

THE THERMAL ECOLOGY OF *SCELOPORUS OCCIDENTALIS*

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By

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## ABSTRACT

### The Thermal Ecology of *Sceloporus occidentalis* Luis Patricio Burgos

With temperatures rising globally, assessing the possible impacts of the changing climate becomes more and more urgent. Ectotherms are excellent indicators of potential climatic ramifications on biodiversity because of their heavy reliance on the environment for their thermoregulation. Studies have historically looked at thermal tolerance values to establish predictive models for population and species extinctions.

In part 1, we looked at recent studies that suggest that thermal tolerance may be a plastic trait and test the effects empirically. Most studies are based on captive lizards acclimated to laboratory conditions that do not necessarily reflect natural environments, and if thermal tolerance is plastic and affected by the recent thermal history of the animal, then the data may not be accurate. We tested the critical thermal maximum of the western fence lizard, *Sceloporus occidentalis*, in all four seasons, both under field (same-day) and short (two-day) lab-acclimated conditions. We found that thermal tolerance varied seasonally, with the lowest values in the summer in both same-day and two-day acclimated lizards. Additionally, we found that the thermal tolerance of lizards tested on the same day was higher in spring than in fall, but two days of acclimation to lab conditions eliminated this difference. We also tested the thermal tolerance of lizards housed at several constant acclimation temperatures for one or three weeks and compared these values to those of lizards housed in a terrarium allowing thermoregulation, and to same-day lizards. While the thermal tolerance of all lab-acclimated lizards was higher than that of same-day lizards, there was no significant difference in thermal tolerance among any of the acclimation treatments. Overall, our results show that thermal tolerance may be plastic in some situations in *S. occidentalis*, but that this species overall shows little plasticity in response to acclimation.

In part 2, we evaluated the thermal environment of *S. occidentalis* using operative temperature models. Using operative temperature models combined with field lizard body temperatures and a lab-determined selected body temperature range, we evaluated the thermal environment of *Sceloporus occidentalis* to identify habitat quality, thermoregulatory effectiveness, and thermal exploitation index. Additionally, we used two predictive climate change models at a 1°C and 2°C increase to project the potential changes in habitat quality in the future. The thermal quality was highest for shady microhabitats, lowest for sunny microhabitats, and intermediate for mixed sunny/shady microhabitats. *S. occidentalis* were able to maintain their body temperatures in their  $T_{set}$  range for 6 hours, indicating the ability to exploit multiple microhabitats. Neither climate change scenario (1°C or 2°C increase) placed *S. occidentalis* at risk of extinction, likely because the coastal field site has a relatively mild climate. However, both scenarios greatly decreased the thermal quality of the environment, causing *S. occidentalis* to lose up to 2.5 hours of activity time per day. This highlights that even animals that inhabit mild climates are likely to experience sub-lethal effects of climate change.

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# **1. How does lab acclimation affect the thermal tolerance of a common heliothermic lizard, *Sceloporus occidentalis*?**

## **1.1 Introduction:**

Models predicting species longevity suggest rising temperatures will cause a loss of biodiversity across taxa based on changes in their daily activity time, growth rates, and adaptive potential. Increased exposure to temperatures approaching the thermal tolerance limits negatively impacts all three of these factors (Deutsch et al. 2008; Huey et al. 2012; Sinervo et al. 2010). As a result, predictive models have relied on thermal tolerance limits as variables that can best be used to evaluate extinction risk (Brusch et al. 2016; Buckley et al. 2015; Pontes-da-Silva 2018; Sinervo et al. 2010).

The lower thermal tolerance limit (Critical Thermal Minimum; CT<sub>min</sub>) and upper thermal tolerance limit (Critical Thermal Maximum; CT<sub>max</sub>) are the upper and lower bounds to the locomotor capacity of ectothermic organisms. Once the animal reaches or surpasses these limits, it is unable to escape lethal conditions; in the wild, reaching these limits would likely lead to death (Cowles & Bogert 1944). These limits have been assessed in a variety of species and taxa; the researchers either cool or heat the organism until it loses locomotor function. In reptiles, this physiological response has historically been characterized by the onset of spasms (OS) or the loss of righting response (LRR); the latter is tested by flipping the animal on its back until it can no longer right itself (Lutterschmidt & Hutchison 1997).

However, there are potential problems with the accuracy of the measurements used to assess CTmax in many studies. These include (1) taking measurements of the body temperature *after* rather than at the time of the OS or LRR (Ballinger & Schrank 1970, Bennett & John-alder 1986, Gvozdik & Castilla 2001), (2) handling the animals during the trial (Huang & Tu 2008), (3) use of inaccurate thermometers and other equipment, and (4) possible plasticity in thermal tolerance. Numerous studies have demonstrated varying degrees of plasticity in CTmax among ectothermic taxa (reviewed in Gunderson & Stillman 2015) and have suggested that an animal's recent thermal history can influence its CTmax (Cuculescu et al. 1998; Das et al. 2004; Manush et al. 2004; Stillman 2003). For example, in two populations of the northern grass lizard, *Takydromus septentrionalis*, which usually experience different thermal environments and concomitantly have different CTmax, acclimation to identical laboratory conditions caused them to converge onto the same CTmax (Yang et al. 2008). This is problematic because most thermal tolerance studies involve housing the organisms in a laboratory setting for at least 24 hours before testing the CTmax (Table S2; Hertz & Nevo 1981; Wilson & Echternacht 1990; Van Damme et al. 1991; Du et al. 2000; Du 2006; Phillips et al. 2015), which has been shown to be enough time for some organisms to acclimate to the laboratory (Ballinger & Schrank 1970; Brattstrom & Lawrence 1962). Laboratory conditions vary from study to study, but lizards are typically housed in small terraria that have a bulb on one end and a hide box on the other to allow the lizards to thermoregulate. If acclimation to laboratory conditions affects the thermal tolerance data obtained in many studies, then the data may not be ecologically relevant. This could impact the accuracy of the predictive extinction models that use these data, as they use thermal

tolerance as a fixed threshold that, once exceeded for a certain period of time, increases the predicted extinction risk of a population or species (Sinervo 2010).

On one hand, standardization by acclimating various populations to lab conditions is useful in uncovering a species-wide CT<sub>max</sub>. However, if acclimation dramatically impacts thermal tolerance, then the natural conditions experienced by the animals will not be reflected in laboratory measurements. Ultimately, free-ranging populations—not laboratory populations—are the organisms that will be impacted by climate change. Until recently, it has proven logistically challenging to collect CT<sub>max</sub> data accurately immediately for wild caught individuals (i.e., in the field) because of constraints involved with collecting data in often remote field sites (e.g., maintaining controlled conditions). Because of the inherent delay between capture and testing, organisms acclimate to laboratory conditions before measurements can be taken. However, a recently developed, field-portable device (GATORS: Gas Analysis, Temperature, and Oxygen Regulation System, see Methods 2.4 and Figure 1.1 below) allows for immediate and highly accurate testing of the CT<sub>max</sub> following capture in the field (see DuBois et al. 2017 and Shea et al. 2016 for description of the previous generation of this device).

Here, we used GATORS to test the hypothesis that thermal tolerance is a plastic trait that is affected by an organism's recent thermal history, and therefore impacted by acclimation to the laboratory. We chose the locally abundant, heliothermic western fence lizard (*Sceloporus occidentalis*) to test this hypothesis. In the first experiment, we collected data on the CT<sub>max</sub> of recently captured lizards and of lizards acclimated to the

laboratory for two days to examine how a two-day acclimation affects thermal tolerance; in addition, we repeated the experiment across all four seasons to examine whether recent thermal history in the field impacts thermal tolerance. In another experiment, we acclimated lizards to different temperatures, over one week and three weeks, to determine whether acclimation time influenced CT<sub>max</sub>; we also compared these to lizards allowed to thermoregulate in a terrarium, where they had access to a gradient of temperatures, which is how lizards are most often housed from capture to testing day in thermal tolerance studies. If the CT<sub>max</sub> is highly plastic and impacted by acclimation, then the CT<sub>max</sub> of freshly captured lizards should differ from that of laboratory acclimated lizards. Furthermore, lizards tested during colder seasons or at lower temperatures should have lower CT<sub>max</sub> than those tested during warmer seasons or at higher temperatures.

## **1.2 Materials and methods:**

**1.2.1 Study species:** We collected a total of 173 lizards from the campus of the California Polytechnic State University, San Luis Obispo. Lizards were caught by hand-held noose and placed into numbered tube socks for transport. After testing of CT<sub>max</sub> (see below), each lizard's post-orbital sinus was bled using heparinized microhematocrit tubes (ClearCRIT 75 mm with self-sealing plug), the tubes were centrifuged in a microcapillary centrifuge (International microcapillary centrifuge Model MB, Needham Heights, Massachusetts, USA), and hematocrit (% red blood cells by volume) was calculated. A separate heparinized tube was used to transfer a small sample of blood to a Hemocue cuvette (HemoCue® Hb 201+, Cypress, California, USA) to test for

hemoglobin (g/dL).

**1.2.2 Experiment 1 – Seasonal and Short-Term Acclimation Tests:** We caught all lizards between 0900-1200 on each season's sampling day (Table S1), with CT<sub>max</sub> trials beginning at 1300. The weather conditions of the week prior to each sampling day were representative of a typical season's weather (Table S1). On a given day, approximately half of the lizards (Spring=20, Summer=15, Fall=27, Winter=8) were tested on the day of capture (= same-day lizards), and the other half (Spring=19, Summer=10, Fall=25, Winter=0) were maintained in their tube sock bags in the lab to acclimate for two days in the laboratory at 18° C (= two-day acclimated lizards). In winter, we only collected data on same-day lizards because we were unable to capture a sufficient quantity and chose to have one group with a sample size of 8 as opposed to two sample sizes of 4. In the other seasons, the two-day acclimated lizards were tested for CT<sub>max</sub> two days after capture, also at 1300. Prior to testing, snout-to-vent length (SVL, ± 0.2 cm), tail length (± 0.2 cm), mass (Pesola® 50g precision scale; ± 0.3 % precision), sex, and gravidity (females gravid or not) were measured and recorded.

**1.2.3 Experiment 2 – Longer Term Acclimation Tests:** We ran six different treatments to test the effects of longer term acclimation. The first four treatments were run during the summer as 7-day trials. The lizards had access to water *ad libitum* but were fasted. The treatments consisted of:

1. Gradient: A terrarium with a gradient of temperatures ranging from 20-40° C, representing typical housing conditions for most laboratory lizards. The gradient

treatment consisted of a 20-gallon terrarium with a 100W UVA/UVB mercury vapor bulb (Lucky Herp, Changzhou Jinxu Special Lighting Technology Co., Changzhou Jiangsu, China) hanging on one side of the terrarium, along with a basking rock and a hide box on the opposite side of the tank.

2. Cold: constant 15° C. Lizards were placed in an environmental chamber set to 15° C with a 12:12 photoperiod, which maintained 15° C body temperature in all lizards. When in the environmental chambers, the lizards were housed in wire mesh cages (7.8 cm wide, 7.3 cm tall, 19.5 cm long) separated from one another with folded paper towels to block them from seeing one another to avoid potential stress due to interaction. Unlike the gradient treatment, these lizards were not given a basking rock or a hide box due to the environmental chamber having a homogenous temperature, regardless of position in the cage.
3. Control: constant 25° C. As #2 above, but at 25° C.
4. Hot: constant 35° C. As #2 above, but at 35° C.

