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Water restriction induces behavioral fight but impairs thermoregulation in a dry-skinned ectotherm

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Abstract

1. Behavioral fight responses to desiccation risk are important to predict the vulnerability of terrestrial animals to climate change and yet, they have received little attention so far. In terrestrial ectotherms, behavioral regulation of the water balance (i.e., hydroregulation) is likely to be plastic and may trade-off with thermoregulation behavior because water loss rates are generally higher in warmer environments and body temperatures.

2. When low water availability and heat stress cause physiological dehydration, we expect to highlight a shift to behavioral water-conservation strategies including changes in activity patterns, microhabitat selection and thermoregulation strategies.

3. Here, we compared the behavior of adult common lizards (*Zootoca vivipara*) in indoor arenas that either had a permanent access to water or underwent a one-week long experimental water restriction.

4. Water-restricted lizards reduced their behavioral activity, selected more often cooler and wetter refuges during daytime, and performed less accurate thermoregulation than control lizards. The activity of water-restricted gravid females shifted towards the cooler and wetter early hours of the day. In addition, they had lower body temperatures and preferred lower body temperatures at the end of the experiment (i.e., thermal depression). Water-restricted lizards suffered from a mild physiological dehydration, water-restricted females had lower mass change compared to control ones, and water-restricted males lost weight. Heat stress was simulated every second day, which led to a range of heat avoidance and water conservation strategies independent from water restriction.

5. Altogether, these results confirm that chronic water restriction and dehydration induce responses towards water conservation that conflict with thermoregulation accuracy.

Introduction

Behavioral plasticity is critical for organisms to cope with both acute and chronic changes in environmental conditions, such as the short-term effects of extreme weather events and long-term consequences of ongoing climate change (Beever et al., 2017; Wong & Candolin, 2015). In the last two decades, a growing number of ecological studies investigated how changes in thermal quality of the environment (Row & Blouin-Demers, 2006; Sears et al., 2016), food availability (Long et al., 2014; Manenti et al. 2013), and predation pressures (McGhee et al. 2013) cause shifts in individual behavior that contribute to ecological responses to environmental changes. In comparison, fewer studies have investigated behavioral responses of terrestrial animals to changes in water availability, especially in ectotherms (Pintor et al. 2016; Pirtle et al. 2019). The behavioral responses allowing organisms to control water inputs and losses can be viewed as components of the hydroregulation behavior (Pintor et al., 2016; Pirtle et al., 2019). Analogous to behavioral thermoregulation (Angilletta, 2009), behavioral hydroregulation must be shaped by a balance between benefits (e.g., ability to reach an optimal hydration state) and costs (e.g., risks of predation or heat stress during water foraging). As most scenarios of global changes predict a higher frequency of drought events as well as average changes in precipitation regimes (Field et al. 2012), understanding drivers and patterns of behavioral hydroregulation and its plasticity has become essential to unravel the multifactorial consequences of global changes on terrestrial organisms (Albright et al., 2017; Kearney & Porter, 2009; Kearney et al. 2018; Pirtle et al., 2019).

Water is a critical resource and a limiting factor for terrestrial animals living in chronically warm and dry environmental conditions such as xeric or semi-xeric environments (Davis & DeNardo, 2009; Kearney et al., 2018; Long et al., 2014; Zylstra et al. 2013) as well as during droughts and warm spells in mesic or tropical habitats (Anderson & Andrade, 2017; Marquis et al. 2008). Thus, terrestrial animals have evolved plastic behavioral responses to cope with spatio-temporal fluctuations in water availability and maintain a homeostatic hydration state. One behavioral strategy against water stress involves long-range movements and dispersal responses (i.e., behavioral flight response) to avoid desiccating environments (Massot et al. 2002; Rozen-Rechels et al., 2018). An alternative behavioral strategy involves shifts in activity patterns (e.g., diel activity) and microhabitat selection without change in home range location (i.e., behavioral fight response; Lorenzon et al. 1999; Pintor et al. 2016). Such behavioral fight responses can improve water balance by limiting water loss and/or by increasing metabolic or dietary water intake. For example, some lizard species reduce their behavioral or locomotor activity during periods of water restriction to reduce evaporative water loss (Davis & DeNardo, 2010; Kearney et al., 2018; Lorenzon et al., 1999; Pirtle et al., 2019) while other species shift their activity towards time periods with moister conditions and a higher availability of free standing water (Davis & DeNardo, 2010; Kearney et al., 2018). Plasticity of microhabitat selection is also critical for some organisms to avoid water stress and it may include selection of shadier basking or retreat sites during periods of activity and differential use of moist and cold shelters during periods of inactivity (e.g., in snakes and lizards, Guillon et al. 2013; Dupoué et al. 2015a; Pintor et al. 2016). So far, we still lack a comprehensive quantification of the drivers and patterns that characterize behavioral hydroregulation in ectotherms, especially dry-skinned vertebrate species, which were thought to be little affected by water conditions until recently (Pintor et al., 2016; Rozen-Rechels et al., 2018). Nonetheless, it is commonly accepted that individual performance of ectotherms are strongly influenced by hydration state (e.g., Anderson and Andrade 2017), and recent mechanistic models highlight the critical role of behavioral hydroregulation as a determinant of water balance across the distribution range of dry-skinned lizard species (Pirtle et al., 2019).

The drivers and mechanisms of behavioral thermoregulation and hydroregulation are usually not independent in wild animal populations because environmental changes in water availability usually correlate with changes in thermal conditions on the one hand (e.g., drought and heat waves, Kelley et al. 2015), and because of the coupling between the water and the heat budget of animals on the other (e.g., Kearney and Porter 2009). This makes it hard to distinguish between both behaviors (e.g., Davis and DeNardo 2009). In general, thermo-hydroregulation behavioral strategies must include the interactive set of behaviors that control both water balance and body temperature. In the framework of thermo-hydroregulation, we predict a behavioral trade-off between water balance and heat budget regulation because some thermoregulation behaviors, such as exposure to sunlight to increase body temperature, also frequently enhance water loss rates in desiccating conditions (e.g., Dupoué et al. 2015a; Lourdais et al. 2017; Pirtle et al. 2019). Thus, water availability not only influences hydroregulation but also thermoregulation, and water restriction can for example reduce basking effort. Mechanisms of thermo-hydroregulation may also be more complex than the sum of thermoregulation and hydroregulation behaviors, especially when desiccation risks occur jointly with heat stress. Empirical studies aiming to disentangle the effects of water availability and temperature, and their consequences for water balance and heat budget, are therefore crucial to fully comprehend thermo-hydroregulation strategies and their mechanisms.

Thermoregulation strategies range from thermoconforming, when organisms conform to their environmental temperature, to perfect thermoregulation when organism maintain a high thermoregulation accuracy (constant body temperature at the preferred level) irrespective of the thermal quality of the habitat (Angilletta, 2009). In a cost-free environment, thermal adaptation models predict that ectotherms should be able to maintain a body temperature very close to their preferred body temperature and that the preferred body temperature should optimize performances and fitness (Angilletta, 2009; Herczeg et al. 2006; Huey & Slatkin, 1976). In an environment with desiccation risks due to suboptimal water inputs relative to standard water loss rates, ectotherms should lower their thermal preferences in order to limit water losses and optimize performances that are constrained by both hydration state and body temperature (Anderson & Andrade, 2017; Ladyman & Bradshaw, 2003). This plastic response is called thermal depression and has been observed in snakes exposed to seasonal or experimental dehydration (Ladyman & Bradshaw, 2003). However, the extent to which dehydration risks influence the accuracy of thermoregulation, defined as the difference between preferred body temperature measured during unconstrained thermoregulation (e.g., a neutral arena) and realized body temperature in a constrained environment

(Angilletta, 2009; Blouin-Demers & Nadeau, 2005; Hertz et al. 1993), is yet to be understood. Dehydration risks may increase the cost of thermoregulation, and thermoregulation accuracy should therefore decrease when low water availability or high water loss rates compromise water balance (Blouin-Demers & Nadeau, 2005; Huey & Slatkin, 1976; Sears & Angilletta, 2015). To our knowledge, no study to date has tested how thermoregulation accuracy responds to dehydration risks (Angilletta, 2009).

In this study, we used a laboratory experiment to quantify the behavioral responses to a chronic water restriction and acute heat stress in the European common lizard *Zootoca vivipara* (Lichtenstein, 1823). This cold-adapted species inhabits mesic environment and is tightly dependent on humid conditions in the wild (Dupoué et al., 2017; Lorenzon et al., 1999; Massot et al., 2002). In previous studies, we have shown that common lizards exposed to dry soil conditions invest more in exploration behaviors (Rozen-Rechels et al., 2018), and juvenile lizards living in dry habitats disperse more from their natal home range (Massot et al., 2002), whereas sub-adults reduce their locomotor activity during a chronic water restriction (Lorenzon et al., 1999). Here, we repeatedly measured daily patterns of individual activity and microhabitat selection as well as body temperature and thermoregulation accuracy during one week in adult individuals, gravid females and males, exposed to a chronic water restriction (Dupoué et al., 2018) compared to individuals having access to water *ad libitum*. To quantify potential interactions between water deprivation and heat stress, we implemented a mid-day heat stress every two days. We also assessed thermal preferences, body mass and hydration state (plasma osmolality) before and after the experiment. Based on an earlier study of activity patterns in sub-adults (Lorenzon et al., 1999), we predicted lower activity rates and/or shifts of activity towards cooler hours in water restricted animals, as well as biases in microhabitat selection towards cooler and wetter shelters. We also expected a thermal depression and a lower accuracy of thermoregulation if water-restriction enhances thermoregulation costs. We predicted that behavioral effects of water deprivation would be amplified during simulated heat stress.

