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

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15 **Keywords:** body temperature; water availability; activity; micro-habitat selection; dehydration;
16 non-avian reptiles

17

18

19 **Abstract**

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

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

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

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

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

41

42

43 **Introduction**

44 Behavioral plasticity is critical for organisms to cope with both acute and chronic changes in
45 environmental conditions, such as the short-term effects of extreme weather events and long-term
46 consequences of ongoing climate change (Beever et al., 2017; Wong & Candolin, 2015). In the last
47 two decades, a growing number of ecological studies investigated how changes in thermal quality
48 of the environment (Row & Blouin-Demers, 2006; Sears et al., 2016), food availability (Long et al.,
49 2014; Manenti et al. 2013), and predation pressures (McGhee et al. 2013) cause shifts in individual
50 behavior that contribute to ecological responses to environmental changes. In comparison, fewer
51 studies have investigated behavioral responses of terrestrial animals to changes in water availability,
52 especially in ectotherms (Pintor et al. 2016; Pirtle et al. 2019). The behavioral responses allowing
53 organisms to control water inputs and losses can be viewed as components of the hydroregulation
54 behavior (Pintor et al., 2016; Pirtle et al., 2019). Analogous to behavioral thermoregulation
55 (Angilletta, 2009), behavioral hydroregulation must be shaped by a balance between benefits (e.g.,
56 ability to reach an optimal hydration state) and costs (e.g., risks of predation or heat stress during
57 water foraging). As most scenarios of global changes predict a higher frequency of drought events
58 as well as average changes in precipitation regimes (Field et al. 2012), understanding drivers and
59 patterns of behavioral hydroregulation and its plasticity has become essential to unravel the
60 multifactorial consequences of global changes on terrestrial organisms (Albright et al., 2017;
61 Kearney & Porter, 2009; Kearney et al. 2018; Pirtle et al., 2019).

62 Water is a critical resource and a limiting factor for terrestrial animals living in chronically warm
63 and dry environmental conditions such as xeric or semi-xeric environments (Davis & DeNardo,
64 2009; Kearney et al., 2018; Long et al., 2014; Zylstra et al. 2013) as well as during droughts and
65 warm spells in mesic or tropical habitats (Anderson & Andrade, 2017; Marquis et al. 2008). Thus,
66 terrestrial animals have evolved plastic behavioral responses to cope with spatio-temporal
67 fluctuations in water availability and maintain a homeostatic hydration state. One behavioral
68 strategy against water stress involves long-range movements and dispersal responses (i.e.,

69 behavioral flight response) to avoid desiccating environments (Massot et al. 2002; Rozen-Rechels et
70 al., 2018). An alternative behavioral strategy involves shifts in activity patterns (e.g., diel activity)
71 and microhabitat selection without change in home range location (i.e., behavioral flight response;
72 Lorenzon et al. 1999; Pintor et al. 2016). Such behavioral flight responses can improve water
73 balance by limiting water loss and/or by increasing metabolic or dietary water intake. For example,
74 some lizard species reduce their behavioral or locomotor activity during periods of water restriction
75 to reduce evaporative water loss (Davis & DeNardo, 2010; Kearney et al., 2018; Lorenzon et al.,
76 1999; Pirtle et al., 2019) while other species shift their activity towards time periods with moister
77 conditions and a higher availability of free standing water (Davis & DeNardo, 2010; Kearney et al.,
78 2018). Plasticity of microhabitat selection is also critical for some organisms to avoid water stress
79 and it may include selection of shadier basking or retreat sites during periods of activity and
80 differential use of moist and cold shelters during periods of inactivity (e.g., in snakes and lizards,
81 Guillon et al. 2013; Dupoué et al. 2015a; Pintor et al. 2016). So far, we still lack a comprehensive
82 quantification of the drivers and patterns that characterize behavioral hydroregulation in ectotherms,
83 especially dry-skinned vertebrate species, which were thought to be little affected by water
84 conditions until recently (Pintor et al., 2016; Rozen-Rechels et al., 2018). Nonetheless, it is
85 commonly accepted that individual performance of ectotherms are strongly influenced by hydration
86 state (e.g., Anderson and Andrade 2017), and recent mechanistic models highlight the critical role of
87 behavioral hydroregulation as a determinant of water balance across the distribution range of dry-
88 skinned lizard species (Pirtle et al., 2019).

89 The drivers and mechanisms of behavioral thermoregulation and hydroregulation are usually not
90 independent in wild animal populations because environmental changes in water availability usually
91 correlate with changes in thermal conditions on the one hand (e.g., drought and heat waves, Kelley
92 et al. 2015), and because of the coupling between the water and the heat budget of animals on the
93 other (e.g., Kearney and Porter 2009). This makes it hard to distinguish between both behaviors
94 (e.g., Davis and DeNardo 2009). In general, thermo-hydroregulation behavioral strategies must

95 include the interactive set of behaviors that control both water balance and body temperature. In the
96 framework of thermo-hydroregulation, we predict a behavioral trade-off between water balance and
97 heat budget regulation because some thermoregulation behaviors, such as exposure to sunlight to
98 increase body temperature, also frequently enhance water loss rates in desiccating conditions (e.g.,
99 Dupoué et al. 2015a; Lourdais et al. 2017; Pirtle et al. 2019). Thus, water availability not only
100 influences hydroregulation but also thermoregulation, and water restriction can for example reduce
101 basking effort. Mechanisms of thermo-hydroregulation may also be more complex than the sum of
102 thermoregulation and hydroregulation behaviors, especially when desiccation risks occur jointly
103 with heat stress. Empirical studies aiming to disentangle the effects of water availability and
104 temperature, and their consequences for water balance and heat budget, are therefore crucial to fully
105 comprehend thermo-hydroregulation strategies and their mechanisms.

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

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

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

147 **Material and methods**

148 ***Study species and sampling***

149 Common lizards of this study were captured in semi-natural captive populations in outdoor
150 enclosures located at CEREEP-Ecotron IleDeFrance research center in France (48° 17' N, 2° 41' E).
151 These enclosures include a permanent and natural grassland vegetation and refuges/basking sites
152 made of rocks and logs. Individuals have a permanent access to free water through artificial ponds
153 which get filled with rain. Enclosures are delimited by plastic walls preventing escapes and ground
154 predation, and covered with nets to prevent avian predation. In 2018, one population was composed
155 of 71 ± 9 (standard deviation SD) individuals, mainly yearlings (50 ± 7 individuals). Food was
156 composed of local wild arthropods or annelids (spiders, worms, crickets) and in quantities that we
157 suppose to be *ad libitum* as we did not observe differential survival rate of individuals compared to
158 year of lower population densities. Mating season usually starts with emergence of females around
159 March-April and lasts around 2 to 4 weeks. Mating occurs between late March and early April,
160 followed by ovulation and fertilization, and a gestation period that lasts two months on average.
161 From May 3rd, 2018 to May 7th, 2018 we captured 72 adult individuals in 10 different 100-m²
162 outdoor enclosures. At capture, lizards were measured for snout-vent length (SVL, with a rule, ± 0.5
163 mm) and body mass (BM, OHAUS, Adventurer, 220 g, ± 1 mg), then placed in individual terraria
164 ($18 \times 11 \times 12$ cm) with a shelter and sterilized peat soil as substratum. This sample was half
165 composed of pregnant females (mean \pm SD; SVL: 64 ± 3 mm; BM: 5.25 ± 0.79 g) and of post-
166 reproductive males (SVL: 58 ± 2 mm; BM: 4.42 ± 0.32 g). Terraria were located in a temperature-
167 controlled room (23°C from 09:00 to 18:00, 16°C at night). Individuals were fed three times a week
168 with 300 ± 10 mg of live house crickets (*Acheta domestica*). In normal housing conditions, water
169 was available *ad-libitum* in a petri dish and sprayed three times a day (09:00, 13:00 and 17:00).
170 After a minimum acclimation period of one week, individuals were sorted in three trial groups (24
171 individuals per group) tested between May 10th to May 20st (trial 1), May 21st to May 31st (trial 2)
172 or June 1st to June 11th (trial 3). Males were randomly attributed to each trial group. Females were

