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BEHAVIORAL ECOLOGY –ORIGINAL RESEARCH

Behavioural hydroregulation protects against acute effects of drought in a dry-skinned ectotherm

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Abstract

During extreme climate events, behavioural thermoregulation may buffer ectotherms from thermal stress and overheating. However, heatwaves are also combined with dry spells and limited water availability, and how much individuals can behaviourally mitigate dehydration risks through microclimate selection remains largely unknown. Herein, we investigated the behavioural and physiological responses to changes in air and microhabitat humidity in a terrestrial ectotherm, the asp viper (*Vipera aspis*). We exposed individuals to a simulated heatwave together with water deprivation for 3 weeks, and manipulated air water vapour density (wet air vs. dry air) and microclimate (wet shelter vs. dry shelter) in a two-by-two factorial design. Dry air conditions led to substantial physiological dehydration and muscle wasting. Vipers exposed to dry air used more often a shelter that offered a moist microclimate, which reduced dehydration and muscle wasting at the individual level. These results provide the first experimental evidence that active behavioural hydroregulation can mitigate specific physiological stress responses caused by a dry spell in an ectotherm. Future studies investigating organismal responses to climate change should consider moisture gradient in the habitat and integrate both hydroregulation and thermoregulation behaviours.

Keywords Behaviour · Humidity gradient · Hydroregulation · Microclimate selection · Water

Introduction

Climate warming poses a major extinction threat to biodiversity, because rising local temperatures force many terrestrial organisms to shift their range, alter their phenology, or expend more energy to maintain homeostasis (Riddell et al. 2019a, b; Urban 2015). These physiological and ecological effects will largely depend on potential buffering by microclimatic conditions (Pincebourde et al. 2016; Potter et al. 2013). Notably, for some terrestrial organisms, vulnerability to global warming depends less on global climate trends than on microclimate conditions at small spatial scales, such as those generated by heterogeneous vegetation and soil layers, and on behavioural strategies allowing exploitation of these microclimate conditions (Potter et al. 2013; Riddell et al. 2021). Thus, to predict ecological consequences of climate change, one should better characterise how organisms respond to microclimatic heterogeneity (Sears et al. 2019; Woods et al. 2021).

In the context of global warming, the risks of overheating are significantly reduced when an organism is capable of fast behavioural responses (Seebacher and Franklin 2005; Terrien et al. 2011). Behavioural thermoregulation allows organisms, especially ectotherms, to exploit spatial variability in thermal conditions to maintain an optimal body temperature despite temporal fluctuations in operative temperature (Angilletta 2009; Fuller et al. 2021). For example, many terrestrial ectotherms typically live in a complex thermal landscape where they can change their posture and coloration, seek shade, use burrows, or shift their daily activity

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patterns to control heat gain, exploit cold and warm microclimates, and ultimately avoid overheating risks (Black et al. 2019; Forget-Klein and Green 2021; Moore et al. 2018). Given this, the most efficient thermoregulators should be better capable of maintaining homeostasis in a warming world (Huey et al. 2012; Kearney et al. 2009). Yet, climate change does not only challenge body temperature regulation but also puts a strong pressure on osmoregulation (i.e., the homeostatic maintenance of salt and body fluids' balance) in terrestrial organisms (Riddell et al. 2019a, b; Rozen-Rechels et al. 2019). For example, avoidance of overheating in desert endotherms involves evaporative cooling, which implies that birds and mammals must actively seek or save water to maintain an optimal body temperature in a warmer climate (Mitchell et al. 2018; Riddell et al. 2019a, b).

In many terrestrial ectotherms, behavioural trade-offs between thermoregulation and osmoregulation exist, because evaporative water loss increases with body temperature and in the microclimate conditions relevant for basking (e.g., Dupoué et al. 2015b; Riddell et al. 2018). In addition, climate warming often involves concurrent changes in temperature, rainfall, and moisture, especially during extreme climate events such as summer hot and dry spells that increase water loss risks (Trenberth et al. 2014; Ummenhofer and Meehl 2017). Organisms should partially buffer the water constraints of climate change by means of behavioural hydroregulation, defined as the range of behaviours that control water gains and water losses and help individuals maintain an optimal hydration state for their activity and functional capacities (Pirtle et al. 2019; Rozen-Rechels et al. 2019). For example, reptiles living under water stress can maintain their water balance by reducing their activity (Davis and Denardo 2010; Kearney et al. 2018) or by selecting cool (Ladyman and Bradshaw 2003; Pintor et al. 2016) or wet microclimates (Dupoué et al. 2015b; Guillon et al. 2014; Lourdais et al. 2017).

A wide range of hydroregulation behaviours have been observed in the wild, from moisture harvest in desert beetles, free-standing water seeking in large mammals to soil moisture preferences in frogs (Rozen-Rechels et al. 2019; Wolcott and Wolcott 2001). Despite that, our knowledge of the buffering capacity of these behavioural mechanisms still remains limited (Pirtle et al. 2019; Rozen-Rechels et al. 2020). Behavioural hydroregulation is most obvious in wet-skinned amphibians (Greenberg and Palen 2021; Lertzman-Lepofsky et al. 2020). Some amphibians can reduce evaporative water loss by means of behavioural changes such as postural adjustments to reduce the cutaneous water-exchange surface, mucus production, or the selection of moist microhabitats (e.g., Lillywhite et al. 1997; Wolcott and Wolcott 2001). There is good evidence that some amphibians perform active behavioural hydroregulation to maintain an optimal hydration state independently from thermoregulation (Mitchell and Bergmann 2016), but whether this behavioural flexibility is efficient during extreme weather events remains unknown. Behavioural data are even more elusive for dry-skin ectotherms due to difficulties disentangling hydroregulation and thermoregulation mechanisms in these organisms (Pintor et al. 2016; Rozen-Rechels et al. 2019). Dry-skin ectotherms often have a strong resistance to evaporative water loss, meaning that their hydration state changes more slowly than body temperature and that shifts caused by behavioural hydroregulation could take place over long time periods. Behavioural hydroregulation could be controlled by the internal hydration status, leading individuals to choose less desiccating microclimates as a water-saving strategy only when chronically stressed (Ladyman and Bradshaw 2003; Rozen-Rechels et al. 2020). On the other hand, terrestrial organisms can perceive moisture gradients through hygrosensing (Chown et al. 2011) and further rely on odours or other cues to locate free-standing water (Serrano-Rojas and Pašukonis 2021; Wood et al. 2021), such that changes in air water vapour pressure conditions may also trigger rapid changes in their behaviours.

Herein, we experimentally studied hydroregulation behaviours independently from body temperature regulation in a temperate ectotherm, the asp viper (*Vipera aspis*). We used climatic chambers to simulate weather conditions akin to those of a summer dry spell. We removed access to water and manipulated air moisture (dry versus wet air conditions) for 3 weeks. Water-deprived vipers were allowed to hydroregulate by providing them with a wet shelter (with a damp substrate) or not hydroregulate by providing them with a dry shelter. We examined the following hypotheses and predictions: first, water deprivation will most negatively impact hydration state in a dry air environment. We predict important mass loss and plasma osmolality increase in these conditions. Second, dehydration should induce compensatory physiological responses to mitigate water constraints. We predict, in particular, patterns of structural muscle catabolism to release bound water (Brusch et al. 2018) and a greater thirst reaction (Dezetter et al. 2021; Dupoué et al. 2014). Third, behavioural selection of wet microclimates should mitigate these effects by reducing evaporative water loss, especially when the simulated air is drier. We predict higher wet shelter use and lower physiological costs in this treatment group. The intensity of shelter use should also negatively correlate with physiological costs at the individual level.

