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TITLE: Hydric Physiology of Lizards

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ABSTRACT

Hydric Physiology of Lizards

Savannah Weaver

Chapter 1: Animals can respond to extreme climate by behaviorally avoiding it, or by physiologically coping with it. We understand behavioral thermoregulation and physiological thermal tolerances, but water balance has largely been neglected. Climate change includes both global warming and changes in precipitation regimes, so improving our understanding of organismal water balance is increasingly urgent. We assessed the hydric physiology of endangered Blunt-nosed Leopard Lizards (Gambelia sila) by measuring cutaneous evaporative water loss (CEWL), plasma osmolality, body mass, and body condition throughout their active season. On average, G. sila had low CEWL that is likely desert-adaptive, and high plasma osmolality that is indicative of dehydration. Given that our study was in a drought year, it is reasonable to believe that every lizard measured was dehydrated to a degree. We hypothesized that throughout the G. sila active season, as their habitat got hotter and drier, G. sila would become increasingly dehydrated and watertight. Instead, CEWL and plasma osmolality showed minimal change for females and nonlinear change for males, which we hypothesize is connected to sex-specific reproductive behaviors and changes in food availability. We also measured thermoregulation and microhabitat use, expecting that more hydrated lizards would have higher body temperature, better thermoregulatory accuracy, and spend more time aboveground. However, we found no effect of CEWL, plasma osmolality, body mass, or body condition on these thermal and behavioral metrics. We posit either that G. sila tolerate dehydration to maintain activity during their brief active season, or that because every lizard was dehydrated due to the drought, they all experienced equally constrained thermoregulation and microhabitat use. Finally, G. sila spend considerable time underground in burrows, and we believe burrows serve as essential hydric, not only thermal, refugia. Our findings suggest that these lizards might benefit from artificial humid refugia and supplemental hydration, especially during drought.

Chapter 2: Testing acclimation plasticity informs our understanding of functional biodiversity and applies to conservation management amidst our rapidly changing climate. While there is a wealth of research on the plasticity of thermal and hydric physiology in response to temperature acclimation, there is a comparative gap for research on acclimation to different hydric regimes, as well as the interaction between water and temperature. We sought to fill this gap by acclimating Western Fence Lizards (Sceloporus occidentalis) to experimental climate conditions (crossed design of Hot or Cool, Dry or Humid) for eight days, and measuring cutaneous evaporative water loss (CEWL), plasma osmolality, hematocrit, and body condition before and after acclimation under common conditions. CEWL changed plastically in response to the different climates, with lizards acclimated to Hot Humid conditions experiencing the greatest increase in CEWL. Change in CEWL among individuals was negatively related to treatment vapor pressure deficit. Plasma osmolality, hematocrit, and body condition all showed greater changes in response to temperature than to humidity or vapor pressure deficit. CEWL and plasma osmolality were positively related across treatment groups.
before acclimation and within treatment groups after acclimation, but the two variables showed different responses to acclimation, suggesting that they are interrelated but governed by different mechanisms. This study is among just a small number of studies that assess more than one metric of hydric physiology and that test the interactive effects of temperature and humidity. Such measurements will be essential for predictive models of activity and survival for animals under climate change.
ACKNOWLEDGMENTS


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CHAPTER 1
INTRODUCTION

Environmental water availability and organismal water requirements determine species energy balance, fitness, and distributions (Kearney et al 2018; Pirtle et al 2019). It is generally understood that organismal evaporative water loss is adapted to environmental aridity (Cox and Cox 2015), yet little is known about most organisms’ water requirements, tolerances, and variability. A better understanding of hydric physiology would lead to new insights about animal behavior, improved modeling of species distributions, and more effective conservation management.

The endangered Blunt-nosed Leopard Lizard (*Gambelia sila*) of the San Joaquin Desert is one such organism with a wealth of knowledge about their life history, ecology, and thermal behaviors (Gaudenti et al 2021; Ivey et al 2020; Montanucci 1965, 1967; Tollestrup 1982, 1983), but no assessment of their hydric physiology. *Gambelia sila* have been extirpated from 95% of their historical range (Williams et al 1998), and the patchy extant populations will increasingly experience hot habitat that limits their behavior (Ivey et al 2020). The hydric physiology and limits of *G. sila* could multiply these likely detrimental thermal effects of climate change, or be the basis their resilience. Thus, for Chapter 1, I set out to quantify the water balance of *G. sila* by measuring their cutaneous evaporative water loss (CEWL) and plasma osmolality, and quantifying variation across individuals and throughout time. I also tested relationships between hydric physiology and thermal ecology and behavior. Water and temperature are inextricably linked (RR), so assessing the patterns and interactions of both hydric and thermal physiologies and
ecologies is crucial to understand organismal behavior and to predict and mitigate the effects of climate change.

Given that physiological plasticity is likely to determine resiliency to climate change (Gunderson and Stillman, 2015; Riddell et al., 2018; Seebacher et al., 2015; Somero, 2010; Stillman, 2003; Urban et al., 2014), I was specifically interested in testing the plasticity of CEWL in response to different climates. However, given that G. sila are endangered, for Chapter 2, I used very common Western Fence Lizards (Sceloporus occidentalis) for an experiment to test the plasticity of CEWL under different vapor pressure deficits, or different levels of dryness of the air. I acclimated S. occidentalis to four different combinations of temperature and humidity, creating a gradient of vapor pressure deficits, and measured the change in CEWL and plasma osmolality. This acclimation study is one of very few to test plasticity of CEWL, and the first to look at the interrelation among different metrics of hydric physiology. The studies presented in this thesis push forward the nascent field of hydric physiology and ecology, with application to the conservation management of G. sila.
CHAPTER 2

HYDRIC PHYSIOLOGY AND ECOLOGY OF AN ENDANGERED DESERT LIZARD

2.1 Introduction

Desert animals live life at the extreme, experiencing severe temperatures and sparse water resources. To avoid such harsh conditions, animals can behaviorally buffer themselves by selecting favorable microhabitats (Sunday et al., 2014), and by limiting their activity to specific times of year (Storey, 2002) or times of day (Abom et al., 2012; DeGregorio et al., 2018). Crocodilians, tortoises, and lungfish all estivate in burrows when under threat of desiccation (Nagy and Medica, 1986; Nagy, 1988; Fishman et al., 1992; Christian et al., 1996). However, behavioral responses to climate are only effective when microhabitat heterogeneity is available (Sears et al., 2016). Thus, physiological limits are what ultimately determine the geographic distribution of species (Kearney et al., 2018). There has been a wealth of research on such thermal limits (Angilletta, 2009; Sinervo et al., 2010; Taylor et al., 2020), but a comparative paucity of research on hydric limits. Climate change is well underway (IPCC, 2021), and hydric costs of thermoregulation are likely the biggest drivers of population declines (Riddell et al., 2019a, 2021). Clearly, it is imperative that we understand the water balance and dehydration tolerances of organisms to draw a complete picture of ecological physiology and to predict how species may or may not cope with climate change. This is especially urgent for threatened and endangered species adapted to arid environments, which are already living under hydric stress.
Blunt-nosed Leopard Lizards (*Gambelia sila*) are endangered desert lizards that have been the subject of a wealth of thermal physiology and ecology research (e.g., Germano, 2019; Ivey *et al.*, 2020; Gaudenti *et al.*, 2021), but no hydric physiology research. Adult *G. sila* are typically active aboveground from April to July each year, with some opportunistic activity in March, September, and October (Montanucci, 1965; Germano and Williams, 2005), and they rely on perennial shrubs, large annual forbs, and mammal burrows to behaviorally buffer themselves from midday and late summer heat (Ivey *et al.*, 2020; Gaudenti *et al.*, 2021). Despite having “apparently no requirement for water” (Ahlborn, 2000), and lower likelihood of occurrence where rainfall is higher (Stewart *et al.*, 2019), studies suggest that *G. sila* fail to reproduce in drought years (Germano and Williams, 2005; Westphal *et al.*, 2016). So, while these lizards may be able to behaviorally buffer themselves from temperature extremes, water restriction appears detrimental. Accordingly, we set out to characterize the hydric physiology of *G. sila*.

To assess hydric physiology, we focused on: cutaneous evaporative water loss (CEWL), which estimates the permeability of the skin to water loss to the environment; plasma osmolality, the concentration of solutes in the blood plasma, which is an estimate of dehydration level; body mass, which indicates how well organisms are eating and drinking; and body condition, which assesses body mass relative to body length. At acute time scales of minutes to hours, exposure to hot or dry conditions can rapidly increase CEWL (Pintor *et al.*, 2016; Vicenzi *et al.*, 2021; Warburg, 1965). Hot or dry conditions increase vapor pressure deficit, the drying power of the air, which drives water down its concentration gradient, from organisms to the environment. The resulting high CEWL
might lead to dehydration, measured as increased plasma osmolality. However, organismal physiology can counteract this by reducing CEWL when exposed to high vapor pressure deficit, a pattern observed at acclimation (Kattan and Lillywhite, 1989; Weaver et al., 2022; Chapter 2) and adaption time scales (Cox and Cox, 2015). Whether high CEWL leads to dehydration, or dehydration triggers reduced CEWL, animals benefit from adjusting their postures to and seeking refugia that decrease the amount of water they lose to their environment (Dezetter et al., 2022; Pirtle et al., 2019). This water conservation can even be more important than thermoregulation and activity for foraging and mating (Davis and DeNardo, 2009; 2010; Pintor et al., 2016). And yet, widespread investigation of these phenomena has only just begun. If we are to conserve endangered G. sila, then understanding the relative importance of water compared to temperature and activity is imperative.

As temperatures increase throughout their active season, G. sila spend progressively more time belowground (Ivey et al., 2020; Gaudenti et al., 2021), but their decreased surface activity could also be related to dehydration (Davis and DeNardo, 2009). Because they are adapted to desert conditions, we hypothesized that G. sila should have extremely low water loss rates (Cox and Cox, 2015), and that variation in these rates among individuals would be impacted by lizards’ hydration. To assess their typical hydration levels and water loss rates, we measured body condition, plasma osmolality, and CEWL of G. sila throughout their active season. We predicted that G. sila would have higher plasma osmolality (e.g., be less hydrated) and lower CEWL compared to mesic species. We also expected that plasma osmolality would increase and CEWL would decrease as their habitat got hotter and drier throughout their active season (Davis
and DeNardo, 2009, 2010). We also predicted the more-hydrated females would have greater reproductive investment. In addition to measuring hydric physiology, we used temperature-sensing radio-transmitters to record body temperature and track microhabitat use. We hypothesized that hydration would constrain thermoregulation, predicting that more hydrated individuals would maintain higher body temperatures, have better thermoregulatory accuracy, and spend more time aboveground (Davis and DeNardo, 2009; Sannolo and Carretero, 2019).

2.2 Methods and Materials

2.2.1 Capture & Identification

In the spring and summer of 2021, we used hand-held lassos to capture 79 (32F, 47M) G. sila (Table 1). Late April and early May mark the beginning of the active season for these lizards, when most G. sila emerge from overwintering in rodent burrows (Montanucci, 1965). Each lizard was PIT-tagged for identification upon recapture (8mm MUSICC Integrated Chip; Avid Identification Systems, Inc, Norco, CA, USA), and a subset of lizards (Table 1) were fitted with VHF temperature-sensing radio transmitters with 16 cm whip antenna (model BD-2T; Holohil Systems Ltd, Carp, Ontario, Canada). Transmitters were attached with epoxy to 2.4 mm diameter metal alloy ball chain collars, which were fitted around lizards’ necks, sized large enough to permit swallowing large prey but small enough that they would not slip over their head, and the ends were clamped shut in a ball chain clasp; transmitter collars were 3-9% of lizards’ mass. Throughout the study, five transmitter signals disappeared and eight lizards lost their transmitter collars; we re-fitted two of the lost collars to different lizards in May (Table
1). Handling of *G. sila* was approved under federal permit TE-166383, a state MOU issued to M. Westphal in 2018, and Cal Poly IACUC #1809.

**Table 2.1.** Number of *Gambelia sila* measured during each measurement period of the study. Numbers in parentheses are the subset of lizards that were fitted with radio-transmitter collars.

<table>
<thead>
<tr>
<th>Measurement Period</th>
<th>Newly captured lizards</th>
<th>Re-captured lizards</th>
<th>Total</th>
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<tr>
<td>April 23-25</td>
<td>67 (39)</td>
<td>-</td>
<td>67</td>
</tr>
<tr>
<td>May 7-8</td>
<td>11 (2)</td>
<td>26 (21)</td>
<td>37</td>
</tr>
<tr>
<td>July 14</td>
<td>1</td>
<td>14 (14)</td>
<td>15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>79</strong></td>
<td><strong>40</strong></td>
<td><strong>119</strong></td>
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### 2.2.2 Hydric Physiology

At each capture or recapture, we collected the following data: lizard mass (Pesola 50-100 g precision scale; ± 0.5 g), snout-vent-length (SVL; ± 1 mm), sex, blood samples either from caudal venipuncture using heparinized needles or from the post-orbital sinus of the right eye using heparinized microhematocrit capillary tubes (Clay Adams, Becton Dickinson, Sparks, MD, USA), and cutaneous evaporative water loss (CEWL; ± g m$^{-2}$ h$^{-1}$) in 3-5 technical replicates on the mid-dorsum using an AquaFlux evaporimeter (model AF200; BioX Systems, London, UK). The AquaFlux measures CEWL as instantaneous movement of water across the skin of a 3 mm diameter area within a closed chamber. When the measurement chamber is pressed against the skin and a closed system is created, accumulated water is removed, then instantaneous water flux is recorded when the reading stabilizes to ± 0.02 g m$^{-2}$ h$^{-1}$. The device has high repeatability (Imhof *et al.*, 2014), and it was calibrated following manufacturer guidelines prior to our study, and before each set of measurements. Additional explanations and uses of the AquaFlux
evaporimeter are available (Imhof et al., 2009; Elkeeb et al., 2010; Lourdais et al., 2017; Weaver et al., 2022). Immediately following CEWL measurements, we recorded lizard body temperature (± 1°C) by inserting a thermocouple into the cloaca (Traceable Pocket-size K-type Thermocouple, model 14-649-81; Thermo Fisher Scientific, Waltham, MA, USA). The evaporimeter used to measure CEWL also recorded ambient temperature (± 0.1°C) and relative humidity (± 0.1 %) at the time of and immediately adjacent to the site of measurement. We used these temperature and relative humidity values to calculate vapor pressure deficit (a measure of the dryness of the air; kPa) at the time of CEWL measurement using the equation:

\[ VPD = 0.611 \times e^{\frac{17.502 T}{T + 246.15}} \times (1 - \frac{RH}{100}) \]

where T is temperature (°C) and RH is percent relative humidity (Campbell and Norman, 1998).