173 sorted according to their SVL such that longer and older females, which usually give birth earlier
174 (Rutschmann et al., 2016), were tested first to avoid a strong variation in gestation stage among
175 trials (trial 1: 68 ± 2 mm; trial 2: 64 ± 1 mm; trial 3: 61 ± 2 mm). Inside each trial group, individuals
176 were randomly attributed to a control treatment or to the water-restricted treatment (Control
177 females: SVL: 64 ± 3 mm; BM: 5.10 ± 0.84 g; Water-restricted females: SVL: 65 ± 3 mm, $F_{1,34} =$
178 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:
179 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,
180 $F_{1,34} = 0.08$, $p = 0.78$). To conform with Animal Welfare recommendations, we ensured all
181 individuals were healthy by checking body condition, behavior and parasite load before the
182 beginning and at the end of the experiment.

183 ***Experimental design***

184 Experiments were performed in open-top arenas (N=12, $78 \times 56 \times 44$ cm) installed in a
185 temperature-controlled room maintained at 20°C during day and night. In a 13th terrarium we
186 measured all micro-habitats thermal characteristics using operative temperature copper models
187 mimicking the lizards thermal properties in which were fixed HOBO® Pro v2 U23-003 (ONSET
188 Cape Cod, Massachusetts) temperature loggers (see Supplementary Information). Arenas were filled
189 with a substratum of dry and sterilized peat soil and equipped with one plastic shelter (Exo-Terra®
190 cave, $7.6 \times 15.2 \times 5.1$ cm) at each corner. At one side of the arena, hereafter called the warm side,
191 two 40W light bulbs were placed above each shelter in order to warm the surface temperature at
192 approximately 35.9 ± 4.2 °C during daytime (between 8 am and 5 pm, similar to a standard early
193 summer activity day, see Supplementary Information and Figure S1), which corresponds to slightly
194 more than the preferred body temperature in common lizards (Gvoždík & Castilla, 2001). The other
195 side of the terrarium, hereafter called the cold side, was maintained at approximately 24.5 ± 2.0 °C
196 during daytime (between 8am and 5pm, see Figure S1), which is well below the preferred body
197 temperature range. At both sides, soil and air inside one shelter was made wet by adding a thin
198 sponge regularly moistened on top of the substrate, whereas the other shelter was kept dry by

199 adding the same kind of sponge without wetting. Due to evaporative cooling effect, temperature in
200 wet shelters was colder than in dry shelters (warm side: 30.0 ± 3.1 °C in wet vs. 32.0 ± 3.7 °C in
201 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, $F_{1,6944} =$
202 2447.7 , $p < 0.0001$; see Figure S1). Yet, all temperature records on the cold side remained within
203 the thermal preference set of the species, while they remained in the set on the warm side. Wet
204 shelters had also higher water density (water vapor pressure calculated from relative humidity
205 measurements with iButton DS1923, Maxim Integrated, San Jose, California following Tieleman et
206 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,
207 $F_{1,1598} = 8108$, $p < 0.0001$; cold side: 17.7 ± 1.2 g.m⁻³ in wet vs. 11.6 ± 1.7 g.m⁻³ in dry, $F_{1,1594} =$
208 6540 , $p < 0.0001$; see Supplementary Information). We randomly assigned a wet or dry treatment to
209 the shelters on each side in each terrarium. In the center of the arena, we placed two large petri
210 dishes (~8 cm of diameter, 0.5cm deep) to store free-standing water. We placed above the middle of
211 the warm side an infrared halogen 50W bulb to optionally change thermal conditions to 40-42°C
212 substrate and air temperature on the warm side, which is close to the critical thermal maximum for
213 this species (Gvoždík & Castilla, 2001). At night, temperatures were of 20.0 ± 1.1 °C. Two UVB
214 30W neon tubes (Reptisun 10.0, white light) provided white light above each arena during daytime.
215 We installed one male and one size-matched female in each arena during each trial. At this time of
216 year, males do not attempt to copulate with gravid, non-receptive adult females, and aggressive
217 interactions among lizards are rare (Fitze et al., 2005). Indeed, we did not observe any aggressive
218 behavior during this study. At day 0, animals were put in the arenas until day 8. Lights bulbs were
219 turned on automatically during daytime every day and infrared halogen bulbs were automatically
220 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
221 warm side. During day 0, arenas were sprayed with water three times (07:30, 14:00, 17:00) to
222 provide mist and petri dishes were constantly filled with water (control treatment). Spraying lasted
223 approximately 5 seconds and was always made by the same observer in the same manner, and
224 aimed at having free water available as droplets that dried quickly. From day 1 to day 8, half of the

225 individuals were exposed to a sub-lethal water restriction during which mist was only provided in
226 early morning (08:00) and no free-standing water was available (droplets in empty petri dishes were
227 dried). Individuals were fed with 200 ± 10 mg of live house crickets (*Acheta domestica*) every day
228 at 10:00. The chronology of the experiment for one experimental group is summarized in Figure S3.

229 ***Pre-manipulation and post-manipulation measurements***

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

243 After thermal preferences measurements, individuals were weighted to calculate body mass change
244 between the beginning and the end of the experiment (ΔBM) and relocated in their home terrarium
245 for an hour or two until approximately 15:00. We then sampled 10 μL of blood from the post-orbital
246 sinus using a standard protocol (Meylan et al., 2003) to assess changes in plasma osmolality. Blood
247 samples were centrifuged at 11,000 g to separate plasma, which was kept frozen at approximately -
248 25°C. Plasma osmolality was determined from two aliquots of plasma diluted in physiological
249 serum using a vapor pressure osmometer (Wescor Vapro® 5520) following (Dupoué et al. 2018).
250 The osmolality was calculated as the average osmolality of the two aliquots corrected by the

251 dilution minus the osmolality of a reference, physiological serum (307 ± 3 mOsm.kg⁻¹). We
252 assessed Δ OSMO by subtracting final osmolality to initial osmolality.

253 ***Behavioral data***

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

264 ***Statistical analyses***

265 Statistical analyses were performed using R version 3.4.4 “Someone to Lean On” (R Core Team,
266 2018). In all cases, the best models were inferred with backward model selection based on log-
267 likelihood ratio tests. We evaluated significance by comparing the best-fit model with a reduced
268 model, from which was subtracted the effect of interest using log-likelihood ratios. We also
269 provided the estimated effect \pm standard error.

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

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

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

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

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

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

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

316 Analyses of shelter selection showed over-dispersion of data that we corrected by using an
317 observation-level random effect (Harrison, 2014).

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

327 **Results**

328 *Change in body mass and plasma osmolality*

329 Body mass change was smaller in water restricted lizards than in controls ($F_{1,66} = 9.50, p = 0.003$)
330 independently from sex (water treatment \times sex: $F_{1,65} = 2.74, p = 0.1$). Males had a lower Δ BM than
331 females (-0.37 ± 0.08 g; $F_{1,66} = 19.7, p < 0.0001$). Thus, we recorded a gain of mass in control
332 females (0.4 ± 0.1 g), no change in BM in water-restricted females (0.1 ± 0.1 g) and control males
333 (0 ± 0.1 g), and a mass loss in water-restricted males (-0.3 ± 0.1 g; Table 1). In addition, water-
334 restricted individuals had a higher osmolality change (i.e., higher plasma dehydration) than control
335 individuals (water treatment: $F_{1,67} = 4.59, p = 0.04$) independently from sex (water treatment \times sex:
336 $F_{1,65} = 0.01, p = 0.91$; sex: $F_{1,65} = 0.05, p = 0.94$, see Table 1).