Material and methods

Study species and sampling

Common lizards of this study were captured in semi-natural captive populations in outdoor enclosures located at CEREEP-Ecotron IleDeFrance research center in France (48° 17’ N, 2° 41’ E). These enclosures include a permanent and natural grassland vegetation and refuges/basking sites made of rocks and logs. Individuals have a permanent access to free water through artificial ponds which get filled with rain. Enclosures are delimited by plastic walls preventing escapes and ground predation, and covered with nets to prevent avian predation. In 2018, one population was composed of 71 ± 9 (standard deviation SD) individuals, mainly yearlings (50 ± 7 individuals). Food was composed of local wild arthropods or annelids (spiders, worms, crickets) and in quantities that we suppose to be *ad* libitum as we did not observe differential survival rate of individuals compared to year of lower population densities. Mating season usually starts with emergence of females around March-April and lasts around 2 to 4 weeks. Mating occurs between late March and early April, followed by ovulation and fertilization, and a gestation period that lasts two months on average. From May 3rd, 2018 to May 7th, 2018 we captured 72 adult individuals in 10 different 100-m^2 outdoor enclosures. At capture, lizards were measured for snout-vent length (SVL, with a rule, ± 0.5 mm) and body mass (BM, OHAUS, Adventurer, 220 g, ± 1 mg), then placed in individual terraria (18 × 11 × 12 cm) with a shelter and sterilized peat soil as substratum. This sample was half composed of pregnant females (mean ± SD; SVL: 64 ± 3 mm; BM: 5.25 ± 0.79 g) and of post-reproductive males (SVL: 58 ± 2 mm; BM: 4.42 ± 0.32 g). Terraria were located in a temperature-controlled room (23°C from 09:00 to 18:00, 16°C at night). Individuals were fed three times a week with 300 ± 10 mg of live house crickets (*Acheta domestica*). In normal housing conditions, water was available *ad-libitum* in a petri dish and sprayed three times a day (09:00, 13:00 and 17:00). After a minimum acclimation period of one week, individuals were sorted in three trial groups (24 individuals per group) tested between May 10th to May 20st (trial 1), May 21st to May 31st (trial 2) or June 1st to June 11th (trial 3). Males were randomly attributed to each trial group. Females were sorted according to their SVL such that longer and older females, which usually give birth earlier (Rutschmann et al., 2016), were tested first to avoid a strong variation in gestation stage among trials (trial 1: 68 ± 2 mm; trial 2: 64 ± 1 mm; trial 3: 61 ± 2 mm). Inside each trial group, individuals were randomly attributed to a control treatment or to the water-restricted treatment (Control females: SVL: 64 ± 3 mm; BM: 5.10 ± 0.84 g; Water-restricted females: SVL: 65 ± 3 mm, $F_{1,34} = 0.66$, $p = 0.42$; BM: 5.51 ± 0.73 g, $F_{1,34} = 0.32$, $p = 0.57$; Control males: SVL: 58 ± 3 mm; BM: 4.43 ± 0.33 g; Water-restricted males: SVL: 57 ± 2 mm, $F_{1,34} = 2.11$, $p = 0.16$; BM: 4.41 ± 0.31 g, $F_{1,34} = 0.08$, $p = 0.78$). To conform with Animal Welfare recommendations, we ensured all individuals were healthy by checking body condition, behavior and parasite load before the beginning and at the end of the experiment.

Experimental design

Experiments were performed in open-top arenas (N=12, 78 × 56 × 44 cm) installed in a temperature-controlled room maintained at 20°C during day and night. In a 13rd terrarium we measured all micro-habitats thermal characteristics using operative temperature copper models mimicking the lizards thermal properties in which were fixed HOBO® Pro v2 U23-003 (ONSET Cape Cod, Massachusetts) temperature loggers (see Supplementary Information). Arenas were filled with a substratum of dry and sterilized peat soil and equipped with one plastic shelter (Exo-Terra® cave, 7.6 × 15.2 × 5.1 cm) at each corner. At one side of the arena, hereafter called the warm side, two 40W light bulbs were placed above each shelter in order to warm the surface temperature at approximately 35.9 ± 4.2 °C during daytime (between 8 am and 5 pm, similar to a standard early summer activity day, see Supplementary Information and Figure S1), which corresponds to slightly more than the preferred body temperature in common lizards (Gvoždík & Castilla, 2001). The other side of the terrarium, hereafter called the cold side, was maintained at approximately 24.5 ± 2.0 °C during daytime (between 8am and 5pm, see Figure S1), which is well below the preferred body temperature range. At both sides, soil and air inside one shelter was made wet by adding a thin sponge regularly moistened on top of the substrate, whereas the other shelter was kept dry by adding the same kind of sponge without wetting. Due to evaporative cooling effect, temperature in wet shelters was colder than in dry shelters (warm side: 30.0 ± 3.1 °C in wet vs. 32.0 ± 3.7 °C in dry, $F_{1,8841} = 580.8$, $p < 0.0001$; cold side: 22.8 ± 1.8 °C in wet vs. 24.6 ± 2.0 °C in dry, $F_{1,6944} = 2447.7$, $p < 0.0001$; see Figure S1). Yet, all temperature records on the cold side remained within the thermal preference set of the species, while they remained in the set on the warm side. Wet shelters had also higher water density (water vapor pressure calculated from relative humidity measurements with iButton DS1923, Maxim Integrated, San Jose, California following Tieleman et al. 2002 procedure) than dry ones (warm side: 23.6 ± 2.1 $g.m^{-3}$ in wet vs. 12.2 ± 2.9 $g.m^{-3}$ in dry, $F_{1,1598} = 8108$, $p < 0.0001$; cold side: 17.7 ± 1.2 $g.m^{-3}$ in wet vs. 11.6 ± 1.7 $g.m^{-3}$ in dry, $F_{1,1594} = 6540$, $p < 0.0001$; see Supplementary Information). We randomly assigned a wet or dry treatment to the shelters on each side in each terrarium. In the center of the arena, we placed two large petri dishes (~8 cm of diameter, 0.5cm deep) to store free-standing water. We placed above the middle of the warm side an infrared halogen 50W bulb to optionally change thermal conditions to 40-42°C substrate and air temperature on the warm side, which is close to the critical thermal maximum for this species (Gvoždík & Castilla, 2001). At night, temperatures were of 20.0 ± 1.1 °C. Two UVB 30W neon tubes (Reptisun 10.0, white light) provided white light above each arena during daytime. We installed one male and one size-matched female in each arena during each trial. At this time of year, males do not attempt to copulate with gravid, non-receptive adult females, and aggressive interactions among lizards are rare (Fitze et al., 2005). Indeed, we did not observe any aggressive behavior during this study. At day 0, animals were put in the arenas until day 8. Lights bulbs were turned on automatically during daytime every day and infrared halogen bulbs were automatically turned on from 11:00 to 14:00 at day 2, day 4, day 6 and day 8 to mimic a mid-day heat stress in the warm side. During day 0, arenas were sprayed with water three times (07:30, 14:00, 17:00) to provide mist and petri dishes were constantly filled with water (control treatment). Spraying lasted approximately 5 seconds and was always made by the same observer in the same manner, and aimed at having free water available as droplets that dried quickly. From day 1 to day 8, half of the individuals were exposed to a sub-lethal water restriction during which mist was only provided in early morning (08:00) and no free-standing water was available (droplets in empty petri dishes were dried). Individuals were fed with 200 ± 10 mg of live house crickets (*Acheta domestica*) every day at 10:00. The chronology of the experiment for one experimental group is summarized in Figure S3.

Pre-manipulation and post-manipulation measurements

In the morning of the day before day 0 and after day 8, we measured thermal preferences of all individuals in a neutral thermal gradient (80 × 15 × 20 cm) filled with a substratum of dried peat soil. We placed a plate of wood on the ground and installed a 40W heat bulb 15 cm above the ground at the warm side of the gradient (50 ± 5.9 °C surface temperature). The cold side of the gradient was maintained at a low temperature (24.3 ± 2.0 °C). A UVB 30W neon tube provided natural, white light conditions above each thermal gradient. Heat bulbs were turned on at 08:00 for one hour before individuals were placed in each thermal gradient for habituation during 30 minutes. At 09:30 and every 20 minutes until 13:10, surface temperature T_p of all lizards was measured using an infrared thermometer (Raytek, Raynger MX2). This measure, strongly correlated with internal body temperature data, provides an estimate of preferred body temperature (Artacho et al., 2013). We then calculated the difference between each record at the end of the experiment and the record made on the same individual at the same time of the day before the experiment, hereafter called ΔT_p.

After thermal preferences measurements, individuals were weighted to calculate body mass change between the beginning and the end of the experiment (ΔBM) and relocated in their home terrarium for an hour or two until approximately 15:00. We then sampled 10 µL of blood from the post-orbital sinus using a standard protocol (Meylan et al., 2003) to assess changes in plasma osmolality. Blood samples were centrifuged at 11,000 *g* to separate plasma, which was kept frozen at approximately -25°C. Plasma osmolality was determined from two aliquots of plasma diluted in physiological serum using a vapor pressure osmometer (Wescor Vapro® 5520) following (Dupoué et al. 2018). The osmolality was calculated as the average osmolality of the two aliquots corrected by the dilution minus the osmolality of a reference, physiological serum (307 ± 3 $mOsm.kg^{-1}$). We assessed ΔOSMO by subtracting final osmolality to initial osmolality.

