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# **Behavioural response to predation risks depends on experimental change in dehydration state in a lizard**

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Running title: Behavioural responses to water and predation stresses combined

## Abstract

1 Optimal regulation of body temperature and water balance is essential for the survival of  
2 terrestrial ectotherms in a changing world. A behavioural trade-off exists between these two  
3 constraints because maintaining a high body temperature usually increases evaporative water  
4 losses. In addition, the evaluation of predation risk is a key factor in behavioural decision for  
5 prey species, and predation threat can cause shift in individual behaviours due to the  
6 modification the cost-benefit balance of thermo-hydroregulation. However, little is known on  
7 how preys integrate these different biotic and abiotic stressors when combined. Here, we  
8 performed an experimental study on the common lizard, a terrestrial ectotherm prey species,  
9 sensitive to water restriction and able to detect specialized predator scents in its environment.  
10 We analysed changes in thermo-hydroregulation behaviours, activity patterns and body  
11 temperature in response to a chronic water stress coupled with simulated punctual occurrences  
12 of predator scents. Water restriction and predator threat had mostly additive effects on lizard  
13 thermoregulation behaviour. They both reduced the time spent basking and thermoregulation  
14 precision. They also had opposite effects on the time spent active, water restriction reducing  
15 activity whereas the presence of predator scents increased it. Yet, we also found an interactive  
16 effect on hydroregulation behaviour, as water restricted lizards showed a wet-shelter  
17 preference only in absence of predator odours. This study demonstrates the existence of some  
18 hydration state dependent behavioural responses to predator threat and suggests that fear of  
19 predators may compromise thermo-hydroregulation and thus prey performances.

20

21 Keywords: thermoregulation - lizards – dehydration – predation risk – reptiles – activity  
22 pattern

23

24 **Significance Statement**

25 In this paper, we show that the fear of predators induces significant changes in the thermo-  
26 hydroregulation behaviours of a widespread terrestrial lizard species, some of which are  
27 influenced by a physiological increase in dehydration induced by an experimental restriction  
28 of water availability. There is a general lack of understanding about how preys respond to  
29 simultaneous changes in biotic and abiotic stressors; in particular, our comprehension of the  
30 non-energetic costs of thermoregulation caused by the presence of predators and the absence  
31 of water in the environment is extremely limited. Our findings indicate that predators have  
32 state-dependent effects on the behaviour of their preys and that joint changes in water  
33 availability and predation risks can compromise the thermo-hydroregulation strategies of their  
34 preys, potentially affecting their **physiological** performances.

Preprint

## 35 **1. Introduction**

36 Water is one of the most essential resources for the survival and reproduction of organisms,  
37 and natural selection has led to several behavioural adaptations and acclimation strategies  
38 enabling terrestrial organisms to cope with predictable and unpredictable reductions of water  
39 availability (Davies, 1982; Chown, Sørensen & Terblanche, 2011; Pirtle, Tracy & Kearney,  
40 2019; Fuller *et al.*, 2021). In ectotherms, thermoregulation is primarily behavioural (Angilletta  
41 Jr, 2009) and there is a potential conflict between water balance and body temperature  
42 regulation because thermoregulation effort and high body temperatures generally increase  
43 evaporative water loss through the skin and during respiration (e.g., Lourdais *et al.*, 2017).  
44 Thus, water constraints influence thermoregulation **accuracy** and dehydration should lead to  
45 “sub-optimal” body temperature in terrestrial ectotherms (Huey & Slatkin, 1976; Anderson &  
46 Andrade, 2017; Rozen-Rechels *et al.*, 2019). Indeed, there is now accumulating evidence that  
47 dehydration is associated with lower field body temperatures during activity (Ladyman &  
48 Bradshaw, 2003), reduction in preferred body temperatures in the laboratory (Sannolo &  
49 Carretero, 2019), decrease of behavioural activity or shifts in the daily and seasonal activity  
50 patterns (Rozen-Rechels *et al.*, 2020). This surge of interest for the understanding of the joint  
51 mechanisms involved in the regulation of body temperature and water balance led to the  
52 development of the thermo-hydroregulation concept (Rozen-Rechels *et al.*, 2019), defined as  
53 the integrated suite of behavioural and physiological processes enabling homeostatic  
54 regulation of both body temperature and hydration state.

55 Biotic interactions such as predation are also a major selective force shaping  
56 behavioural strategies. The detection of specialized predators in the habitat can induce  
57 significant behavioural changes in their prey (Clinchy, Sheriff & Zanette, 2013). Anti-  
58 predator behavioural responses include temporal and spatial shifts in activity, changes in  
59 dispersal behaviour or differential investment in vigilance effort of the prey (Brown, Laundre

60 & Gurung, 1999; Martin, 2011). However, to date, little is known about the combined  
61 influences of dehydration and predation risks on thermo-hydroregulation behaviours and  
62 whether these two stressors have additive or interactive effects on thermoregulation. Yet, this  
63 is highly relevant to patterns and processes of thermo-hydroregulation because these two  
64 constraints should influence jointly the non-energetic costs of thermo-hydroregulation  
65 behaviours and these effects should be driven by their spatiotemporal variations in natural  
66 populations. For example, simultaneously avoiding predation and fulfilling thermo-  
67 hydroregulation needs may be in conflict, as shown in recent studies of ecological interactions  
68 between ungulates and their predators in semi-arid areas, such as African savannahs (Veldhuis  
69 *et al.*, 2019). In these habitats, large predators are attracted by waterholes such that water  
70 dependent ungulate species also experience a higher exposure to predation risks, leading to  
71 temporal or spatial shifts in their thermo-hydroregulation strategies (Valeix *et al.*, 2009b).  
72 Another possibility includes shifts in social behaviour in response to water deprivation that  
73 increase the risks of predation, such as huddling behaviours in amphibians (Rohr & Madison,  
74 2003). In such situations, the benefits of thermo-hydroregulation responses to water  
75 deprivation may be cancelled by a simultaneous increase in predation risks (Rohr & Madison,  
76 2003).

77 In addition to interactions between predation risks and water availability caused by their  
78 level of complementation in the landscape, preys may react to predation risks differently  
79 depending on their hydration state. This is especially likely in organisms that can tolerate a  
80 wide range of osmotic states and display large variations in their hydration status, such as  
81 many terrestrial ectotherms (Lillywhite, 2016). According to classical behavioural models  
82 (Lima, 1998), if a trade-off exists between avoiding predators and getting necessary water  
83 resources, dehydrated individuals should be more prone to take risks in the presence of  
84 predators than well-hydrated individuals. Alternatively, since the dehydration state of the

85 individual also influences the potential costs and benefits of thermoregulation in a risky  
86 environment (Rozen-Rechels *et al.*, 2019), anti-predator responses may involve changes in  
87 thermoregulation, such as reduction in behavioural activity or changes in micro-habitat  
88 selection. Changes in body condition induced by chronic dehydration could also influence an  
89 individual's response to the fear of a predator, which is generally condition-dependent (e.g. in  
90 lizards, Martín & López, 1999; Martín, López & Cooper Jr, 2003).