337 *Change in thermal preferences and thermoregulation accuracy*

338 The preferred body temperatures were most of the time lower after the experiment than before with
339 an average $\Delta T_p = -1.70 \pm 0.20$ °C, and only nine individuals out of 72 had positive ΔT_p records
340 (Table 1, Figure 1A). ΔT_p was significantly higher in males than in females (sex: $F_{1,69} = 12.75, p =$
341 0.0007). Control individuals had a constant ΔT_p across times of day, while water-restricted
342 individuals ΔT_p dropped approximately by 0.12 ± 0.05 °C every 20 minutes with time of day (time
343 of the day: $F_{1,779} = 2.83, p = 0.09$; time of the day \times water treatment: $F_{1,779} = 6.63, p = 0.01$). At
344 midday (13:10, end of our test), water-restricted lizards had a ΔT_p lower of 1.30 ± 0.45 °C than
345 controls irrespective of sex (Figure 1A; water treatment: $F_{1,69} = 8.36, p = 0.005$; water treatment \times
346 sex: $F_{1,69} = 0.64, p = 0.43$).

347 Surface body temperature records during the experiment were influenced significantly by a
348 three-way interaction of D_{exp} , water treatment and sex ($D_{exp} \times$ water treatment \times sex: $F_{1,3258} = 4.61,$
349 $p = 0.03$; Figure 1B). The body temperature of control females decreased by 0.19 ± 0.05 °C.day⁻¹
350 during the experiment, but it decreased twice as fast in water-restricted females (contrast = $0.24 \pm$
351 0.07 °C.day⁻¹; $t_{3258} = -3.37, p = 0.0008$). No such effect was found in males (Figure 1B) but the
352 body temperature was higher of 2.13 ± 0.41 °C in males than in females ($t_{66} = 5.15, p < 0.0001$).

353 Thermoregulation inaccuracy was influenced significantly by the water treatment in
354 interaction with D_{exp} ($D_{exp} \times$ water treatment: $F_{1,3260} = 5.17, p = 0.02$; Figure 1C). Thermoregulation
355 inaccuracy of control individuals did not change through time ($t_{3260} = 0.55, p = 0.58$). On the
356 contrary, thermoregulation became less accurate with D_{exp} in water-restricted individuals (contrast:
357 $+0.08 \pm 0.03^{\circ}\text{C}\cdot\text{day}^{-1}, t_{3260} = 2.27, p = 0.02$), irrespective of sex ($D_{exp} \times$ water treatment \times sex: $F_{1,3258}$
358 $< 0.01, p = 1$). On average, males were slightly less accurate in their thermoregulation than females
359 ($0.3 \pm 0.1^{\circ}\text{C}$, sex: $F_{1,69} = 4.25, p = 0.04$; sex \times water treatment: $F_{1,68} = 0.34, p = 0.56$).

360 *Behavioral flexibility*

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

370 Activity rate was also influenced by a three-way interaction between sex, water treatment and
371 D_{exp} ($\chi^2 = 3.96, \text{df} = 1, p = 0.046$, Figure 3A). Females reduced their activity across time of the
372 experiment ($z = -2.40, p = 0.02$) irrespective of water treatment ($z = 0.19, p = 0.85$). On the contrary,
373 control males did not change their activity rate while water-restricted males reduced it (Figure 3A).
374 Lizards also reduced their basking effort during the experiment ($\chi^2 = 12.8, \text{df} = 1, p = 0.003$; Figure
375 3B) and males basked more than females ($\chi^2 = 4.45, \text{df} = 1, p = 0.03$). Water treatments did not
376 influence basking effort (water treatment: $\chi^2 = 3.25, \text{df} = 1, p = 0.07$; water treatment \times D_{exp} : $\chi^2 =$
377 $0.01, \text{df} = 1, p = 0.92$; water treatment \times sex: $\chi^2 = 0.12, \text{df} = 1, p = 0.72$). These results on
378 emergence time and activity patterns thus imply that water-restricted females shifted their activity

379 towards the earliest hours of the day (08:00: ~20% active at D0 vs. ~50% active at D7; 10:00: ~50%
380 active at D0 vs. ~25% active at D7, see Figure 3).

381 Warm vs. cold shelter selection was significantly influenced by the two-way interactions of
382 water treatment with D_{exp} ($\chi^2 = 6.47$, $df = 1$, $p = 0.01$) and with sex ($\chi^2 = 4.26$, $df = 1$, $p = 0.04$).
383 Control individuals tend to shift towards warmer shelters across the experiment ($z = 1.94$, $p =$
384 0.053) while water-restricted individuals shifted progressively to cooler shelters (contrast: $-0.23 \pm$
385 0.09 day^{-1} ; $z = -2.55$, $p = 0.01$). In addition, at day 0, there was no difference in shelter selection
386 between water-restricted females and control individuals ($z = 0.38$, $p = 0.23$), however, control
387 males selected colder shelters than water-restricted males (Figure 3C). Regarding wet shelter
388 selection in the warm side of the terrarium, we found that water-restricted individuals selected the
389 wetter and cooler shelter on average (contrast: 1.43 ± 0.39 ; water treatment: $\chi^2 = 12.4$, $df = 1$, $p =$
390 0.0004 ; water treatment $\times D_{exp}$: $\chi^2 = 2.41$, $df = 1$, $p = 0.12$; water treatment \times sex: $\chi^2 = 1.41$, $df = 2$,
391 $p = 0.49$; Figure 3D). Irrespective of water treatment, individuals also shifted on average their
392 shelter selection towards the wetter shelter as the time of the experiment proceeded ($\chi^2 = 7.22$, $df =$
393 1 , $p = 0.007$, Figure 3D).

394 *Effects of simulated heat stress on behavior*

395 In this section, we focus on patterns and effects of water-restriction that are different from those
396 described during days without heat stress in the previous section, but Table S1 summarizes all best
397 models for heat stress simulation. Between 11:00 and 14:00, heat stress significantly reduced
398 behavioral activity and basking effort (Figure 4A and 4B), but this reduction was independent from
399 the water restriction treatment (activity: $\chi^2 = 2.72$, $df = 1$, $p = 0.10$; basking: $\chi^2 = 0.63$, $df = 1$, $p =$
400 0.73). Heat stress simulation also reduced the selection of warm shelters in females ($z = -3.30$, $p =$
401 0.001) but not in males (post-hoc test: $z = -1.5$, $p = 0.13$), independently from the water restriction
402 treatment (Figure 4C). During simulated heat stress, individuals more often selected the wet and
403 cool shelter (contrast: 0.56 ± 0.19 ; $z = 8.86$, $p = 0.003$; Figure 4D), but this was again independent
404 from the water restriction treatment ($\chi^2 = 1.15$, $df = 1$, $p = 0.28$). Last, there was a tendency for

405 water-restricted individuals to select the wet and cool shelters more often than control individuals
406 with D_{exp} (Figure 4D), but this trend was not significant ($\chi^2 = 3.22$, $df = 1$, $p = 0.07$).

