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BASIC AND APPLIED ECOLOGY

Research article

Water deprivation drives intraspecific variability in lizard heat tolerance

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Abstract

Quantifying intraspecific variation in heat tolerance is critical to understand how species respond to climate change. In a previous study, we recorded variability in critical thermal maxima (CT_{max}) by 3 °C among populations of small Iberian lizard species, which could substantially influence predictions of climate-driven activity restriction. Here, we undertake experiments to examine

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whether we could reproduce similar levels of heat-tolerance variability in response to water deficit. We hypothesized that deprivation of drinking water should increase variability in CT_{max} between populations more than deprivation of food under the theoretical expectation that the variation of the more limiting resource must trigger stronger variation in physiological performance. We measured CT_{max} after manipulating availability of live prey and drinking water in two populations of an arid and a mesic lizard species from the Iberian Peninsula. We quantified a mean CT_{max} across all studied lizards of $44.2\text{ }^{\circ}\text{C} \pm 0.2\text{ SE}$ for the arid species and $41.7\text{ }^{\circ}\text{C} \pm 0.3\text{ SE}$ for the mesic species. Using multimodel inference, we found that water deprivation (combined with food supply) caused population differences in CT_{max} by 3 to 4°C which were two to three times wider than population differences due to food deprivation (combined with water supply) or to food and water provision. To highlight the need for more thermo-hydroregulatory research, we examined bias in research effort towards thermal *versus* hydric environmental effects on heat tolerance through a systematic literature review. We show that environmental temperature has been used five times more frequently than precipitation in ecological studies of heat tolerance of terrestrial species. Studies linking thermal tolerance of ectotherms to the interplay of air temperature and water availability are needed in the face of projected increases in aridity and drought in the 21st century, because the balance of body temperature and water resources are functionally interlinked.

Keywords

climate change; CT_{max} ; drought; ecophysiology; macroecology; phenotypic variance; precipitation; specialization; thermo-hydroregulation

Life – at least life as we know it – should not exist without liquid water, even if energy conditions are optimal and all other essential matter conditions are non-limiting (O'Brien 2006)

Introduction

Anthropogenic climate change is causing an expansion of drylands (Huang, Yu, Guan, Wang & Guo 2016) since the beginning of the 20th century (Marvel, Cook, Bonfils, Durack, Smerdon et al.

2019). Current climate model projections indicate that aridification (Park, Jeong, Joshi, Osborn, Ho et al. 2018) and increased frequency and severity of droughts (Trenberth, Dai, van der Schrier, Jones, Barichivich et al. 2013) will be intensified in a ‘business-as-usual’ emission scenario and represent one of the major climate hazards to human societies and ecosystem services (Mora, Spirandelli, Franklin, Lynham, Kantar et al. 2018). Consequently, since many species are bound to experience increased dehydration stress, understanding how water availability affects their thermal tolerance is crucial (Kearney, Simpson, Raubenheimer & Kooijman 2013; Mitchell, Boardman, Clusella-Trullas & Terblanche 2017; Rozen-Rechels, Dupoué, Lourdais, Chamaillé-Jammes, Meylan et al. 2019).

Globally, water-temperature dynamics act as an eco-evolutionary force shaping biotic interactions, species ranges and diversity (O'Brien 2006), and precipitation drives evolutionary change more strongly than temperature in plants and animals (Siepielski, Morrissey, Buoro, Carlson, Caruso et al. 2017). As the climate changes, Bonebrake and Mastrandrea (2010) project that decreased precipitation and elevated temperature should depress population fitness in high and low altitudes, respectively, while the margins of the southern or warmer ranges of many species in the Northern Hemisphere might be determined by precipitation more strongly than by temperature (Sunday, Bates & Dulvy 2012; Thomas & Lennon 1999). Additionally, clines of thermal performance (Clusella-Trullas, Blackburn & Chown 2011) and species richness (Latimer 2007) have been shown to be best predicted by geographical variation in water availability (mediated largely by rainfall), or its interplay with environmental temperatures (Hawkins, Field, Cornell, Currie, Guégan et al. 2003; Kellermann, Overgaard, Hoffmann, Fløjgaard, Svenning et al. 2012; Razgour, Persey, Shamir & Korine 2018). In that respect, precipitation outperforms environmental temperature in predictions of lizard activity, habitat requirements and/or distributional ranges (Kearney, Munns, Moore, Malishev & Bull 2018; Sousa-Guedes, Arenas-Castro & Sillero 2020), can accelerate population extirpation where rainfall fluctuates considerably (McLaughlin, Hellmann, Boggs & Ehrlich 2002), multiplies extinction risk relative to predictions based on

climate warming alone (McCain & Colwell 2011; Saltr , Chadoeuf, Peters, McDowell, Friedrich et al. 2019) and, in combination with temperature, drives habitat preferences leading species to switch to open agricultural land in wet regions and the shelter of forests in dry regions (Frishkoff, Karp, Flanders, Zook, Hadly et al. 2016). Those broad macroecological patterns need to be examined functionally, with a focus on interactions among components of climate and species traits, so species' abilities to cope with climatic shifts and varying access to free-standing water are adequately understood (Rozen-Rechels et al. 2019).

