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1 **Microhabitat humidity rather than food availability drives**
2 **thermo-hydroregulation responses to drought in a lizard**

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30 Authors’ contributions:

31 Théo Bodineau, Jean-François Le Galliard, Chloé Chabaud and Olivier Lourdais conceived
32 the ideas and designed methodology; Théo Bodineau, Chloé Chabaud, Simon Agostini,
33 Beatriz Decencière, Sandrine Meylan and Jean-François Le Galliard participated to lizard
34 captures and breeding; Théo Bodineau and Jean-François Le Galliard collected and analysed
35 the data; Théo Bodineau led the writing of the manuscript with Jean-François Le Galliard. All
36 authors contributed critically to the drafts and gave final approval for publication.

37

38

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40 The authors declare no competing or financial interests.

41

42 Data accessibility:

43 Data will be deposited in Zenodo after acceptance.

44

45

46 **Abstract**

47 1. The regulation of energy, water and thermal balance involves integrated processes that
48 should drive ecological responses of ectotherms to climate change. Functional trade-offs
49 between thermoregulation and hydroregulation are exacerbated during hot or dry spells, but
50 how microhabitat hydric properties and trophic resource availability influence these trade-offs
51 remains unknown.

52 2. Here, we investigated the effects of microhabitat humidity and food availability on thermo-
53 hydroregulation strategies in the ground-dwelling common lizard (*Zootoca vivipara*) during a
54 simulated hot and dry spell event. We exposed lizards to a five-day long acute water
55 restriction in hot conditions in the laboratory and manipulated hydric quality of the retreat site
56 (wet or dry shelter) as well as food availability (*ad libitum* food or food deprivation).

57 3. Water restriction and food deprivation caused physiological responses such as muscle
58 catabolism and mobilization of caudal energy reserves. Lizards also developed behavioural
59 strategies to conserve water or energy via decreased thermoregulation effort, higher shelter
60 use and increased eye closure behaviours through time.

61 4. These physiological and behavioural changes were importantly buffered by the presence of
62 a wet shelter but not by food availability. A wet retreat site reduced the behavioural conflicts
63 between thermoregulation and hydroregulation, allowed lizards to maintain a better condition
64 and reduced physiological dehydration. Instead, food intake did not play a major role in the
65 regulation of hydration state and increased behavioural conflicts between thermoregulation
66 and hydroregulation.

67 5. A better consideration of thermo-hydroregulation behaviours and microhabitat hydric
68 quality is required to address ectotherm responses to future climate change.

69 Keywords: behaviour, food, microhabitat, shelter, thermo-hydroregulation, trade-off, water

70 **Introduction**

71 Behavioural thermoregulation can mitigate the impact of climate warming in ectotherms
72 (Gunderson & Leal, 2015; Kearney, 2013). Recent empirical studies and mechanistic models
73 indicate that the behavioural capacity of ectotherms to buffer consequences of climate
74 warming will be constrained by water availability in the environment, which may further
75 exacerbate the deleterious effects of heat stress (Kearney et al., 2018; Rozen-Rechels et al.,
76 2020; Sannolo & Carretero, 2019). A proximate conflict exists between regulation of water
77 balance and thermoregulatory behaviours, since basking and maintenance of high body
78 temperatures typically increase water loss rates and eventually lead to dehydration (Anderson
79 & Andrade, 2017; Dmi'El, 2001). Conversely, dehydration risks can lead to water
80 conservation strategies such as reduced activity, thermal depression, diurnal or nocturnal
81 selection of cool and wet microhabitats or minimisation of time spent with the eyes open,
82 which can compromise energy acquisition, body condition and survival (e.g. Davis &
83 DeNardo, 2009; Le Galliard et al., 2021; Rozen-Rechels et al., 2020; Sannolo & Carretero,
84 2019). Despite the relevance of this functional trade-off for behavioural patterns of activity or
85 microhabitat selection, we have limited knowledge of environmental factors that influence
86 conflicts between thermoregulation and hydroregulation (Dezetter et al., 2023).

87 The availability and overall quality of microhabitats should importantly influence
88 behavioural trade-offs between thermoregulation and hydroregulation. In terrestrial
89 ectotherms, wet and cold microhabitats provide thermal shelters to escape extreme surface
90 temperatures (Beck & Jennings, 2003; Moore et al., 2018). They also reduce evaporative
91 water loss, dehydration stress and physiological alterations caused by dehydration such as
92 muscle wasting (Dezetter et al., 2023). Yet, only few studies have quantified the effects of
93 microhabitat quality on thermo-hydroregulation strategies during hot and dry weather event
94 (Dezetter et al., 2023). Food resources should also influence thermo-hydroregulation
95 strategies because investment in these behaviours should be constrained by energy intake
96 according to the cost-benefit model of thermoregulation (Angilletta Jr., 2009; Huey & Slatkin,

97 1976). Food intake could influence hydroregulation behaviours if it allows to recoup some of
98 the energetic costs of hydroregulation and because food can provide dietary water, a scenario
99 which we called the "food for water hypothesis" (Chabaud et al., 2023). This hypothesis has
100 been little tested so far in terrestrial ectotherms and some studies indicate that they are more
101 dependent on free water rather than dietary water to regulate their water balance (Chabaud et
102 al., 2023; Lillywhite, 2017; Perez et al., 2021; Wright et al., 2013).

103 Here, we designed an experiment to quantify the effects of food availability and
104 microhabitat humidity on thermo-hydroregulation strategies, as well as body condition and
105 whole-organism energetics, during a hot and dry weather event simulated in the laboratory in
106 the common lizard *Zootoca vivipara* (Lichenstein, 1823). This lacertid is found in cool and
107 moist environments and is highly sensitive to water restriction (Herczeg et al., 2003; Rozen-
108 Rechels et al., 2020). We simulated a summer drought with water restriction and manipulated
109 food availability (*ad libitum* food vs. no food) as well as the humidity of a surface shelter
110 (access to a wet shelter or to a dry shelter). In the same time, we quantified the behavioural
111 adjustments of the lizards as well as their body condition, plasma osmolality and energy-
112 related plasma metabolites. We examined the following four hypotheses and predictions.
113 First, water and food restriction should challenge homeostasis and compromise the energy and
114 water balance of lizards. We predict a decrease in body condition in response to water
115 restriction and food deprivation, an increase in plasma osmolality in response to water
116 restriction and a reduction in circulating glucose and triglycerides caused by food deprivation.
117 Second, compensatory physiological mechanisms should occur under conditions of water and
118 food restriction. We predict muscle catabolism and mobilisation of caudal lipid reserves to
119 release amino acids, lipids, and bound water and recoup some of the missing inputs of food
120 and water (Brusch et al., 2018, 2020, 2022). Third, behavioural responses should mitigate
121 water and energy constraints. We predict a decrease in selected body temperatures and an
122 increase in shelter use in response to water and food restrictions. Frequency of water