Behavioral data

From day 0 to day 8, we observed the behavior of lizards with a focal sampling every 30 minutes from 08:00 to 17:00. At each sampling time, we observed whether the individual was "active" (i.e. outside the shelter) or not (i.e. in the shelter or buried in the soil). When active, we recorded when the individual was basking (not moving under the heat spot), moving, eating, drinking, or immobile but not basking. When the individual was active, we measured the surface body temperature of its dorsum using an infrared thermometer as detailed above. When the individual was not visible, we searched under its shelter by having a quick look, and, when the lizard was still not found, we assumed that it was buried into the soil and did not disturb it. We made a total of 12,312 focal observations of the 72 lizards and 3,334 temperature recordings during the 3 trials and 9 days of each trial.

Statistical analyses

Statistical analyses were performed using R version 3.4.4 "Someone to Lean On" (R Core Team, 2018). In all cases, the best models were inferred with backward model selection based on log-likelihood ratio tests. We evaluated significance by comparing the best-fit model with a reduced model, from which was subtracted the effect of interest using log-lokelihood ratios. We also provided the estimated effect ± standard error.

Differences in ΔBM and ΔOSMO were assessed with ANCOVAs using linear models with the two-way interaction between treatment and sex as well as the additive effects of the trial group as fixed effects. In the case of ΔBM, we also added the initial mass as a fixed effect as mass change should be relative to the size of the animal.

We tested the variations of the behaviors and thermoregulation statistics of interest using ANCOVAs fitted with linear mixed models with the lme function from the *nlme* package (Pinheiro & Bates, 2006) and generalized linear mixed models with the glmer function from the *lme4* package

(Bates et al., 2015). In order to analyze the effect of water restriction on thermal preference, we first compared ΔT_p at each time of day between control and water-restricted individuals using a linear mixed-effect model with the three-way interaction between time of day, treatment and sex as fixed effects. To calculate contrasts, we set the intercept of the model to the last observation of the day (i.e. time of day = 13:10). We assessed inter-group variation by considering the experimental group as a fixed effect and we took inter-individual variability into account by defining an individual level random effect.

Based on our thermal preference data and body temperature, we inferred thermoregulation inaccuracy at each record by calculating the absolute difference between body temperature record and thermal preference (Hertz et al., 1993; Sears et al., 2016). To infer thermal preference corresponding to the same day as the one of each record, we assumed a linear change (increase or decrease depending on each individual) of preferred body temperature (measured in the thermal gradient) from before to after the experiment. Then, to analyze the effect of water restriction on body temperature and thermoregulation inaccuracy, we ran linear mixed-model regressions including as fixed effects the three-way interaction between sex, treatment and the number of days since the beginning of the experiment (hereafter referred to as D_{exp}) as well as the trial group as an additive effect. To account for potential differences among arenas (Figure S2) and individuals, we used lizard identity nested in the corresponding arena as random effects in the following models ran on behavioral data.

We focused our analyses of behavioral data on emergence time (i.e. time of first activity each day), behavioral budget and shelter choice. First, we tested the effect of water restriction on emergence time using the Cox survival model from the package *coxme* (Therneau, 2018). The survival model analyses the expected time spent before an event happened. Each lizard has a constant per-unit-time probability to emerge (death event) until the end of the day during which we consider that it never went out (“survived”; 7.8% of our observations). The model estimates the mean time of emergence.

We implemented in this model a three-way interaction of D_{exp}, treatment and sex as fixed effect as well as the trial group as an additive effect.

We then tested the influence of water restriction on four behavioral items: i.e., proportion of time spent active, proportion of time spent basking, proportion of time spent in hot vs. cold shelters, and proportion of time spent in wet hot shelters vs. dry hot shelters. We did not analyze the proportion of time spent in wet cold shelters vs. dry cold shelters as individuals spent between 80% to 95% of their time in the warm side. For each behavioral item, we counted the number of occurrences per day and analyzed proportions using generalized linear mixed models with binomial errors, comparing (i) the number of times an individual was active to the number of observations in the day (19), (ii) the number of times it was basking to the number of time it was active, (iii) the number of time it selected a hot shelter to the number of time it selected a cold shelter, and (iv) the number of time it selected a wet hot shelter to the number of time it selected a dry hot shelter. We included the three-way interaction of D_{exp}, treatment and sex as fixed effects as well as the trial group as an additive effect. We restricted this analysis to days without heat stress (D0, D1, D3, D5, D7). Analyses of shelter selection showed over-dispersion of data that we corrected by using an observation-level random effect (Harrison, 2014).

In order to analyze the effect of heat stress and water restriction, we calculated the behavioral budget of lizards from 11:30 to 14:00, which corresponds to the time of day when heat stress was simulated. We analyzed the interaction between heat stress and water restriction considering all days from D0 to D8. We tested the three-way interaction of heat stress and water restriction with sex and D_{exp}, as well as the trial group as an additive effect. Since the distribution of the shelter selection traits (warm vs. cold shelter selection and wet vs. dry warm shelter selection) was close to bimodal, we did not analyze the proportion of shelter use but instead compared a bimodal variable in each case (i.e., lizard in the warm shelter all the time vs. other cases, lizard in the wet warm shelter all the time vs. other cases).

Results

Change in body mass and plasma osmolality

Body mass change was smaller in water restricted lizards than in controls ($F_{1,66} = 9.50$, $p = 0.003$) independently from sex (water treatment × sex: $F_{1,65} = 2.74$, $p = 0.1$). Males had a lower ΔBM than females (-0.37 ± 0.08 g; $F_{1,66} = 19.7$, $p < 0.0001$). Thus, we recorded a gain of mass in control females (0.4 ± 0.1 g), no change in BM in water-restricted females (0.1 ± 0.1 g) and control males (0 ± 0.1 g), and a mass loss in water-restricted males (-0.3 ± 0.1 g; Table 1). In addition, water-restricted individuals had a higher osmolality change (i.e., higher plasma dehydration) than control individuals (water treatment: $F_{1,67} = 4.59$, $p = 0.04$) independently from sex (water treatment × sex: $F_{1,65} = 0.01$, $p = 0.91$; sex: $F_{1,65} = 0.05$, $p = 0.94$, see Table 1).

Change in thermal preferences and thermoregulation accuracy

The preferred body temperatures were most of the time lower after the experiment than before with an average $\Delta T_p = -1.70 \pm 0.20$ °C, and only nine individuals out of 72 had positive ΔT_p records (Table 1, Figure 1A). ΔT_p was significantly higher in males than in females (sex: $F_{1,69} = 12.75$, $p = 0.0007$). Control individuals had a constant ΔT_p across times of day, while water-restricted individuals ΔT_p dropped approximately by 0.12 ± 0.05 °C every 20 minutes with time of day (time of the day : $F_{1,779} = 2.83$, $p = 0.09$; time of the day × water treatment: $F_{1,779} = 6.63$, $p = 0.01$). At midday (13:10, end of our test), water-restricted lizards had a ΔT_p lower of 1.30 ± 0.45 °C than controls irrespective of sex (Figure 1A; water treatment: $F_{1,69} = 8.36$, $p = 0.005$; water treatment × sex: $F_{1,69} = 0.64$, $p = 0.43$).

Surface body temperature records during the experiment were influenced significantly by a three-way interaction of D_{exp}, water treatment and sex (D_{exp} × water treatment × sex: $F_{1,3258} = 4.61$, $p = 0.03$; Figure 1B). The body temperature of control females decreased by 0.19 ± 0.05 °C.day^{-1} during the experiment, but it decreased twice as fast in water-restricted females (contrast = 0.24 ± 0.07 °C.day^{-1}; $t_{3258} = -3.37$, $p = 0.0008$). No such effect was found in males (Figure 1B) but the body temperature was higher of 2.13 ± 0.41 °C in males than in females ($t_{66} = 5.15$, $p < 0.0001$).

Thermoregulation inaccuracy was influenced significantly by the water treatment in interaction with D_{exp} (D_{exp} × water treatment: $F_{1,3260} = 5.17$, $p = 0.02$; Figure 1C). Thermoregulation inaccuracy of control individuals did not change through time ($t_{3260} = 0.55$, $p = 0.58$). On the contrary, thermoregulation became less accurate with D_{exp} in water-restricted individuals (contrast: +0.08 ± 0.03°C.day^{-1}, $t_{3260} = 2.27$, $p = 0.02$), irrespective of sex (D_{exp} × water treatment × sex: $F_{1,3258} < 0.01$, $p = 1$). On average, males were slightly less accurate in their thermoregulation than females (0.3 ± 0.1°C, sex: $F_{1,69} = 4.25$, $p = 0.04$; sex × water treatment: $F_{1,68} = 0.34$, $p = 0.56$).

Behavioral flexibility

On average, an individual was seen active 5.2 ± 0.1 times a day and emerged for the first time of the day between 9:30 and 10:00, with ca. 50% of individuals active by 08:30 (see Figure 2). Lizards were seen in a shelter 66 ± 1% of the day and selected a warm refuge 88 ± 1 % of the time, which was also wet 69 ± 1% of the time. When active, a lizard was basking on average 79 ± 1% of the time. Emergence time was influenced significantly by the three-way interaction between water treatment, D_{exp} and sex ($\chi^2 = 18.23$, df = 1, $p < 0.0001$; Figure 2). Control females emerged later during daytime ($z = -3.92$, $p < 0.0001$). On the contrary, water-restricted females emerged significantly earlier than control ones with D_{exp} (contrast: 0.26 ± 0.05 day^{-1}; $z = 3.59$, $p = 0.0003$) as well as control males ($z = 3.23$, $p = 0.001$) and water-restricted males ($z = 2.50$, $p = 0.01$).