Materials and methods

Study species

The asp viper is a typical sit-and-wait foraging and capital breeding snake distributed over southwestern Europe in sunny and warm microhabitats (Lourdais et al. 2002). This diurnal species is an active thermoregulator that maintains a high body temperature (30–33 °C) during daily activity (Lorioux et al. 2013; Lourdais et al. 2017). A functional trade-off exists between thermoregulatory needs and water loss in asp vipers: behavioural thermoregulation implies the use of warm microclimates, which increase evaporative water loss rates (Dupoué et al. 2015b; Guillon et al. 2014; Lourdais et al. 2017). Behavioural selection of warm and moist basking locations helps gravid females to mitigate the conflict between thermal needs and water loss (Lourdais et al. 2017). Access to water can be limited in natural habitats, especially during summer dry spells, which are increasing in frequency and intensity over the range distribution of this species (Spinoni et al. 2018). This capital breeder and semi-arid adapted species can tolerate long periods of fast and water deprivation, but at the cost of lower body reserves, enhanced emaciation and physiological stress, and therefore potential reduction in future fitness (Dupoué et al. 2015a; Lorioux et al. 2016). Herein, we used 42 non-reproductive adults (27 females and 15 males) from a captive colony originating from western France and maintained at the Centre d'Etudes Biologiques de Chizé, France.

Manipulation of hydric conditions and air temperature

Our experimental design consisted of a strictly defined manipulation of hydric conditions independently from air temperature. We used four controlled environment chambers (Vötsch VP 600, Balingen, Germany) allowing realistic simulations of daily fluctuations in air temperature and humidity. Asp vipers were deprived of water for 3 weeks, which corresponds to a moderate duration for a dry spell in natural conditions (Perkins et al. 2012). The daily temperature cycle mimicked body temperature recorded during a typical heatwave with daytime temperature of 32 °C for 14 h and nighttime temperature of 26 °C for 5 h, with temperature either gradually increasing or decreasing for the remaining 5 h (Lourdais et al. 2013). We next manipulated air humidity (dry air vs. wet air) and microclimate (dry shelter vs. wet shelter) using a two-by-two factorial design. First, we compared two contrasted ambient water vapour pressure deficit (VPD) to expose individuals to either desiccant ($VPD = -3.10 \pm 0.08$ kPa in dry air; Dupoué et al. 2015b) or humid conditions ($VPD = -1.43 \pm 0.08$ kPa in wet air). Second, we manipulated microclimate conditions by providing either a dry or a wet shelter. Shelters consisted of small, custom-made plastic containers (15 cm diameter, 11 cm height; one entrance of 3 cm diameter on the side) filled with an artificial sponge and microfiber cloths. Once accustomed, vipers will readily use shelters in the laboratory. The sponge and microfiber cloths were changed every 3 days and maintained dry in the dry shelters but damp in the wet shelters to enhance air humidity. In dry shelters, VPD were similar to the ambient conditions and therefore offered no humidity gradient. In turn, wet shelters provided high and constant humidity ($VPD = -0.61 \pm 0.09$ kPa), which simulates natural underground microhabitats that are typically moist with a stable and high humidity throughout the day. The differential between wet shelter and ambient air VPD was, respectively, of 2.49 kPa and 0.82 kPa for dry and wet air. Temperatures inside the shelters were similar to ambient temperatures. Note that we observed no water condensation inside the plastic shelters so drinking water was deemed extremely unlikely.

Experimental design

Prior to the experiment, for the purpose of acclimation to general maintenance conditions, we housed all individuals for 1 week in climatic chambers using individual plastic boxes ($35 \times 20 \times 17$ cm) with one dry shelter, a sheet of newspaper as a substrate and a natural day light cycle (circa 12:12). During this period, vipers had *ad-libitum* access to drinking water and were exposed to a standard daily temperature cycle (6 h daytime at 30 °C, 13 h nighttime at 22 °C, and mean VPD of -1.20 ± 0.10 kPa). Then, we randomly allocated individuals to each treatment group while ensuring a balanced sex ratio. Each individual was placed in a climatic chamber with the corresponding air humidity treatment (dry air or wet air) and we applied the following protocol over 24 days: from Day 0 to Day 2 (3 days), we provided all individuals with a dry shelter to quantify baseline shelter use; from Day 3 to Day 20 (18 days), we exposed each individual to its microclimate treatment (dry shelter or wet shelter) to quantify the combined effects of air humidity and microclimate; from Day 21 to Day 23 (3 days), we provided all individuals with a wet shelter to test for change in shelter use in individuals previously exposed to dry shelters; and finally, on Day 24, we provided all individuals with water *ad-libitum* to assess post-treatment water intake from drinking. We then transferred them to standard housing conditions. We did not feed the snakes to avoid confounding effects of digestion and energy intake on body mass (Stahlschmidt et al. 2015).

Variables collected and parameters measured

Shelter use over time

Every day from Day 0 to Day 23, we recorded the position of each individual three times per day during daytime (9:00, 13:00, and 17:00). Individual position in the box was categorised as either using the shelter or being outside. First, we considered the cumulative shelter use from Day 0 to Day 2 (proportion of shelter use out of 9 observations) to record the baseline shelter use. To investigate responses to combined air humidity and microclimate treatments (Day 3 to Day 20), we first examined the cumulative shelter use during that period (proportion of shelter use out of 54 observations). Second, we considered more specifically changes in daily shelter use (proportion of shelter use out of the three daily observations) over time (Day 0 to Day 20). We also calculated an individual shelter use score over a 3-day period between Day 3 and Day 20 (total number of times inside the shelter per 9 observations, 6 repetitions) to investigate the relation between shelter use behaviour and body mass changes (recorded every 3 days, see below). Finally, we also calculated cumulative shelter use from Day 21 to Day 23 (proportion of shelter use out of nine observations) to investigate wet shelter use at the end of the treatment.

Changes in body mass and muscle condition

In the absence of food intake, body mass (BM) change provides a non-invasive proxy of the whole-body hydration state in snakes (Dupoué et al. 2014). We therefore weighed (±0.1 g) all individuals every 3 days to assess BM changes during the treatment period, and then weighed them again at the end of Day 24 to assess mass uptake and rehydration after the manipulation. From this, we calculated total ΔBM as the difference between BM at Day 20 and at Day 0. We also analysed mass changes over each 3 day period during the combined manipulation of air humidity and microclimate (Day 3 to Day 20, six repetitions per individual) to investigate the relation between individual shelter use and body mass changes during that period. Finally, post-treatment water intake was inferred from the mass difference between Day 24 and Day 23.

Bound water is a significant component of all body tissues that can be mobilised to support water demands during periods of water deprivation, especially from wet protein of muscles in reptiles (Brusch et al. 2018). In snakes, skeletal muscles can be mobilised to support water requirements (Brusch et al. 2018). To quantify skeletal muscles loss during the treatment period, we measured tail width (TW ± 0.01 mm, average from triplicates) on Day 0 and on Day 20 using an electronic pressure-sensitive specimeter (Absolute Digimatic, Mitutoyo, Japan) at the position of the sixth subcaudal scale (Lorioux et al. 2016). We calculated ΔTW as the difference between mean TW at Day 20 and Day 0.

Changes in plasma osmolality

We collected blood samples on Day 0 and Day 20 to assay plasma osmolality, which measures the whole-body electrolyte and water balance (Peterson 2002). We sampled individuals in a random order by sex and treatments and collected 150 μL of blood through cardiocentesis, using a 1 mL syringe and a heparinized 29-gauge needle. We immediately centrifuged the blood at 2000*g*, separated plasma from the red blood cells and stored the samples at − 28 °C. Plasma osmolality (± 1 mOsm kg^{-1}) was measured from 10 μL duplicates (intraindividual CV: 0.36%) using a vapour pressure osmometer (model 5500, Wescor, Logan, UT, USA). We calculated ΔOsmolality as the difference between mean plasma osmolality before and after manipulation.