91 In **many** terrestrial ectotherms, thermoregulation plays an important role in predator  
92 avoidance because high locomotor performances allowing prey to escape from their predators  
93 are closely related to an optimal regulation of body temperature (Landry Yuan *et al.*, 2021).  
94 However, behavioural thermoregulation often involves increased activity (i.e., shuttling  
95 behaviours) and preferential use of open habitats (i.e., basking behaviours), which can draw  
96 the attention of predators and increase predation risks. Therefore, there is a proximate trade-  
97 off between active thermoregulation strategies and the exposure to predation (Angilletta Jr,  
98 2009). For example, terrestrial **reptiles** can adjust their escape tactics and flight initiation  
99 distances in relation to their body temperature and opportunities for optimal basking in their  
100 environment (Cooper, 2009). Furthermore, reptiles often use burrows or crevices as shelters to  
101 protect themselves from predators and they will use differentially these shelters depending on  
102 their thermal quality and their perception of predation risks (Amo, López & Martín, 2004;  
103 Lориoux, Lisse & Lourdais, 2013). Generally, the perception of predators triggers a decrease  
104 in behavioural activity and may reduce the accuracy of thermoregulation (Downes, 2001;  
105 Herczeg *et al.*, 2008; Angilletta Jr, 2009; Lориoux *et al.*, 2013). These organisms thus provide  
106 relevant models to address interaction between predation risks, thermoregulation and water  
107 constraints, even though this facet has been poorly studied.

108 Here, we set up a laboratory experiment **with** a ground-dwelling lizard (the common  
109 lizard, *Zootoca vivipara*) to quantify changes in thermo-hydroregulation behaviours when

110 animals are exposed to simultaneous changes in dehydration risks and the threat of predation.  
111 Common lizards are widespread lizards from cold and wet habitats across Eurasia and they  
112 are preyed upon by a diversity of generalist and specialist predators, including snakes such as  
113 the adder (Prestit, 1971) and the smooth snake (Drobenkov, 2014). Previous studies have  
114 demonstrated that there is an overall reduction in thermoregulation effort for animals under  
115 water stress (Rozen-Rechels *et al.*, 2020). Common lizards and their **snake** predators rely on  
116 chemical cues in their environment to detect each other, and previous studies have shown that  
117 common lizards can recognize scent from a specialized snake predator and differentiate it  
118 from the scent of food or of conspecifics (Thoen, Bauwens & Verheyen, 1986; Van Damme  
119 *et al.*, 1990). Here, we examined the following hypotheses. First, we expect a reduction in the  
120 proportion of time spent basking (and thus being more vulnerable to predators) and **a delay in**  
121 **emergence time** for individuals confronted to scents of their predators. Lizards could also  
122 lower their body temperature (Martin & Huey, 2008; Anderson & Andrade, 2017) and/or shift  
123 toward more thermo-conformity under stressful conditions as predicted by Huey & Slatkin,  
124 1976. We also expect adult males to be more prone to take risks than females, as it has been  
125 suggested in this species by Antczak *et al.*, 2019. Second, we posit that lizards confronted to  
126 predator scents in addition to water stress will respond differently than well-hydrated lizards,  
127 as antipredator behaviours are often condition dependent. **Dehydrated lizards would take**  
128 **greater risks, searching for water despite the presence of predators, and thus being more**  
129 **active than expected. Additionally, they may reduce body temperature to limit water**  
130 **loss, even though it impacts their sprint performance when encountering predators.**

131 To test these hypotheses, we submitted lizards to two different conditions including a  
132 control with access to *ad libitum* drinking water and a treatment group with restricted access  
133 to drinking water, hereafter referred to as the water treatment. Before and after this  
134 manipulation, we recorded lizards' behaviours and activity, as well as their body temperature,



135 under two conditions of predator threat (absence or presence of predator scents in the  
136 environment). This experimental design allows us to detect a potential interactive effect  
137 between the two stressors.

## 138 **2. Material and methods**

### 139 2.1. Studied species and acclimation conditions

140 The common lizard *Zootoca vivipara* is a small lacertid lizard (Reptilia: Lacertidae; adult  
141 snout-vent length 50-70mm) with a wide Euro Siberian distribution (Surget-Groba *et al.*,  
142 2006). It inhabits mesic environments such as humid grasslands and peat bogs and is highly  
143 sensitive to water deprivation due to its high standard water loss rates (Lorenzon *et al.*, 1999;  
144 Massot *et al.*, 2002; Dupoué *et al.*, 2017). The species is mainly preyed by birds and snakes  
145 including the adder (Viperidae, *Vipera berus*) and several species of Colubridae (Prestit, 1971;  
146 Steen, Løw & Sonerud, 2011; Drobenkov, 2014). Common lizards used in this study were  
147 captured after the reproduction period (in early July 2019) in semi-natural populations  
148 maintained in 10 fenced, outdoor enclosures in CEREEP-Ecotron IleDeFrance in Saint Pierre  
149 lès Nemours, France (48°17'N, 2°41'E) without natural predators. We captured 72 adults ( $\geq 2$   
150 years old) with a 1:1 sex ratio, measured their snout-vent length with a plastic ruler ( $\pm$   
151 0.5mm), and weighted them for body mass ( $\pm 1$ mg). Lizards were then placed in individual  
152 terraria (18 × 11 × 12cm) for acclimation in a temperature-controlled room (23°C from 8:00  
153 to 19:00, 15°C otherwise) and fed with living crickets (*Acheta domestica*) *ad libitum*. During  
154 acclimation, drinking water was available *ad libitum* in a water cup and terraria were sprayed  
155 3 times a day with water to maintain a wet environment.

### 156 2.2 Experimental design

157 After a one-week acclimation period, lizards were divided into 3 trial groups (24 individuals  
158 per group, 12 females and 12 males) tested successively. Inside each group, individuals were  
159 paired in couple (**one male and one female of similar body size**) and behavioural

160 observations were recorded on pairs of individuals. The day before the first behavioural  
161 observations, each pair was transferred to a neutral arena ( $79 \times 57 \times 42\text{cm}$ ) in a temperature-  
162 controlled room maintained at  $25^\circ\text{C}$  between 8:00 am and 5:30 pm. Arenas were equipped  
163 with a substratum of sterilized peat soil, a water cup and, on one side, two 40W light bulbs  
164 placed above two artificial shelters in order to provide them with basking spots and refuges in  
165 the so-called “hot zone” of the arena and a retreat site without refuge in the “cold part” of the  
166 arena. One shelter was maintained humid at ground level with a wet sponge, whereas the  
167 other one was maintained dry with a dry sponge. In addition, a UV neon tube (Reptisun 10.0,  
168 white light) provided UV enhanced light above each arena during daytime. Mean temperature  
169 in wet shelter was slightly lower than in dry shelter (wet:  $27.1^\circ\text{C} \pm 3.4 \text{ SD}$  ; dry:  $27.9^\circ\text{C} \pm 4.2$   
170 **SD**), and mean relative humidity was much higher in the wet one (wet:  $54.4\% \pm 10.4 \text{ SD}$ ; dry:  
171  $35.4\% \pm 9.4 \text{ SD}$ ). On the cold side of the arena (without the heating lamps above), ground  
172 temperatures stayed below *Zootoca vivipara* preferred temperature all day long whereas the  
173 hot part offered optimal temperatures for thermoregulation (temperature during behavioural  
174 trial: cold mean =  $26.5^\circ\text{C}$ , maximum =  $28.5^\circ\text{C}$  ; hot mean =  $35.4^\circ\text{C}$ , maximum =  $39.8^\circ\text{C}$ ,  
175 Gvoždík & Castilla, 2001).

