



SYMPOSIUM

The Shape of Water: Physiological Adaptations to Habitat Aridity in the Ornate Tree Lizard (*Urosaurus ornatus*)

Jhan C. Salazar^{*,1} and Donald B. Miles^{*,†,2}

^{*}Department of Biology, Washington University, St. Louis, MO 63130, USA; [†]Department of Biological Sciences, Ohio University, Athens, OH 45701, USA

From the symposium “Feel the flow: how water movement shapes organisms and ecosystems” presented at the virtual annual meeting of the Society for Integrative and Comparative Biology, January 16–March 31, 2024.

¹E-mail: jhancsalazar@wustl.edu

²E-mail: milesd@ohio.edu

Synopsis Deserts have always amazed researchers due to their high diversity of habitats, where plant and animal species have been able to adapt and diversify, even when these areas impose several constraints on an organism's activity patterns. In particular, deserts support several lizard species adapted to the thermal and water restrictions found in such biomes. Although several studies have attempted to understand how lizard species might respond to water deficits or droughts in deserts, few have addressed how these responses might vary along a latitudinal gradient. This raises the question of whether physiological buffering of the organism or the climatic environment affects water loss in lizards. Here, we used six populations of *Urosaurus ornatus* to test whether water loss is influenced more by the intrinsic physiology of the lizard or by the climatic niche. We found that water loss is primarily influenced by the climatic niche of the lizard. However, future studies should focus on how microclimatic variables can influence water loss in organisms found across large latitudinal gradients.

Resumen Los desiertos siempre han fascinado a los investigadores debido a su alta diversidad de hábitats, donde las especies de plantas y animales han podido adaptarse y diversificarse, incluso cuando estas áreas imponen varias restricciones a los patrones de actividad de los organismos. En particular, los desiertos albergan varias especies de lagartos adaptadas a las restricciones térmicas e hídricas que se encuentran en estos biomas. Aunque varios estudios han intentado entender cómo las especies de lagartos pueden responder al déficit de agua o la sequía en los desiertos, pocos han abordado cómo esto podría variar a lo largo de un gradiente latitudinal. Esto plantea la pregunta de si el amortiguamiento fisiológico del organismo o el entorno climático afecta la pérdida de agua en los lagartos. Aquí, utilizamos seis poblaciones de *Urosaurus ornatus* para probar si la pérdida de agua está influenciada por la fisiología intrínseca del lagarto o por el nicho climático. Encontramos que la pérdida de agua está influenciada principalmente por el nicho climático del lagarto. Sin embargo, estudios futuros deberían centrarse en cómo las variables microclimáticas pueden influir en la pérdida de agua en organismos encontrados a lo largo de grandes gradientes latitudinales.

Introduction

The natural world is governed by two types of interactions that dictate the distribution of species: biotic (e.g., parasitism, predation, and competition) and abiotic (e.g., latitude, altitude, humidity, water access, and temperature; Janzen 1967; Holt 2003; Ghalambor et al. 2006; Zamora-Camacho et al. 2013). In the last decade, species have been threatened by rising temperatures and water stress due to climate change. There is substantial

interest in understanding how organisms may respond to temperature variation. In particular, intense research has focused on how rising temperatures are changing patterns of precipitation, floods, sea levels, the intensity of El Niño and La Niña, and frequency, intensity, and duration of droughts (Danin 2017; Iwami et al. 2017; Nordvelle et al. 2017; Fang and Yu 2020; Wang et al. 2022). A worrisome outcome of a reduction in rainfall is water stress. In terrestrial species, the availability of

water is crucial for survival and reproduction (Masó et al. 2020; Dezetter et al. 2021). This is particularly true for ectothermic species because this group of organisms depends on ambient temperatures to regulate their body temperature, which in turn affects physiological performance (Huey 1982). Water is essential for this group of organisms because their thermoregulatory behavior can be disrupted when it comes to water availability and water balance (Rozen-Rechels et al. 2020a, 2020b, 2021). Changes in ambient temperature and therefore water availability due to climate change could significantly affect their survival (e.g., Bogert 1949; Mautz 1982; Lorenzon et al. 1999; Gunderson and Stillman 2015; Ortega et al. 2016; Sinclair et al. 2016).

Species with behavioral adaptations and acclimatization strategies to survive in warm and dry environments also face challenges imposed by the interaction between thermal and hydric stress. One of the most commonly used strategies involves behavioral flexibility. Some species use movement to avoid warm temperatures by seeking refuge in shady microhabitats in dry environments; a behavior known as behavioral flight response or antipredator behavior (Rozen-Rechels et al. 2020a). This response can involve changing microhabitat selection without altering habitat location. Another behavioral strategy that organisms employ to avoid water stress is adjusting their activity time (Lorenzon et al. 1999; Pintor et al. 2016; Rozen-Rechels et al. 2020a). In the face of high temperatures and water stress, the ability of ectothermic organisms to maintain their body temperatures at a physiological optimum, while avoiding hydric stress becomes compromised. Thus, organisms may select microhabitats that have lower resource availability, but a higher probability to avoid overheating or excessive water loss. The reduction in food intake coupled with body temperatures below optimal to reduce water loss may result in lower survivorship and decreased reproductive success (Lorenzon et al. 1999; Rozen-Rechels et al. 2020a). The severe consequences of thermal and hydric stress can also be exacerbated by changes in the habitat (Chown et al. 2011).

Furthermore, species inhabiting different regions face varying degrees of water stress. In tropical environments, previous studies have shown that species exhibit little response to changes in water availability, and the heritability of desiccation resistance is also low compared to temperate species (Hoffman et al. 2003; Breshears et al. 2005; Hoffmann 2010). However, water availability also affects thermoregulatory effectiveness (Chukwuka et al. 2020; Rozen-Rechels et al. 2021), especially in arid regions, where the climate is typically warm with low relative humidity. In these areas, organisms are frequently exposed to continuous

heat and water stress. Desert species have developed strategies to avoid high temperatures and water scarcity by retreating to microhabitats with lower temperatures and reduced risk of water loss (Chukwuka et al. 2020). Thus, species inhabiting more xeric environments are predicted to exploit habitats that favor lower body temperatures when active. Concomitant differences thermal traits would include lower thermal preferences and a narrower thermal breadth (i.e., the difference between CT_{min} and CT_{max}). These potential shifts should favor water economy.

We use thermal and hydric data to address the question of whether climatic factors or thermoregulatory traits affect water loss in ectotherms. We measured multiple thermal traits that are associated with thermoregulation and should affect the ability of lizards to avoid hydric stress (e.g., Sannolo et al. 2019). We included the WorldClim variables because previous studies found a strong effect of transepidermal water loss (TEWL) with precipitation, whereas hydration status was affected by temperature (Chabaud et al. 2022).

In our study, we utilized the ornate tree lizard (*Urosaurus ornatus*), an insectivorous small-bodied lizard with a broad distribution spanning northern Utah south to northern Mexico (Miles 1994; Haenel 2007). We examined six populations of *U. ornatus* across four Western US states (Utah, New Mexico, Arizona, and Texas), specifically chosen for the variation in environments and habitats. Our central question addresses whether water loss and hydration levels in lizards at different latitudes involve physiological compensation or are associated with population-specific climatic conditions. Physiological compensation would be evident with a trade-off between individuals that have high field active body temperatures or high thermal preferences and TEWL or hydration state. Maintaining elevated T_b 's should favor less leaky skin and enhanced water conservation. We expect that water loss and hydration are correlated with temperature and precipitation. Warmer populations are anticipated to experience higher water loss and less hydration compared to colder populations. Conversely, we anticipate a negative correlation between water loss, hydration, and precipitation variables. In environments with higher water availability, species are expected to experience less water loss and thus be more hydrated.