123 conservation behaviour such as eye closure behaviours should also increase in response to
124 dehydration. If wet shelter reduces water losses, lizards provided with wet shelter should use
125 it more and dehydration should be negatively correlated with the frequency of shelter use
126 (Dezetter et al., 2023). Fourth, deleterious effects of water restriction and the behavioural
127 trade-off between thermoregulation and hydroregulation should be weaker when shelters are
128 optimal (i.e., wet microclimatic conditions) and when food is available if food provides a net
129 dietary water intake.

130 **Material and methods**

131 **Study species and sampling**

132 Between April 21st and May 24th 2021, we captured 60 adult male European common lizards
133 (*Zootoca vivipara*) inside semi-natural populations maintained in outdoor enclosures at
134 CEREEP-Ecotron IleDeFrance. Lizards were captured in three sequential capture sessions to
135 generate three sequential batches of 20 lizards. Prior to experiments, we acclimated lizards for
136 a minimum of five days in standard laboratory conditions (see Rozen-Rechels et al., 2020) in
137 order to standardise habituation to laboratory conditions and food satiety.

138 **Experimental conditions and study design**

139 The experiment was performed in 20 neutral arenas lit automatically between 8:30 am and 6
140 pm by a UVB-enhanced neon tube and halogen heating bulbs in order to generate a thermal
141 gradient (operative temperatures from hot to cold spot: $35.8\text{ }^{\circ}\text{C} \pm 7.5\text{ SD}$ to $22.1\text{ }^{\circ}\text{C} \pm 2.5$
142 SD). Each arena was filled with 3 cm of peat and water-filled Petri dishes to provide lizards
143 with free-standing water. In the cold side of the arena, we installed a “shelter” that was
144 thermally sub-optimal for basking but had optimal hydric conditions or not depending on
145 treatment conditions in our study (see Supplementary figures S1 and S2). We manipulated
146 both food availability (*ad libitum* feeding with 200 ± 20 mg of crickets per day for the control
147 treatment or no feeding) and humidity of the shelter (access to a wet shelter for the control
148 treatment or access to a dry shelter) in a balanced full-factorial experimental design. Each

149 batch of 20 lizards was divided into four experimental groups formed by the factorial
150 combination of the two treatments. The study was repeated three times independently with
151 our three batches of lizards. During the course of the experiment (see Supplementary figure
152 S3), lizards spent seven successive days alone in the same arena including one day of
153 acclimation (called “day -1”) when lizards were maintained under control conditions with
154 water, *ad libitum* food, wet shelter, and three water sprays per day. During “day 0”, we
155 observed lizards under the same control conditions as during the acclimation day. At the end
156 of this first day, we removed water cups from all arenas, removed crickets for the food-
157 deprived arenas, and replaced the wet shelter with a dry shelter for dry shelter treatment
158 group. From day 1 to day 5, a limited amount of water was provided to the lizards at 8:30 am
159 by spraying arenas with water for five seconds. This manipulation was done to generate a
160 chronic, sub-lethal water restriction similar to exposure to several days of warm and dry
161 weather in natural conditions (Dupoué et al., 2020). After the last behavioural observation on
162 day 5, all individuals were provided with water and food *ad libitum*.

163 **Pre- and post-experimental measurements**

164 At the beginning and at the end of the experiment (day -1 and day 5), lizards were bled using
165 a standard protocol (see Meylan et al., 2003) to measure plasma osmolality, circulating
166 triglyceride and glucose levels in plasma in order to quantify metabolic changes during the
167 experiment. Plasma osmolality (an indicator of hydration status in species lacking a salt gland
168 which increases in dehydrated individuals) was measured using a Vapro® vapour pressure
169 osmometer and circulating triglyceride and glucose levels were measured in plasma by
170 colorimetric assays (Triglyceride Colorimetric Assay kit and Glucose Colorimetric Assay kit,
171 ref. 10010303 and 10009582, Cayman Chemical, USA). At the beginning (day -2) and at the
172 end of the experiment (day 5), lizards were weighed using a precision scale (PX 323 Pioneer,
173 OHAUS Corp., USA) and scanned using a flatbed scanner (CanoScan LiDE 700F, Canon) to
174 measure tail width at the 9th sub-caudal scale row using ImageJ software (Version 1.52v), as a

175 proxy for tail reserves. Hind limb thickness was measured to the nearest 0.01 mm on both legs
176 using a spessimeter (ID-C1012BS, Mitutoyo, Japan).

177 **Behavioural measurements**

178 We recorded body temperature, basking behaviours (increased exposure to radiation from
179 heat bulbs or not) and the position of lizard (inside or outside the shelter) for 6 days (day 0 to
180 day 5) with an instantaneous sampling every 30 minutes from 9 am to 5 pm (i.e., 17 readings
181 during the day). Surface body temperature was recorded using an infrared thermometer
182 (Raytek, Raynger MX2). We then calculated body temperature T_b using a calibration curve
183 (see Supplementary figure S4). When the lizard was visible, we noted whether its eyes were
184 open or closed. When the lizard was not visible, the roof of the shelter was slowly lifted to
185 note the presence or not of the lizard in the shelter.