407 **Discussion**

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

424 Overall, our observations are consistent with the prediction that common lizards unable to disperse
425 adopt behavioral fight strategies to conserve more water and mitigate the consequences of the
426 simulated mild but chronic water restriction on water balance (Lorenzon et al., 1999; Pirtle et al.,
427 2019). The behavioral fight syndrome included a diminution of locomotor and behavioral activity, a
428 change in daily activity patterns and some shifts of shelter selection strategies. The dominant
429 behavioral response to water restriction in females was a shift of their behavioral activity towards
430 the first hours of the day (Figure 2), a time period of the day that is cooler and wetter on average

431 and when free standing water is available in the form of dew in both treatments (Figure S1). The
432 earlier activity of water-restricted females could thus be a strategy to forage for water and perform
433 standard activities in conditions in which desiccation risk is minimized. This result is consistent
434 with with those of empirical studies and quantitative models suggesting that changes in water
435 availability can be a dominant driver of seasonal or spatial changes in daily activity patterns in
436 squamate reptiles, and more generally in terrestrial ectotherms (Davis & DeNardo, 2010; Kearney
437 et al., 2018; Wells, 2010).

438 In our experimental set-up, shelters in the warm side of the terrarium were at an operative
439 temperature of approximately 30-32°C, which is close to females' but slightly below males'
440 preferred body temperature (Table 1). These shelters thus allow individuals to regulate body
441 temperature without effort and under low overheating risks, which is critical to perform optimally
442 some slow and temperature-dependent activities such as digestion (Stevenson, 1985). Cold side
443 shelters were, however, suboptimal for thermoregulation with temperatures ranging around 23-
444 25°C. This explains the high rate of warm shelter use (>75%) in our study. Wet shelters were also
445 three times less desiccating (vapor pressure deficit: 0.98 mbar in warm wet shelters, 0.36 mbar in
446 cold ones) than dry ones (3.04 mbar in warm dry shelters, 1.5 mbar in cold ones) and differed on
447 average only by 2°C temperature difference due to evaporative cooling. We therefore suggest that
448 the differential use of wet over dry shelters is much more likely driven by hydroregulation than
449 thermoregulation behavior. Thus, water restricted individuals shifted their hydroregulation behavior
450 by increasing the use of wetter shelters, which is consistent with a strategy aiming at reducing
451 dehydration rate. At the same time, they used more often cool shelters, which are suboptimal for
452 thermoregulation but, on average, half less desiccating than warm ones. This shift suggests that
453 conditions of water restriction can trigger hydroregulation strategies critical for the maintenance of
454 water balance at the expense of thermoregulation strategies, as suggested by Pintor et al. (2016). In
455 natural conditions, we suspect that ecological responses to drought and water restriction will
456 critically depend on the presence and distribution of cold and wet shelters or vegetation in the

457 lizards' habitat (Pirtle et al., 2019). The distribution of such patches in the landscape in
458 complementation to other resources will be a predictor of the ability of organisms to cope with
459 changing moisture conditions through habitat selection (Sears & Angilletta, 2015; Sears et al.,
460 2016).

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

474 Unexpectedly, we did not observe any interactive effect of water restriction and heat stress on
475 behavior. This could be explained by a lack of statistical power, as heat stress was too strong in our
476 set-up, or because hydration status does not influence sensitivity to heat stress in common lizards
477 (see Figure S1C). Behavioral responses to a simulated midday heat stress were consistent with
478 overheating avoidance, probably to avoid the risk of reaching maximum critical thermal limits and
479 to reduce evaporative water loss through skin and respiration. During the simulated heat stress,
480 lizards hide more often and seek more often cooler microhabitats which offered more optimal
481 thermal conditions (close to thermal preferences) than the warm side (see Figure S1C), a result
482 predicted by theoretical models of thermoregulation (Angilletta, 2009; Porter et al., 1973). They

483 also selected wet shelters more often, independently from their hydration status, thus suggesting
484 that heat stress induced water-conservation behaviors. However, because wet shelters were also
485 significantly cooler during the heat stress, it is difficult to tell if this behavioral shift was a sign of
486 behavioral hydroregulation or thermoregulation. We were surprised by the absence of interaction
487 between the water balance status and the sensitivity to heat stress. Our study however suggests that
488 heat stress not only influences thermoregulation behaviors, but also water-related habitat selection
489 patterns.

490 Some behavioral effects of water restriction were different between sexes which might also simply
491 be the consequence of being gravid (all females expect one finally laid). Only females showed
492 strong differences in activity patterns in response to water restriction but both sexes adjusted their
493 microhabitats selection behaviors the same way. In both treatments, gravid females had lower
494 thermal preferences and lower active body temperatures. This average shift is consistent with
495 previous observations of thermal depression during gestation in the common lizard, suggesting that
496 thermal conditions for embryonic development are optimized around 29-30°C at the end of
497 gestation (Gvoždík & Castilla, 2001; Le Galliard et al., 2003). We found minor changes in thermal
498 preferences in males only but the effects of water restriction on thermal preferences were similar in
499 both sexes. In sharp contrast, the active body temperature of males was not influenced by water
500 restriction and remained relatively high during the experiment. Altogether, these results support the
501 hypothesis that thermoregulation behaviors are more sensitive to water restriction in females than in
502 males, most likely because higher water needs during pregnancy select for stronger water-
503 conservation strategies in pregnant females than in non-reproductive individuals (Dupoué et al.,
504 2015b; Dupoué et al., 2018). In viviparous ectotherms, pregnancy leads to an increased demand for
505 water (Dupoué et al., 2016, 2018; Dupoué et al., 2015b), and pregnant females could alter their own
506 water balance to protect their embryos from hydric stress (Dupoué et al., 2016, 2018). In addition,
507 the trade-off between thermoregulation and hydroregulation is likely higher in pregnant females
508 because pregnancy induces morphological and physiological changes that increase standard water

509 loss rates (Lorioux et al., 2013). As females and males faced similar physiological dehydration, we
510 speculate that plasticity of thermoregulation behaviors is critical for females to override the water
511 conflict with their embryos and maintain a constant survival and reproductive effort despite their
512 stronger reliance on water (as seen in Dupoué et al., 2018). We can not however conclude if these
513 differences are sex-specific, the consequence of being gravid or both of them.

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

528 Our study is the first experimental test of the effect of dehydration risk on thermoregulation
529 accuracy and it confirms the prediction of less accurate thermoregulation in water-restricted
530 environments. Since thermal conditions were constant across days and between treatments (see
531 Figure S1), we thus demonstrated unambiguously that the non-energetic costs of thermoregulation
532 due to risk of desiccation can impair thermoregulation efficiency, even in a simple laboratory set-up
533 in which thermoregulation is presumably less costly on average than in natural conditions
534 (Angilletta, 2009; Huey & Slatkin, 1976; Sears & Angilletta, 2015). The effects of water restriction

535 on thermoregulation accuracy highlight potential behavioral conflicts between thermoregulation and
536 hydroregulation and suggest that water conservation might be prioritized over thermoregulation and
537 optimization of the energy budget in ectotherms (Basson et al., 2017; Herczeg et al., 2003). Our
538 study thus emphasizes that costs of hydroregulation on thermoregulation are critical in
539 understanding the responses of ectotherms to environmental changes.

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542 All applicable international, national, and/or institutional guidelines for the care and use of animals
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546 A7743117-341-1 delivered by the Préfecture de Seine-et-Marne. Animals were captured and
547 manipulated under authorization 2007-198-005 delivered by the Préfecture de Seine-et-Marne.

548 **Data Accessibility**

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

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690 **Tables**

691

692 **Table 1** Summary of individual characteristics before and after the experiment in male (M) and
 693 female (F) common lizards from water-restricted (WR) and control groups. Values are average from
 694 raw data \pm standard error. Preferred body temperature was calculated from means of multiple
 695 records at different times of the day (see methods). Treatment effects on PBT were marginal ($F_{1,69} =$
 696 3.01 , $p = 0.09$), whatever the sex (water treatment \times sex: $F_{1,68} = 0.64$, $p = 0.43$).