176 The experimental design consisted in 2 days of behavioural observations prior to  
177 manipulation, 8 days of manipulation of water availability, and 2 days of observations after  
178 this manipulation period (see Figure 1). First, behavioural observations (see below) were  
179 performed with lizards maintained in standard conditions (including *ad libitum* water) for  
180 each pair with a random succession of each of the two predator trial groups (“control” trial  
181 group with no scent or “odor” trial group with predator scents added in the arena) during one  
182 day each. We referred to this sequential test as the “test day sequence” variable and our  
183 sequential test was motivated by the need to avoid habituation of lizards to scents since they  
184 were tested against predator scents only during one day (Parsons *et al.*, 2018). The odor trial

185 group was obtained by combining scents of two natural predators of the common lizards. We  
186 used a standardized quantity of shed skins of the adder (*Vipera berus*) **that we cut into pieces**  
187 **and combined with the substrate layer before spreading it out in the arena. In** addition,  
188 we used shelters installed in a live smooth snake's (*Coronella austriaca*) terrarium for at least  
189 24h, in order to imitate a realist situation as these two predators can be sympatric. Shed skins  
190 were obtained from adders (N = 40) captured in Western France and temporarily maintained  
191 in captivity **at Centre for Biological Studies of Chizé, France** (Dezetter *et al.*, 2021). The  
192 smooth snakes (N = 2) were captured in natural habitats at CEREEP-Ecotron in late June  
193 2019. Next, after these two days, lizards were put back in their individual terrarium for 8 days  
194 without odors of predators. Water treatment (control *ad libitum* or restricted water) was  
195 randomly attributed to each pair following standard protocols: in control conditions, each  
196 terrarium had a water cup filled with drinking water in the morning whereas we removed the  
197 water in restricted conditions (Dupoué *et al.*, 2018). In addition, we reduced water spray to  
198 once every day in restricted conditions instead of 3 times a day in control conditions. Lizards  
199 were fed with living cricket *Acheta domestica* throughout the experiment. Finally, after these  
200 8 days manipulation period, pairs of lizards were placed back in the neutral arena (**with an**  
201 **empty water cup for the water restricted lizards**) for 2 additional days of behavioural  
202 observations involving a new random sequence of each of the two predator trial groups (see  
203 Figure 1).

### 204 2.3. Behavioural observations

205 During each observation trial (see Figure 1), we quantified the behaviour of lizards with a  
206 focal sampling survey every 30 min from 08:30 to 17:00. At each focal sampling, we  
207 observed if the individual was “active” (i.e. visible to the observer) or “inactive” (i.e., hidden  
208 in a shelter or buried in the soil). When the individual was not visible, we searched gently  
209 under the shelters and noted if it was found under the wet or dry one. When the lizard was still

210 not found, we assumed that it was buried into the soil and did not disturb it. In this case, the  
211 individual was reported as “hidden”. When it was “active”, we further recorded whether the  
212 individual was basking (immobile position, **flatten body** oriented towards light bulb under  
213 the hot spot), **moving** (active movement of any kind) or drinking. We reported in which part  
214 of the arena the individual was located (either on the hot part or the cold part away from the  
215 hot spots and shelters). We also measured from the same distance the surface body  
216 temperature on the back of each active lizard using an infrared thermometer (Raynger MX2,  
217 Raytek) following standard protocols and previous work showing a strong correlation  
218 between surface and core body temperature (Chabaud *et al.*, 2022).

#### 219 2.4. Plasma osmolality assays

220 We measured body mass and collected blood samples from the post-orbital sinus using 1-2  
221 micro-capillary tubes (ca. 20-40  $\mu\text{l}$  whole blood) before and after the 8 days of manipulation  
222 to measure plasma osmolality, which provides a standardized assay of physiological  
223 dehydration. Samples were centrifuged at 11,000 rpm for 5 min to separate plasma from red  
224 blood cells. Plasma samples (approx. 10-15  $\mu\text{l}$ ) were immediately frozen at  $-28^{\circ}\text{C}$  until further  
225 analyses in the lab. Then, plasma osmolality was determined using a vapor pressure  
226 osmometer (model Vapro 5600, ELITechGroup) with the protocol described in Wright *et al.*,  
227 2013 and adjusted to small plasma volumes (Dupoué *et al.*, 2017). Before analyses, plasma  
228 was diluted (1:3) in standard saline solution (Osmolarity =  $280 \text{ mOsm.l}^{-1}$ ) to obtain 2  
229 duplicates per sample (CV = 1,5%). Mean value from the 2 duplicates was used in subsequent  
230 analyses. Due to their manipulation, a few samples could not be analysed so we only got 55  
231 out of 72 values for osmolality change, randomly distributed among the sex and water  
232 treatment.

#### 233 2.5. Statistical analyses

234 Statistical analyses were performed using R statistical software (version 3.6.3; R Core Team  
235 2020). First, intra-individual changes in osmolality and body mass during the manipulation  
236 were compared with ANCOVA on linear models including effects of treatment, sex and their  
237 interaction and the additive effects of initial value (of osmolality or body mass) and of trial  
238 group. Second, inter- and intra-individual variation in different behavioural items and in body  
239 temperature was analysed with different statistical methods depending on the behavioural  
240 item. We first summed the number of times the behaviour was recorded each day relative to  
241 the total number of focal sampling in the day, and calculated relative frequency for 5  
242 behavioural items: (1) the proportion of surveys spent active, (2) the proportion of active  
243 surveys spent basking, (3) the choice of a wet shelter (proportion of surveys seen in the wet  
244 shelter among surveys in a shelter), (4) the proportion of surveys spent hidden, and (5) the  
245 proportion of surveys spent in hot zone. These behavioural items were analysed with  
246 generalized linear mixed models using the *glmer* function from the ‘lme4’ package (Bates *et*  
247 *al.*, 2014) with a binomial family and a logit link. We added the experimental arena and the  
248 individual as random factors to account for the inter-individual variability and for potential  
249 differences among arenas. Next, body temperatures were analysed with linear mixed models  
250 using the *lme* function from the ‘nlme’ package (Pinheiro *et al.*, 2006). In all cases, we tested  
251 the tree way interaction between predator scent trial (absence or presence), water treatment  
252 (water restricted or water control) and time period (before or after the manipulation), in  
253 addition to additive effects of sex, trial group and test day sequence (day 1 or day 2). Finally,  
254 we analysed emergence hour (first time of the day the individual was observed “active”) with  
255 a mixed effect Cox model using the *coxme* function of package ‘coxme’ (Therneau, 2012).  
256 This model assumes that the emergence time of the lizard can be best described by a  
257 proportional hazards model with a single random intercept per group. We fitted the model  
258 with additive fixed effects of the predator scent trial, water treatment and time period and

259 their three-way interactions and additive fixed effects of sex, test day sequence and trial  
260 group. Random intercept effects were included for arena and individual groups, respectively.

