



ELSEVIER

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Thermal tolerance varies with age and sex for the nonnative Italian Wall Lizard (*Podarcis siculus*) in Southern California

Heather E.M. Liwanag^{a,*}, Daniel Haro^a, Breanna Callejas^b, Gloria Labib^b, Gregory B. Pauly^c

^a Department of Biological Sciences, California Polytechnic State University, San Luis Obispo, CA, United States

^b Department of Biology, Adelphi University, Garden City, NY, United States

^c Section of Herpetology and Urban Nature Research Center, Natural History Museum of Los Angeles County, Los Angeles, CA, United States

ARTICLE INFO

Keywords:

Critical thermal maximum
Critical thermal minimum
Nonnative species
Preferred temperature
Thermal adaptation
Thermal preference
Thermal tolerance breadth
Thermoregulation
Evaporative water loss

ABSTRACT

Temperature has a substantial effect on both the physiology and behavior of ectothermic animals such as lizards. Physiology and behavior can also be influenced by ontogenetic and sex differences, but these effects are largely understudied in lizards. We examined ontogenetic and sex-based differences in thermal tolerances, preferred temperature, and temperature-dependent evaporative water loss rates in Italian Wall Lizards, *Podarcis siculus*, collected from an introduced population near Los Angeles, California, USA that were acclimated to laboratory conditions. *Podarcis siculus* has been introduced to multiple localities in the USA and the Mediterranean region and has demonstrated remarkable ability to adapt to novel climatic conditions. In the California population, adults of both sexes had a higher critical thermal maximum (CT_{max}) than juveniles, and adult females had a lower critical thermal minimum (CT_{min}) than juveniles and adult males. Thus, adult females had a significantly wider thermal breadth ($CT_{max} - CT_{min}$) compared to adult males and juveniles. Mass-specific evaporative water loss was higher in juveniles compared to adult males at intermediate temperatures. There was no significant difference among groups for preferred temperature. This implies that thermal tolerance, a physiological characteristic, varies with age and sex for this population, whereas thermal preference, a behavioral characteristic, does not. Interestingly, CT_{min} for all age and sex classes was above temperatures likely experienced by some nonnative populations in winter, suggesting individuals need to find urban thermal retreats. These results add to the growing literature demonstrating that thermal tolerances and breadths can vary between sexes and across age classes in squamate species.

1. Introduction

Body temperature is an important physiological factor that affects the performance of ectothermic animals (Stevenson, 1985). An ectotherm's body temperature profoundly influences the performance of physiological systems such as locomotion (Huey et al., 1989), digestion (van Marken Lichtenbelt, 1992), and growth (Angilletta et al., 2004). Furthermore, ectotherms are known to exhibit a positive relationship between body temperature and physiological variables such as metabolic rate and evaporative water loss (Andrews and Pough, 1985; Zucker, 1980). As a result, the body temperatures that ectotherms experience will directly affect energetic and osmotic balance in these animals.

Thermal tolerances and performance vary widely from species to species depending on a variety of influences, including both abiotic factors (e.g., temperature or environmental moisture) (Clusella-Trullas

et al., 2011; Deutsch et al., 2008) and biotic factors (e.g., phylogenetic patterns and diet) (Clusella-Trullas and Chown, 2014; Tejpal et al., 2014). Thermal tolerances and performance can also vary intraspecifically, with differences among populations, among age classes, or between the sexes. In general, juvenile ectotherms tend to be less tolerant of environmental stressors, including temperature changes (Gilbert and Lattanzio, 2016; Tang et al., 2013). In addition, the sex of an ectothermic organism may potentially influence its temperature tolerance (Beal et al., 2014; Lailvaux and Irschick, 2007; Lailvaux et al., 2003; Winne and Keck, 2005) and desiccation tolerance (Gilbert and Lattanzio, 2016). Although thermal tolerances and breadths are critically linked to understanding organismal physiology, behavior, and ecology, and thermal tolerances can vary within species, relatively few studies have tested for ontogenetic and sex-based differences in thermal tolerances. More such studies are needed before general patterns can be assessed.

* Corresponding author.

E-mail address: hliwanag@calpoly.edu (H.E.M. Liwanag).

<https://doi.org/10.1016/j.jtherbio.2018.10.010>

Received 8 March 2018; Received in revised form 4 October 2018; Accepted 13 October 2018

Available online 19 October 2018

0306-4565/© 2018 Elsevier Ltd. All rights reserved.

Introduced species are especially important model organisms for examining thermal tolerances. Although species typically have preferred temperatures and are unlikely to deviate substantially from those temperatures, introduced species sometimes thrive in habitats that may expose them to temperatures outside of those experienced in the native range (Matzek, 2012). One such introduced species is *Podarcis siculus*, the Italian Wall Lizard, which has become established in areas such as the central and northeastern United States, where the climate is drastically different from the mild, Mediterranean climate found in their native range of Italy and the Adriatic Coast (Burke et al., 2002; Donihue et al., 2014; Kolbe et al., 2013). The Italian Wall Lizard's ability to thrive in diverse climates may be attributable to broad thermal tolerances, rapid evolutionary adaptation, acclimatization, or some combination of the three. Here, we ask whether the remarkable eurythermy of *P. siculus* also varies among sexes and age classes within the same population. The objectives of this study were: (1) to determine the thermal tolerances, thermal preferences, and temperature-dependent evaporative water loss (EWL) rates of *P. siculus* collected from an introduced population in Southern California and (2) to examine whether thermal tolerances, thermal preferences, and/or EWL differ among sexes or age classes after prolonged acclimation to laboratory conditions, including constant temperature. In addition to gaining a better understanding of how thermal physiology may vary across age classes and sexes, these results will also allow future research examining the thermal biology of introduced *P. siculus* populations inhabiting dramatically different climates.