Blood samples were stored on ice for transport to the lab, then centrifuged in a micro-hematocrit centrifuge (model IEC MB; Damon IEC Division, Thermo Fisher Scientific) for two minutes, and percent hematocrit (± 1%) was recorded. Plasma osmolality (± 3 mmol kg\(^{-1}\)) was measured in 1-3 technical replicates on a vapor pressure osmometer (VAPRO, model 5600; Wescor, ELITech, Logan, UT, USA) within 48 hours of blood collection.

To suggest whether G. sila are adapted to their desert environment, we compared their CEWL and plasma osmolality to Western Fence Lizards (Sceloporus occidentalis), a mesic lizard measured following the same methods (Weaver et al., in revision). For S. occidentalis, we measured CEWL in 5 technical replicates on the mid-dorsum using the same evaporimeter. CEWL of both G. sila and S. occidentalis was measured on the same
day of capture, within a few hours of capture. Blood from *S. occidentalis* was collected from the post-orbital sinus using microhematocrit tubes, and samples were processed and plasma osmolality measured in the same way as for *G. sila*.

In April and May, we also assessed gravidity of 19 radio-collared females and the approximate stage of their egg development by palpating the lower abdomen. Gravidity was not assessed for females that were not radio-collared, as they were typically small, and we assumed them to be reproductively inactive. We examined egg development including clutch and egg size using an ultrasound (Sonosite M-Turbo with a HFL50x/ 15-6Mhz transducer; FUJIFILM Sonosite Inc, Bothell, WA). All measurements and samples were taken within 2-3 hours of capture, with the exception of some females being ultrasound and some lizards being given supplemental hydration (see next paragraph) the morning following capture. Immediately after measurements, lizards were released at their location of capture.

In an attempt to experimentally alter hydration via water supplementation and to investigate willingness of *G. sila* to drink water, we assigned radio-collared lizards to either water treatment (N=22, 11M:11F) or sham treatment (N=19, 10M:9F). Following measurements, lizards in the water treatment group were kept in plastic bins (42x27x16 cm) for ~1 hr with 2-3-inches of water in which they could easily stand and lean down to drink. Lizards in the sham treatment group were kept in the same bins, for the same amount of time, but with dry newspaper substrate to control for the effects of handling. For some water treatment lizards, we also tried dripping water into their mouths using a plastic dropper. The same handling was done for sham treatment lizards, but with no
water. We recorded individual mass before and after water/sham treatment as a proxy for how much water individuals drank, if at all.

2.2.3 Thermal Ecology & Behavior

We radio-tracked lizards between 0700 to 1800 daily from the time they were fitted with collars through mid-July using VHF receivers (TR-8 Handheld Scanning Receivers; Telonics Inc., Mesa, AZ, USA) fitted with antennas (Yagi; Communications Specialists, Orange, CA, USA). Due to dropped transmitter collars and lost signals, we tracked 39 of the 41 collared lizards for a mean ± SD of 45 ± 38 observations over 45 ± 29 days per lizard. We recorded the microhabitat use of each lizard each time we observed it as one of the following: “Burrow,” when lizards were underground inside burrows engineered by Giant Kangaroo Rats (*Dipodomys ingens*), including lizards that could not be seen and those that were visible deep inside the burrow; “Full Shade,” when lizards were under the shade of a shrub and the entire body was shaded; “Partial Shade,” when lizards were under shade but part of the body was in the sun; and “Open,” when lizards were aboveground in full sun, not under the shade of a shrub, including when at the mouth or apron of a burrow but still visible from above.

We also recorded field active body temperature (*T*<sub>b</sub>) with a stationary 3 m tall solar-powered omni-antenna (model RA-6B; Telonics, Mesa, AZ, USA; RemotePro 2.5 W Solar Power System; Tycon Systems, Bluffdale, UT, USA; Eldora 10P solar panel; Vikram Solar Ltd, West Bengal, India) and receiver with data acquisition system (TR-5 Option 320; Telonics). This receiver system logged the temperature-sensitive inter-pulse interval of the signal from each transmitter collar within its 300 m range every 5-10
minutes, and those values were converted to degrees Celsius based on manufacturer-provided transformation curves. The transmitter collars were externally attached to the lizard, so $T_b$ refers to surface body temperature, and may overestimate core body temperature when lizards are basking in the sun, but surgically implanting transmitters is not feasible for endangered lizards. At the end of the study in July, we excavated 11 of the estivating lizards and recaptured the three lizards that were still active aboveground. Only these 14 lizards survived to the end of the active season and/or still had detectable radio-transmitter signals; there were three other lizards that we tracked to the end of the study, but we were unable to excavate them and remove their collars. Throughout the season, we found 13 dropped transmitter collars, nine of which may have simply fallen off the lizard, and four of which showed clear signs of depredation. We lost the signal of 11 transmitter collars, which could be due to dead batteries or lizards that were carried away by avian predators.

2.2.4 Climate Conditions

At our study site, we recorded temperature and relative humidity 1 m inside 11 burrows every 30 minutes (HOBO External Temperature/RH Sensor Data Loggers, model MX2302A; Onset Computer, Bourne, MA, USA) throughout the study. We also obtained hourly ambient temperature, relative humidity, wind speed, and precipitation from a weather station 3.7 km due east of the study site (station ID CXXC1; Natural Resources Conservation Service, 2021).
2.2.5 Statistical Analysis

We used linear regression (LR) to assess whether our measures of hydric physiology—CEWL, plasma osmolality, hematocrit, body mass, and body condition—were interrelated. We also used LRs to quantify the effect of lizard body temperature, ambient temperature, and ambient vapor pressure deficit at the time of measurement on CEWL. Lizards ranged from small yearlings to large adults, so we calculated body condition as scaled mass index (g’; Peig and Green, 2009) using a scaling equation derived from our April measurements. Body condition is an assessment of relative body mass for a given lizards’ body length; for gravid female lizards, body condition is expected to be high and mostly represent reproductive progress rather than body condition alone. Based on the lizards with repeat measurements, SVL did not change throughout the study (linear mixed-effect model with individual lizard ID as a random effect: estimate = 0.1, t = 0.7, p = 0.5). To determine whether our supplemental hydration for radio-collared lizards was successful, we ran LR and compared the marginal mean change in mass during the 1 hr hydration treatment for hydration versus sham lizards. To test differences in plasma osmolality, CEWL, and temperature and vapor pressure deficit at the time of CEWL measurement between *G. sila* and *S. occidentalis*, we ran LR for each variable with species as the explanatory variable, then we calculated the effect estimates as marginal means and confidence intervals.

To assess how hydric physiology changed throughout the *G. sila* active season, we used linear mixed-effect models (LMMs) to quantify how CEWL, plasma osmolality, hematocrit, body mass, and body condition differed across measurement periods, with individual lizard ID as a random effect to account for repeated measurements (1-3
measurements per lizard). We tested interactions of measurement period with supplemental hydration treatment and lizard sex. For CEWL, we tested the addition of covariates ambient temperature and vapor pressure deficit at the time of CEWL measurement. To assess whether the likelihood of a female being gravid was related to CEWL, plasma osmolality, hematocrit, body mass, or body condition, we used generalized linear models (GLMs) with a binomial distribution. Individual lizard ID was not included as a random effect because it accounted for a negligible amount of variation.

For each lizard, we took a subset of daily $T_b$ data between the 80-90th percentiles, then calculated daily “high $T_b$” as the mean of that subset. We used the 80-90th percentiles instead of actual daily maximum $T_b$, and extreme surface body temperature values from each lizard (>2 SD away from individual mean) were omitted (<5% of all points), because the highest temperature values for body surface temperatures tend to be inflated due to the lizard basking and exposing their temperature-sensing radio-collar directly to the sun. We calculated thermoregulatory accuracy by subtracting $T_{set}$ (Gaudenti et al., 2021; Ivey et al., 2020) from each instance of daytime $T_b$ (between 0700 to 1900; as in Ivey et al., 2020) and taking the absolute value (Hertz et al., 1993), so zero refers to perfect accuracy, $T_b$ within $T_{set}$, and greater values refer to $T_b$ further from the $T_{set}$ range. Microhabitat use was calculated for individual lizards and for all radio-tracked lizards overall as the proportion of total observations that were in each microhabitat; we also calculated proportion of time aboveground (in Open or Shade microhabitats) versus belowground (in Burrow microhabitat).

To assess the effect of hydric physiology on high $T_b$, thermoregulatory accuracy, and proportion of time spent aboveground, we paired the April and May hydric
physiology measurements from each lizard with their average behavior and thermal ecology during each 11-day time interval following that hydric physiology measurement period. We ran a LMM on each relationship with individual lizard ID as a random effect. Clustering the data into 11-day time intervals was arbitrary, and simply fit with the hydric physiology measurement periods. We followed the same 11-day time interval clustering pattern to look at how microhabitat use and climate changed throughout the active season, and to be able to draw connections to hydric physiology measurement periods. To assess whether the probability that a lizard would be found belowground (in Burrow microhabitat) changed throughout the active season, we ran a generalized linear mixed-effects model with time interval, lizard sex, and their interaction as explanatory variables, a binomial distribution, and individual lizard ID as a random effect.

We calculated vapor pressure deficit (Campbell and Norman, 1998) for all recorded temperature and relative humidity values for both burrow and weather station data with the same equation outlined above. For each burrow in which we measured microclimate, we calculated the daily mean temperature, relative humidity, and vapor pressure deficit, then took the average of those daily means for each 11-day time interval throughout the active season, calculated from at least six days of data. To assess how burrow microclimate changed throughout the active season, we used LMMs to quantify how daily means differed across time intervals, with burrow ID as a random effect.

For the local aboveground climate, we calculated the average daily mean daytime and nighttime temperature, relative humidity, vapor pressure deficit, and wind speed for each 11-day interval throughout the active season. Daytime was defined as the time between sunrise and sunset at our study site (Global Monitoring Laboratory, 2021).
Burrow microclimate was not different between daytime and nighttime for a given burrow or time interval. We also used the local weather station to get the annual cumulative winter precipitation from December to March for 2018-2021. All statistics and figures were done in R v4.2.2 (R Core Team, 2022) using tidyverse workflow (Wickham, 2022). We used the lm function for linear models; the lmer function in the lmerTest package for LMMs (Kuznetsova et al., 2020); the anova function for type 3 sum of squares values with Kenward-Roger degrees of freedom; and the emmeans and pairs functions in the emmeans package for marginal means, confidence intervals, and their pairwise differences (Lenth et al., 2022). Plots were made with ggplot2 (Wickham et al., 2022). Functions without specified packages are from base R. χ² tests are type 2 with a Pearson distribution. Data and code are archived on Zenodo (will publish and create DOI after review).

2.3 Results

2.3.1 Hydric Physiology

On average, lizards in the supplemental hydration group gained 0.37 g during treatment while lizards in the sham group lost 0.77 g (t = -3.9, p = 0.0002; F(1,59) = 15.46, p = 0.0002), which amounted to average gains of 1% body mass for supplemental hydration lizards versus losses of 2% body mass during the treatment hour (t = -3.7, p = 0.0005; F(1,59) = 13.41, p = 0.0005). However, we did not observe lizards drinking water in the treatment bins, and lizards refused to drink from the plastic dropper. There was no difference in the direction or amount of change in CEWL, plasma osmolality, or lizard mass throughout the study based on the hydration treatments given in April and May (all
|t| < 1, p > 0.4). We also detected no differences in high Tb, thermoregulatory accuracy, or microhabitat use based on supplemental hydration (all |t| < 1.7, p > 0.1). Thus, for all other analyses, the data for the different treatments are pooled.

Compared to more-mesic Western Fence Lizards (Sceloporus occidentalis), on average, G. sila had half the rate of CEWL: 10.4 ± 0.9 g m$^{-2}$ h$^{-1}$ versus 20.8 ± 0.8 g m$^{-2}$ h$^{-1}$ (marginal means ± 95% confidence interval; Fig 1; Weaver et al., in revision). Gambelia sila were also less hydrated on average, with plasma osmolality 368 ± 2 mmol kg$^{-1}$ compared to 348 ± 2 mmol kg$^{-1}$ for S. occidentalis (Fig 1A). Also, we found this difference in CEWL despite G. sila being measured at higher temperatures (29.4 ± 0.4°C versus 27.2 ± 0.4°C; Fig 1B) and vapor pressure deficits (3.3 ± 0.1 kPa versus 2.0 ± 0.1 kPa; Fig 1C).
Figure 2.1. Cutaneous evaporative water loss (CEWL) of desert Blunt-nosed Leopard Lizards (*Gambelia sila*) captured in the Carrizo Plain National Monument, CA versus mesic Western Fence Lizards (*Sceloporus occidentalis*) captured throughout the campus of California Polytechnic State University, San Luis Obispo, CA (Weaver et al., in revision). CEWL is compared relative to hydration (plasma osmolality; A), and ambient temperature (B) and vapor pressure deficit (VPD; C) at the time of CEWL measurement. Each small point represents a measurement of an individual lizard taken shortly after their capture; the data for *G. sila* include 1-3 measurements per individual. Large points represent marginal means. 95% confidence intervals were removed because they were so small that they were obscured by the mean points. CEWL, plasma osmolality, and temperature and VPD at the time of measurement were all significantly different between species (all p < 0.0001).