261 For each analysis, we built a full model and checked its assumptions with graphical  
262 analyses of residuals and predictions, for example to test the Gaussian and homoscedastic  
263 distribution of residuals. For *glmer* models, we also performed goodness-of-fit tests to confirm  
264 the absence of overdispersion. For Cox models, we used a graphical test of proportional hazards  
265 provided with the package ‘survminer’ (Kassambara *et al.*, 2017). Data were slightly over-  
266 dispersed only for one behavioural item (proportion of surveys spend hidden,  $\chi^2 = 407$ ,  
267  $p < 0.0001$ ), so we used an observation-level random effect to correct it as recommended in this  
268 situation (Harrison, 2014). Then, starting with the full model, the best model was selected using  
269 stepwise removal of non-significant effects based on standard *F* statistics computed with the  
270 *Anova* function from package ‘car’ (Fox & Weisberg, 2011). Results are shown as means  $\pm$ SE  
271 unless otherwise stated. If the odor trials have an effect on behavioural items, it should appear  
272 in the models as a mean effect independently of time period, as it is designed as a one-time  
273 effect. On the other hand, the water treatment is designed as a chronic manipulation, and its  
274 potential effect should therefore appear as an interactive effect of water treatment and the time  
275 period. In particular, we expect no difference between water treatment groups before the  
276 manipulation and potential contrasts after the manipulation (see Figure 1). Finally, interactive  
277 effects of water restriction and predator scents presence should appear as a three-way  
278 interaction between water treatment, time period and predator scent trial.

### 279 **3. Results**

#### 280 3.1. Effect of water deprivation on plasma osmolality and body mass

281 Water restriction influenced significantly body mass change, with a negative effect on females  
282 (water treatment  $\times$  sex:  $F_{1,64} = 9.65$ ,  $p = 0.0028$ ), water restricted females losing on average  
283  $0.12 \pm 0.03$  g (**3% of their initial body mass**) whereas control females gained  $0.04 \pm 0.02$  g

284 (1% of their initial body mass ; estimate=  $-0.16 \pm 0.04$ ,  $p=0.0009$ ). However, water treatment  
285 did not impact males (estimate= $0.05 \pm 0.05$ ,  $p=0.32$ ), male body masses remained constant. We  
286 also found an effect of trial group, animals from trial 2 losing more body mass than the two  
287 other trials ( $F_{2,64} = 6.73$ ,  $p=0.002$ ). In addition, water restriction increased osmolality by an  
288 average of  $17 \text{ mOsm.kg}^{-1}$  in male and female lizards, whereas non-restricted ones decreased  
289 their osmolality by an average of  $2 \text{ mOsm.kg}^{-1}$  (water treatment:  $F_{1,55} = 14.63$ ,  $p=0.0003$ ;  
290 initial value:  $F_{1,55} = 65.03$ ,  $p < 0.001$ ).

### 291 3.2. Changes in thermo-hydroregulation behaviours

292 A lizard was reported active on average  $8.4 \pm 0.2$  times a day out of 18 observations. Activity  
293 probability increased significantly in the presence of predator scents prior to the water  
294 manipulation ( $\chi^2 = 13.23$ ,  $df = 1$ ,  $p = 0.006$ , Figure 2) but this difference was smaller after the  
295 water manipulation since lizards were slightly less active in presence of odours after the 8-  
296 days manipulation period than before (time period  $\times$  predator scent trial:  $\chi^2 = 3.96$ ,  $df = 1$ ,  
297  $p=0.05$ ). Activity also decreased through time with water restriction compared to the control  
298 treatment (time period  $\times$  water treatment:  $\chi^2 = 22.96$ ,  $df = 1$ ,  $p < 0.0001$ , Figure 2)  
299 independently from predator scent treatment. Other factors influencing the proportion of time  
300 spent active include test day sequence as lizards were less active on the second day of  
301 observation trials ( $\chi^2 = 20.95$ ,  $df = 1$ ,  $p < 0.0001$ ).

302 When lizards were active, the time they spent basking under the hot spot, immobile and  
303 exposed was influenced additively by our two experimental constraints as the time spent  
304 basking decreased in presence of predator scents (predator scent trial:  $\chi^2 = 5.34$ ,  $df = 1$ ,  
305  $p=0.02$ , estimate =  $-0.37 \pm 0.16$ ,  $p=0.02$ ) and in the water restricted group (time period  $\times$   
306 water treatment:  $\chi^2 = 3.97$ ,  $df = 1$ ,  $p=0.05$ , estimate =  $-0.46 \pm 0.23$ ,  $p=0.046$ ). We also found an  
307 effect of time period alone ( $\chi^2 = 10.63$ ,  $df = 1$ ,  $p=0.001$ ) since lizards spent more time basking  
308 after than before the manipulation and an overall effect of trial group ( $\chi^2 = 7.96$ ,  $df = 2$ ,

309 p=0.019). In addition, when lizards were active, they were situated in the hot part of the arena  
310 on average  $11.4 \pm 0.2$  times a day. The predator odour trials influenced the time spent in the  
311 hot zone ( $\chi^2 = 15.99$ ,  $df = 1$ ,  $p < 0.0001$ ) but not the water treatment. Test day sequence also  
312 impacted the time spent in hot zone ( $\chi^2 = 6.87$ ,  $df = 1$ ,  $p = 0.009$ ) as well as the trial group ( $\chi^2 =$   
313  $7.35$ ,  $df = 2$ ,  $p = 0.025$ ). Lizards were less often seen on the hot part of the arena in presence of  
314 predator odours and during the second day of the observation sequences.

315 Lizards were found on average  $3.8 \pm 0.2$  times a day under a shelter and only slightly  
316 more than half of the time under the wet shelter ( $2.18 \pm 0.15$  times a day). The water  
317 treatment influenced the proportion of time lizards spent under the wet shelter differently  
318 between the predator scent trials (time period  $\times$  water treatment:  $\chi^2 = 22.92$ ,  $df = 2$ ,  $p < 0.0001$ ;  
319 time period  $\times$  water treatment  $\times$  predator scent trial:  $\chi^2 = 14.11$ ,  $df = 2$ ,  $p = 0.0009$ , Figure 3).  
320 In the water restricted group, lizards were seen more often under the wet shelter than in  
321 control group when no predator odour was present (estimate =  $1.04 \pm 0.51$ ,  $p = 0.044$ ). Wet  
322 shelter preference was not found when shelters and the substrate had predator odours.