Recent developments highlight that the heat tolerance of vertebrate ectotherms is highly variable within the distribution of individual species (Clusella-Trullas & Chown 2014). Critically, intraspecific variability in heat tolerance can severely alter predictions of the impacts of climate change on those animals and therefore needs to be investigated in detail (Herrando-P rez, Ferri-Y  nez, Monasterio, Beukema, Gomes et al. 2019; Sinclair, Marshall, Sewell, Levesque, Willett et al. 2016; Valladares, Matesanz, Guilhaumon, Ara jo, Balaguer et al. 2014). Access to free-standing water represents one of the key drivers by which species might modify their heat tolerance to cope with thermal stress. This notion has been recently branded as 'thermo-hydroregulation' whereby the regulation of body temperature and water balance are functionally integrated at the organismal and population levels and bound to experience behavioural and physiological trade-offs (Rozen-Rechels et al. 2019). Where thermo-hydroregulation and heat tolerance have been studied in controlled experiments using model invertebrates, there are contrasting results depending on study species, choice of heat-tolerance metric, and experimental setup (Chown, S rensen & Terblanche 2011; Mitchell et al. 2017). For instance, in vinegar flies (*Drosophilidae*) tolerance to high temperatures decreased from humid to dry conditions despite that its heritability peaked in arid conditions (Bubliy, Kristensen, Kellermann & Loeschcke 2012). By contrast, considering that animals can obtain water via food resources and directly drinking, fruit flies (*Tephritidae*) deprived of access to water showed decreased heat knockdown time, but only if combined with food deprivation, and treatments did not affect the expression profiles of heat-shock proteins (Mitchell et al. 2017). Given

that insects are highly sensitive to variation in rainfall and water availability, owing to their small body size that drives a high surface area to volume ratio, and thus high susceptibility to evaporative water loss and limited capacity to carry water (Chown et al. 2011), it could be argued that these experiments should bear limited relevance for terrestrial ectotherms.

Lizards constitute a zoological group that has historically attracted a great deal of ecophysiological and evolutionary research (Camargo, Sinervo & Sites 2010; Clusella-Trullas & Chown 2014; Wollenberg Valero, Marshall, Bastiaans, Caccone, Camargo et al. 2019). In small species, water supply has been recognized as a major source of experimental bias in measuring thermal traits (Camacho & Rusch 2017). Lorenzon, Clobert, Oppliger and John-Alder (1999) found that individuals of the viviparous lizard *Zootoca vivipara* subject to water shortage had reduced growth rates and activity and selected for lower body temperatures, and argued that “...there is population variability in phenotypic plasticity with respect to water availability in the habitat”. In previous research with Iberian lacertids, we have reported variability in critical thermal maxima (CT_{max}) by up to 3 °C between populations of the same species. Such a magnitude of population-level variation in heat tolerance (relative to cold tolerance) might indicate that heat tolerance is finely tuned to climatic variation, going unnoticed in global studies using macroclimatic predictors (Herrando-Pérez, Monasterio, Beukema, Gomes, Ferri-Yáñez et al. 2020). Most importantly, choosing one representative population for the heat tolerance of a full species therefore becomes problematic in macro-ecological and physiological studies addressing climate impacts on hundreds to thousands of species (Herrando-Pérez et al. 2019).

Here we examine whether we can experimentally reproduce similar levels of intraspecific variation in CT_{max} (3 °C) by modifying the availability of drinking water as a proxy for the extreme hydrological conditions the populations of a lizard species might experience in Iberia (Araújo, Thuiller & Pearson 2006; Le Galliard, Massot, Baron & Clobert 2012; Sousa-Guedes et al. 2020). Because dietary water can be an important source of water for reptiles (Congdon, Ballinger & Nagy 1979; Nagy 1972), we teased apart the effects of drinking water and food on CT_{max} by letting two

populations of two Iberian lizards have access to either or both resources. Under the theoretical expectation that the magnitude of variation of a driver of physiological stress should trigger proportional variation in physiological performance and/or specialization (Orlando & Guillelte 2001; Vamosi, Armbruster & Renner 2014), we hypothesize that deprivation of the more limiting resource for thermal performance (i.e., drinking water) should cause greater difference in heat tolerance between populations of a species than a less limiting resource (i.e., dietary water or food) (Murphy & Denardo 2019; Wright, Jackson & DeNardo 2013). We concur with others (Pirtle, Tracy & Kearney 2019) that research efforts addressing the role of water in the thermal biology of species needs to be emphasized, as current studies focusing on climate impacts on biodiversity are heavily biased towards thermal effects. We support our claim by quantifying the frequency with which (i) air temperatures *versus* precipitation have been correlated with heat tolerance of terrestrial fauna and (ii) different metrics of thermal performance have been correlated with water resources for vertebrate ectotherms in the primary literature.

Materials and methods

We sampled two populations of each of two species of Iberian lizards, and measured their CT_{max} in response to availability of food and/or water in laboratory conditions. We detail our sampling, experimental and statistical approach in the next three subsections, and conclude by describing our literature review.

Sampling

Our two study species (Fig. 1) were the spiny-footed lizard *Acanthodactylus erythrurus* and the common wall lizard *Podarcis muralis* (Family Lacertidae). We selected them because they represent extremes of adaptation to xeric (*A. erythrurus*) *versus* mesic (*P. muralis*) environments in Iberian reptiles (Speybroeck, Beukema, Bok, Van Der Voort & Velikov 2016), and are widespread and abundant across most of their range. The Iberian distribution of both species is enveloped by a

gradient of precipitation where sites with the highest rainfall coincide with lowest Winter (*A. erythrurus*) or Summer (*P. muralis*) temperatures (Appendix A: Fig. 2).