Activity rate was also influenced by a three-way interaction between sex, water treatment and D_{exp} ($\chi^2 = 3.96$, df = 1, p = 0.046, Figure 3A). Females reduced their activity across time of the experiment ($z = -2.40$, $p = 0.02$) irrespective of water treatment ($z = 0.19$, $p = 0.85$). On the contrary, control males did not change their activity rate while water-restricted males reduced it (Figure 3A). Lizards also reduced their basking effort during the experiment ($\chi^2 = 12.8$, df = 1, $p = 0.003$; Figure 3B) and males basked more than females ($\chi^2 = 4.45$, df = 1, $p = 0.03$). Water treatments did not influence basking effort (water treatment: $\chi^2 = 3.25$, df = 1, p = 0.07; water treatment × D_{exp}: $\chi^2 = 0.01$, df = 1, $p = 0.92$; water treatment × sex: $\chi^2 = 0.12$, df = 1, $p = 0.72$). These results on emergence time and activity patterns thus imply that water-restricted females shifted their activity towards the earliest hours of the day (08:00: ~20% active at D0 vs. ~50% active at D7; 10:00: ~50% active at D0 vs. ~25% active at D7, see Figure 3).

Warm vs. cold shelter selection was significantly influenced by the two-way interactions of water treatment with D_{exp} ($\chi^2 = 6.47$, df = 1, $p = 0.01$) and with sex ($\chi^2 = 4.26$, df = 1, $p = 0.04$). Control individuals tend to shift towards warmer shelters across the experiment ($z = 1.94$, $p = 0.053$) while water-restricted individuals shifted progressively to cooler shelters (contrast: -0.23 ± 0.09 day^{-1}; $z = -2.55$, $p = 0.01$). In addition, at day 0, there was no difference in shelter selection between water-restricted females and control individuals ($z = 0.38$, $p = 0.23$), however, control males selected colder shelters than water-restricted males (Figure 3C). Regarding wet shelter selection in the warm side of the terrarium, we found that water-restricted individuals selected the wetter and cooler shelter on average (contrast: 1.43 ± 0.39; water treatment: $\chi^2 = 12.4$, df = 1, $p = 0.0004$; water treatment × D_{exp}: $\chi^2 = 2.41$, df = 1, $p = 0.12$; water treatment × sex: $\chi^2 = 1.41$, df = 2, $p = 0.49$; Figure 3D). Irrespective of water treatment, individuals also shifted on average their shelter selection towards the wetter shelter as the time of the experiment proceeded ($\chi^2 = 7.22$, df = 1, $p = 0.007$, Figure 3D).

Effects of simulated heat stress on behavior

In this section, we focus on patterns and effects of water-restriction that are different from those described during days without heat stress in the previous section, but Table S1 summarizes all best models for heat stress simulation. Between 11:00 and 14:00, heat stress significantly reduced behavioral activity and basking effort (Figure 4A and 4B), but this reduction was independent from the water restriction treatment (activity: $\chi^2 = 2.72$, df = 1, $p = 0.10$; basking: $\chi^2 = 0.63$, df = 1, $p = 0.73$). Heat stress simulation also reduced the selection of warm shelters in females ($z = -3.30$, $p = 0.001$) but not in males (post-hoc test: $z = -1.5$, $p = 0.13$), independently from the water restriction treatment (Figure 4C). During simulated heat stress, individuals more often selected the wet and cool shelter (contrast: 0.56 ± 0.19; $z = 8.86$, $p = 0.003$; Figure 4D), but this was again independent from the water restriction treatment ($\chi^2 = 1.15$, df = 1, $p = 0.28$). Last, there was a tendency for water-restricted individuals to select the wet and cool shelters more often than control individuals with D_{exp} (Figure 4D), but this trend was not significant ($\chi^2 = 3.22$, df = 1, $p = 0.07$).

Discussion

Our experiment quantified changes in behavior and thermoregulation accuracy in response to a chronic water restriction in a terrestrial ectotherm. As expected, water-restricted lizards displayed a significantly higher osmolality than control individuals indicating that water restriction induced physiological dehydration. This is corroborated by water-restricted females constant weight contrary to control ones, and water-restricted males losing body mass relative to controls, consistently with similar measurements in previous ecophysiological studies of water restriction in common lizards (Dupoué et al., 2018). At the same time, water-restricted gravid females shifted slightly their thermal preferences (measured in a neutral arena) towards lower temperatures than control females (by ca. 1°C) and also had lower body temperatures in water-restricted conditions (by ca. 2°C). Interestingly, in both males and females, water restriction decreased thermoregulation accuracy with time more than controls. Our results also revealed that water-restricted females shifted their activity to the early morning hours, whereas water-restricted males reduced their average activity. Water-restricted individuals also shifted microhabitat selection to cooler and wetter shelters. In addition, simulated heat stress strongly reduced activity and basking rate, and enhanced microhabitat preference for cooler and wetter shelters but, unexpectedly, this effect did not depend on water restriction treatment.

Overall, our observations are consistent with the prediction that common lizards unable to disperse adopt behavioral fight strategies to conserve more water and mitigate the consequences of the simulated mild but chronic water restriction on water balance (Lorenzon et al., 1999; Pirtle et al., 2019). The behavioral fight syndrome included a diminution of locomotor and behavioral activity, a change in daily activity patterns and some shifts of shelter selection strategies. The dominant behavioral response to water restriction in females was a shift of their behavioral activity towards the first hours of the day (Figure 2), a time period of the day that is cooler and wetter on average and when free standing water is available in the form of dew in both treatments (Figure S1). The earlier activity of water-restricted females could thus be a strategy to forage for water and perform standard activities in conditions in which desiccation risk is minimized. This result is consistent with with those of empirical studies and quantitative models suggesting that changes in water availability can be a dominant driver of seasonal or spatial changes in daily activity patterns in squamate reptiles, and more generally in terrestrial ectotherms (Davis & DeNardo, 2010; Kearney et al., 2018; Wells, 2010).

In our experimental set-up, shelters in the warm side of the terrarium were at an operative temperature of approximately 30-32°C, which is close to females' but slightly below males' preferred body temperature (Table 1). These shelters thus allow individuals to regulate body temperature without effort and under low overheating risks, which is critical to perform optimally some slow and temperature-dependent activities such as digestion (Stevenson, 1985). Cold side shelters were, however, suboptimal for thermoregulation with temperatures ranging around 23-25°C. This explains the high rate of warm shelter use (>75%) in our study. Wet shelters were also three times less desiccating (vapor pressure deficit: 0.98 mbar in warm wet shelters, 0.36 mbar in cold ones) than dry ones (3.04 mbar in warm dry shelters, 1.5 mbar in cold ones) and differed on average only by 2°C temperature difference due to evaporative cooling. We therefore suggest that the differential use of wet over dry shelters is much more likely driven by hydroregulation than thermoregulation behavior. Thus, water restricted individuals shifted their hydroregulation behavior by increasing the use of wetter shelters, which is consistent with a strategy aiming at reducing dehydration rate. At the same time, they used more often cool shelters, which are suboptimal for thermoregulation but, on average, half less desiccating than warm ones. This shift suggests that conditions of water restriction can trigger hydroregulation strategies critical for the maintenance of water balance at the expanse of thermoregulation strategies, as suggested by Pintor et al. (2016). In natural conditions, we suspect that ecological responses to drought and water restriction will critically depend on the presence and distribution of cold and wet shelters or vegetation in the lizards' habitat (Pirtle et al., 2019). The distribution of such patches in the landscape in complementation to other resources will be a predictor of the ability of organisms to cope with changing moisture conditions through habitat selection (Sears & Angilletta, 2015; Sears et al., 2016).

Interestingly behavioral adjustments were not efficient enough to counter physiological dehydration since lizards in this study suffered from a slight increase in plasma osmolality and decrease in body mass change as in earlier laboratory experiments (Dupoué et al., 2018). The range of measured changes in plasma osmolality in this study indicates a mild physiological dehydration (Dupoué et al., 2018), and plasma osmolality levels of water-restricted individuals were also high compared to baseline levels recorded in a range of natural populations with contrasted access to water (Dupoué et al., 2017). In addition, we found gradual changes in behavioral responses of lizards in this experiment, which suggests that they are mostly driven by changes in water balance instead of water availability *per se*. Indeed, if lizards responded to water availability *per se*, behavioral responses would be immediate while hydration state would likely change gradually during the course of the experiment (Davis & DeNardo, 2009; Dupoué et al., 2015a). We conclude that water balance status is a cue to individual behavior changes, thus implying that behavioral fight traits are condition-dependent behaviors (Buchanan, 2000).

Unexpectedly, we did not observe any interactive effect of water restriction and heat stress on behavior. This could be explained by a lack of statistical power, as heat stress was too strong in our set-up, or because hydration status does not influence sensitivity to heat stress in common lizards (see Figure S1C). Behavioral responses to a simulated midday heat stress were consistent with overheating avoidance, probably to avoid the risk of reaching maximum critical thermal limits and to reduce evaporative water loss through skin and respiration. During the simulated heat stress, lizards hide more often and seek more often cooler microhabitats which offered more optimal thermal conditions (close to thermal preferences) than the warm side (see Figure S1C), a result predicted by theoretical models of thermoregulation (Angilletta, 2009; Porter et al., 1973). They also selected wet shelters more often, independently from their hydration status, thus suggesting that heat stress induced water-conservation behaviors. However, because wet shelters were also significantly cooler during the heat stress, it is difficult to tell if this behavioral shift was a sign of behavioral hydroregulation or thermoregulation. We were surprised by the absence of interaction between the water balance status and the sensitivity to heat stress. Our study however suggests that heat stress not only influences thermoregulation behaviors, but also water-related habitat selection patterns.