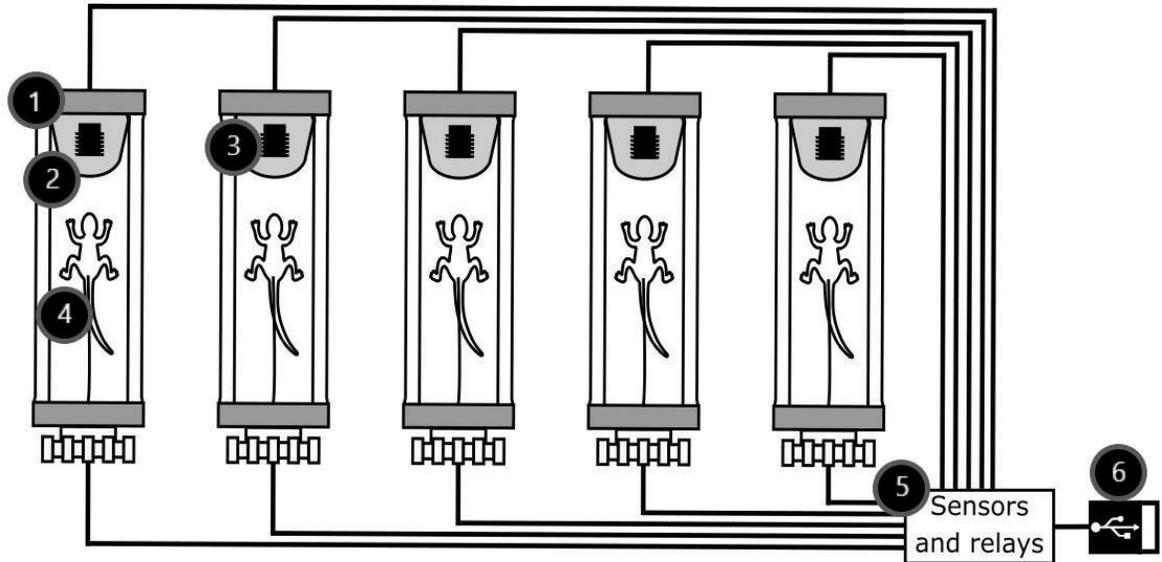
The other two treatments were 3-week trials. The lizards in these trials were fed daily and had access to water *ad libitum*. These treatments are part of an unrelated study, and we took advantage of the acclimation treatments to measure the CT<sub>max</sub> of lizards acclimated under somewhat different conditions than in treatments 1- above.

5. Extended Control: constant 25° C. As #3 above, but for 3 weeks.
6. Extended Hot: constant 25° C for 1 week, then increased to constant 35° C for the following 2 weeks.

**1.2.4 Measuring CT<sub>max</sub>:** CT<sub>max</sub> was assessed using GATORS (Figure 1.1). The

multiplex system is designed to heat the air inside five lizard chambers at a steady rate of  $1^{\circ}\text{C}$  per minute, while allowing the researcher to independently flip each chamber to observe when the lizard reaches its  $\text{CT}_{\text{max}}$ . Each of the five independent cylindrical acrylic animal chambers (18 cm length, 4 cm diameter) are encased by a larger acrylic chamber (25 cm length, 10 cm diameter). The inner chamber is capped with a grooved handle made of ABS 3D printer plastic that can rotate the inner chamber without removing it from the outer chamber. The outer chamber is heated evenly using a nose cone that contains a Peltier device, used for heating or cooling, and a fan that circulates the air. The outer chamber's temperature is the ambient temperature ( $T_a$ ) and is reported to the Arduino by a mounted digital temperature sensor (Adafruit I2C MCP9808 temperature sensor, New York, New York, USA). The Arduino is programmed to turn the Peltier device on or off, depending on the temperature reported by the digital temperature sensor. The heated air circulates into the inner chamber through openings on the grooved handle that are contained within the outer chamber, so no heated air is lost to the environment before it has reached the lizard. The air circulates out of the inner chamber through the opposite end of the chamber, which has a mesh covering so the lizard cannot enter the nose cone. The lizard's body temperature ( $T_b$ ) is reported to the Arduino through a resistance temperature detector ( $\pm 0.1\%$ , Honeywell platinum RTD HEL-700 series, Golden Valley, Minnesota, USA) inserted into the cloaca and held in place with medical tape. The Arduino regulates each chamber's temperature independently from the others to ensure the heating rate is constant for each chamber whether or not the other chambers are in use.

Prior to data collection, the  $T_b$  of the lizards were standardized at  $30^\circ\text{C}$  ( $\pm 1^\circ\text{C}$ ) within the GATORS internal chambers. Then the outer chamber heated at  $1^\circ\text{C}/\text{min}$ , as reported and modulated by the Arduino microcontroller. One person monitored the computer while three other observers monitored the lizards. The procedure mimicked a single-blind setup, where the computer operator could see the lizards' temperatures, but the lizard observers could not, to reduce bias. As the temperatures increased and the lizards began to exhibit thermoregulatory behaviors (e.g., mouth gaping and heavy panting) the observers began to flip the lizards by rotating the internal chambers, once every 30 seconds. When the lizards could no longer right themselves, despite vigorous shaking of the internal chamber, the LRR command was input into the Arduino serial monitor input on the computer, which records the time and temperature (both  $T_a$  and  $T_b$ ) at which the lizard lost its righting response, representing the  $CT_{\text{max}}$ . Hereafter, we refer to the LRR as the  $CT_{\text{max}}$ . Once the lizards reached  $CT_{\text{max}}$ , they were immediately removed from the chamber and cooled down by blowing on them. No lizards died during the experiments.



**Figure 1.1: Simplified Schematic of the Gas Analysis, Temperature, and Oxygen Regulation System (GATORS).** This field-portable system can be used to test CT<sub>max</sub> and CT<sub>min</sub>, as well as metabolic rate via flow-through respirometry, and supports the use of different oxygen concentrations. Thermal maxima and minima are reached with the use of a Peltier device (1) that can increase or decrease temperature based on the direction of current flow. The heated/cooled air is then circulated around the outer chamber using a small fan (2) located in the nose cone of each chamber. The ambient temperature is detected using digital temperature sensors (3) in the outer chamber, and the body temperatures of the lizards are recorded using resistance temperature detectors (4) inserted into the cloacae. Both temperatures are reported to the Arduino circuit board that contains the sensors and relays (5) that form a feedback loop to keep the chambers heating/cooling at their set rate. The live readout and command input are recorded on the computer interface (6).

**1.2.5 Data Analysis:** Data were analyzed using JMP Pro version 12 software. For both experiments, data were analyzed using ANCOVA examining how treatment, sex, SVL, mass, tail length, and hematocrit affected CT<sub>max</sub>. If the ANCOVA was significant, a Tukey HSD post-hoc test was performed. All data are presented as the mean  $\pm$  1 SEM with a significance level of  $\alpha = 0.05$ .

For Experiment 1 (seasonal and short-term acclimation), we could not quantitatively

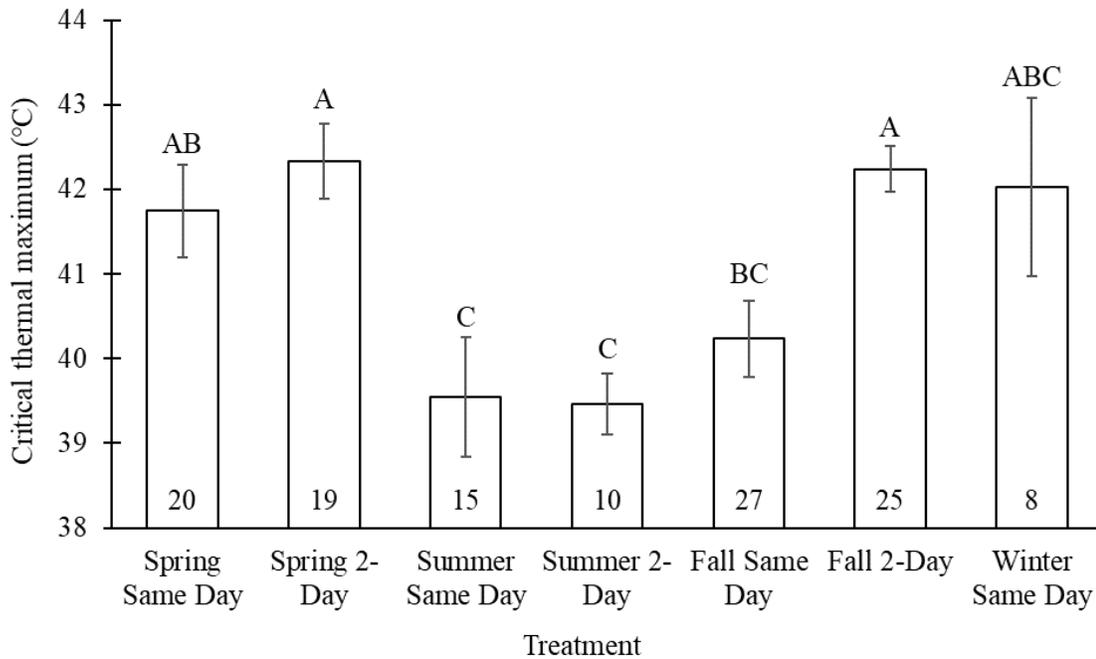
evaluate the effects of season and treatment or their interaction because winter did not have a two-day treatment; instead, we created a single “treatment” component, which is comprised of the season and the day of testing (same-day or two-day). This gave us seven different treatments for analysis in Experiment 1: spring same-day, spring two-day, fall same-day, fall two-day, summer same-day, summer two-day, and winter same-day. The fall data (same-day and two-day) are aggregates of the 2016 and 2017 fall seasons because we replicated the experiment for this season only; they were not significantly different and were pooled for analysis. We also ran an ANOVA examining the variation of hematocrit across treatment groups.

For Experiment 2 (longer term acclimation), we analyzed the six treatments described in section 1.2.c above along with the summer same-day treatment from Experiment 1, which served as a control. Hemoglobin was excluded from the analysis, as its explanatory power was low because of the strong correlation with hematocrit ( $r = 0.68$ ). One data point with a low CTmax of 30° C from Experiment 1 (control group) was excluded from analysis because it was the only data point to match the standardization temperature for GATORS.

## **1.3 Results**

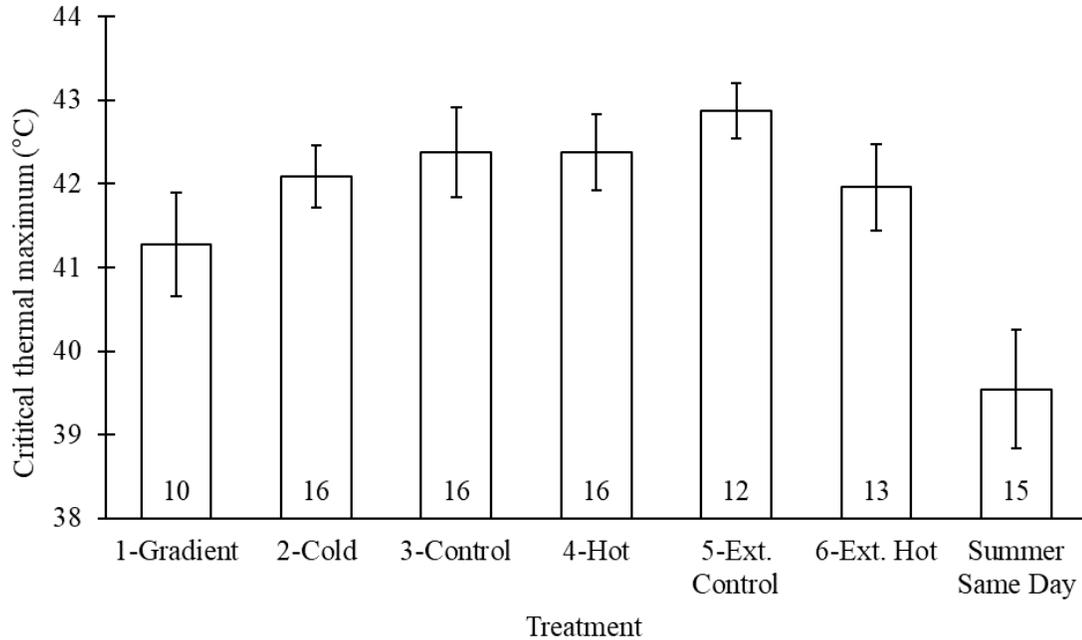
**1.3.1 Experiment 1 - Seasonal and Short-Term Acclimation Tests:** Treatment significantly impacted CTmax ( $F_6 = 5.55$ ,  $p < 0.001$ ), and field-active (same-day) CTmax was significantly higher in spring than in summer (Fig. 1.2). The lab acclimated (two-

day) CTmax was higher in both spring and fall than in summer (Fig. 1.2). Fall lizards that were tested on the same day had different CTmax values than those tested after a two-day acclimation (Fig. 1.2). CTmax in the summer was not impacted by lab acclimation. No other covariates were significant (all  $p > 0.20$ ), but hematocrit showed a trend for a positive, but non-significant relationship with CTmax ( $p = 0.10$ ).