Methodology

Study sites

We collected data from lizards captured at six different sites in the Western USA: (1) Black Gap Wildlife Management Area, Texas (Black

Gap–29°35′25.44″N, 102°54′2.088″W, 622m); (2) Sevilleta National Wildlife Refuge, San Jon, New Mexico (Sev–34°17′59.46″N, 106°37′45.228″W, 1774m); (3) Saguaro National Park, Tucson, Arizona (Sag–32°13′55.776″N, 110°41′23.172″W, 837m); (4) Molino Canyon, Santa Catalina Mountains, Arizona (Molino–32°19′48.18″N, 110°41′40.524″W, 1259 m); (5) Big Wash, near Chloride, Arizona (Big Wash–35°27′7.344″N, 114°10′14.16″W, 1864 m); and (6) Steinaker Lake, Vernal, Utah (Vernal–40°30′45″N, 109°31′24.9996″W, 1680 m). The sites differ in vegetation and climate. Black Gap is situated in the Chihuahuan Desert with the most precipitation occurring in the summer. The Sevilleta site is an ecotone between Chihuahuan Desert and mixed Oak-Juniper woodland. The population at Saguaro inhabits a mesquite bosque. The site has two rainy seasons: a winter season and summer monsoons. Both seasons are followed by a period of drought. Molino Canyon is an Oak woodland/arid grassland habitat. Lizards have access to water at this site. Big Wash consists of an Oak-Juniper woodland intermixed with sage. The Vernal site is situated in a Juniper-sage habitat. We used population as a proxy for latitude.

Natural history of *U. ornatus*

The ornate tree lizard (*U. ornatus*) is a short-lived, small-bodied species in the family Phrynosomatidae. This species is distributed along the west and east coasts of central México, east to the Edwards plateau, Texas, west to southern Arizona, and has its northern range limit in northern Utah/southern Wyoming. Its eastern range limit is western Colorado and central New Mexico. Depending on the locality, tree lizards may occupy trees, rocks, or, in some populations lizards will be use both. All lizards in the study were saxicolous, with the exception of the lizards at Saguaro, which were exclusively arboreal.

Capture and morphological measurements

We sampled lizards from the six sites in the southwestern US between May and June 2021. At each site, we searched for lizards between one hour after local sunrise to 13:00–14:00 and again at ~16:30–19:00. Lizard activity declines after midday, but we surveyed in the late afternoon to verify activity patterns. We captured lizards using a fishing pole with a loop affixed at the tip. We capture 84 individuals in total (44 females and 40 males).

Immediately after capture (~30–60 s), we measured the body temperature (T_b) of each lizard using a quick-reading cloacal thermometer (Miller and Weber). We also recorded air temperature. Substrate temperatures at the site of capture were recorded using an infrared

thermometer (Amprobe IR-750). Each lizard was placed in a cotton mesh bag and transported to a field laboratory for further measurements.

In the lab, we determined the sex of each lizard (males had enlarged post-anal scales) and measured snout-vent length (SVL, mm) to the nearest 0.5 mm and mass (to the nearest 0.01 g).

Thermal traits: preferred body temperature, critical thermal minimum, and critical thermal maximum

We measured the preferred body temperature (T_{pref}) using a linear photo-thermal gradient. The gradient consisted of eight lanes: 1.2 m L x 0.2 m W x 0.25 m H. The walls were made of a plastic-coated wood, which limited the ability of lizards to climb out of the gradient. At one end, we suspended 250 W infrared heat lamp and the other had ice packs, which resulted in a temperature range of ~25–50°C. We housed lizards in individual terraria at a temperature of 27°C for one hour prior to placing them into the thermal gradient. We affixed a 36 g Type T thermocouple to the venter anterior of the cloaca. We used medical tape to secure the thermocouple in place. Each thermocouple was connected to an Omega 8-channel data acquisition module. We allowed the lizards to move freely along each lane for a period of 15 min. We then recorded the T_b of lizards every minute for a period of 90 min. The gradient was in a room illuminated by fluorescent lights and cooled with air conditioning. All measurements were made during the activity period of the species.

CT_{min} and CT_{max} measured following Gilbert and Miles (2019). For CT_{min} , lizards were placed in a plastic tub in an ice-water bath. We affixed a thermocouple to the venter anterior of the cloaca affixed with medical tape. The body temperature was decreased by 1°C per minute. When the movement of the lizard became sluggish, we began turning the lizard on its back. The temperature at which the lizard lost its righting response was recorded as the critical thermal minimum. The following day, we measured CT_{max} . We placed a lizard in a plastic tub and suspended an infrared heat lamp from above. Lizards had a heating rate of ~1°C per minute. Once the lizard manifested a panting response, we began turning the lizard on its back. The temperature at which the lizard lost its righting response was recorded as the critical thermal maximum. We calculated the thermal breadth (T_{br}) as $CT_{max} - CT_{min}$. We calculated the Thermal Safety Margin (TSM) as $CT_{max} - T_{pref}$.

TEWL

We measured trans-epidermal water loss with a hand-held, closed chamber device (Delfin Vapometer®, Delfin Technologies LTD, Finland). The Vapometer measures

instantaneous movement of water across the skin. The open port of the Vapometer was pressed against the skin of the lizard and a reading obtained within 10–20 s. We took three measurements from the throat, belly, and dorsum (at the midback). We used the average of the three measurements as our estimate of TEWL. Measurements are expressed in $\text{g} \times \text{m}^{-2} \times \text{h}^{-1}$.

Water loss

We used a gravimetric method for measuring water loss. We used the initial mass at capture as M_{t0} . We then put each lizard in a separate plastic tub. Each tub had a lid perforated with holes to allow air flow. A paper towel lining was placed on the floor of the tub. We held the lizards in the tub overnight for a period of 8 h and weighed again designated as mass at M_{t8} . The rate of water loss was $(M_{t0} - M_{t8})/8 \text{ h}$. Lizards that defecated overnight were not used in the analysis.

Plasma osmolality/hydration

Prior to releasing the lizards, we obtained a blood sample via the post-orbital sinus. We used a heparinized capillary tube to collect 60 μL of blood. We immediately centrifuged the blood to separate the plasma from the packed red blood cells. We kept the plasma samples at -20°C and later store at -80°C until we conducted the analysis. Our estimate of hydration status was obtained by quantifying the concentration of solutes in the blood plasma. We used a Vapro Vapor Osmometer model 5600 (ELITech, Logan, UT) to obtain the plasma osmolality. Because plasma osmolality is a measure of the concentration of blood solutes, animals with high values are presumed to be dehydrated; we recorded this for four out of the six populations.

Environmental data

We used the WorldClim database to extract environmental variables for each of the georeferenced individuals (Fick and Hijmans 2017). These WorldClim layers have a spatial resolution of 1 km^2 and summarize thermal averages, extremes, and ranges for temperature and precipitation. We included the WorldClim variables because previous studies have found that TEWL is affected by precipitation or temperature (Chabaud et al. 2022).