We caught, by noosing, 15 males from each of two populations of the two study species (Fig. 1; Appendix A: Fig. 1), namely (i) *A. erythrurus* from El Saler (Valencia, Eastern Spain: coastal dehesa combining dunes and Mediterranean scrub) and Chapinería (Madrid, Central Spain: inland dehesa combining dry grassland with sparse holm oak trees); and (ii) *P. muralis* from Peñagolosa (Castellón, Eastern Spain: mountain springs in temperate, mixed forest) and El Portalet (Huesca, The Pyrenees: rocky outcrops in peatland). Sampling took place in July 2015 (El Saler, Peñagolosa) and 2016 (Chapinería, El Portalet). We have observed *P. muralis* drinking directly from streams in the two study sites during Spring and Summer. As to *A. erythrurus*, lizards might have rare access to drinking water in a dune-dominated habitat (El Saler) and perhaps from puddles after storms in Chapinería, while dew drinking is unlikely because individuals become active in sunny days at ~10:00 AM to 12:00 PM at the two study sites when dew has already evaporated. The latter species is mostly inactive on cloudy days (Belliure 2015).

Experiments

Once in the lab, each lizard was housed in an individual terrarium, and acclimated to captivity from days 1 to 15 following capture. On day 1 of every study year, the batch of 30 terraria was positioned in three contiguous rows in a greenhouse at ambient temperature and moisture covered by a sunshade 2 meters above ground level. The sunshade allowed sun exposure in different parts of the setting during different times of the day; preliminary data from empty terraria over a two-week period showed that daily maximum temperatures within terraria closely followed maximum temperatures at local meteorological stations (Spanish Agency of Meteorology, www.aemet.es), i.e., a maximum of 35 °C when terraria were under shade and a minimum of 30 °C when exposed to direct sunlight. Each terrarium had a substrate of coconut fibre and included a ceramic brick for shelter and thermoregulation. Lizards emerged from their shelter from 9:30 to 11:00 AM

(conditional on cloudiness), and we then provided them daily with spring water (a full Petri dish placed in the lower-left corner of each terrarium) along with live prey (dropped at the upper side of each terrarium). Based on captivity experiments with lacertids (Herrando-Pérez et al. 2019), we gave each lizard a daily meal of three prey items, systematically providing the same combination of mealworms (*Tenebrio molitor*), waxworms (*Galleria mellonella*) and house crickets (*Acheta domesticus*) for each individual.

On day 15, all individuals had weights similar to those of their day of capture and consumed their prey items daily. Thereafter, from day 16 to 26, we allocated each lizard from each population randomly to one of three treatments (number of lizards per population and treatment = 5), namely provision of (i) food *and* water as in the acclimation phase (control treatment), (ii) only food or (iii) only water. To avoid edge effects in the experimental setting, we randomized the position of all terraria daily from day 1 to 26. Two *P. muralis*/Portalet individuals in the ‘food only’ treatment escaped from their terraria prior to CT_{max} estimation. A sample size of 3 males per population should accurately capture the mean population CT_{max} estimated from larger sample sizes in our study species (Herrando-Pérez et al. 2019), and is within the range of published work (e.g., Beal, Lattanzio & Miles 2014; Muñoz, Langham, Brandley, Rosauer, Williams et al. 2016). To demonstrate the latter statistically, we applied a retrospective power analysis (Dean & Voss 1999) to our linear models (detailed below).

We determined CT_{max} on day 27 after capture through the dynamic method described by Lutterschmidt and Hutchison (1997). Following the protocol of previous research (Herrando-Pérez et al. 2019), and for each study population, we carefully inserted a thermocouple 1 to 2 cm into the cloaca of each lizard — to avoid internal damage, the apical sensor was coated with a minute drop of nail polish (left to dry out overnight) and the thermocouple was further coated with pure vaseline during the experiments. The thermocouple was wired to a HH-25TC Omega® Thermometer via a 1 m-long cable (made up of flexible polytetrafluoroethylene). For each lizard, we fastened a 2×5 cm² strip of parafilm to secure the distal part of the cable (equipped with the thermocouple)

ventrally against the cloaca. Thereafter, lizards were kept some 30 cm below a red-heat lamp, and body temperatures were raised by $1\text{ }^{\circ}\text{C} \times \text{min}^{-1}$. Throughout each trial, three of us in conjunction (JB, FFY, SHP) monitored body temperature and behaviour, and ultimately measured CT_{max} as the cloacal temperature at which each lizard lost its righting response (Lutterschmidt & Hutchison 1997).

We did not take pretreatment measurements of CT_{max} because they would impact the fitness of the study individuals in our experiments and could introduce noise in the acclimatization period had the individuals experienced different thermal histories. For example, lizards can undergo short-term heat hardening after being exposed to their CT_{max} (Gilbert & Miles 2019; Phillips, Muñoz, Hatcher, Macdonald, Llewelyn et al. 2016) and thermal assays can have lasting effects on the fitness that are never assessed because of the short-term nature of those assays (Ribeiro, Camacho & Navas 2012). Nonetheless, our estimates of CT_{max} (see Results) fell within those observed for other Iberian populations of the same species, with heat tolerance varying from 44 to 47 °C for *A. erythrurus* (5 to 7 males per population), and from 42 to 44 °C for *P. muralis* (5 males per population) (Herrando-Pérez et al. 2019). We prevented physiological/physical damage by holding lizards (up to their necks) in a water bath (20-25 °C) immediately after each thermal shock, ultimately attaining 100 % survival. We provided lizards with food and water *ad libitum* daily from day 27 (right after the CT_{max} estimation) to 31, and all individuals were released at their point of capture on day 32 from the onset of experiments. Latitude and longitude of study sites and body sizes and CT_{max} per lizard individual are presented in Appendix B.