2. Materials and methods

2.1. Study population and husbandry

We examined lizards from a nonnative *P. siculus* population in coastal San Pedro, Los Angeles County, California. Deichsel et al. (2010) identified the source of the population as a local homeowner who collected four males and three females from Taormina on Sicily and then released them into an urban Southern California backyard in 1994. The population has expanded dramatically from that single yard and now covers an area with a radius 350–700 m from the original release point (Pauly, unpublished data). Annual surveys (2013–2017) indicate that the population continues to expand rapidly across this urban residential neighborhood with extremely high densities of lizards (> 50 individuals per house lot) in some areas (Pauly, unpublished data). This Southern California population and populations in the native range all occur in a Mediterranean climate (Table A1).

In October 2014, 28 Italian Wall Lizards were collected: 9 adult males (mean mass \pm SE = 6.87 \pm 0.65 g; mean SVL \pm SE = 68.00 \pm 2.31 mm), 7 adult females (4.72 \pm 0.36 g; 61.29 \pm 3.38 mm), and 12 juveniles (2.82 \pm 0.20 g; 47.67 \pm 1.31 mm). Surface air temperatures at this site during October typically range between 10 °C and 18 °C (Arguez et al., 2012). Each lizard was housed in an individual 9.5-litre plastic terrarium, with tree bark (Zilla bark blend, Franklin, WI) as substrate for burrowing. The room was maintained at 28 °C with a space heater (Lasko, Philadelphia, PA) to acclimate the lizards to a common thermal environment (Burke et al., 2002; but see Discussion for recommendation against prolonged housing at constant temperature in future research). Animals were maintained on a 12:12 h light:dark cycle. The substrate was sprayed with water on a daily basis to maintain humidity. Lizards were fed five crickets twice weekly, and were supplied with water *ad libitum*. Animals were maintained in the laboratory setting for a minimum of 3 months prior to measurements to minimize any potential effects of recent thermal history of individuals. *Podarcis siculus* is sexually dimorphic for a number of characters; adults were identified to sex based on examination of body size, body shape, head shape, color pattern, and size of femoral pores. In October, adult males in this population are experiencing recrudescence and females are quiescent with egg-laying ending in August (Pauly and Goldberg, unpublished data).

None of the adult females were gravid at the time of testing. Juveniles measured 40–54 mm SVL at the time of testing, below the minimum size at sexual maturity for males and females in this population, which is 55 mm for females and 57 mm for males (Pauly and Goldberg, unpublished data). Due to limitations imposed by our animal use protocols, the same individuals were reused for multiple measurements. Lizards were provided a recovery time of at least 1 mo between measurements of different variables (thermal preferences, thermal tolerances, EWL). One of the juvenile lizards died after its preferred temperature had been determined but prior to determination of thermal tolerances. The animals were transferred to a new housing facility prior to the measurement of EWL, and more lizards died (7 juveniles, 3 adult females) after this transfer. We waited an additional month after the transfer to ensure the remaining animals were of good health prior to EWL measurements.

Prior to each temperature-based measurement, each lizard was weighed to the nearest 0.001 g with a digital balance (Practicum 213, Sartorius, Bohemia, NY). A flexible, 40-gauge type K thermocouple (Omega Engineering, Stamford, CT) was inserted into the lizard's cloaca and securely taped with medical tape. Lizard body temperatures were monitored continuously with a four-channel thermocouple reader (Model RDXL4SD, Omega Engineering, Norwalk, CT). Thermocouples were cleaned and sanitized with ethanol between trials. All trials were conducted in the daytime, which is appropriate for a diurnal species.

2.2. Thermal tolerances

To secure the lizard during thermal tolerance trials, a short strand of dental floss (Johnson and Johnson Reach brand) was taped to the venter of the lizard using a piece of medical tape, placed carefully to avoid impeding limb movement. This strand of dental floss was then attached to a small piece of cardboard (10.5 cm \times 3.0 cm) to anchor the lizard in place. The lizard was placed within an open 11-litre plastic Rubbermaid roughneck container (40.1 cm L \times 26.0 cm W \times 17.7 cm H), and the cardboard anchor was taped securely to the side of the container. Lizard body temperature was manipulated using an environmental chamber (Precision model 818 Plant Growth Chamber, Thermo Scientific, Somerset, NJ). The thermocouple wires were carefully threaded through the door seal and attached to the thermometer, allowing for the lizard's internal body temperature to be monitored while the animal was in the chamber. The measurement of thermal tolerance was randomized, such that approximately half of the lizards were tested for minimum tolerance first, and the other half for maximum tolerance first. To minimize stress on the lizards, a recovery time of at least 48 h separated the measurement of minimum and maximum thermal tolerances for each lizard.

Thermal tolerances were measured by slowly altering the temperature of the testing chamber and then measuring the loss of righting reflex (Cowles and Bogert, 1944; Huey and Kingsolver, 1989; Huey and Stevenson, 1979; Lutterschmidt and Hutchison, 1997). To assess critical thermal minimum (CT_{min}), the secured lizard was placed in an environmental chamber kept at 2 °C. Once the lizard's internal body temperature reached 14 °C, its righting ability was tested. Using a pair of 11" bamboo (OXO brand) cooking tongs, the lizard was gently placed on its back and allowed to flip itself back onto its venter. This process was repeated at every 1 °C drop in body temperature, until it took more than 3 s for the lizard to right itself. If a lizard did not right itself readily, its hind limbs were gently pinched using small metal forceps to induce a righting response. If a righting response was seen after agitation, testing would continue. Although maintaining chamber temperature at 2 °C was enough to cause most of the lizards' internal body temperatures to drop successfully, some lizards required a lower environmental temperature to induce a sufficient drop in body temperature. In those cases, the chamber's temperature was carefully reduced at 1 °C intervals until a decrease in lizard body temperature was induced. In some trials, the chamber reached almost –2 °C before CT_{min} could be assessed, while lizard body temperature neared 5–11 °C. Once a lizard

did not right itself after agitation, its internal body temperature was recorded as CT_{\min} . The lizard was then removed from the chamber and righting ability was reassessed after body temperature increased by 1 °C. If the lizard flipped itself readily after warming 1 °C, the initially recorded CT_{\min} was considered valid. If after warming 1 °C, the lizard still could not right itself readily or after agitation, righting ability was reassessed at additional 0.5 °C intervals as the lizard continued to warm. CT_{\min} was recorded as the final temperature at which the lizard was unable to right itself. Once CT_{\min} was determined, the lizard was allowed to warm to room temperature before being placed in its individual cage. The rate of warming in most cases was approximately 1 °C per minute.

