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## ► To cite this version:

Théo Bodineau, Chloé Chabaud, Beatriz Decencière, Simon Agostini, Olivier Lourdais, et al.. Microhabitat humidity rather than food availability drives thermo-hydroregulation responses to drought in a lizard. *Oikos*, 2024, 2024 (6), 10.1111/oik.10535 . hal-04738801

HAL Id: hal-04738801

<https://cnrs.hal.science/hal-04738801v1>

Submitted on 15 Oct 2024

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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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13

14 Running title: Effects of microhabitat quality and food availability on thermo-hydroregulatory  
15 strategies of a dry-skinned ectotherm

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22 Acknowledgement:

23 The authors thank Damien Lopez Santin and Anouk Pellerin who contributed to behavioural  
24 and physiological data acquisition. This work was supported by an Agence Nationale de la  
25 Recherche grant (ANR-17-CE020013, ‘AQUATHERM’) to J.-F. Le Galliard. Théo  
26 Bodineau’ PhD grant is funded by the Ministère de l’Enseignement Supérieur et de la  
27 Recherche. This work has benefited from human and technical resources provided by the  
28 CEREEP-Ecotron IleDeFrance (CNRS/ENS UMS 3194).

29

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

39 Conflict of Interest:

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 21<sup>st</sup> and May 24<sup>th</sup> 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  $200 \pm 20$  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 9<sup>th</sup> 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   |
| <b>Variables</b>                                |                      |                       |                       |                    |
| $\Delta$ body mass (g)                          | $-0.22 \pm 0.05^a$   | $-0.65 \pm 0.05^b$    | $-0.67 \pm 0.05^b$    | $-1.04 \pm 0.05^c$ |
| $\Delta$ limb thickness (mm)                    | $-0.07 \pm 0.02^a$   | $-0.14 \pm 0.02^{ab}$ | $-0.15 \pm 0.02^{bc}$ | $-0.21 \pm 0.02^c$ |
| $\Delta$ tail width (mm)                        | $-0.14 \pm 0.11^a$   | $-0.43 \pm 0.11^{ab}$ | $-0.47 \pm 0.11^{ab}$ | $-0.68 \pm 0.11^b$ |
| $\Delta$ [triglycerides] (mg.dL <sup>-1</sup> ) | $256.4 \pm 28.9^a$   | $-57.2 \pm 28.5^b$    | $378.3 \pm 29.7^c$    | $-47.8 \pm 29.6^b$ |
| $\Delta$ [glucose] (mg.dL <sup>-1</sup> )       | $49.5 \pm 11.0^{ab}$ | $24.1 \pm 11.0^b$     | $71.8 \pm 11.0^a$     | $8.22 \pm 11.8^b$  |