Statistical analyses

Throughout, all analyses and graphics were done in the *R* environment (*R* Core Team 2020). Our focus was on intraspecific variability in heat tolerance, rather than on interspecific patterns (see Garland & Adolph 1994). We tested the hypothesis that drinking-water deprivation should cause greater differences in heat tolerance between populations of a species than deprivation of food (see

Introduction). So for each of the two study species separately, and with CT_{max} as the common response, we contrasted the strength of statistical evidence for five linear models using the function *lm* in the *R* package *stats* (R Core Team 2020), namely: (i) $CT_{max} \sim 1$ (null model): CT_{max} is indistinguishable among populations and treatments, i.e., identical intercept or mean CT_{max} across populations; (ii) $CT_{max} \sim pop$: CT_{max} is population-dependent, i.e., different population intercepts irrespective of treatment, where *pop* = population identity; (iii) $CT_{max} \sim treat$: CT_{max} is treatment-dependent, i.e., different treatment intercepts irrespective of population identity, where *treat* = treatment; (iv) $CT_{max} \sim pop+treat$: different population and treatment intercepts but the effect of one predictor on CT_{max} does not depend on the effect of the other predictor (additive model); and (v) $CT_{max} \sim pop*treat$: different population and treatment intercepts and the effect of one predictor on CT_{max} depends on the effect of the other predictor (interaction model). Effectively, models (ii) and (iii) represent a one-way ANOVA, and models (iv) and (v) represent a two-way ANOVA, while the output of *lm* is directly transferable to an ANOVA table using the *anova* function in *stats*. Because individuals might adapt differently to captivity, we replicated our analyses above for a model set including ‘body condition’ (Schulte-Hostedde, Zinner, Millar & Hickling 2005: residuals of the linear regression of body weight on snout-to-vent length) of each individual on day 26 of the experiment as an additional predictor in all models.

We ranked model support, based on the Akaike’s Information Criterion adjusted to finite sample size (AIC_c) (Sugiura 1978), by means of model probabilities ($wAIC_c$, which are scaled to a 0 to 1 interval) and evidence ratios of $wAIC_c$ of the top-ranked model to the $wAIC_c$ of every other model in the set (Burnham & Anderson 2002). We assumed Gaussian errors (corroborated in the saturated model [$CT_{max} \sim pop*treat$] by means of Q-Q and fit-versus-residual plots), and confirmed variance homogeneity using Bartlett’s test (Bartlett 1937). We quantified the power of the best supported model in the *R* package *powerMediation* (Qiu 2018) based on our sample size (3 to 5 males per *population* \times *treatment*, see above), a *p* value of 0.05 (probability of the data given that

the null hypothesis is true), and an effect size of 3 (*A. erythrurus*) to 4 °C (*P. muralis*) — see Results.

Literature review

We carried out two literature reviews using strings of key words combined by Boolean operators in *Scopus* covering the period 1970 to 2019 (see full details in Appendix C). The results of both reviews were plotted in bipartite-network graphs using the *R* package *igraph* (Csárdi & Nepusz 2006). While our focus is on drinking-water availability, the effects of both internal hydration status and free-standing water availability are partly conflated in our review of the ecological literature, and should not be confused.

In the first review ('general literature review', *Scopus* accessed on 01/05/2019), we asked whether authors documenting a relationship between climate and animal heat tolerance had chosen (i) climatic metrics of temperature and/or precipitation and (ii) particular taxa. We combined expressions of heat tolerance widely used across terrestrial invertebrates and vertebrates (i.e., 'ctmax' OR 'heat tolerance' OR 'thermal maximum' OR 'thermal maxima' OR 'upper critical temperature' OR 'upper lethal temperature' OR 'upper thermal' (but excluding some taxa-specific expressions like 'heat coma' mainly used in arthropod research) with the wildcard *climat* — we excluded the terms 'temperature' or 'precipitation' so as to prevent a bias for either metric. We restricted our search to the main-stream ecological journals, namely *Ecology* ($N = 7$ papers matching our search criteria), *Ecology Letters* ($N = 6$), *Functional Ecology* ($N = 22$), *Journal of Animal Ecology* ($N = 4$), *Oecologia* ($N = 14$) and *Oikos* ($N = 6$), along with *Global Ecology and Biogeography* ($N = 6$) given its coverage of large-scale ecological patterns. We excluded journals focused on thermal biology (e.g., *Journal of Thermal Biology*) because they would have introduced directional bias towards studies using temperature predictors. In total, we collated a set of 64 target papers. For each target paper, we recorded the taxonomic group studied (amphibia, arthropoda, aves, mammalia, reptilia) and the broad climate metric being used (temperature, precipitation). We

then counted as ‘one case’ each time a climate metric had been used at least once in a statistical analysis for one of our study taxa, giving a total of 95 ‘cases’ across the 64 target papers. A total of 61 papers contributed 1 to 3 cases, and 3 papers contributed 4, 5 and 8 cases. For instance, a paper using temperature data for reptiles would amount to 1 case, a paper using precipitation data for birds and reptiles would include 2 cases, and a paper using precipitation and temperature data for birds and reptiles would amount to 4 cases or *climate* \times *taxa* combinations.

In the second *Scopus*-based literature review (‘focal literature review’), we quantified the frequency with which herptile heat tolerance (our main focus) has been predicted from variation in environmental water (experimental dehydration, humidity/moisture, lentic/lotic water bodies, precipitation/rainfall) in studies focusing on the interplay of hydric and thermal herptile physiology. We first used a pilot string of key words to identify the most common terms referring to metrics of thermal performance and water resources (*Scopus* accessed on 01/04/2018). Then, we used those terms to do a second browsing of papers (06/04/2019), giving a total of 56 target papers. From each target paper, we extracted the name of the study species and locality, and of the metrics of water and temperature used in quantitative analyses. We finally counted the number of times (‘cases’) a metric of thermal physiology had been predicted from a metric of environmental water, resulting in 53 cases across the 56 target papers. A total of 49 papers contributed 1 to 3 cases, and 7 papers contributed 4 to 6 cases. For instance, a paper predicting preferred body temperature from experimental dehydration treatments would include 1 case, and a paper predicting cold and heat tolerance through a moisture gradient would include 2 cases. We lastly classified and counted all cases where a thermal or hydric variable had been used as response or predictor in quantitative analyses.