186 **Statistical analyses**

187 Analyses were performed with R software version 4.2.0 (R Core Team, 2022). We used
188 mixed models to control for the fully randomised design of our experiment where we
189 attributed different individuals from different treatments to the same arena three times (three
190 experimental batches). We also controlled for inter-individual variability by including a
191 random effect of individual identity. For each analysis, we built a full model, checked its
192 assumptions and performed a goodness-of-fit test. Non-significant effects were then
193 eliminated using a stepwise model selection procedure starting from the full model. First, we
194 calculated intra-individual changes in body mass, in average limb thickness, in tail width, in
195 plasma osmolality, in triglyceride and in glucose concentration by subtracting the final values
196 measured after the experiment from the initial values measured before the experiment. To
197 explain these morphological and physiological changes, we fitted linear mixed models
198 (Pinheiro et al., 2012) with experimental batch, the initial centred value of the morphological
199 or physiological trait and interactive effects of the two experimental treatments as fixed
200 effects. For the analysis of the change in plasma osmolality, we added the individual-centred

201 daytime shelter use rate during the experiment (calculated as the number of observations
202 where the lizard was in the shelter divided by the total number of daytime observations during
203 the experiment) in interaction with the shelter and food treatments as fixed effects to test the
204 relationship between shelter use and dehydration. To study thermo-hydroregulation
205 behaviours, we counted the number of times each day out of 17 observations where lizards
206 exhibited basking behaviour, when they closed their eyes, and when they were inside the
207 shelter. We analysed basking, eye closure behaviour and shelter use as success-failure events
208 with generalised linear mixed models assuming a binomial distribution and a logit link using
209 the *glmer* function from the *lme4* package (Bates et al., 2014). T_b was analysed using linear
210 mixed models. We analysed these four behavioural traits with the experimental batch and the
211 factorial, linear or quadratic effects of time (number of days since start of the experiment) in
212 interaction with the shelter and food treatments as fixed effects.

213 **Results**

214 All lizards lost condition during the experiment, as manifested by a decrease of body mass,
215 limb thickness and tail width (Table 1). Mass, limb thickness and tail width losses were
216 additively influenced by shelter treatment and food treatment. Food restricted lizards and
217 lizards provided with dry shelter lose more body mass, limb thickness and tail thickness than
218 others. We also found an overall increase in plasma osmolality for all experimental groups
219 indicating strong physiological dehydration ($+57.09 \text{ mOsmol.kg}^{-1} \pm 5.6 \text{ SE}$). Dehydration was
220 influenced by the interaction between shelter use rate and shelter treatment but food treatment
221 did not affect dehydration. On average, dehydration was greater in lizards provided with a dry
222 shelter compared to a wet shelter. Surprisingly, dehydration was positively correlated with
223 daily shelter use in lizards provided with a wet shelter, whereas it was not correlated in lizards
224 provided with a dry shelter (see Figure 1B). In addition, fed lizards showed a significant
225 increase in plasma glucose concentration relative to food-deprived lizards, and a greater
226 increase in circulating plasma triglyceride concentration was observed in fed than food-

227 deprived lizards and also to some extent in lizards from the dry shelter group than from the
228 wet shelter group (Table 1).

229 During water restriction, daytime shelter use increased and was influenced by the
230 interaction between time, shelter treatment and food treatment (Figure 1A). In food-deprived
231 animals, daytime shelter use increased similarly in dry and wet shelter treatment groups,
232 whereas in fed lizards, daytime shelter use increased more strongly in lizards provided with a
233 dry shelter. Average daily T_b and basking behaviours of lizards decreased through time as the
234 number of days of water restriction increase but these behavioural changes were only
235 influenced by the shelter treatment (Figure 2A, B, C, D). The diminution of T_b and basking
236 behaviours was more pronounced in lizards provided with a dry shelter. Finally, we found
237 strong effects of shelter treatment in interaction with time on the ocular behaviour of lizards.
238 Eye closure rate increased remarkably more in lizards provided with a dry shelter compared to
239 lizards with a wet shelter (Figure 2E).

240 **Discussion**

241 During the experiment, all lizards showed significant increase in plasma osmolality which
242 indicates physiological dehydration (Peterson, 2002; Rozen-Rechels et al., 2020) and the
243 food-deprived group had lower circulating triglyceride concentrations and blood glucose
244 levels suggesting fasting patterns consistent with previous studies on lipid or glucose
245 metabolism in reptiles (McCue, 2007; Moon et al., 1999; Price, 2017). Concurrently, water
246 and food restrictions led to reductions in body mass, hind limb width, and tail width. In snakes
247 and birds, muscle wasting from the tail and limbs is caused by protein catabolism and is
248 associated with a significant release of protein-bound water to improve the water status during
249 water restriction (Brusch et al., 2018; Gerson & Guglielmo, 2011). In lizards, the tail is also a
250 reserve organ consisting mostly of muscle and lipids stored as triglycerides (Avery, 1974;
251 Price, 2017). Thus, our data suggest that fasting and dehydration caused mobilisation of

252 caudal lipid reserves and muscle wasting to support water, energy, and amino acid
253 requirements when water and dietary intakes are insufficient (Cherel et al., 1992).

254 At the same time, lizards developed behavioural strategies during the water restriction
255 period including a lower basking effort, a lower body temperature and a higher eye closure
256 rate, and those responses were more strongly expressed in the dry shelter treatment. Our study
257 is the first experimental demonstration of an adjustment of eye closure behaviour in response
258 to hydric stress. In dry-skinned ectotherms with an eyelid, eye closure behaviour should play
259 an important role in organismal hydroregulation because the eye surface is poorly resistant to
260 water loss and may represent a significant proportion of the body surface (Pirtle et al., 2019;
261 Waldschmidt & Porter, 1987). In addition, eye opening is important for predators' detection
262 and visual interactions with conspecifics so hydric stress may decrease vigilance and increase
263 risks of predation. Altogether, these behavioural responses indicate a trade-off between heat
264 and water balance regulation mediated by the hydric costs of thermoregulation (Rozen-
265 Rechels et al., 2020). Hydric quality of the shelter rather than food availability was the main
266 modulator of these thermo-hydroregulation strategies and an important buffer against the
267 deleterious effects of water restriction. Presence of a wet shelter at day and night limited very
268 strongly dehydration, muscle and lipid reserves mobilisation, thermal depression and the
269 increase of eye closure rate, which can play an important role in organismal hydroregulation
270 (Pirtle et al., 2019; Waldschmidt & Porter, 1987). These findings highlight the importance of
271 shelter and wet microhabitat quality in the behavioural responses of ectotherms to future
272 changes in climate conditions (Beck & Jennings, 2003; Moore et al., 2018).

