long as they consistently seek refuge under shrubs or in burrows to cool off. However, the current hours of restriction (ranging from 5-10 hours per day depending on the metric used, Figure 1.4) are extremely high (Sinervo *et al.*, 2010), suggesting that these lizards may already be dramatically restricted by high temperatures. The number of restriction hours may be slightly overestimated because we used external radio-transmitters, which may read higher  $T_b$  than the actual internal  $T_b$ , especially when they are basking in the sun. However, lizards spend a small quantity of time in the sun during this hot time of year (Figure 1.1b), so our overestimates are likely to be minor. The high number of restriction hours, along with factors like extreme aridity, might explain why *G. sila* enter aestivation and why they have such a short active season (Germano *et al.*, 1994). Clearly, *G. sila* is adapted to hot, arid environments, as evidenced by its ability to be active at high ambient temperatures (Germano, 2019), its high thermal tolerance, and its persistence in desert ecosystems. However, how long will it be before ambient temperatures become high enough that lizards cannot physiologically and behaviorally mitigate them?



Unsurprisingly, anthropogenic climate change is likely to exacerbate the already hot climate in the San Joaquin Desert and impact *G. sila*. Our models predict that as temperatures increase, *G. sila* will continue to lose hours of activity because of microhabitat temperatures surpassing  $T_{\text{pant}}$  (and theoretically also their  $CT_{\text{max}}$ ),  $VT_{\text{max}}$ , and  $T_{\text{set}}$ . The restriction hours for  $T_{\text{pant}}$  and  $VT_{\text{max}}$  were similar because the temperatures for  $T_{\text{pant}}$  and  $VT_{\text{max}}$  were similar (Figure 1.3, Camacho *et al.*, 2018). As global temperatures continue to rise, there will be a resulting shift in the distribution of local species populations and changes in timing of activity (Parmesan & Yohe, 2003; Root *et al.*, 2003; Sinervo *et al.*, 2017). However, at the rate of climate change occurring, lizards may not be capable of responding to increasing temperatures. On the one hand, having shade-providing shrubs in this heterogeneous habitat may aid in the resilience of this species to a rising climate (Germano, 2019). On the other hand, behavioral thermoregulation (for example, use of shrub shade) can actually *prevent* lizards from adapting to climate change because higher thermal tolerance is not being selected for (Huey *et al.*, 2003; Buckley *et al.*, 2015). Furthermore, the projected changes in biophysical models by 1 and 2°C ignore the spatial heterogeneity of the environment (Sears *et al.*, 2011), and actual changes could be very different because microhabitat temperatures will increase at different rates than ambient temperatures. Our data show that conditions inside burrows, which have the lowest temperatures during midday, will exceed the  $T_{\text{set}}$  of *G. sila* with a 2 °C increase in temperatures by the end of the century. Notably, this relies on temperatures of biophysical models placed 1 m into a burrow, and it is possible that lizards could move deeper into burrow systems to maintain preferred temperatures. Future studies will examine depth and complexity of kangaroo rat burrow

systems. If burrows cannot provide an adequate thermal buffer to lizards in the future, then lizards will experience an increase in energy expenditure throughout the day without the available time to forage. This additional energy expenditure in the face of climate change will exacerbate the potential for decreased energy for reproduction and growth (Sears *et al.*, 2011; Sinervo *et al.*, 2017). Clearly, evidence-based and proactive management of kangaroo rat burrows and shade-providing shrubs are essential to the persistence of *G. sila* in the Carrizo Plain in the future. If nothing is done to mitigate the effects of climate change and make important decisions about the management of this habitat, the extirpation of this population and potentially extinction of the entire species is a distinct possibility.

## **2. Using Temperature-Based Activity Estimation to Predict Surface Activity and Microhabitat Use of an Endangered Heliothermic Lizard**

### **2.1 Introduction**

Refugia constitute a major resource for terrestrial organisms because they provide protection from predators (Martín & López, 2004; Manicom *et al.*, 2008), provide escape from extreme temperatures (Schwarzkopf & Alford, 1996; Polo & López, 2005), and can buffer animals from extreme aridity and precipitation events (Bulova, 2002; Burda *et al.* 2007; Ivey *et al.*, in press). However, essential activities like mate-searching and feeding typically require surface activity in xerophilic animals (Krause *et al.*, 2000; Martín & Pilar, 1999; Amo *et al.* 2007; Munguia *et al.* 2017), and consequently these animals can experience trade-offs between refugia use and surface activity when conditions are harsh (Webb & Whiting, 2005; Davis *et al.* 2008). Animals inhabiting arid environments face risk of extinction due to the increased temperatures and longer periods without precipitation induced by climate change (Archer & Predick, 2008; Barrows, 2011) force these animals to seek refuge more frequently and potentially reduce their ability to obtain resources (Buckley *et al.*, 2015; Grimm-Seyfarth *et al.*, 2017). Heliothermic (sun-basking) lizards are a group that is particularly at-risk (Sinervo *et al.*, 2010) because they already thermoregulate at high temperatures (Cowles & Bogert, 1944; Huey, 1982) and further increases in ambient temperatures will force them into refugia. These species also have very little plasticity in thermal tolerance because they are adept at behaviorally thermoregulating by shuttling among the sun, shade, and refugia (Gunderson & Stillman, 2015) and therefore have a low potential for adapting to higher temperatures (Huey,

1982; Huey *et al.*, 2003; Angilletta, 2009; Muñoz & Losos, 2018). These species are thus excellent candidates to use in order to examine and understand how shifts in climatic events will impact organisms that rely on their thermal environment and aid us in understanding how we can use temperature to model their activity.