**Figure 1.2: Mean Critical Thermal Maximum Values (CTmax; Measured as Loss of Righting Response)  $\pm$  1 SEM for *Sceloporus occidentalis* across Season and with Short-term (2-day) Lab Acclimation.** Sample sizes are listed at the bases of the bars. Groups with different letters are significantly different, as determined by a Tukey's post-hoc test ( $\alpha = 0.05$ ).

**1.3.2 Experiment 2 - Longer Term Acclimation Tests:** Only mass was significant ( $F_1 = 4.54$ ,  $p = 0.04$ ), with larger lizards having a lower CTmax (Fig. S1). No other factors or covariates were significant (all  $p > 0.15$ ), including treatment (Fig. 1.3).



**Figure 1.3: Mean Critical Thermal Maxima Values (CTmax; Measured as Loss of Righting Response)  $\pm$  1 SEM for *Sceloporus occidentalis* after Varying Lab Acclimation Treatments (see Section 2.3, above). Sample sizes are listed at the bases of the bars. None of the groups are significantly different.**

#### 1.4 Discussion:

The purpose of this study was two-fold: (1) to use a new, highly accurate methodology for measuring thermal tolerance to investigate whether housing lizards in the laboratory may have inadvertently compromised thermal tolerance data in previous studies, where the conditions may have not reflected what animals in nature experience and (2) to assess the level of plasticity in thermal tolerance in a small, heliothermic lizard (*S. occidentalis*). Overall, our experiments provided mixed support for the hypothesis that thermal tolerance variables (CTmax in our study) are affected by recent thermal environmental conditions. On one hand, the difference between the fall same-day and fall two-day acclimated lizards in Experiment 1 suggests that CTmax is plastic and can respond to environmental conditions within days, potentially hours. On the other hand, lizards in

Experiment 2 were housed for longer periods at constant temperatures and did not display any differences in CTmax among drastically different temperature treatments, suggesting CTmax is not very plastic in *S. occidentalis*. Overall, plasticity appears to be low in this species, but we have uncovered the potential for plasticity to impact CTmax data as measured under standard protocols.

In Experiment 1, we expected to see differences in the CTmax values, where lizards caught in the warmer seasons (spring and summer) would have higher CTmax values than lizards caught in the cooler seasons (fall and winter). We did observe differences, but not in the ways that we had expected. Although the sample size in winter was too low and variation too high for inference, we found that lizards collected in the spring had the same CTmax as lizards collected in the fall, but lizards collected in summer demonstrated lower CTmax than the other seasons. Although the CTmax of lizards collected and measured on the same day during spring and fall did not differ, we found that in the two-day laboratory acclimation treatments, the values were much closer to each other and fall lizards had a significantly higher thermal tolerance. This implies that housing lizards under laboratory conditions, even for just two days, could potentially mask naturally occurring differences that may be attributed to the recent thermal history of the lizards. Other studies have shown similar results: housing conspecific lizards from different populations in the same laboratory conditions caused the CTmax of those populations to converge onto similar values, but these studies used longer acclimation times than we did (Table S1; Layne & Claussen 1981, Yang et al. 2008). Despite using acclimation times that were much shorter, we show here that lab housing can mask differences in CTmax,

which is congruent with previous studies that found acclimation can occur rapidly, as quickly as 6 hours under warm temperature regimes and 24 hours for cooler temperatures in salamanders (Hutchison 1961, Lutterschmidt & Hutchison 1997).

Neither acclimation to the lab nor the hot summer temperatures explain the unexpectedly low CT<sub>max</sub> values in both summer treatments. One potential explanation is dehydration, which can decrease CT<sub>max</sub> (Plummer et al. 2003). In this case, we would expect lizards collected in summer to have exhibited a significantly higher hematocrit (indicating lower hydration), but hematocrit did not explain a significant amount of variation in the CT<sub>max</sub> values ( $p=0.10$ , section 1.3.a), nor did it differ between treatments ( $F_{6,6}=1.366$ ,  $p = 0.236$ ). Other potential explanations for the lower values in both summer treatments include factors that indirectly affect thermal tolerance and are affected by higher temperatures, like faster gut passage times (McConnachie & Alexander 2004; Van Damme et al. 1991) and higher metabolic rates (Garland et al. 1987; Tsuji 1988) leading to an empty gut, which is associated with a lower CT<sub>max</sub> (Larson 1961).

The low summer values might not reflect a direct response of thermal tolerance to the hot environment, but rather an indirect response in the form of a trade-off due to other physiological functions (Portner et al. 2001, Dunbar et al. 2007, Jones & Berkelmans 2011). This can be seen in other aspects of their thermal physiology when gravid, such as an altered preferred body temperature (Le Galliard et al. 2003) and increased basking time (Schwarzkopf & Shine 2006). Clearly, replication of the experiment in summer along with more data collection on variables like hydration and evaporative water loss

would allow us to better assess why the CTmax values are low in summer. Nonetheless, it is worth noting that very few studies collect CTmax data on animals over time, and simply assume that the data collected at the time of the experiment are representative of the species' CTmax, suggesting that plasticity in CTmax could impact the accuracy of data collected in thermal tolerance studies.

In contrast to Experiment 1, the data from Experiment 2 did not support our hypothesis that acclimation and recent thermal history affects the thermal tolerance of lizards.

Lizards acclimated at 15° C, 25° C, or 35° C and lizards allowed to thermoregulate in a gradient did not differ in CTmax values, regardless of acclimation time and whether they were fed or fasted. Our treatments encompassed a full range of potential experimental conditions that might typically be experienced by lab-housed lizards, and yet CTmax was relatively static. Notably, the CTmax of all the treatment groups acclimated for 1-3 weeks did not differ from one another but were higher than the CTmax for lizards collected and tested on the same day (summer same-day). Again, this shows that data collected on laboratory-housed animals may not accurately reflect the data that would be collected had the animals been tested immediately upon capture.

In addition, our data differ from most studies on lizards, which show that thermal tolerance increases as acclimation temperature increases (Lowe & Vance 1955; Huang et al. 2006; Hong et al. 2009). However, our results agree with the only other acclimation study on *S. occidentalis*, which showed that acclimation temperature has no effect on thermal tolerance (Kour & Hutchison 1970). Gunderson and Stillman (2015) conducted a

broad-scale analysis of plasticity in thermal tolerance of ectotherms that used the acclimation response ratio (ARR) as a measure of plasticity (where  $ARR = \Delta \text{ Thermal tolerance} / \Delta \text{ Acclimation temperatures}$ , see Table S2). Comparing the ectotherm values to those in our study and in Kour and Hutchison (1970), *S. occidentalis* finds itself in the unique position of being one of two species sharing the lowest ARR in CT<sub>max</sub> of all ectotherms, with the other being *Ectemnorhinus marioni*, the Antarctic weevil (Klok & Chown 2003). This value is 0.01, indicating a negligible effect of acclimation on plasticity.

As the ability to thermoregulate effectively increases, the need for plasticity decreases. Plasticity may be related to the variation and extremity of the conditions of the population's environment, where higher plasticity may result when the ability to thermoregulate is diminished. For example, aquatic organisms living in thermally homogenous environments tend to have high plasticity, whereas terrestrial organisms in thermally heterogenous microhabitats that allow for behavioral thermoregulation tend to have low plasticity (Gunderson & Stillman 2015). Perhaps being a behaviorally thermoregulating heliotherm inhabiting the relatively stable and mild habitat of the California Central Coast means that local *S. occidentalis* are buffered from extreme heat (Kearney et al. 2009; Ruiz-Aravena 2014) and therefore have lowered needs for a plastic response. If *S. occidentalis* can utilize solar radiation and behavioral shuttling between sun and shade to maintain its preferred body temperature in a mild climate (see Chapter 2), then perhaps selection for plasticity has been mild. Nonetheless, other heliothermic lizards have exhibited much higher plastic responses than *S. occidentalis* (Table S2).

In Chapter 2, we show that *S. occidentalis* displays a high thermoregulatory effectiveness: the ability to thermoregulate in a given environment. This could mean that despite sharing a similar thermoregulatory strategy (heliothermy, as opposed to thermoconformity), *S. occidentalis* is exploiting microhabitats better than other heliotherms and further lowering its need for a physiologically plastic response. To further investigate this, we could measure the plasticity, as measured by ARR, of *S. occidentalis* that live in harsher climatic conditions and compare them to those of coastal lizards at our field site. Also, we could measure ARR in multiple populations and species as a function of thermoregulatory effectiveness (Hertz et al. 1993) as well as general thermoregulatory strategy (heliothermy vs thermoconformity) in multiple species to gain a deeper insight into drivers behind the evolution of plasticity. Thermoregulatory effectiveness would serve a dual purpose in this analysis: to investigate the thermal habitat available to the organism as well as determining how well they are exploiting it. Thus, the goal of the comparison would be to identify whether the behavioral ability to maintain a favorable body temperature mitigates the need to employ a physiologically plastic response. We urge future researchers that study thermal tolerance to also determine the thermoregulatory effectiveness of their study species (and vice versa) to examine this trend, as it requires the pairing of the two variables (Figure S2).

This information is critical for conservation: as an active thermoregulator, *S. occidentalis* depends on the environment, especially solar radiation, to regulate its internal body temperature. Environmental increases in temperature will affect reproduction, ontogeny,

and development, and it is unlikely that lizards will be able to overcome these barriers. For example, a study involving a congeneric species examined maternal temperature effects on nesting behavior and embryonic thermal sensitivity and found that behavioral adaptations required to overcome these stressors are not present (Telemeco et al. 2017). Also, transgenerational plasticity has not been found to confer advantages in most reptiles and amphibians (Donelson et al. 2017), despite showing promise in aquatic vertebrates where it has been shown to increase offspring growth rate (Salinas & Munch 2012) and reproductive rate (Donelson et al. 2016). Because of the increased exposure to CT<sub>max</sub> as well as to the physiological effects stated above, *S. occidentalis* and other heliotherms with low plasticity may be especially susceptible to the impacts of rapid climate change due to their reliance on behavior rather than the ability to adapt physiologically (Stillman 2003). With increasing temperatures, behavioral thermoregulation will not be sufficient to maintain body temperature below CT<sub>max</sub> and physiological adaptations will be required (Chapter 2).

### **1.5 Conclusion:**

Thermal tolerance is a useful variable to use in models that estimate species longevity, but most studies look at organisms that have been lab acclimated, which has the potential to change the values of CT<sub>max</sub> that animals may exhibit in the field due to plasticity (Hoffmann et al. 2013; Yang et al. 2008). We did not find that the effect was due to the thermal environment the lizards were kept in during their housing, as hypothesized, but rather an unidentified factor related to captivity that was impacting the CT<sub>max</sub> measurements. Other species likely reflect this impact of laboratory housing (Table S2),

though it has not been widely tested due to the constraints of collecting and testing animals on the same day. This study finds that even in *S. occidentalis*, a lizard with a low plastic response, there is a quantifiable effect of laboratory housing that could skew or bias data away from what would be collected from lizards that have been in natural conditions.