Some behavioral effects of water restriction were different between sexes which might also simply be the consequence of being gravid (all females expect one finally laid). Only females showed strong differences in activity patterns in response to water restriction but both sexes adjusted their microhabitats selection behaviors the same way. In both treatments, gravid females had lower thermal preferences and lower active body temperatures. This average shift is consistent with previous observations of thermal depression during gestation in the common lizard, suggesting that thermal conditions for embryonic development are optimized around 29-30°C at the end of gestation (Gvoždík & Castilla, 2001; Le Galliard et al., 2003). We found minor changes in thermal preferences in males only but the effects of water restriction on thermal preferences were similar in both sexes. In sharp contrast, the active body temperature of males was not influenced by water restriction and remained relatively high during the experiment. Altogether, these results support the hypothesis that thermoregulation behaviors are more sensitive to water restriction in females than in males, most likely because higher water needs during pregnancy select for stronger water-conservation strategies in pregnant females than in non-reproductive individuals (Dupoué et al., 2015b; Dupoué et al., 2018). In viviparous ectotherms, pregnancy leads to an increased demand for water (Dupoué et al., 2016, 2018; Dupoué et al., 2015b), and pregnant females could alter their own water balance to protect their embryos from hydric stress (Dupoué et al., 2016, 2018). In addition, the trade-off between thermoregulation and hydroregulation is likely higher in pregnant females because pregnancy induces morphological and physiological changes that increase standard water loss rates (Lorioux et al., 2013). As females and males faced similar physiological dehydration, we speculate that plasticity of thermoregulation behaviors is critical for females to override the water conflict with their embryos and maintain a constant survival and reproductive effort despite their stronger reliance on water (as seen in Dupoué et al., 2018). We can not however conclude if these differences are sex-specific, the consequence of being gravid or both of them.

We can not also exclude that competition between individuals in the same terrarium would have affected our results. We did not observe any aggressive behavior among individuals, but we did not continuously assess the behavior of individuals nor we can conclude at the absence of avoidance behaviors. Competition for thermal resources is a well-known process (Downes and Shine 1998; Angilletta 2009) and we might thus suppose that such competition might also occur for microhabitats according to their moisture. The absence of sex-difference in thermoregulation accuracy, which is expected to change with competition pressure, makes us think that competition between females and males could be neglected. We also measured that water-restricted individuals seemed to spend more time together than separated in shelters, especially in the wet and hot shelters, while control individuals spent as much time together than separated (see Supplementary Information). This is contradictory with the hypothesis that females and males are in competition for microhabitats in our set-up; to properly conclude, this study should have been pursued with more treatments on social conditions in the terrarium (e.g., solitary individuals or same sex individuals).

Our study is the first experimental test of the effect of dehydration risk on thermoregulation accuracy and it confirms the prediction of less accurate thermoregulation in water-restricted environments. Since thermal conditions were constant across days and between treatments (see Figure S1), we thus demonstrated unambiguously that the non-energetic costs of thermoregulation due to risk of desiccation can impair thermoregulation efficiency, even in a simple laboratory set-up in which thermoregulation is presumably less costly on average than in natural conditions (Angilletta, 2009; Huey & Slatkin, 1976; Sears & Angilletta, 2015). The effects of water restriction on thermoregulation accuracy highlight potential behavioral conflicts between thermoregulation and hydroregulation and suggest that water conservation might be prioritized over thermoregulation and optimization of the energy budget in ectotherms (Basson et al., 2017; Herczeg et al., 2003). Our study thus emphasizes that costs of hydroregulation on thermoregulation are critical in understanding the responses of ectotherms to environmental changes.

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Data Accessibility

Analyses reported in this article can be reproduced using the data provided at publication on *Dryad* (*provided after acceptance*).

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Tables

Table 1 Summary of individual characteristics before and after the experiment in male (M) and female (F) common lizards from water-restricted (WR) and control groups. Values are average from raw data ± standard error. Preferred body temperature was calculated from means of multiple records at different times of the day (see methods). Treatment effects on PBT were marginal ($F_{1,69}$ = 3.01, $p = 0.09$), whatever the sex (water treatment × sex: $F_{1,68}$ = 0.64, $p = 0.43$).

Trait	**Sex**	**Before experiment**		**After experiment**	
		Control	**WR**	**Control**	**WR**
BM (g)	F	5.4 ± 0.2	5.5 ± 0.1	5.9 ± 0.2	5.6 ± 0.1
	M	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.7 ± 0.1
OSMO (mOsm.kg^{-1})	F	327 ± 6	320 ± 7	340 ± 6	352 ± 10
	M	337 ± 9	335 ± 7	352 ± 7	369 ± 7
Preferred body temperature (°C)	F	31.9 ± 0.2	32.1 ± 0.2	30.0 ± 0.1	29.3 ± 0.1
	M	34.2 ± 0.1	34.8 ± 0.1	33.4 ± 0.2	33.6 ± 0.2

Figure legends

Figure 1 Effects of chronic water restriction on **(A)** the intra-individual change in daytime preferred body temperature measured in a neutral thermal gradient before and after the experiment (ΔT_p), **(B)** the body temperature of lizards in the test arenas where water restriction was performed (average from each day) and **(C)** the thermal accuracy of lizards in their test arenas (average from each day) calculated like the difference between body temperature and preferred body temperature. Points are means and error bars are standard errors from raw data. Curves are best-predicted regression lines from models (see main text) with sex and water-restriction treatment as covariates. F: females, M: males, WR: water-restricted.

Figure 2 Proportions of active individuals according to the daytime at day 0 **(A)**, day 2 **(B)**, day 7 **(C)** and day 8 **(D)** of the experiment**.** Lines are non-parametric lowess regressions per treatment and sex group. Points are means and error bars are standard errors from raw data. Data from day 0 and day 7 correspond to days without heat stress, whereas data from day 2 and day 8 correspond to days with heat stress simulated from 11:00 to 14:00. Note the graphical shift in activity patterns for water-restricted animals (solid lines) as time of the experiment proceeds. F: females, M: males, WR: water-restricted individuals.

Figure 3 Effects of chronic water restriction on **(A)** the probability of being active vs. inactive, **(B)** the probability of basking when being active (i.e., basking effort), **(C)** the probability of selecting a shelter in the warm vs. in the cold side and **(D)** the probability of selecting a wet shelter in the warm side vs. a dry shelter in warm side, according to the number of days since the start of the experiment. Data and results of best models are reported for measurement days without heat stress between 11:00 and 14:00 to facilitate comparison with Figure 4. Points are means and error bars are standard errors from raw data. Curves are best-predicted regression lines from models (see main text) with sex and water-restriction treatment as covariates. F: females, M: males, WR: water-restricted. The amplitude of the y-axis (probability) was not presented from 0 to 1 to allow for a better readability of the variations.

Figure 4 Effects of chronic water restriction and daily heat stress on **(A)** the probability of being active vs. inactive, **(B)** the probability of basking when being active, **(C)** the probability of selecting a shelter in the warm side vs. in the cold side and **(D)** the probability of selecting a wet shelter in the warm side vs. a dry shelter in warm side. Data and results of best models are reported for measurement days without heat stress between 11:00 and 14:00 when heat stress was simulated. Grey zones indicate days with heat stress. Points are means and error bars are standard errors from raw data. Curves are best-predicted regression lines from the selected models (see main text) with sex and water-restriction treatment as covariates. In panel **B**, we observe a decrease of basking effort in water-restricted females during heat stress days that seems to become stronger with the number of days of the experiment. Still, the three-way interaction between water restriction treatment, sex and number of days since the beginning of the experiment was not significant (χ^2 = 2.15, df = 1, p = 0.14). F: females, M: males, WR: water-restricted.