273 Contrary to earlier studies (Dezetter et al., 2023; Pintor et al., 2016), lizards with
274 access to a wet shelter did not use it more often than the ones with a dry shelter and we found
275 a positive relationship between individual dehydration state and the daytime wet shelter use
276 rate, whereas we predicted the opposite relationship since wet shelter should reduce
277 evaporative water loss rates. These results suggest condition-dependent wet shelter use, for

278 example if lizards decided to use the wet shelter below a certain dehydration level, and
279 highlight the potential thermoregulatory costs of hydroregulation behaviours. Behavioural
280 selection of a wet microclimate inside the shelter allowed lizards to maintain a better
281 hydration state more efficiently but also involved the selection of a thermally suboptimal
282 microhabitat. Thus, wet shelter use had a strong opportunity and energetic cost because it was
283 detrimental to thermoregulatory activities essential for energy intake such as prey capture and
284 assimilation rates (Van Damme et al., 1991), which potentially explains why this behaviour
285 was less frequent in weakly dehydrated individual lizards (which privileged thermoregulation
286 activities) than in strongly dehydrated individuals (which privileged hydroregulation
287 activities). On the other hand, individual dehydration state was not correlated with the
288 daytime dry shelter use rate because a dry shelter does not effectively reduce water losses and
289 dehydration contrary to a wet shelter.

290 Food availability did not influence mean daily thermoregulation effort contrary to the
291 predictions of the cost-benefit model of thermoregulation and results of some earlier studies
292 (Brown & Griffin, 2005; Huey & Slatkin, 1976). Thus, common lizards have limited
293 behavioural acclimation responses to energy imbalance, maybe because fasting was too short
294 or because physiological responses to fasting were prioritized over behavioural responses.
295 Finally, in accordance to a recent study (Chabaud et al., 2023), none of our findings support
296 the “food for water hypothesis” because *ad libitum* food conditions did not reduce
297 dehydration nor the morphological and behavioural impacts of water restriction. On the
298 contrary, shelter use in the presence of dry shelter and *ad libitum* food was more frequent,
299 which suggests that food consumption and processing require a good hydration status and thus
300 food availability increases reliance on free standing water, as suggested for several snake
301 species and a carnivorous lizard (Lillywhite, 2017; Murphy & DeNardo, 2019; Wright et al.,
302 2013). Therefore, our experiment indicates that food acquisition can exacerbate the conflict
303 between thermoregulation and hydroregulation, most likely because behavioural optimisation

304 of food capture, processing and digestion implies behavioural selection of elevated body
305 temperatures or increased basking effort, which are water costly thermoregulation behaviours.
306 An alternative explanation is that feeding itself increases water requirements or that prey
307 capture and digestion requires a good hydration state as seen in some carnivorous snakes
308 (Lillywhite, 2017). Thus, food-deprived lizards use dry and wet shelters similarly, whereas in
309 the presence of food, lizards with a dry shelter must spend more time inside the shelter to
310 regulate their hydration state than lizards with a wet shelter.

311 **Conclusions**

312 Our study challenges previous studies that have only focused on the effects of temperature or
313 water availability on thermoregulatory behaviours of ectotherms. Food intake did not play a
314 major role in regulating the hydration status of this insectivorous species but food intake
315 influenced water balance regulation by increasing a behavioural trade-off between
316 thermoregulatory and hydroregulatory activities. Our findings demonstrate the urgent need to
317 take into account the dual effect of warming and drought events, behavioural interactions
318 between thermoregulation and hydroregulation and the buffering role of microclimatic
319 conditions to understand the ecological and evolutionary responses of ectotherms to climate
320 change (Moore et al., 2018).

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464 **Table 1.** Summary statistics of morphological and physiological changes in 60 males *Zootoca*
465 *vivipara* from the four treatment groups (n = 15 per group). Morphological and physiological
466 changes were calculated by subtracting the initial value measured at the beginning of the
467 experiment from the final value measured at the end of the experiment. Shelter and food
468 treatments had an additive effect on change in body mass ($F_{1,33} = 82.95$, $p < 0.0001$; $F_{1,33} =$
469 69.41 , $p < 0.0001$ respectively), in limb thickness ($F_{1,37} = 18.15$, $p = 0.0001$; $F_{1,37} = 10.15$, $p =$
470 0.003), in tail width ($F_{1,36} = 9.96$, $p = 0.003$; $F_{1,36} = 6.39$, $p = 0.02$) and in the concentration of
471 circulating triglycerides ($F_{1,33} = 4.61$, $p = 0.04$; $F_{1,33} = 152.5$, $p < 0.0001$). Circulating glucose
472 concentrations were influenced by food treatment ($F_{1,33} = 14.64$, $p = 0.0005$) but not by shelter
473 treatment ($F_{1,33} = 0.13$, $p = 0.71$). Data are provided as mean (\pm SE) for each treatment group
474 and different letters indicate significant differences between groups ($p < 0.05$; Tukey's HSD
475 post hoc test).

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Treatment groups	Wet shelter		Dry shelter	
	Food	Food deprivation	Food	Food deprivation
Variables				
Δ body mass (g)	-0.22 ± 0.05^a	-0.65 ± 0.05^b	-0.67 ± 0.05^b	-1.04 ± 0.05^c
Δ limb thickness (mm)	-0.07 ± 0.02^a	-0.14 ± 0.02^{ab}	-0.15 ± 0.02^{bc}	-0.21 ± 0.02^c
Δ tail width (mm)	-0.14 ± 0.11^a	-0.43 ± 0.11^{ab}	-0.47 ± 0.11^{ab}	-0.68 ± 0.11^b
Δ [triglycerides] (mg.dL ⁻¹)	256.4 ± 28.9^a	-57.2 ± 28.5^b	378.3 ± 29.7^c	-47.8 ± 29.6^b
Δ [glucose] (mg.dL ⁻¹)	49.5 ± 11.0^{ab}	24.1 ± 11.0^b	71.8 ± 11.0^a	8.22 ± 11.8^b