Studying how climate change influences vulnerable ectotherms relies on robust methods for collecting continuous data on body temperature and microhabitat use. However, small, heliothermic lizards provide a logistical challenge in terms of continuous sampling. Most studies employ the “grab and jab” technique, in which a lizard is captured, and a point sample of its body temperature is collected via a cloacal thermometer. Point-sampling of body temperature is highly biased in that it provides a small number of data points reflecting only those time periods in which animals are active and researchers can access them (Taylor *et al.*, 2004). Furthermore, tracking small individuals over time is difficult due to limitations in radio-transmitter size and battery life. Even if telemetry is possible, tracking these animals on a regular basis over time presents financial and logistical challenges. Researchers might be able to accurately predict activity and microhabitat use based on body temperature data for small, heliothermic lizards in arid, hot environments if those data were collected continuously and subjected to robust validation. This method, known as temperature-based activity estimation (TBAE), has been tested in a large-bodied lizard and snake (Davis *et al.* 2008). TBAE predicted surface activity 96% of the time in the lizard (Gila monster, *Heloderma suspectum*), which forages actively on the surface, but only 66% of the time in the snake (Western Diamond-backed Rattlesnake, *Crotalus atrox*), which tends to hide in shade and therefore thermoconforms more than the Gila monster. In this study, we

investigated whether TBAE would successfully predict not just surface activity, but also microhabitat use, in a smaller, heliothermic lizard.

We evaluated the efficacy of TBAE in estimating surface activity and microhabitat use in the blunt-nosed leopard lizard *Gambelia sila*, a federally endangered lizard found in a few isolated populations in the hot and arid San Joaquin Valley and Carrizo Plain in California, USA (IUCN 2019; Germano *et al.*, 2005; Germano & Rathbun, 2016; Stewart *et al.*, 2019). Substantial financial resources are invested annually in studying this species, in efforts to inform management plans for its protection and recovery. *Gambelia sila* may be dramatically impacted by climate change in the coming years (Ivey *et al.*, 2020), although they may be able to shift activity patterns to mitigate warming (Germano, 2019). Nevertheless, documenting its thermal ecology and activity patterns represents an essential component of its continued assessment and management strategy. Here we tested the hypothesis that TBAE can accurately predict surface activity and microhabitat use in blunt-nosed leopard lizards. In doing so, we evaluated the following three key predictions: (1) TBAE predicts whether a lizard is underground or surface active, (2) TBAE predicts microhabitat use such as sun, shade, or burrow, and (3) TBAE predicts the time of day that a lizard first emerges from its overnight refugium. This study also assesses continuous body temperature data as a less intrusive and cost-effective means to monitor sensitive species.

## **2.2 Materials and Methods**

### *2.2.1 Study Species and Sites*

The study took place in the Elkhorn Plain in the Carrizo Plain National Monument, California, USA at two different sites. The first site (a “shrubbed” site) has

sparsely distributed *Ephedra* shrubs throughout the terrain (35.117998° -119.629063°). The second site (a “shrubless” site) lacks *Ephedra* shrubs or any other ground cover and is located 6.1 km SW of the shrubbed site (35.117998° -119.629063°). The Elkhorn Plain experiences arid summers (average high 30 - 40 °C) and cool winters (average low 5 - 9 °C, Germano *et al.*, 2005, Raws; USA Climate Archive, 2019). Both sites are dominated by Giant Kangaroo Rat (*Dipodomys ingens*) precincts with extensive burrow networks that provide important refugia for *Gambelia sila* (Ivey *et al.*, 2020). TBAE analyses of surface activity and microhabitat use were performed using data from the shrubbed site in 2018. Analyses of the timing of morning emergence were performed using data from both sites in 2019 (see *Predicting Emergence Time* below).

Adult blunt-nosed leopard lizards were captured by hand-held lasso in early May 2018 at the shrubbed site (N = 30), and in late April/early May 2019 at the shrubbed (N = 20) and shrubless (N = 20) sites. Lizards were fitted with VHF temperature-sensitive radio-transmitter collars (Holohil model BD-2T, Holohil Systems Ltd., Carp, ON, Canada) following the methods of Germano and Rathbun (2016). We recorded standard morphometrics (mass, SVL, sex, gravidity, tail length, and tail status), released lizards at their sites of capture, and subsequently tracked lizards 1-3 times per day using a VHF receiver and Yagi antenna (R-1000 Telemetry Receiver, Communications Specialists, Inc., Orange, CA, USA). During each tracking event, behavioral observations, microhabitat description, GPS coordinates, and a timestamp were recorded. In July (the end of their active period), lizards were recaptured, radio-transmitters were removed, and lizards were released at their location of capture to estivate for the remainder of the summer.