The critical thermal maximum (CT_{\max}) was assessed in a similar manner as CT_{\min} . The environmental chamber was initially set at 39 °C. Assessment of righting ability began once lizard body temperature reached 36 °C, and was reassessed at each 1 °C increase in body temperature. Because the higher temperatures caused lizards to move vigorously, lizards were flipped by hand if the animals could no longer be manipulated with the wooden tongs. Although surface temperature of the experimenter could potentially influence lizard body temperature, any effect should be minimal because human body temperature is close to the experimental temperatures for CT_{\max} trials. A constant 39 °C was not sufficient to cause internal body temperature to reach CT_{\max} for most lizards. Therefore, the chamber temperature was increased in 1 °C intervals until an additional rise in lizard body temperature was observed. Because CT_{\max} is often close to the lethal maximum (Krenek et al., 2012), the chamber temperature was increased incrementally to reduce the risk of mortality. In many cases, the chamber temperature neared 50 °C before CT_{\max} could be assessed. In these scenarios, lizard body temperature neared 43–44 °C. None of the lizards died during the measurement of CT_{\max} . Once CT_{\max} was recorded, the lizard was removed from the chamber and righting ability was reassessed after body temperature decreased by 1 °C. If the lizard flipped itself readily after cooling 1 °C, the initially recorded CT_{\max} was considered valid. If after cooling 1 °C, the lizard still could not right itself readily or after agitation, righting ability was reassessed at additional 0.5 °C intervals as the lizard cooled. CT_{\max} was recorded as the final temperature at which the lizard was unable to right itself. Once CT_{\max} was determined, the lizard was allowed to cool to room temperature before being placed in its individual cage. The rate of cooling in most cases was approximately 1 °C per minute. The method used for assessing CT_{\max} and CT_{\min} is similar to that used in another lizard thermal biology study (Leal and Gunderson, 2012).

2.3. Preferred temperature

Preferred temperature (T_{pref}) was measured using a linear temperature gradient (163 cmL × 46 cmW) divided into four lanes, with a temperature range from 10 °C to 40 °C along its length. White dividers were placed between lanes so that lizards could not see each other, to reduce the potential effects of visual cues on spacing. A 1-cm thick plastic sheet was placed above the gradient to restrict air flow between lanes and reduce potential effects of neighboring scents on spacing. The cold end of the gradient was maintained with cold water circulated through piping beneath the metal floor of the gradient, using a circulating water bath (SD07R-20, PolyScience, Niles, IL) set to 10 °C. The warm end of the gradient was maintained by electric heat strips beneath the metal floor of the gradient. The bottom of the gradient was covered with reptile sand (Zoo Med, San Luis Obispo, CA) so that the lizards were not in direct contact with the metal floor. The gradient was encased in a Plexiglas chamber lined with Drierite (W.A. Hammond, Xenia, OH) to reduce condensation. Each lizard was fitted with a flexible, 40-gauge type K thermocouple (Omega Engineering, Stamford, CT) inserted into the lizard's cloaca and securely taped with medical

tape, and placed into a separate lane of the gradient. Four lizards were tested simultaneously, and lizard body temperatures were monitored continuously with a four-channel thermocouple reader (Model RDXL4SD, Omega Engineering, Stamford, CT). Body temperatures were recorded every 15 min for 2 h, and T_{pref} was determined as the average body temperature within the 50% interquartile of the last hour (Hertz et al., 1993; Huey, 1982). Because time of day can influence T_{pref} (Carretero et al., 2006; Cowgell and Underwood, 1979), all trials were conducted at the same time of day (late morning) for consistency.

2.4. Evaporative water loss

Evaporative water loss (EWL) was measured as mass lost over time at 10 °C, 20 °C, 30 °C, and 40 °C. One trial was performed per temperature for a total of four trials, in a random order with respect to experimental temperature. Lizards were fasted one week prior to each trial in order to minimize the probability of defecation during the trial. To minimize lizard movement during the trials we placed lizards in fiberglass (18 × 16 mesh count, 0.011ga diameter) pouches measuring 11 cm × 14 cm, giving lizards only enough space to turn within the pouch. Each lizard was placed inside a pouch and weighed to the nearest 0.001 g using a digital balance (Practicum 213, Sartorius, Bohemia, NY); this was recorded as the starting mass. The lizards (in pouches) were placed atop individual petri dishes on three separate shelves inside an environmental chamber for 7.5 h. Relative humidity within the chamber scaled linearly with temperature ($RH = 34.4 - 0.63 T$, $R^2 = 0.87$), measuring 28%, 21.7%, 15.4%, and 9.1% at 10 °C, 20 °C, 30 °C, and 40 °C respectively. Maximum airflow at the ventilation window within our chamber was 1.4 km h^{-1} , and was undetectable at shelf heights. Lizards were re-weighed every 1.5 h and their positions were rotated among the shelves to control for any inconsistency of temperature, humidity, or airflow within the chamber. Any animal that urinated or defecated into the petri dish was eliminated from the trial. In addition, one adult male managed to escape from the pouch during the 30 °C trial. Evaporative water loss was calculated as the net loss in body mass over the duration of the trial (Mautz, 1982; Pintor et al., 2016; Withers et al., 2000). This rate was divided by initial lizard mass to obtain a mass-specific rate of water loss.