477 Δ = intra-individual change

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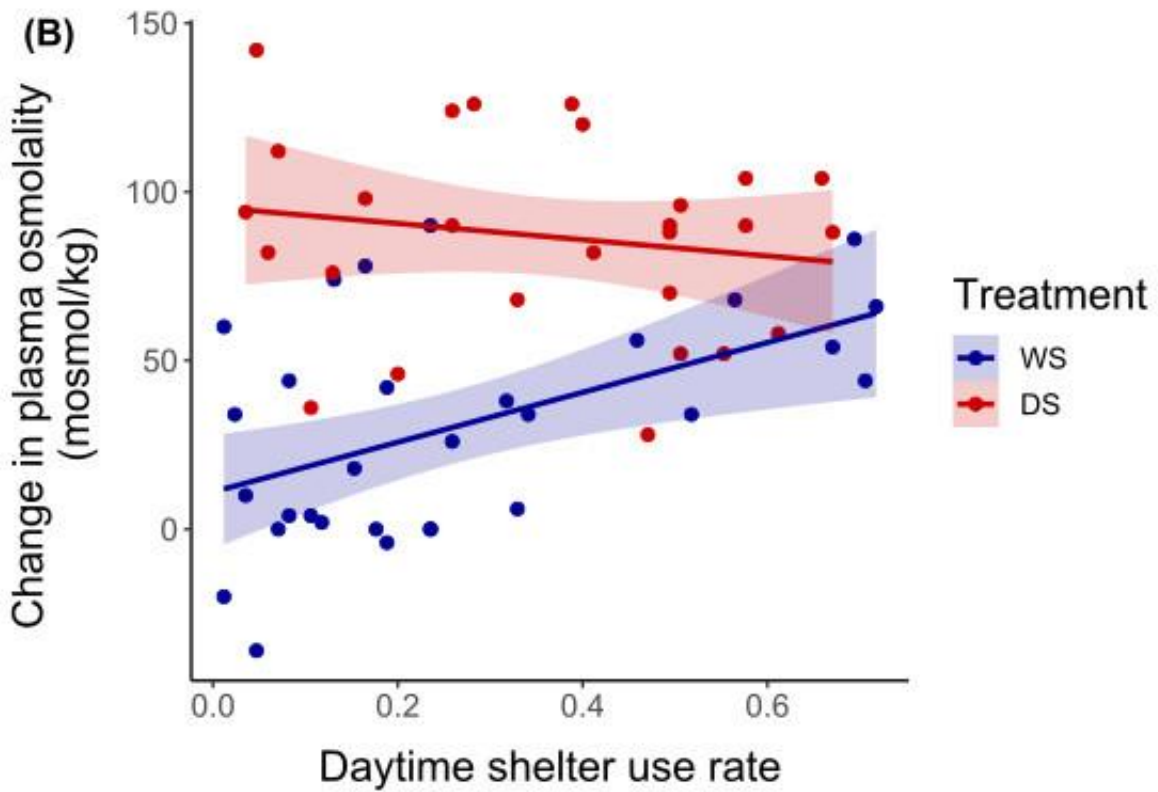
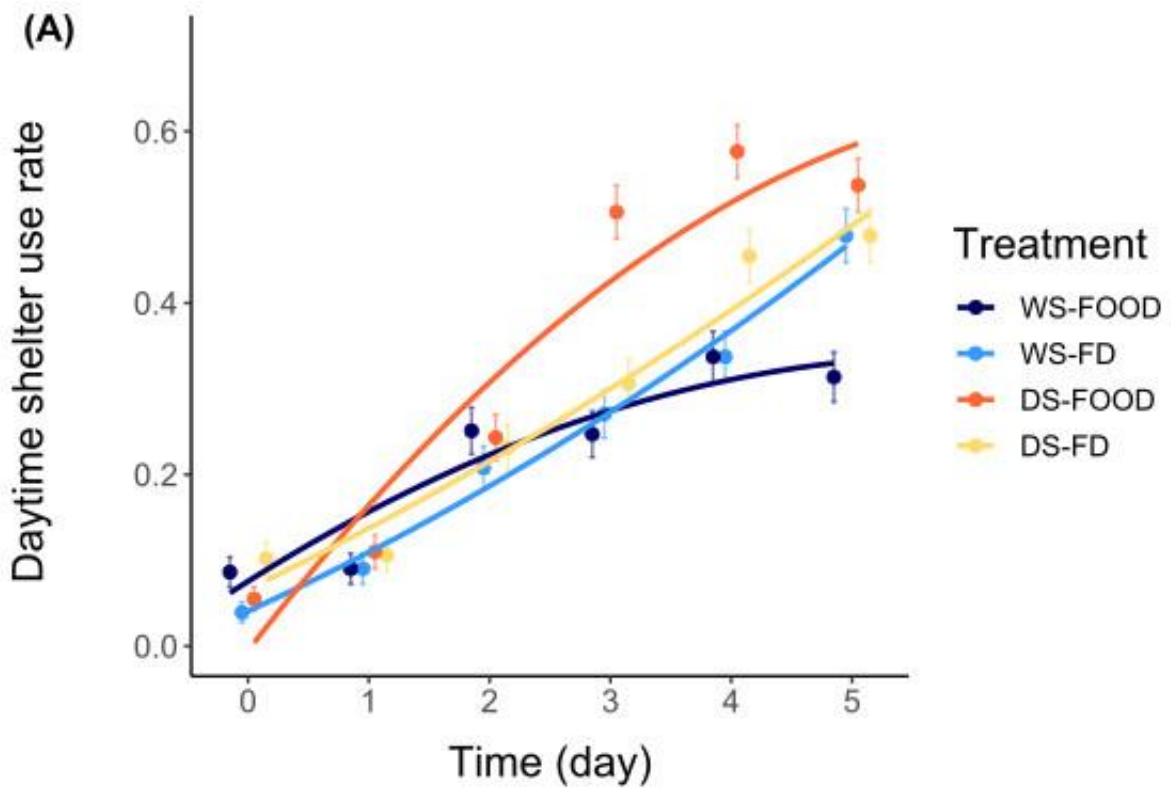
480 **Figure legends**

481 **Figure 1.** A. Interactive effects of shelter and food treatment on daytime shelter use as a
482 function of time (number of days since the start of the experiment). B. Plasma osmolality
483 change during the experiment as a function of daytime shelter use and shelter treatment.
484 Colours in panel A represent experimental groups formed by the factorial combination of the
485 two treatments (WS-FOOD (dark blue): wet shelter and *ad libitum* feeding, WS-FD (light
486 blue): wet shelter and food deprivation, DS-FOOD (orange): dry shelter and *ad libitum*
487 feeding, DS-FD (yellow): dry shelter and food deprivation). Points represent average daily
488 shelter use (\pm SE) and solid lines represent the average shelter use adjusted with a quadratic
489 time effect. We found a significant effect of the interaction between time, shelter treatment
490 and food treatment on shelter use ($\chi^2_1 = 8.68$, $p = 0.003$). Colours in (B) represent lizards with
491 access to wet shelter (WS (dark blue)) or dry shelter (DS (red)). Points represent individual
492 shelter use score during the experiment and a positive change in osmolality indicates
493 physiological dehydration. The change in plasma osmolality was influenced by the interaction
494 between shelter use rate and shelter treatment ($F_{1,30} = 4.44$, $p = 0.04$) independently of food
495 treatment ($F_{1,30} = 2.27$, $p = 0.14$). Dehydration was positively correlated with daytime shelter
496 use in lizards with access to wet shelter ($F_{1,10} = 7.13$, $p = 0.02$) but it was not correlated in
497 lizards with access to dry shelter ($F_{1,8} = 3.68$, $p = 0.09$). Daytime shelter use rates were
498 calculated as the number of observations where the lizard was in the shelter divided by the
499 total number of daytime observations of the day in (A) or of the experiment in (B). Water
500 restriction begins on day 1.