### 2.2.2 Body Temperature ( $T_b$ )

Body temperatures ( $T_b$ ) of lizards were continually recorded (~ every 5-10 min) as the temperature of the radio-collar via relay to a Telonics TR-5 receiver with data acquisition system (Telonics Option 320) and 10ft tall omni antenna (Telonics model RA-6B). Since transmitters record surface temperature and not core  $T_b$ , it is important to acknowledge that our  $T_b$  measurements are estimates of actual  $T_b$ . We programmed the system to log the interpulse intervals for each radio-transmitter about every ten minutes, and used manufacturer-provided calibration equations to convert interpulse intervals to  $T_b$ . Data were checked by visual inspection for any clearly skewed temperatures, which were consequently removed from the set.

### 2.2.3 Characterizing the Thermal Habitat: Air Temperature and Biophysical Models

We characterized the thermal habitat at the shrubbed site in 2018 using two methods: air temperatures and temperatures of biophysical models. First, we downloaded data from the RAWS weather station at Cochora Ranch (station ID: CXXC1), 3.7 km due east of the shrubbed site, to use as a proxy for air temperature ( $T_{air}$ ). Second, we deployed biophysical models from July 1-19 (N = 6 in the sun, N = 6 in the shade of *Ephedra* shrubs, N = 6 in burrows) following the methods of Ivey *et al.* (2020). Briefly, the models consisted of 1" (2.5cm) diameter copper pipes fitted with a Thermochron iButton (DS1921G-F5), filled with water, and secured with PVC caps screwed onto the male copper ends. Total length of the models was 15.3 cm. Models placed above ground were fitted with "legs" made from copper wiring to prop models on one end, mimicking a lizard basking. Models placed in burrows did not have legs.

#### 2.2.4 Temperature-Based Activity Estimation (TBAE)

First, we used the difference between  $T_{\text{air}}$  and  $T_{\text{b}}$  to predict when a lizard was surface active or below ground. When lizards are above ground, their  $T_{\text{b}}$  often exceeds  $T_{\text{air}}$  as they bask in the sun (= positive temperature differential). We tested positive temperature differentials of 2, 4, 6, 8, 10, 12, and 14 °C to determine which differential best predicted when lizards were surface active. One researcher created a spreadsheet with the  $T_{\text{b}}$  of each lizard at each of its radio-telemetry fixes, plus data on its activity (above or below ground). We used the “IF THEN” function in Microsoft Excel to predict whether the animal was above or below ground based on the positive temperature differential. For example, if  $T_{\text{b}}$  was above  $T_{\text{air}}$  by 2 °C, then the lizard was predicted to be above ground; if not, it was predicted to be below ground. After making the predictions, we merged predicted and actual data to examine how the various positive temperature differentials impacted accuracy of our predictions.

Next, we used the temperatures of biophysical models to estimate microhabitat use and surface activity. The average hourly temperatures of each biophysical model (sun, shade, burrow) during blunt-nosed leopard lizard’s active hours (700 - 1900) were plotted against each lizard’s  $T_{\text{b}}$  the same day, and a researcher blind to the lizard lizard’s actual microhabitat predicted its microhabitat based on the following criteria (modified from Davis *et al.*, 2008): (1) Lizards were predicted to be in the open if their  $T_{\text{b}}$  was equal to or higher than the temperature of the models in the open; (2) lizards were predicted to be under shrubs if their  $T_{\text{b}}$  was equal to or higher than the temperature halfway between those of the models in burrows and under shrubs, but lower than models in the open; (3) lizards were predicted to be in burrows if their  $T_{\text{b}}$  was lower than the temperature



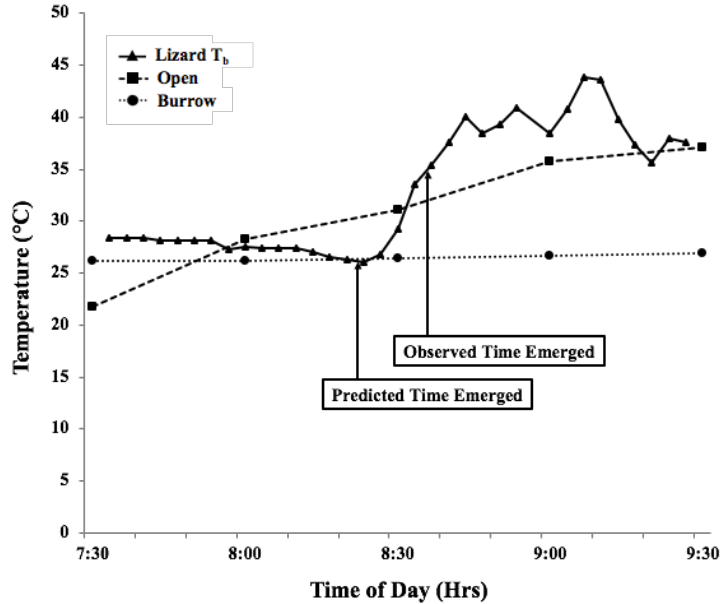
halfway between the models in burrows and under shrubs. Predictions of lizards in the open and under shrubs were combined to constitute above-ground predicted activity, and predictions of lizards in burrows constituted below-ground predicted activity. Next, the blind predictions were compared to actual observations, and the proportions correctly predicted were calculated. We used a two-proportion Z-test in JMP® (v. Pro 14) to compare the efficacy of the two methods of TBAE ( $T_{\text{air}}$  versus biophysical models) for predicting above and below ground activity.