Statistical analyses

All statistical analyses were conducted using R version 4.3.2 (R Core Team 2023). Figures were made with the *ggplot2* package (Wickham 2009). To determine if the variation in thermohydration traits among

populations of *U. ornatus* persisted after accounting for body mass, we conducted an analysis of covariance (ANCOVA), with body mass as a covariate. An analysis of variance (ANOVA) was used to test whether CT_{\min} , CT_{\max} , T_b , T_{pref} , T_{br} , water loss, and hydration differ among populations. Next, we employed a linear model analysis to test if there was a relationship between water loss in the throat, belly, and back with total water loss. To explore how environmental variables affect the thermal physiology data (CT_{\min} , CT_{\max} , T_b , T_{pref} , T_{br} , and thermal safety margin) of *U. ornatus*, we estimated a Pearson correlation between all the climatic variables and the thermal data for each individual. Lastly, to test whether the intrinsic physiology of the lizard or the climatic niche were affecting the water loss in *U. ornatus*, we calculated the correlation between water loss and WorldClim and thermo-physiological variables.

Results

Body mass had no influence on the physiological traits (Supplementary data; Table S1), but the location of each population influenced the physiological results. We found significant differences among the populations for CT_{\min} ($F_{5,57} = 5.33$; $P < 0.001$), CT_{\max} ($F_{5,37} = 3.01$; $P = 0.02$), T_b ($F_{5,64} = 5.34$; $P < 0.001$), T_{br} ($F_{5,36} = 6.77$; $P < 0.001$), hydration level ($F_{2,27} = 4.46$; $P < 0.0001$), and water loss ($F_{3,30} = 8.40$; $P < 0.001$) (Supplementary data; Table S1).

For T_{pref} , there was an interaction between population and body mass ($F_{5,58} = 3.38$; $P = 0.01$; Supplementary data; Table S1). The significant interaction term suggests that there are population differences in T_{pref} that are conditional on body mass. The pattern is driven by Vernal lizards having the lowest values for body mass and lowest average T_{pref} . In contrast, lizards from Saguaro were larger and had the highest estimate for T_{pref} . For thermal safety margin, there was no difference among populations when controlling for body mass (Fig. 1; Supplementary data; Table S1).

We found a strong effect of geographic location on CT_{\min} ($F_{1,5} = 10.58$, $P < 0.0001$), CT_{\max} ($F_{1,5} = 7.28$, $P < 0.0001$), T_b ($F_{1,5} = 7.69$, $P < 0.0001$), and T_{br} ($F_{1,5} = 12.22$, $P < 0.0001$). Specifically, the Black Gap population—the southernmost population—exhibited the most significant differences among the different thermal traits (Fig. 2; Table 1). For CT_{\min} , the population that is located in the northern limit (Vernal) has the lowest CT_{\min} , while the population in the southern limit (Black Gap) has the highest CT_{\min} . In contrast, we found that the population in the southern limit had the lowest CT_{\max} (Black Gap), while the population north of Black Gap have the warmest

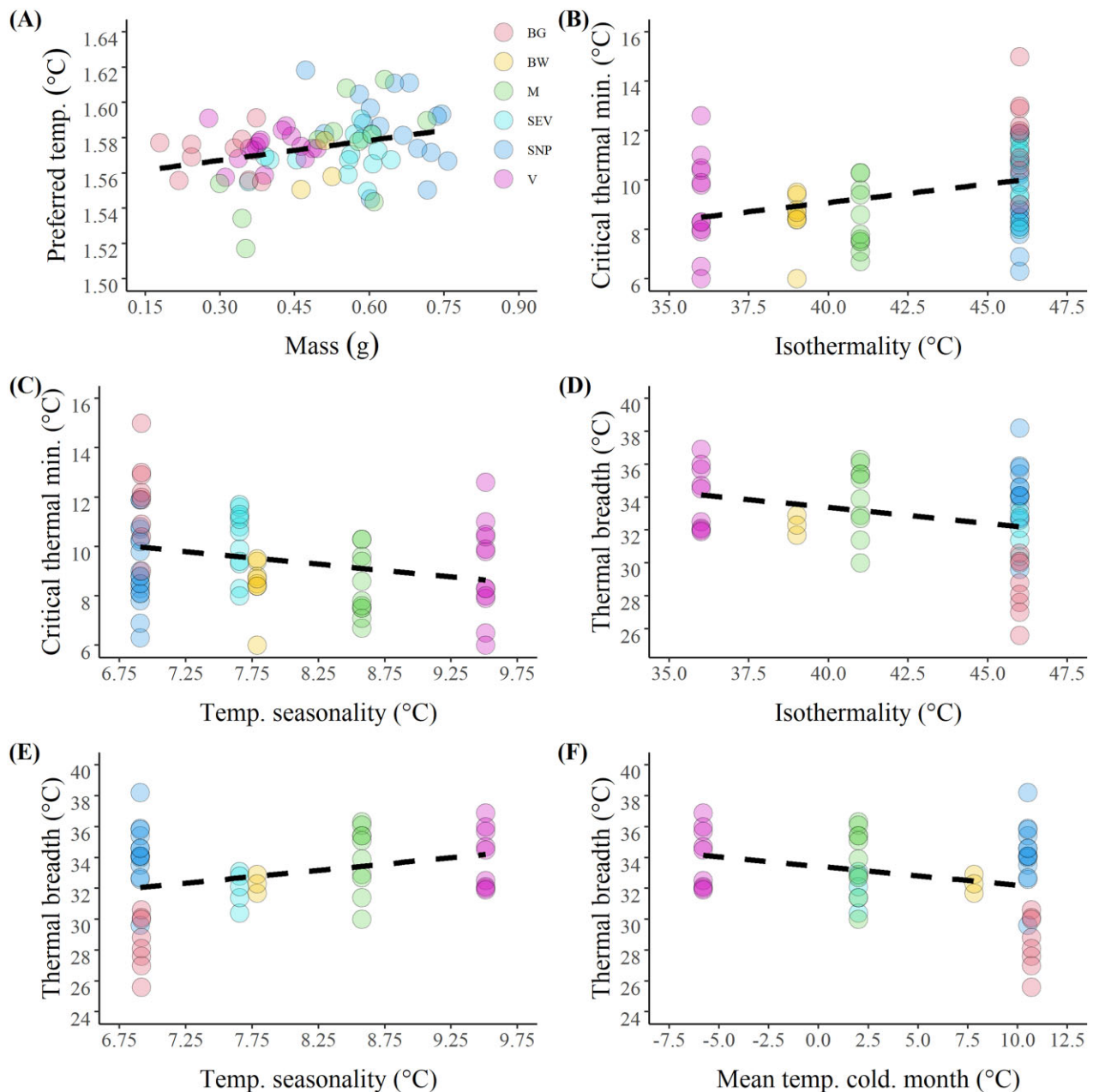


Fig. 1 Relationship between species between thermal traits and body size (mass) or WorldClim variables. **(A)** Relationship between species between mass and T_{pref} ; this is shown in a logarithmic scale. Relationship between CT_{min} and **(B)** isothermality (Bio 3) and **(C)** temperature seasonality (Bio 4). Relationship between thermal breadth (T_{br}) and **(D)** isothermality, **(E)** temperature seasonality, and **(F)** mean temperature of the coldest quarter (Bio 11). Each sample site has a letter code where: V for Vernal, SEV for Sevilleta National Wildlife Refuge, BW for Big Wash, M for Molino Basin, SNP for Saguaro National Park, and BG for Black Gap Wildlife Management Area. The dotted line represents the slope of the regression analysis. Each dot represents an individual. Populations are color coded.