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

697

698 **Figure legends**

699

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

708

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

716

717 **Figure 3** Effects of chronic water restriction on **(A)** the probability of being active vs. inactive, **(B)**
718 the probability of basking when being active (i.e., basking effort), **(C)** the probability of selecting a
719 shelter in the warm vs. in the cold side and **(D)** the probability of selecting a wet shelter in the warm
720 side vs. a dry shelter in warm side, according to the number of days since the start of the
721 experiment. Data and results of best models are reported for measurement days without heat stress
722 between 11:00 and 14:00 to facilitate comparison with Figure 4. Points are means and error bars are
723 standard errors from raw data. Curves are best-predicted regression lines from models (see main

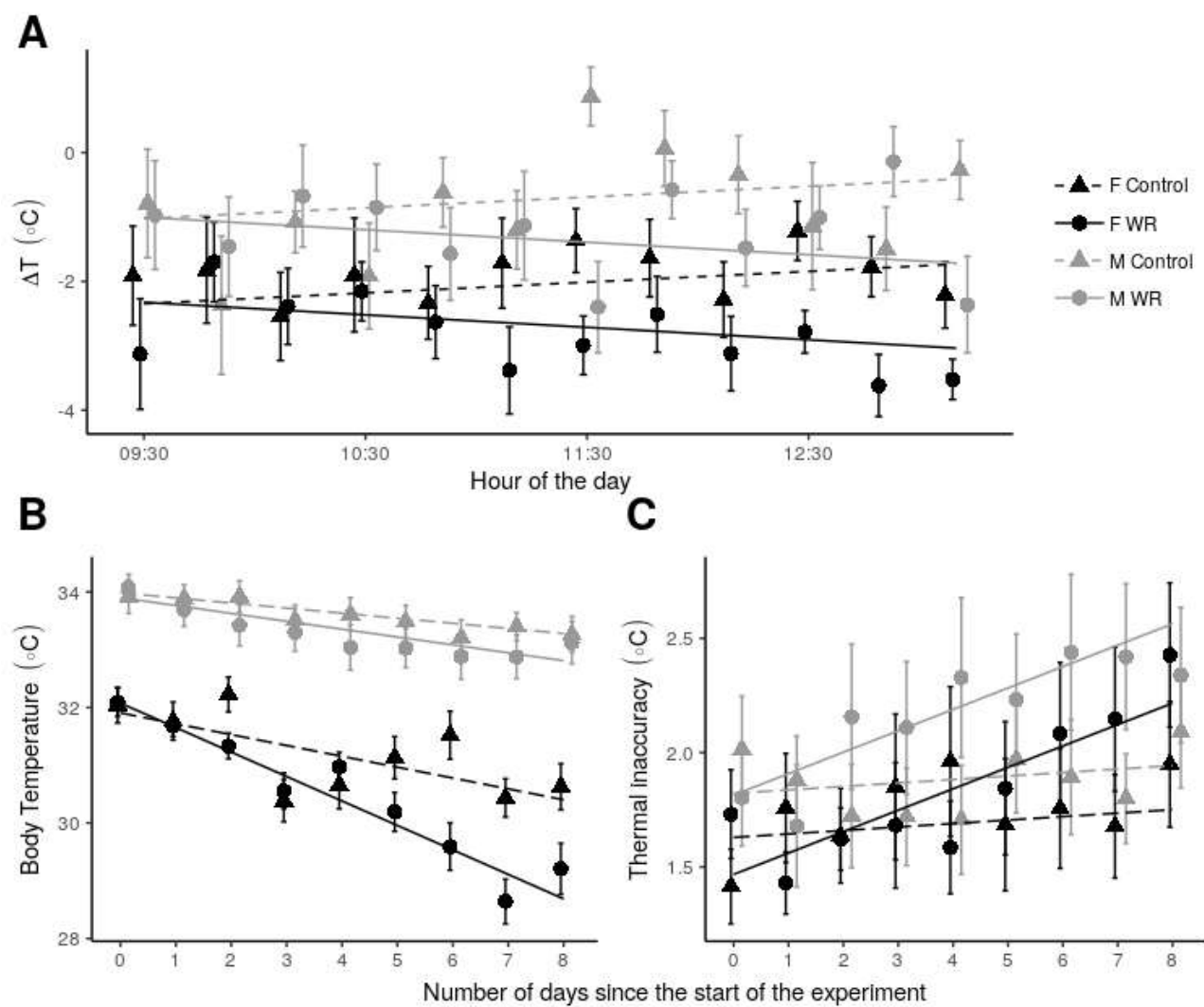
724 text) with sex and water-restriction treatment as covariates. F: females, M: males, WR: water-
725 restricted. The amplitude of the y-axis (probability) was not presented from 0 to 1 to allow for a
726 better readability of the variations.

727

728 **Figure 4** Effects of chronic water restriction and daily heat stress on **(A)** the probability of being
729 active vs. inactive, **(B)** the probability of basking when being active, **(C)** the probability of selecting
730 a shelter in the warm side vs. in the cold side and **(D)** the probability of selecting a wet shelter in the
731 warm side vs. a dry shelter in warm side. Data and results of best models are reported for
732 measurement days without heat stress between 11:00 and 14:00 when heat stress was simulated.
733 Grey zones indicate days with heat stress. Points are means and error bars are standard errors from
734 raw data. Curves are best-predicted regression lines from the selected models (see main text) with
735 sex and water-restriction treatment as covariates. In panel **B**, we observe a decrease of basking
736 effort in water-restricted females during heat stress days that seems to become stronger with the
737 number of days of the experiment. Still, the three-way interaction between water restriction
738 treatment, sex and number of days since the beginning of the experiment was not significant ($\chi^2 =$
739 2.15, $df = 1$, $p = 0.14$). F: females, M: males, WR: water-restricted.