2.5. Statistical analyses

Thermal tolerances (CT_{\min} and CT_{\max}) and thermal breadth ($CT_{\max} - CT_{\min}$) were compared among sexes and age classes using ANCOVA, with mass as a covariate. Similarly, T_{pref} was compared among sexes and age classes using ANCOVA, with mass and gradient lane as covariates. Non-significant covariates were removed sequentially from the model, and pairwise comparisons were performed using a Tukey test. Total EWL was log transformed to achieve normality and compared among sexes and age classes using a mixed linear model. Temperature, sex/age class, and the interaction between the two factors were included as fixed effects, log transformed mass was included as a covariate, and individual lizard ID was included as a random effect. To detect any differences in mass-specific EWL, we divided total EWL by the initial mass of each lizard and analyzed log transformed mass-specific EWL with temperature. Sex/age class, and the interaction between the two factors were included as fixed effects, while including individual lizard ID as a random effect. When significant interactions were present we compared independent means by pairwise comparisons using the 'multcomp' package in the R environment (Hothorn et al., 1979). P values were corrected for multiple tests using the Holm method (Holm, 1979). Unless otherwise specified, numerical values are presented as mean ± SEM. Where unspecified, $P < 0.05$ was considered statistically significant. Mixed models were performed using the statistical software JMP (Version 12.0, SAS Institute Inc., Cary, NC).

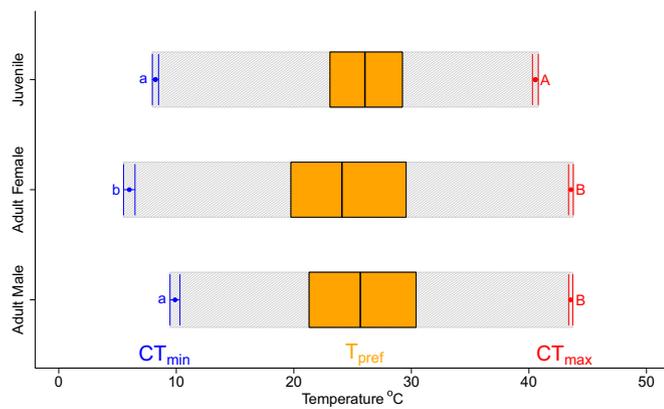


Fig. 1. Thermal tolerance and preferred temperature for *P. siculus* according to age and sex classes. Mean critical thermal minimum (CT_{min} ; \pm SEM) is represented in blue at the left edge of each bar; different lowercase letters represent significant differences in CT_{min} among groups ($n = 11$ juveniles, 7 adult females, 9 adult males). Mean critical thermal maximum (CT_{max} ; \pm SEM) is represented in red at the right edge of each bar; different uppercase letters represent significant differences in CT_{max} among groups ($n = 11$ juveniles, 7 adult females, 9 adult males). Interquartile range of the last body temperature selected by lizards is represented by the orange box toward the middle of each bar. Preferred temperatures (T_{pref} ; black band within the orange box) were not significantly different among groups ($n = 12$ juveniles, 7 adult females, 9 adult males). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

CT_{min} was significantly different among groups ($F_{2,23} = 20.489$, $P < 0.001$), with no significant effect of body mass ($P = 0.761$) in the full model (Fig. 1; Table A2). Adult females had a significantly lower CT_{min} compared to juvenile lizards ($P = 0.005$) and adult males ($P < 0.001$). The difference between juvenile CT_{min} and adult male CT_{min} approached significance, but was not statistically significant after adjustment of the probability statistic using the Tukey-Kramer method ($P = 0.05$) (Fig. 1). CT_{max} was significantly different among groups ($F_{2,23} = 38.073$, $P < 0.001$), with no significant effect of body mass ($P = 0.877$) in the full model (Table A2). Juveniles had a significantly lower CT_{max} compared to adult females ($P < 0.001$) and adult males ($P < 0.001$), but adults did not differ significantly from each other ($P = 0.993$) (Fig. 1). Thermal tolerance breadth ($CT_{max} - CT_{min}$) was significantly different among groups ($F_{2,24} = 35.528$, $P < 0.001$), with no significant effect of body mass ($P = 0.731$) in the full model (Table A2). Adult females had a significantly broader thermal tolerance compared to juveniles ($P < 0.001$) and adult males ($P < 0.001$). Although there was no significant difference in thermal tolerance breadth between juveniles and adult males ($P = 0.395$), the breadth of adult males was shifted towards higher temperatures relative to juveniles (Fig. 1). Our novel method of allowing recovery for CT_{min} and CT_{max} resulted in a change in recorded CT_{min} for one juvenile from 7 °C to 8.5 °C. Using the original value for statistical analysis does not change the significance of our ANCOVA results nor the Tukey test results for CT_{min} or thermal tolerance breadth comparisons.

There was no significant difference in the preferred temperature among groups ($F_{2,21} = 0.321$, $P = 0.729$), and no effect of body mass ($P = 0.549$) or gradient lane ($P = 0.622$) in the full model or as independent predictor variables (Fig. 1; Table A2). Animals were observed shuttling between opposite ends of the gradient, but were generally aligned in similar positions within the gradient by the end of each trial.