CT_{max} (Saguaro National Park—SNP). There was no difference among populations for T_{pref} ($F_{1,5} = 1.62$, $P = 0.17$; Fig. 2; Table 1); there was a population difference, when modulated by size ($F_{5,58} = 3.38$; $P = 0.01$; Supplementary data; Table S1). We found a strong, significant difference on water loss ($F_{1,3} = 7.24$, $P = 0.0006$; Fig. 3; Table 2) and hydration ($F_{1,3} = 3.80$, $P = 0.0003$; Fig. 3; Table 2) among the sites. Lizards from

Saguaro and Vernal had lower rates of water loss than those from Molino Canyon.

Climate predictors of thermal physiological

We found significant climate predictors (Supplementary data; Table S2) for only CT_{min} and T_{br} . The critical thermal minimum was positively related to isothermality (Bio 3), but negatively related

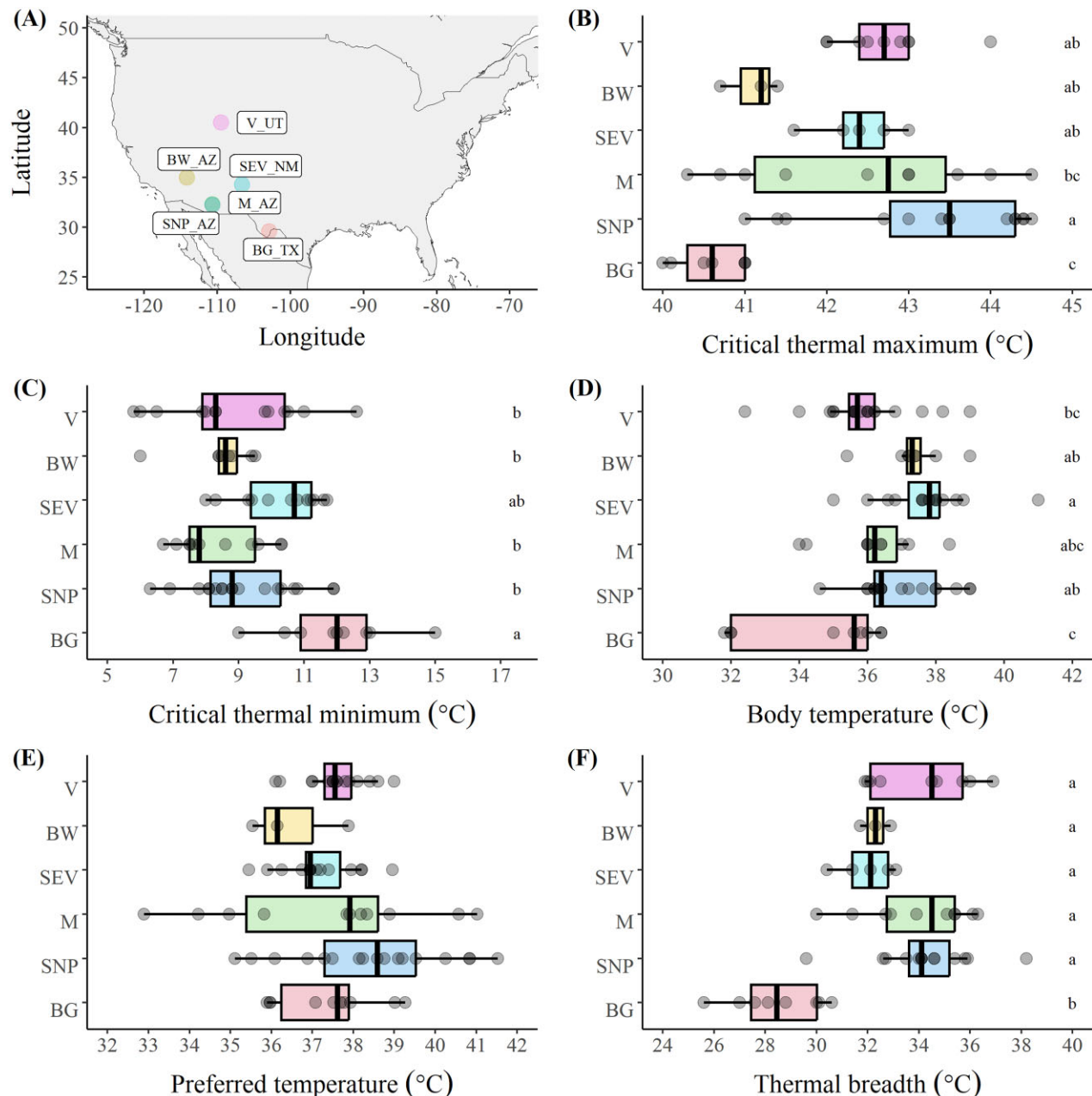


Fig. 2 (A) Sample sites: here, we show the name of each site and the acronym of the state where it is located (UT—Utah, NM—New Mexico, AZ—Arizona, and TX—Texas). Population differences for (B) critical thermal maximum (CTmax), (C) critical thermal minimum (CTmin), (D) body temperature (Tb), (E) preferred temperature (Tpref), and (F) thermal breadth (Tbr). Each sample site has the same letter code as Fig. 1. The small letters a, b, and c indicate if the thermal trait values statistically differ (different letter) or not (same letter). The boxes are colored according to the sample site where the lizards were measured. We included data from males and females. Box is 25–75 percentile, horizontal line in the box is median (black bar), and vertical line (bars) is the box 1.5 times IQR (interquartile range).

with temperature seasonality (Bio 4). While T_{br} had a positive association with temperature seasonality and negative correlations with annual mean temperature (Bio 1), isothermality (Bio 3), and mean temperature of coldest quarter (Bio 11; Fig. 1; Supplementary data; Fig. S1).

Physiological and climatic predictors of water loss

We found that none of the thermal physiological traits were good predictors of water loss (Supplementary Table S3). However, several environmental variables were strongly correlated with water loss

Table 1 Elevation (m), body temperature (T_b ; °C), preferred temperature (T_{pref} ; °C), critical thermal minimum (CT_{min} ; °C) and maximum (CT_{max} ; °C), and thermal breadth (T_{br} ; °C) for each population

Population	Elevation	T_b	T_{pref}	CT_{min}	CT_{max}	T_{br}
BW	1864	37.33 ± 1.01 (8)	36.52 ± 1.22 (3)	8.46 ± 1.08 (8)	41.10 ± 0.60 (3)	32.30 ± 0.60 (3)
BG	622	34.56 ± 2.01 (9)	37.41 ± 1.21 (10)	11.92 ± 1.72 (9)	40.24 ± 0.80 (9)	28.48 ± 1.73 (8)
M	1259	36.16 ± 1.31 (10)	37.33 ± 2.57 (11)	8.40 ± 1.30 (11)	42.41 ± 1.46 (10)	33.92 ± 2.12 (10)
SEV	1774	37.71 ± 1.35 (15)	37.14 ± 0.92 (15)	10.27 ± 1.27 (12)	42.38 ± 0.53 (5)	31.96 ± 1.09 (5)
SNP	837	36.99 ± 1.22 (17)	38.43 ± 1.88 (17)	9.15 ± 1.57 (18)	43.29 ± 1.22 (14)	34.23 ± 1.96 (14)
V	1680	35.86 ± 1.41 (20)	37.58 ± 0.77 (16)	8.85 ± 2.06 (13)	42.72 ± 0.61 (9)	34.03 ± 1.95 (9)

Mean and standard deviation values are shown; numbers in parentheses indicate sample size. Each sample site has a letter code where: V for Vernal, SEV for Sevilleta National Wildlife Refuge, BW for Big Wash, M for Molino Basin, SNP for Saguaro National Park, and BG for Black Gap Wildlife Management Area..