477  $\Delta$  = intra-individual change

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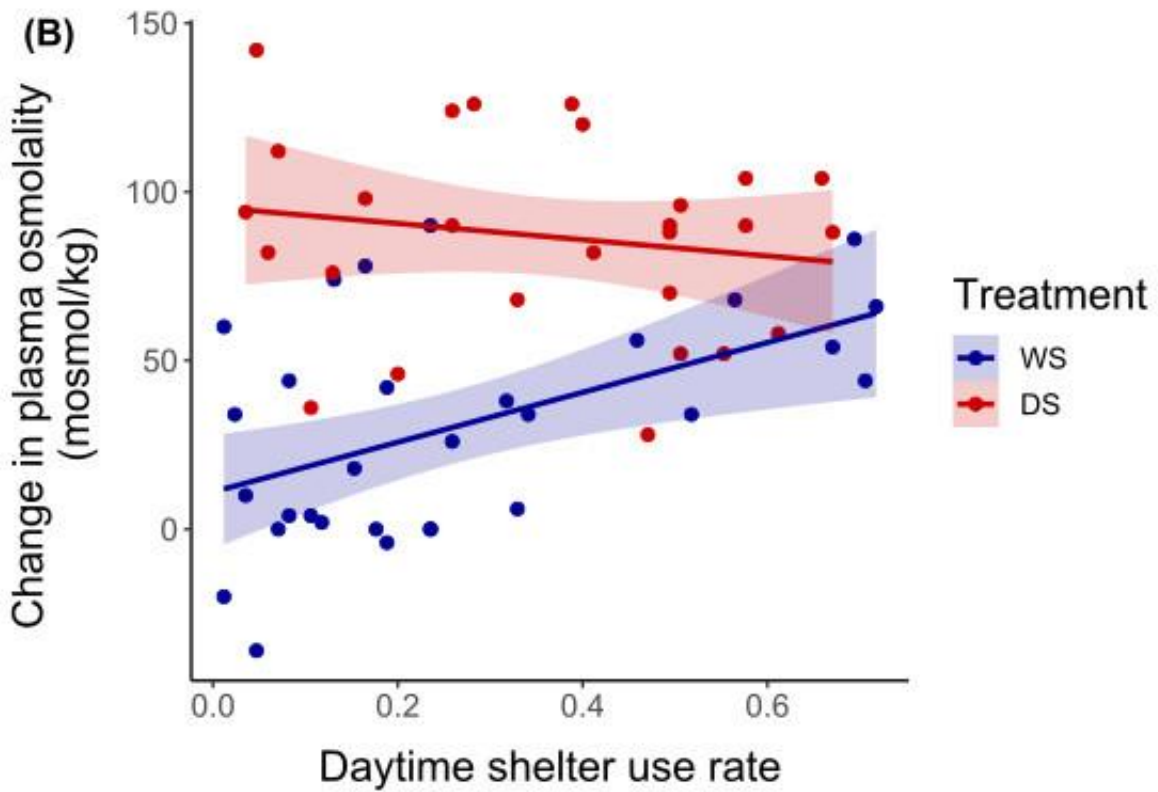
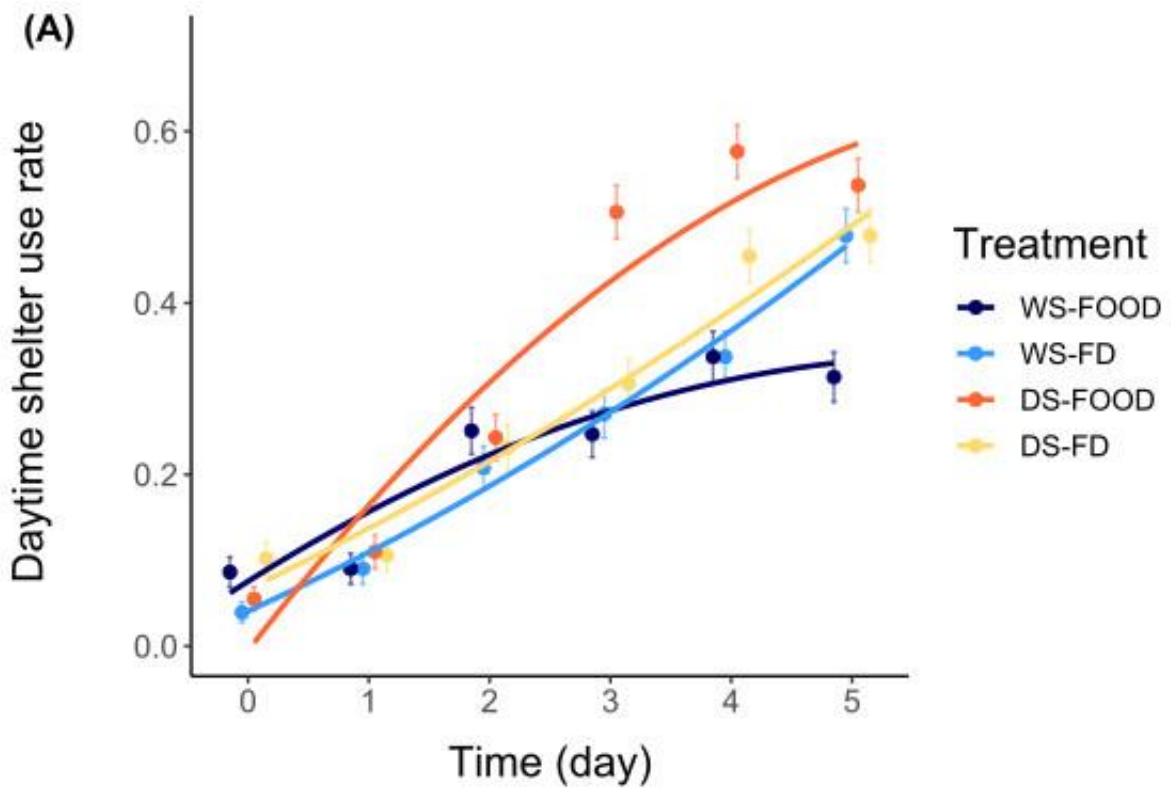
## 480 **Figure legends**