Results

Physiological metrics

We found that the overall mean CT_{max} among all individuals and treatments was on average 2.5 °C higher for the (arid-adapted) spiny-footed lizard *Acanthodactylus erythrurus* ($44.2\text{ }^{\circ}\text{C} \pm 0.2\text{ SE}$) than for the (mesic-adapted) common rock wall lizard *Podarcis muralis* ($41.7\text{ }^{\circ}\text{C} \pm 0.3\text{ SE}$) as previously observed with other Iberian populations of both species (see Materials and methods).

We contrasted statistical evidence for five linear models (Table 1) capturing whether CT_{max} varied with population identity and/or experimental treatment (provision of food and water, only food, only water) for each species separately. The interaction model explained 56 to 73 % of the variation in CT_{max} , and was 1 to 9 orders of magnitude more likely than the four alternative models (Table 1). The former model supports that differences in CT_{max} between populations depended on treatment (Fig. 2). Thus, lizards having access to food and water, or only water, showed between-population differences in mean CT_{max} of only 1 (*A. erythrurus*) to 2 °C (*P. muralis*), but deprivation of water combined with food supply more than doubled population-level differences in mean CT_{max} by up to 3 to 4 °C, respectively (Fig. 2).

For both species, and for sample sizes of 3 to 5 males per *population* \times *treatment*, our two-way ANOVA interaction model had a statistical power (i.e., probability of the data if the null hypothesis is false) of 0.83 (*A. erythrurus*) and 0.97 (*P. muralis*). Controlling for lizard body condition resulted in equivalent statistical support for, and variance explained by, the interaction model (Appendix A: Table 1), with population differences in heat tolerance peaking in the only treatment where lizards had no access to drinking water.

Publication metrics

Among the 64 ecological papers investigating heat tolerance and including quantitative analysis of climate metrics ('general literature review', see Materials and methods), ectotherms (arthropoda, amphibians, reptiles) and endotherms (birds, mammals) were used in 88 % and 14 % of the papers (Fig. 3), respectively. Across those papers, quantitative analyses ('cases', see Materials and methods) using environmental temperature data (84 %) outnumbered those using precipitation data

(16 %) (Fig. 3), and those proportions were of similar magnitude for papers published after 2010 (Appendix C).

On the other hand, from a total of 56 papers reporting correlations between thermal physiology and water resources for herptiles ('focal literature review', see Materials and methods), 40 predicted a physiological thermal metric from a water-related metric (Table 2; Appendix A: Fig. 3). The most used responses were (preferred) body temperature (60 % of the cases) and heat tolerance (19 %), respectively, while the most popular predictors were dehydration and moisture gradients and wet *versus* dry seasonality (25 to 30 % each). Across statistical correlations of any sort, body temperature (23 %) and evaporative water loss (26 %) were the most common 'responses', while temperature and moisture gradients predominated as 'predictors' (29 %) (Table 2; Appendix A: Fig. 3).

Overall, the average published ecophysiological paper is one looking into temperature effects on arthropods (Fig. 3), particularly insects. This indicates the importance of insects to agriculture and human health, less restrictive ethical considerations for undertaking thermal experiments for invertebrates than for vertebrates, ease of laboratory rearing, as well as the much higher species richness of the former. When water variables are included in ecophysiological studies of ectotherms (Table 2), the prevailing focus is on how species manage their water balance and thermal performance in order to respond to environmental change once water has been assimilated in their bodies, often by monitoring and/or modelling evaporative water loss.

Discussion

Understanding how thermal tolerance varies within and among species has long been recognized as a critical area of ecophysiological research (Brattstrom 1968; Congdon et al. 1979; Hertz, Arima, Harrison, Huey, Losos et al. 2013) and is now of crucial importance to predict how ectotherms might respond to ongoing and future climate change (Clusella-Trullas & Chown 2014; Herrando-Pérez et al. 2019; Sinclair et al. 2016). Our experiments indicate that deprivation of drinking water

can accentuate the variation of heat tolerance between populations of ectothermic species by up to 4 °C — which exceeds our previous estimates of heat-tolerance shifts (3 °C) among populations of species of Iberian lacertids (Herrando-Pérez et al. 2020). Such population differences can lead to differences in species-level estimates of restricted activity by more than 20 days (Herrando-Pérez et al. 2019). For terrestrial ectotherms from the Northern Hemisphere, 4 °C variation in CT_{max} represents about 20 % of the interspecific variation in CT_{max} , and 50 % the total interspecific variation in CT_{max} previously recorded for the latitudinal band of the Iberian Peninsula (estimated from Bennett, Calosi, Clusella-Trullas, Martínez, Sunday et al. 2018), and thus requires careful examination in macrophysiological, biogeographical and evolutionary research.