CEWL, plasma osmolality, body mass, and body condition of *G. sila* all showed temporal variation, with different patterns based on lizard sex (Table 2). Female lizards experienced no change in CEWL or plasma osmolality throughout the active season (Figs 2A, 2B). CEWL for male lizards was significantly lower in May than it was for April or July (Fig 2A), and plasma osmolality for male lizards was significantly lower in July than in May (Fig 2B). The relative differences by sex and measurement period in CEWL and
plasma osmolality were the same when assessed only in lizards with repeated measurements, with some statistical differences: plasma osmolality also differed between sexes in May ($t = -3.8$, $p = 0.0003$); and male plasma osmolality differed between April and July ($t = 2.5$, $p = 0.04$). Covariates temperature and vapor pressure deficit at the time of CEWL measurement were nonsignificant when added to the CEWL model, whether our entire dataset or only repeat measures were used (LMM on full dataset: $SS_{\text{temp}} = 9$, $F(1,110)_{\text{temp}} = 0.8$, $p_{\text{temp}} = 0.4$; $SS_{\text{VPD}} = 19$, $F(1,110)_{\text{VPD}} = 1.8$, $p_{\text{VPD}} = 0.2$; compare to Table 2).

### Table 2.2. Linear mixed-effect model results for how much of the variation in cutaneous evaporative water loss (CEWL), plasma osmolality, body mass, and body condition of *Gambelina sila* is explained by measurement period (month) throughout the active season, lizard sex, and their interaction.

<table>
<thead>
<tr>
<th>Hydric Response Variable</th>
<th>Explanatory Variable</th>
<th>SS</th>
<th>F statistic (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEWL (g m$^{-2}$ h$^{-1}$)</td>
<td>Month</td>
<td>120</td>
<td>5.63 (2,85)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>34</td>
<td>3.22 (1,99)</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>Month*Sex</td>
<td>47</td>
<td>2.2 (2,85)</td>
<td>0.12</td>
</tr>
<tr>
<td>Plasma Osmolality (mmol kg$^{-1}$)</td>
<td>Month</td>
<td>6749</td>
<td>5.83 (2,92)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>1441</td>
<td>2.49 (1,88)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Month*Sex</td>
<td>770</td>
<td>0.67 (2,92)</td>
<td>0.52</td>
</tr>
<tr>
<td>Body Mass (g)</td>
<td>Month</td>
<td>388</td>
<td>11.72 (2,50)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>53</td>
<td>3.19 (1,102)</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>Month*Sex</td>
<td>83</td>
<td>2.5 (2,50)</td>
<td>0.092</td>
</tr>
<tr>
<td>Body Condition (g$^{'}$)</td>
<td>Month</td>
<td>400</td>
<td>18.58 (2,80)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>12</td>
<td>1.08 (1,103)</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Month*Sex</td>
<td>138</td>
<td>6.42 (2,80)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Abbreviations: SS = partial sum of squares, obtained using type 3 ANOVA with Kenward-Roger degrees of freedom; Month = measurement period (April, May, or July); Sex = lizard sex (Male, Female); Month*Sex = interaction effect.
Figure 2.2. Cutaneous evaporative water loss (CEWL; A), plasma osmolality (B), and body condition (C; Peig and Green, 2009) of *Gambelia sila* differed across measurement periods throughout their active season and based on lizard sex. Large points with error bars represent marginal means ± 95% confidence intervals. Each small point represents a measurement on an individual lizard. Sample sizes are noted on the x-axis. Different letters denote significant pairwise differences between measurement periods for a given sex (p < 0.0001 for females; p < 0.04 for males). Asterisks denote significant pairwise differences between sexes for a given measurement period (p < 0.04). See Table 2 for linear mixed-effects model results. These means are on the full dataset from our study, which includes 1-3 measurements on any given lizard; means based only on data from lizards with 2-3 repeat measurements showed the same patterns.
On average, the body condition of female lizards was higher in May than in April or July, to be expected due to our inclusion of gravid females, while male lizards had consistent body condition from April to May, then decreased in July (Fig 2C). The pattern for mass (not shown in a Figure) was the same as body condition for females; for males, the only difference was that mass was not different between April and July ($t = 1.2, p = 0.4$). Mass was significantly different between sexes in July ($t = -2.1, p = 0.04$), but not April or May (both $|t| < 1.6, p > 0.1$). When assessed only in lizards with repeated measurements, we found similar trends: males again had the same decrease in body condition in July ($t = 2.7, p = 0.03$), but no temporal change in mass (all $|t| > 1.2, p > 0.08$); female body condition showed the same pattern (Fig 2C); female mass differed between all measurement periods (all $|t| > 2.5, p < 0.05$); and the only difference between sexes was body condition in May ($t = 2.9, p = 0.005$).

Of the 19 radio-collared female lizards we ultra-sounded in April and May, 12 were gravid at one or both measurement periods (Fig 3). Each gravid female had 3-5 eggs. The number of eggs a female had was positively correlated with her SVL ($F(1,6) = 19.70, p = 0.004$) and body mass ($F(1,6) = 14.79, p = 0.008$). For the females that were gravid by May, regardless of whether they were gravid in April, CEWL did not change from April to May ($t = -0.6, p = 0.6$). Conversely, plasma osmolality ($t = 2.9, p = 0.03$), body mass ($t = 3.5, p = 0.01$), and body condition ($t = 2.7, p = 0.04$) all increased from April to May. These changes in gravid females mirror the average change for all female lizards in this study, which includes the 16 small females for which gravidity was not assessed (Fig 2). The probability of a female being gravid was not affected by their CEWL rates, plasma osmolality, or body condition (all $X^2 < 1.6$, all $p > 0.2$). The positive
effect of body mass on the likelihood of a female being gravid was marginally nonsignificant (Fig 3).

![Gravidity and body mass of female Gambelia sila at the beginning of their active season. Each point represents an individual female. Standalone points are females with only one measurement. Points connected by a line represent females with repeat measurements. Greater body mass was related to a higher probability of a female being or becoming gravid ($\chi^2(1) = 3.5, p = 0.06$).](image)

**Figure 2.3.** Gravidity and body mass of female *Gambelia sila* at the beginning of their active season. Each point represents an individual female. Standalone points are females with only one measurement. Points connected by a line represent females with repeat measurements. Greater body mass was related to a higher probability of a female being or becoming gravid ($\chi^2(1) = 3.5, p = 0.06$).

When we assessed the interrelation of hydric physiology metrics, plasma osmolality explained a significant amount of the variation in CEWL, with a marginally nonsignificant interaction with measurement period (Fig 4). CEWL could also be explained by negative relationships with hematocrit ($F(1,114) = 9.86, p = 0.002$) or body condition ($F(1,116) = 3.89, p = 0.05$), but had no relationship with lizard mass ($F(1,116) = 1.44, p = 0.2$). In turn, variation in plasma osmolality could be explained by positive relationships with hematocrit ($F(1,114) = 15.07, p < 0.001$) or body condition ($F(1,114) = 5.30, p = 0.02$), but had no relationship with lizard mass ($F(1,114) < 0.001, p = 1$).

Hematocrit and body condition were positively related for female lizards, but had no relationship for male lizards (direct effect: $F(1,112) = 9.03, p = 0.003$; interaction effect: $F(1,112) = 7.97, p = 0.005$).
Figure 2.4. Relationship between cutaneous evaporative water loss (CEWL) and plasma osmolality of *Gambelia sila* based on measurement period throughout their active season. Each small point is a different lizard at a given time point. Lines represent linear regression. The relationship between CEWL and plasma osmolality was significant ($F(1,110) = 5.07, p = 0.03$), and the interaction effect of measurement period was marginally nonsignificant ($F(2,110) = 2.77, p = 0.07$).

### 2.3.2 Thermal Ecology & Behavior

The proportion of time lizards spent aboveground was not affected by CEWL ($F(1,3) = 6.1, p = 0.1$), plasma osmolality ($F(1,8) = 0.5, p = 0.5$), body mass ($F(1,22) = 0.6, p = 0.5$), or body condition ($F(1,23) = 0.3, p = 0.6$). High $T_b$ was positively related to body condition ($F(1,41) = 4.3, p = 0.04$), but the effect was extremely small (Fig 5). High $T_b$ was not related to CEWL ($F(1,42) = 0.3, p = 0.6$), plasma osmolality ($F(1,47) = 3.0, p = 0.09$), or body mass ($F(1,42) = 1.5, p = 0.2$). Thermoregulatory accuracy was positively related to plasma osmolality ($F(1,18) = 7.3, p = 0.01$), but the effect was again extremely small (Fig 5). Thermoregulatory accuracy was not explained by CEWL ($F(1,48) = 0.8, p$
= 0.4), body mass (F(1,39) = 1.6, p = 0.2), or body condition (F(1,46) = 2.6, p = 0.1).

None of these relationships differed based on lizard sex.

![Correlation heatmap of Gambelia sila microhabitat use (as the proportion of time spent aboveground), high surface body temperatures (T_b), and thermoregulatory accuracy based on cutaneous evaporative water loss (CEWL), plasma osmolality, body mass, and body condition (Peig and Green, 2009). Color indicates correlation coefficient. Numbers on the grid represent slopes from each linear mixed-effects model. Asterisks denote significant relationships (p < 0.05).](image)

**Figure 2.5.** Correlation heatmap of *Gambelia sila* microhabitat use (as the proportion of time spent aboveground), high surface body temperatures (T_b), and thermoregulatory accuracy based on cutaneous evaporative water loss (CEWL), plasma osmolality, body mass, and body condition (Peig and Green, 2009). Color indicates correlation coefficient. Numbers on the grid represent slopes from each linear mixed-effects model. Asterisks denote significant relationships (p < 0.05).

The probability of a lizard being found belowground between 0700 to 1800 h was significantly different among time intervals throughout the active season ($\chi^2(6) = 283.7$, p < 0.0001) and based on sex ($\chi^2(1) = 5.3$, p = 0.02; interaction: $\chi^2(6) = 20.3$, p = 0.002).

From May 10 to June 11, females were significantly more likely to be in burrows than males (Fig 6). Microhabitat use did not differ between sexes for any other time period (p > 0.06). For both sexes, the probability of being belowground during the day significantly increased throughout the active season (Fig 6).
2.3.3 Climate Conditions

Throughout the active season, temperature and vapor pressure deficit increased and relative humidity decreased for burrow microclimates and for local ambient climate (Fig 7). Wind speed did not consistently directionally change throughout the season. Cumulative local winter precipitation was 99 mm in 2018, 193 mm in 2019, 114 mm in 2020, and 74 mm in our study year, 2021. The local winter rainfall immediately prior to the *G. sila* active season of our study in 2021 was half the prior 3-year average.
Figure 2.7. Temperature (A), relative humidity (B), and vapor pressure deficit (VPD; C) in burrow microhabitat (circles) versus local aboveground climate (triangles) where Gambelia sila lizards were studied. Circles with error bars represent the marginal means ± 95% confidence intervals for average microclimate across burrows. Each small gray circle represents an individual burrow. Triangles represent the average of daily mean local climate during the day (red upward triangle) and at night (blue downward triangle). Different letters denote significant pairwise differences in burrow microclimates based on time interval (p < 0.03). Vertical dashed lines show when hydric physiology measurements were taken.
2.4 Discussion

2.4.1 Hydric Physiology

We found that *G. sila* are watertight relative to more-mesic lizards and tolerant of higher plasma osmolality. On average, CEWL for *G. sila* was much lower than for more-mesic *S. occidentalis* measured using the same methods (Fig 1; Weaver *et al.*, in revision). We cannot directly compare to additional studies due to differences in measurement methods and ambient conditions, emphasizing the need of a comparison of the different methods for measuring evaporative water loss. High temperature and vapor pressure deficit lead to increased CEWL at acute time scales of minutes to hours (Warburg, 1965; Cooper and Withers, 2008; Riddell *et al.*, 2019b; Vicenzi *et al.*, 2021; Davis *et al.*, in review), but *G. sila* had lower CEWL than *S. occidentalis* despite being measured at higher temperature and vapor pressure deficits (Figs 1B, 1C), suggesting that *G. sila* have adaptively low CEWL that helps them reduce water loss to their arid environment. This supports the hypothesis that decreased skin permeability to water loss is necessary for inhabiting arid environments (Cox and Cox, 2015). We also observed plasma osmolality values 320-440 mmol kg\(^{-1}\) for *G. sila* (Fig 2B). Another desert lizard, the Gila Monster (*Heloderma suspectum*), had plasma osmolality ~290 mmol kg\(^{-1}\) when hydrated and ~360 mmol kg\(^{-1}\) when dehydrated (Davis and DeNardo, 2007), indicating that most of the *G. sila* we measured were dehydrated to some degree. Such dehydration was to be expected given that our study occurred during a drought year (US Drought Monitor, 2021), but it is still impressive that these lizards can tolerate such high plasma osmolality values. The animals with the highest extracellular fluid osmolality are marine osmoconformers, but those animals usually only tolerate a small range of plasma
osmolality values (Willmer et al., 2005). Not only do G. sila tolerate relatively high plasma osmolality compared to another desert lizard, but the interindividual variation we measured in plasma osmolality indicates that they also tolerate extreme fluctuation in plasma osmolality. Tolerance of such variability in plasma osmolality likely benefits G. sila because they can save the energy and water that would have been used to maintain a narrow range of values. But, it also likely necessitates potentially costly coping mechanisms. The adaptations and mechanisms that animals have in order to maintain plasma osmolality within a narrow range are widely recognized (Willmer et al., 2005), but more effort should go to understanding how animals, especially desert reptiles, tolerate large changes in, and high values of, plasma osmolality.