Figure 1

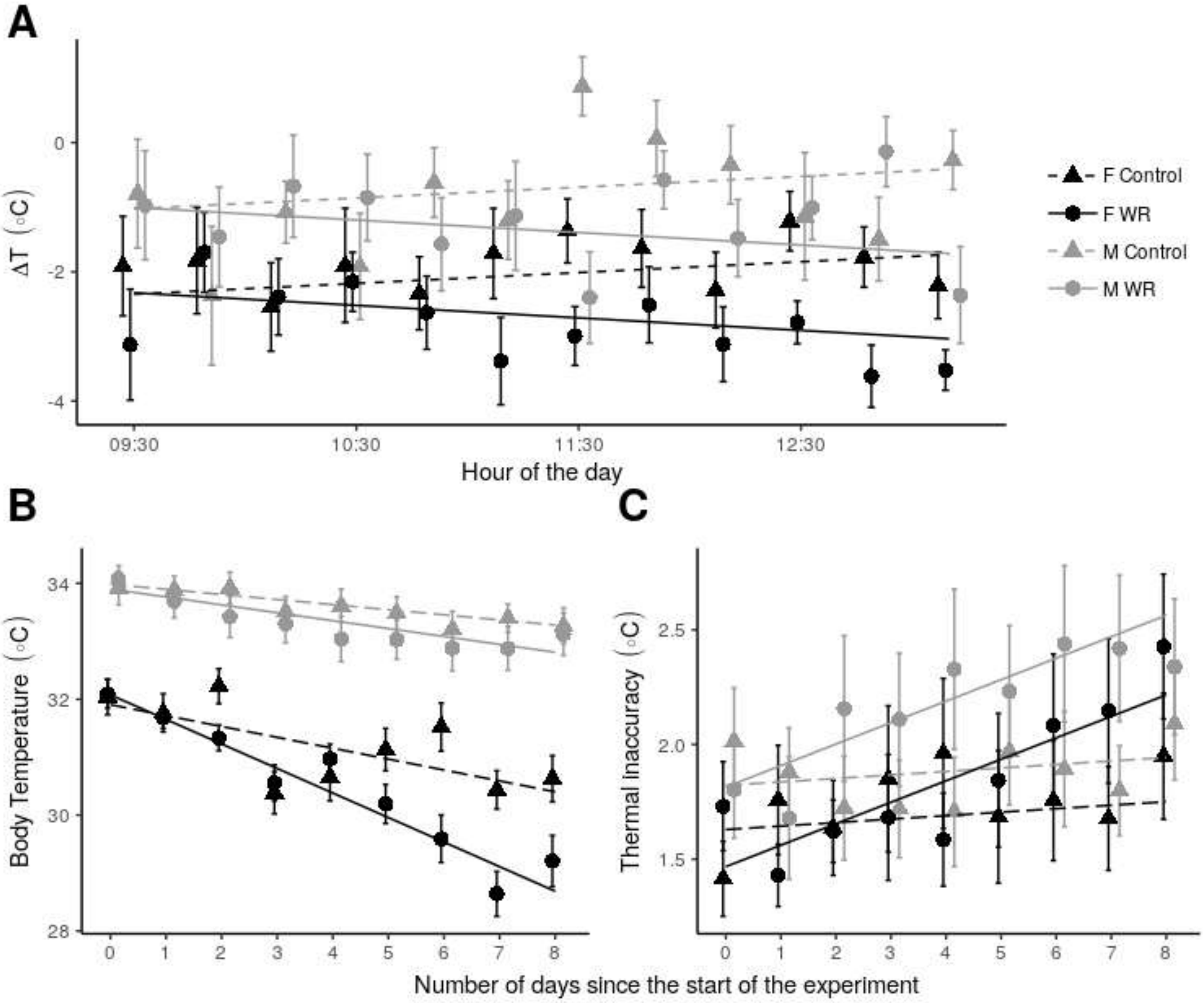

A
ΔT (◦C)
0
-2
-4
09:30
10:30
11:30
12:30
Hour of the day
F Control
F WR
M Control
M WR
B
Body Temperature (◦C)
34
32
30
28
C
Thermal Inaccuracy (◦C)
2.5
2.0
1.5
0
1
2
3
4
5
6
7
8
Number of days since the start of the experiment

Figure 2

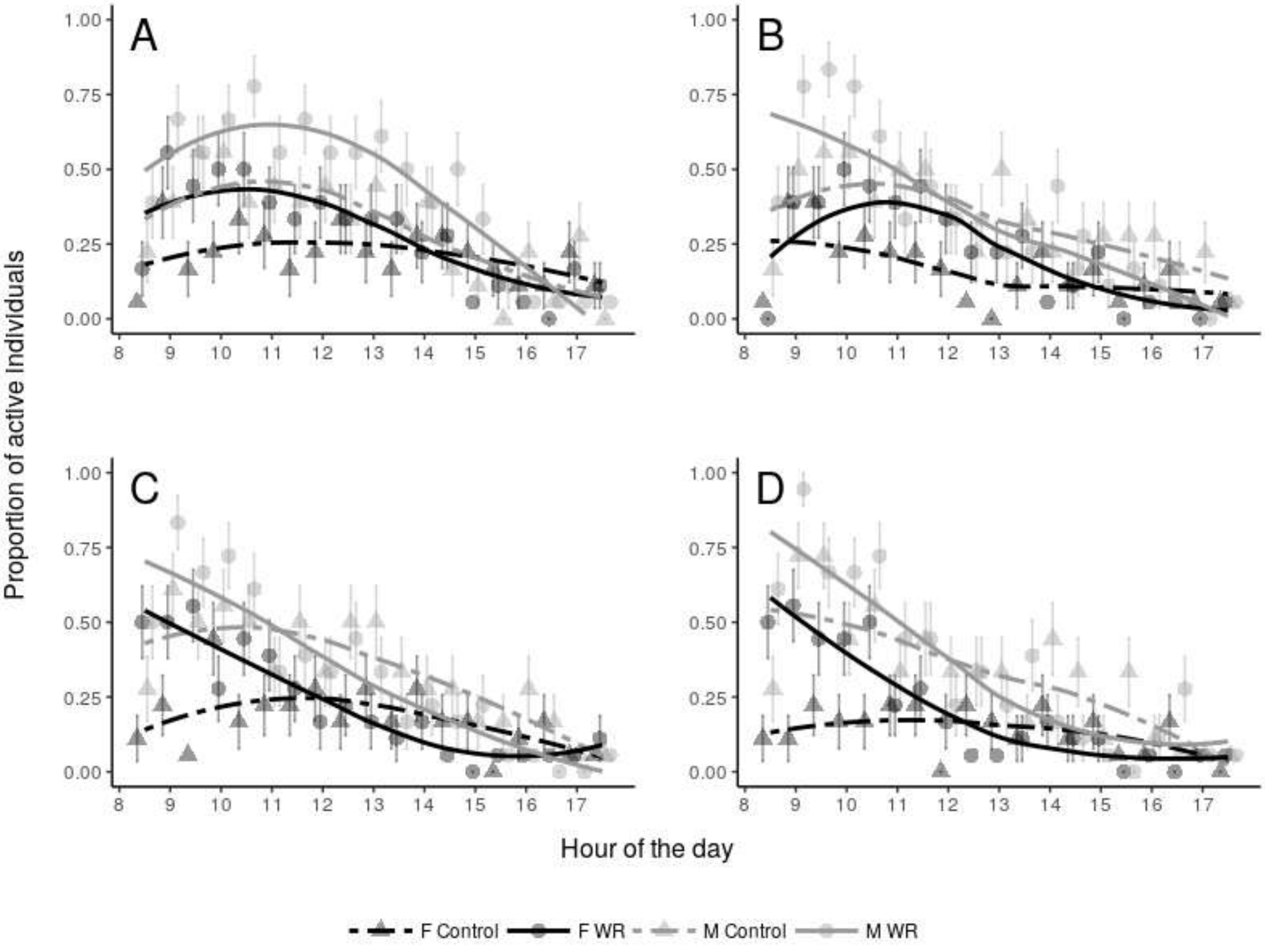

A
B
C
D
1.00
0.75
0.50
0.25
0.00
8
9
10
11
12
13
14
15
16
17
Proportion of active individuals
Hour of the day
F Control
F WR
M Control
M WR