323 Lizards were hidden in the soil on average  $5.8 \pm 0.2$  times a day. Water treatment did  
324 not explain the variation in the number of times they were buried (time period  $\times$  water  
325 treatment:  $\chi^2 = 2.12$ ,  $df = 2$ ,  $p = 0.34$ ) but the interaction between the predator scent and time  
326 period did (predator scent trial:  $\chi^2 = 0.42$ ,  $df = 1$ ,  $p = 0.52$ ; time period  $\times$  predator scent trial:  $\chi^2$   
327 =  $5.38$ ,  $df = 1$ ,  $p = 0.02$ ), lizards being more often buried in presence of predator odours after  
328 the manipulation. The other factor influencing burrowing included test day sequence ( $\chi^2 =$   
329  $26.70$ ,  $df = 1$ ,  $p < 0.0001$ ), lizards being more often buried on day 2 than on day 1 of  
330 behavioural trials. We found no effect of sex on either of the thermo-hydroregulation  
331 behaviours.

### 332 3.3. Change in emergence time



333 On average, most lizards emerged between the 2<sup>nd</sup> and the 3<sup>rd</sup> observation of the day (9:00 to  
334 9:30 am) with 50% of individuals being active by 9:30 am (Figure 4). Emergence time was  
335 influenced significantly by the predator scent presence and its interaction with time period  
336 (predator scent trial:  $\chi^2 = 8.79$ ,  $df = 1$ ,  $p=0.003$ ; time period  $\times$  predator scent trial:  $\chi^2 = 9.29$ ,  $df$   
337  $= 1$ ,  $p=0.0023$ ). Lizards emerged slightly later in the presence than in the absence of predator  
338 scents (Figure 4), but this was less the case after the manipulation irrespective of the water  
339 treatment group. We also found an effect of the test day sequence ( $\chi^2 = 6.29$ ,  $df = 1$ ,  $p=0.01$ ),  
340 with lizards emerging slightly earlier on the second consecutive day of observation than on  
341 the first. The water treatment had no detectable effect on the emergence time (time period  $\times$   
342 water treatment:  $\chi^2 = 0.77$ ,  $df = 1$ ,  $p=0.38$ ). We found no effect of sex on emergence time.

### 343 3.4. Change in body temperature

344 Mean surface body temperature during activity was  $34.3 \pm 0.1$  °C. The mean body temperature  
345 during the day was slightly reduced by the presence of predator odours ( $\chi^2 = 4.2$ ,  $df = 1$ ,  
346  $p=0.04$ , Figure 5) but only marginally by the water restriction (water treatment  $\times$  time period:  
347  $\chi^2 = 2.8$ ,  $df = 1$ ,  $p=0.09$ ). Time period alone ( $\chi^2 = 7.2$ ,  $df = 1$ ,  $p=0.007$ ) and test day sequence  
348 ( $\chi^2 = 9.49$ ,  $df = 1$ ,  $p=0.002$ ) also influenced the body temperature, lizards having a higher  
349 mean body temperature after the manipulation, but a lower one on the second consecutive day  
350 of observation than on the first. We found no effect of sex on the mean body temperature.

## 351 **4. Discussion**

352 Our experiment was designed to study the simultaneous effects of water availability and  
353 predator threat on thermo-hydroregulation behaviours and activity patterns. We used male and  
354 female adult lizards but found no effect of sex on **any** of the behavioural variables, maybe  
355 because the manipulation period was outside reproduction season for this species and  
356 different trade-offs between water needs and fear of predation between sexes occur only when  
357 reproduction is at stake (Dupoué *et al.*, 2018; Rozen-Rechels *et al.*, 2020). We simulated a

358 mild water deprivation using a chronic stress experiment over several days, whereas predation  
359 threat was simulated as a one-time effect using behavioural trials in both presence or absence  
360 of predator scents during one day. Thus, our design contrasted the short term responses of  
361 well-hydrated and dehydrated animals to a punctual occurrence of predator scents, as might  
362 occur in natural populations when predators **traverse the home range** of several preys and  
363 patrol more distances than their preys. Thanks to this experimental design, the behaviours of  
364 animals were tested before and after water manipulation, and we could control for inter-  
365 individual variation in mean behaviour, including thermo-hydroregulation temperaments  
366 (Cote & Clobert, 2006), to strengthen the statistical power. In addition, a chronic exposure to  
367 predator scents without direct physical encounters would have been less relevant as animals  
368 can learn that the risk is not associated with danger and behavioural responses will thus fade  
369 with time (Parsons *et al.*, 2018). We indeed found that some behavioural traits were  
370 influenced by a slight effect of time period, possibly explained by habituation to odours of  
371 predators; for example, predator scent effects on wet shelter use and emergence time were  
372 weaker after the 8-day water manipulation. **Lizards also exhibited decreased activity and**  
373 **were less frequently found in the hot zone on the second day of trials, with increased**  
374 **burying behaviour and lower body temperatures. This pattern suggests that lizards**  
375 **became accustomed to the arena environment and their paired partner, resulting in**  
376 **reduced exploration and overall activity, which subsequently impacted the other**  
377 **observed effects.**

378 Our study reveals interesting results on how predator threat affects activity and  
379 emergence time in this terrestrial ectotherm. The presence of predator scents increased  
380 activity rate but in the meantime it decreased basking rate during activity. Increased activity  
381 can be associated with a higher level of vigilance and also more frequent escape attempts (via  
382 scratching behaviours, see Kawamoto, Le Galliard & Badiane, 2021), but this was not

383 correlated with a better thermoregulation. On the contrary, lizards spent less time basking,  
384 which is consistent with the observed reduction of mean active body temperature in presence  
385 of predation threat. Overall, this suggests that the presence of predator scents led to less  
386 optimal thermoregulation. Previous works on other lizard species also demonstrated a  
387 reduction of basking behaviours when a predation risk was perceived, both in laboratory  
388 experiments (Downes, 2001; Robert & Thompson, 2007) and in the field (Lister & Aguayo,  
389 1992). The lower basking effort and less accurate thermoregulation in the presence of  
390 predators scents also conform with predictions of the cost-benefit model of thermoregulation  
391 (Huey & Slatkin, 1976) and other similar studies on ectotherms (Herczeg *et al.*, 2008;  
392 Gvoždík, Černická & Van Damme, 2013). These behavioural responses may represent a  
393 significant fitness cost over the long term if maintenance of an optimal body temperature is  
394 important for the detection of future threat or foraging efficiency (Amo *et al.*, 2004). For  
395 example, a reduction of basking effort had consequences on juvenile growth rates in one study  
396 due to the reduced time spent in temperature-dependent activities such as foraging and  
397 digestion (Downes, 2001).

398 We also found that lizards emerged later in presence of predators scents, which might  
399 be a strategy to shift their activity toward periods of the day with a more limited predator  
400 activity or to reduce overall daytime activity period. This shift of the daily activity pattern is  
401 interesting given that smooth snakes emerge earlier in the morning during the hottest months  
402 of the year (de Bont, van Gelder & Olders, 1986), so emerging later could be a strategy for  
403 common lizards to limit encounters with this specialized predator. Prey often need to adjust  
404 their behavioural decisions and activity patterns according to geographic **or temporal**  
405 variation in predation risks (reviewed in Lima & Dill, 1990). This shift is particularly  
406 important to study for lizards in natural conditions as during the summertime, when drought is

407 combined with high temperatures, activity in the morning is favoured to avoid heat stress and  
408 dehydration (Rozen-Rechels *et al.*, 2020).