Statistical analyses

We conducted all analyses using the R software version 3.6.3 (R Core Team 2020). To investigate the effects of treatments on behavioural hydroregulation, we first built a generalized linear model with a binomial error distribution and a logit link function to examine cumulative shelter use (success = total number of observation when shelter was used; failure = total number of observation when shelter was not used) between Day 3 to Day 20., We used sex, air humidity, microclimate treatment, and the interaction between both treatments as explanatory factors in the full model. This full model was built with *glm* function of the *stats* package (R Core Team 2020) and fitted well with the data (overdispersion test: $X^2 = 43.9$, dispersion coefficient = 1.1, $df = 39$, $P = 0.27$). We used two similar models to analyse the baseline shelter use before the start of the microclimate manipulation (between Day 0 and Day 2) and the wet shelter use at the end of the treatment (between Day 21 and Day 23). Because of significant overdispersion of the binomial distribution in these two data sets, we compared treatment group using a betabinomial approach (Harrison 2015). The betabinomial model for proportions was fitted with *betabin* function of the *aod* package (Lesnoff and Lancelot 2012). We further investigated repeated measures through time of daily shelter use according to treatments between Day 0 and Day 20 using a generalized linear mixed-effects model with a binomial error distribution (success = number of observations per day when shelter was used; failure = number of observation per day when shelter was not used) and a logit link function. We included sex as a fixed factor, and air humidity, microclimate treatment, time (number of days) and their three-way interaction as explanatory variables. Individual identity was included as a random term to control for repeated measures on the same individuals. Because data were skewed toward zero at Day 0 and shelter use was identical between treatment groups at the beginning of the manipulation, we forced a similar intercept for the four treatments in our full model. This model was built with glmer function of the lme4 package (Bates et al. 2015) and fitted well with the data (overdispersion test: $X^2 = 816.4$, dispersion coefficient = 0.9, $df = 896$, $P = 0.92$). In all cases, we performed backward elimination model selection starting from the full models to remove non-significant effects using likelihood ratio tests. We report here P values from Wald tests based on z-score of the parameter estimates of the models.

To analyse physiological and morphological responses, we used linear models fitted with the *lm* function (*stats* package) and univariate linear mixed-effects models fitted with the *lme* function of the *nlme* package (Pinheiro et al. 2020). We performed backward elimination model selection starting from the full models after checking that the residuals of our models fulfilled a Gaussian distribution using Shapiro–Wilk tests and had homogeneous variances across treatment groups. We further explored statistical differences between treatment groups using pairwise post hoc Tukey tests (*emmeans*, package *emmeans*; Lenth 2018). Regarding morphological and osmolality data, we first built independent linear models to confirm that there were no treatment differences in BM, TW or plasma osmolality at the beginning of the experiment. Models included the initial value as the dependent variable and air humidity, microclimate treatment, and their interaction as fixed effects. There was no difference in BM, TW, and plasma osmolality among treatments before the manipulation (all $P > 0.05$, Table S1 and S2). To investigate the effects of the treatments on body mass loss over time, we used a mixed-effects model for repeated measures with BM as the dependent variable, sex as a fixed factor, air humidity treatment, microclimate treatment, time (i.e., days on which individuals were weighed) and their three-way interaction as explanatory variables. Individual identity was included as a random, intercept term.

We next built three independent linear models to investigate the effects of the treatments on total ΔBM, ΔTW, and ΔOsmolality and post-treatment ΔBM. We included air humidity, microclimate treatment, and their interaction as explanatory variables, sex as a categorical factor, and the initial value as a linear covariate. We further examined whether ΔBM, ΔTW, and post-treatment ΔBM were correlated with ΔOsmolality or final osmolality using linear regressions. To determine whether ΔOsmolality or final osmolality better explained physiological changes, we used the Akaike Information Criterion to compare models (*AIC*, package *stats*). We also investigated whether post-treatment ΔBM was correlated with total ΔBM using a similar model construction and comparison.

We also wanted to investigate the relationship between wet shelter use and mass change of snakes. To achieve this, we considered mass changes across individuals in the dry air treatment with access to wet shelter and applied the statistical methods from van de Pol and Wright (2009).We calculated an individual-centred shelter use score at each 3-day period for each individual. We built a linear mixed-effects model with the change in body mass per 3-day period as the dependent variable and shelter use score together with individual-centred shelter use score as explanatory variables. Individual identity was set as a random factor.

Results

Quantification of hydroregulation behaviour

At the onset of the study (from Day 0 to Day 2), there was no difference in shelter use among treatment groups (air humidity: $z = -0.55$, $P = 0.58$; Microclimate treatment: $z = 0.55$, $P = 0.58$). During the cumulated manipulation period (from Day 3 to Day 20), the total proportion of shelter use was influenced by the interaction between air humidity and microclimate treatment ($z = -2.1, P = 0.03$). Supporting our prediction, shelter use was mainly observed in vipers maintained in dry air with a wet shelter, while other individuals almost never selected the shelter (Table 1). The daily shelter use gradually increased over time in the dry air and wet microclimate treatment group, while it remained stable and low in other groups (air humidity × microclimate treatment × time: $z = -2.1, P = 0.03$; Fig. 1a). When all individuals were provided with a wet shelter at the end of the experiment (from Day 21 to Day 23), the proportion of shelter use was then additively influenced by air humidity ($z = -3.5, P < 0.001$) and former microclimate treatments ($z = -2.4, P = 0.02$). This was due to a behavioural shift where vipers maintained in the dry microclimate treatment rapidly increased their use of the shelter when it became wet (Fig. 1a).

Physiological and morphological changes across treatments

Vipers significantly lost body mass over time during the experiment ($F_{1,290} = 1674.8, P < 0.001$, Fig. 1b), and air humidity and microclimate treatments interactively influenced BM loss (air humidity × microclimate treatment × time: $F_{1,290} = 26.2, P < 0.001$). Vipers from the dry air and dry shelter lost BM at a much higher rate than those maintained in other conditions (Fig. 1b). In the dry air treatments, vipers with access to a wet shelter lost BM at a slower rate than those with a dry shelter. The total ΔBM over the experimental period was also influenced by the interaction between both treatment types ($F_{1,37} = 4.5, P = 0.04$; Table 1, mean ΔBM = – 13.2 ± 5.5% of initial BM).

Table 1 Summary statistics of the traits measured on asp vipers from each treatment group

Variables	Experimental treatments				
	Wet air		Dry air		
	Wet shelter ($n = 10$)	Dry shelter ($n = 11$)	Wet shelter ($n = 11$)		Dry shelter ($n = 10$)
	Mean ($\pm$SE)	Mean ($\pm$SE)	Mean ($\pm$SE)		Mean ($\pm$SE)
Cumulative shelter use (%)	7.96 (4.64) ^{A}	4.71 (3.48) ^{A}	58.58 (6.90) ^{B}	*63.33 (5.53)* ^{B}	10.92 (3.57) ^{A}
Δ Body mass—Day20 (g)	– 15.31 (1.23) ^{A}	– 14.37 (0.93) ^{A}	– 18.73 (1.05) ^{AB}	*– 18.26 (1.04)* ^{A}	– 22.77 (1.49) ^{B}
Δ Plasma osmolality (mOsm.kg^{-1})	30.15 (4.17) ^{A}	31.45 (5.12) ^{A}	45.50 (8.96) ^{AB}	*38.55 (6.26)* ^{A}	68.60 (11.87) ^{B}
Δ Tail width (mm)	– 0.21 (0.09) ^{A}	– 0.48 (0.13) ^{AB}	– 0.46 (0.11) ^{AB}	*– 0.43 (0.12)* ^{AB}	– 0.81 (0.15) ^{B}
Post-treatment water intake (g)	10.19 (1.01) ^{A}	12.94 (1.19) ^{AB}	17.63 (1.62) ^{BC}	*16.63 (1.40)* ^{BC}	21.84 (1.66) ^{C}

Values written in italic are from the Dry air and Wet shelter group when excluding the individual that almost never selected the shelter (see details in main text). Different superscript letters are used to show significant differences between treatment groups for each variable

Plasma osmolality increased in all groups indicating physiological dehydration, and the increase in osmolality was influenced by sex ($F_{1,37} = 23.7, P < 0.001$), air humidity treatment ($F_{1,37} = 15.5, P < 0.001$) but only marginally by microclimate treatment ($F_{1,37} = 2.9, P = 0.09$). Individuals had a higher increase in osmolality in dry conditions, and tended to have a lower increase in osmolality when a wet shelter was available (Table 1). However, one male from the dry air and wet microclimate treatment almost never used the wet shelter (shelter use score of 10%). Excluding this individual, we found that ΔOsmolality was significantly influenced by the interaction between air humidity and microclimate treatments ($F_{1,35} = 6.6, P = 0.01$) and by sex ($F_{1,35} = 23.5, P < 0.001$). Increase in plasma osmolality was highest in vipers maintained in dry air with a dry shelter and lowest and similar in the other three groups (Table 1). Overall, males had a higher increase in plasma osmolality than females. Tail width change ΔTW was additively influenced by air humidity ($F_{1,38} = 5.7, P = 0.02$, Fig. 2a) and microclimate treatments ($F_{1,38} = 5.6, P = 0.02$, Fig. 2b). Vipers had increased TW loss in dry air conditions and when they only had access to a dry shelter (Table 1; Fig. 2a).