## **2. Modeling the current and future thermal environment of the heliothermic lizard, *Sceloporus occidentalis***

### **2.1 Introduction:**

Ectotherms depend on the environment to keep their bodies at temperatures that facilitate physiological functions. Ectotherms rely on favorable habitats for regulation of body temperature ( $T_b$ ) which affects most physiological processes, from sprint speed to digestive efficiency (Hertz et al. 1983, Van Damme et al. 1991, Ji et al. 1996). When favorable habitats are unavailable, ectotherms are at a disadvantage because they must actively compensate or lose performance efficiency. The ability to thermoregulate allows many ectothermic organisms to maintain optimal body temperatures even if certain microhabitats become thermally unfavorable (Grover 1996, Díaz 1997, Goller et al. 2014). Reptiles can regulate their  $T_b$  behaviorally by increasing evaporative water loss via gaping the mouth or by changing their body posture to modulate the amount of heat gained or lost through conduction and solar radiation (Porter & James 1979). They can also regulate  $T_b$  physiologically by aggregating or dispersing melanin to regulate heat absorption (Clusella-Trullas et al. 2008) or by shunting blood to the skin to heat or cool more effectively (Bartholomew & Tucker 1963). A common method of regulating  $T_b$  is moving among various microhabitats in a heterogeneous thermal environment to keep the  $T_b$  within their selected temperature range (Bogert 1959, Myhre & Hammel 1969, Huey 1974).

Operative temperature models (OTMs) have been used for over 35 years to analyze an animal's thermal environment because they provide better insight into the temperatures

available to an animal than using only ambient (i.e., air or ground) temperature (Bakken & Gates 1975, Bakken et al. 1985, Bakken 1992, Hertz et al. 1993). With sound experimental design and accurate calibration (Walsberg & Wolf 1996), OTMs can be useful tools for examining multiple facets of thermoregulation. OTMs are commonly used to calculate thermoregulatory effectiveness (E) of an organism in an environment (Hertz et al. 1993) as well as the extent to which they are exploiting it ( $E_x$ ; Christian & Weavers 1996). Many studies have used these indices to investigate the relationship between an animal's thermoregulatory behavior and the thermal quality of various microhabitats. In general, organisms select the microhabitat that allows them to be within their selected temperature range ( $T_{set}$ ) for the greatest amount of time (Diaz 1997, Scheers & Van Damme 2002, Row & Blouin-Demers 2006).

Recently, OTMs have been used to evaluate extinction risk in lizards by calculating activity time. Activity time is typically calculated as the amount of time that the lizard thermal habitat is under the critical thermal maximum (CTmax) temperature threshold (Sinervo et al. 2010); however, time within the selected temperature range ( $T_{set}$ ) is also often used (Pontes-da-Silva et al. 2018). These extinction risk models suggest that losing more than 3.85 hours a day of activity time is associated with a greater risk of extinction (Sinervo et al. 2010, Bruschi et al. 2016).

This study focused on the western fence lizard (*Sceloporus occidentalis*), a common heliothermic basking lizard with a broad geographic range, spanning most of the western continental United States. Past studies using OTMs to study *S.occidentalis* have focused

mostly on the temporal microhabitat use compared to congeneric *Sceloporus* lizards (Mcginnis 1970, Adolph 1990, Grover 1996). The thermal quality of the environment and activity patterns have been evaluated in *Sceloporus* lizards that inhabit thermally stressful desert environments (Grant & Dunham 1988). Clearly, activity is limited by extreme temperatures in these habitats, and climate change will likely exacerbate this. However, *S. occidentalis* was chosen as the focus of this study to observe how climate change will impact the activity of a common species of lizard inhabiting a mild environment.

We set out to investigate the thermal environment available to *S. occidentalis* and make predictions for the effects of climate change on that thermal environment (see section 2.2.1 for climate details for our study area). Using OTMs, we evaluated three different microhabitats commonly used by *S. occidentalis* – sun, shade, and mixed (partial sun and shade) – using various thermal indices. It is important to keep in mind that because of *S. occidentalis*' heliothermic nature, the sunny microhabitat is where they are expected to spend the majority of their daily activity time in. We also compared the OTM measurements to field body temperature data ( $T_b$ ) to calculate the thermal exploitation index (Christian and Weavers 1996). Then, we looked at two global climate change scenarios, the representative concentration pathways (RCPs), that evaluate future climate based on greenhouse gas emissions. We used RCP scenarios 4.5 and 8.5 (Cal-adapt) to predict the loss of daily activity time and assess extinction risk under each scenario.

## **2.2 Methods:**

### **2.2.1 Study Site**

This study was conducted over a four-month period, from June to September 2017, at a site located 1.6 km northeast of Cuesta College on the California central coast (35°20'43.4"N 120°43'55.5"W). This area has a mild, Mediterranean climate with average highs of 24-26°C and average lows of 10-12°C during the summer months (Weatherspark). In this habitat, *S. occidentalis* is commonly found basking on oak trees and rock outcrops.

### **2.2.2 Operative Temperature Models ( $T_e$ )**

To obtain operative temperatures in several microhabitats ( $T_e$ ; Bakken & Gates 1975), we constructed eight hollow copper casts that matched the shape and size of adult *S. occidentalis* using a methodology modified from Bakken & Gates (1975). We used a silicone molding kit (Smooth-on Ecoflex 00-30) to make a wax cast utilizing a deceased lizard that was adjusted to resemble a lizard standing on its two front limbs, as posture can affect the temperatures reported by the models (Porter et al. 1979). Before pouring the wax, we placed a Thermochron iButton (DS1921G-F5) in the hollow body, so that when the wax was melted out of the model, the iButton remained within. Once the models were cast in copper and the entirety of the wax was melted out, they were tested against live lizards in the full range of the thermal gradient (see below) to test their accuracy. All eight models were found to be within  $\pm 1^\circ\text{C}$  of the live lizard body temperature after 120 seconds of being in the same location.

The models were placed in three different microhabitats on rock outcrops: “Sun” (n=3), on rock surfaces that received sun for the entire duration of the day; “Shade” (n=3),

crevices in large rock piles that were fully removed from the sun at all times of the day; “Mixed” (n=2), areas that were partially exposed to the sun and partly in shade, such as under a bush on a rock. We used a quick dry epoxy (Loctite quick-set epoxy, Henkel Corp., USA) on the limbs to affix each model to its location. Models were deployed during the warmest time of year for the San Luis Obispo area (Figure 2.S1), from June 14, 2017 to September 25, 2017. The models were monitored daily to check that they remained undamaged.

### **2.2.3 Live lizard temperatures ( $T_b$ )**

*S. occidentalis* (N = 128 samples) were captured by hand-held noose on site between the hours of 0900 and 1500 in the months of July and August 2017 to obtain live lizard temperatures. We did not mark lizards when capturing, so we were unable to account for whether we collected repeat temperature measurements on the same lizard, but we do not expect that any significant individual differences in  $T_b$  influenced our results. When collecting cloacal temperatures (Fischer Scientific traceable thermometer type k,  $\pm 0.3^\circ\text{C}$ ), we immediately probed the lizards within 30 seconds and limited our skin contact with the lizard to ensure the most accurate readings possible.

### **2.2.4 Selected body temperature ( $T_{set}$ )**

The selected body temperature range of *S. occidentalis* (n=28) was determined using a 1.17 m thermal gradient (10°C to 50°C) with four 6.35 cm wide lanes. Each lane was separated by a 1.17 m long, 19 cm tall plastic divider that prevented the lizards from seeing or interacting with each other. The bottom of the gradient consisted of a thermally

conductive metal plate covered in 1.27 cm of sand (ZooMed ReptiSand, San Luis Obispo, California). It was heated using electrical heat tape (Thermolyne BSAT101-020 Heating Tape, ThermoFisher Scientific, Waltham, Massachusetts) that covered half (0.59 m) of the underside of the metal plate, and the cool temperatures were attained using a water-cooling system that circulated cooled water under 0.38 m of the metal plate using copper piping (Polyscience benchtop chiller, LM series, Polyscience, Niles, Illinois).

A flexible thermocouple (Bead Probe TPK-01-40G, TECPEL Co., Taipei, Taiwan) was inserted into each lizard's cloaca and secured with medical tape around the base of the tail to monitor  $T_b$ , which was recorded every 5 minutes by a thermal logger (OMEGA RDXL4SD). Each trial ran for 3 hours, the first half of which was an acclimation period used to habituate the lizards to the thermal gradient, and the second half was the data collection period. The lizards were not handled during the trial and were only visually monitored every half hour to ensure minimal interference. Two trials were run per day on different lizards: morning, from 1100-1400 and afternoon, from 1400-1700. This was done because the thermal gradient only had 4 lanes and we wanted to test as many lizards on the same day. We recorded sex, snout-to-vent length (SVL;  $\pm 2$  cm), tail length ( $\pm 2$  cm), mass (Pesola® 50g precision scale;  $\pm 0.3$  % precision), and gravidity (female gravid or not) prior to testing.

### **2.2.5 Data Analysis**

#### ***Selected body temperature***

The  $T_{set}$  data were analyzed using JMP Pro version 12 software.  $T_{set}$  was designated as

the median value for the last 90 minutes of the 3-hour trial, per lizard ( $T_{\text{set-individual}}$ ), where the average individual variance had decreased to  $0.87^{\circ}\text{C}$  from  $1.41^{\circ}\text{C}$  in the first 90 minutes. We ran an ANCOVA that examined the effects of sex, SVL, mass, lane (in the gradient), and time (morning or afternoon) on  $T_{\text{set-individual}}$ . We did not include gravidity in our analyses because none of the females were gravid. To determine the  $T_{\text{set}}$  range for *S. occidentalis* at our field site, we took the median  $T_{\text{set}}$  value of the  $T_{\text{set-}}$  individual values and used a 40% and 60% quartile range.

### ***Indices of Thermoregulation***

When calculating indices of thermoregulation, we only included operative temperatures ( $T_e$ ) from the hours of 1100-1800, as those were the average daylight hours during the time of the study. Additionally, we were only able to collect  $T_b$  from 0900-1500 due to logistical constraints, but the calculations for the indices require a  $T_b$  value for each hour calculated, which meant that we were missing values for some of *S. occidentalis*' active hours. To solve this, we extrapolated the  $T_b$  data by taking the average of the  $T_b$  values and treating those as the 1600-1800 values for the indices.