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507 **Figure 2.** Effects of experimental treatments on thermo-hydroregulation behaviours of male
508 *Zootoca vivipara*. Mean body temperature (A, B), mean basking rate (C, D), and eye closure
509 rate (E) of lizards as a function of day and experimental treatment (WS: wet shelter, DS: dry
510 shelter, FOOD: food *ad libitum*, FD: food deprivation). Points and error bars represent
511 temperature, basking rate, and average daily eye closure rate of lizards (\pm SE). Basking and
512 eye closure rates was calculated as the number of observations where the lizard exhibited
513 basking behaviour or had its eyes closed divided by the total number of observations of the
514 day. Water restriction begins on day 1. Shelter treatment in interaction with time significantly
515 influenced mean body temperature ($F_{1,2514} = 11.04$, $p < 0.0001$), mean basking rate ($\chi^2_5 =$
516 45.35 , $p < 0.001$), and eye closure rate ($\chi^2_5 = 17.05$, $p < 0.001$) in contrast to food treatment (p
517 > 0.05 for all).

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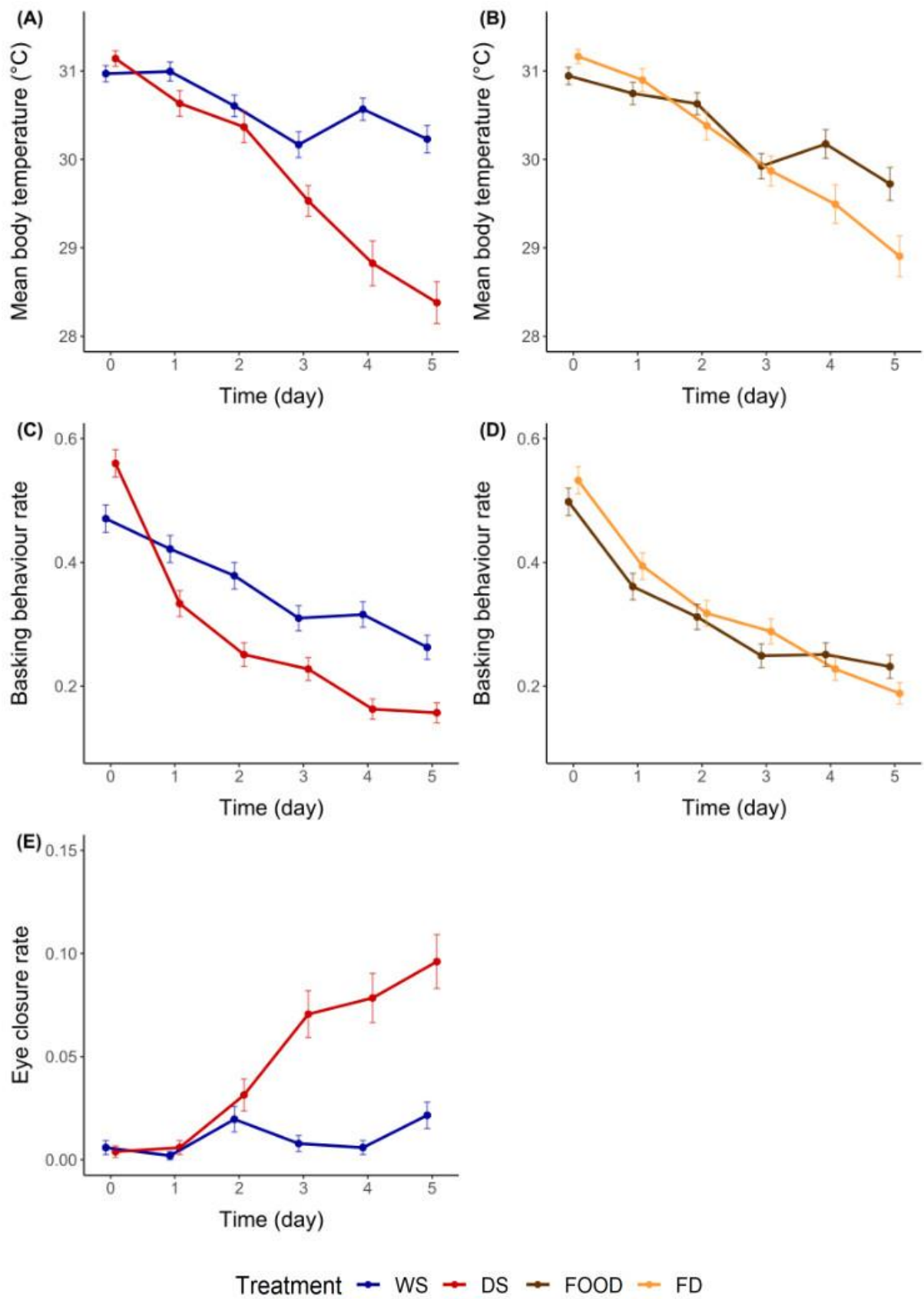
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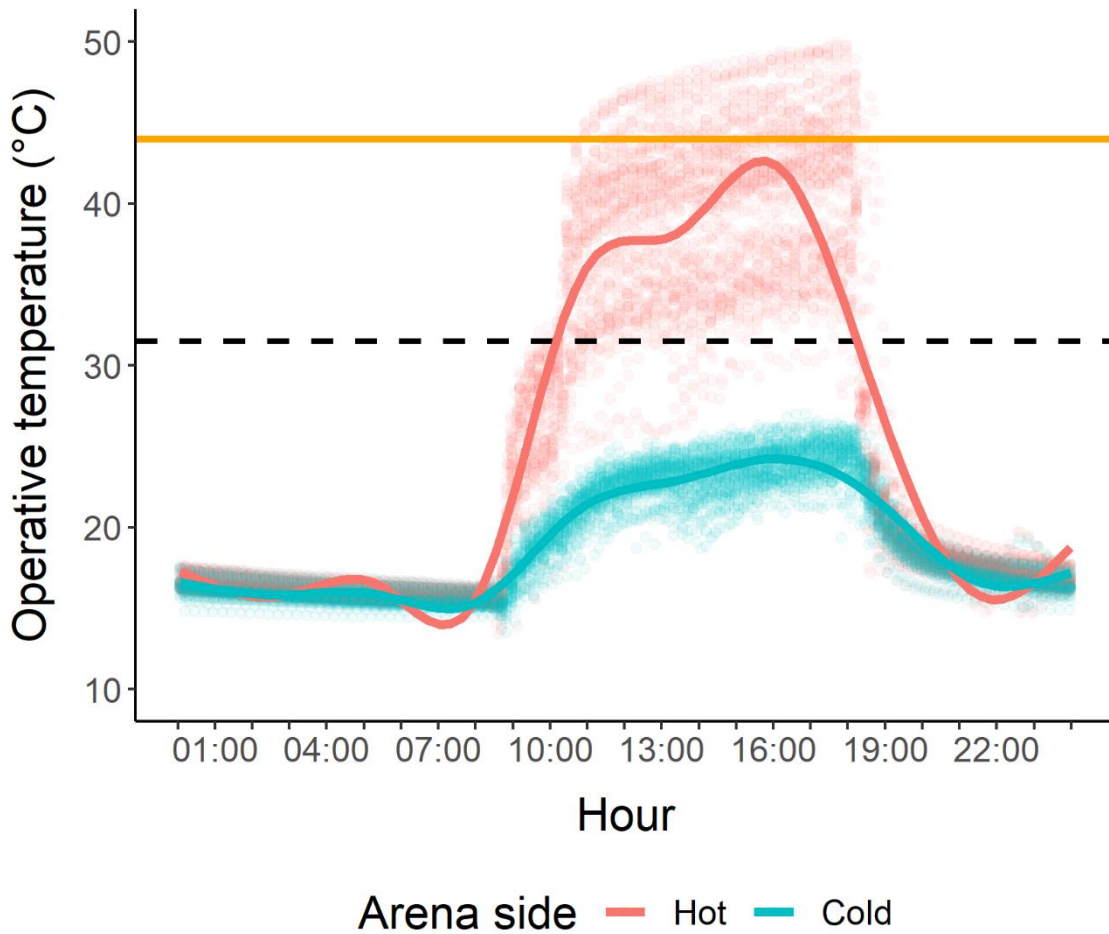
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530 **Supplementary Information**