We hypothesized that throughout the active season for G. sila, as spring shifted to summer and their habitat got hotter and drier (Fig 7), these lizards would become progressively more dehydrated and more watertight. We expected plasma osmolality to consistently increase as lizards dehydrated, and CEWL to consistently decrease to help lizards conserve water. Instead, we observed relatively consistent body condition, plasma osmolality, and CEWL (Fig 2). The observed patterns may be related to prey abundance and the differences in life history between males and females. After emergence in April, lizards can gorge themselves on relatively abundant arthropod prey, improving body condition and hydration. Although the abundance of arthropods was lower during this study than other years due to the drought, within a season, arthropods are generally most abundant in April and early May. As the season progresses, arthropods begin to disappear, and by July, lizards have lost some body condition (Fig 2C), potentially due to the lack of food. Despite the assumed hydric gain of feeding on arthropods, male G. sila
had lower CEWL and higher plasma osmolality in May than in April and July (Figs 2A, 2B), and no change in body condition from April to May (Fig 2C). Given that males must search for mates and defend territory (Montanucci, 1965; Tollestrup, 1983), and that this was a drought year (US Drought Monitor, 2021) with relatively low arthropod abundance, male *G. sila* might have used more water than they could replenish with their diet, resulting in the increased plasma osmolality and decreased CEWL in May (Figs 2A, 2B). Then, it is extremely odd that CEWL increased and plasma osmolality decreased from May to July (Figs 2A, 2B). By June, due to the seasonal changes of the Carrizo Plain and the drought (US Drought Monitor, 2021), the study site was desiccated to a crisp, and there were hardly any living arthropods. Considering that male *G. sila* also decreased body condition from May to July (Fig 2C), they could be catabolizing muscle to maintain hydration (Brusch *et al*., 2018), or the changes could be due to a lack of solutes from the decrease in arthropod prey late in the season. We cannot attribute our findings to either of these mechanisms, but *G. sila* clearly have adaptations to help maintain hydration.

Many females were gravid (Fig 3), and gravidity was somewhat related to body size, as shown in previous studies of *G. sila* (Montanucci, 1965, 1967). Changes in body condition for females could be due to additive effects of feeding and egg development: as more resources are put towards egg development between April to May, females become heavy with eggs. Then, by July, females lay eggs and lose weight (Fig 2C). Feeding should result in water gain, and egg development water loss, counteracting each other when it comes to hydration, potentially leading to the consistent plasma osmolality and CEWL values observed for females (Figs 2A, 2B). Water restriction did not affect
reproductive output in viviparous lizards (Dupoué et al., 2017), suggesting that female *G. sila* likely develop and lay eggs despite drought, such as during our study. However, dehydrated females have dehydrated eggs (Brusch et al., 2019) and less viable offspring (Dupoué et al., 2017), so hatching success, rather than maternal reproductive investment, may be the mechanism underlying lack of recruitment for *G. sila* in drought years (Westphal et al., 2016).

Unsurprisingly, each of the hydric physiology metrics we assessed were correlated. Plasma osmolality and CEWL had a strong relationship, but the direction of that relationship differed depending on when hydric physiology was assessed. In April and July, there was a slight positive relationship, while in May, there was a strong negative relationship (Fig 4). The difference in this correlation among time periods could relate to the changes in CEWL and plasma osmolality throughout the active season, especially for males, which made up most of our sample size (Fig 2). This negative relationship suggests that when lizards are dehydrated, measured as high plasma osmolality, they become more watertight, reducing CEWL (Figs 2A, 2B, 4), like observations in toads (Anderson et al., 2017; Senzano and Andrade, 2018). Dehydration minimizes the ability to evaporatively cool in another desert lizard (DeNardo et al., 2004), but evaporative water loss could also be reduced to maintain hydration (Gerson et al., 2019). We cannot say based on our study whether *G. sila* reduce CEWL to maintain hydration or have decreased CEWL due to dehydration. However, the sex differences we found suggest that because males are more active (Fig 6), they become dehydrated (Fig 2B), and reduced CEWL follows (Figs 2A, 4). This emphasizes the need to investigate how and why different metrics of organismal water balance are interrelated.
2.4.2 Thermal Ecology

Although there was variability in hydric physiology across individual lizards and measurement periods (Figs 2, 4), hydric physiology did not correlate with any thermal ecology metrics (Fig 5). We predicted that more hydrated lizards (with low plasma osmolality) would maintain higher body temperatures, have better thermoregulatory accuracy, and spend more time aboveground (Ladyman and Bradshaw, 2003; Davis and DeNardo, 2009; Sannolo and Carretero, 2019), but we were unable to detect any hydric constraints on thermoregulation and microhabitat use. Basking and maintaining activity may be so important to these heliothermic lizards that the hydric cost does not matter. Their tolerance of dehydration, shown in their high plasma osmolality values, could be due to the necessity of foraging and mating within the short active season these lizards are limited to. Rather than hydration constraining thermoregulation, as seen in other lizards (Davis and DeNardo, 2009; Sannolo and Carretero, 2019; Rozen-Rechels et al., 2020b, 2020a), *G. sila* might tolerate dehydration to maintain thermoregulation. We might have found no effect of hydric physiology on reproductive output for the same reason. These desert-adapted lizards might need to reproduce and maintain activity, no matter the hydric cost, because their window of opportunity is only a 3-4-month active season. However, if *G. sila* are adapted to readily tolerate dehydration, it is even more puzzling why we did not observe somewhat linear decreases in CEWL (Fig 2A) and increases in plasma osmolality (Fig 2B) throughout the active season while *G. sila* were spending time aboveground during the day (Fig 6) and their habitat got hotter and drier (Fig 7).
An alternative explanation for why we detected no relationships between hydric physiology and thermal ecology (Fig 5) is that this is an artifact of all lizards in this study being dehydrated during a drought year. All radio-tracked lizards may have been spending equally high proportions of time in burrows due to dehydration (Fig 6). Our entire study could represent one end of the relationship we hypothesized: that dehydration constrains thermoregulation (Rozen-Rechels et al., 2019). In our study, most of the lizards were likely dehydrated compared to wetter years (Fig 2), and thus potentially thermally constrained, but perhaps if hydrated lizards were compared to dehydrated lizards, we would observe the predicted hydric constraints on thermal ecology. This is exactly why we attempted to give some lizards water, but G. sila seemed not to drink. Supplemental hydration treatment successfully led to a 1% increase in body mass, assumed to be from water intake, but it was apparently not enough to lead to measurable differences in hydric physiology at later measurement periods.

2.4.3 Behavior & Climate

For most of their active season, G. sila spent most of their time aboveground. Then in mid-June, they more than doubled the amount of daytime spent in burrows, and they continued to increase the proportion of time spent belowground through the end of our study (Fig 6). The increased proportion of time spent in burrows (Fig 6) qualitatively parallels the increased heat and dryness as the active season goes on (Fig 7). This shift could be due to universally decreased activity, or a shift to more crepuscular activity, which we did not survey for. Other studies posit that this microhabitat use pattern, observed at both seasonal and daily scales, is for thermoregulation (Ivey et al., 2020;
Gaudenti et al., 2021). Indeed, the sudden increase in time spent belowground in burrows corresponded to a sudden increase in local aboveground temperatures (Figs 6, 7A), but vapor pressure deficit, the desiccation pressure of the air, increased similarly (Fig 7). Compared to local aboveground daytime conditions, vapor pressure deficit was consistently lower at night aboveground and at all times in burrows (Fig 7C). While lizards certainly use burrows to avoid thermal extremes aboveground, our data indicate that burrows are likely helpful to G. sila water balance. Regardless of whether their shift in microhabitat selection throughout the season is due strictly to increased burrow usage or due to a shift in their timing of activity, the behavioral shift we measured would have hydric benefits. By selecting less-desiccating microhabitats, G. sila can decrease the amount of water lost to their environment (Seebacher and Alford, 2002; Dezetter et al., 2022). Burrows could even be a source of water: cloacal gaping is one method of evaporative cooling in lizards (DeNardo et al., 2004), so perhaps an everted cloaca could take water up from the substrate, such as in a damp burrow. This could also explain the increased mass during treatment in supplementally hydrated lizards, despite their seeming absence of drinking. From May 10 to June 11, females spent more time in burrows than males (Fig 6), which could be related to egg laying behavior, and possibly a factor that led to less change in CEWL and plasma osmolality throughout the active season for females compared to males (Figs 2A, 2B). Microhabitat selection could be equally or more important for water balance as it is for thermoregulation (Guillon et al., 2014; Pintor et al., 2016; Lourdais et al., 2017), and understanding the hydric needs of these endangered lizards will be essential for their conservation.
2.4.4 Conclusion

Our assessment of *G. sila* water balance could show that these lizards are incredibly desert-adapted, or that they are dangerously water-stressed. These desert lizards have adaptively low CEWL (Figs 1, 2A), and more dehydrated lizards are more watertight (Fig 4), suggesting that *G. sila* are adapted to conserve water. CEWL and plasma osmolality were relatively consistent throughout these lizards’ active season (Figs 2A, 2B), despite their environment getting hotter and drier (Fig 7), and we detected no effect of hydric physiology on thermoregulation or microhabitat selection (Fig 5). Our data indicate that either *G. sila* tolerate dehydration to maintain their usual behavior, or all *G. sila* in this study were equally dehydrated and thermally and behaviorally constrained. In either case, there is certainly a limit to this dehydration tolerance; a single drought year may not be detrimental, but repeated drought years are more likely to surpass their limits (Dodd, 1993; Selwood *et al.*, 2015). Given that this study took place in a drought year (US Drought Monitor, 2021), we do not know what CEWL and plasma osmolality would be for hydrated *G. sila*, and future studies should assess both ideal hydration and lethal desiccation limits for these lizards. While our supplemental hydration did not lead to a measurable change in hydric physiology, supplemental feeding of gut-loaded arthropods could be more likely to lead to changes in hydration and behavior. Or, misting enclosures, rather than providing standing water, could more closely simulate their natural water resources and be more likely to lead to drinking. Selection of microhabitats as hydric versus thermal refugia should also be explicitly tested. Artificial refugia and supplemental water and feeding could be reasonable conservation interventions, especially during drought.
CHAPTER 3

HYDRATION AND EVAPORATIVE WATER LOSS OF LIZARDS CHANGE IN RESPONSE TO TEMPERATURE AND HUMIDITY ACCLIMATION

1.1 Introduction

Organisms exhibit adaptations to changing conditions over generations (Hochachka and Somero, 1968; Muñoz et al., 2014; Somero, 2010) and plasticity in response to changes in conditions over time and across seasons (Domínguez-Guerrero et al., 2021; Toolson and Hadley, 1979). Examining how physiological traits and mechanisms are influenced by acclimation expands our understanding of complex eco-physiological interactions. Further, knowledge of these patterns and their underlying mechanisms is crucial for predicting and mitigating the impacts of the climate crisis on wild organisms (Gunderson and Stillman, 2015; Huey et al., 2012; Kearney et al., 2018; Riddell et al., 2018; Seebacher et al., 2015; Somero, 2010; Stillman, 2003; Urban et al., 2014).

Water and temperature are two interrelated drivers of physiological adaptations and acclimatory responses in terrestrial animals (Bodensteiner et al., 2020; Cox and Cox, 2015; Hochachka and Somero, 2002; Rozen-Rechels et al., 2019; Tracy and Walsberg, 2001). In extreme heat, both endotherms and ectotherms use evaporative cooling to thermoregulate (DeNardo et al., 2004; Fuller et al., 2021; Pirtle et al., 2019). However, osmotic homeostasis in terrestrial organisms is challenged by water loss to the environment, which can lead to facultative hyperthermia in birds (Gerson et al., 2019) and reduced activity (Davis and DeNardo, 2009) and low selected temperatures in lizards (Pintor et al., 2016; Sannolo and Carretero, 2019). Water is also a primary limiting factor
of the geographic distribution of wild animals (Dunkin et al., 2013; Kearney et al., 2018), yet the amount of research into hydric physiology lags far behind the wealth of information on thermal physiology (Rozen-Rechels et al., 2019; Taylor et al., 2020).

The skin is a primary site of osmoregulation (Pirtle et al., 2019), and cutaneous evaporative water loss can be much greater than respiratory water loss (Cohen, 1975; McKechnie and Wolf, 2004). When the environment is dry, low ambient water vapor pressure physically acts to draw water down its concentration gradient, from organisms, across their skin, and into the environment (Campbell and Norman, 1998; Monteith and Campbell, 1980). Thus, within a species, evaporative water loss tends to be higher when the vapor pressure deficit is higher, such as in low relative humidity (Cooper and Withers, 2008; Pintor et al., 2016; Shoemaker and Nagy, 1977; Warburg, 1965; Willis et al., 2011) and at high temperatures (Dmi’el, 2001; Dmi’el and Tel-Tzur, 1985; Riddell et al., 2019; Toolson and Hadley, 1979; Vicenzi et al., 2021). In this way, low relative humidity and high temperature physically act together through vapor pressure deficit to increase evaporative water loss. However, the skin can resist extreme drying, especially in animals adapted to avoid desiccation in arid environments. Some animals can withstand changes in temperature and humidity with no changes in evaporative water loss (Cooper and Withers, 2014; Eto et al., 2017; Withers and Cooper, 2014). There is also evidence that the skin becomes more resistant to water loss when vapor pressure deficit increases (Riddell and Sears, 2015), indicating that some organisms physiologically counteract vapor pressure deficit to help maintain osmotic homeostasis.

Increased habitat aridity correlates with decreased evaporative water loss rates in mammals (Muñoz-Garcia et al., 2016; Tracy and Walsberg, 2000; Tracy and Walsberg,
2001), birds (Haugen et al., 2003; Muñoz-Garcia and Williams, 2005; Tieleman and Williams, 2002), and reptiles (Bentley and Schmidt-Nielsen, 1966; Claussen, 1967; Cloudsley-Thompson, 1965; Cloudsley-Thompson, 1971; Cohen, 1975; Dmi’el, 2001; Duvdevani and Borut, 1974; Gunderson et al., 2011; Mautz, 1982; Moen et al., 2005; Munsey, 1972; Winne et al., 2001). In this way, cutaneous resistance to water loss helps maintain organismal water balance despite the osmotic stress of a high vapor pressure deficit. Such adaptation of hydric physiology has enabled animals to inhabit extremely dry desert environments (Cox and Cox, 2015). While we know the immediate physical effects of vapor pressure deficit, and we know that organisms can physiologically adapt to counterbalance vapor pressure deficit on an evolutionary time scale, much less is known about how evaporative water loss changes in response to humidity and temperature on an acclimation time scale. Rapid global change, including dramatically altered thermal and hydric regimes, is testing the physiological limits of wild animals, highlighting the importance of understanding the extent to which animals can plastically respond to our currently changing climate.