Benefits of hydroregulation and post-treatment water intake

Focusing on the dry air and wet shelter treatment, we found that body mass loss was negatively correlated with the individual-centred shelter use score ($F_{1,65} = 26.1, P < 0.001$). Higher behavioural selection of the wet shelter led to reduced body mass loss (Fig. 3). Post-treatment water intake was additively influenced by air humidity ($F_{1,37} = 32.5, P < 0.001$, Fig. 2c) and microclimate treatments ($F_{1,37} = 8.7, P = 0.005$, Fig. 2d). Vipers exposed to dry air conditions and vipers with access to a dry shelter drank more on Day 24 (Table 1; Fig. 2c).

Correlations between physiological responses

Overall, BM loss was positively correlated with both ΔOsmolality ($F_{1,39} = 55.9, P < 0.001$, Fig. S1a) and final osmolality ($F_{1,39} = 4.99, P < 0.001$), but most influenced by ΔOsmolality (Table S8). TW loss was also positively correlated with both ΔOsmolality ($F_{1,39} = 17.9, P < 0.001$, Fig. S1b) and final osmolality ($F_{1,39} = 19.2, P < 0.001$; Fig. S6) with similar strength of correlation (Table S8). Post-treatment water intake was positively correlated with ΔOsmolality ($F_{1,39} = 66.0, P < 0.001$, Fig.S1c), final osmolality ($F_{1,39} = 80.0, P < 0.001$), and ΔBM ($F_{1,39} = 34.3, P < 0.001$), but most influenced by final osmolality (Table S8).

Discussion

We provide the first experimental evidence that behavioural hydroregulation can mitigate physiological consequences of a simulated dry spell in a terrestrial ectotherm. Wet shelters were used more frequently when individuals were exposed to dry air conditions. This pattern was gradual, reaching 75% of the time by the end of the manipulation, compared to less than 10% in other treatment groups. This demonstrates microclimate selection based on water vapour gradient independently from temperature, thus providing unambiguous evidence of behavioural hygroregulation sensu stricto (Mitchell and Bergmann 2016; Wolcott and Wolcott 2001). This behaviour allowed individuals to attenuate mass loss and dehydration risks in more desiccating conditions.

Concurrent changes in morphology, plasma osmolality, and post-treatment drinking behaviour all indicated that more desiccating air led to more severe physiological dehydration. Body mass loss throughout the treatment period was higher in individuals exposed to dry air and these individuals also had the greatest increase in plasma osmolality.

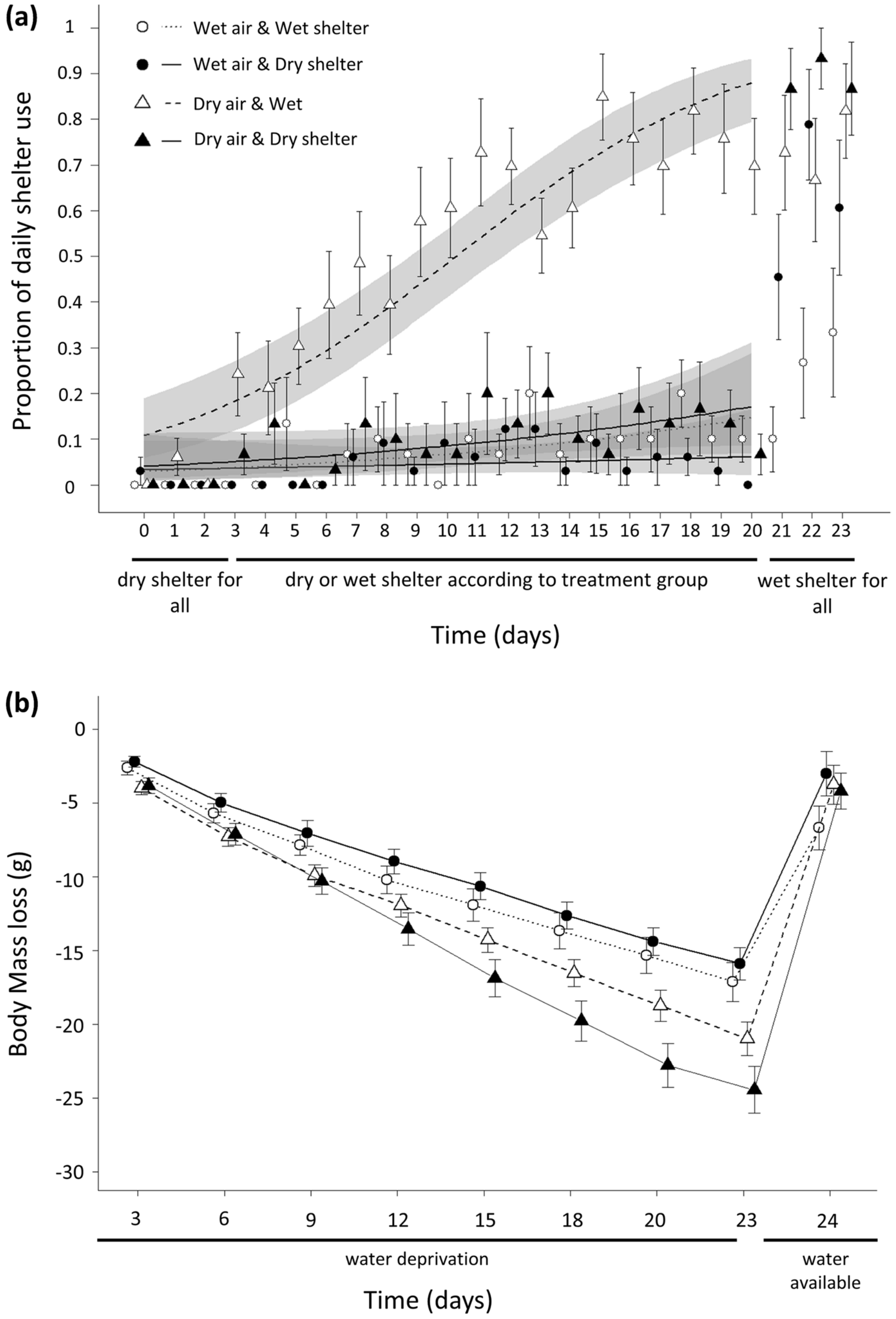

Fig. 1 Effects of experimental treatments on behavioural hydroregulation and body mass loss. **a** Changes in daily shelter use (proportion of shelter use out of the three daily observations) over time in each treatment group. Points are mean values with error bars representing ± 1 SE. The predictions of the best logistic regression model (curves) were fitted with the data from Day 0 to Day 20, together with their 95% confidence intervals (shaded background). Vipers had access to either a dry or a wet shelter and were maintained in dry or wet air from Day3 to Day20. All individuals had access to a dry shelter from Day0 to Day2, and to a wet shelter from Day21 to Day23. **b** Average body mass loss (g) loss through time (every 3 days) in each treatment group. All individuals were deprived of drinking water from Day0 to Day 23. On Day24, all individuals were provided with drinking water *ad-libitum*. Error bars represent ± 1 SE

Muscle loss and post-treatment water intake were greater in individuals exposed to dry air, and both traits were correlated with physiological dehydration, here assessed by final osmolality values. Therefore, dry air conditions increased muscle catabolism, probably to release the bound water that makes up a significant portion of skeletal muscles (Brusch et al. 2018) and resulted in increased thirst levels (Edwards et al. 2021; Lillywhite et al. 2019). Dehydration-induced muscle catabolism can be beneficial to support water needs and reduce the immediate survival risks associated with hyperosmolality in vertebrates (Brusch et al. 2018; Gerson and Guglielmo 2011; McCue et al. 2017). However, such compensatory mechanisms directly alter structural muscles and associated locomotor capacities (Lorioux et al. 2016; Lourdais et al. 2004). It is well known that dehydration can impair locomotor performance as demonstrated in amphibians and some lizards (Greenberg and Palen 2021; Anderson and Andrade 2017; Wilson et al. 1989). Although we did not measure behavioural capacities or functional properties such as locomotion or foraging, the high levels of dehydration and muscle mobilization observed in some of our snakes are most likely associated with impaired locomotor abilities.