Overall, our study attempts (i) to connect with the large body of ecophysiological studies undertaken by the middle of the 20th century (reviewed by Cowles & Bogert 1944; Lillywhite 2006) on the interaction of water and thermal physiology, and (ii) to promote the ongoing reunification of ecology with physiology (Gaston, Chown, Calosi, Bernado, Bilton et al. 2009) under climate change. The challenges faced by macrophysiology (Chown & Gaston 2016), and their application to understanding mechanisms underlying broad-scale ecological patterns of endotherms and ectotherms (Buckley, Hurlbert & Jetz 2012), concentrate on species responses to environmental temperatures. Fewer studies (by a factor of 5 in our literature review; Fig. 3) have assessed the extent to which ectotherm thermal tolerance and stress vary as a function of water availability in the environment, and much fewer considered how an organism's water balance might interact with thermoregulatory behaviour and stress resistance (Pirtle et al. 2019; Rozen-Rechels et al. 2019; see Introduction). This knowledge gap represents an impediment for the functional analyses and understanding of large-scale ecophysiological patterns, but also provides opportunity to contribute novel ecological information to multidisciplinary drought research in a changing climate (Ault 2020). Indeed, the organismal responses to drought should be regarded as a form of biological drought index to track departures from mean conditions. Climatologists are striving to narrow the uncertainty of drought and precipitation predictions under climate change (Ault 2020), and

ecologists should endeavor to improve the mechanistic links between water availability and the fitness of organisms (Bonebrake & Mastrandrea 2010; McCain & Colwell 2011; Rozen-Rechels et al. 2019) at the population and species levels.

We currently ignore the extent by which spatial mosaics of heat tolerance (Sears, Raskin & Angilletta 2011) are determined by water availability. Thermodynamic niche models partly pursue that goal by acknowledging that the responses of organisms to their environments must be mediated by food-temperature-water interactions in a spatially-explicit context (Kearney et al. 2013). In a ground-breaking contribution using those models, Kearney et al. (2018) found that growth and body condition of the sleepy lizard *Tiliqua rugosa* (given spatially constant metrics of thermal performance) could be best predicted by assuming that individuals had access to drinking water relative to dietary water and rainfall, while the dry-continental distribution margins of this Australian species mostly responded to water limitation. These authors conclude that “...water can be more important than temperature in constraining the activity, habitat requirements, and distribution limits of terrestrial ectotherms”. Indirectly, Kearney et al. (2018) imply that precipitation can alter the amounts of humidity and drinking water in the environment, and all of these three factors can have strikingly different effects on the physiology of organisms (Dupoué, Rutschmann, Le Galliard, Clobert, Blaimont et al. 2018; Lorenzon et al. 1999).

The factors shaping thermo-hydroregulatory strategies are expected to be complex, so we provide in the following a few examples (see also Introduction). Low precipitation can markedly augment the impacts of temperature extremes on fitness (Wang, Zeng, Li, Bi & Du 2016) and curtail the microhabitat quality of thermal refugia (Scheffers, Edwards, Diesmos, Williams & Evans 2014). For instance, lizards can prefer tree-shaded spots in dry periods but sun-exposed microhabitats with larger foraging areas after storms (Ryan, Latella, Giermakowski, Snell, Poe et al. 2016), and wet over dry refuges as temperature increase (Pintor, Schwarzkopf & Krockenberger 2016) or cold over warm refuges under water restriction (Rozen-Rechels, Badiane, Agostini, Meylan & Le Galliard 2020). In temperate mesic-adapted lizards, males are smaller and experience

higher survival than females with increased humidity (Romero-Diaz, Breedveld & Fitze 2017).

Additionally, lizards can accentuate evaporative water loss in mesic environments (Belasen, Brock, Li, Chremou, Valakos et al. 2017) and lower basal hormonal levels, critical to modulate daily and seasonal activity, when faced with shortage of free-standing water (Dupoué et al. 2018).

Behavioural adjustments can relax selective pressure at the expense of adaptation of thermal tolerance in the long-term (Buckley, Ehrenberger & Angilletta 2015), but might incur fitness costs and further widen differences in heat tolerance between populations of species exposed to contrasting water resources. Across the board, it is important to recognize that ectotherm ecophysiological research exclusively looking at experimental air-temperature effects does not neglect drinking water *per se* but supply it to individuals *ad libitum* so that it does not affect the interpretation of results.

Disentangling the relative contribution of phenotypic plasticity (e.g., seasonal acclimatization) and genetic adaptation to intraspecific variation in heat tolerance variation remains one of the main challenging areas for future ecophysiological research (discussed by Diamond 2017; Herrando-Pérez et al. 2019). Compared to many vertebrate endotherms, most herptiles are characterized by limited dispersal and, consequently, they are highly constrained to track future climate changes (Araújo & Pearson 2005), thereby prompting local ecophysiological responses for persistence. In that context, drinking-water availability can act as an evolutionary driver and as an amplifier of ectotherm physiological plasticity. Additionally, thermal and hydric requirements might conflict with or trade off in lizard microhabitat selection and activity patterns and complicate ectotherm responses to climate change (Pirtle et al. 2019). This seems a critical area of future development including experimental settings where we can discriminate ecophysiological responses to varying temperature and drinking-water conditions, and scaling up those responses at broad spatial scales. For example, the size and number of scales partly determines evaporative water loss, varies with aridity and precipitation both intra- and inter-specifically (Calsbeek, Knouft & Smith 2006; Oufiero, Gartner, Adolph & Garland 2011; Soulé 1966; Wegener, Gartner & Losos 2014),

and could therefore modify lizard heat tolerance and also be subject to natural selection for varying water conditions.

