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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 (≥ 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 (± 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 × 57 × 42cm) in a temperature-
162 controlled room maintained at 25°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°C ± **3.4 SD** ; dry: 27.9°C ± **4.2**
170 **SD**), and mean relative humidity was much higher in the wet one (wet: 54.4% ± **10.4 SD**; dry:
171 35.4% ± **9.4 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°C, maximum = 28.5°C ; hot mean = 35.4°C, maximum = 39.8°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 μl 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 μl) were immediately frozen at -28°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 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 ± 0.03 g (**3% of their initial body mass**) whereas control females gained 0.04 ± 0.02 g

284 (1% of their initial body mass ; estimate= -0.16 ± 0.04 , $p=0.0009$). However, water treatment
285 did not impact males (estimate= 0.05 ± 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 mOsm.kg^{-1} in male and female lizards, whereas non-restricted ones decreased
289 their osmolality by an average of 2 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 ± 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 ± 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 ± 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 ± 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 ± 0.2 times a day under a shelter and only slightly
316 more than half of the time under the wet shelter (2.18 ± 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 ± 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 ± 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: χ^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 2nd and the 3rd 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 ± 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⁻¹ 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.

491 **8. References**

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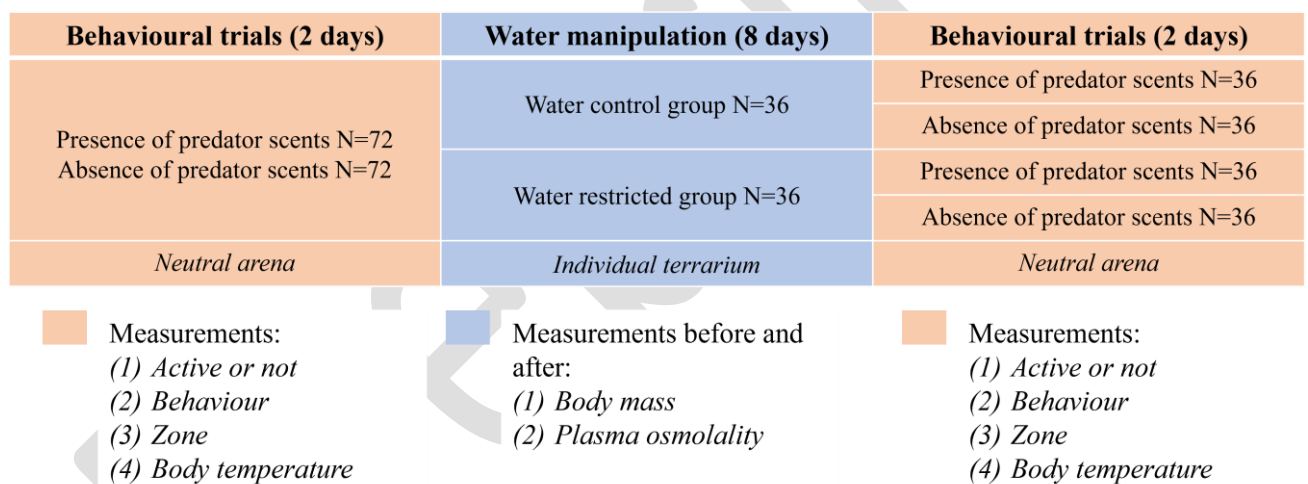
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677 **Figures and legend**