Figure 3

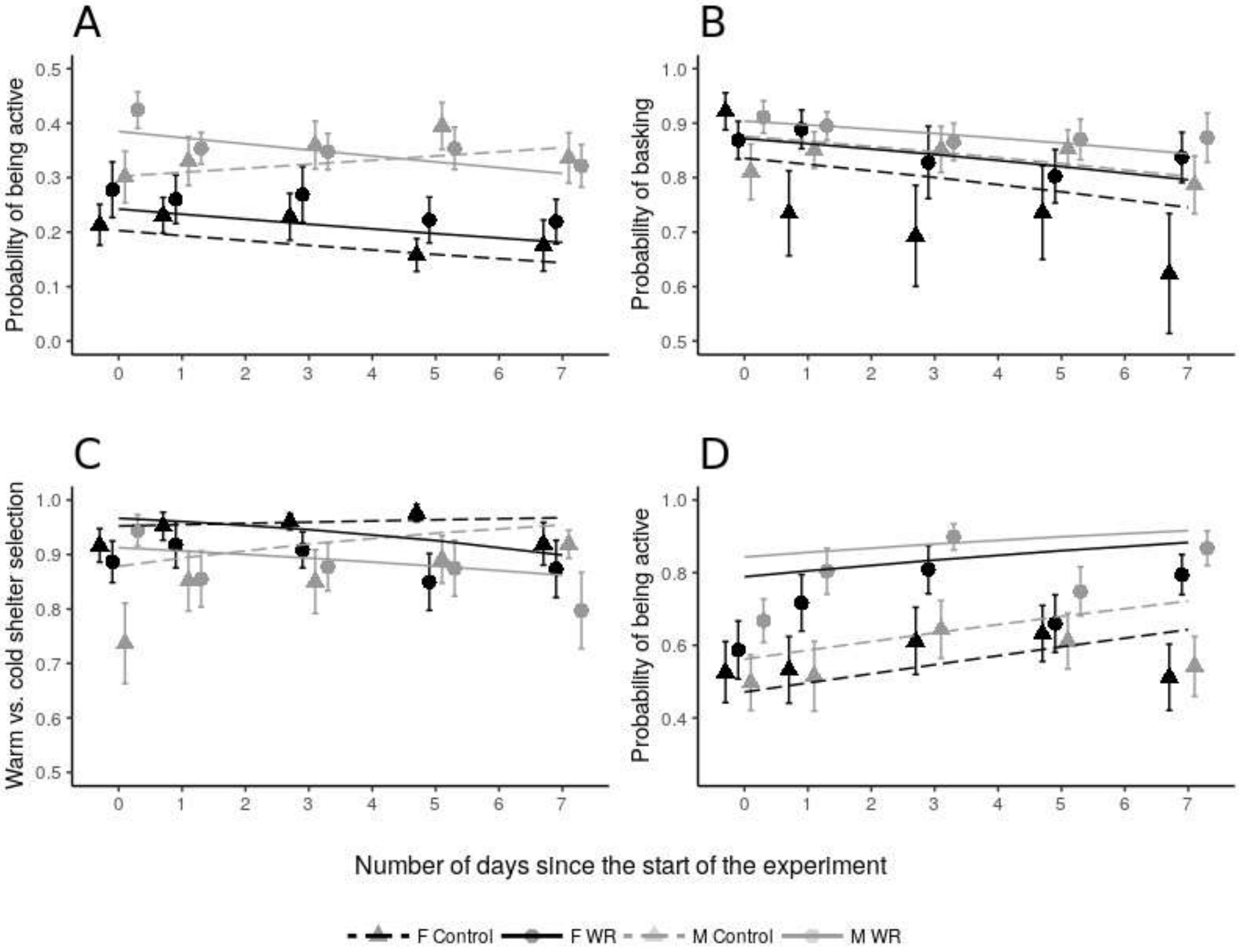
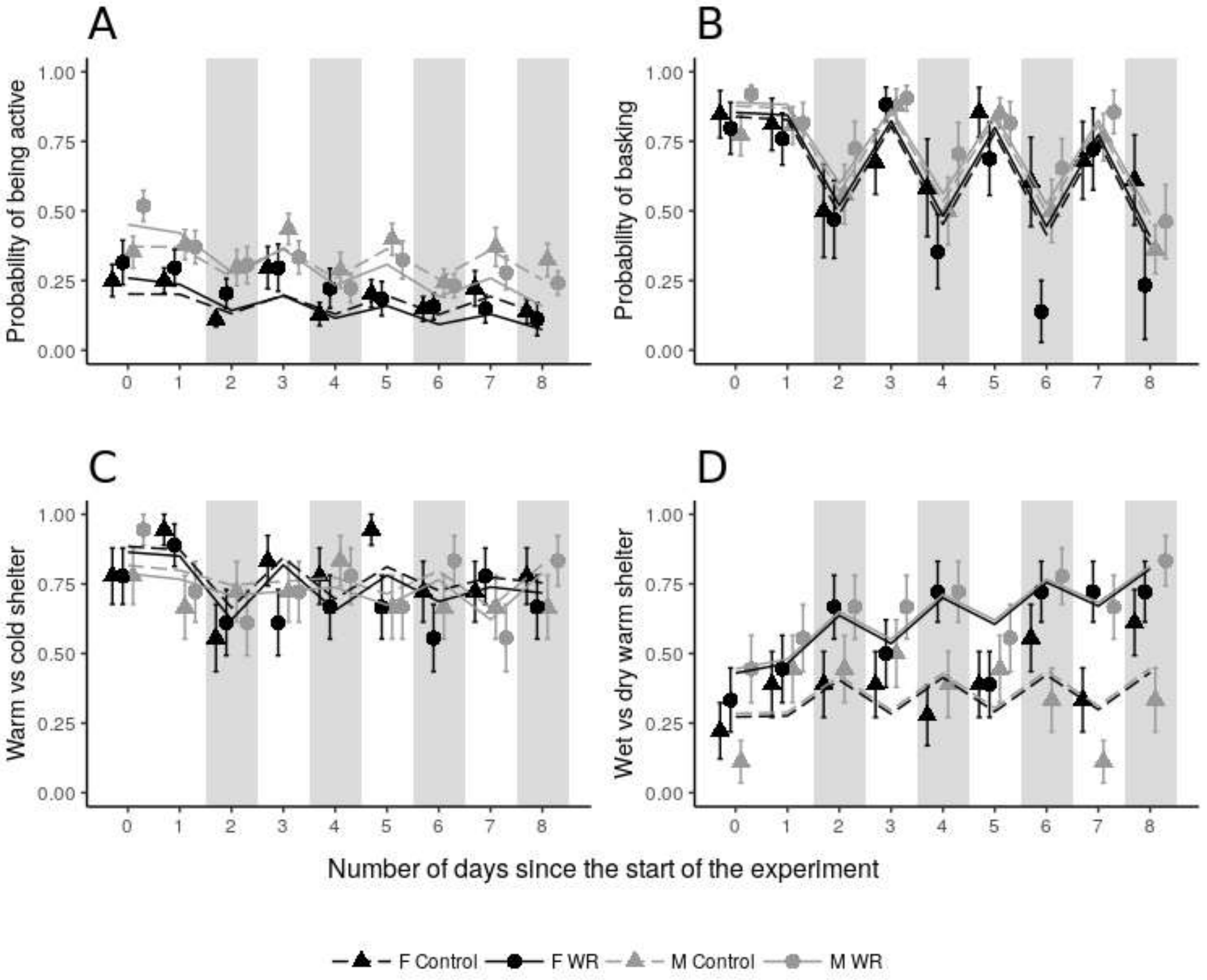

A
Probability of being active
0.0
0.1
0.2
0.3
0.4
0.5
B
Probability of basking
0.5
0.6
0.7
0.8
0.9
1.0
C
Warm vs. cold shelter selection
0.5
0.6
0.7
0.8
0.9
1.0
D
Probability of being active
0.4
0.6
0.8
1.0
0
1
2
3
4
5
6
7
Number of days since the start of the experiment
F Control
F WR
M Control
M WR

Figure 4


A
Probability of being active
B
Probability of basking
C
Warm vs cold shelter
D
Wet vs dry warm shelter
0.00
0.25
0.50
0.75
1.00
0
1
2
3
4
5
6
7
8
Number of days since the start of the experiment
F Control
F WR
M Control
M WR

Supplementary Information 1

In order to measure the operative temperature of a lizard in micro-habitat, we equipped an experimental arena with 10 temperature probes from 5 HOBO® Pro v2 U23-003 (ONSET Cape Cod, Massachusetts) temperature loggers. Each probe was fitted inside a copper tube (1cm diameter, 6cm long) painted in a mix of brown and green color and with black and light lines in order to mimic the reflectance properties of a common lizard. The probe was fixed inside the tube without touch the copper by using pipette tips that were cut at the right diameter. We measured the operative temperature inside and on top of each shelter as well as in the middle of the terrarium every 5 minutes. We averaged this temperature over 30 minutes periods to have calculate the average operative temperature in the environment (Figure S1).
We also placed in the center of each arena a temperature and humidity data logger (iButton DS1923, Maxim Integrated, San Jose, California). We measured average temperature and relative humidity every 5 minutes (see Figure S2).

Figure S1

Daily variation of operative temperature during days without (A and B) and with (C and D) heat stress between 11:00 and 14:00. A and C are record from shelters on the hot side shelters and middle of the arena i, B and D are for cold side shelters. Jittered points correspond to raw operative temperature values, lines are prediction from a smooth loess regression.

Figure S2

Boxplot of the temperature (A) and relative humidity (H) measures in the 13 terraria. Boxes represent the range from the 25% to the 75% quantile. Bold lines in the boxes are for the median value. Points are marginal value from the calculated distribution.