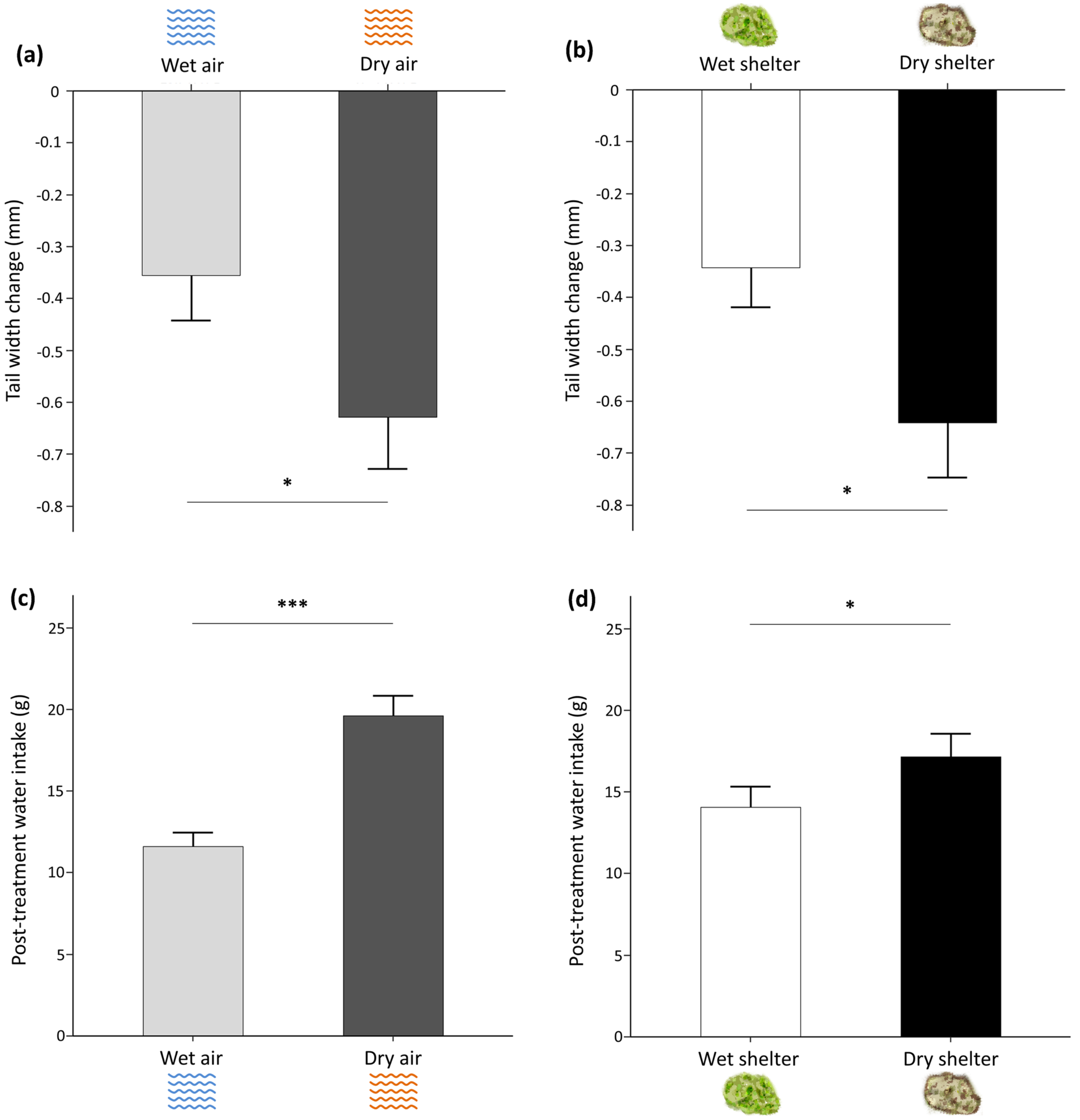

Fig. 2 Additive effects of air humidity and microclimate treatment on changes in tail width (mm) throughout the experiment (**a**; **b**) and on post-treatment water intake (g) following access to drinking water on Day24 (**c**; **d**). Error bars represent ± 1 SE

Altogether, these results indicate that a more desiccating air in the absence of free-standing water caused greater dehydration, likely through increased evaporative water loss (Dupoué et al. 2015b). Such hygrometric constraints are likely even more prominent at high ambient temperatures, because desiccation risks rise exponentially with temperature (increased magnitude of VPD, see Riddell et al. 2019a, b) and may be even stronger during key life-history events when thermal requirements are elevated such as gestation in female vipers (see Dupoué et al. 2015b; Stahlschmidt et al. 2011).

Supporting our initial predictions, wet shelter use buffered the effects of dry air conditions on physiological dehydration. When a wet shelter was available, individuals had

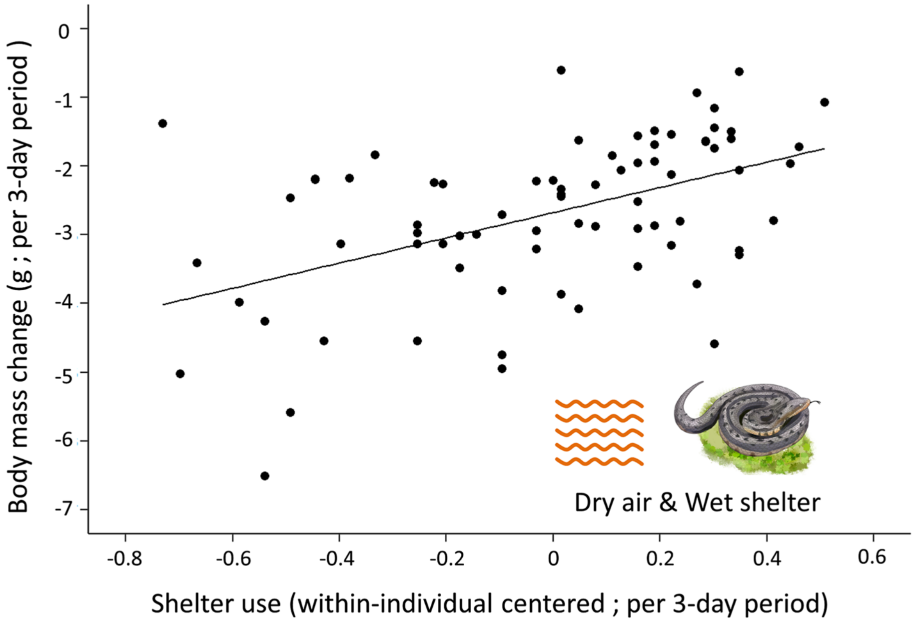

Fig. 3 Correlation between body mass change (g) and individual-centred shelter use score per 3-day period within the dry air and wet shelter treatment group (six repetitions per viper from Day 3 to Day 20 of the manipulation). The individual-centred score quantifies intraindividual variation in shelter use (positive values indicate stronger shelter use than the mean score of the individual). Drawing credits: Sydney Hope

on average a lower body mass loss especially in dry air, tended to have a lower increase in plasma osmolality, and had a lower post-treatment water intake. Interestingly, when we excluded one viper whose behaviour was not responsive to wet shelter availability, the influence of a wet microclimate on osmolality and body mass changes was only significant under the dry air condition. Individuals with access to a wet shelter also had lower muscle loss rates compared to those with a dry shelter, which suggests that they had less of a need to catabolize muscle protein. In addition, within the dry air and wet shelter treatment, the more a viper used the shelter, the less body mass it lost. Behavioural selection of a moist microclimate thus allowed vipers to attenuate dehydration risks and specific associated morphological responses. Altogether, snakes that had access to a wet shelter could thus mitigate the direct performance costs possibly induced by hyperosmolality (Greenberg and Palen 2021; Anderson and Andrade 2017), and also minimised the indirect performance costs of muscle catabolism (Brusch et al. 2018; Lorioux et al. 2016).