There is a wealth of evidence supporting the plasticity of thermal and hydric physiology in response to temperature acclimation, especially for ectotherms that depend on the environment to regulate body heat. Lizards acclimated to higher temperatures may have thermal preferences that are lower (Ryan and Gunderson, 2021; Wilhoft and Anderson, 1960) or higher than lizards acclimated to cooler temperatures (Ryan and Gunderson, 2021). In lizards, the critical thermal maximum and/or minimum can be impacted by acclimation temperature (Llewelyn et al., 2018), by egg incubation temperature (Abayaratna et al., 2019; Llewelyn et al., 2018), or by acute heat shock
There is also evidence of acclimation at the molecular level: cuticle lipid fluidity is an adapted trait based on habitat temperature (Hochachka and Somero, 2002), and grasshoppers raised in hot conditions experienced lipid phase change at higher temperatures (Gibbs et al., 1991). For hydric physiology, acclimation to high temperatures leads to decreased evaporative water loss in birds (Muñoz-Garcia et al., 2008; but see McKechnie and Wolf, 2004), lizards (Vicenzi et al., 2021), and salamanders (Riddell et al., 2019). Animals from Drosophila to lizards that are incubated at higher temperatures, then raised in common temperatures after hatching, show higher desiccation rates as adults than individuals incubated at lower temperatures (Llewelyn et al., 2018; Parkash et al., 2014). Finally, on a short time scale of only two hours of acclimation, warm-acclimated lizards had significantly lower evaporative water loss than cool-acclimated lizards (Oufiero and Van Sant, 2018), whereas a similar study found the opposite effect for toads (Senzano and Andrade, 2018).

There is comparatively less research into how thermal and hydric physiology respond to hydric acclimation. This could be due to the comparable difficulty of modulating and measuring hydric factors: whereas environmental temperature can be changed and measured directly, environmental water could refer to water vapor, free-standing drinking water, or water content from the diet and metabolism, each posing a unique set of methodological challenges. Most studies modulate drinking water or relative humidity, or harness environmental differences in aridity. For example, drinking water restriction led to lower thermal preference and evaporative water loss in lizards (Rozen-Rechels et al., 2020). Rats with access to drinking water and high humidity had significantly higher evaporative water loss than rats without (Tracy and Walsberg, 2001).
Acclimation to humid conditions resulted in significantly higher evaporative water loss than acclimation to dry conditions for birds (Clement et al., 2012; Muñoz-Garcia et al., 2008), lizards (Kattan and Lillywhite, 1989; Kobayashi et al., 1983; Weaver et al., 2022), and snakes (Moen et al., 2005), indicating the potential for adaptive plasticity that aligns with the evolutionary pattern among species discussed above. However, strong, rapid acclimation effects are not universal (Gunderson et al., 2011; Tieleman and Williams, 2002; Tieleman et al., 2003) and may depend on the specific habitat a population or species inhabits (Clement et al., 2012). Finally, while thermal ecology and physiology metrics have become extraordinarily streamlined (Taylor et al., 2020), evaporative water loss measurements are typically crudely estimated based on mass loss or using hygrometry. Both estimates largely preclude our understanding of how different tissues (e.g., skin, eyes, respiration, cloaca) contribute to evaporative water loss. Thus, our aim was to assess the effects of temperature and humidity acclimation on several metrics of hydric physiology, and to focus specifically on the skin as a major organ of evaporative water loss.

In this study, we investigated the acclimatory capacity of cutaneous evaporative water loss (CEWL) and related changes in hydration for a lizard in response to temperature and relative humidity. We also assessed the effect of climatic and organismal factors on individual variation in hydration and CEWL. This experiment is among the first to test plasticity in response to both temperature and humidity, and to incorporate several metrics of hydric physiology. We used a factorial design with high and low temperatures and humidities, each resulting in a unique vapor pressure deficit, to assess the acclimation capacity of skin resistance to water loss, measured as CEWL. CEWL was
measured at common conditions before and after exposure to different climate conditions, with any differences therefore representing physiological acclimation. Our null hypothesis was that we would measure no change in CEWL, and that hydration and body condition would change based solely on the vapor pressure deficit to which lizards were exposed, with lizards exposed to the highest vapor pressure deficit losing the most water. Our alternative hypothesis was that CEWL would change in response to experimental climate conditions, and lizards exposed to the highest vapor pressure deficit would have the lowest CEWL at the end of the experiment. To assess this, we acclimated Western Fence Lizards (*Sceloporus occidentalis*) to four different climate treatments and examined the acclimation plasticity of CEWL and related changes in plasma osmolality, hematocrit, and body condition. If skin permeability can acclimate to experimental climate conditions, then we expect to find a negative relationship between treatment vapor pressure deficit and CEWL that parallels the adaptation to aridity discussed above.

1.2 Materials and Methods

1.2.1 Baseline Measurements

We used hand-held lassos to capture 138 adult male *S. occidentalis* throughout the campus of the California Polytechnic State University in San Luis Obispo, California, USA on five dates in June-August of 2021. In the lab, we recorded snout-vent length (SVL; ± 0.5 mm) and mass (± 0.5 g), drew blood from the postorbital sinus of the right eye using self-sealing heparinized micro-hematocrit capillary tubes (Clay Adams, Becton Dickinson, Sparks, MD, USA), and measured CEWL (g m$^{-2}$ h$^{-1}$) using a handheld AquaFlux evaporimeter (model AF200; BioX Systems, London, UK). The AquaFlux
measures CEWL as trans-epidermal water loss of a 3 mm diameter area within a chamber sealed against the skin, thus excluding the influence of immediate external physical factors such as ambient vapor pressure deficit. It measures the instantaneous movement of water across the skin, enabling CEWL measurements that specifically answer questions related to physiological acclimation of the skin. Additional explanations of the AquaFlux evaporimeter are available (Lourdais et al., 2017; Weaver et al., 2022). Blood samples were centrifuged in a micro-hematocrit centrifuge (model IEC MB; Damon IEC Division, Thermo Fisher Scientific, Waltham, MA, USA) for two minutes, then hematocrit (± 1%) was recorded. Plasma osmolality (± 3 mmol kg\(^{-1}\)) was measured in 1-4 technical replicates using a vapor pressure osmometer (VAPRO, model 5600; Wescor, ELITech, Logan, UT, USA). CEWL was measured in 4-6 technical replicates in the same region of the mid-dorsum while lizards were held by their forelimbs to minimize conductive heat gain from the researcher’s hands. Immediately after measuring CEWL, we recorded lizard internal body temperature (± 1°C) by inserting a thermocouple into the cloaca (Traceable Pocket-size K-type Thermocouple, model 14-649-81; Thermo Fisher Scientific). All procedures were approved by Cal Poly IACUC (protocol #2103) and permitted by the California Department of Fish and Wildlife.

### 1.2.2 Experimental Acclimation

We ranked lizards by mass to systematically assign individuals to one of four climate treatments: Cool Humid, Hot Humid, Cool Dry, or Hot Dry. Ordering lizards by mass then alternating their assignment ensured equivalent average starting mass among treatment groups (mean ± s.d.: Cool Humid: 11.6 ± 1.4 g; Hot Humid: 11.6 ± 1.7 g; Cool
Dry: 11.8 ± 1.6 g; Hot Dry: 12.0 ± 1.7 g). We ran the experiment in five separate trials, with a total of 134 lizards completing acclimation (two lizards died during acclimation and two lizards were removed from treatment due to extreme rates of mass loss). Lizards were kept in individual transparent plastic containers (25 x 15 x 13 cm) in an environmental chamber (Low Temperature Illuminated Incubator, model 818; Thermo Fisher Scientific) maintained at ~24°C for the Cool treatment, which we estimate to be the low end of ambient temperatures when *S. occidentalis* are active, and ~35°C for the Hot treatment, which is the thermal preference for *S. occidentalis* (McGinnis, 1966; Wilhoft and Anderson, 1960), with a 12:12 photoperiod (Table 1). Every lizard had a sponge in their container, which was kept wet for lizards in Humid treatments, maintaining relative humidity ~80%, and kept dry for lizards in the Dry treatments, resulting in relative humidity of ~34% for Hot Dry and ~18% for Cool Dry (Table 1). Compared to 24-hour weather recorded on the Cal Poly campus (Irrigation Training and Research Center, 2021) during the active season of *S. occidentalis*, only the Hot Humid treatment conditions are extremely different from what these lizards might experience in the wild (Fig. 1). Acclimation temperature and humidity inside six of the containers for each trial group were recorded every 30 minutes (HOBO External Temperature/RH Sensor Data Loggers, model MX2302A; Onset Computer, Bourne, MA, USA). Actual humidity and temperature varied slightly among trials, demonstrating the importance of recording actual experimental conditions in replicates despite using identical chambers and methods. Therefore, we included trial as a random factor in our acclimation models to account for this variation. Containers had 14 holes, each 4 mm in diameter, along the top for air flow and a wire partition to prevent direct access to the sponges. Containers
were kept tilted so any pooled water from sponges was behind the wire partition and could not be accessed by the lizards.

**Table 3.1.** Acclimation humidity, temperature, and vapor pressure deficit for each treatment group (mean ± 1 s.d.), and the number of lizards exposed to those conditions. VPD was calculated using the mean relative humidity and temperature values.

<table>
<thead>
<tr>
<th>Climate Acclimation Treatment</th>
<th>Relative Humidity (%)</th>
<th>Temperature (°C)</th>
<th>Vapor Pressure Deficit (kPa)</th>
<th>Lizards (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cool Humid</td>
<td>78.3 ± 19.6</td>
<td>23.8 ± 1.6</td>
<td>0.6</td>
<td>34</td>
</tr>
<tr>
<td>Hot Humid</td>
<td>80.9 ± 12.5</td>
<td>34.9 ± 1.1</td>
<td>1.1</td>
<td>33</td>
</tr>
<tr>
<td>Cool Dry</td>
<td>17.9 ± 7.4</td>
<td>24.4 ± 1.3</td>
<td>2.5</td>
<td>33</td>
</tr>
<tr>
<td>Hot Dry</td>
<td>34.1 ± 8.4</td>
<td>35.5 ± 0.9</td>
<td>3.8</td>
<td>34</td>
</tr>
</tbody>
</table>
Figure 3.1. Experimental acclimation conditions compared to the range of potential temperature, relative humidity, and vapor pressure deficit (VPD) that *Sceloporus occidentalis* might experience in the wild. The large, colored points represent the average treatment conditions during the eight days of acclimation (Table 3.1). These are overlaid on a series of small points representing daytime weather data collected ~1m above the ground in an open area every 15 minutes by the California Polytechnic State University Irrigation Training and Research Center (2021), for the active season of *S. occidentalis* from March through September 2021. Sunrise and sunset times used to determine daytime hours were obtained from the Global Monitoring Laboratory (2021).

In addition to the baseline measurements taken after capture on day 0 of acclimation, we again recorded mass on days 4-8, drew blood on days 4 and 8, and measured CEWL on day 8. All CEWL measurements for all treatment groups, before and after acclimation, were taken at common lab conditions: 26.7 ± 0.8°C (mean ± s.d.) and 46.7 ± 6.8% relative humidity, which is most similar to conditions in the Cool and Dry treatment. Because measurements were taken under common conditions and the evaporimeter measures instantaneous water flux across the skin, CEWL assesses skin permeability to water loss. Any changes in CEWL measured for a given lizard are
therefore due to physiological acclimation plasticity. Even so, ambient temperature and humidity in the lab varied slightly on a given measurement day, so we also examined whether lizard body temperature, ambient temperature, and ambient vapor pressure deficit at the time of measurement impacted post-acclimation CEWL. Lizards were fasted during acclimation, then after the final experiment measurements on day 8, they were given two days to recover with ad libitum access to crickets and water in glass terraria with a heat lamp, basking rocks, and shelters. We again measured mass and drew blood after this short recovery opportunity. We toe-clipped lizards to prevent recapture in later trials, then released lizards at their sites of capture.

1.2.3 Statistical Analysis

We investigated both the baseline variability and the acclimation plasticity of CEWL, plasma osmolality, and hematocrit. To quantify the drivers of baseline variability, we used linear models to investigate the following predictor variables: mass; SVL; body condition; temperature, vapor pressure deficit, solar radiation, and wind speed at the time of capture. Because the range of weather values for one date was completely outside the range for the other dates, we only use data from the dates with a similar distribution of weather data in these models. The model for hematocrit was a simple linear model because it did not vary across capture dates, but plasma osmolality and CEWL did significantly differ across capture dates, thus linear mixed-effects models (LMMs) were run with capture date as a random effect. The full LMM for plasma osmolality also included hematocrit because we predicted that plasma volume would affect plasma osmolality. The full LMM for CEWL also included: cloacal temperature,
ambient temperature, and vapor pressure deficit at the time of CEWL measurement; the amount of time (hours) between capture and measurement; hematocrit; and plasma osmolality. We included these variables because we predicted that hydration would affect CEWL, and we wanted to assess whether CEWL varied with ambient conditions. We started with the full model for each response variable, dropped terms to eliminate multicollinearity, then continued backwards model selection based on Akaike’s Information Criterion (AIC) and sum of squares values until the null model was reached. Models were then ranked using ∆AIC, and we present the top models with ∆AIC < 2 and the fewest number of variables.