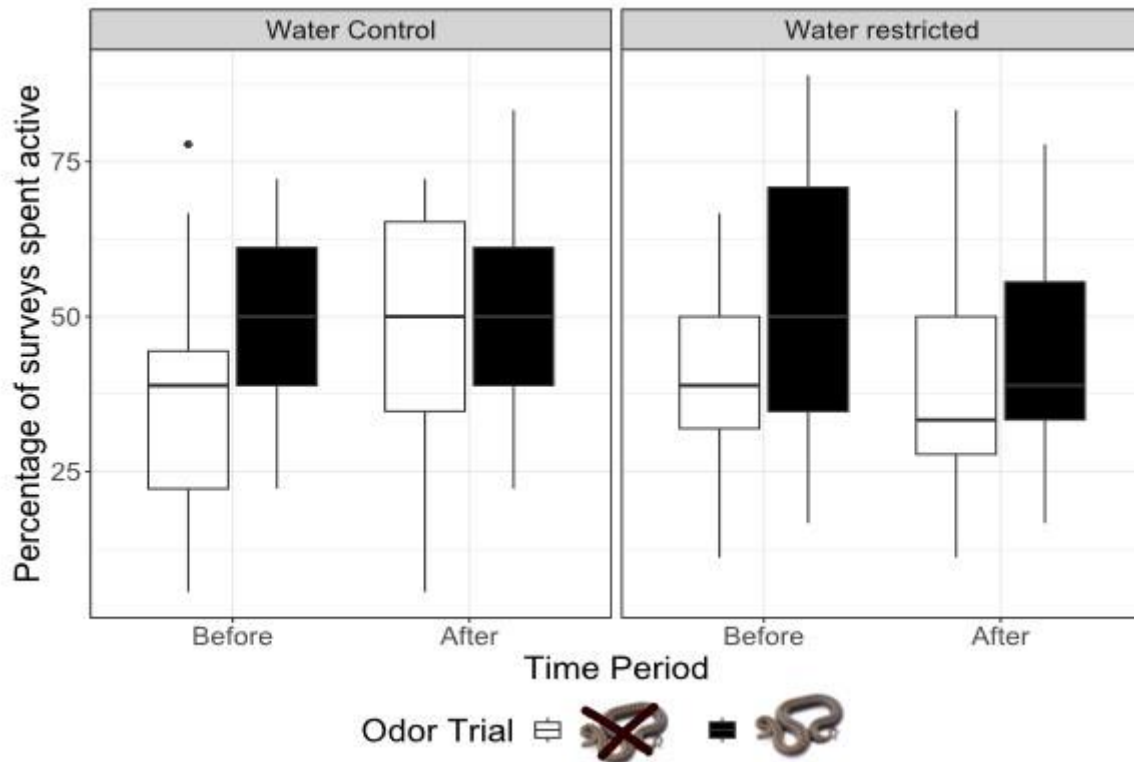
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679 **Fig.1** Timeline of the experimental design including behavioural trials with and without
680 predator odours (2 days), then a chronic water treatment during 8 days (water restriction or
681 water control) followed by a repetition of behavioural 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:

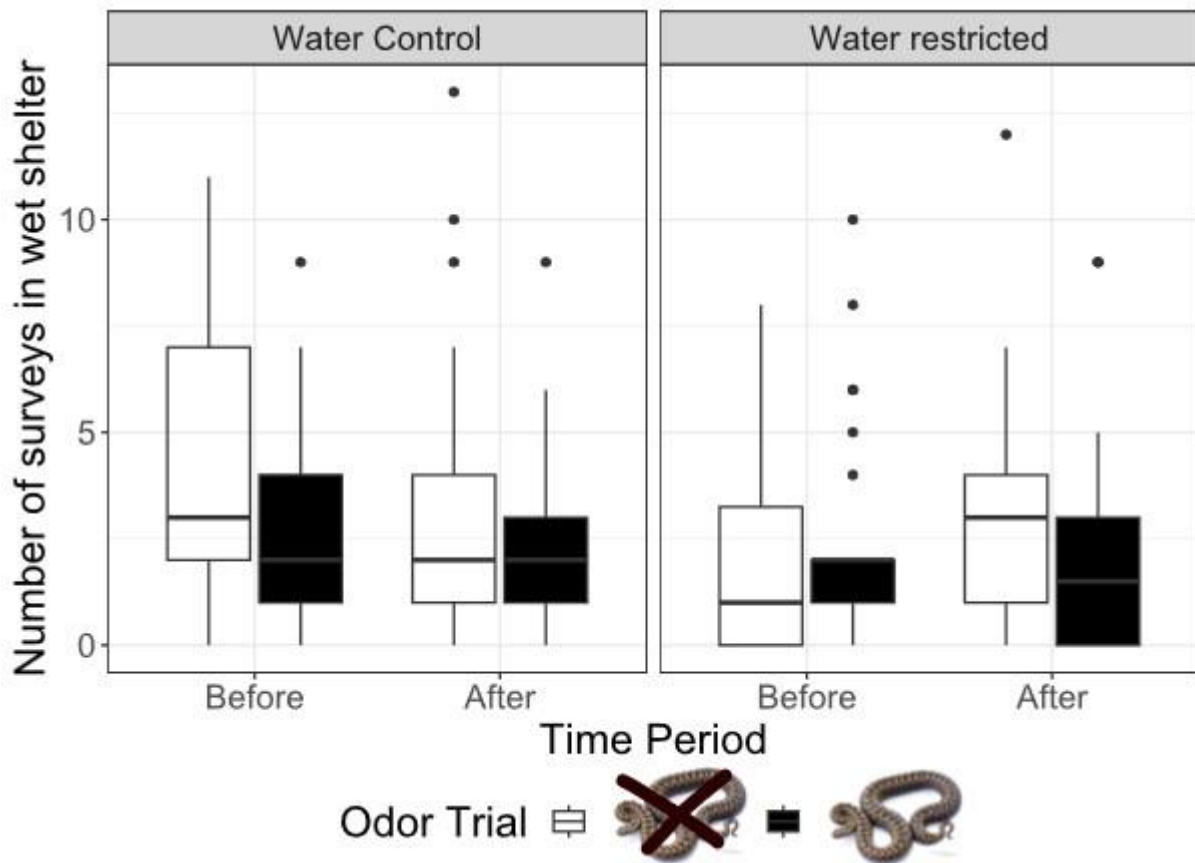
688 water control lizards on the left panel and water restricted lizards on the right panel.

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

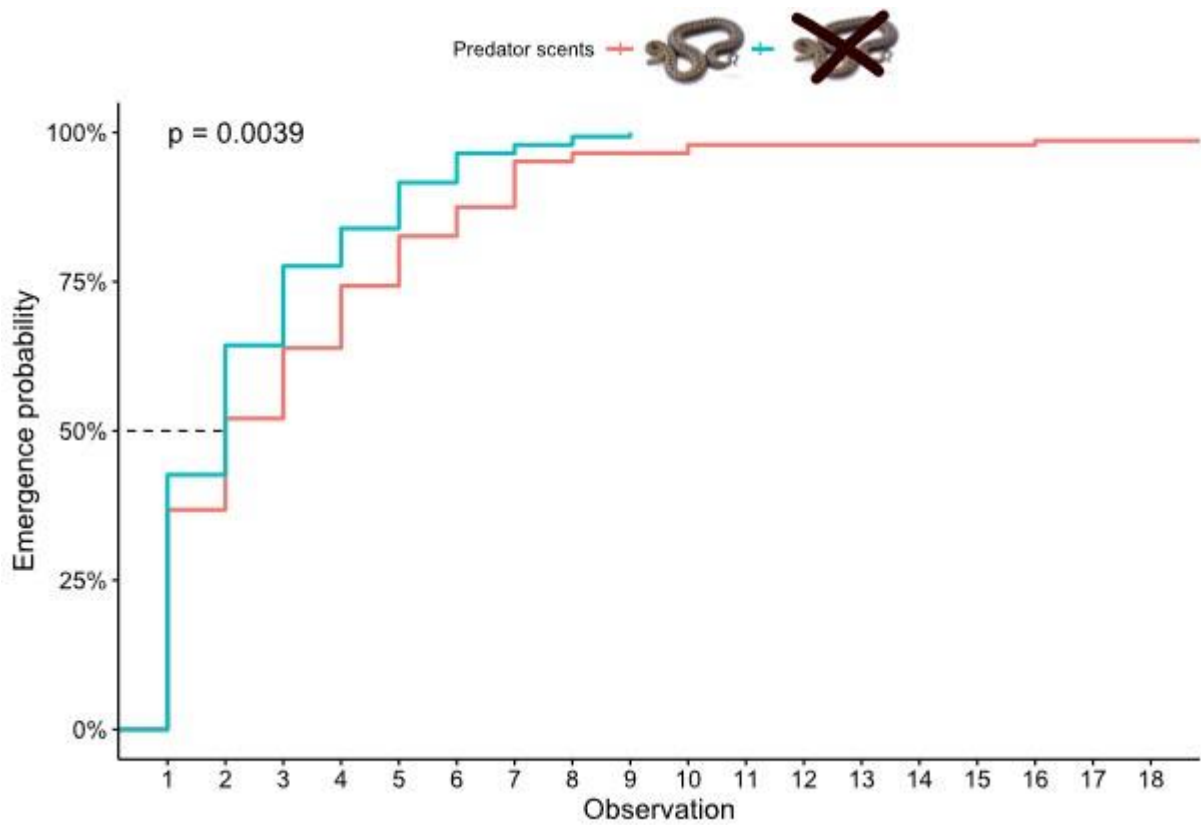
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694 **Fig.3** Number of observations in wet shelter between 8:30am and 5:00pm depending of water
 695 treatment groups: water control lizards on the left panel and water restricted lizards on the
 696 right panel. Behavioural trials are separated according 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