### *2.2.5 Predicting Emergence Time*

In 2019, a new set of biophysical models were deployed ( $N = 4$  in the sun,  $N = 4$  in burrows) at both the shrubbed and shrubless sites. We used blunt-nosed leopard lizard  $T_b$  and biophysical model temperatures to estimate morning emergence time of lizards at each site. Each day from June 23 to July 14, 2019, we haphazardly selected two lizards as focal animals. Before dawn, two researchers each radio-tracked one focal animal and waited at least 4 meters away from the lizard's burrow with binoculars posed on the burrow entrance. We recorded the emergence time in two ways: (1) the time of day when the lizard's head was first visible emerging from the burrow, and (2) the time of day when the lizard's entire body and tail had emerged from the burrow. June and July conditions are extremely hot and arid, and sometimes lizards do not emerge from burrows at all. If lizards did not emerge by the time  $T_{\text{air}}$  reached 29.5 °C, the observation was abandoned and that lizard was not included as a data point. Observations took place at both the shrubbed ( $N = 10$  lizards) and shrubless ( $N = 10$  lizards) sites. Two lizards observed at the shrubbed site were too far from the receiver for associated  $T_b$  data to be

collected, so the final sample for TBAE was 18 individual lizards (no repeat observations).

To predict emergence time using TBAE, a researcher blind to a lizard's actual emergence time plotted the lizard's  $T_b$  data and the biophysical model temperatures from that site for the duration of an emergence observation and predicted the lizard's emergence time as the time point immediately preceding a distinct increase in the slope of  $T_b$  (Figure 2.1). Predicted emergence times were then compared with observed emergence times, and the absolute value of the difference in predicted and observed emergence times (for both emergence criteria: head and entire body) was calculated; this value (in minutes) represents how close our predicted emergence time was to the actual emergence time. We compared observed emergence times (minutes after sunrise) of all lizards observed ( $N = 20$ , head only and full body) between the shrubbed and shrubless sites using Student's t-tests; all data were normally distributed and had homogenous variances. The sample size for head emergence was 20 and for full emergence was 18 (two lizards failed to fully emerge from their burrows after one hour).

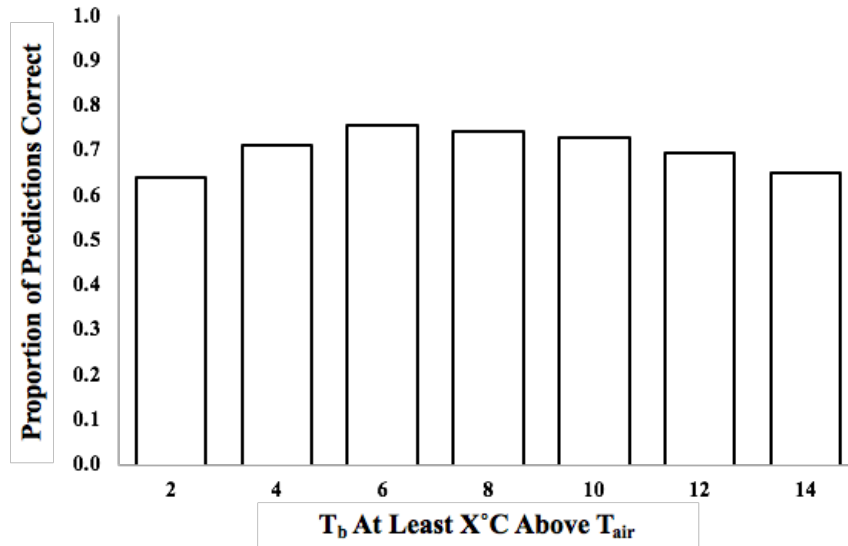


**Figure 2.1: Methodology used to predict morning emergence time of *Gambelia sila*.** Emergence was predicted as the time of day immediately preceding a distinct upward slope in the lizard's  $T_b$  (triangles and solid line) based on the reasoning that it would take several minutes for the radio-transmitter to heat in the sun. The rising  $T_b$  was also typically associated with departure from burrow biophysical model temperatures (circles and dotted line) and approach of open (sun) biophysical model temperatures (squares and dashed line). This was then compared to the observed emergence time, where the lizard's head first appeared outside its burrow. The average difference between observed and predicted emergence time was 11 minutes and 37 seconds.