409 In addition, our investigation of behavioural changes in water restricted animals  
410 mostly confirmed our predictions and previous works on this species (Rozen-Rechels *et al.*,  
411 2020). Dehydration was on average mild in manipulated lizards, with small relative decrease  
412 in body mass and small relative increase in plasma osmolality compared to maximum  
413 sustainable values that common lizards can tolerate under laboratory conditions (up to 25-30  
414 % body mass decline and up to 50-70 mOsm.kg<sup>-1</sup> increase, see Dupoué *et al.*, 2020). Despite  
415 this, dehydrated lizards were significantly less active and had lower basking rates than water  
416 control ones, which led to a less accurate thermoregulation given the trend, albeit not  
417 significant, for lower body temperatures in the treatment group. Dehydrated lizards also  
418 increased their use of the wet shelter, probably as a hydroregulation strategy to reduce  
419 evaporative water loss rates and conserve more water (Dezetter, Le Galliard & Lourdais,  
420 2022). Thus, our data confirmed the existence of a behavioural trade-off between  
421 thermoregulation and hydroregulation, as predicted for terrestrial ectotherms in Rozen-  
422 Rechels *et al.*, 2019 and observed in several recent studies (e.g., Greenberg & Palen, 2021;  
423 Nervo *et al.*, 2021). However, these behavioural adjustments did not allow the water restricted  
424 lizards to totally compensate for the lack of water, as their plasma osmolality slightly  
425 increased compared to non-restricted ones.

426 From our independent analysis of six behavioural traits and body temperature, we  
427 found interactive effects of these two constraints on only one behaviour, which suggests that  
428 the two stressors had primarily additive effects on thermoregulation and activity patterns.  
429 These conclusions run against our hypotheses that anti-predator responses could depend on  
430 hydration state or that thermo-hydroregulation responses are dependent on the fear of  
431 predation, as might be the case for example when one environmental constraint has dominant

432 effects on behavioural plasticity and is given priority over the other (Rozen-Rechels *et al.*,  
433 2019). Water stress and the fear of predators additively impacted activity and  
434 thermoregulation behaviours, but sometimes in an opposite way. Indeed, as the presence of  
435 predator scents in the environment increased activity rate, chronic water stress decreased it;  
436 yet, we found no interactive effect. However, water stress and the fear of predators  
437 interactively impacted the use of wet shelter, as dehydrated animals used it more often only in  
438 absence of predator scents. In our experimental design, resting under the wet shelter  
439 represents a good strategy to optimize both temperature, since the shelter is located in the hot  
440 zone closed to the basking area, as well as water balance, since a wet shelter allows lizards to  
441 reduce evaporative water losses thanks to the high ambient relative humidity (Pintor,  
442 Schwarzkopf & Krockenberger, 2016). However, we used shelters with or without fresh  
443 scents of a specialized predator (the smooth snake) implying that shelters were potentially  
444 perceived as a danger for dehydrated lizards and priority was given to other, safer behavioural  
445 responses to dehydration than shelter use. Thus, these results suggest that a behavioural  
446 response to limit dehydration while maintaining a high body temperature (hot shelter use) was  
447 modified by the risk of predation (Angilletta, Niewiarowski & Navas, 2002). Similar results  
448 on habitat selection were found in geckos, with the avoidance of retreat site with predator  
449 scents at the expense of thermoregulation (Downes & Shine, 1998). These results highlight  
450 that anti-predator behaviours can have consequences on the regulation of crucial parameters  
451 such as body temperature and water balance. If the starvation-predation risk trade-off has  
452 been studied in other ectotherm species (e.g., Bennett, Pereira & Murray, 2013), few studies  
453 have considered how predation risk can influence the non-energetic costs to the maintenance  
454 of water balance (Valeix *et al.*, 2008, 2009a). We need a deeper understanding of the  
455 interactive effects of these two constraints in more ectothermic species.

456

457 In conclusion, we found mostly additive effects of water and predator threat on  
458 thermoregulation behaviours, but an interactive effect was observed for the use of shelter that  
459 shift from optimal to sub-optimal in the presence of predators odours. This change in shelter  
460 use driven by both water restriction and perception of predations risks could have  
461 consequences for thermo-hydroregulation in natural conditions including shelter use during  
462 daytime in response to hot temperatures but also shelter use during night time when lizards  
463 need to rest at lower body temperatures (Rutschmann *et al.*, 2021). In turn, ecological effects  
464 of water restriction should be enhanced in prey species and their predators when they use the  
465 same habitat (Valeix *et al.*, 2009a) as it is the case here for common lizards and *Vipera berus*,  
466 which live both in the same wet microhabitats (Guillon *et al.*, 2014). Our results suggest that  
467 hydroregulation may be critical in lizard populations with a lot of adders as the fear of  
468 predation can affect optimal hydroregulation strategies, and “the ecology of fear” (Clinchy *et*  
469 *al.*, 2013) should be taken into account when making predictions about the life history  
470 strategies of lizards facing changes in climate conditions.

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## 478 **6. Compliance with ethical standards**

479 **Conflict of interest** The authors have no competing or financial interests to declare that are  
480 relevant to the content of this article.

481 **Ethical approval** The experimental procedures involving vertebrate animals were carried out  
482 in accordance with institutional guidelines and ethical standards, under license from the  
483 French Ministry of the Environment (permits APAFIS#25252-2020042722118884). Research  
484 protocol was approved by the Darwin ethics committee on animal experimentation, regarding  
485 animal welfare.

486 **Informed consent** Informed consent was obtained from all individual participants included in  
487 the study.

## 488 **7. Data availability statement**

489 Data generated during the current study is available as a supplementary material and will be  
490 deposit in a Zenodo repository after acceptance.

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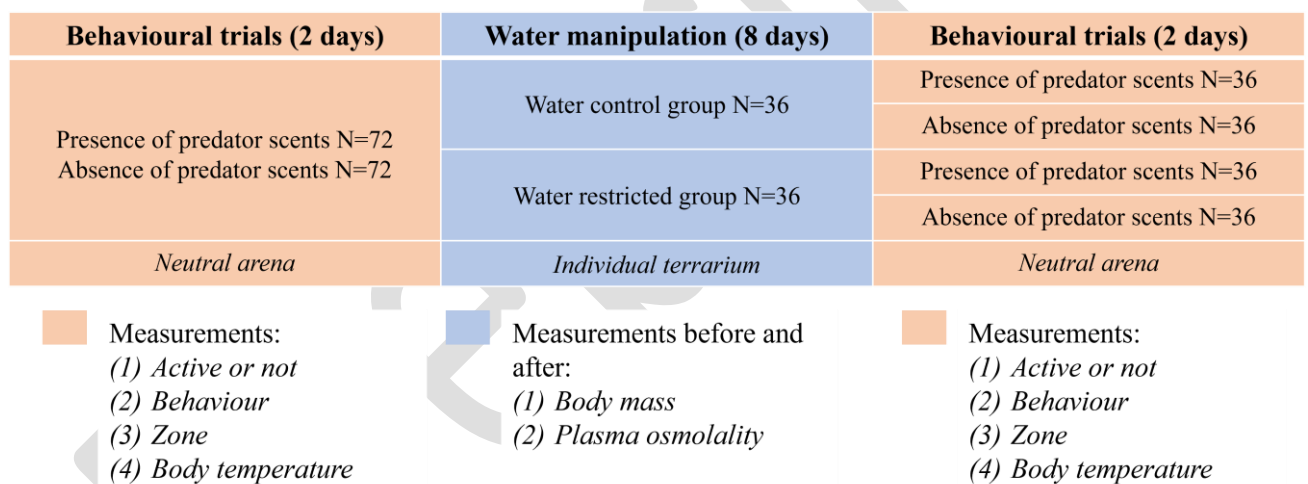
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677 **Figures and legend**