Table 2 Hydration (mOsm/kg), thermal safety margin (TSM), water loss in the throat (throat; $gm^{-2}h^{-1}$), water loss in the belly (belly; $gm^{-2}h^{-1}$), throat water loss in the back (back; $gm^{-2}h^{-1}$), and transepidermal water loss (TEWL; $gm^{-2}h^{-1}$) for each population

Population	Hydration	TSM	Throat	Belly	Back	TEWL
BW	482.00 ± 43.84 (2)	NA	13.90 ± 4.61 (9)	15.78 ± 3.94 (9)	19.19 ± 6.17 (9)	NA
BG	NA	3.04 ± 1.17 (8)	25.03 ± 6.68 (7)	29.49 ± 14.56 (7)	25.74 ± 16.23 (7)	0.0034 ± 0.0022 (6)
M	NA	5.51 ± 2.49 (9)	16.59 ± 6.03 (9)	20.90 ± 10.04 (7)	36.25 ± 16.25 (9)	0.004 ± 0.0015 (11)
SEV	376.50 ± 37.05 (12)	4.64 ± 0.89 (5)	19.07 ± 7.71 (14)	31.00 ± 10.47 (14)	30.69 ± 10.34 (14)	NA
SNP	474.50 ± 69.30 (10)	4.72 ± 2.29 (13)	14.33 ± 4.28 (16)	22.99 ± 11.62 (16)	34.74 ± 12.00 (16)	0.0018 ± 0.0008 (14)
V	468.00 ± 130.79 (7)	4.94 ± 0.63 (9)	9.54 ± 2.97 (19)	16.67 ± 5.75 (19)	19.51 ± 6.29 (18)	0.0022 ± 0.00074 (8)

Mean and standard deviation values are shown; numbers in parentheses indicate sample size. Each sample site has a letter code where: V for Vernal, SEV for Sevilleta National Wildlife Refuge, BW for Big Wash, M for Molino Basin, SNP for Saguaro National Park, and BG for Black Gap Wildlife Management Area.

(Supplementary data; Table S3). The most notable associations were positive correlations with mean diurnal temperature range (Bio 2), temperature seasonality (Bio 4), and temperature annual range (Bio 7) (Fig. 3; Supplementary data; Table S3). Water loss was negatively correlated with mean temperature of the coldest quarter (Bio 11) and precipitation (Bio 12, 13, 16, 18, and 19; Fig. 3; Supplementary data; Fig. S2; Table S3). The population in the more seasonal environment and highest latitude (e.g., Vernal) had higher values of water loss compared to the Saguaro site, which has lower seasonality values. This suggests that as the days get warmer, lizards tend to lose more water, while as the temperature gets colder, they tend to lose less water. Additionally, we found that total water loss was strongly related with TEWL in the throat ($F_{1,28}$, $P = 0.008$, $R^2 = 0.22$; Supplementary data; Table S4) compared to other parts of the body (back and belly; Supplementary data; Fig. S3; Table S4).

Discussion

In this study, we evaluated the effects of latitude and the thermal environment on water loss in populations of *U. ornatus* along a latitudinal gradient spanning

~1400 km. We found significant differences among the populations for four thermal traits (CT_{min} , CT_{max} , T_b , and T_{br}). One thermal trait, preferred temperature (T_{pref}), exhibited a significant mass-population interaction. This suggests that differences in thermal preference are tied to variations in body mass. Our findings show that both latitude and the thermal environment of lizards do not correlate with water loss and hydration. A subset of climatic variables showed a correlation with water loss in lizards. Of the 19 WorldClim variables, nine are correlated to some degree with water loss (Supplementary data; Table S3). Particularly the mean diurnal temperature range (Bio 2; Fig. 3) and mean temperature of the coldest quarter (Bio 11; Fig. 3).

One striking result from this study is that mass is not involved in water loss or in most physiological variables, and only T_{pref} is affected by mass (Fig. 1A), suggesting that as mass increases, T_{pref} increases. The smallest lizards were captured in Vernal, UT, and these had the lowest T_{pref} . The larger individuals were from Saguaro, and they had higher values for T_{pref} . This is an intriguing result, as typically CT_{min} and CT_{max} are affected by body size more than other thermal traits (Gunderson 2024), rather than T_{pref} (but see