## 2.3 Results

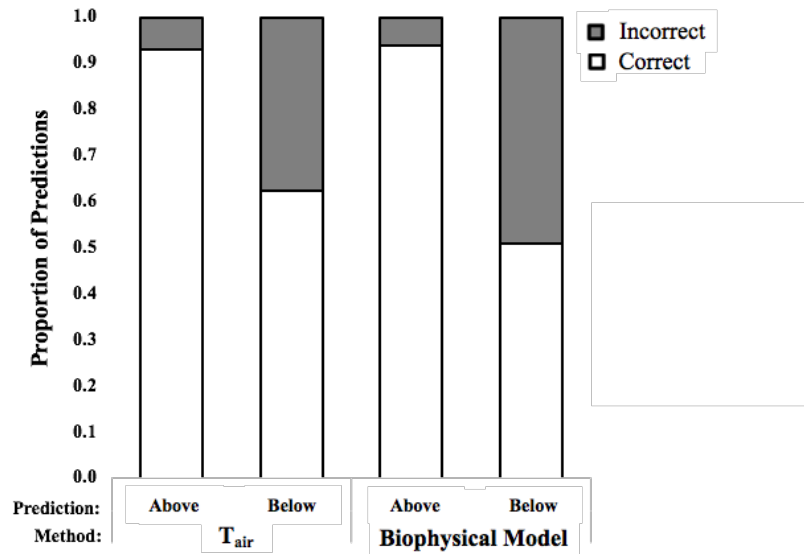
### 2.3.1 Temperature Based Activity Estimation (TBAE)

The proportion of observations of blunt-nosed leopard lizards correctly predicted to be above ground based on the criteria that  $T_b$  is at least  $X$  °C (where  $X = 2, 4, 6, 8, 10, 12, \text{ or } 14$  °C) above  $T_{\text{air}}$  ranged from 0.64 (2 °C) to 0.76 (6 °C). Thus, we correctly predicted surface versus below ground activity 76% of the time when using the criterion that they are above ground if  $T_b$  exceeds  $T_{\text{air}}$  by at least 6 °C (Figure 2.2).



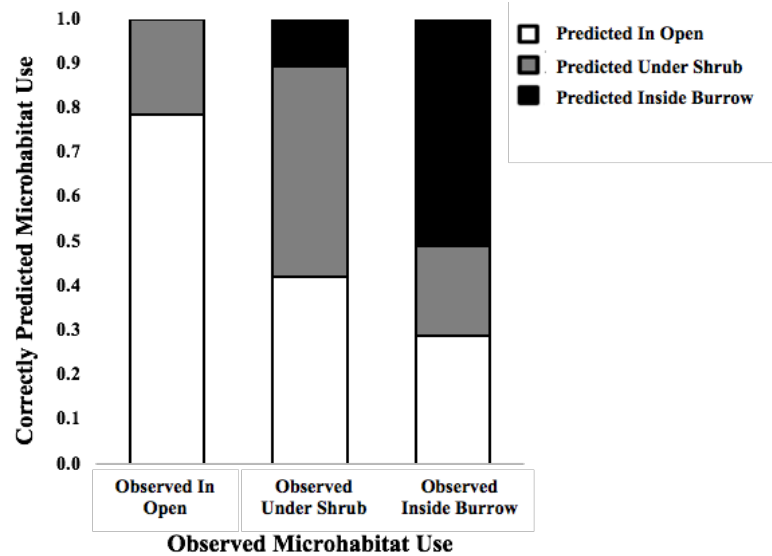
**Figure 2.2: Using air temperature to predict surface activity versus below ground refuge use of *Gambelia sila* resulted in accurate predictions 64-76% of the time overall.** Predictions were maximized (76% correct) using the criterion that lizards are above ground when their body temperatures ( $T_b$ ) are at least 6 °C above the air temperature ( $T_{\text{air}}$ ).

Using TBAE to predict surface activity versus burrow occupancy, we found that calculation using  $T_{\text{air}}$  (75.7% correct overall) was superior to calculation using biophysical models (60.5% correct overall,  $Z = 3.43$ ,  $p = 0.0003$ ; Figure 2.3). We did not find a significant difference in accuracy of above-ground predictions using the two methods; with both methods, observations predicted to be above ground were correct about 93% of the time ( $Z < 0.001$ ,  $p = 1.00$ ). We found a significant difference in proportion of successful predictions for below-ground observations, with  $T_{\text{air}}$  (62% correct) significantly outperforming biophysical models (51% correct,  $Z = 1.78$ ,  $p = 0.037$ ). Predicting activity using biophysical models overestimated the time above ground specifically by misidentifying many lizards as being in the open when they were actually in burrows.



**Figure 2.3: Temperature-based activity estimation resulted in accurate prediction of above-ground activity by *Gambelia sila* more often than accurate prediction of below ground (burrow) occupation.** Using air temperature ( $T_{air}$ ) to predict below-ground occupation was superior to using biophysical model temperatures when predicting below ground occupation. For both methods, ~93% of observations predicted to be above ground were correct, whereas 62% (using  $T_{air}$ ) and 51% (using biophysical models) were correct for below-ground predictions.