Figure S1

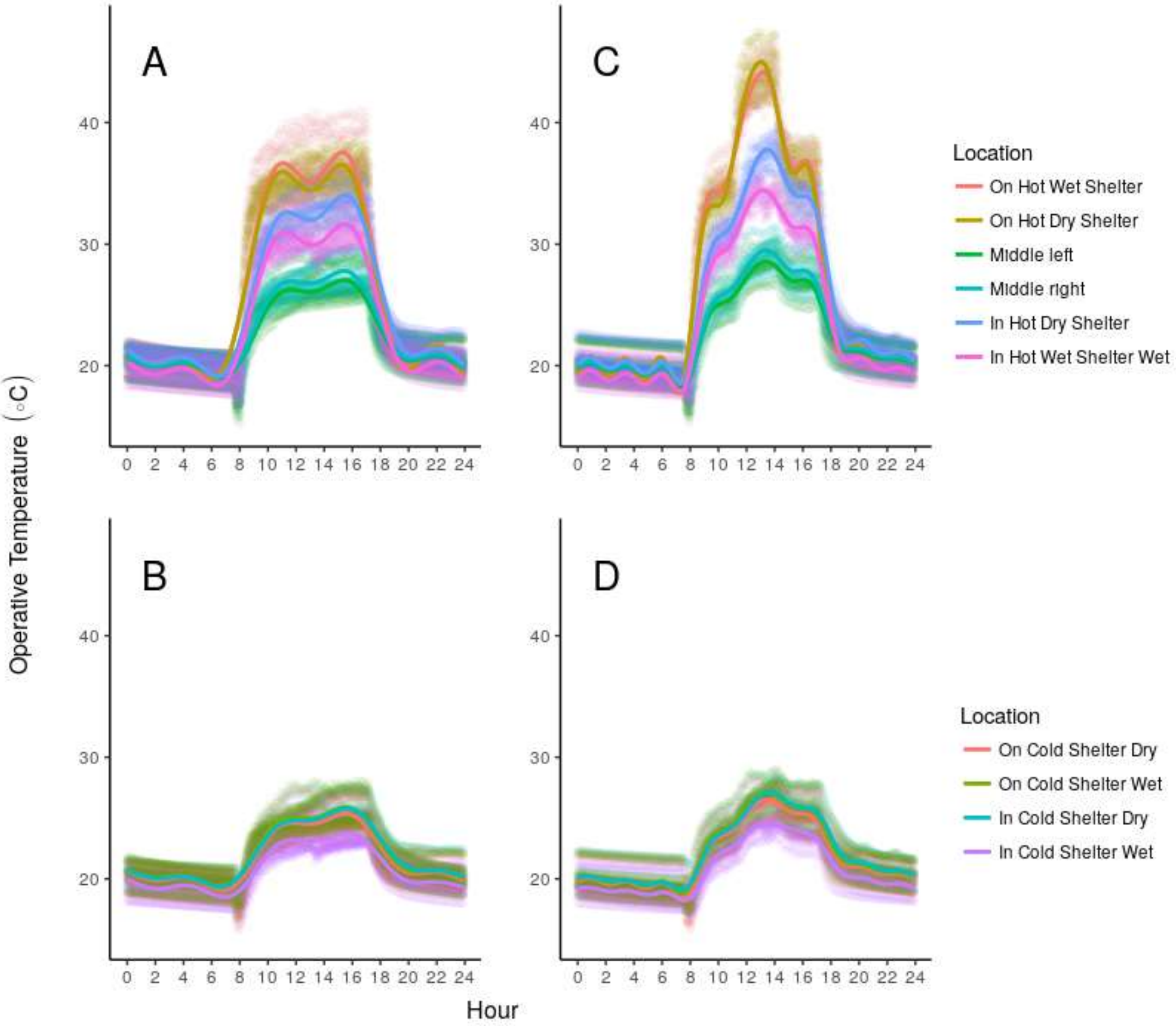
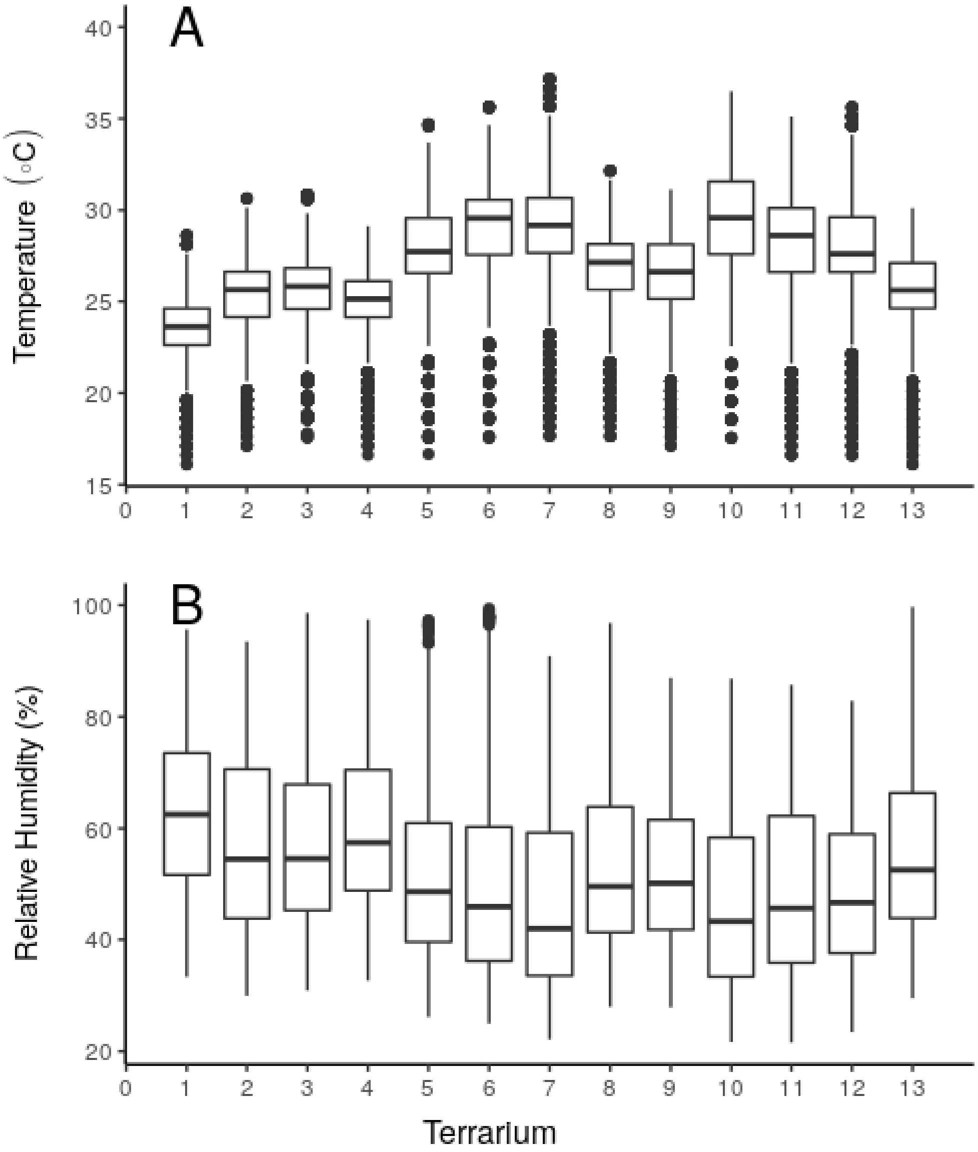

A
C
B
D
Operative Temperature (∘C)
Hour
40
30
20
0 2 4 6 8 10 12 14 16 18 20 22 24
Location
On Hot Wet Shelter
On Hot Dry Shelter
Middle left
Middle right
In Hot Dry Shelter
In Hot Wet Shelter Wet
Location
On Cold Shelter Dry
On Cold Shelter Wet
In Cold Shelter Dry
In Cold Shelter Wet

Figure S2


A
Temperature (∘C)
40
35
30
25
20
15
B
Relative Humidity (%)
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Terrarium

Supplementary In formation 2

From May 6th to May 9th 2019, we ran the same experimental conditions in two experimental terraria. Between 11:00 and 14:00 of May 7th and May 9th we implemented a heat stress. We measured air relative humidity and temperature every 5 minutes in each shelter by fixing a thermo-hygrochron on the shelter wall above the sponge (iButton DS1923, Maxim Integrated, San Jose, California). We calculated water vapor pressure for each log following Tieleman et al. (2002) methods.

Tieleman BI, Williams JB, Buschur ME. 2002. Physiological adjustments to arid and mesic environments in larks (Alaudidae). Physiological and Biochemical Zoology. 75(3):305–313. doi:10.1086/341998.

Figure S3 Chronology of the experiment for one experimental group.

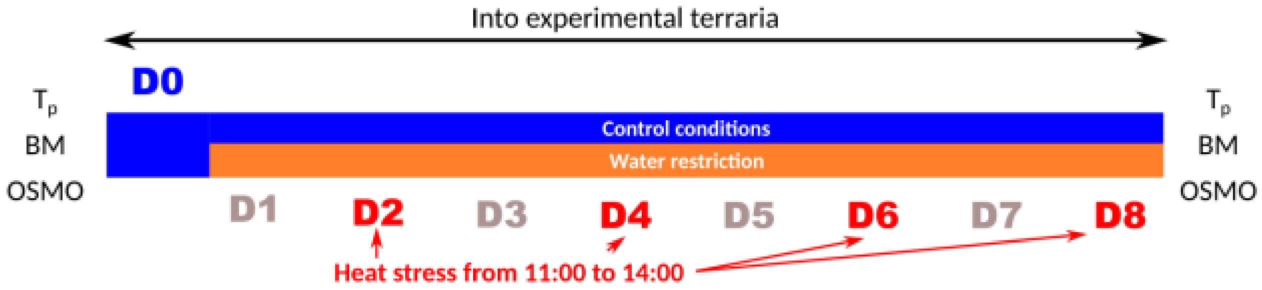


Table S1 Description of the results of the best models explaining activity, basking, selection of hot vs. cold shelters and selection of wet hot vs. dry hot shelters with the effects of heat stress. With χ^2 the value of the chi-square test, df the number of degree of freedom associated and *p* is the two-sided p value.
HS I: heat stress treatment;WR: water restriction treatment.

Behavior	**Fixed effect**	χ^2	**df**	***p***
Activity	HS	33.95	1	< 0.0001
	Sex	11.41	1	0.0007
	WR * D_{exp}	13.85	1	0.0002
Basking	D_{exp}	6.61	1	0.01
	HS	91.33	1	< 0.0001
Hot vs. cold shelter	HS * D_{exp}	5.55	1	0.02
	HS * Sex	6.07	1	0.01
Wet vs. dry hot shelter	HS	8.83	1	0.003
	WR	19.14	1	< 0.0001
	D_{exp}	5.01	1	0.03

Supplementary Information 3

In order to evaluate possible avoidance behaviors between the two individuals in the same terrarium, we counted the number of times individuals were in the same shelter and the number of times both individuals were in a shelter but not the same one. We then compared the probability of being together vs. being separated in a shelter according to the water treatment with a MANOVA fitted with a generalized linear mixed model with a binomial error, also adding the group as a fixed effect and the couple as a random effect.

The treatment did not explained significantly variations in the probability that two individuals stayed together in the same shelter, we however highlighted a tendency that water-restricted individuals had higher probability to be together in the same shelter than separated ($\chi^2_{1,5} = 3.6$, $p = 0.06$, estimate ± SE: 0.38 ± 0.20) compared to control individuals that had the same probability to be together or separated (0.11 ± 0.20). It seems that in the case of water-restriction, individuals remained together mainly in warm and wet shelters (Figure S4).

Figure S4 Number of times we observed individuals together and in what shelter when both of them were in a shelter at the same time. Colors represent the shelter selected by the female (same as the males when together). Toge. = together; sep. = seperated.