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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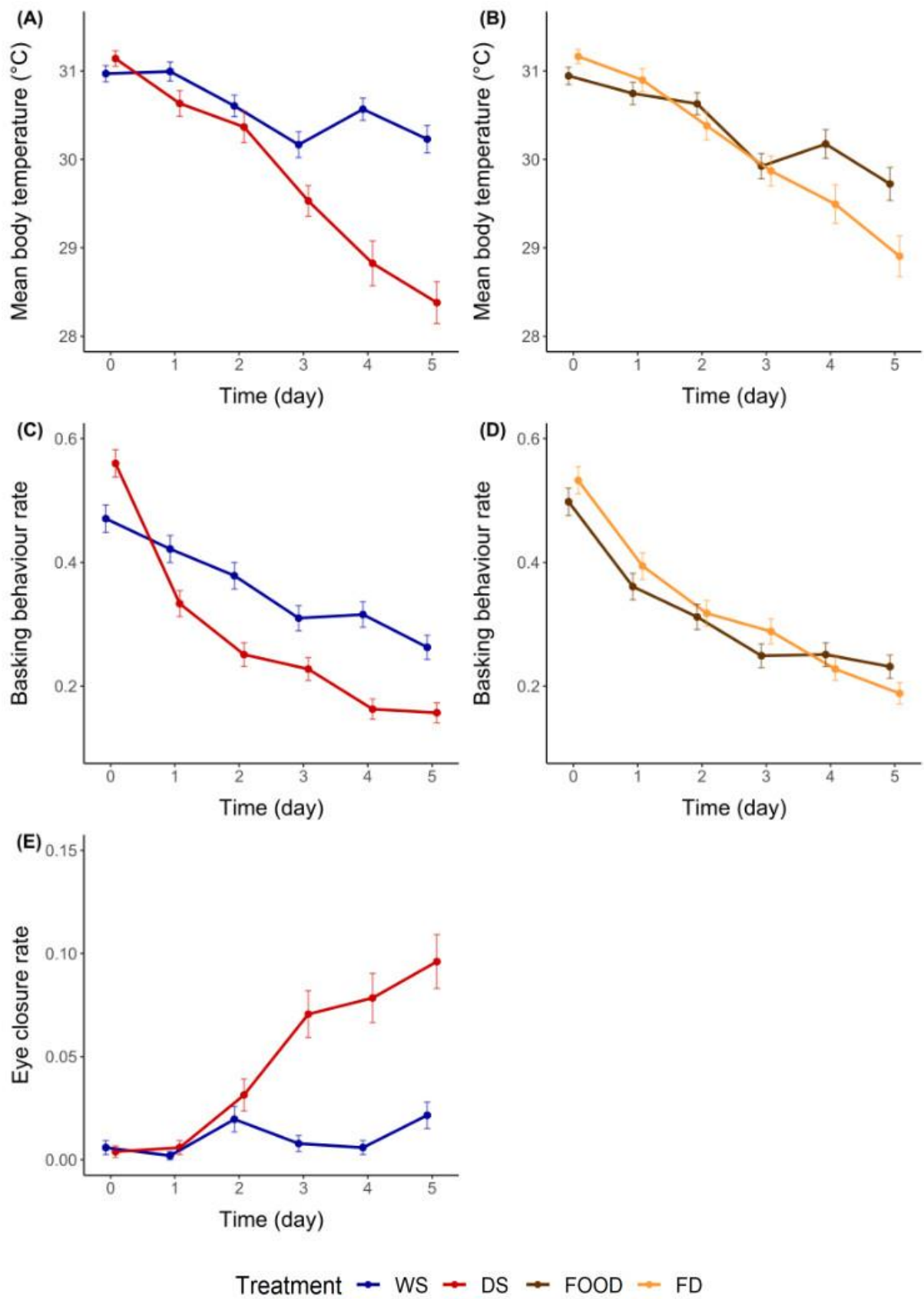