We used weather conditions at the time of capture in our models of baseline variability as simplistic estimates of the conditions the lizard was experiencing immediately before its capture and measurements, to test for effects of macroclimate on baseline hydric physiology. We obtained weather data from the Cal Poly Irrigation Training and Research Center (2021), which is recorded every 15 minutes from ~1 m above the ground in an open area, and we interpolated values for every minute to pair each lizard’s capture time to the ambient temperature, humidity, wind speed, and solar radiation at the time of capture. Humidity was recorded by the weather station as percent relative humidity, and we converted this to vapor pressure deficit (kPa) using the equation:

\[
VPD = 0.611 \times e^{\frac{17.502}{T+245.97}} \times (1 - \frac{RH}{100})
\]

where T is temperature (°C) and RH is percent relative humidity (Campbell & Norman 1998).

To determine the effect of experimental climate acclimation, we used LMMs to quantify how time in acclimation treatment, acclimation humidity treatment, acclimation
temperature treatment, and treatment vapor pressure deficit affected changes in each of
CEWL, plasma osmolality, hematocrit, and body condition. Due to the physical
relationship among relative humidity, temperature, and vapor pressure deficit (Campbell
& Norman 1998; Riddell et al 2019), the variables were highly collinear and could not be
compared in the same models. Thus, for each response variable, we created three
independent models: one with binary humidity treatment (Humid/Dry), one with binary
temperature treatment (Hot/Cool), and one with numeric vapor pressure deficits of each
treatment group (0.6 kPa for Cool Humid, 1.1 kPa for Hot Humid, 2.5 kPa for Cool Dry,
and 3.8 kPa for Hot Dry). Vapor pressure deficit was calculated based on mean
temperature and humidity for each treatment group using the same equation as above. We
only measured CEWL before and after acclimation, so we calculated Δ CEWL as the
difference between CEWL measured after the experiment versus before for each lizard.
The LMMs for ΔCEWL included trial as a random effect. Plasma osmolality, hematocrit,
and body condition were measured three or more times throughout the experiment, so the
LMMs included the interaction of time in acclimation (numeric days) and nested random
effects of trial and individual lizard ID. We used root mean squared error (RMSE), R²,
and AIC values to compare models.

We quantified the effect size of acclimation by creating LMMs for each of
CEWL, plasma osmolality, hematocrit, and body condition with time in acclimation
(numeric days), treatment group (categorical Cool Humid, Hot Humid, Cool Dry, Hot
Dry), and their interaction as predictors. The LMM for CEWL included trial as a random
effect, and the other LMMs included nested random effects of trial and individual lizard
ID. We used these models to estimate the marginal means of each linear trend. We also
created LMMs for the day 8 measurements of each response variable after the experiment, with categorical treatment as the predictor and trial as a random effect. These models were used to estimate the marginal means for each treatment group at the end of acclimation.

Body temperature is typically a covariate of CEWL, so we created a LMM of the day 8 CEWL measurements with body temperature at measurement, categorical treatment, and their interaction as predictors, with trial as a random effect; this will determine the impact of body temperature at the time of measurement versus acclimation effects on CEWL. We ran the same model for ambient temperature and vapor pressure deficit at the time of measurement for the same reason. We also used two-sided t-tests to determine whether Δ CEWL values were significantly different from zero. Due to the visual nonlinearity in plasma osmolality values throughout the experiment, for each treatment group, we ran an ANOVA followed by Tukey’s HSD to quantify pairwise differences in plasma osmolality among experiment days within treatment groups. To determine whether there was a relationship between plasma osmolality and CEWL post-acclimation, we created LMMs using the day 8 data for each treatment group, with trial as a random effect. We created an additional LMM for Δ CEWL with numerical vapor pressure deficit across trials and treatments as the predictor to assess how vapor pressure deficit influenced the observed changes in CEWL across individual lizards. We tested both linear and polynomial effects of vapor pressure deficit on Δ CEWL. None of the models we present meaningfully deviate from linear regression conditions of linearity, normality, and equal error variance.
To assess whether there was an effect of the recovery opportunity, we calculated \( \Delta \) plasma osmolality, \( \Delta \) hematocrit, and \( \Delta \) body condition as the difference between measurements taken after the recovery period versus after acclimation and versus before acclimation for each lizard. The difference between post-recovery and post-acclimation measures whether there was any recovery at all, and the difference between post-recovery and pre-acclimation measures whether lizards recovered back to their baseline. We used two-sided t-tests to determine whether the changes during recovery were significantly different from zero.

Models used a cleaned dataset consisting of averaged technical replicates for plasma osmolality and CEWL. Any outliers from technical replicate groups that were identified based on boxplot distributions were removed. For plasma osmolality, all values except one extreme outlier were retained. Most CEWL technical replicate groups (per lizard per date) had one outlier omitted, and the maximum number of measurements omitted per group was two. All data analyses for plasma osmolality and CEWL used the average of the remaining replicates after outlier exclusion. We also removed one CEWL outlier at the measurement level for a lizard that was actively shedding and had CEWL far above the normal measurement range, because ecdysis is associated with exceptionally high evaporative water loss (Bogert and Cowles, 1947; Claussen, 1967; Cohen, 1975). We removed 10 plasma osmolality measurements due to a technical error with the osmometer.

Body condition was calculated with the scaled mass index (Peig and Green, 2009) using SVL and mass at capture (day 0), then using later mass measurements to observe change in body condition throughout the experiment. No outliers, high leverage, or
influential points had to be removed to improve generalizability of the equation. We report body condition in mass units, relativized by lizard length, and we refer to it as g'.

All statistics and figures were done in R v4.2.2 (R Core Team, 2022) using tidyverse workflow (Wickham, 2022). We used the lm function for simple linear models, the vif function in the car package for variance inflation factor values to assess multicollinearity (Fox et al., 2022), the lmer function in the lme4 package for LMMs (Bates et al., 2022), the lmer function in the lmerTest package for LMM p-values (Kuznetsova et al., 2020), the aictab function in the AICcmodavg package for ranking models using AIC (Mazerolle, 2020), the drop1 and anova functions for AIC and sum of squares values to conduct model selection, the r.squaredGLMM function in the MuMIn package for R² values (Bartón, 2022), the emmeans, emtrends, and pairs functions in the emmeans package for effect sizes (Lenth et al., 2022), the aov and TukeyHSD functions for pairwise differences in plasma osmolality, and the t.test function for t-tests of the effect of recovery. Plots were made with ggplot2 (Wickham et al., 2022). Functions without specified packages are from base R. All sum of squares values presented are type 1 with Kenward-Roger degrees of freedom. Data and code were archived on Zenodo (will publish and create DOI after review).

1.3 Results

1.3.1 Baseline Variability

Variability in baseline CEWL upon capture was best explained by a LMM that included plasma osmolality (Fig. 2), the ambient temperature at the time of CEWL measurement, and the hours between capture and measurement for a given lizard (Table
2). Increases in plasma osmolality were accompanied by small increases in CEWL, and plasma osmolality explained a significant amount of the variation in baseline CEWL (Fig. 2; Table 2). Among lizards, as temperature at the time of measurement increased, CEWL increased (Table 2). Longer time between capture and measurement related to increased CEWL, but holding time did not explain a significant amount of the variation in baseline CEWL, despite its retention in the top models (Table 2). Equally good models (Δ AIC < 2) explaining the variability in baseline CEWL also included the variables solar radiation at the time of capture (LMM: estimate = 0.008, SE = 0.004, F(1, 89) = 4.61, p = 0.03), wind speed at the time of capture (LMM: estimate = 0.65, SE = 0.40, F(1, 66) = 2.62, p = 0.1), and vapor pressure deficit at the time of CEWL measurement (LMM: estimate = -50.00, SE = 18.46, F(1, 23) = 7.34, p = 0.01).
Table 3.2. Best linear models to quantify the variability of baseline cutaneous evaporative water loss (CEWL), plasma osmolality, and hematocrit of *Sceloporus occidentalis* (n=112) upon capture.

<table>
<thead>
<tr>
<th>Response</th>
<th>Predictor</th>
<th>Estimate</th>
<th>SE</th>
<th>Type</th>
<th>df</th>
<th>F statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEWL (g m(^{-2}) h(^{-1}))</td>
<td>Intercept</td>
<td>-160.09</td>
<td>32.68</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Plasma Osmolality (mmol kg(^{-1}))</td>
<td>0.09</td>
<td>0.02</td>
<td>104</td>
<td>1, 91</td>
<td>11.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature at Measurement (°C)</td>
<td>5.54</td>
<td>1.19</td>
<td>198</td>
<td>1, 91</td>
<td>22.56</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Hold Time (hr)</td>
<td>0.33</td>
<td>0.26</td>
<td>14</td>
<td>1, 91</td>
<td>1.55</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Random Effect: Capture Date</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Osmolality (mmol kg(^{-1}))</td>
<td>Intercept</td>
<td>302</td>
<td>19.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mass (g)</td>
<td>1.36</td>
<td>0.81</td>
<td>615</td>
<td>1, 92</td>
<td>3.53</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>Temperature at Capture (°C)</td>
<td>1.7</td>
<td>0.73</td>
<td>925</td>
<td>1, 93</td>
<td>5.31</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Random Effect: Capture Date</td>
<td>18.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>Intercept</td>
<td>20.46</td>
<td>5.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body Condition (g')</td>
<td>1.29</td>
<td>0.46</td>
<td>121</td>
<td>1, 95</td>
<td>5.26</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>Wind Speed at Capture (mph)</td>
<td>1</td>
<td>0.24</td>
<td>390</td>
<td>1, 95</td>
<td>16.96</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Figure 3.2. Relationship between cutaneous evaporative water loss (CEWL) and plasma osmolality of *Sceloporus occidentalis* (n=138) upon capture. Each point represents an individual lizard. Line represents a simple linear regression (F(1, 136) = 16.67, p < 0.0001, R\(^2\) = 0.1). Plasma osmolality was a significant variable in the linear mixed-effects model describing variability in baseline CEWL (Table 3.2).
The best LMM to explain variability in baseline plasma osmolality included the significant positive effect of temperature at the time of capture, and a marginally nonsignificant positive effect of lizard mass (Table 2). An equally good model for plasma osmolality also included lizard SVL (LMM: estimate = 0.97, SE = 0.63, F(1, 91) = 2.39, p = 0.1). The best model predicting variability in hematocrit included lizard body condition and wind speed at capture, both with significant positive effects on baseline hematocrit (Table 2). An equally good model for hematocrit also included the effect of solar radiation at capture (simple linear model: estimate = 0.004, SE = 0.005, F(1,94) = 0.81, p = 0.37). Across lizard capture dates of this and a preceding study (Weaver et al. 2022), mean daily weather values significantly changed over time only for temperature (simple linear model: estimate = 0.05, SE = 0.004, F(1, 152) = 128.17, p < 0.0001) and wind speed (simple linear model: estimate = -0.005, SE = 0.002, F(1, 152) = 7.43, p = 0.007).

1.3.2 Acclimation Plasticity

The best model to explain ∆ CEWL during acclimation was the humidity treatment model (Table 3). Lizards in Humid treatments had the greatest increases in CEWL following acclimation: on average, CEWL increased 1.92 g m$^{-2}$ h$^{-1}$ day$^{-1}$ for lizards in the Hot Humid treatment, versus 1.18 for Cool Humid lizards, 0.46 for Hot Dry lizards, and 0.45 for Cool Dry lizards (Fig. 3). ∆ CEWL was significantly greater than zero for each treatment group (t-tests: Cool Humid: estimate = 9.51, t(32) = 8.75, p < 0.0001; Hot Humid: estimate = 15.35, t(32) = 8.73, p < 0.0001; Cool Dry: estimate = 3.59, t(32) = 2.75, p = 0.01; Hot Dry: estimate = 3.70, t(33) = 2.51, p = 0.02). ∆ CEWL
had a significant negative linear relationship with treatment vapor pressure deficit (Fig. 4), and a polynomial model did not do a better job of explaining the relationship (Δ AIC < 2).

Table 3.3. Comparison of linear mixed-effects models that use either humidity treatment, temperature treatment, or vapor pressure deficit (VPD) to quantify the changes in cutaneous evaporative water loss (CEWL), plasma osmolality, hematocrit, and body condition (scaled mass index; Peig and Green, 2009) of *Sceloporus occidentalis* (n=134) in response to eight days of acclimation to experimental climate conditions (Table 3.1).

<table>
<thead>
<tr>
<th>Response</th>
<th>Model*</th>
<th>RMSE†</th>
<th>R²</th>
<th>AICc</th>
<th>Δ AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ CEWL (g m⁻² h⁻¹)</td>
<td><strong>Humidity</strong></td>
<td>7.11</td>
<td>0.21</td>
<td>918.19</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>VPD</td>
<td>7.46</td>
<td>0.15</td>
<td>932.20</td>
<td>14.01</td>
</tr>
<tr>
<td></td>
<td>Temp</td>
<td>8.24</td>
<td>0.02</td>
<td>955.40</td>
<td>37.21</td>
</tr>
<tr>
<td>Plasma Osmolality (mmol kg⁻¹)</td>
<td><strong>Time * Temp</strong></td>
<td>22.73</td>
<td>0.02</td>
<td>3583.06</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Time * Humidity</td>
<td>22.83</td>
<td>0.01</td>
<td>3586.70</td>
<td>3.64</td>
</tr>
<tr>
<td></td>
<td>Time * VPD</td>
<td>22.76</td>
<td>0.01</td>
<td>3587.55</td>
<td>4.49</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td><strong>Time * Temp</strong></td>
<td>3.35</td>
<td>0.36</td>
<td>2396.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Time * Humidity</td>
<td>3.38</td>
<td>0.34</td>
<td>2409.60</td>
<td>13.35</td>
</tr>
<tr>
<td></td>
<td>Time * VPD</td>
<td>3.38</td>
<td>0.34</td>
<td>2414.08</td>
<td>17.83</td>
</tr>
<tr>
<td>Body Condition (g')</td>
<td><strong>Time * Temp</strong></td>
<td>0.21</td>
<td>0.29</td>
<td>605.72</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Time * VPD</td>
<td>0.24</td>
<td>0.19</td>
<td>798.40</td>
<td>192.68</td>
</tr>
<tr>
<td></td>
<td>Time * Humidity</td>
<td>0.26</td>
<td>0.17</td>
<td>912.18</td>
<td>306.46</td>
</tr>
</tbody>
</table>

* Model of Δ CEWL included trial as a random effect. Other models included nested random effects of trial and individual lizard ID.
† RMSE = root mean squared error.
Figure 3.3. Plasticity of cutaneous evaporative water loss (CEWL) of *Sceloporus occidentalis* (n=134) in response to eight days of acclimation to experimental climate conditions (Table 3.1). Vapor pressure deficit for each treatment group is noted in the legend. CEWL was measured on days 0 and 8. Points represent raw means ± 1 s.e.m. Lines connect the means for each treatment group.
Figure 3.4. Relationship between the change in cutaneous evaporative water loss (ΔCEWL) of *Sceloporus occidentalis* (n=134) and vapor pressure deficit during eight days of acclimation to experimental climate conditions (Table 3.1). Vapor pressure deficit was calculated for each treatment group and trial (n=20). Each point represents an individual lizard. Line represents a simple linear regression (estimate = -2.96, SE = 0.59, F(1, 131) = 24.82, p < 0.0001, R^2 = 0.15).