- 1) Thermal Quality ( $d_e$ ):** The average difference between  $T_{\text{set}}$  and  $T_e$ . Values closer to zero indicate a high thermal quality and values further from zero indicate a low thermal quality.  $T_e$  values that were within the  $T_{\text{set}}$  range were given a value of 0. If  $T_e$  values were above the  $T_{\text{set}}$  range, the difference was calculated between the upper limit of  $T_{\text{set}}$  and the  $T_e$  value. Conversely, if  $T_e$  values were below the  $T_{\text{set}}$  range, the difference was calculated between the lower limit of  $T_{\text{set}}$  and the  $T_e$  value (Hertz et al. 1993). Thermal quality was calculated for each hour within the

time range per microhabitat (Figure S5), then averaged to give one value per microhabitat for the thermoregulatory effectiveness analysis (see #3 below).

$$d_e\text{HABITAT} = \text{average } | (T_{\text{set}} - T_e) |$$

- 2) Thermoregulatory Accuracy ( $d_b$ ):** The average difference between  $T_b$  and  $T_{\text{set}}$ . This -index compares body temperatures of free-ranging lizards to the laboratory-obtained selected body temperature (Hertz et al. 1993). The  $d_b$  was calculated for each hour within the time range (Figure 2.S3) then averaged to get one value for the thermoregulatory effectiveness analysis.

$$d_b = \text{average } | (T_{\text{set}} - T_b) |$$

- 3) Thermoregulatory effectiveness ( $E$ ):** Calculated as the difference between 1 and the quotient of  $d_b$  and  $d_e$ . Values for this index range between 1 and 0, where 1 indicates a larger thermoregulatory effectiveness (Hertz et al. 1993). The larger  $d_e$  becomes in relation to  $d_b$ , the close the value approaches 1.

$$E\text{HABITAT} = 1 - (d_b/d_e)$$

- 4) Thermal exploitation index ( $E_x$ ):** The proportion of  $T_b$  measurements that fell within  $T_{\text{set}}$  for times where  $d_e$  is also 0 (Christian and Weavers 1996). Each  $d_e$  was compared to  $d_b$  within the 1100-1800 time range and the time exploited (hours  $d_b$  was in  $T_{\text{set}}$ ) was divided by the time possible (hours each  $d_e$  was in  $T_{\text{set}}$ ) and multiplied by 100. The resulting quotient was  $E_x$ . The closer the value is to 100, the greater the thermoregulatory performance.

$$\frac{(\text{Hours when } T_b = T_{set})}{(\text{Hours when any } T_e = T_{set})} * 100$$

### ***Climatic predictions and restricted activity time***

To predict how lizards may be affected by warming climates, we used two representative concentration pathway (RCP) climate scenarios, 4.5 and 8.5 (Cal-adapt). RCP 4.5 is a scenario in which emissions peak around the year 2040, then steadily decline. RCP 8.5 assumes emissions rise gradually, but steadily through 2050, then plateau around 2100. Using the “modeled projected annual mean” tool, we found the year in which the annual average temperatures at our field site had increased 1°C from the 2017 average. We repeated this for the 2°C difference as well. To make predictions, we estimated a 1°C change in yearly average to be equivalent to a 1°C change in  $T_e$ . Restricted activity time was quantified as the amount of time any of the  $T_e$  surpassed the 41.5 °C  $CT_{max}$  of *S. occidentalis* (Chapter 1).

## **2.3 Results:**

### ***Selected body temperature***

The ANCOVA showed that none of the factors measured (sex, SVL, mass, lane, and time) significantly affected  $T_{set}$ -individual ( $F_{8,27} = 0.93$ ,  $p=0.51$ ). The median, 40%, and 60% quartiles of  $T_{set}$ -individual yielded a  $T_{set}$  range of 32.74 – 34.98 °C, with a median value of 33.45 °C.

### ***Indices of Thermoregulation***

During their active hours (1100-1800), *S. occidentalis* had access to  $T_e$ 's between ~30 °C

and 40 °C, on average (Figure 2.1). The “sun”  $T_e$  average was consistently over 2 °C above  $T_{set}$ , making “sun” the microhabitat with the lowest thermal quality ( $d_e$ ; Table 1, Figure S2). The “shade” and “mixed”  $T_e$ ’s were similar to each other in thermal quality but differed in that the “shade”  $T_e$ ’s approached the lower value of  $T_{set}$  during mid-day and the “mixed” model  $T_e$ ’s approached the upper value of  $T_{set}$  during mid-day (Figure 1). Thermoregulatory accuracy ( $d_b$ ) showed *S. occidentalis* maintained  $T_b$  within  $T_{set}$  for 6 hours on average (Table 1; Figure S6), and  $T_b$  below  $CT_{max}$  at all observed hours and within  $T_{set}$  at all but one (Table 1).

**Table 1.1: Indices of Thermoregulation for *S. occidentalis* in Microhabitats at a Central California Site.** Thermal quality ( $d_e$ ), thermoregulatory effectiveness (E), and thermoregulatory exploitation ( $E_x$ ) indices are listed for each microhabitat (sun, mixed, and shade). Live lizards were able to thermoregulate in their selected body temperature ( $T_{set}$ ) range for all the hours during which that range was available.

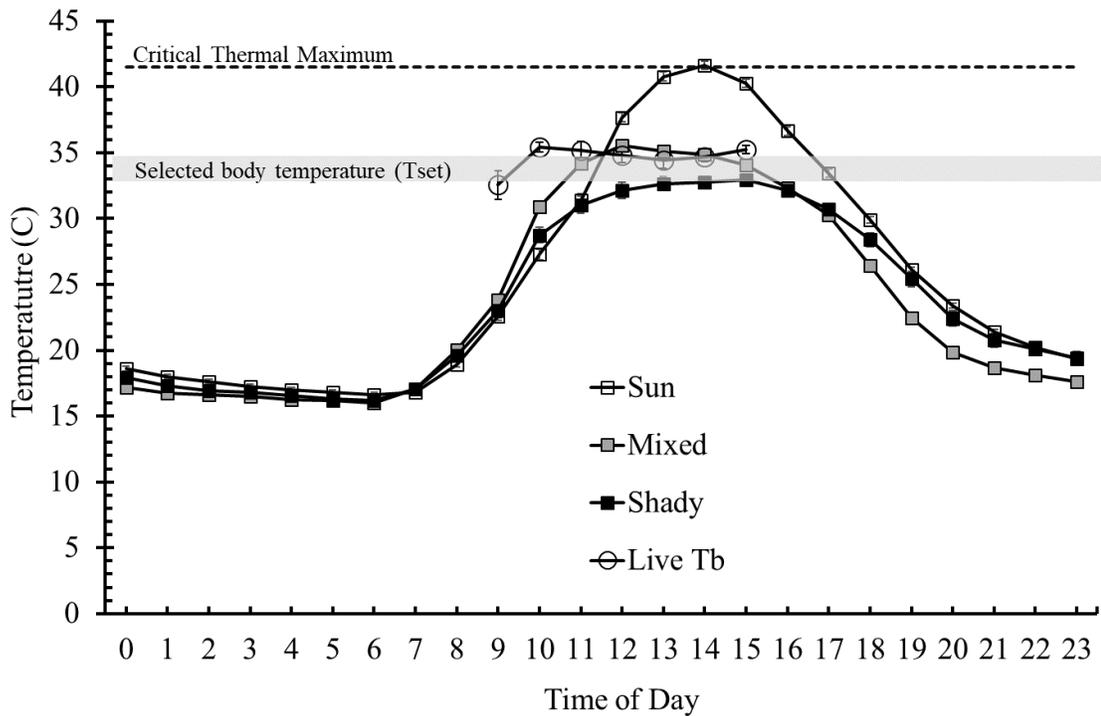
Habitat	Thermal Quality ( $d_e$ )	E	Microhabitat time in $T_{set}$ (hr)	Lizard time in $T_{set}$ (hr)	$E_x$ – Time spent/ range possible
Sun	3.29	0.98	1	6	600%
Mixed	1.25	0.95	3	6	200%
Shade	1.17	0.95	2	6	300%

### *Climatic predictions and restricted activity time*

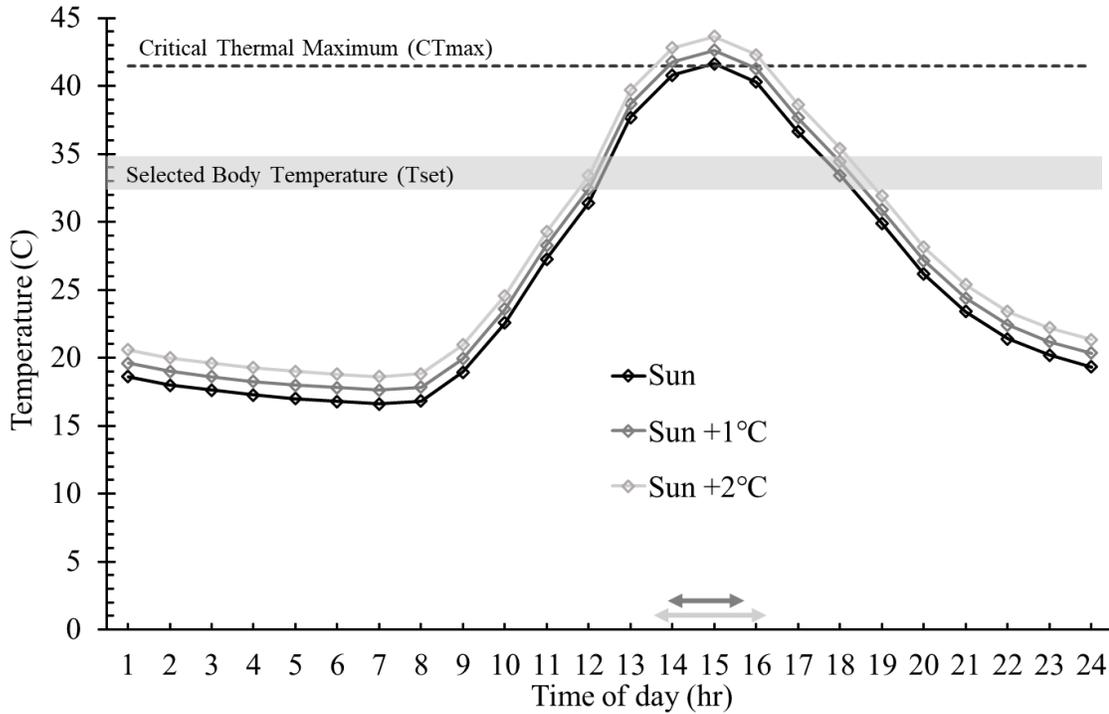
In the RCP 4.5 scenario, our field site will have increased 1 °C from its 21.8 °C 2017 annual average to 22.8 °C by 2048, and to 23.8 °C by 2082. For the RCP 8.5 scenario, our field site will have increased 1 °C to 22.8 °C by 2062 and to 23.8 °C by 2076.

Assuming equivalent warming across all microhabitats, a 1 °C increase in temperatures will cause the “sun” model  $T_e$ ’s to exceed the  $CT_{max}$  of 41.5 °C for 2.05 hours, and a 2

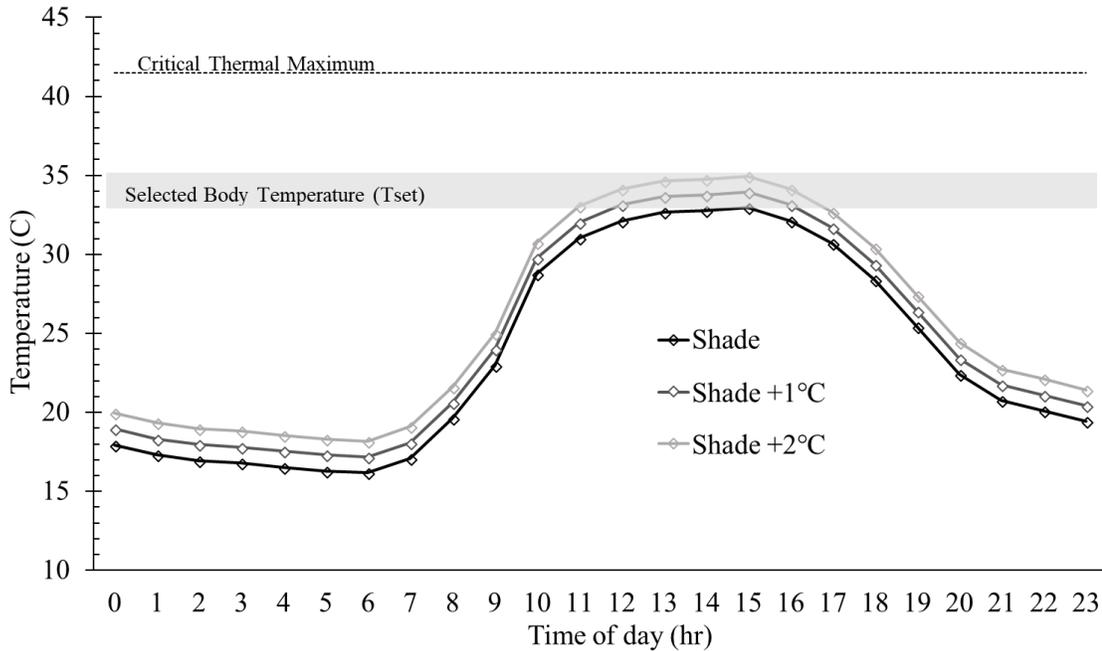
°C increase will cause  $T_e$ 's to exceed 41.5 °C for 2.5 hours (Figure 2.1). Both the 1 °C and 2 °C increases would shift the “shade” model  $T_e$ 's into the  $T_{set}$  range for most of *S. occidentalis*' activity hours: 4 hours for the 1 °C increase and 6 hours for the 2 °C increase (Figure 2.3). We did not continue past a 2 °C increase as the RCP climate scenarios did not reach a 3 °C increase within the century, and as we progress further from the present, the predictions become increasingly inaccurate.