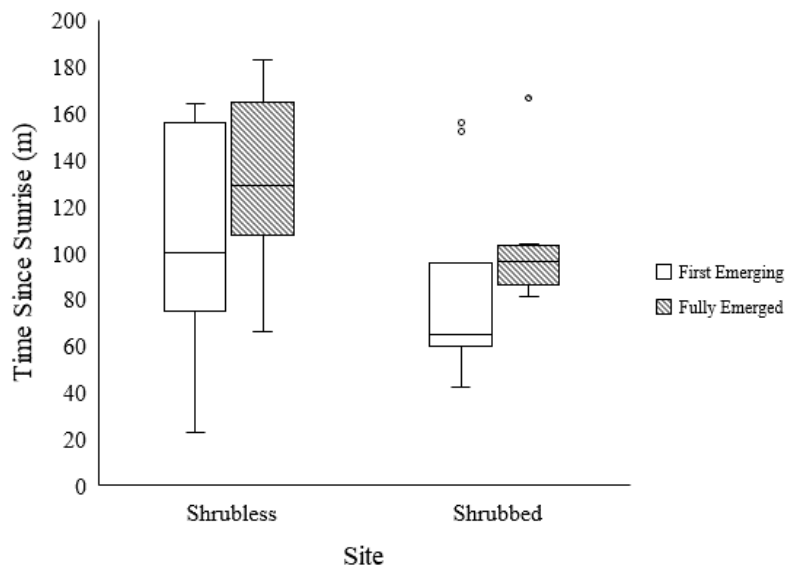
Of the 147 radio-telemetry fixes in 2018, 114 (77.6%) were in burrows, 19 (12.9%) were under shrubs, and 14 (9.5%) were in the open in the sun. Figure 4 shows the relative success predicted based on biophysical model data. When lizards were found in a given microhabitat, TBAE correctly predicted they were in that habitat with varying accuracy (79% correct when in the open, 47% when under shrubs, and 51% when inside burrows).



**Figure 2.4: Proportion of correctly predicted observations of microhabitat use of *Gambelia sila* using temperature-based activity estimation based on biophysical model temperatures.** Lizard microhabitat use was predicted correctly most often when they were in the open.

### 2.3.2 Predicting Emergence Time

In summer 2019, lizards began emerging (head out of burrow) at about 0745 (no difference between shrubbed and shrubless sites in emergence time as minutes after sunrise:  $t_{18} = 1.28$ ,  $p = 0.22$ ), and were fully emerged (body and tail out of burrow) by about 0813 (lizards at the shrubless site tended to emerge later than lizards at the shrub site:  $t_{16} = 2.11$ ,  $p = 0.051$ , Figure 5). The difference between the predicted emergence and observed emergence (head out of burrow) was  $11:37 \pm 01:57$  (mm:ss). Of the 18 observations, 8 were an underestimation of predicted emergence and 10 were an overestimation. The difference between the predicted emergence and observed full emergence was  $27:00 \pm 02:31$  (mm:ss). Of the 18 observations, all predictions underestimated the time of lizards full emergence.



**Figure 2.5: *Gambelia sila* emergence times (minutes after sunrise) at two sites (shrubless and shrubbed).** Initial emergence (head out of burrow) time did not differ between shrubbed and shrubless sites. Lizards at the shrubless site tended to fully emerge (whole body and tail) earlier than lizards at the shrubbed site.

## 2.4 Discussion

In this study, we found partial support for the hypothesis that TBAE can be used to describe and model activity for a small, heliothermic, endangered lizard. First, TBAE accurately predicted surface activity in blunt-nosed leopard lizards about 93% of the time when either  $T_{\text{air}}$  from a nearby weather station or biophysical model temperatures are used. However, accuracy in predicting below ground (burrow) occupancy is much lower (51-62% using biophysical models and  $T_{\text{air}}$ , respectively). Second, using biophysical model data, TBAE accurately predicts microhabitat use in 79% of observations in which lizards are in the sun, 47% of those in the shade, and 51% of those in burrows. Finally, the temperature-based prediction of the time of day that a lizard first emerges from its overnight refugium was only about 11 minutes away from the actual time it began

emerging, and about 30 minutes from when it fully emerged. The heliothermic nature of these lizards combined with the use of external, temperature-sensitive radio-transmitters that rapidly heat when exposed to solar radiation is thus a viable tool to remotely monitor lizard activity with far less investment in labor force than is typically used.

A central goal of radio-telemetry monitoring studies is to quantify surface activity and microhabitat use in sensitive species like blunt-nosed leopard lizards (i.e., Westphal *et al.*, 2018), and how they may be impacted by abiotic conditions such as weather and biotic variables such as prey abundance, predator behavior, and others. We have shown that TBAE correctly estimates surface activity 93% of the time for blunt-nosed leopard lizards, a value very similar to the 96% accuracy rate obtained for TBAE of gila monsters by Davis *et al.* (2008). Both blunt-nosed leopard lizards and gila monsters are active foragers and are therefore likely to be exposed to a range of environmental temperatures as they forage, which can alter their  $T_b$  enough in comparison to their underground refugia to facilitate TBAE. Furthermore, since blunt-nosed leopard lizards are heliothermic lizards, their exposure to solar radiation should further help distinguish their surface-active  $T_b$  from their  $T_b$  when inside burrows (Stevenson, 1985; Xiang *et al.*, 1996). In contrast, TBAE failed to predict surface activity as accurately in an ambush-foraging rattlesnake (66% accuracy, Davis *et al.*, 2008) because its body temperature in the shade of its ambush site was not sufficiently distinguishable from its body temperature inside a refugium. TBAE is therefore a potentially valuable method for researchers interested in estimating activity of actively foraging species that are expected to be exposed to relatively high temperature variation in their environment.