Body temperature at the time of measurement had a small effect on post-acclimation CEWL measurements (LMM: sum of squares (SS) = 231, F(1, 116) = 6.25, p = 0.01), but treatment group explained most of the variation (LMM: SS = 3115, F(3, 122) = 28.12, p < 0.0001), and the effect of body temperature did not differ among treatment groups (LMM interaction effect: SS = 28, F(3, 123) = 0.25, p = 0.8). We measured similarly minor effects for both ambient temperature (LMM ambient temperature effect: SS = 320, F(1, 59) = 9.56, p = 0.003; treatment effect: SS = 3464, F(3, 121) = 34.47, p < 0.0001; interaction effect: SS = 65, F(3,122) = 0.65, p = 0.6) and vapor pressure deficit at the time of measurement (LMM ambient vapor pressure deficit effect: SS = 91, F(1,32) = 2.67, p = 0.1; treatment effect: SS = 3536, F(3,122) = 34.49, p = 0.0001; interaction
effect: SS = 153, F(3,121) = 1.49, p = 0.2). The relationship between CEWL and plasma osmolality remained post-acclimation only for lizards in the Cool Dry treatment (LMM: estimate = 0.07, SE = 0.03, F(1, 28) = 4.88, p = 0.04; other treatment groups p > 0.05).

Change in plasma osmolality was best explained by the model including time in acclimation and acclimation temperature (Table 3). Although plasma osmolality is visually nonlinear (Fig. 5A), the pairwise differences between measurement days for each treatment group were nonsignificant (all p > 0.05). On average, plasma osmolality increased 1.59 mmol kg\(^{-1}\) day\(^{-1}\) for lizards in the Hot Dry treatment, 0.48 for Hot Humid, 0.13 for Cool Dry, and decreased 0.65 for lizards in the Cool Humid treatment (Fig. 5A).
Figure 3.5. Changes in plasma osmolality (A), hematocrit (B), and body condition (C; scaled mass index: Peig and Green, 2009) of Sceloporus occidentalis (n=134) during eight days of acclimation to experimental climate conditions (Table 3.1); followed by two days of a recovery opportunity with ad libitum food and water. Vapor pressure deficit for each treatment group is noted in the legend. Plasma osmolality (A) and hematocrit (B) were measured on days 0, 4, 8, and 10. Body condition (C) was measured on days 0, 4, 5, 6, 7, 8, and 10. Acclimation (days 0-8) and recovery (days 8-10) are separated by vertical dashed lines. Points represent raw means ± 1 s.e.m. Lines represent linear regressions for changes during the acclimation period only.
Changes in hematocrit and body condition throughout acclimation were also best explained by the model including time in acclimation and acclimation temperature (Table 3). Hematocrit decreased in all lizards throughout acclimation, with lizards in Hot treatments decreasing slightly faster than lizards in Cool treatments: on average, hematocrit decreased 1.55% day$^{-1}$ for lizards in the Hot Humid treatment, versus 1.36 for Hot Dry, 1.17 for Cool Humid, and 1.14 for lizards in the Cool Dry treatment (Fig. 5B). All lizards also decreased in body condition throughout acclimation: on average, body condition decreased 0.30 g$\cdot$ day$^{-1}$ for lizards in the Hot Dry treatment group, 0.24 for Hot Humid, 0.16 for Cool Dry, and 0.12 for Cool Humid (Fig. 5C).

At the completion of the experiment, CEWL was highest for lizards in the Hot Humid treatment, followed by lizards exposed to Cool Humid conditions; lizards exposed to Dry conditions had the lowest final CEWL, with no difference between the two Dry treatment groups (Fig. 6A). Plasma osmolality did not differ among treatment groups at the completion of the experiment (Fig. 6B). Post-acclimation hematocrit and body condition were impacted only by temperature treatment: both hematocrit and body condition were significantly higher for lizards exposed to Cool conditions than for lizards exposed to Hot conditions (Figs. 6C & 6D).
Figure 3.6. Final values of cutaneous evaporative water loss (CEWL; A), plasma osmolality (B), hematocrit (C), and body condition (D; scaled mass index; Peig and Green, 2009) of *Sceloporus occidentalis* (n=134) after eight days of acclimation to experimental climate conditions (Table 3.1). Vapor pressure deficit for each treatment group is noted on the x-axes. Data presented were collected on day 8, at the conclusion of the acclimation experiment, before the recovery opportunity. Each small point represents an individual lizard. Large points represent marginal means ± 95% confidence intervals. For each variable, treatment groups with the same letter were not significantly different based on a pairwise comparison of the marginal means.

After the 2-day recovery opportunity, plasma osmolality continued to increase (t-test: estimate = 10.05, t(121) = 3.48, p = 0.0007; Fig. 5A) and hematocrit continued to decrease (t-test: estimate = -2.06, t(126) = -4.21, p < 0.0001; Fig. 5B). Body condition did recover compared to post-acclimation values (t-test: estimate = 0.45, t(131) = 6.68, p
< 0.0001), but it remained lower than pre-acclimation (t-test: estimate = -1.14, t(131) = - 11.54, p < 0.0001; Fig. 5C).

1.4 Discussion

1.4.1 Baseline Variability

There was sufficient variation in hydric physiology among individual lizards for several significant relationships to be detected. Most notably, CEWL had a significant positive relationship with plasma osmolality (Fig. 1; Table 2), whereas we had predicted a negative relationship such that lower hydration (high plasma osmolality) would be associated with water conservation (low CEWL), as indicated in toads (Anderson and Andrade, 2017). The positive relationship we detected suggests that lower hydration is related to high CEWL, which could theoretically be maladaptive. When CEWL was relativized by the ambient temperature or vapor pressure deficit during that measurement, the slopes of the relationship across and within capture dates did not change. Thus, although the temperature at the time of CEWL measurement was also a significant predictor of CEWL (Table 2), it does not affect the relationship between CEWL and plasma osmolality. Another possibility is that lizards with higher CEWL have higher plasma osmolality as a direct result of their higher water loss rates, which would explain the positive relationship we observed. The relationship detected between CEWL and plasma osmolality in this study is small (Table 2), and it applies to well-hydrated lizards at the time of capture, where a causal, positive relationship between CEWL and osmolality is reasonable. This relationship was not detected previously (Weaver et al., 2022), but our study is among very few that measured both hydration state and
evaporative water loss rates (see also Dupoué et al., 2017b; Kattan and Lillywhite, 1989). Future studies should incorporate measures of both hydration and water loss to further assess their relationship.

The best models of baseline CEWL also included relationships with ambient temperature and vapor pressure deficit at the time of measurement, the time between capture and measurement, and solar radiation and wind speed at the time of capture. That CEWL was explained better by temperature and vapor pressure deficit at the time of measurement than by temperature and vapor pressure deficit at the time of capture suggests that CEWL is extremely labile. Also, the effect of the time between capture and measurement was retained in the best models explaining CEWL, suggesting that the stress of capture and being held in the lab or a related factor could increase CEWL (Table 2). Although the temperature and humidity change between capture and measurement was relatively small, other studies have indeed found changes in evaporative water loss in response to temperature within two hours (Oufiero and Van Sant, 2018; Senzano and Andrade, 2018). Previously, we detected small effects of ambient temperature and vapor pressure deficit at the time of measurement on CEWL (Weaver et al., 2022), but those relationships were opposite of what we detected in this study. A potential explanation is that average time between capture and measurement differed between the studies: 3.0 h for this study versus 2.4 h for Weaver et al. (2022). In this study, we found a positive effect of ambient temperature during measurement on CEWL (Table 2), though the range of ambient temperatures only spanned 3°C. There are two possible reasons why ambient temperature at the time of measurement was a better predictor of CEWL than body temperature: CEWL may be impacted more by skin surface temperature than by core
body temperature, and skin temperature could be closer to ambient temperature than to core body temperature; and/or the thermocouple recorded body temperature to the nearest 1°C while the evaporimeter recorded ambient temperature to the nearest 0.1°C, potentially causing an issue with resolution. The positive effect of ambient temperature on CEWL that we found is consistent with the positive relationships found by other studies (e.g., Dmi’el, 2001; Vicenzi et al., 2021) where temperatures spanned 10-20°C, and our study extends this by showing that changes as small as 3°C can meaningfully impact CEWL. We hypothesize that the fluidity of lipids in the skin are responsible for this relationship because they are an established mechanism of altered CEWL (Lillywhite, 2006; Williams et al., 2012), and cuticle lipid phase is temperature dependent (Gibbs, 2002; Rourke and Gibbs, 1999). There was also a negative effect of vapor pressure deficit at measurement on CEWL in the other top models. However, the range of vapor pressure deficit experienced by lizards on a given measurement day was <0.5 kPa, so this is not likely to translate to a meaningful biophysical relationship. Positive relationships with wind speed and solar radiation at the time of capture were included in other top models for CEWL, but the effect estimates were small.

Variability in baseline plasma osmolality and hematocrit were also significantly explained by ambient conditions at the time of capture (Table 2), though additional research will be necessary to elucidate the mechanisms behind the relationships we detected. Temperature at capture was the only weather condition that explained a significant amount of the variability in baseline plasma osmolality (Table 2), while in a previous study, plasma osmolality was impacted by other conditions including vapor pressure deficit, wind speed, and solar radiation (Weaver et al., 2022). Higher
temperatures are likely to increase metabolism and total evaporative water loss, thereby decreasing hydration and increasing plasma osmolality. Lizard mass also explained some variability in plasma osmolality, with higher plasma osmolality for larger lizards (Table 2). In mammals, plasma protein concentrations scale positively with mass (Kjeld and Ólafsson, 2008), but to our knowledge this has not been studied in lizards. We found a significant positive effect of body condition on hematocrit in both studies (Table 2; Weaver et al., 2022). This makes sense because hematocrit is known to have positive relationships with body size and mass in *S. occidentalis* (Dunlap, 1995a; Dunlap, 1995b; Dunlap, 2006), perhaps because erythropoiesis is stimulated by testosterone (Parida et al., 2014), which is higher in larger male *Sceloporus* (Halliday et al., 2014). High wind speed was related to increased hematocrit (Table 2), which could be via a dehydrating effect that has been shown experimentally (Anderson and Andrade, 2017; Senzano and Andrade, 2018). Finally, the small, positive effect of solar radiation on hematocrit in the equally good model could also be due to a dehydrating effect because lizards are more likely to bask when solar radiation is high, and subsequent high body temperatures may necessitate evaporative cooling. However, we did not find effects of wind speed and solar radiation on plasma osmolality in this study. We expected plasma osmolality and hematocrit to be correlated measures of hydration, but there are clearly other mechanisms at play.

1.4.2 Acclimation Plasticity

Our results indicate that exposure to certain climatic conditions can affect lizard osmoregulation, and that skin resistance to water loss demonstrates an acclimatory
response based on vapor pressure deficit. We measured physiological changes in response to humidity and temperature within just eight days. CEWL for Humid-acclimated lizards increased 2-4x that for Dry-acclimated lizards (Fig. 3), a result consistent with previous humidity acclimation studies (Clement et al., 2012; Kattan and Lillywhite, 1989; Kobayashi et al., 1983; Moen et al., 2005; Muñoz-Garcia et al., 2008; Weaver et al., 2022). \( \Delta \) CEWL was explained best by the humidity treatment to which lizards were exposed (Table 3), suggesting that humidity exerts an influence on skin permeability to water loss. It is possible that binary humidity treatment explained \( \Delta \) CEWL better than the numeric vapor pressure deficit model because humidity affects the skin in ways not measured through physical vapor pressure deficit. Lizards in the Hot Humid treatment had the largest increase in CEWL, but were not exposed to the lowest vapor pressure deficit (Fig. 3). While higher CEWL for Hot-acclimated lizards could be related to additive effects of heat and humidity, CEWL was measured at ~27°C, which was much cooler than the Hot acclimation temperature. Thus, neither evaporative cooling (Case, 1972; Fuller et al., 2021) or skin lipid phase state (Gibbs, 2002; Williams et al., 2012), could explain the especially high \( \Delta \) CEWL for lizards in the Hot Humid group. Alternatively, lizards in the Hot Humid treatment may have exhibited the greatest acclimation response (Fig. 3) because they were exposed to extreme conditions that differ from what they experience in the wild (Fig. 1). The large increases in CEWL we measured are surprising, assuming that it would be beneficial to conserve water in all conditions. High humidity could be a cue that water is replaceable, and if skin resistance to water loss is energetically costly, reducing skin resistance might be beneficial. Studies have yet to quantify the energetics of skin permeability or to test how humidity might
signal skin permeability changes. We predicted that CEWL would decrease for the lizards acclimated to Dry conditions, but it slightly increased (Fig. 3). It is possible that the Dry conditions in this experiment did not increase vapor pressure deficit enough for CEWL to decrease, but the Dry treatment humidity values were at the low end of relative humidity these lizards experience in the wild (Fig. 1). A previous study successfully elicited a decrease in CEWL for lizards exposed to Dry conditions (Weaver et al., 2022), but they measured post-acclimation CEWL at the same temperature as the acclimation temperature. The measurement temperature in this study was higher than the Cool and lower than the Hot acclimation temperatures, and that temperature difference could influence CEWL (Dmi’el, 2001; Riddell et al., 2019; Vicenzi et al., 2021). While we did find a relationship between post-acclimation CEWL and body temperature at the time of measurement, this effect was tiny compared to the effect of treatment. Due to slightly different ambient conditions across measurements, body temperatures varied by about 5°C at measurement. However, acclimation treatment explained 13x more variation in post-acclimation CEWL values than did body temperature at the time of measurement. Ambient temperature and vapor pressure deficit at the time of measurement also varied, but acclimation treatment again explained 10x and 38x more variation in post-acclimation CEWL, respectively. CEWL values at the end of the experiment reflect the same pattern as the acclimation response (Figs. 3 & 6A). Overall, these results support our hypothesis that CEWL is plastic in response to climate acclimation.