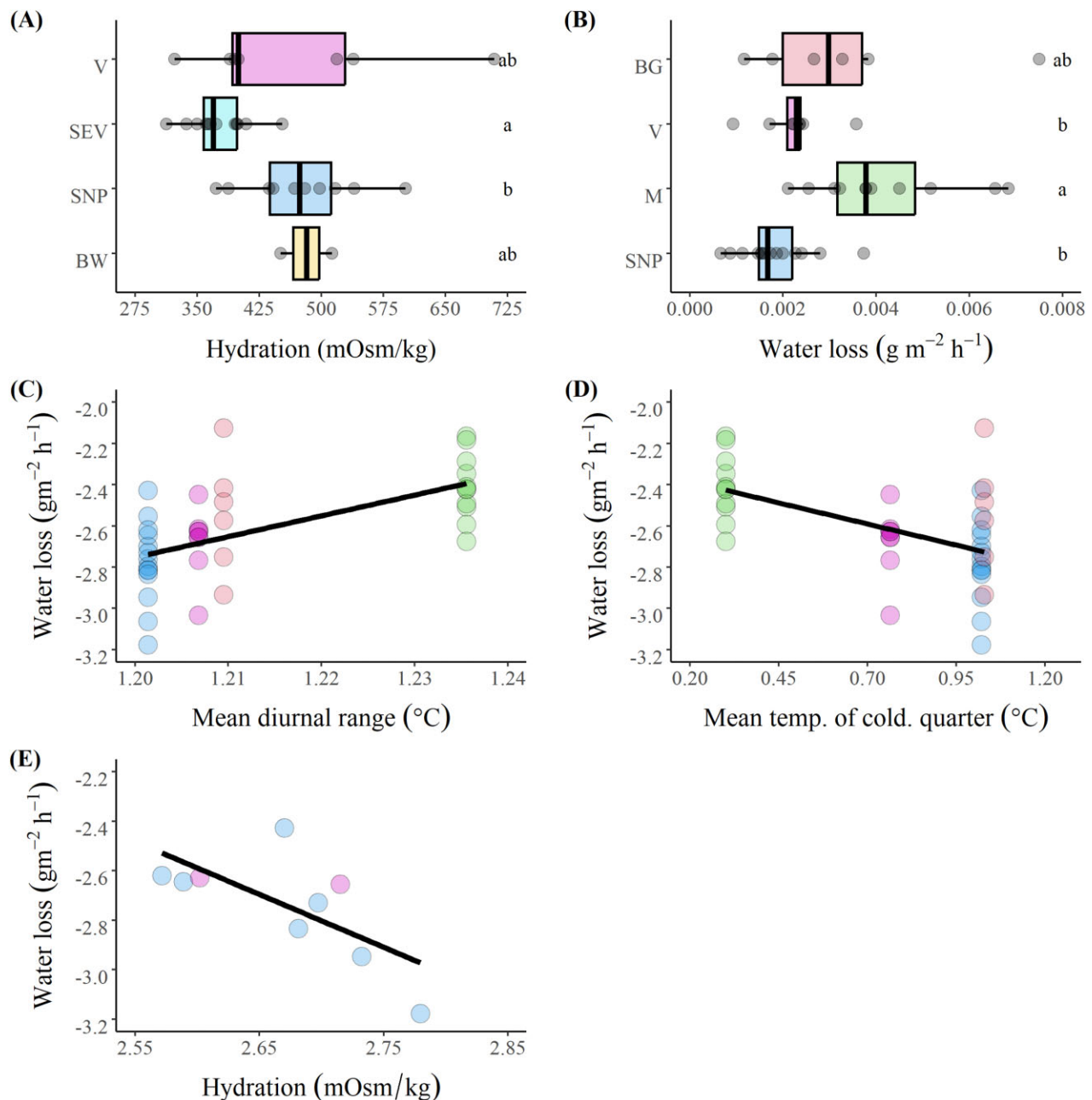


Fig. 3 Population difference for (A) hydration, and (B) TEWL. Each sample site has the same letter code as Fig. 1. Relationship between species between water loss and (C) mean diurnal temperature range (Bio 2), (D) mean temperature of the coldest quarter (Bio 12), and (E) hydration; these three relationships are in logarithmic scale. The small letters a, b, and c indicate if the thermal trait values differ (different letter) or not (same letter) statistically. The boxes are colored according to the sample site where the lizards were measured. We included data from males and females. Boxes are 25–75 percentile, horizontal line in the box is median (black bar), and vertical line (bars) is the box 1.5 times IQR (interquartile range).

Thompson et al. 2023). A possible explanation for why larger individuals might exhibit a higher T_{pref} is that heavier individuals take longer to reach their T_{pref} due to requiring more energy to increase their internal temperature. Consequently, they may prefer warmer T_{pref} . Additionally, their smaller surface-area-to-volume ratio could lead to a lower rate of heat loss through heat transfer with the surrounding environment, potentially

incentivizing the setting of a higher T_{pref} (Porter and Gates 1969; Gunderson 2024).

We observed differences in T_{pref} among populations that depended on body mass (Fig. 2E). The pattern of limited variation in T_{pref} has been found in several other lizard groups (e.g., Garcia-Porta et al. 2019; S'khifa et al. 2022). Our findings suggest that thermoregulatory behavior in *U. ornatus* may be influenced by body

mass dependent of geographic locality. Nevertheless, we did not find clinal variation in T_{pref} . In addition, a recent study observed that hydration and water loss are tightly correlated (Weaver et al. 2024); we find the same pattern (Fig. 3E, Supplementary data; Table S4). Hydration is not correlated with any thermo-physiological traits (Weaver et al. 2024); we observed no relationship between thermal physiological traits and hydration (Fig. 3E, Supplementary data; Table S4).

For CT_{min} , CT_{max} , and T_b , different latitudinal patterns are commonly observed (Stellatelli et al. 2022). We found that CT_{max} increases with latitude while CT_{min} decreases (Sunday et al. 2011; Sunday et al. 2012). However, we noticed substantial variation within each population, which opens the door for future studies that take into account thermal limits and latitude in lizards (Fig. 1B, C). Additionally, those three thermal traits are highly correlated with mean annual temperature (Bio 1), suggesting the sensitivity of species to changes in environmental temperature. However, thermal limits cannot infinitely increase or decrease, necessitating species to find alternative ways to mitigate warming temperatures, often through behaviors such as seeking thermal refugia or changing their activity time.

Water loss is usually correlated with body mass, with water loss increasing as mass increases (Addo-Bediako et al. 2001; Cox and Cox 2015; Sannolo et al. 2019; Albaladejo-Robles et al. 2022; Le Galliard et al. 2021). However, we observed that water loss is uncorrelated with body mass (see Supplementary data; Table S3). In contrast, we found that water loss is highly correlated with environmental variables, specifically mean diurnal temperature range (Bio 2) and mean temperature of the coldest quarter (Bio 11; see Fig. 3; Supplementary data; Table S3). An increase in mean diurnal temperature range leads to increased water loss, while an increase in mean temperature of the coldest month decreases water loss. Our results suggest that the Bio 2 variable poses a critical threat to populations of *U. ornatus*. In this scenario, as global temperatures rise, the mean diurnal temperature range is also likely to increase, resulting in increased water loss in *U. ornatus*. Therefore, this lizard species, like others in different parts of the globe, will be at risk (Huey et al. 2009; Huey et al. 2010). Additionally, the population with the highest rate of water loss, Molino Basin, has free water available nearly year-round (Fig. 3). This pattern was also observed in the European common lizard (*Zootoca vivipara*) (Dupo  e et al. 2017). Common lizards from drier sites had lower rates of water loss than individuals from sites with access to free water, a pattern similar to what we observed in the ornate tree lizard. The other sites, Vernal and Saguaro are located along an alluvial fan

lacking free water (Fig. 3). In addition, we found that water loss is also correlated with the WorldClim related to precipitation (see Supplementary data; Table S3), suggesting that water availability can influence patterns of water loss (Chabaud et al. 2022).

Moreover, we found that water loss in *U. ornatus* is correlated with TEWL at the throat (see Supplementary data; Table S4). In other lizard species, researchers have found the throat is used for thermoregulation when experiencing high temperatures (e.g., Tattersall and Gerlach 2005). However, no study has investigated which specific body part is related to water loss, though other studies have looked at water loss in the eyes and the head (Mautz 1980; Mautz 1982; Waldschmidt and Porter 1987; Blamires and Christian 1999). This study represents the first investigation in lizards where we measured water loss in different parts of the body and determined which part is correlated with overall water loss.

For instance, in bearded dragons (*Pogona vitticeps*), gaping—a movement in the mouth and throat—has been observed to aid in lowering body temperature (Tattersall and Gerlach 2005). This gaping behavior promotes evapotranspiration, consequently reducing body temperature (T_b ; Tattersall and Gerlach 2005). Future studies should focus on the mechanisms and significance of throat water loss not only in *U. ornatus* but also in other lizard species.

Climate change is affecting organisms worldwide, especially those nearing their thermal maximum or facing water stress (Huey et al. 2009; Huey et al. 2010; Danin 2017; Iwami et al. 2017; Nordvelle et al. 2017; Fang and Yu 2020; Wang et al. 2022). When warming temperatures and water stress are combined, the risk of ecological and physiological impacts from climate change amplifies (Lertzman-Lepofsky et al. 2020). It's crucial to recognize that organisms can respond to climate change in various ways: by shifting their habitat range in terms of latitude or elevation (Parmesan et al. 1999; Mont  ano-Centellas et al. 2023), adapting to the new warm conditions (Bradshaw and Holzapfel 2006; Llewelyn et al. 2018; Sun et al. 2021), changing their thermoregulatory behavior (Kearney et al. 2009; Mu  oz et al. 2014), or face local extirpation or global extinct (Sinervo et al. 2010; Pontes-da-Silva et al. 2018; Wiens et al. 2019).

Our results suggest that *U. ornatus*, a desert-dwelling lizard, is likely to be affected by warming temperatures and water stress induced by climate change. Additionally, we observed changes in CT_{max} among populations along a latitudinal gradient (see Fig. 1B), indicating a strong effect of latitude on heat tolerance. Despite this, none of the WorldClim variables correlated with

CT_{max}. Consequently, while *U. ornatus* faces threats from increasing temperatures, the extent of these threats remains uncertain. Given the challenges posed by water loss to organisms, it is especially important to understand how the interplay between thermal physiology and water use can help predict the responses of *U. ornatus* to climate change.