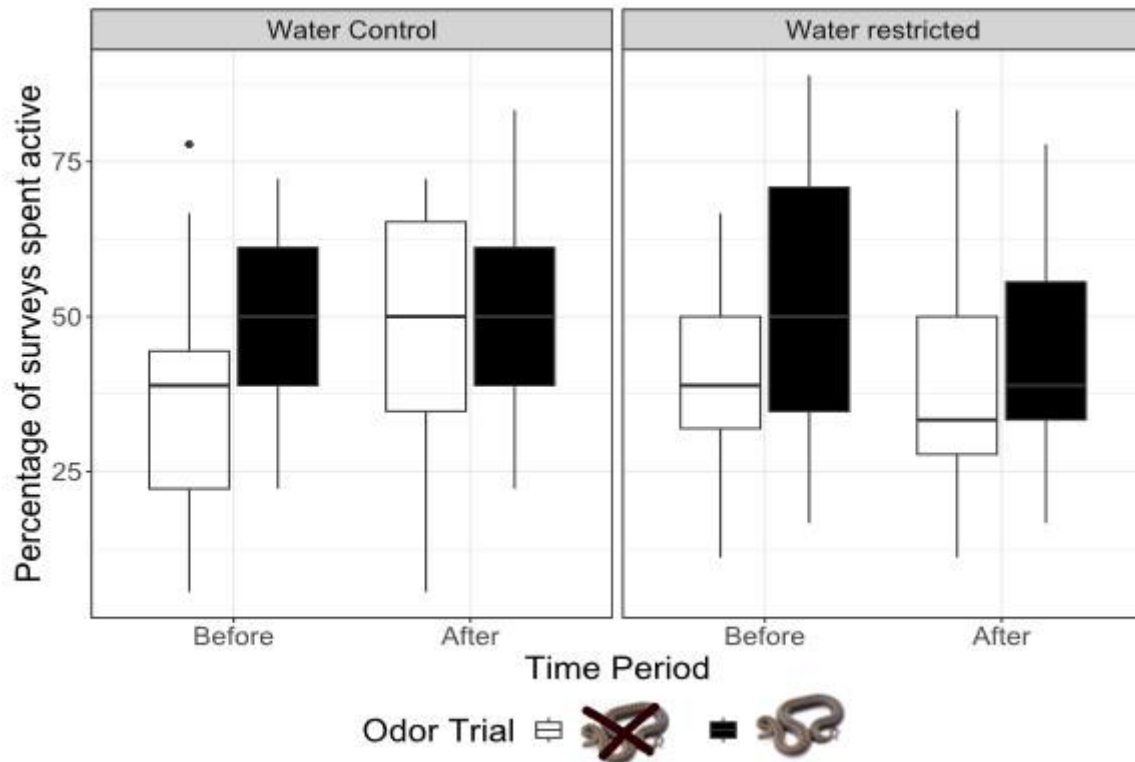
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679 **Fig.1** Timeline of the experimental design including behavioural trials with and without  
 680 predator odours (2 days), then a chronic water treatment during 8 days (water restriction or  
 681 water control) followed by a repetition of behavioural trials with and without predator odours  
 682 (2 days). **Lizards were transferred to the arena on the day before the observations began.**

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687 **Fig.2** Proportion of time spent active between 8:30am and 5:00pm in water treatment groups:

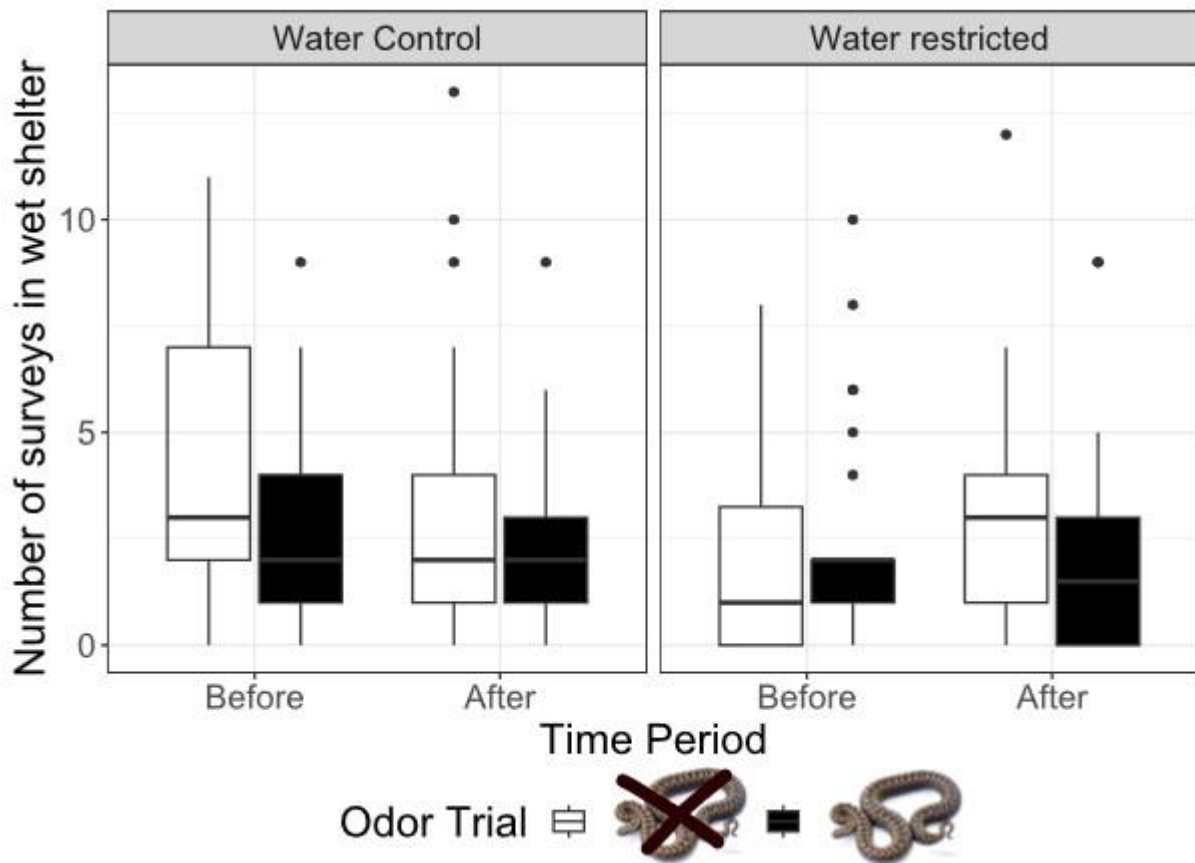
688 water control lizards on the left panel and water restricted lizards on the right panel.