To assess how vapor pressure deficit influenced skin permeability to water loss, we used the full variation in vapor pressure deficit across treatments and trials to explain differences in Δ CEWL among lizards. We found a significant negative effect of vapor
pressure deficit on $\Delta$ CEWL (Fig. 4), which aligns with the evolutionary pattern of reduced evaporative water loss in arid environments that has been measured in other reptiles (i.e., Dmi’el, 2001; Gunderson et al., 2011). Because the evaporimeter we used measures instantaneous skin permeability and not total water lost over an extended period, our results provide strong evidence that lizard skin physiologically acclimated to our climate treatments. Lower CEWL under dry conditions is an adaptive response that will prevent dehydration, and it has been implicated in the evolution that enabled transitions to arid environments (Cox and Cox, 2015). In reptiles, scale size, density, and morphology likely play a role in skin resistance to water loss (Calsbeek et al., 2006; Sakich and Tattersall, 2021; Wishingrad and Thomson, 2020), but scale structure is not likely to change within individuals during an eight-day acclimation period. Changes in CEWL within individuals are mostly attributed to the concentration and composition of lipids in the skin (Cox et al., 2008; Haugen et al., 2003), and lipids also play a role in CEWL differences among populations and species (Lillywhite, 2006; Williams et al., 2012). Changes in blood flow have been implicated for more acute changes in CEWL (Marder and Raber, 1989), and these changes could theoretically persist during measurement if acclimation involved mechanisms like angiogenesis or changes in blood volume.

For changes in plasma osmolality, temperature was a more influential variable than humidity or vapor pressure deficit (Table 3). Plasma osmolality may be influenced more by temperature than humidity because temperature has a direct effect on metabolism (Angilletta, 2009) and therefore overall water loss. The lizards in our experiment had no drinking water to replenish what was used, so plasma osmolality
increased, similar to other results showing increased plasma osmolality in response to water restriction (Dupoué et al., 2017a). Although temperature explained most of the change in plasma osmolality, there were also differences based on humidity: within temperature treatments, plasma osmolality increased more for lizards exposed to Dry conditions (Fig. 5A). Change in plasma osmolality across treatment groups was not directly related to the vapor pressure deficit lizards experienced (Fig. 5A), but it is possible that within temperature treatments, respiratory evaporative water loss was minimized in Humid conditions (Lovegrove et al., 2014; Warburg, 1965). Notably, plasma osmolality was not statistically different between any of the treatment groups at the end of the acclimation experiment (Fig. 6B). Although temperature had a significant effect on plasma osmolality change throughout the experiment (Table 3), the changes were relatively small, and values did not differ across days within a treatment group. On average, we measured 1-13 mmol kg\(^{-1}\) change in plasma osmolality over the course of acclimation, while we found \(S.\ occidentalis\) to have a natural plasma osmolality range of \(~100\) mmol kg\(^{-1}\) among individuals. Wild \(S.\ occidentalis\) experience lower and much more variable temperatures than in this experiment (Fig. 1). Considering our relatively extreme experimental climates and the naturally high variation in plasma osmolality, we hypothesize that meaningful changes in plasma osmolality might take longer than 8-10 days to observe.

We expected plasma osmolality and CEWL to be linked in their response to experimental climate conditions. But, we did not find evidence to support this, as CEWL changed more in response to humidity and plasma osmolality changed more in response to temperature. It is possible that the two hydric physiology factors are ruled by different
mechanisms: water and food consumption could primarily affect plasma osmolality while ambient climate could primarily affect CEWL (Kattan and Lillywhite, 1989). Hydration seems to be impacted by metabolism (Angilletta, 2009), and while there is evidence that respiratory evaporative water loss is also tied to metabolism (Welch and Tracy, 1977; Wolf and Walsberg, 1996), CEWL might not be. Although CEWL and plasma osmolality showed different responses to experimental acclimation, we found a relationship between their baseline values (Fig. 2). The same positive trend held for CEWL and plasma osmolality values after acclimation, but the relationship was only significant for one treatment group. As discussed above, this could be driven by CEWL rates that impact plasma osmolality, rather than osmolality constraining CEWL rates. For either causal direction, there could also be a time lag effect that complicates measuring the relationship. In our study, plasma osmolality was maintained despite dehydrating conditions (Fig. 6B), which could be enabled by fat or muscle catabolism (Brusch et al., 2018), thereby enabling acclimation effects of CEWL unconstrained by short-term dehydration. Organismal water could also be moderated through water resorption from feces and behavioral modifications like closing the eyes and mouth (Karasov, 1983; Pirtle et al., 2019; Waldschmidt and Porter, 1987).

Our experiment provides insights into how hematocrit and body condition might be impacted by various climatic conditions. Because lizards were fasted, hematocrit and body condition declined in all individuals (Figs. 5B & 5C), but our design allowed us to tease out the effects of exposure to different climates by comparing values among treatment groups. Both hematocrit and body condition were more influenced by temperature than humidity or vapor pressure deficit (Table 3). All lizards experienced a
significant decrease in hematocrit throughout the experiment (Figs. 5B), likely due to some combination of fasting, lack of natural sunlight which contributes to erythropoiesis via vitamin D (Smith and Tangpricha, 2015), and possibly a blood draw frequency exceeding net erythropoiesis. The decrease in hematocrit was temperature-dependent: Hot-acclimated lizards decreased hematocrit significantly faster than Cool-acclimated lizards (Fig. 3B, 4C), potentially relating to higher metabolism as discussed above for plasma osmolality. However, the models with humidity or vapor pressure deficit accounted for nearly as much of the change in hematocrit as did temperature (Table 3).

Temperature treatment explained more of the change in body condition than humidity treatment or vapor pressure deficit (Table 3). Lizards acclimated to Hot conditions decreased body condition 1.5 to 2.5x that for lizards acclimated to Dry conditions (Fig. 5C), and at the completion of the experiment, body condition was significantly lower for Hot-acclimated lizards than Cool-acclimated lizards (Fig. 6D). The decline in body condition we observed is at least partially due to fasting during the experiment, with the greater decline in Hot-acclimated lizards likely due to the elevated metabolism they experienced. This could also be related to fat and muscle catabolism to maintain the consistent plasma osmolality values we measured (Fig. 6B; Brusch et al., 2018). In conjunction with temperature, fasting, and catabolism, water loss might also contribute to decline in body condition. Numerous studies use mass loss rate as an estimate of evaporative water loss (Galliard et al., 2021; Žagar et al., 2022). While we measured CEWL based on instantaneous skin permeability to water loss, actual water lost throughout the duration of the experiment might be better estimated with body condition decline. In other words, CEWL measures the instantaneous water flux across the skin,
While change in body condition represents the cumulative amount of water lost. Even though skin permeability to water loss can change (Fig. 3), lizards likely still lost more water when experiencing high vapor pressure deficits (Fig. 5C). Other studies should initiate tests to determine the relationship between instantaneous skin permeability to water loss versus cumulative water loss and mass loss, especially because each is referred to as evaporative water loss.

When lizards were given a recovery opportunity with *ad libitum* water and food after the experiment, we observed lizards eating, basking, and soaking in the water dish. Body condition significantly increased (Fig. 5C), which is evidence of drinking, eating, or both. However, plasma osmolality continued to increase (Fig. 5A), which suggests that there was not enough time to assimilate the water. Alternatively, the water content of the crickets may have been low, thereby requiring the lizards to use water for digestion (Murphy and DeNardo, 2019). The continued decrease in hematocrit suggests a time lag between restoration of feeding and erythropoiesis, or that these heliothermic lizards require full access to thermal and basking gradients to recover from a week of fasting and several blood draws (Fig. 3B).

### 1.4.3 Conclusion

Humidity and temperature acclimation led to significant changes in CEWL and related effects on hydration in a lizard in only eight days. Our data support our hypotheses that water loss is plastic, and that acclimation parallels adaptation across species. These results suggest that plasticity in CEWL could be highly adaptive in the face of climate change. However, we did not measure the rapidity or reversibility of these
acclimation changes, or whether plasticity differs among *S. occidentalis* populations based on habitat, and these studies will also be essential to inform conservation management. We found some support for our hypothesis that plasma osmolality would be affected by exposure to different climates, but we think that temperature-dependent effects of metabolism had the greatest impact on osmolality, hematocrit, and body condition. Finally, baseline variability in CEWL was significantly explained by plasma osmolality, but the two variables responded differently to experimental climate acclimation. Future studies should incorporate both variables to determine how their interplay might affect physiological responses to environmental change. The mismatch we observed between CEWL and plasma osmolality suggests that they are controlled at least in part by separate mechanisms. The organismal-level results presented here have molecular, ecological, and evolutionary bases which should continue to be investigated to further our understanding of functional biodiversity and to improve conservation management in the face of the climate crisis.
CHAPTER 4

CONCLUSION

For Chapter 1, all *G. sila* we measured were potentially dehydrated, as every plasma osmolality measurement we took was much higher than values for other hydrated desert lizards. We believe that we measured relatively little variability in hydric physiology throughout time and found no relationships between hydric physiology and thermal ecology because all lizards we measured were dehydrated to some degree. If a similar study were to be conducted when there is not an ongoing drought, and a range of hydrated and dehydrated *G. sila* could be measured, we still hypothesize that hydric physiology would change throughout time relative to local climate and that hydration would constrain thermal ecology. Nearing the end of their active season, *G. sila* doubled the amount of daytime they spent underground in burrow refugia, and this corresponds to an increase in the vapor pressure deficit these lizards would have been subjected to aboveground during the day. *Gambelia sila* could be selecting less-desiccating burrow microhabitats to reduce the amount of water lost to their environment, therefore preventing further dehydration. In addition to the potential behavioral regulation of *G. sila* to minimize CEWL when they are exposed to high vapor pressure deficit, for Chapter 2, we found that the CEWL of *S. occidentalis* was plastic in response to different vapor pressure deficits. *Sceloporus occidentalis* that were exposed to low vapor pressure deficit increased CEWL much more than those exposed to high vapor pressure deficit, which mirrors the negative relationship of CEWL with habitat aridity across species.

While we found a negative relationship between CEWL and plasma osmolality for *G. sila* in Chapter 1, we found a positive relationship for *S. occidentalis* in Chapter 2.
We expected to consistently find a negative relationship between CEWL and plasma osmolality; dehydrated lizards (high plasma osmolality) should minimize their water loss (low CEWL). Our data on *G. sila* match this prediction. Conversely, we measured high CEWL when plasma osmolality was high for *S. occidentalis*. It is possible that in these mesic lizards, CEWL is not as strongly regulated, and *S. occidentalis* can dehydrate to maintain CEWL because there is water available in their environment. Thus, the relationships among different hydric physiology metrics could depend on the environment a given species inhabits, as well as its specific water requirements and adaptations. Our data support this idea: on average, *G. sila* had significantly higher plasma osmolality and lower CEWL than *S. occidentalis*.

Finally, to assess whether the differences we found between *G. sila* and *S. occidentalis* are likely to represent environmental adaptations, we also measured CEWL of Aegean Wall Lizards (*Podarcis erhardii*), another Mediterranean-climate lizard inhabiting a niche similar to *S. occidentalis*. We measured CEWL of *P. erhardii* on their day of capture, within a few hours of capture, in 5 technical replicates on the mid-dorsum using the same evaporimeter and methods as for *G. sila* and *S. occidentalis*. We again used LR, followed by pairwise comparison of marginal means, to assess differences in measurements between species. As expected, the CEWL of *P. erhardii* and *S. occidentalis* were similar, and both species had CEWL higher than *G. sila* (Fig Z). All three species we measured support the hypothesis that CEWL rates are adaptive to the environment organisms inhabit, with arid species having the lowest CEWL. But, as climate change progresses, all three species are likely to experience prolonged droughts.
and heatwaves, calling for further investigation of hydric limits and plasticity to inform conservation management.

**Figure 4.1.** Cutaneous evaporative water loss (CEWL) of desert Blunt-nosed Leopard Lizards (*Gambelia sila*) captured in the Carrizo Plain National Monument, CA, USA; Mediterranean-climate Western Fence Lizards (*Sceloporus occidentalis*) captured throughout the campus of California Polytechnic State University, San Luis Obispo, CA, USA (Weaver et al., in revision); and Mediterranean-climate Aegean Wall Lizards (*Podarcis erhardii*) captured on Naxos Island, Cyclades, Greece. CEWL is compared relative to ambient temperature (A) and vapor pressure deficit (VPD; B) at the time of CEWL measurement. Each small point represents a measurement of an individual lizard taken shortly after their capture; the data for *G. sila* include 1-3 measurements per individual. Large points represent marginal means. 95% confidence intervals were removed because they were so small that they were obscured by the mean points. The temperature and VPD at the time of measurement were significantly different between each pairwise species comparison (all $|t| > 9.2$, $p < 0.0001$). CEWL was equivalent between *S. occidentalis* and *P. erhardii* ($t = 0.2$, $p = 0.97$), and both species had significantly greater CEWL than *G. sila* (both $|t| > 16.6$, $p < 0.0001$), despite being measured at lower temperatures and vapor pressure deficits.
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