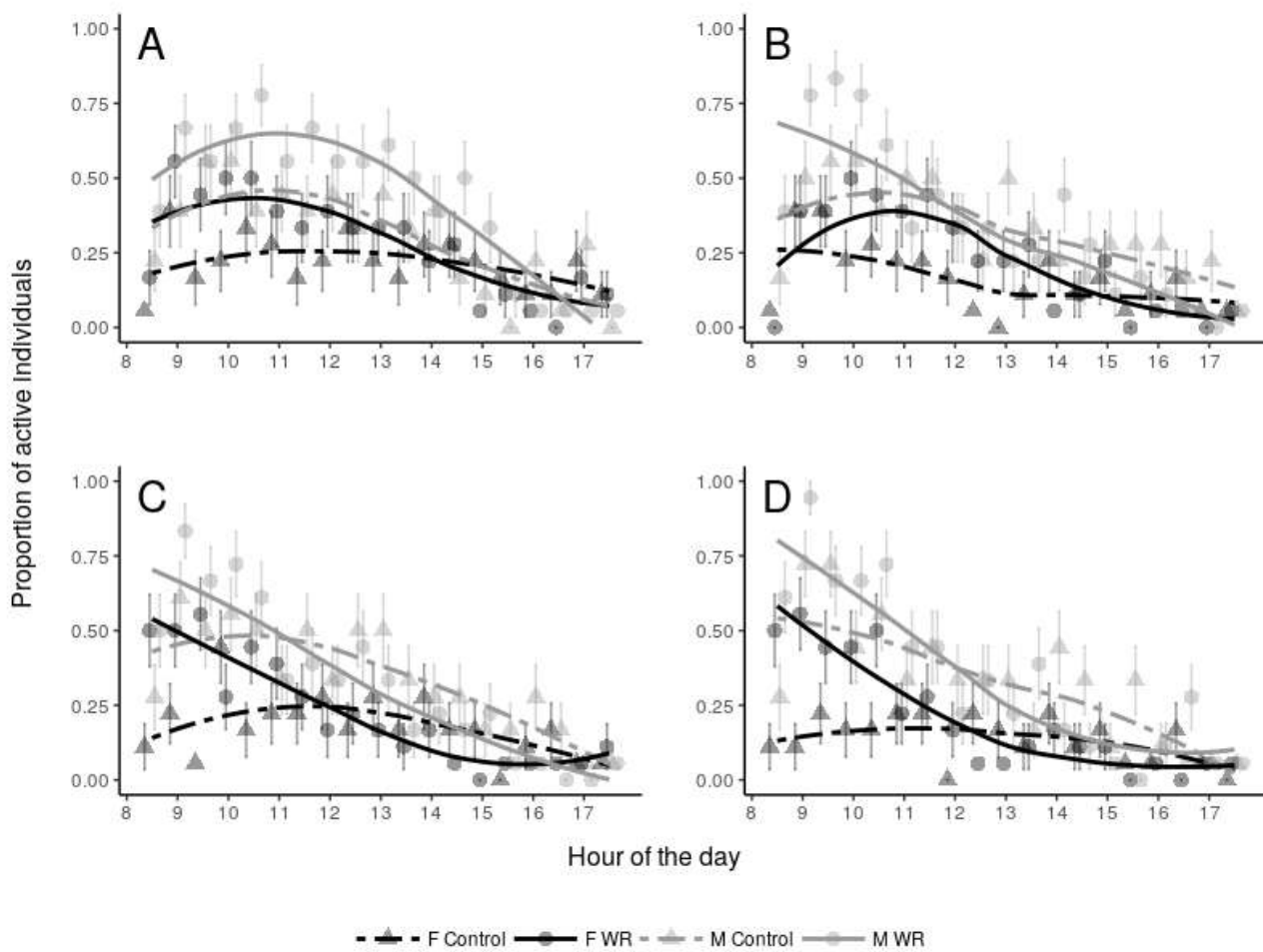
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741 **Figure 1**



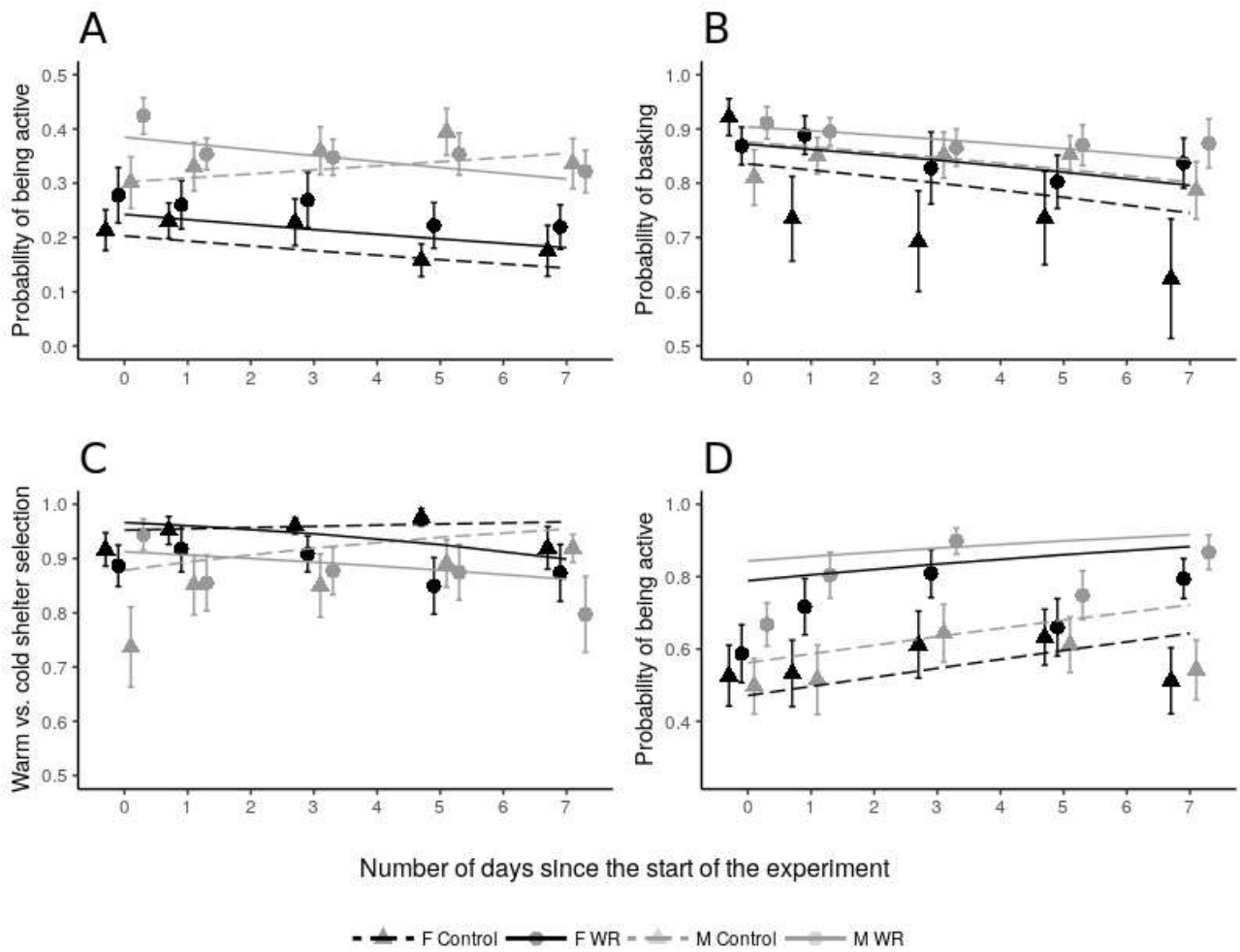
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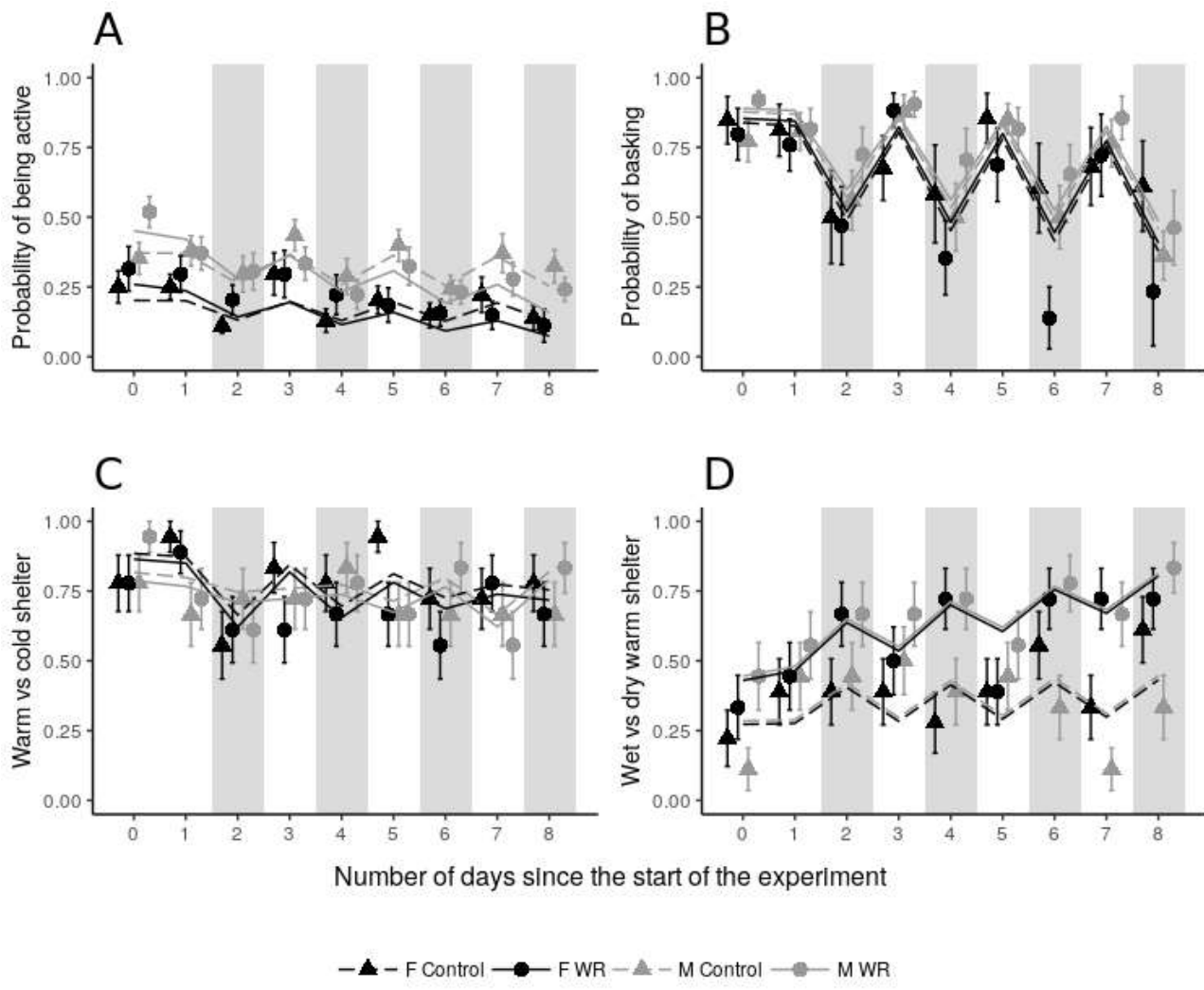
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Supplementary Information 1