We also found significant differences among individuals in their wet shelter use irrespective of treatment conditions and sex suggesting wide variation in hydroregulation behaviours likely exist. Within natural populations, such variation might translate in individual differences in physiological sensitivity to extreme climate events. Since flexible hydroregulation behaviours buffer physiological consequences of desiccating air, they should be beneficial for populations facing more frequent and intense dry spells, such as those located at the warm edge of the distribution. At the end of the experiment, most vipers that had been housed with a dry shelter immediately selected the wet shelter at a frequency similar to that of vipers that were maintained with a wet shelter and dry air for several days. This sudden shift suggests that these snakes had reached a significant dehydration threshold, which induced an immediate behavioural response once a wet microclimate was available.

Hygrosensing and behavioural hydroregulation have been previously observed in dry-skinned ectotherms both in the field (Guillon et al. 2014; Lourdais et al. 2017) and in laboratory conditions (Dupoué et al. 2015b; Stahlschmidt and Denardo 2010; Stahlschmidt et al. 2011). Our findings extend these earlier observations and highlight that microclimate selection within a "hygrometric landscape" caused by spatial heterogeneity in humidity promotes water balance regulation and minimises the costs of dehydration. We posit that such behavioural adjustments are frequent in terrestrial ectotherms and call for a better characterisation of microclimate moisture gradients in the context of climate change (Pincebourde et al. 2016; Woods et al. 2021). Our results indicate that the use of moist microhabitats may allow ectotherms to avoid desiccation during extreme climate events, similar to the key role of behavioural thermoregulation in limiting exposure to acute thermal extremes and overheating (Huey and Tewksbury 2009; Kearney et al. 2009). For example, following the classic terminology for thermoregulation, some species may actively "hygroregulate", while others may behave as "hygroconformer". The former may efficiently seek microclimates minimising the deviance to optimal moisture (Moore et al. 2018; Székely et al. 2018; Lourdais et al. 2017), which would allow individuals to maintain their hydration state, performance, and activity (Greenberg and Palen 2021; Anderson and Andrade 2017). However, their behavioural capacity to do so ultimately depends on habitat “hygrometric quality”. This emphasizes the critical importance of the availability of moist microhabitats for dry-skinned ectotherms, which can decrease with anthropogenic disturbances (Gorissen et al. 2017) or climate change (Dundas et al. 2021). In addition, we posit that a behavioural capacity to maintain optimal hygrometric conditions may dampen long-term exposure to dehydration risks along gradients of climate or habitat aridity. This may reduce selection on physiological tolerance to dehydration, similar to the concept of the “Bogert effect” for thermoregulatory behaviour (Muñoz 2021). More generally, the occurrence of behavioural hygroregulation may have implications for our understanding of the evolution of ecological niches of organisms (Encarnación-Luévano et al. 2021; Farallo et al. 2020; Woods et al. 2021).

Understanding and predicting organismal responses to climate change are currently a major challenge in ecology and conservation (Kearney et al. 2018a; Riddell et al. 2019a, b). Our experimental study demonstrates that hygrometric constraints and behavioural hydroregulation capacity must be considered in this context. Recent individual-based models have used energy and heat budgets to predict fine-scale movements within microhabitats in response to thermal microclimate conditions (Malishev et al. 2018; Sears et al. 2016), but those approaches often overlooked water constraints. Future mechanistic models should include the effects of air moisture and behavioural hydroregulation on the water budget of organisms (see Huang et al. 2020; Peterman and Semlitsch 2014). One challenge to this approach is that, in contrast to behavioural thermoregulation where individuals generally immediately adjust their behavioural activity in response to well-known temperature thresholds or thermal performance curves (Angilletta 2009), behavioural hygroregulation likely changes more gradually in response to a shift in individual hydration state. The physiological costs of desiccant conditions should also vary among species depending on their resistance to water loss, thus influencing the timing of behavioural changes. For example, some dry-skinned ectotherms may adjust their hydroregulation behaviour over days or weeks, as shown here, while most wet-skinned ectotherms may adjust over minutes or hours (Peterman and Semlitsch 2014; Székely et al. 2018). In addition, empirical data suggest that the performance costs of dehydration increase abruptly only after a significant dehydration threshold is reached in amphibians (Cheuvront and Kenefick 2014; Greenberg and Palen 2021). Such nonlinear, threshold effects should trigger more or less rapid behavioural adjustment depending on the shape of hydric performance response curve (Edwards et al. 2021; Lillywhite et al. 2019). Future studies should therefore examine the strength and shape of the relationships between dehydration and performance capacities for which we currently lack data (Rozen-Rechels et al. 2019).

In conclusion, our study emphasizes the importance of air humidity, as well as behavioural hydroregulation, when investigating the effects of climate change on ectotherms. Microhabitat quality and heterogeneity appear to be essential to support behavioural responses and mitigate deleterious effects of extreme weather events. However, current habitat simplifications associated with human activities are likely to compromise these important components of microhabitat (Guiller et al. 2022).

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Author contributions MD, J-FLG, and OL conceived the ideas and designed methodology. MD and OL carried all experimental procedures and collected the data. MD led the data analyses and writing of the manuscript together with J-FLG and OL, and all authors contributed critically in result interpretation and manuscript writing. All authors gave final approval for publication.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing or financial interests.

Ethical approval All experimentation procedures with animals listed in this study were approved by an independent ethical committee (Apafis#23238-2019121010574211_V3).

Consent to participate Not applicable.

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References

Anderson RC, Andrade DV (2017) Trading heat and hops for water: dehydration effects on locomotor performance, thermal limits, and thermoregulatory behavior of a terrestrial toad. Ecol Evol 7:9066–9075. https://doi.org/10.1002/ECE3.3219

Angilletta MJ (ed) (2009) Thermal adaptation. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198570875.001.1

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Soft 67(1):1–48. https://doi.org/10.18637/jss.v067.i01

Black IRG, Berman JM, Cadena V, Tattersall GJ (2019) Behavioral thermoregulation in Lizards: strategies for achieving preferred temperature. Behav Lizards. https://doi.org/10.1201/9781498782739-2

Brusch GA, Lourdais O, Kaminsky B, DeNardo D (2018) Muscles provide an internal water reserve for reproduction. Proc Biol Sci. https://doi.org/10.1098/RSPB.2018.0752

Cheuvront SN, Kenefick RW (2014) Dehydration: physiology, assessment, and performance effects. Compr Physiol 4:257–285. https://doi.org/10.1002/CPHY.C130017

Chown SL, Sørensen JG, Terblanche JS (2011) Water loss in insects: an environmental change perspective. J Insect Physiol 57:1070–1084. https://doi.org/10.1016/J.JINSPHYS.2011.05.004

Davis JR, Denardo DF (2010) Seasonal patterns of body condition, hydration state, and activity of gila monsters (*Heloderma suspectum*) at a Sonoran Desert Site. J Herpetol 44:83–93. https://doi.org/10.1670/08-263.1

Dezetter M, Le Galliard JF, Guiller G, Guillon M, Leroux-Coyau M, Meylan S, Brischoux F, Angelier F, Lourdais O (2021) Water deprivation compromises maternal physiology and reproductive success in a cold and wet adapted snake Vipera berus. Conserv Physiol. https://doi.org/10.1093/CONPHYS/COAB071

Dundas SJ, Ruthrof KX, Hardy GESJ, Fleming PA (2021) Some like it hot: Drought-induced forest die-off influences reptile assemblages. Acta Oecologica 111:103714. https://doi.org/10.1016/J.ACTAO.2021.103714