The reality is that the whole Mediterranean basin is currently exposed to the synergistic effects of drought and warming. Thus, low soil moisture in Spring and early Summer are amplifying the incidence of heatwaves in the hottest months (Dasari, Salgado, Perdigao & Challa 2014; Russo, Gouveia, Dutra, Soares & Machado Trigo 2019), and their magnitude is projected to increase across most of Iberia (Dasari et al. 2014). In drylands and during droughts, herptiles might rely on rapid adaptive and/or plastic responses for survival, particularly in the south-west of Europe where many reptiles are projected to lose climate space due to the combination of increased warming and decreased precipitation (Araújo et al. 2006; Le Galliard et al. 2012). Range-restricted species such as small lizards are predicted to experience higher precipitation seasonality than broad-range species (Li, Li, Sandel, Blank, Liu et al. 2015). Those climate shifts might be critical for population persistence in the periphery of the thermal niche of species (Lancaster 2016; Prieto-Ramirez, Pe'er, Rödder & Henle 2018; Valladares et al. 2014) and across altitudinal gradients (McCain & Colwell 2011).

Conclusions

We contend that local populations of small herptiles are constrained to adjust their thermoregulatory, feeding and reproductive behaviour to the availability of a limiting resource such as drinking water (Dupoué, Blaimont, Rozen-Rechels, Richard, Meylan et al. 2020; Dupoué, Rutschmann, Le Galliard, Miles, Clobert et al. 2017; Le Galliard et al. 2012; Murphy & Denardo 2019; Peterson 1996). While global concerns have arisen because (i) ectotherms are narrowing their thermal safety margins as a result of a warming climate (Sunday, Bates, Kearney, Colwell, Dulvy et al. 2014) and (ii) predators relying on invertebrates, such as many reptiles like those studied here, are facing massive declines of their arthropod prey (Lister & Garcia 2018; Sánchez-Bayo & Wyckhuys 2019), increased aridification (Park et al. 2018; Trenberth et al. 2013) reducing the

frequency or magnitude of pulses of free-standing water could pose even greater threat (Murphy & Denardo 2019; Wright et al. 2013).

Terrestrial (micro)habitat quality can be severely constrained by the lack of environmental water, and in turn increase species extinction risk much more strongly than previously thought relative to aquatic species (Pinsky, Eikeset, McCauley, Payne & Sunday 2019). Thermal refugia might be physically available but too dry for herptiles to maintain a homeostatic water balance and avoid physiological stress. From first principles, body temperature and water balance are functionally integrated for ectotherms to attain physiological homeostasis (Rozen-Rechels et al. 2019), so it is unsurprising that small herptile species that tolerate narrow ranges of environmental temperatures are those that also tolerate narrow ranges of precipitation regimes, and *vice versa* (Bonetti & Wiens 2014). Due to the temperature-precipitation interactions imposed by multidimensional climate fluctuations (Garcia, Cabeza, Rahbek & Araújo 2014; VanDerWal, Murphy, Kutt, Perkins, Bateman et al. 2013) and contrasting eco-evolutionary dynamics in the cold *versus* hot range margins of species distributions (Calosi, Bilton, Spicer, Votier & Atfield 2010; Nadeau & Urban 2019), we argue that a ‘cold-hot’ axis of covariation of thermal performance and thermoregulatory behaviour (Goulet, Thompson, Michelangeli, Wong & Chapple 2017) needs to be conceptualized against a ‘dry-wet’ axis to fully represent the thermal niche of ectotherm species and how they can tolerate and/or adapt to climate change.

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Authors' contributions

SHP and DRV conceived the idea and designed methodology. JB, SHP and MPB sampled lizards in the wild. JB and SHP maintained lizards in captivity. JB, SHP and FFY co-estimated CT_{max} in the lab. JB, MPB and SHP reviewed the literature. SHP did statistical analyses, and wrote the first draft of the manuscript. All authors contributed critically to the drafts, gave final approval for publication and declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found at XXX.

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Table 1. Probabilities, evidence ratios, and variance explained for linear models equating critical thermal maxima (CT_{max}) as a function of population (*pop*), treatment (*treat*) or their interaction. The study includes two populations of the spiny-footed lizard *Acanthodactylus erythrurus* (Chapinería, El Saler) and the common wall lizard *Podarcis muralis* (Peñagolosa, Portalet) in the Iberian Peninsula, each subject to three experimental treatments (provision of both food and water, only food, or only water).

Species	Models	Model probability ($wAIC_c$) *	Evidence ratio (ER) *	Variance explained
Spiny-footed lizard	$CT_{max} \sim pop \times treat$	0.99	1.0	55.8
	$CT_{max} \sim pop + treat$	6.6E-03	1.5E+02	43.0
	$CT_{max} \sim pop$	1.7E-03	5.7E+02	42.2
	$CT_{max} \sim treat$	5.8E-07	1.7E+06	< 1
	$CT_{max} \sim 1$	2.8E-07	3.6E+07	-
Common wall lizard	$CT_{max} \sim pop \times treat$	0.99	1.0	73.3
	$CT_{max} \sim pop + treat$	1.1E-02	8.9E+01	66.3
	$CT_{max} \sim pop$	4.9E-06	2.0E+05	46.1
	$CT_{max} \sim treat$	7.0E-08	1.4E+07	23.9
	$CT_{max} \sim 1$	5.2E-10	1.9E+09	-

* Model probabilities ($wAIC_c$) and evidence ratios (= $wAIC_c$ of top-ranked model to $wAIC_c$ of any given model) obtained through the Akaike Information Criterion adjusted for finite sample size (AIC_c), and variance explained (coefficient of determination) estimated by linear models with Gaussian errors. Sample size = 5 male adults per population and treatment, except 3 males for the only-food treatment for *P. muralis* from Portalet.

Table 2. Frequency of relationships (‘cases’, see Materials and methods) of water- and temperature-related metrics of herptile physiology in response to water and temperature predictors in 56 target papers from the ecophysiological literature (focal literature review). Percentages (%) estimated for responses and predictors separately.