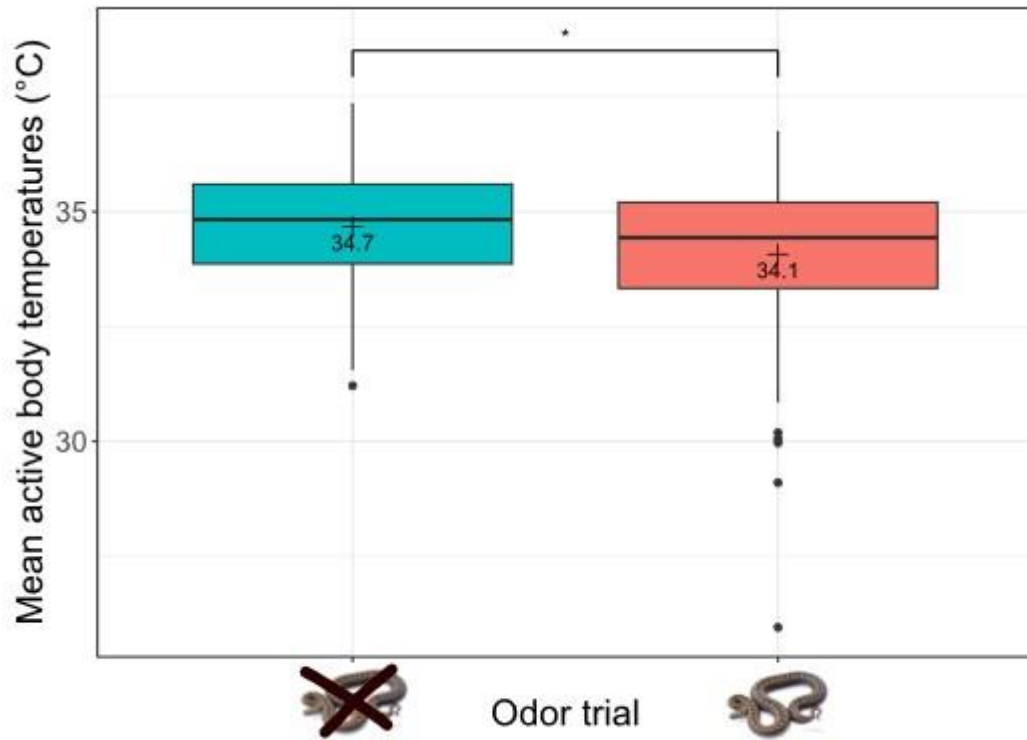
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702 **Fig.4** Emergence time depending on the presence (red line) or absence (blue line) of predator
 703 scents from observation 1 starting at 8:30am to observation 18 ending at 5:00pm. Emergence
 704 was scored every 30 mins. Representation are Kaplan-Meier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