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

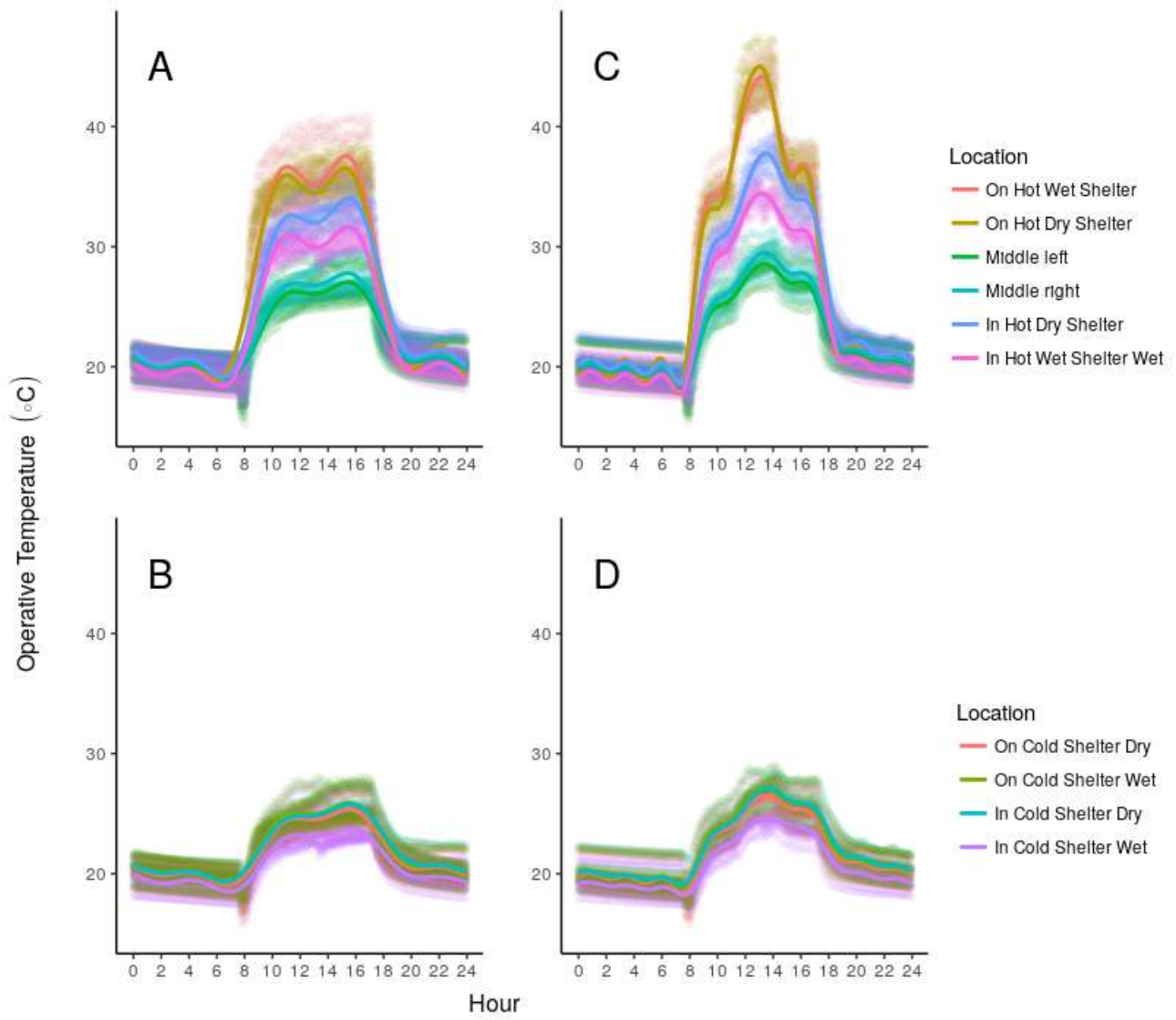
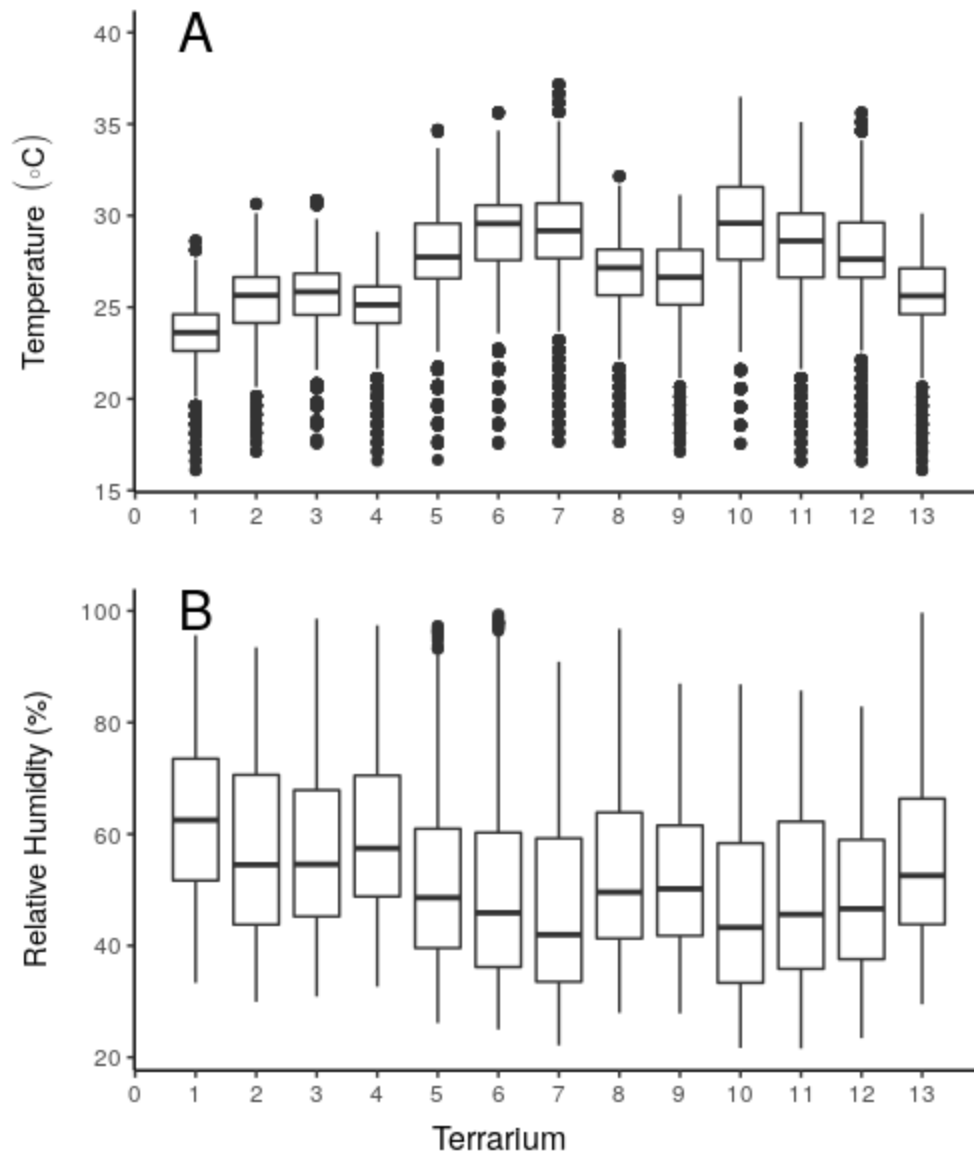