Our study represents an initial step towards understanding the factors influencing species responses to climate change. By measuring several physiological traits and WorldClim variables, we uncovered associations between them and possible consequences to global warming. However, there remains much to explore and test in future studies. Specifically, future research should delve into how microclimatic variables influence the thermal physiology of *U. ornatus*, as well as the roles of thermoregulatory behavior and altitude in adapting to latitude and environmental conditions.

Concluding remarks

Our study aimed to investigate the impact of intrinsic physiology and climatic factors on water loss in *U. ornatus* populations across a 1400 km latitudinal gradient. Although significant differences in thermal traits were observed among populations, they were not correlated with water loss. However, certain climatic variables, notably mean diurnal temperature range and mean temperature of the coldest quarter, showed correlations with water loss. Interestingly, while body mass did not correlate with water loss or most physiological traits, an exception was temperature preference (T_{pref}), which increased with mass.

Furthermore, we observed that CT_{min} decreased and CT_{max} increased with latitude, indicating sensitivity to environmental temperature changes. Water loss was not associated with other physiological traits or body mass. Sites with free water exhibited higher water loss rates, with environmental variables influencing these rates. Notably, throat water loss correlated with overall water loss, suggesting a potential role in thermoregulation.

Our study underscores the threat posed by climate change to *U. ornatus* and emphasizes the importance of understanding the interaction between latitude and the thermal environment in predicting species responses to climatic change. We recommend further research to explore the effects of microclimatic variables on thermal physiology and investigate the role of thermoregulatory behavior and altitude in adapting to environmental conditions.

Author contributions

Jhan C. Salazar: Conceptualization, Methodology, Investigation, Formal Analysis, Writing Original and

drafted the manuscript, and Donald B. Miles: Conceptualization, Methodology, Investigation, Resources, Formal, and Writing – review and editing. All authors contributed to the discussion of the results and provided their contribution to the manuscript.

Acknowledgments

We want to thank Jonathan B. Losos for providing his insightful comments on the manuscript.

Funding

JCS was supported by funding from the Human Frontier of Science Program (HFSP—RGP0030/2020; Dr. Jonathan B. Losos). DBM acknowledges support from the National Science Foundation (DEB 1950636), the Ohio University Research Committee, and Clark Way Harrison Visiting Professor fellowship..

Supplementary data

Supplementary data available at *ICB* online.

Conflict of interest

We declare no competing interests.

Data availability

The full dataset we used is available at: <https://doi.org/10.6084/m9.figshare.26086630.v1>.

References

- Addo-Bediako A, Chown SL, Gaston KJ. 2001. Revisiting water loss in insects: A large-scale view. *J Insect Physiol* 47:1377–88.
- Albaladejo-Robles G, Rodríguez N, Rodríguez-Concepción B, Nogales M, Vences M. 2022. Limited ecophysiological variation in the Canary Island lizard *Gallotia galloti* (Oudart, 1839) across an elevational range of over 3500 m (Squamata: Lacertidae). *Herpetol Notes* 15:87–96.
- Blamires SJ, Christian KA. 1999. Seasonal water loss of the lizard *Lophognathus temporalis* in the wet-dry tropics of northern Australia. *Amphib Reptilia* 20:211–5.
- Bogert CM. 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution* 3:195–211.
- Bradshaw WE, Holzapfel CM. 2006. Evolutionary response to rapid climate change. *Science* 312:1477–8.
- Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J et al. 2005. Regional vegetation die-off in response to global-change-type drought. *Proc Natl Acad Sci USA* 102:15144–8.
- Chabaud C, Berroneau M, Berroneau M, Dupoué A, Guillon M, Viton R, Gavira RSB, Clobert J, Lourdais O, Le Galliard J-F. 2022. Climate aridity and habitat drive geographical variation in morphology and thermo-hydroregulation strategies of a widespread lizard species. *Biol J Linn Soc* 137:667–85.

- Chown SL, Sørensen JG, Terblanche JS. 2011. Water loss in insects: an environmental change perspective. *J Insect Physiol* 57:1070–84.
- Chukwuka CO, Monks JM, Cree A. 2020. Heat and water loss versus shelter: a dilemma in thermoregulatory decision making for a retreat-dwelling nocturnal gecko. *J Exp Biol* 223:jeb231241.
- Cox CL, Cox RM. 2015. Evolutionary shifts in habitat aridity predict evaporative water loss across squamate reptiles. *Evolution* 69:2507–16.
- Danin T. 2017. Projected increases in hurricane damage in the united states: the role of climate change and coastal development. *Ecol Econ* 138:186–98.
- Dezetter M, Le Galliard JF, Guiller G, Guillon M, Leroux-Coyau M, Meylan S, Brischoux F, Angelier F, Lourdaïs O. 2021. Water deprivation compromises maternal physiology and reproductive success in a cold and wet adapted snake *Vipera berus*. *Conserv Physiol* 9:coab071.
- Dupoué A, Rutschmann A, Le Galliard JF, Miles DB, Clobert J, Denardo DF, Brusch GA, Meylan S. 2017. Water availability and environmental temperature correlate with geographic variation in water balance in common lizards. *Oecologia* 185:561–71.
- Fang S-W, Yu J-Y. 2020. Contrasting transition complexity between El Niño and La Niña: observations and CMIP5/6 models. *Geophys Res Lett* 47:e2020GL088926.
- Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *Intl J Clim* 37:4302–15.
- García-Porta J, Irisarri I, Kirchner M, Rodríguez A, Kirchhof S, Brow JL, MacLeod A, Turner AP, Ahmadzadeh F, Albaladejo G et al. 2019. Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nat Commun* 10:4077.
- Ghalambor CK. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr Comp Biol* 46:5–17.
- Gilbert AL, Miles DB. 2019. Antagonistic responses of exposure to sublethal temperatures: adaptive phenotypic plasticity coincides with a reduction in organismal performance. *Am Nat* 194:344–55.
- Gunderson AR, Stillman JH. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc Biol Sci* 282:1–8.
- Gunderson AR. 2024. Disentangling physiological and physical explanations for body size-dependent thermal tolerance. *J Exp Biol* 227:jeb245645.
- Haanel GJ. 2007. Phylogeography of the tree lizard, *Urosaurus ornatus*: responses of populations to past climate change. *Mol Ecol* 16:4321–34.
- Hoffmann AA, Hallas RJ, Dean JA, Schiffer M. 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* 301:100–2.
- Hoffmann AA. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *J Exp Biol* 213:870–80.
- Holt RD. 2003. On the evolutionary ecology of species' ranges. *Evol Ecol Res* 5:159–78.
- Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Álvarez Pérez HJ, Garland T. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proc Biol Sci* 276:1939–48.
- Huey RB, Losos JB, Moritz C. 2010. Are lizards toast? *Science* 328:832–3.
- Huey RB. 1982. Temperature, physiology, and ecology of reptiles. In: Gans C, Pough FH, editors. *Biology of the Reptilia*, vol. 12. New York: Academic Press. p. 25–74.
- Iwami Y, Hasegawa A, Miyamoto M, Kudo S, Yamazaki Y, Ushiyama T, Koike T. 2017. Comparative study on climate change impact on precipitation and floods in Asian river basins. *HRL* 11:24–30.
- Janzen DH. 1967. Why Mountain passes are higher in the tropics. *Am Nat* 101:233–49.
- Kearney M, Shine R, Porter WP. 2009. The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proc Natl Acad Sci USA* 106:3835–40.
- Le Galliard J-F, Chabaud C, De Andrade DOV, Brischoux F, Carretero MA, Dupoué A, Gavira RSB, Lourdaïs O, Sannolo M, Van Dooren TJM. 2021. A worldwide and annotated database of evaporative water loss rates in squamate reptiles. *Glob Ecol Biogeogr* 30:1938–50.
- Lertzman-Lepofsky GF, Kissel AM, Sinervo B, Palen WJ. 2020. Water loss and temperature interact to compound amphibian vulnerability to climate change. *Glob Chang Biol* 26:4868–79.
- Llewelyn J, Macdonald SL, Moritz C, Martins F, Hatcher A, Phillips BL. 2018. Adjusting to climate: acclimation, adaptation and developmental plasticity in physiological traits of a tropical rainforest lizard. *Integr Zool* 13:411–27.
- Lorenzon P, Clobert J, Oppliger A, John-Alder H. 1999. Effect of water constraint on growth rate, activity and body temperature of yearling common lizard (*Lacerta vivipara*). *Oecologia* 118:423–30.
- Masó G, Ozgul A, Fitze PS. 2020. Decreased precipitation predictability negatively affects population growth through differences in adult survival. *Am Nat* 195:43–55.
- Mautz WJ. 1980. Factors influencing evaporative water loss in lizards. *CBPA* 67:429–37.
- Mautz WJ. 1982. Correlation of both respiratory and cutaneous water losses of lizards with habitat aridity. *J Comp Physiol B* 149:25–30.
- Miles DB. 1994. Population differentiation in locomotor performance and the potential response of a terrestrial organism to global environmental change. *Am Zool* 34:422–36.
- Montaño-Centellas F, Fuentes AF, Cayola L, Macía MJ, Arellano G, Loza MI, Nieto-Ariza B, Tello JS. 2023. Elevational range sizes of woody plants increase with climate variability in the Tropical Andes. *J Biogeogr* 00:1–13.
- Muñoz MM, Stimola MA, Algar AC, Conover A, Rodríguez AJ, Landestoy MA, Bakken GS, Losos JB. 2014. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proc R Soc B* 281:1–9.
- Nordkvelle J, Rustad SA, Salmivalli M. 2017. Identifying the effect of climate variability on communal conflict through randomization. *Clim Change* 141:627–39.
- Ortega Z, Mencia A, Pérez-Mellado V. 2016. Are mountain habitats becoming more suitable for generalist than cold-adapted lizards thermoregulation? *PeerJ* 4:e2085.
- Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thomas CD, Descimon H, Huntley B, Kaila L, Kullberg J, Tammaru T et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* 399:579–83.
- Pintor AFV, Schwarzkopf L, Krockenberger AK. 2016. Hydoregulation in a tropical dry skinned ectotherm. *Oecologia* 182:925–31.