**Figure 2.1: Average Hourly Operative and Lizard Body Temperatures over the Course of a Day.** The average of the live temperatures is portrayed as a dotted line across all hours. Selected body temperature ( $T_{set}$ ) is a range from 32.74-34.98 °C. Critical thermal maximum is the dashed line at 41.5 °C. Error bars are  $\pm 1$  SEM.



**Figure 2.2: Climate Projections for the “Sun” Operative Temperatures at Both a 1 °C Increase and a 2 °C Increase, Compared to its Current State.** A 1 °C increase results in a loss of ~2 hours of activity time and a 2 °C increase results in a loss of ~2.5 hours of activity time. The current selected body temperature and critical thermal maximum are also displayed. The arrows on the x-axis indicate the hours in which the  $T_e$  exceeds  $CT_{max}$ , the darker arrow represents the 1 °C increase and the lighter arrow represents the 2 °C increase.



**Figure 2.3: Climate Projections for the “Shade” Operative Temperatures at Both a 1 °C Increase and a 2 °C Increase, Compared to its Current State.** While the microhabitat is at no risk of surpassing the critical thermal maximum, it will shift into Tset range for 4 hours at 1 °C increased and 6 hours for 2 °C increased.

#### 2.4 Discussion:

By comparing  $T_e$ 's from OTMs and  $T_{bs}$  from field-active lizards to the lab-determined thermal variables  $T_{set}$  and  $CT_{max}$ , we were able to evaluate the current thermoregulatory performance of *S. occidentalis* in their thermal habitat. The thermal habitat was comprised of three microhabitats: the open “sun” microhabitat, brush “mixed” microhabitat, and fully covered “shade” microhabitat, each of which had different  $T_e$ 's during the day. We were also able to use local climate change scenarios to forecast the thermal habitat at a 1 °C and 2 °C increase and combined those data with models of extinction risk to estimate local extinction risk. These data aid our understanding on the thermoregulatory strategy of *S. occidentalis* as well as the effects of increasing temperatures on a lizard that is common and lives in a mild thermal habitat, rather than one that inhabits a less favorable thermal habitat.

*S. occidentalis* likely maintain their  $T_b$  within the  $T_{set}$  range behaviorally by shuttling back and forth between microhabitats, rather than relying on physiological adaptations. There were only 4 hours during the day during which at least one of the microhabitat  $T_e$ 's were within the  $T_{set}$  range (Figure 2.1, Figure S5). Yet, *S. occidentalis* were able to thermoregulate in the  $T_{set}$  range for 6 hours (Table 1), indicating a high thermoregulatory performance under the thermal exploitation index –  $E_x$ . When looking at each of the microhabitat  $T_e$ 's, their relationship to  $T_{set}$  individually, and strictly using  $E_x$ , it is difficult to understand how the lizards are outperforming the “total” of the microhabitats. However, by looking at the temperature range available (Figure 2.1), it becomes clear that the lizards are using all three microhabitats to maintain their field-active  $T_b$ s, as each microhabitat is either above or below the  $T_{set}$  range (when not in  $T_{set}$ ), and when used in conjunction, can yield more hours in  $T_{set}$  than the total available in each individual microhabitat. In a study that used biotelemetry to collect data on body temperature and behaviors, *S. occidentalis* changed their microhabitat choice depending on the daily temperature; especially on hot days, lizards actively used the shade to reduce their  $T_b$ 's (Mcginnis 1970). Other studies have also shown that in cooler, montane habitats, *Sceloporus* lizards are still able to maintain a similar  $T_b$  to those living in a desert habitat by exploiting microhabitats (Adolph 1990), which further supports the idea that *S. occidentalis* are primarily behavioral thermoregulators.

Currently, *S. occidentalis* living in mild, coastal habitats like our field site can take advantage of thermally desirable microhabitats to stay within their  $T_{set}$  and below their

CTmax. Nonetheless, as temperatures increase, thermal quality of the environment and available temperatures will change. A recent study on Mexican *Sceloporus* lizards estimated that if daily activity time is restricted by more than 3.85 hours, a species is at risk of going extinct, based on the “existence/persistence status” of other local *Sceloporus* lizards (Sinervo et al. 2010). For the sake of simplicity, we used that value at various temperature changes and found that *S. occidentalis* is not at risk of local extinction at either predicted temperature increase if daily activity time is measured as the time any microhabitat exceeds the CTmax (Figure 2.2). While studies mainly use CTmax as the main restricting variable when determining daily activity time, as we did above, some studies have opted to use time within  $T_{set}$ . Both variables are important and the use of one or the other may depend on the thermoregulatory strategy of the study species. CTmax is a logical threshold as it is the temperature that when exceeded is a risk for death (Cowles & Bogert 1944), thus lizards are unlikely to risk surpassing it. On the other hand,  $T_{set}$  is important for physiological processes and may be equally restrictive to the survival of generations if not maintained (Telemeco et al 2017).

For *S. occidentalis*, if daily activity time is evaluated using the amount of time where  $T_b$  is within  $T_{set}$ , then both the 1 °C and 2 °C increases will severely limit daily activity time, as the only microhabitat that allows the maintenance of  $T_{set}$  during daily active hours is the shade habitat (Figure 2.3). This would mean that during a large portion of their mating season, *S. occidentalis* would be forced to primarily inhabit the shaded habitat, drastically limiting the opportunity for reproductive and territorial displays. Studies provide support for the cost-benefit model of thermoregulation, which proposes that as

organisms become less and less capable of thermoregulating at their  $T_{set}$ , they shift from an active, heliothermic strategy to being increasingly passive thermoconformers (Huey & Slatkin 1976, Herczeg et al. 2006). However, this scenario is unlikely, congeneric *Sceloporus* lizards during times of competition, such as for mates, thermoregulate around their  $CT_{max}$ , even risking exceeding it, rather than thermoregulating around their  $T_{set}$  (Rusch & Angilletta 2017). This provides support for using  $CT_{max}$  as the determinant of daily activity in *S. occidentalis* rather than  $T_{set}$ .

It is important to note that despite failure to exceed the threshold for local extinction under predicted climatic changes, the habitats will decrease in thermal quality and affect other aspects of lizard physiology and ecology. These include sub-optimal nesting behavior (Telemeco et al. 2017), altered hatchling growth rate (Sinervo & Adolph 1989), and early testicular regression during seasonal gonadal development (Marion 1982). Furthermore, metabolic rate and food consumption both increase as temperature increases (Niewiarowski & Waldschmidt 1992, Van Damme et al. 1991), but foraging opportunities will be limited as daily activity time decreases.

Like any methodology, the use of OTMs is not without its limitations. For example, OTMs are unable to account for the spatial distribution (e.g. distance between microhabitats) of temperatures within an environment, which can play a large part in which microhabitats an organism uses for thermoregulation (Sears et al. 2016, Sears & Angilletta 2015). Our study was mostly limited to seeing how *S. occidentalis* escape heat, as it took place during the summer months, but a closer look at the data revealed an

interesting observation: as the temperatures increased in the morning, *S. occidentalis* were able to heat more quickly than all the OTMs (Time of day 0900, Fig. 1.1). This could be attributed to changes in thermal conductive capacity, as live lizards can change their posture, whereas the OTMs cannot. A flattened lizard can gain heat directly from the substrate, but the OTMs, due to the posture in which they were constructed, are limited mostly to thermal convection (Porter & James 1979). OTMs also lack the capacity for physiological regulation that would be available to the lizards, like vasodilation and vasoconstriction.

Our use of the thermoregulatory effectiveness index (E) in this study has drawbacks. We used E to evaluate three microhabitats that comprise the thermal environment to which *S. occidentalis* has access, but E treats each microhabitat as an individual calculation. As a result, the index reports that lizards are most effective in the sun microhabitat (Table 1). However, as previously stated, *S. occidentalis* shuttle between microhabitats and so E may not truly reflect the actual thermoregulatory effectiveness in that microhabitat. Our results indicate that as thermal quality decreases, thermoregulatory effectiveness increases (Table 1), but this may be due to the limitations of the index to properly evaluate each microhabitat individually. Interestingly, this trend has been shown experimentally in a non-heliothermic lizard *Sphenodon punctatus*, the tuatara, where researchers manipulated thermal habitat quality in the lab and found that in their low thermal quality habitat treatment, thermoregulatory effectiveness was highest (Besson & Cree 2010).

*S. occidentalis* are effective thermoregulators in mild, Mediterranean habitats, taking advantage of their access to the various thermal microhabitats that the terrain provides. They can maintain their  $T_b$  within  $T_{set}$  and are presently at no risk of exceeding their  $CT_{max}$  in any climate change scenario for this century. Although neither of the climate scenarios we looked at meet the extinction criterion that previous papers have proposed, *S. occidentalis* may lose their capacity to thermoregulate, at least partially, due to the shifts in the temperatures available in each microhabitat.

Our results show that there will be a quantifiable negative effect on the thermoregulation of ectothermic reptiles. In addition, there will likely also be indirect effects on foraging, reproduction, and other aspects of their ecology and physiology. This is particularly worrying because extinction risk no longer only includes lizards that live in harsh, desert climates, but rather a broader set of species that includes those that live in mild, favorable habitats.