Total EWL significantly increased with temperature across all groups ($F_{3,36.7} = 112.080$, $P < 0.001$; Fig. A1). Mass was not a significant covariate of total EWL in our model ($F_{1,21.7} = 3.399$, $P = 0.079$); we report the effects of mass at each temperature in the

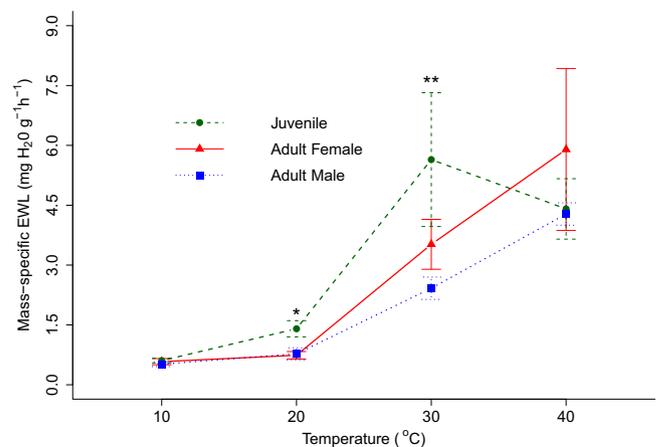


Fig. 2. Mean \pm SEM mass-specific evaporative water loss rates ($\text{mg H}_2\text{O g}^{-1} \text{h}^{-1}$) for *P. siculus* juveniles (green circles with dashed line), adult females (red triangles with solid line), and adult males (blue squares with dotted line). Significance between juveniles and adult males is denoted by asterisks as follows: * $P < 0.05$, ** $P < 0.01$. Significance was determined using pairwise comparisons of means corrected for multiplicity using the Holm method. Sample sizes are as follows: 10 °C: $n = 4$ juveniles, 4 adult females, 8 adult males; 20 °C: $n = 3$ juveniles, 4 adult females, 8 adult males; 30 °C: $n = 4$ juveniles, 4 adult females, 5 adult males; 40 °C: $n = 4$ juveniles, 4 adult females, 8 adult males. Reduced sample sizes within a group are due to the animal escaping from the pouch (1 adult male) or urination or defecation during that trial, rather than attrition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Supplementary materials (Table A3). Mass-specific EWL was significantly different among groups ($F_{2,13.4} = 4.934$, $P = 0.025$) Fig. 2. Specifically, mass-specific EWL was 81% higher in juveniles compared to adult males at 20 °C (corrected $P = 0.041$) and 133% higher in juveniles compared to adult males at 30 °C (corrected $P = 0.006$; Fig. 2, Table A4).

4. Discussion

Sexual maturation and sex-specific hormonal changes can alter physiological phenotypes in lizards (Cox et al., 2008; Kubička et al., 2017; Schořáľková et al., 2017). Studies of thermal physiology in lizards have historically focused only on adult males, to remove the possibility of hormonal or other sex mediated differences, or have not taken sex into account at all (Huey and Pianka, 2007). However, in order to understand thermal physiology in a species, it is crucial that sex-specific differences be taken into account (Huey and Pianka, 2007; Lailvaux, 2007). Equally important, and even less understood, is the potential for differences in thermal physiology between age classes as found in several recent studies (Xu and Ji, 2006; Tang et al., 2013; Gilbert and Lattanzio, 2016). Importantly, ontogenetic and sex-based differences in thermal physiology can be combined with behavioral and life-history studies to understand mechanisms driving the evolution of thermal physiology.

We found that, after lab acclimation to constant thermal conditions, critical thermal temperatures and thermal breadth varied between sexes and across age classes in an introduced population of *P. siculus*, which normally experiences a Mediterranean climate similar to that experienced by populations in the native range of this species (Table A1). Of particular note is that while all sex and age categories had a similar thermal preference, 1) adult females had a lower CT_{min} than adult males and juveniles; 2) juveniles had a lower CT_{max} than adults; and 3) as a result of these differences, adult females had a broader thermal tolerance than adult males and juveniles. Because these differences were found after lizards had been acclimated to similar

conditions, they are more likely to be due to inherent and potentially adaptive differences between male and female adults.

When we compare our findings to work on other lizard species, we find that a lower CT_{min} in adult females compared to adult males, as we found in *P. siculus*, is unusual. In previous studies for which differences in thermal tolerance between sexes of other lizard species have been found, adult females had higher CT_{min} , and/or lower CT_{max} , and consequently narrower tolerance breadths compared to adult males (Ji et al., 1996; Lailvaux et al., 2003; Lailvaux and Irschick, 2007). We would expect differences in thermal tolerance to be correlated with microhabitat use. Activity data have been published for *P. siculus* in New York (Burke and Ner, 2005), but sex-specific activity data have not been recorded. Adult male and adult female *P. siculus* from the California population were commonly found near each other during sampling, but a formal study of habitat use and basking behavior would be necessary to rule out differing microhabitat use as a potential explanation for the observed differences in CT_{min} between the sexes. Alternatively, it is possible that CT_{min} differs between the sexes due to physiological differences (e.g., due to sex-specific hormones), and the animals' habitat selection may reflect their physiological limits. More work is needed to clarify the proximal mechanisms behind our observed differences in CT_{min} .

Juveniles having a lower CT_{max} than adults could also relate to differential microhabitat use; habitat overlap between age classes is minimal in the introduced range of *P. siculus* in Southern California. Based on four years of field surveys in this urban neighborhood, adults are typically observed in areas with vertical structures such as walls, fences, and planters, especially where cracks and holes provide retreats. Juveniles are typically found near areas used by adults but tend to remain in or adjacent to lawn areas and vegetated planters. Intraspecific habitat partitioning has been well documented in other lizard species, and often is attributed to adult male presence decreasing juvenile survival, which forces juveniles into lower quality microhabitats (Keren-Rotem et al., 2006; Delaney and Warner, 2017). *Podarcis siculus*, especially adult males, are well documented to engage in cannibalistic behavior (e.g., Burke and Mercurio, 2002; Capula and Aloise, 2011), and a dietary analysis using preserved *P. siculus* from the San Pedro population revealed adult males cannibalizing juvenile lizards (Pauly, unpublished data). Thus, juveniles likely need to avoid areas inhabited by adult males. Because juveniles do not use the prominent basking sites used by adults and spend more time in more vegetated areas, it may be unnecessarily costly for juveniles to maintain a high CT_{max} .