- Pontes-da-Silva E, Magnusson WE, Sinervo B, Caetano GH, Miles DB, Colli GR, Diele-Viegas LM, Fenker J, Santos JC, Werneck FP. 2018. Extinction risks forced by climatic change and intraspecific variation in the thermal physiology of a tropical lizard. *J Therm Biol* 73:50–60.
- Porter WP, Gates DM. 1969. Thermodynamic equilibria of animals with environment. *Ecol Monogr* 39:227–44.
- R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rozen-Rechels D, Badiane A, Agostini S, Meylan S, Le Galliard J-F. 2020. Water restriction induces behavioral fight but impairs thermoregulation in a dry-skinned ectotherm. *Oikos* 129:572–84.
- Rozen-Rechels D, Farigoule P, Agostini S, Badiane A, Meylan S, Le Galliard J-F. 2020 Short-term change in water availability influences thermoregulation behaviours in a dry-skinned ectotherm. *J Anim Ecol* 89:2099–110.
- Rozen-Rechels D, Rutschmann A, Dupoué A, Blaimont P, Chauveau V, Miles DB, Guillon M, Richard M, Badiane A, Meylan S et al. 2021. Interaction of hydric and thermal conditions drive geographic variation in thermoregulation in a widespread lizard. *Ecol Monogr* 91: e01440.
- S'khifa A, Carretero MA, Harris DJ, Slimani T. 2022. Eco-physiological conservativeness and size-mediated plasticity in the High Mountain Lizard *Atlantolacerta andreanskyi* confirm its vulnerability to climate change. *Salamandra* 58: 139–50.
- Sannolo M, Civantos E, Martín J, Carretero MA. 2020. Variation in field body temperature and total evaporative water loss along an environmental gradient in a diurnal ectotherm. *J Zool* 310:221–31.
- Sinclair BJ, Marshall KE, Sewell MA, Levesque DL, Willett CS, Slotsbo S, Dong Y, Harley CDG, Marshall DJ, Helmuth BS et al. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol Lett* 19:1372–85.
- Sinervo B, Méndez-De-La-Cruz F, Miles DB, Heulin B, Bastiaans E, Villagrán-Santa Cruz M, Lara-Resendiz R, Martínez-Méndez N, Calderón-Espinosa ML, Meza-Lázaro RN et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328:894–9.
- Stellatelli OA, Vega LE, Block C, Rocca C, Bellagamba P, Dajil JE, Cruz FB. 2022. Latitudinal pattern of the thermal sensitivity of running speed in the endemic lizard *Liolaemus multimaculatus*. *Integr Zool* 17:619–37.
- Sun BJ, Ma L, Wang Y, Mi CR, Buckley LB, Levy O, Lu HI. 2021. Latitudinal embryonic thermal tolerance and plasticity shape the vulnerability of oviparous species to climate change. *Ecol Monogr* 91:e01468.
- Sunday JM, Bates AE, Dulvy NK. 2011. Global analysis of thermal tolerance and latitude in ectotherms. *Proc R Soc B Biol Sci* 278:1823–30.
- Sunday JM, Bates AE, Dulvy NK. 2012. Thermal tolerance and the global redistribution of animals. *Nat Clim Change* 2:686–90.
- Tattersall GJ, Gerlach RM. 2005. Hypoxia progressively lowers thermal gaping thresholds in bearded dragons, *Pogona vitticeps*. *J Exp Biol* 208:3321–30.
- Thompson A, Kapsanaki V, Liwanag HEM, Pafilis P, Wang JJ, Brock KM. 2023. Some like it hotter: differential thermal preferences among lizard color morphs. *J Therm Biol* 113: 103532.
- Waldschmidt SR, Porter WP. 1987. A model and experimental test of the effect of body temperature and wind speed on ocular water loss in the lizard *Uta stansburiana*. *EEP* 60: 678–86.
- Wang Y, Huang G, Hu K, Tao W, Li X, Ging H, Gu L, Zhang W. 2022. Asymmetric impacts of El Niño and La Niña on the Pacific–South America teleconnection pattern. *JCLI* 35:1825–38.
- Weaver SJ, Axsom IJ, Peria L, McIntyre T, Chung J, Telemeco RS, Westphal MF, Taylor EN. 2024. Hydric physiology and ecology of a federally endangered desert lizard. *Conserv Physiol* 12:coae019.
- Wickham H. 2009. ggplot2. Elegant Graphics for Data Analysis. London: Springer.
- Wiens JJ, Camacho A, Goldberg A, Jezkova T, Kaplan ME, Lambert SM, Miller EC, Streicher JW, Walls RL. 2019. Climate change, extinction, and Sky Island biogeography in a montane lizard. *Mol Ecol* 28:2610–24.
- Zamora-Camacho FJ, Reguera S, Moreno-Rueda G, Pleguezuelos JM. 2013. Patterns of seasonal activity in a Mediterranean lizard along a 2200 m altitudinal gradient. *J Therm Biol* 38: 64–9.