531 **Figure S1**

532 Daily variation of operative temperature on the hot and cold side of the thermal gradient. Jittered
533 points correspond to raw operative temperature values. Smoothing with a generalized additive
534 model was applied to visualize the nonlinear patterns (solid blue and red line). Thermal
535 preferences (black dashed line) and upper critical thermal limit (orange solid line) of *Zootoca*
536 *vivipara* (Gvoždík & Castilla, 2001) are represented.



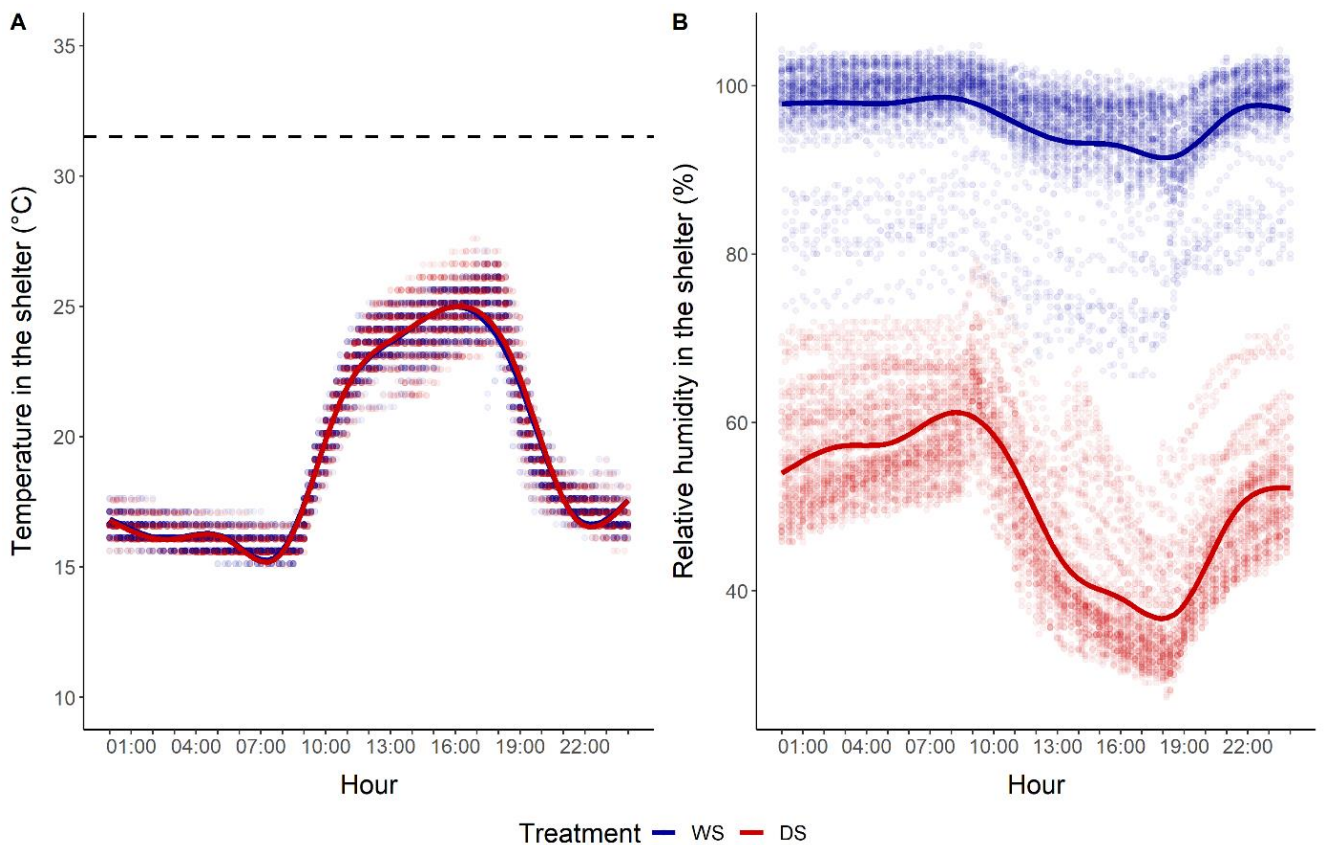
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539 **Figure S2**