689 Behavioural trials are separated according to the presence (black) or absence (white) of

690 predator scents in the environment. Data are reported for measurements “before” the start of

691 the water restriction manipulation, and “after” the end of the water restriction manipulation

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694 **Fig.3** Number of observations in wet shelter between 8:30am and 5:00pm depending of water

695 treatment groups: water control lizards on the left panel and water restricted lizards on the

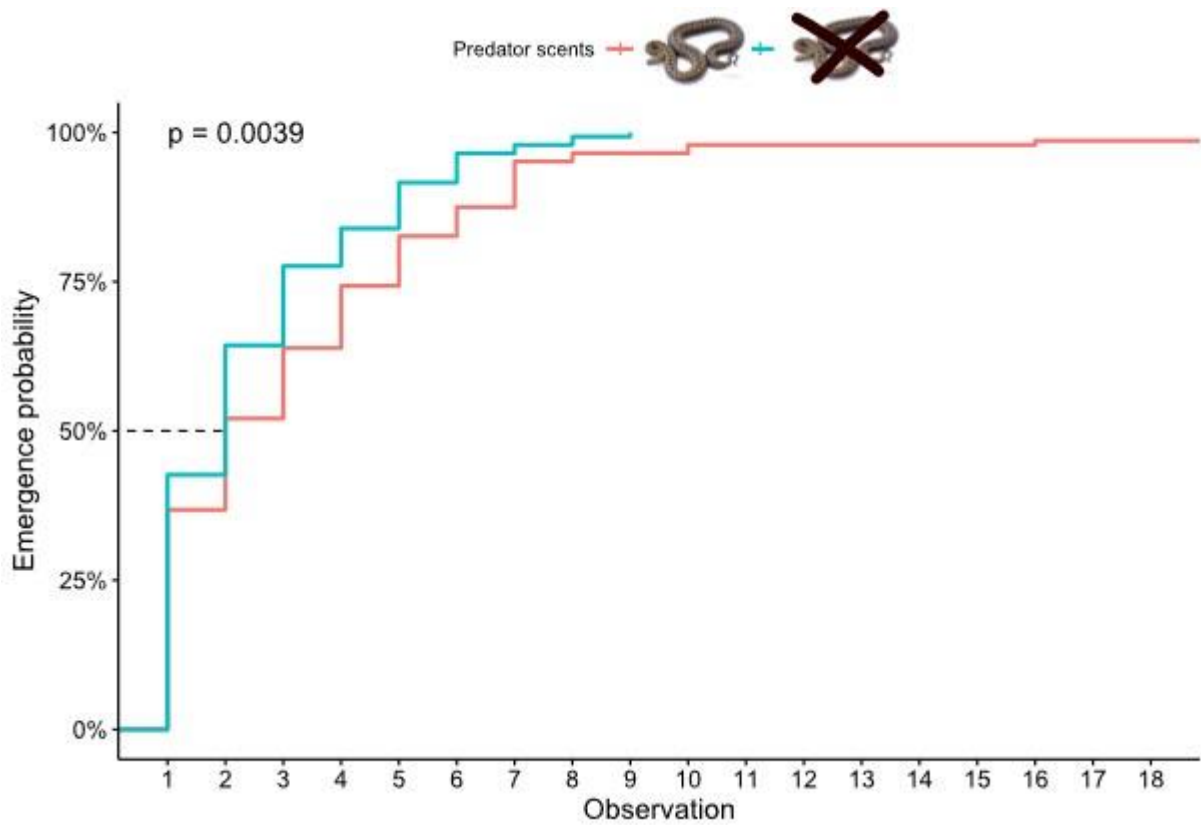
696 right panel. Behavioural trials are separated according to the presence (black) or absence

697 (white) of predator scents in the environment. Data are reported for measurements “before”

698 the start of the water restriction manipulation, and “after” the end of the water restriction

699 manipulation

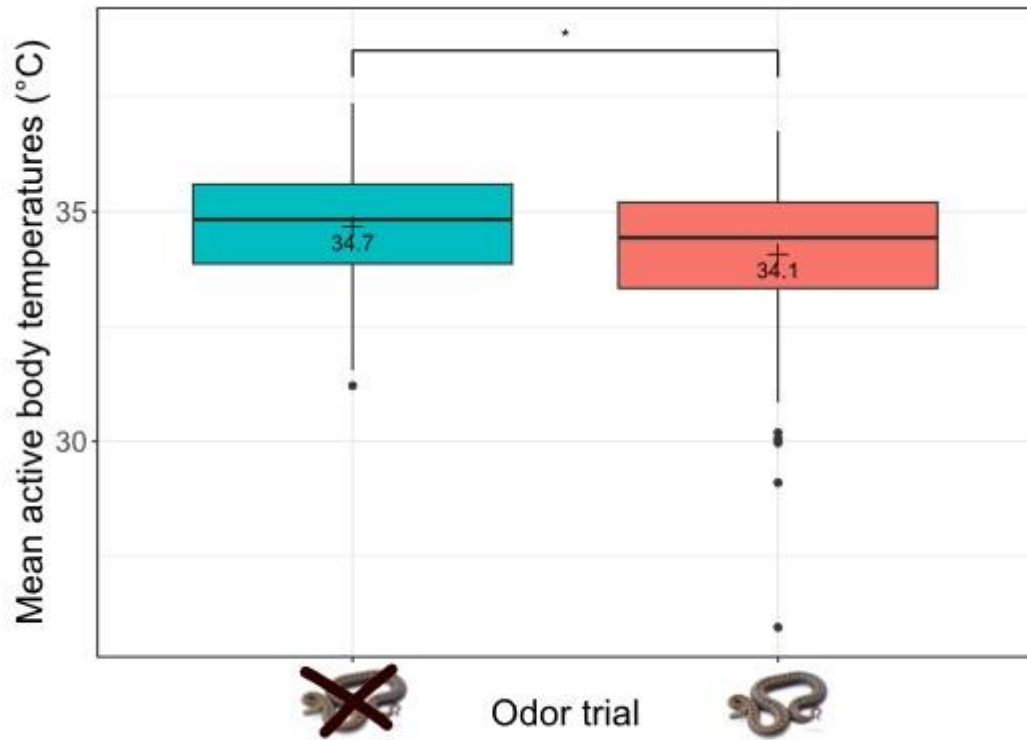
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702 **Fig.4** Emergence time depending on the presence (red line) or absence (blue line) of predator  
 703 scents from observation 1 starting at 8:30am to observation 18 ending at 5:00pm. Emergence  
 704 was scored every 30 mins. Representation are Kaplan-Meier survival curves, produced with  
 705 *survfit* function

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707

708 **Fig.5** Boxplot of body temperature during the reported active observations depending on the  
 709 absence (blue) or presence (pink) of predator scents in the arena. The plus sign represents  
 710 mean value, written just below, and horizontal line is the median value, N=72 individuals  
 711 observed two times each in both odor trials