The value of TBAE lies in its use of  $T_b$  data that are collected by an automated system and therefore do not require direct researcher sampling. In other words, researchers could deploy radio-transmitters on lizards, radio-track them as needed for the goals of their particular study, but allow TBAE to collect the data necessary for estimating surface activity. This could significantly save on time and resources by reducing personnel investment in radio-telemetry. An alternate method for collecting data on animal surface activity uses light level geolocators, which record the intensity of blue light (Wilmers *et al.*, 2015) primarily as a means of tracking migration in birds (Lisovski *et al.*, 2019); but can also be externally attached to lizards or other terrestrial wildlife to log diel exposure to light. Choosing TBAE versus light level geolocators will depend on the goals and budget of the study, the secrecy and recapture rate of individuals of the species, and other factors. One advantage of TBAE over light level geolocators is that  $T_b$  data are collected continually by an automated receiver, whereas geocator tags must be retrieved from animals to be downloaded (Lisovski *et al.*, 2019). Any animals lost (e.g., to predation) represent lost data. Furthermore, in most studies on rare species like blunt-nosed leopard lizards, we would expect that researchers would already be using radio-telemetry to facilitate repeated observations of known individuals, so it is typically simpler and far less expensive to choose temperature-sensitive radio-telemetry over light level geolocators. On the other hand, light level geolocators would work very well in recording surface activity in systems where it is feasible to attach them to a large sample of animals with a high recapture rate.

TBAE was not as accurate when predicting below-ground activity. This limitation was primarily because TBAE misidentified certain observations as being in the open

when they were actually in burrows. Heliothermic lizards like blunt-nosed leopard lizards maintain their  $T_b$  within a narrow range, typically within or near their laboratory-measured preferred  $T_b$  range, by shuttling between sun and shade (Lortie *et al.*, 2015; Westphal *et al.*, 2018; Germano, 2019; Ivey *et al.*, 2020). When a lizard moves from the sun into a burrow, its measured  $T_b$  could remain more than 6 °C above  $T_{air}$  or biophysical model temperature for a short period of time; if lizards are tracked within that period of time, then TBAE would incorrectly assign them as being above ground. TBAE correctly predicted below-ground activity 62% of the time when using  $T_{air}$  and 51% of the time when using biophysical models. We expected that biophysical models would be more accurate than  $T_{air}$  because models are in the exact same field sites and mimic the size and shape of lizards to facilitate realistic heat exchange with the environment, whereas  $T_{air}$  data merely represent air temperatures from a nearby weather station. The fact that  $T_{air}$  was a better estimate could be the result of several factors. First, when making predictions using  $T_{air}$ , we had two categories to choose from: above or below ground. In contrast, when making predictions using biophysical model temperatures, we had three categories (open, shrub, and burrow, with open and shrub predictions then combined into above-ground predictions). In the latter case, predicting “shrub” use for a lizard that was actually underground because its temperature was intermediate between the two could result in overprediction of above-ground activity; if we only had the options of assigning it to above or below ground, we may have accurately assigned it as below ground. In other words, if we had assigned only above or below ground categories using biophysical models like we did for  $T_{air}$ , then the two methods may have provided more comparable predictions. Alternatively, the lower accuracy of biophysical models may reflect model

design and radio-transmitter construction. Our radio-transmitters are on collars and therefore measure external temperature, not deep  $T_b$  of lizards, and the temperatures should change rapidly when exposed to sun. In contrast, our biophysical models are constructed with internal data loggers immersed in water, which may introduce lag time for temperature changes due to high thermal inertia. Additionally, Giant Kangaroo Rat burrows are complicated in terms of depth, chamber size, and soil type, likely creating a labyrinth of thermal heterogeneity underground (Kay & Whitford, 1978) that is not captured by our biophysical models placed one meter inside burrows. The superior performance of  $T_{air}$  is good news because it means that researchers could simply download data from a nearby weather station rather than constructing biophysical models.  $T_{air}$  data collected from a mini weather station deployed at the actual field site could provide even more accurate data. In summary, TBAE using  $T_{air}$  as a reference is a highly accurate means of estimating surface activity, but its ability to predict when lizards are underground during daytime hours is more limited.

To predict microhabitat (burrow, shade, or open) use, TBAE using biophysical models accurately predicted microhabitat use for 79% of the observations when the lizard was in the open (sun), 47% of the observations in the shade, and 51% of the observations inside the burrows. Of the observations for blunt-nosed leopard lizards in the open, 100% of all predictions were above ground (79% correctly predicted in the sun and 21% wrongly predicted to be under the shade of a shrub) and in no cases were lizards predicted to be underground. Accuracy of predictions for shade and burrows were lower, probably for several reasons. First, as described above, blunt-nosed leopard lizards shuttle among these three microhabitats regularly (Ivey *et al.*, 2020), and an animal's

temperature at a given radio-telemetry fix could be impacted by the microhabitat it occupied shortly before being observed. Second, the temperatures of biophysical models in the shade and in burrows are necessarily more similar to one another than either is to the temperature of models in the open that are exposed to solar radiation, so error in assigning shade or burrow microhabitat in TBAE (Figure 2.4) is expected.