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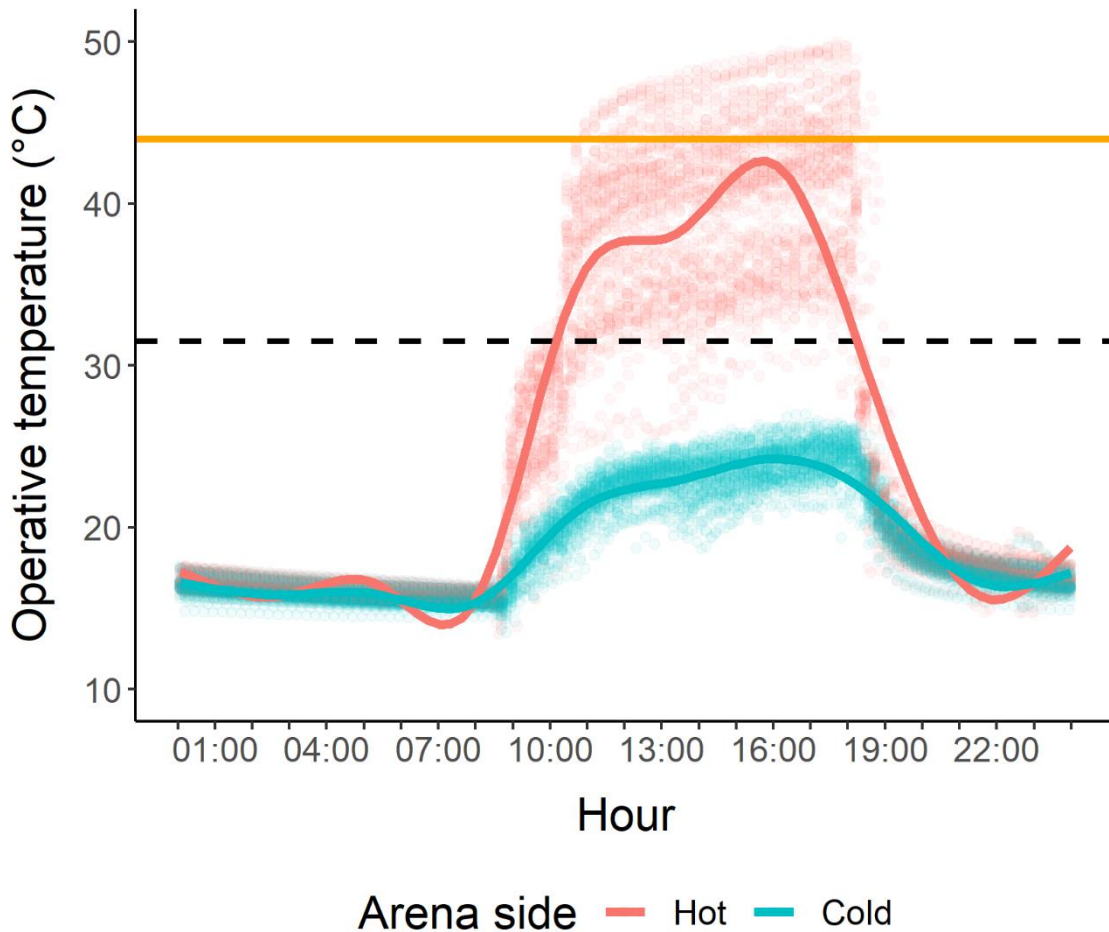
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530 **Supplementary Information**