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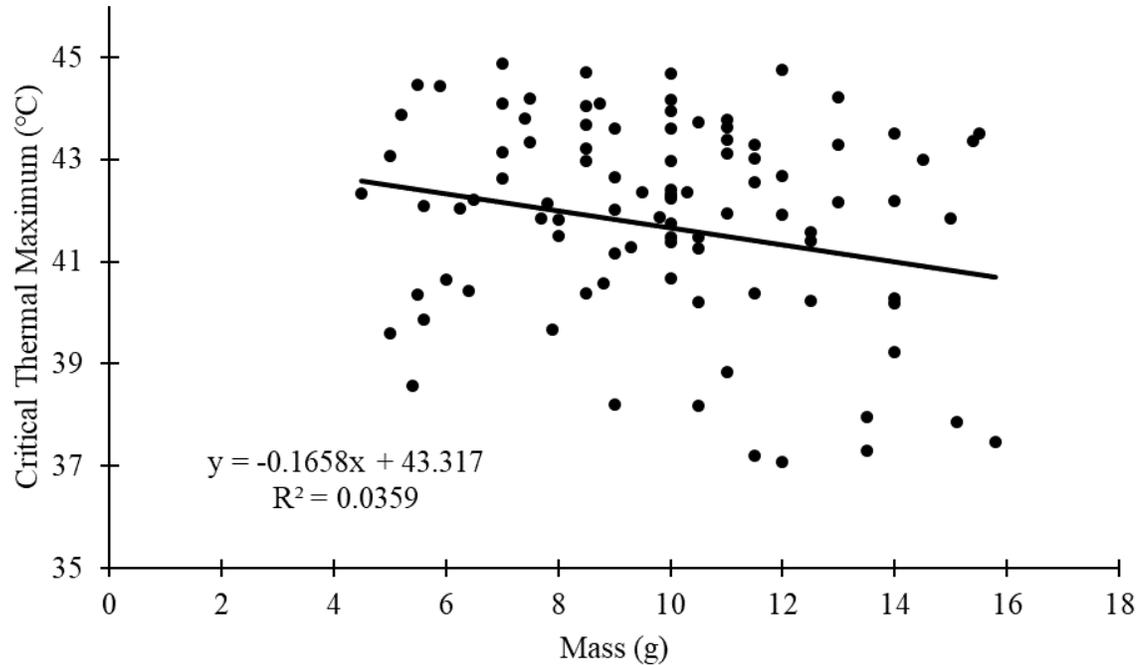
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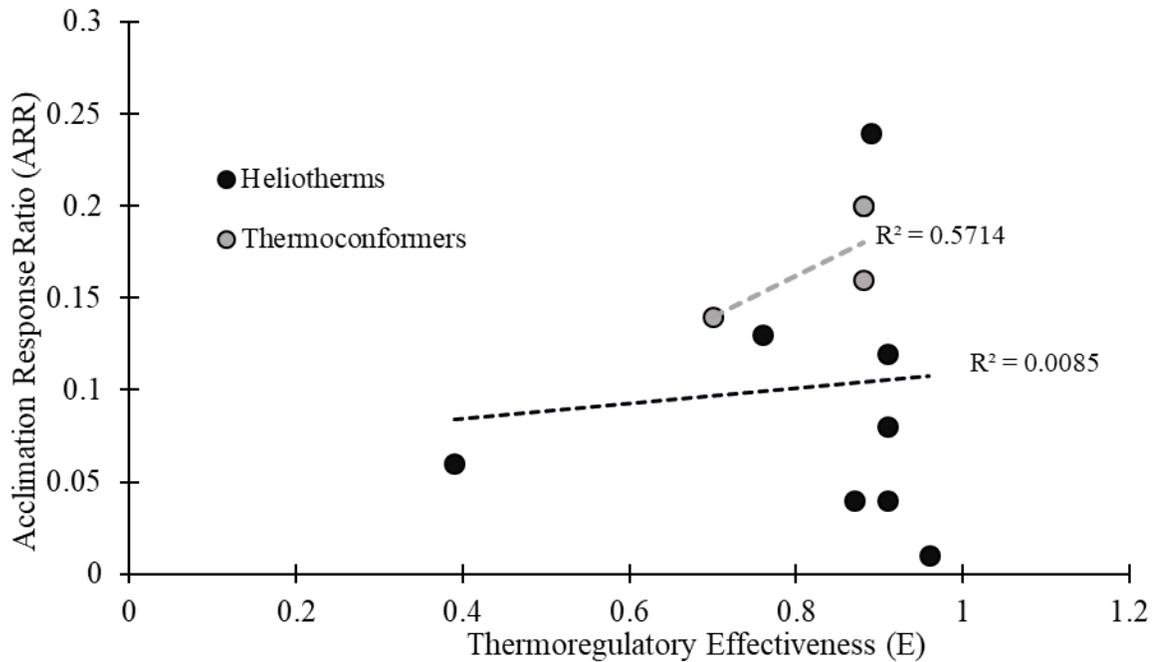
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## Appendices

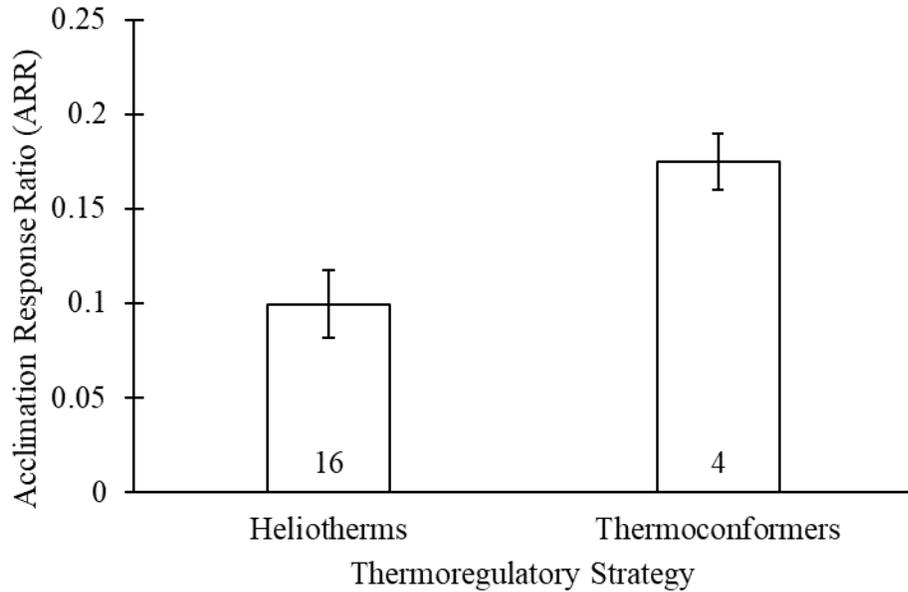
### Appendix A. Supplemental figures and tables:



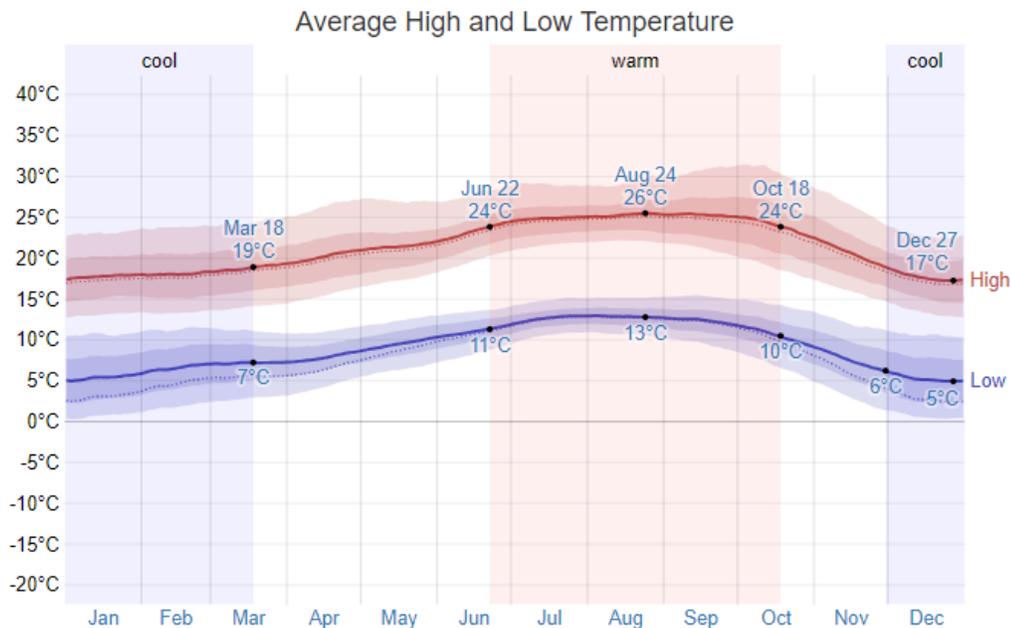
**Figure S1: Critical Thermal Maximum vs Mass for the Lizards in Experiment 2 – Longer-Term Acclimation Trials.** Mass had a significant relationship ( $F_1 = 4.54$ ,  $p = 0.04$ ) with critical thermal maximum, where larger lizards had lower Critical Thermal Maxima than smaller lizards.



**Figure S2: Lizard Acclimation Response Ratio (ARR) vs Thermoregulatory Effectiveness (E) Grouped by Thermoregulatory Strategy.** There is no relationship between plasticity and effectiveness, but thermoconformers generally have a higher plasticity than heliotherms ( $p=0.057$ , see Fig. S3). Not all lizards that have published ARR values have published E values, thus the difference in sample size between this figure and figure S3 (Zheng et al. 2013, Hong et al. 2009, Huang & Tu 2008, Yang et al. 2008, Huang et al. 2006, Corn 1971, Kour and Hutchison 1970, Murrish & Vance 1968, Lowe and Vance 1955).

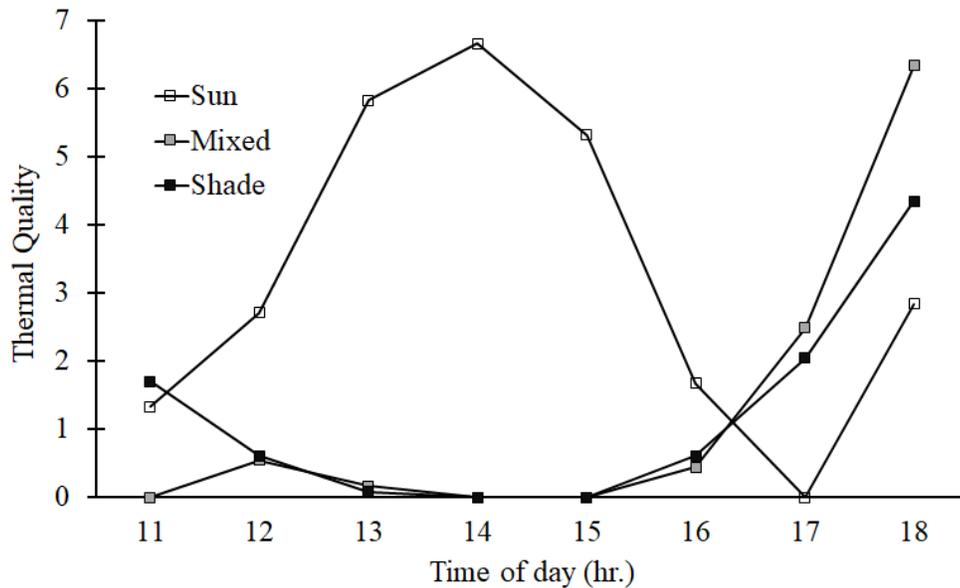


**Figure S3: Mean Acclimation Response Ratio (ARR)  $\pm$  1 SEM for Heliotherms Compared to Thermoconformers.** The means are not statistically different, as determined by a t-test ( $p=0.057$ ). Sample sizes are listed at the bases of the bars (Shu-Ran et al. 2017, Lara-Resendiz et al. 2015, Zheng et al. 2013, Hong et al. 2009, Wright 2009, Sartorius 2002, Corn 1971, Kour and Hutchison 1970, Lowe and Vance 1955).

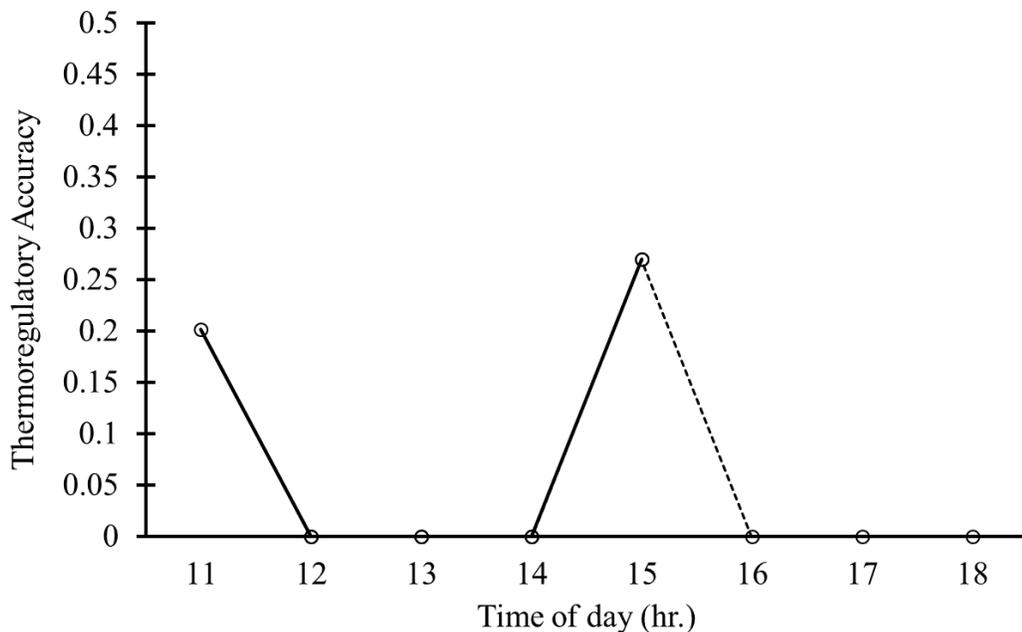


The daily average high (red line) and low (blue line) temperature, with 25th to 75th and 10th to 90th percentile bands. The thin dotted lines are the corresponding average perceived temperatures.