The beginning of lizard emergence in the morning was predictable to within roughly 11 minutes, which supports the utility of TBAE as a means of remotely collecting data on morning emergence. Lizards at the shrubbed and shrubless sites began to emerge at approximately the same time, and lizards at the shrubbed site fully emerged slightly earlier in the day than lizards at the shrubless site. In the absence of shade-providing plants, lizards at the shrubless site may be more reliant on the protection offered by their overnight burrows than lizards at the shrubbed site, which can take advantage of shrubs for thermoregulation and protection from avian predators. Lizards began emerging from burrows at about 0745 hours and were fully emerged by 0830. These times agree with those reported by Germano (2019), who compiled times at which lizards are active throughout the active season. These data are informative for practical use by managers; for example, California Department of Fish and Wildlife recently revised its guidelines for blunt-nosed leopard lizard survey protocols based on these emergence times (CDFW, 2019). As midday temperatures increase due to climate change, we may see lizards begin to emerge earlier in the morning, retreat to burrows earlier in the afternoon, and rely more heavily on plants for shade (Germano, 2019), which could potentially buffer blunt-nosed leopard lizards from experiencing the rising

temperatures. TBAE conducted annually would allow this prediction to be tested with reliability and with less effort than that required to radio-track lizards at dawn each day.

While animals must and should still be radio-tracked to obtain data relevant to the particular question being asked and to validate TBAE and delineate its limitations (as we have done here), adding TBAE to a radio-telemetry project could substantially improve inference about animal activity patterns and microhabitat use while minimizing researcher effort and expense. For examples, researchers could radio-track every other day or every third day rather than 2-3 times per day as is typical in studies of blunt-nosed leopard lizards. We urge researchers to consider how adopting TBAE might augment their studies. TBAE has been used for a variety of applications ranging from studying maternal thermoregulation (Stahlschmidt *et al.*, 2012) to examining usage of artificial refugia versus natural refugia in sympatric species (Lelièvre *et al.*, 2010). TBAE can reduce the stress that endangered species experience by limiting interactions with researchers in the field. Harnessing the power of temperature to predict animal activity proves to be a useful resource to augment surveys and radio-telemetry studies, and it will aid managers and researchers in determining how to improve protocols for surveying and studying these species in the future while minimizing the stress placed on these sensitive species.

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## Appendices

### A. Supplementary Figures and Tables

**Table S1. Morphometric data of the *G.sila* individuals in this study, during initial capture, and where applicable, upon final capture.**

<i>Lizard ID</i>	<i>SVL (cm)</i>	<i>Sex</i>	<i>Initial Gravidity</i>	<i>Final Gravidity</i>	<i>Initial Mass (g)</i>	<i>Final Mass (g)</i>	<i>Mode of Final Capture</i>
4	10.5	f	yes	no	34	30.43	excavated
9	9.4	f	yes	no	35.5	26.85	lasso
12	9.3	m	N/A	N/A	24.5	22.65	excavated
12B*	9.4	f	yes	N/A	37	28.76	excavated
13†	11.3	m	N/A	N/A	44.5	36.69	excavated
16	10.3	f	no	no	33	27.52	excavated
19†	10.5	m	N/A	N/A	46.5	36.65	excavated
20†	10.8	f	yes	no	41.5	33.69	excavated
22	9.7	f	yes	no	40	28.94	excavated
23*†	10.8	f	yes	no	32.5	14.20	lasso
25	10.5	m	N/A	N/A	46.5	35.50	excavated
26†	10.5	m	N/A	N/A	45.5	37.34	excavated
30	10.3	m	N/A	N/A	46	33.79	lasso
31	10.1	m	N/A	N/A	43.5	32.73	excavated
39	10.2	f	yes	no	40.5	32.24	lasso
40	9.5	f	no	no	35.5	27.54	excavated
44*	9.8	m	N/A	N/A	36	32.70	excavated
56	10	m	N/A	N/A	41.5	33.73	excavated
64*	10.4	m	N/A	N/A	40.5	32.48	excavated
73	10.2	m	N/A	N/A	44	32.90	excavated

<b>80</b>	10.6	f	no	no	35	31.75	excavated
<b>93</b>	11.2	m	N/A	N/A	48	38.95	excavated
<b>99</b>	9.2	f	no	no	25	22.79	excavated
<b>100*</b>	9.8	f	yes	no	34	29.00	excavated
<b>130</b>	10.1	m	N/A	N/A	38.5	24.83	lasso
<b>200†</b>	10.3	m	N/A	N/A	41	32.86	lasso
<b>1337*</b>	10.7	m	N/A	N/A	50	41.79	excavated

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Note: Asterisks (\*) indicate lizards that were not used in  $T_{\text{set}}$  trials. Daggers (†) indicate lizards not used in  $T_{\text{pant}}$  trials.