Dupoué A, Angelier F, Lourdais O, Bonnet X, Brischoux F (2014) Effect of water deprivation on baseline and stress-induced corticosterone levels in the Children's python (*Antaresia childreni*). Comp Biochem Physiol Part A Mol Integr Physiol 168:11–16. https://doi.org/10.1016/j.cbpa.2013.11.001

Dupoué A, Brischoux F, Angelier F, Denardo DF, Wright CD, Lourdais O (2015a) Intergenerational trade-off for water may induce a mother-offspring conflict in favour of embryos in a viviparous snake. Funct Ecol. https://doi.org/10.1111/1365-2435.12349

Dupoué A, Stahlschmidt ZR, Michaud B, Lourdais O (2015b) Physiological state influences evaporative water loss and microclimate preference in the snake Vipera aspis. Physiol Behav. https://doi.org/10.1016/j.physbeh.2015.02.042

Edwards M, Sheehy CM, Fedler MT, Lillywhite HB (2021) Thirst and drinking in North American watersnakes (Nerodia spp.). J Exp Biol. https://doi.org/10.1242/JEB.241414

Encarnación-Luévano A, Peterson AT, Rojas-Soto OR (2021) Burrowing habit in Smilisca frogs as an adaptive response to ecological niche constraints in seasonally dry environments. Front Biogeogr. https://doi.org/10.21425/F5FBG50517

Farallo VR, Muñoz MM, Uyeda JC, Miles DB (2020) Scaling between macro- to microscale climatic data reveals strong phylogenetic inertia in niche evolution in plethodontid salamanders. Evolution (NY). 74:979–991. https://doi.org/10.1111/EVO.13959

Forget-Klein É, Green DM (2021) Toads use the subsurface thermal gradient for temperature regulation underground. J Therm Biol. https://doi.org/10.1016/J.JTHERBIO.2021.102956

Fuller A, Mitchell D, Maloney SK, Hetem RS, Fonsêca VFC, Meyer LCR, van de Ven TMFN, Snelling EP (2021) How dryland mammals will respond to climate change: the effects of body size, heat load and a lack of food and water. J Exp Biol. https://doi.org/10.1242/JEB.238113

Gerson AR, Guglielmo CG (2011) House sparrows (Passer domesticus) increase protein catabolism in response to water restriction. Am J Physiol Regul Integr Comp Physiol. https://doi.org/10.1152/AJPREGU.00701.2010/ASSET/IMAGES/LARGE/ZH60041175230003.JPEG

Gorissen S, Greenlees M, Shine R (2017) A skink out of water: impacts of anthropogenic disturbance on an Endangered reptile in Australian highland swamps. Oryx 51:610–618. https://doi.org/10.1017/S0030605316000442

Greenberg DA, Palen WJ (2021) Hydrothermal physiology and climate vulnerability in amphibians. Proc R Soc B. https://doi.org/10.1098/RSPB.2020.2273

Guiller G, Legentilhomme J, Boissinot A, Blouin-Demers G, Barbraud C, Lourdais O (2022) Response of farmland reptiles to agricultural intensification: collapse of the common adder Vipera berus and the western green lizard Lacerta bilineata in a hedgerow landscape. Anim Conserv. https://doi.org/10.1111/ACV.12790

Guillon M, Guiller G, DeNardo D, Lourdais O (2014) Microclimate preferences correlate with contrasted evaporative water loss in parapatric vipers at their contact zone. Can J Zool 92:81–86. https://doi.org/10.1139/cjz-2013-0189

Harrison XA (2015) A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. PeerJ 3:e1114

Huang SP, Kearley RE, Hung KW, Porter WP (2020) Evaporative water loss simulation improves models' prediction of habitat suitability for a high-elevation forest skink. Oecologia 192:657–669. https://doi.org/10.1007/S00442-020-04597-W

Huey RB, Tewksbury JJ (2009) Can behavior douse the fire of climate warming? Proc Natl Acad Sci 106:3647–3648. https://doi.org/10.1073/PNAS.0900934106

Huey RB, Kearney MR, Krockenberger A, Holtum JAM, Jess M, Williams SE (2012) Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos Trans R Soc B Biol Sci 367:1665–1679. https://doi.org/10.1098/rstb.2012.0005

Kearney M, Shine R, Porter WP (2009) The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. Proc Natl Acad Sci 106:3835–3840. https://doi.org/10.1073/PNAS.0808913106

Kearney MR, Munns SL, Moore D, Malishev M, Bull CM (2018a) Field tests of a generalectotherm niche model show how water can limit lizard activity and distribution. Ecol Monogr 88:672–693. https://doi.org/10.1002/ECM.1326

Ladyman M, Bradshaw D (2003) The influence of dehydration on the thermal preferences of the Western tiger snake, Notechis scutatus. J Comp Physiol B 173:239–246. https://doi.org/10.1007/s00360-003-0328-x

Lenth R (2018) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.0. https://cran.r-project.org/package=emmeans

Lertzman-Lepofsky GF, Kissel AM, Sinervo B, Palen WJ (2020) Water loss and temperature interact to compound amphibian vulnerability to climate change. Glob Chang Biol 26:4868–4879. https://doi.org/10.1111/gcb.15231

Lesnoff M, Lancelot R (2012) aod: Analysis of Overdispersed Data. R package version 1.3.2. http://cran.rproject.org/package=aod

Lillywhite HB, Mittal AK, Garg TK, Agrawal N (1997) Integumentary structure and its relationship to wiping behaviour in the common

Indian tree frog, *Polypedates maculatus*. J Zool 243:675–687. https://doi.org/10.1111/J.1469-7998.1997.TB01969.X

LillywhiteSheehySandfoss HBCMMR III, Crowe-Riddell J, Grech A (2019) Drinking by sea snakes from oceanic freshwater lenses at first rainfall ending seasonal drought. PLoS One 14:e0212099. https://doi.org/10.1371/JOURNAL.PONE.0212099

Lorioux S, Vaugoyeau M, Denardo DF, Clobert J, Guillon M, Lourdais O (2013) Stage dependence of phenotypical and phenological maternal effects: insight into squamate reptile reproductive strategies. Am Nat 182:223–233. https://doi.org/10.1086/670809

Lorioux S, Angelier F, Lourdais O (2016) Are glucocorticoids good indicators of pregnancy constraints in a capital breeder? Gen Comp Endocrinol 232:125–133. https://doi.org/10.1016/j.ygcen.2016.04.007

Lourdais O, Bonnet X, Shine R, DeNardo D, Naulleau G, Guillon M (2002) Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. J Anim Ecol 71:470–479. https://doi.org/10.1046/j.1365-2656.2002.00612.x

Lourdais O, Brischoux F, DeNardo D, Shine R (2004) Protein catabolism in pregnant snakes (*Epicrates cenchria* maurus Boidae) compromises musculature and performance after reproduction. J Comp Physiol B 174:383–391. https://doi.org/10.1007/s00360-004-0424-6

Lourdais O, Guillon M, Denardo D, Blouin-Demers G (2013) Cold climate specialization: adaptive covariation between metabolic rate and thermoregulation in pregnant vipers. Physiol Behav 119:149–155. https://doi.org/10.1016/j.physbeh.2013.05.041

Lourdais O, Dupoué A, Guillon M, Guiller G, Michaud B, DeNardo DF (2017) Hydric "Costs" of reproduction: pregnancy increases evaporative water loss in the snake *Vipera aspis*. Physiol Biochem Zool 90:663–672. https://doi.org/10.1086/694848

Malishev M, Bull CM, Kearney MR (2018) An individual-based model of ectotherm movement integrating metabolic and microclimatic constraints. Methods Ecol Evol 9:472–489. https://doi.org/10.1111/2041-210X.12909

McCue MD, Sandoval J, Beltran J, Gerson AR (2017) Dehydration causes increased reliance on protein oxidation in mice: a test of the protein-for-water hypothesis in a mammal. Physiol Biochem Zool 90:359–369. https://doi.org/10.1086/690912

Mitchell A, Bergmann PJ (2016) Thermal and moisture habitat preferences do not maximize jumping performance in frogs. Funct Ecol 30:733–742. https://doi.org/10.1111/1365-2435.12535