Similar to our findings, other studies have reported no differences in T_{pref} between adult male and adult female lizards (Van Damme et al., 1990; Qu et al., 2011). However, our T_{pref} values were lower than previously reported values of laboratory-determined T_{pref} for *P. siculus*, which range from 28.4 °C to 38.8 °C (Avery, 1978; Ortega et al., 2016). This discrepancy may be due to the relatively short period of measurement in our study (2 h) compared to these older studies (5–9 h). More recent work on *P. siculus* from the San Pedro, CA population yielded T_{pref} values averaging 34.5 °C after 4 h in the thermal gradient (Haro and Liwanag, unpublished data), which is within the range reported by previous studies. Alternatively, different populations may shift their T_{pref} in response to acclimatization to different climatic conditions; both of the previous studies examined lizards from study sites that are several degrees warmer than San Pedro in similar seasons. Another consideration is the time of day during which the measurements were conducted, as T_{pref} can shift throughout the day, often peaking in the afternoon (Cowgell and Underwood, 1979; Tosini and Menaker, 1995; Ellis et al., 2006).

Though we cannot definitively predict the pattern of T_{pref} for non-acclimated *P. siculus*, the similarities we found among age classes suggest that juvenile and adult *P. siculus* may benefit from thermoregulation at similar temperatures. Because an organism is expected to seek to operate at or near its thermal optimum (Huey, 1982) we then expect potential competition for optimal habitat to occur between age classes

(Sagonas et al., 2017). As previously stated, dietary analyses show that adult males directly reduce juvenile survival through cannibalism in this population of *P. siculus*. Future work should incorporate juvenile and adult operative temperature models to investigate if adult males impose an energetic cost on juveniles by limiting optimal habitat use for thermoregulation. Additionally, measurement of field active body temperature of lizards in this population would provide insight into the thermoregulatory efficiency of these lizards.

Total EWL did not differ between age classes when mass was taken into account, suggesting that differences in mass-specific EWL were due to higher surface area to volume ratios in juveniles, rather than ontogeny *per se* (Mautz, 1982). However, the higher rates of mass-specific EWL observed in juvenile lizards compared to adult males at 20 °C and 30 °C are still ecologically relevant, as they suggest a higher desiccation rate per gram of tissue for juveniles. Interestingly, these differences were not observed at 40 °C, suggesting the juveniles may have employed behavioral strategies (e.g., postural changes, eye closing) to reduce EWL at this most challenging temperature, or that the aridity of the chamber induced increased skin resistance (Mautz, 1980). The differences we did observe occurred within the range of preferred temperatures found in this study, which could lead to conflict between preferred temperatures and water retention in juveniles. This may partially explain why juveniles tend to be found in or near lawn areas, which would be more humid than exposed basking sites. However, the relative contribution of adult male presence, predator presence, available diet, and hydration would need to be investigated to fully understand what determines juvenile habitat use.

Interestingly, regardless of sex and life stage, all of the critical thermal minima measured in our study are well above winter air temperatures experienced by nonnative populations of *P. siculus* now established in Kansas and the Northeastern US (Burke et al., 2002; Kolbe et al., 2013; Donihue et al., 2014). Lizards in these nonnative populations, like those at high elevation sites in the native range, use winter hibernacula (Burke et al., 2002). Lizards in the Southern California population, however, can be active year round. In hibernacula, *P. siculus* can supercool to –5 °C, although they are unlikely to survive if ice begins to form (Burke et al., 2002). Future research will examine whether *P. siculus* now thriving in these colder climates have different thermal tolerances than the Southern California population examined here.

One limitation of our study is the acclimation of the lizards to a constant temperature (28 °C), with no opportunity for thermoregulation within the laboratory. We recommend against future use of this husbandry approach, as it limits comparison of our findings to the wild population, in addition to potentially contributing to the deaths of some lizards. Prolonged exposure to constant temperatures even 1–2 °C above the preferred temperature range results in appetite decline and weight loss in some lizard species (Licht, 1965). Regimes of constant temperatures can also be detrimental at the lower end of preferred temperatures (Shine, 1983). Given that thermoregulation does not necessarily occur at a single fixed temperature (Barber and Crawford, 1977; Mathies and Andrews, 1997), some of the deaths observed during this study may have been prevented by providing lizards with a constant thermal gradient and diel temperature cycle (Pough, 1991 and references therein). Although this limits our ability to directly translate the reported values to field active lizards in the wild, our results do show that there are persistent differences across age classes and adult sexes in the thermal biology of *P. siculus*. These results highlight the need for more studies examining the roles of ontogeny and sex in the thermal biology of ectotherms. As more studies accumulate and are linked to differences in behavior and natural history, it will be possible to investigate factors that drive the evolution of thermal tolerances. We also echo other authors' recent sentiments that the identification of physiological patterns across ontogeny can help us better understand how ectotherms will respond to changing climates (Gilbert and Lattanzio, 2016; Levy et al., 2016).

Acknowledgements

We thank Heidi Beswick, Rachele Brown, Jim Hall, Jack Kelley, Avery Kirschbaum, Hayden Kirschbaum, Kenneth Kirschbaum, Brett Schiller, and Danielle Tran for assistance with animal collection. Thanks to Katherine Holst and Simone Mangini for assistance with data collection. We also thank Bobby Espinoza, John Hunter, Roger Sanguino, and Nicole Wong for assistance with animal husbandry. Lizards were collected under permit from the California Department of Fish and Wildlife (SCP-4307) to GBP. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. All animal use protocols were evaluated and approved by the Institutional Animal Care and Use Committees at Adelphi University and California Polytechnic State University.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2018.10.010.