Figure S2

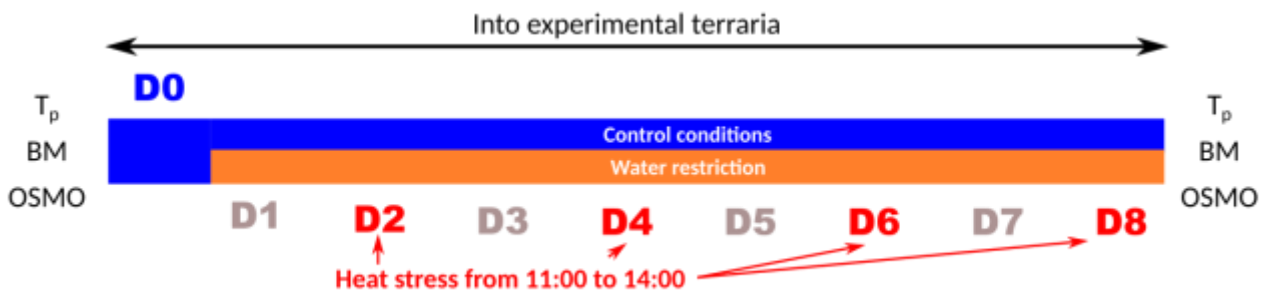


Supplementary In formation 2

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](https://doi.org/10.1086/341998).

1 **Figure S3** Chronology of the experiment for one experimental group.
2



3

4 **Table S1** Description of the results of the best models explaining activity, basking, selection of hot
 5 vs. cold shelters and selection of wet hot vs. dry hot shelters with the effects of heat stress. With χ^2
 6 the value of the chi-square test, df the number of degree of freedom associated and p is the two-
 7 sided p value.

8 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

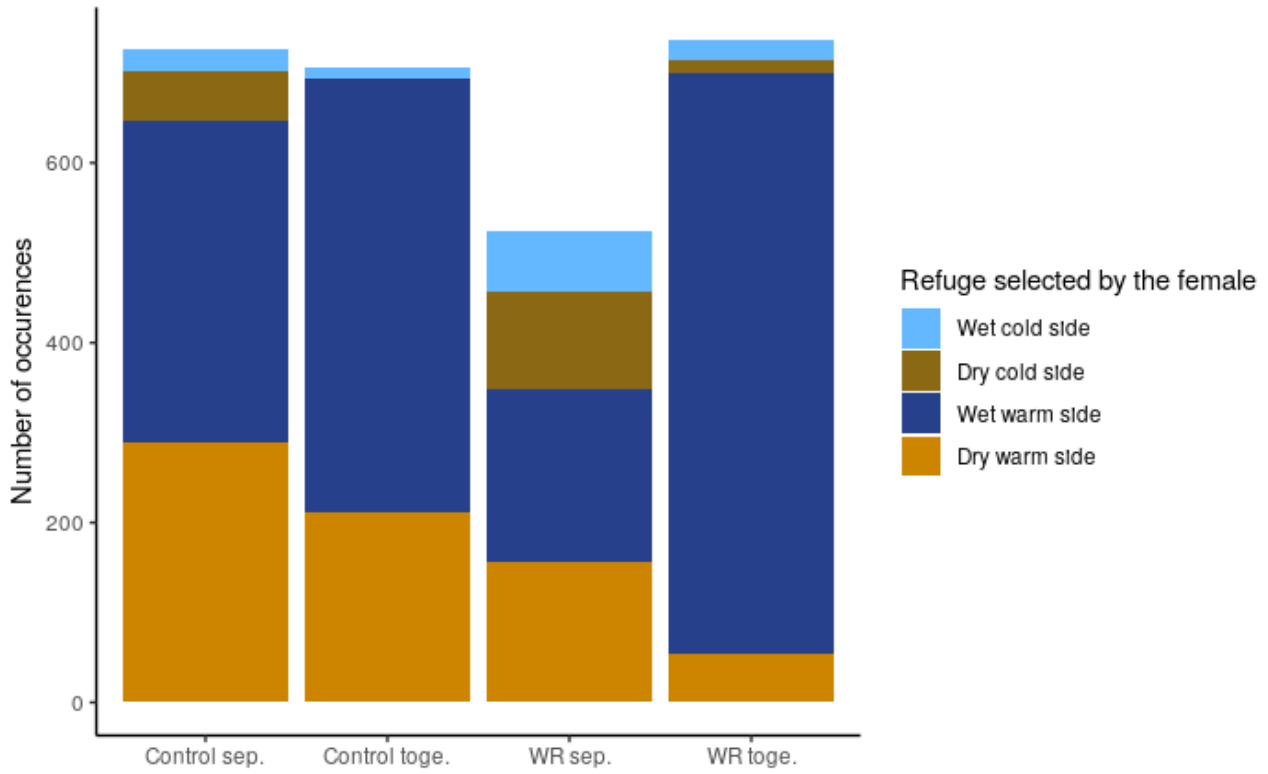
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10 **Supplementary Information 3**

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

17 The treatment did not explained significantly variations in the probability that two individuals
18 stayed together in the same shelter, we however highlighted a tendency that water-restricted
19 individuals had higher probability to be together in the same shelter than separated ($\chi^2_{1,5} = 3.6, p =$
20 0.06 , estimate \pm SE: 0.38 ± 0.20) compared to control individuals that had the same probability to
21 be together or separated (0.11 ± 0.20). It seems that in the case of water-restriction, individuals
22 remained together mainly in warm and wet shelters (Figure S4).

23 **Figure S4** Number of times we observed individuals together and in what shelter when both of
24 them were in a shelter at the same time. Colors represent the shelter selected by the female (same as
25 the males when together). Toge. = together; sep. = seperated.
26



27
28