Mitchell D, Snelling EP, Hetem RS, Maloney SK, Strauss WM, Fuller A (2018) Revisiting concepts of thermal physiology: predicting responses of mammals to climate change. J Anim Ecol 87:956–973. https://doi.org/10.1111/1365-2656.12818

Moore D, Stow A, Kearney MR (2018) Under the weather?—The direct effects of climate warming on a threatened desert lizard are mediated by their activity phase and burrow system. J Anim Ecol 87:660–671. https://doi.org/10.1111/1365-2656.12812

Muñoz MM (2021) The Bogert effect, a factor in evolution. Evolution (NY). https://doi.org/10.1111/EVO.14388

Perkins SE, Alexander LV, Nairn JR (2012) Increasing frequency, intensity and duration of observed global heatwaves and warm spells. Geophys Res Lett 39:20714. https://doi.org/10.1029/2012GL053361

Peterman WE, Semlitsch RD (2014) Spatial variation in water loss predicts terrestrial salamander distribution and population dynamics. Oecologia 176:357–369. https://doi.org/10.1007/S00442-014-3041-4

Peterson CC (2002) Temporal, population, and sexual variation in hematocrit of free-living desert tortoises: correlational tests of causal hypotheses. Can J Zool 80:461–470. https://doi.org/10.1139/z02-021

Pincebourde S, Murdock CC, Vickers M, Sears MW (2016) Fine-scale microclimatic variation can shape the responses of organisms to global change in both natural and urban environments. Integr Comp Biol 56:45–61. https://doi.org/10.1093/ICB/ICW016

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2020) An introduct nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–150, https://CRAN.R-project.org/package=nlme.ion to dplR. https://doi.org/10.1016/j.dendro.2008.01.002

Pintor AFV, Schwarzkopf L, Krockenberger AK (2016) Hydroregulation in a tropical dry-skinned ectotherm. Oecologia 182:925–931. https://doi.org/10.1007/s00442-016-3687-1

Pirtle EI, Tracy CR, Kearney MR (2019) Hydroregulation ; A neglected behavioral response of lizards to climate change ?, in: Behavior of Lizards. CRC Press, pp 343–374. https://doi.org/10.1201/9781498782739-12

Potter KA, Arthur Woods H, Pincebourde S (2013) Microclimatic challenges in global change biology. Glob Chang Biol 19:2932–2939. https://doi.org/10.1111/GCB.12257

R Core Team (2020) R: a language and environment for statistical computing. pdfs.semanticscholar.org.

Riddell EA, McPhail J, Damm JD, Sears MW (2018) Trade-offs between water loss and gas exchange influence habitat suitability of a woodland salamander. Funct Ecol 32:916–925. https://doi.org/10.1111/1365-2435.13030

Riddell EA, Iknayan KJ, Wolf BO, Sinervo B, Beissinger SR (2019a) Cooling requirements fueled the collapse of a desert bird community from climate change. Proc Natl Acad Sci 116:21609–21615. https://doi.org/10.1073/PNAS.1908791116

Riddell EA, Roback EY, Wells CE, Zamudio KR, Sears MW (2019b) Thermal cues drive plasticity of desiccation resistance in montane salamanders with implications for climate change. Nat Commun 10:4091. https://doi.org/10.1038/s41467-019-11990-4

Riddell EA, Iknayan KJ, Hargrove L, Tremor S, Patton JL, Ramirez R, Wolf BO, Beissinger SR (2021) Exposure to climate change drives stability or collapse of desert mammal and bird communities. Science (80–) 371:633–636. https://doi.org/10.1126/SCIENCE.ABD4605

Rozen-Rechels D, Dupoué A, Lourdais O, Chamaillé-Jammes S, Meylan S, Clobert J, Le Galliard J (2019) When water interacts with temperature: ecological and evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. Ecol Evol 9:10029–10043. https://doi.org/10.1002/ece3.5440

Rozen-Rechels D, Badiane A, Agostini S, Meylan S, Le Galliard J (2020) Water restriction induces behavioral fight but impairs thermoregulation in a dry-skinned ectotherm. Oikos 129:572–584. https://doi.org/10.1111/oik.06910

Sears MW, Angilletta MJ, Schuler MS, Borchert J, Dilliplane KF, Stegman M, Rusch TW, Mitchell WA (2016) Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. Proc Natl Acad Sci USA 113:10595–10600. https://doi.org/10.1073/pnas.1604824113

Sears MW, Riddell EA, Rusch TW, Angilletta MJ (2019) The world still is not flat: lessons learned from organismal interactions with environmental heterogeneity in terrestrial environments. Integr Comp Biol 59:1049–1058. https://doi.org/10.1093/ICB/ICZ130

Seebacher F, Franklin CE (2005) Physiological mechanisms of thermoregulation in reptiles: a review. J Comp Physiol B 175:533–541. https://doi.org/10.1007/s00360-005-0007-1

Serrano-Rojas SJ, Pašukonis A (2021) Tadpole-transporting frogs use stagnant water odor to find pools in the rainforest. J Exp Biol. https://doi.org/10.1242/JEB.243122

Spinoni J, Vogt JV, Naumann G, Barbosa P, Dosio A (2018) Will drought events become more frequent and severe in Europe? Int J Climatol 38:1718–1736. https://doi.org/10.1002/joc.5291

Stahlschmidt Z, Denardo DF (2010) Parental behavior in Pythons is responsive to both the hydric and thermal dynamics of the nest. J Exp Biol 213:1691–1695. https://doi.org/10.1242/jeb.041095

Stahlschmidt ZR, Brashears J, DeNardo DF (2011) The role of temperature and humidity in python nest site selection. Anim Behav 81:1077–1081. https://doi.org/10.1016/J.ANBEHAV.2011.02.024

Stahlschmidt ZR, Jodrey AD, Luoma RL (2015) Consequences of complex environments: temperature and energy intake interact to influence growth and metabolic rate. Comp Biochem Physiol 187:1–7. https://doi.org/10.1016/j.cbpa.2015.04.007

Székely D, Cogălniceanu D, Székely P, Denoël M (2018) Dryness affects burrowing depth in a semi-fossorial amphibian. J Arid Environ 155:79–81. https://doi.org/10.1016/J.JARIDENV.2018.02.003

Terrien J, Perret M, Aujard F (2011) Behavioral thermoregulation in mammals: a review. Front Biosci (landmark Ed.) 16:1428–1444. https://doi.org/10.2741/3797

Trenberth KE, Dai A, Van Der Schrier G, Jones PD, Barichivich J, Briffa KR, Sheffield J (2014) Global warming and changes in drought. Nat Clim Chang. https://doi.org/10.1038/nclimate2067

Ummenhofer CC, Meehl GA (2017) Extreme weather and climate events with ecological relevance: a review. Philos Trans R Soc B Biol Sci. https://doi.org/10.1098/rstb.2016.0135

Urban MC (2015) Accelerating extinction risk from climate change. Science (80–) 348:571–573. https://doi.org/10.1126/SCIENCE.AAA4984

van de Pol M, Wright J (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. Anim Behav 77:753–758. https://doi.org/10.1016/J.ANBEHAV.2008.11.006

Wilson BS, Wilson BS, Havel PJ (1989) Dehydration reduces the endurance running capacity of the lizard uta stansburiana. Copeia 1989:1052. https://doi.org/10.2307/1445998

Wolcott TG, Wolcott DL (2001) Role of behavior in meeting osmotic challenges. Integr Comp Biol 41:795–806. https://doi.org/10.1093/ICB/41.4.795

Wood M, Chamaillé-Jammes S, Hammerbacher A, Shrader AM (2021) African elephants can detect water from natural and artificial sources via olfactory cues. Anim Cogn 2021:1–9. https://doi.org/10.1007/S10071-021-01531-2

Woods HA, Pincebourde S, Dillon ME, Terblanche JS (2021) Extended phenotypes: buffers or amplifiers of climate change? Trends Ecol Evol 36:889–898. https://doi.org/10.1016/J.TREE.2021.05.010