**Figure S4: Average Highs and Lows for San Luis Obispo County, CA. Late-June to Mid-October is the Warm Season for the Area.** This figure is taken from [www.weatherspark.com](http://www.weatherspark.com).

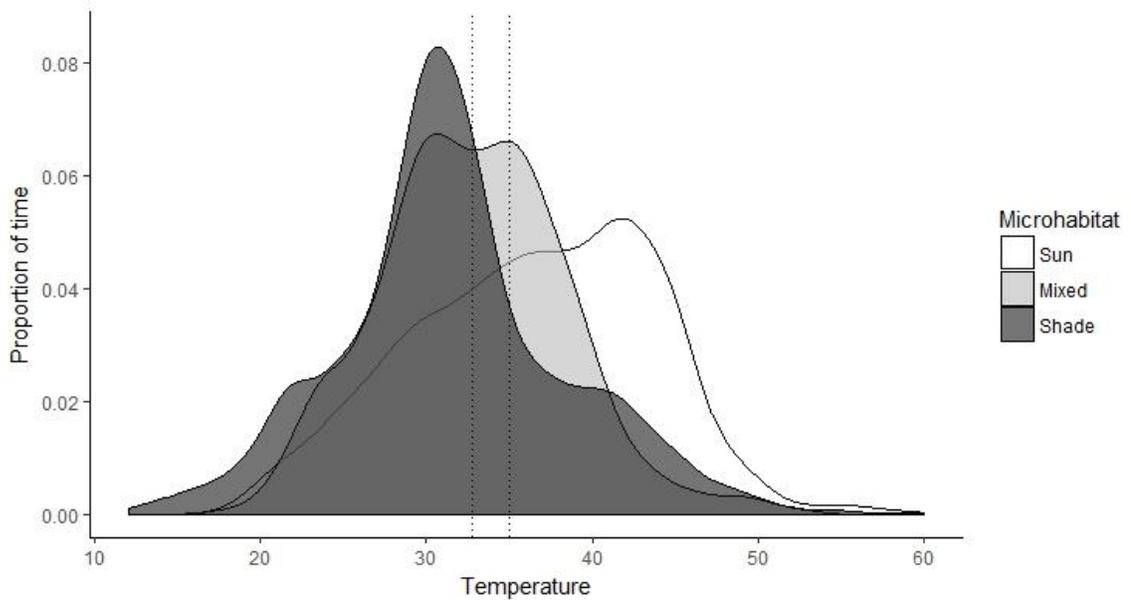


**Figure S5: Thermal quality (de) Plotted against Time of Day for *Sceloporus occidentalis* Active Hours (1100-1800).** Selected temperature (Tset) is represented here by the x-axis. A  $d_e$  value of 0 means the average operative temperature in that habitat fell within the Tset range. As the values move away from 0, the quality of the habitat decreases.

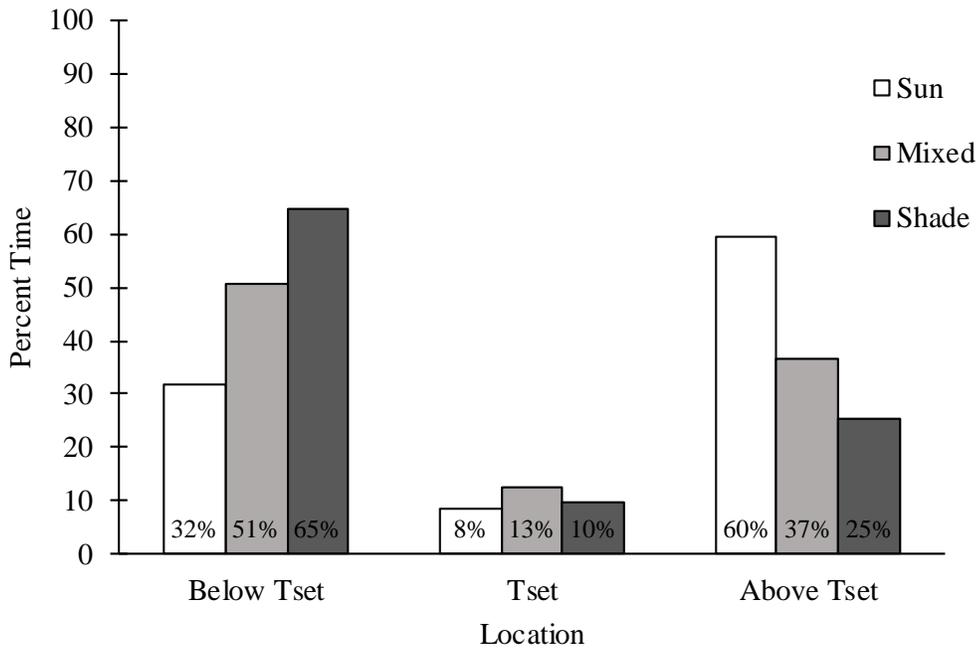


**Figure S6: Thermoregulatory Accuracy (db) Plotted Against Time of Day for *Sceloporus occidentalis* Active Hours (1100-1800).** The dotted line that begins after 1500 indicates that the hours 1600-1800 are not collected data, but rather a prediction based on the average temperature of the live lizards. The x-axis represents the selected temperature ( $T_{set}$ ). A  $d_b$  value of 0 means that the lizard body temperature was within the

$T_{set}$  range. As values move away from 0, the accuracy of thermoregulation decreases.



**Figure S7: A Density Plot that Maps the Proportion of Time our *Sceloporus occidentalis* Operative Temperature Models Spent at Different Temperatures ( $T_e$ ) for All Data Points Between the Hours 1100-1800.** The “sun” model  $T_e$ ’s were at temperatures  $>40$  °C most of the time and the “shade” model  $T_e$ ’s were typically around 30 °C, whereas the “mixed” model  $T_e$ ’s were somewhere in between. The dotted lines represent the selected temperature range ( $T_{set}$ ) found in the lab.



**Figure S8: The Distribution of Operative Temperatures ( $T_e$ ) in Relation to the Selected Temperature Range ( $T_{set}$ ) for each Microhabitat, Between the Hours 1100-1800.** “Sun” model  $T_e$ ’s are above  $T_{set}$  60% of the time and “shade” model  $T_e$ ’s are below

Tset 65% of the time.

**Table S1: Monthly Temperature Averages for the Capture Dates for Experiment 1 - Seasonal and Short-Term Acclimation Tests.** The temperatures were taken from the nearest weather station: San Luis Obispo County Regional Airport (Weather Underground). The capture dates were planned to be representative of the seasonal temperatures.

Season	Test date	Monthly average max. temperature (C)	Monthly average temperature (C)	Monthly average min. temperature (C)
Fall	October 22+ 24, 2016	27	19	16
	December 1 +3, 2017	19	14	9
Winter	February 15, 2017	18	13	7
Spring	May 20 +22, 2016	18	17	13
Summer	August 4+6, 2017	24	20	18

**Table S2: Variation in the Acclimatory Response Ratio (ARR) in Lizard Species Based on Thermoregulatory Strategy (Heliothermy or Thermoconformity)**

(Modified from Table S1 from Gunderson and Stillman 2015). ARR of CTmax is an index of plasticity, where higher ARR's indicate higher plasticity. Note that *Sceloporus occidentalis* has the lowest plastic response (0.01) of all the lizards shown here. The thermoregulatory strategy column (HT – Heliothermic, TC – Thermoconformer) is derived from Table 1 in Sinervo et al. 2010. Multiple temperatures listed in the acclimation time column indicate multiple temperature treatments. Asterisks by the acclimation time indicate that even though the acclimation time is listed as the number in the column, the lizards were held in lab housing for an extended time prior to testing. Asterisks by the thermoregulatory effectiveness (E) indicate the value was taken to be equal of congeneric species that had the value listed.

Species	TR strategy	Acclimation Time (Days)	Acclimation Temp. (°C)	Photo-period (Hr:Hr)	CTmax ARR	ARR Reference	Thermo Effect. (E)	E Reference
<i>Sceloporus occidentalis</i>	HT	14	15/25/35	16:8	-0.01	Kour and Hutchison 1970	NA	NA
<i>Sceloporus occidentalis</i>	HT	7	15/25/35	12:12	0.01	This study	0.96	Chapter 2
<i>Takydromus formosanus</i>	HT	14	10/20/30	12:12	0.03	Huang & Tu 2008	NA	NA
<i>Eremias argus</i>	HT	21 *	28/33/38	14:10	0.04	Hong et al. 2009	0.87	Shu-Ran et al. 2017
<i>Phrynocephalus versicolor</i>	HT	21	28/33/38	NA	0.04	Zheng et al. 2013	0.91*	Shu-Ran et al. 2017
<i>Takydromus hsuehshanensis</i>	HT	14	10/20/30	12:12	0.05	Huang & Tu 2008	NA	NA
<i>Takydromus stejnegeri</i>	HT	14	10/20/30	12:12	0.05	Huang & Tu 2008	NA	NA
<i>Phrynosoma cornutum</i>	HT	14	15/25/35	16:8	0.06	Kour and Hutchison 1970	0.39	Lara-Resendiz et al. 2015
<i>Sphenomorphus taiwanensis</i>	HT	14	10/20/30	NA	0.06	Huang et al. 2006	NA	NA

<i>Phrynocephalus guinanensis</i>	HT	21	28/33/38	NA	0.08	Zheng et al. 2013	0.91*	Shu-Ran et al. 2017
<i>Uta mearnsii</i>	HT	8.5 *	12/35	12:12	0.11	Murrish & Vance 1968	NA	NA
<i>Phrynocephalus vlangalii</i>	HT	21	28/33/38	NA	0.12	Zheng et al. 2013	0.91*	Shu-Ran et al. 2017
<i>Urosaurus ornatus</i>	HT	1-2 OR 8	22-26 OR 35	NA	0.13	Lowe and Vance 1955	0.76	Sartorius 2002
<i>Anolis sagrei</i>	TC	7 *	20/30	NA	0.14	Corn 1971	0.70	Wright 2009
<i>Takydromus septentrionalis</i>	HT	21 *	20/25/35	12:12	0.15	Yang et al. 2008	NA	NA
<i>Anolis carolinensis</i>	TC	14	15/25/35	16:8	0.16	Kour and Hutchison 1970	0.88	Claussen 1981
<i>Anolis distichus</i>	TC	7 *	20/30	NA	0.2	Corn 1971	0.88*	Claussen 1981 and Wright 2009
<i>Eremias brenchleyi</i>	HT	21*	28/33/38	14:10	0.2	Hong et al. 2009	0.88*	Shu-Ran et al. 2017
<i>Xantusia vigilis</i>	TC	14	15/25/35	16:8	0.2	Kour and Hutchison 1970	NA	NA
<i>Sphenomorphus incognitus</i>	HT	14	15/20/30	NA	0.22	Huang et al. 2006	NA	NA
<i>Eremias multiocellata</i>	HT	21*	28/33/38	14:10	0.24	Hong et al. 2009	0.89	Shu-Ran et al. 2017