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.



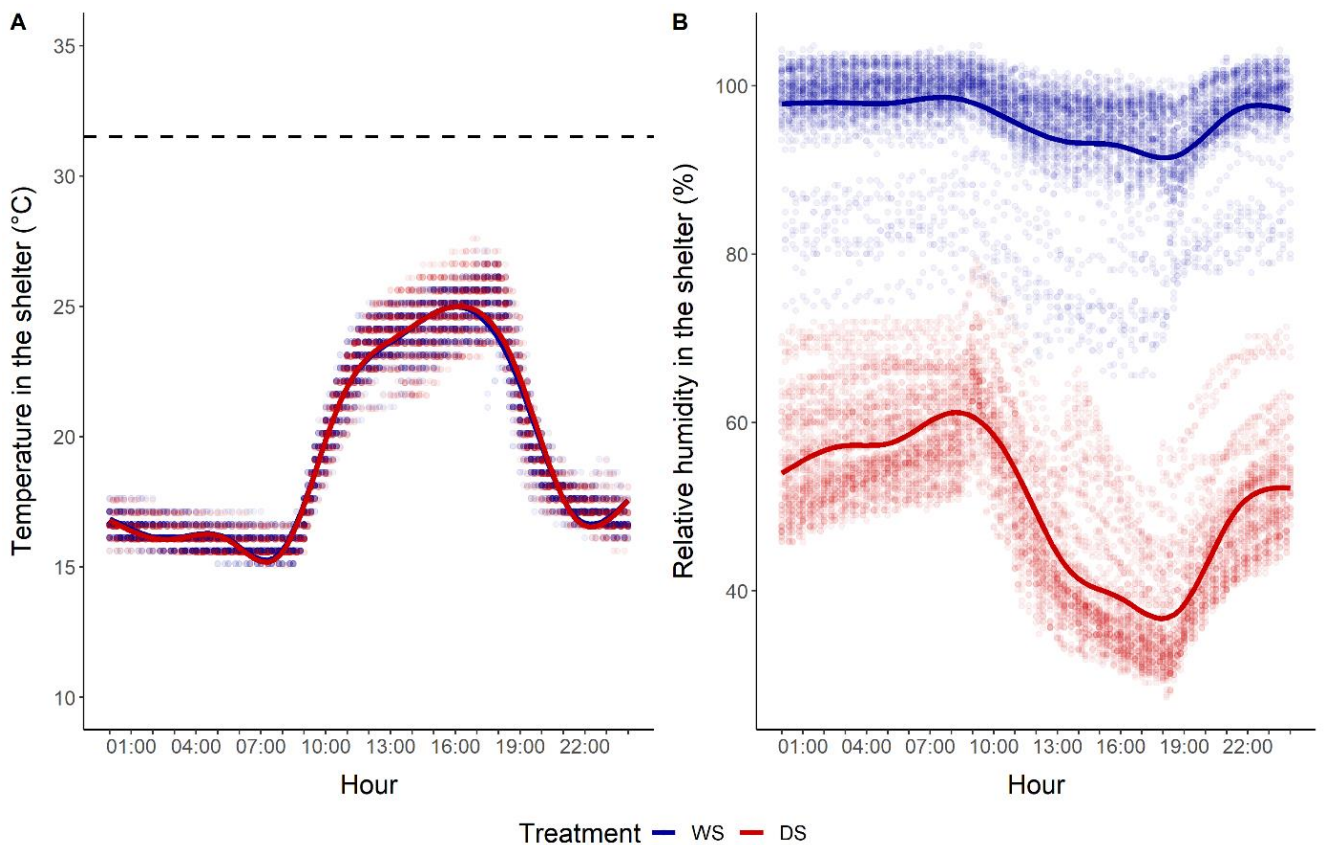
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539 **Figure S2**