540 Daily variation of temperature (A) and relative humidity (B) in shelters as a function of shelter
541 treatment (WS: wet shelter, DS: dry shelter). Jittered points correspond to raw values, red and
542 blue lines are predictions from a generalized additive model. Thermal preference of *Zootoca*
543 *vivipara* (Gvoždík & Castilla, 2001) is represented by a black dashed line. Mean temperature
544 in the shelter did not differ significantly between dry and wet treatments ($F_{1,16123} = 2.28$, $p =$
545 0.95).

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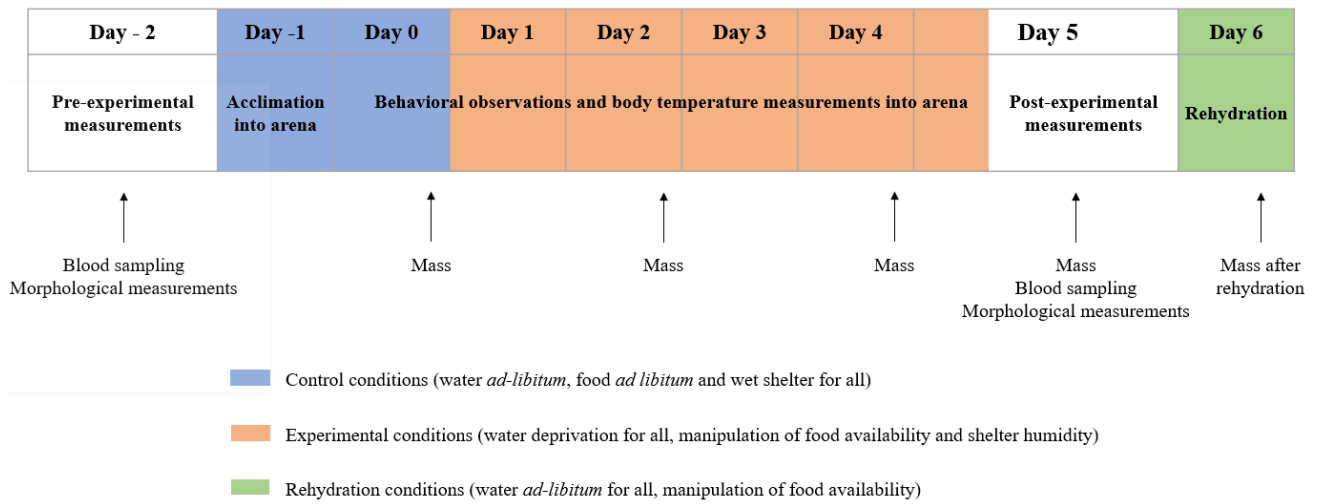
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551 **Figure S3**

552 Chronology of the experiment for each experimental batch.



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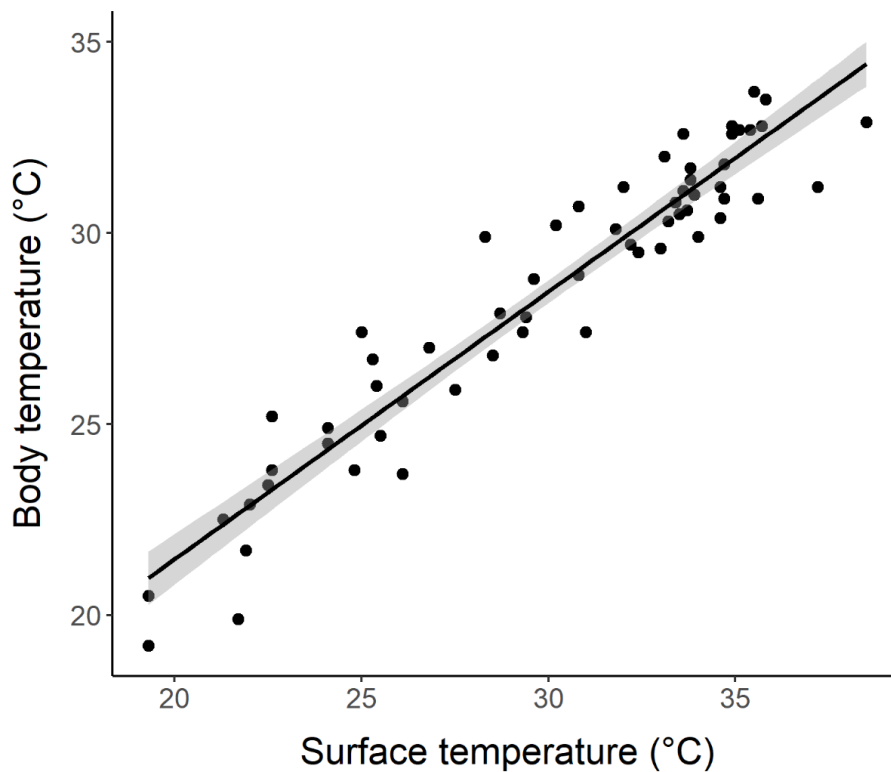
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570 **Figure S4**

571 Relationship between internal body temperature measured with a K-type thermocouple
572 inserted in the cloaca and surface body temperature measured with an infrared thermometer.
573 Surface body temperatures and cloacal temperatures were measured consecutively for each
574 individual using an infrared thermometer (Raytek, Raynger MX2) and a K-type thermocouple
575 (HI 935005N, Hanna) after the last behavioural record of day 0. The linear regression (with
576 95% confidence interval) is plotted against raw data. Cloacal temperatures were strongly
577 correlated with surface temperatures ($N=60$, $T_b = 7.46 (\pm 0.90) + 0.70 (\pm 0.03) \times T_{IR}$, $R^2 =$
578 0.91).



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