References

- Andrews, R.M., Pough, F.H., 1985. Metabolism of squamate reptiles: allometric and ecological relationships. *Physiol. Zool.* 58, 214–231.
- Angilletta Jr, M.J., Steury, T.D., Sears, M.W., 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integr. Comp. Biol.* 44, 498–509.
- Arguez, A., Durre, I., Applequist, S., Vose, R.S., Squires, M.F., Yin, X., Heim Jr, R.R., Owen, T.W., 2012. NOAA's 1981–2010 US climate normals: an overview. *Bull. Am. Meteorol. Soc.* 93, 1687–1697.
- Avery, R.A., 1978. Activity patterns, thermoregulation and food consumption in two sympatric lizard species (*Podarcis muralis* and *P. sicula*) from central Italy. *J. Anim. Ecol.* 47, 143–158.
- Barber, B.J., Crawford Jr, E.C., 1977. A stochastic dual-limit hypothesis for behavioral thermoregulation in lizards. *Physiol. Zool.* 50, 53–60.
- Beal, M.S., Lattanzio, M.S., Miles, D.B., 2014. Differences in the thermal physiology of adult Yarrow's spiny lizards (*Sceloporus jarrovi*) in relation to sex and body size. *Ecol. Evol.* 22, 4220–4229.
- Burke, R.L., Mercurio, R.J., 2002. Food habits of a New York population of Italian Wall Lizards, *Podarcis sicula* (Reptilia, Lacertidae). *Am. Midl. Nat.* 147, 368–375.
- Burke, R.L., Ner, S.E., 2005. Seasonal and diel activity patterns of Italian Wall Lizards, *Podarcis sicula campestris*, in New York. *Northeast. Nat.* 12, 349–360.
- Burke, R.L., Hussain, A.A., Storey, J.M., Storey, K.B., 2002. Freeze tolerance and supercooling ability in the Italian Wall Lizard, *Podarcis sicula*, introduced to Long Island, New York. *Copeia* 2002, 836–842.
- Capula, M., Aloise, G., 2011. Extreme feeding behaviours in the Italian wall lizard, *Podarcis siculus*. *Acta Herpetologica* 6, 11–14.
- Carretero, M.A., Marcos, E., de Prado, P., 2006. Intraspecific Variation of Preferred Temperatures in the NE form of *Podarcis hispanica*. Mainland and Insular Lacertid Lizards: A Mediterranean Perspective. Firenze University Press, Florence, pp. 55–64.
- Clusella-Trullas, S., Chown, S.L., 2014. Lizard thermal trait variation at multiple scales: a review. *J. Comp. Physiol. B* 184, 5–21.
- Clusella-Trullas, S., Blackburn, T.M., Chown, S.L., 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *Am. Nat.* 177, 738–751.
- Cowgell, J., Underwood, H., 1979. Behavioral thermoregulation in lizards: a circadian rhythm. *J. Exp. Zool.* 210, 189–194.
- Cowles, R.B., Bogert, C.M., 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* 83, 261–296.
- Cox, R.M., Zilberman, V., John-Alder, H.B., 2008. Testosterone stimulates the expression of a social color signal in Yarrow's spiny lizard, *Sceloporus jarrovi*. *J. Exp. Zool. Part A: Ecol. Genet. Physiol.* 309, 505–514.
- Deichsel, G., Nafis, G., Hakim, J., 2010. *Podarcis siculus* (Italian wall lizard) USA: California. *Herpetol. Rev.* 41, 513–514.
- Delaney, D.M., Warner, D.A., 2017. Adult male density influences juvenile microhabitat use in a territorial lizard. *Ethology* 123, 157–167.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* 105, 6668–6672.
- Donihue, C.M., Lambert, M.R., Watkins-Colwell, G.J., 2014. Geographic distribution: USA: Connecticut: Fairfield County: *Podarcis sicula*. *Herpetol. Rev.* 45, 661–662.
- Ellis, D.J., Firth, B.T., Belan, I., 2006. Circadian rhythm of behavioral thermoregulation in the sleepy lizard (*Tiliqua rugosa*). *Herpetologica* 62, 259–265.
- Gilbert, A.L., Lattanzio, M.S., 2016. Ontogenetic variation in the thermal biology of Yarrow's spiny lizard, *Sceloporus jarrovi*. *PLoS One* 11, e0146904.
- Hertz, P.E., Huey, R.B., Stevenson, R.D., 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* 142, 796–818.
- Holm, S., 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6, 65–70.
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., Hothorn, M.T., 2017. Package 'multcomp'. Accessed through <http://cran.statsfu.ca/web/packages/multcomp/multcomp>.
- Huey, R.B., 1982. Temperature, Physiology, and the Ecology of Reptiles. *Biology of the Reptilia*. Academic Press, London, pp. 25–91.
- Huey, R.B., Kingsolver, J.G., 1989. Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* 4, 131–135.
- Huey, R.B., Pianka, E.R., 2007. Lizard thermal biology: do genders differ? *Am. Nat.* 170, 473–478.
- Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* 19, 357–366.
- Huey, R.B., Peterson, C.R., Arnold, S.J., Porter, W.P., 1989. Hot rocks and not-so-hot rocks: retreat-site selection by garter snakes and its thermal consequences. *Ecology* 70, 931–944.
- Ji, X., Du, W.G., Sun, P.Y., 1996. Body temperature, thermal tolerance and influence of temperature on sprint speed and food assimilation in adult grass lizards, *Takydromus septentrionalis*. *J. Therm. Biol.* 21, 155–161.
- Keren-Rotem, T., Bouskila, A., Geffen, E., 2006. Ontogenetic habitat shift and risk of cannibalism in the common chameleon (*Chamaeleo chamaeleon*). *Behav. Ecol. Sociobiol.* 29, 723–731.
- Kolbe, J.J., Lavin, B.R., Burke, R.L., Rugiero, L., Capula, M., Luiselli, L., 2013. The desire for variety: Italian Wall Lizard (*Podarcis siculus*) populations introduced to the United States via the pet trade are derived from multiple native-range sources. *Biol. Invasions* 15, 775–783.