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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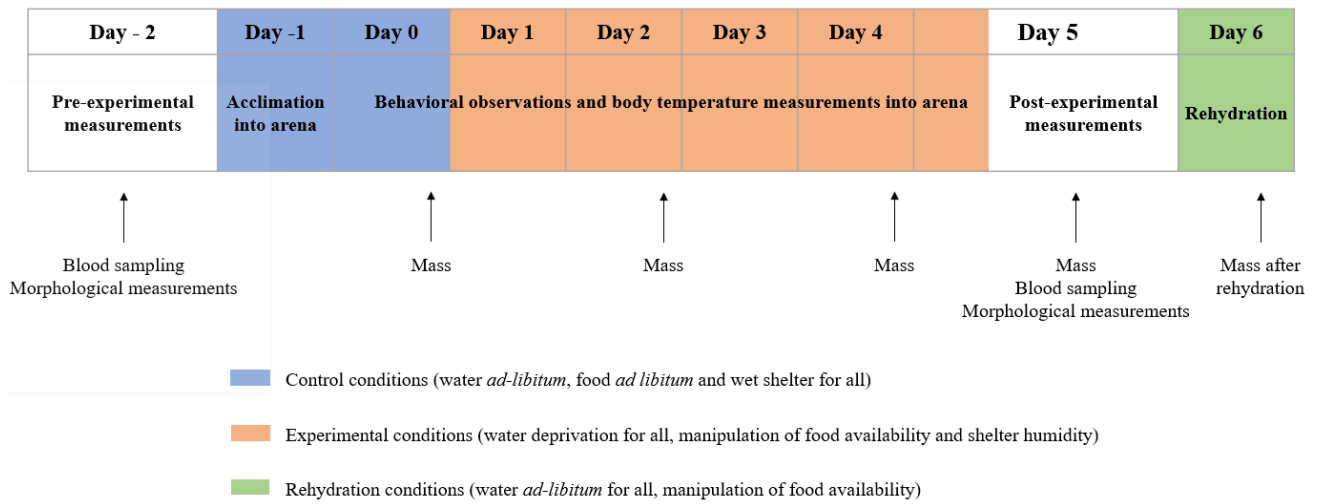
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551 **Figure S3**

552 Chronology of the experiment for each experimental batch.



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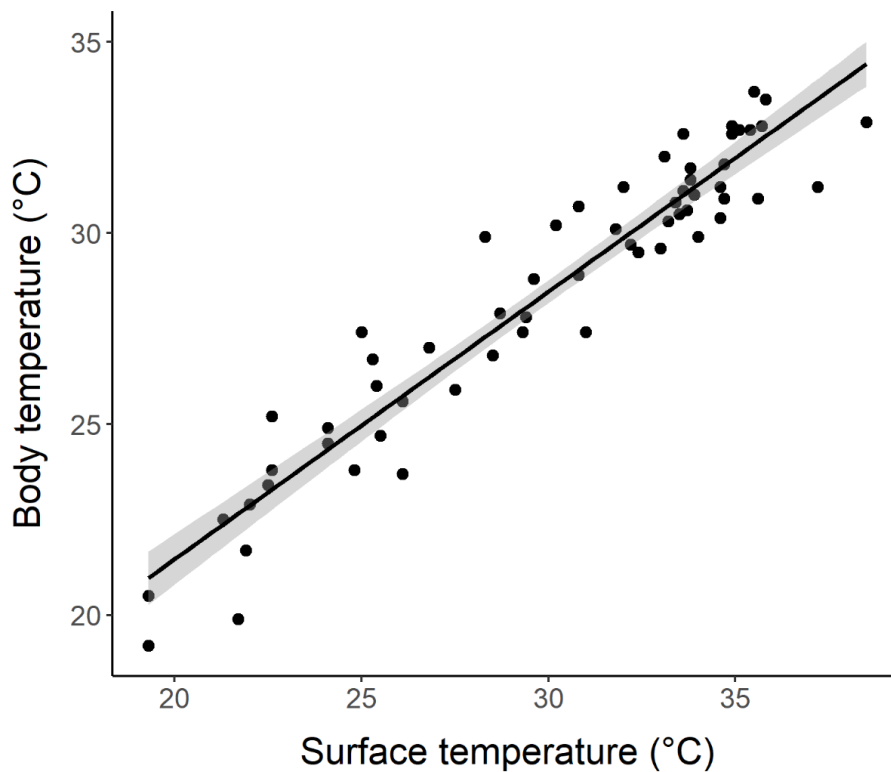
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570 **Figure S4**

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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