Statistical treatment	Variable type	Variable	Frequency	%
Response	Temperature	Body temperature	21	24
		Preferred body temperature	15	17
		Heat tolerance	12	14
		Cold tolerance	6	7
		Operative temperature	4	5
	Water	Evaporative water loss	23	26
		Body dehydration	4	5
	Other	Running speed	1	1
		Heart rate	2	2
Predictor	Temperature	Temperature gradient	25	29
		Body temperature	2	2
	Water	Moisture gradient	20	24
		Body dehydration	18	21
		Wet-dry seasonality	16	19
		Evaporative water loss	4	5

Fig. 1. Adult males of the two study species: the spiny-footed lizard *Acanthodactylus erythrurus* and the common wall lizard *Podarcis muralis* along with the four study localities in the Iberian Peninsula. Photo credits: Salvador Herrando-Pérez.

Fig. 2. Critical thermal maxima (CT_{max}) of two populations from two Iberian species of lacertid lizards, including the spiny-footed lizard *Acanthodactylus erythrurus* (A) and the common wall lizard *Podarcis muralis* (B) in response to three experimental treatments (from left to right): provision of food and water, only food, and only water. Bars quantify mean $CT_{max} \pm SE$. Sample size = 5 male adults per population and treatment, except 3 males for only-food treatment for *P. muralis* from Portalet.

Fig. 3. Frequency of correlations between climate (air temperature *versus* precipitation) and heat tolerance of terrestrial fauna in the ecological literature (general literature review = 64 papers, 95 cases; thickest link = 36 cases, thinnest link = 2 cases).

Fig. 1



Fig. 2

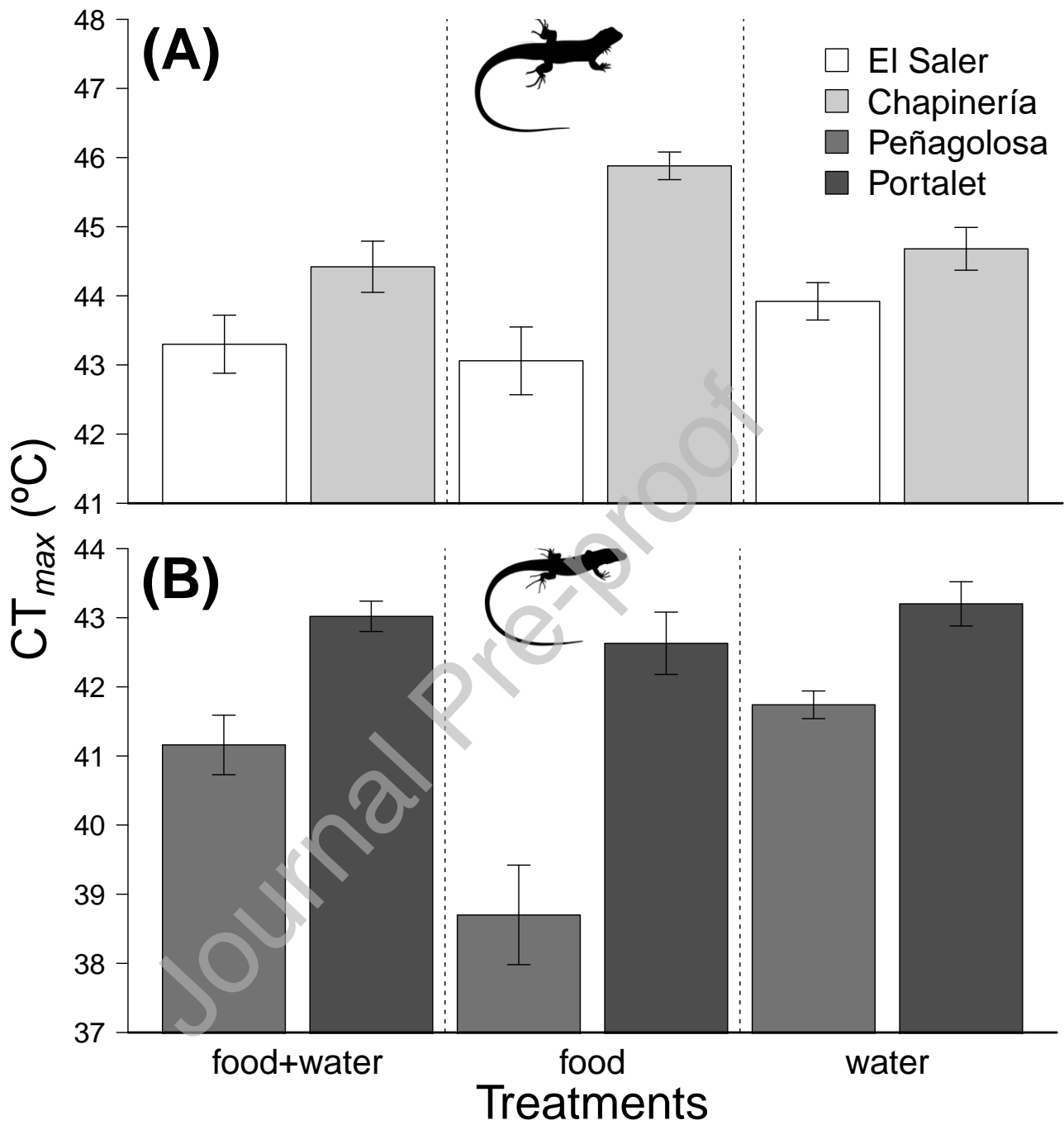


Fig. 3