- Krenek, S., Petzoldt, T., Berendonk, T.U., 2012. Coping with temperature at the warm edge—patterns of thermal adaptation in the microbial eukaryote *Paramecium caudatum*. *PLoS One* 7, e30598.
- Kubička, L., Schořálková, T., Červenka, J., Kratochvíl, L., 2017. Ovarian control of growth and sexual size dimorphism in a male-larger gecko. *J. Exp. Biol.* 220, 787–795.
- Lailvaux, S.P., 2007. Interactive effects of sex and temperature on locomotion in reptiles. *Integr. Comp. Biol.* 47, 189–199.
- Lailvaux, S.P., Irschick, D.J., 2007. Effects of temperature and sex on jump performance and biomechanics in the lizard *Anolis carolinensis*. *Funct. Ecol.* 21, 534–543.
- Lailvaux, S.P., Alexander, G.J., Whiting, M.J., 2003. Sex-based differences and similarities in locomotor performance, thermal preferences, and escape behaviour in the lizard *Platysaurus intermedius wilhelmi*. *Physiol. Biochem. Zool.* 76, 511–521.
- Leal, M., Gunderson, A.R., 2012. Rapid change in the thermal tolerance of a tropical lizard. *Am. Nat.* 180, 815–822.
- Levy, O., Buckley, L.B., Keitt, T.H., Angilletta, M.J., 2016. Ontogeny constrains phenology: opportunities for activity and reproduction interact to dictate potential phenologies in a changing climate. *Ecol. Lett.* 19, 620–628.
- Licht, P., 1965. The relation between preferred body temperatures and testicular heat sensitivity in lizards. *Copeia* 1965, 428–436.
- Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and critique. *Can. J. Zool.* 75, 1561–1574.
- Mathies, T., Andrews, R.M., 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? *Funct. Ecol.* 11, 498–507.
- Matzek, V., 2012. Trait values, not trait plasticity, best explain invasive species' performance in a changing environment. *PLoS One* 7, e48821.
- Mautz, W.J., 1980. Factors influencing evaporative water loss in lizards. *Comp. Biochem. Physiol. Part A* 67, 429–437.
- Mautz, W.J., 1982. Patterns of Evaporative Water Loss. *Biology of the Reptilia*. Academic Press, London, pp. 443–481.
- Ortega, Z., Mencía, A., Pérez-Mellado, V., 2016. Thermal ecology of *Podarcis siculus* (Rafinesque-Schmalz, 1810) in Menorca (Balearic islands, Spain). *Acta Herpetol.* 11, 127–133.
- Pintor, A.F., Schwarzkopf, L., Krockenberger, A.K., 2016. Hydroregulation in a tropical dry-skinned ectotherm. *Oecologia* 182, 925–931.
- Pough, F.H., 1991. Recommendations for the Care of Amphibians and Reptiles in Academic Institutions 33. National Academy Press, Washington D.C., pp. 58–59.
- Qu, Y., Li, H., Gao, J., Su, X., Ji, X., 2011. Thermal preference, thermal tolerance and the thermal dependence of digestive performance in two Phrynocephalus lizards (Agamidae), with a review of species studied. *Curr. Zool.* 57, 684–700.
- Sagonas, K., Kapsalas, G., Valakos, E., Pafilis, P., 2017. Living in sympatry: the effect of habitat partitioning on the thermoregulation of three Mediterranean lizards. *J. Therm. Biol.* 65, 130–137.
- Schořálková, T., Kratochvíl, L., Kubička, L., 2017. Temporal organization: a novel mechanism of hormonal control of male-typical sexual behavior in vertebrates. *Physiol. Behav.* 170, 151–156.
- Shine, R., 1983. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* 57, 397–405.
- Stevenson, R.D., 1985. The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* 126, 362–386.
- Tang, X.L., Yue, F., He, J.Z., Wang, N.B., Ma, M., Mo, J.R., Chen, Q., 2013. Ontogenetic and sexual differences of thermal biology and locomotor performance in a lacertid lizard *Eremias multiocellata*. *Zoology* 116, 331–335.
- Tejpal, C.S., Sumitha, E.B., Pal, A.K., Murthy, H.S., Sahu, N.P., Siddaiah, G.M., 2014. Effect of dietary supplementation of L-tryptophan on thermal tolerance and oxygen consumption rate in *Cirrhinus mrigala* fingerlings under varied stocking density. *J. Therm. Biol.* 41, 59–64.
- Tosini, G., Menaker, M., 1995. Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *J. Biol. Rhythms* 10, 248–255.
- Van Damme, R., Bauwens, D., Castilla, A.M., Verheyen, R.F., 1990. Comparative thermal

- ecology of the sympatric lizards *Podarcis tiliguerta* and *Podarcis sicula*. *Acta Oecologica* 11, 503–512.
- van Marken Lichtenbelt, W.D., 1992. Digestion in an ectothermic herbivore, the green iguana (*Iguana iguana*): effect of food composition and body temperature. *Physiol. Zool.* 65, 649–673.
- Winne, C.T., Keck, M.B., 2005. Intraspecific differences in thermal tolerance of the diamondback watersnake (*Nerodia rhombifer*): effects of ontogeny, latitude, and sex. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.* 140, 141–149.
- Withers, P.C., Aplin, K.P., Werner, Y.L., 2000. Metabolism and evaporative water loss of Western Australian geckos (Reptilia: sauria: Gekkonomorpha). *Aust. J. Zool.* 48, 111–126.
- Xu, X.F., Ji, X., 2006. Ontogenetic shifts in thermal tolerance, selected body temperature and thermal dependence of food assimilation and locomotor performance in a lacertid lizard, *Eremias brenchleyi*. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.* 143, 118–124.
- Zucker, A., 1980. Procedural and anatomical considerations of the determination of cutaneous water loss in squamate reptiles. *Copeia* 1980, 425–439.