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1 Hydric status influences salinity-dependent water selection in frogs from
2 coastal wetlands

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4

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20

21 **Abstract.**

22 The environment is heterogeneous across spatial and temporal scales, and the
23 behavioural responses required to adjust individuals' needs to resource availability
24 across such variable environments should be under selective pressure. Coastal
25 wetlands are characterized by a diversity of habitats ranging from fresh- to salt
26 water; and individuals occurring in such complex habitats need to adjust their
27 habitat use based on their osmotic status. In this study, we experimentally tested
28 whether an amphibian species (*Pelophylax* sp.) occurring in coastal wetlands was able
29 to discriminate and select between different salinity concentrations (0, 4, 8 and 12 g.l-
30 ¹) and whether hydric status (hydrated *versus* dehydrated) influenced salinity-
31 dependent water selection. We found that frogs selected water based on salinity
32 differentially between hydrated and dehydrated individuals, with the later favoring
33 lower salinities likely to improve their osmotic status. Interestingly, we highlighted
34 the ability of frogs to select lower salinity before having access to water, suggesting
35 that frogs can assess water salinity without actual contact. In coastal wetlands where
36 salinity of water bodies can dynamically vary through space and time, such
37 behavioural osmoregulation process is potentially a key factor affecting individual
38 movements, habitat choice and thus species distribution. Our study further
39 highlights the importance of salinity-dependent habitat heterogeneity and especially
40 the presence of freshwater environments as structuring factors for the amphibian
41 community.

42 **Keywords**

43 Activity, Amphibian, Dehydration, Osmotic status, Habitat selection, Salinity

45 **1. Introduction**

46

47 The environment is heterogeneous across spatial and temporal scales. Such variation
48 affects species distribution according to the availability of resources through
49 processes of habitat selection [1]. Habitat selection by a species can also vary
50 according to environmental characteristics (e.g., climate, predation pressure) [2-4],
51 life-history stages (e.g., age, growth, reproduction) [1,5-7], physiological status (e.g.,
52 energy budget, immune status, water balance) [8,9], or a combination thereof [1]. As
53 a consequence, the ability of individuals to assess habitat quality should strongly
54 influence their performance and survival, and the behavioural responses required to
55 adjust individual needs to resource availability are expected to be under strong
56 selective pressure [1].

57

58 Freshwater is a vital resource that influences the ecology and behaviour of animal
59 species, especially in environments where water is restricted or unavailable [10,11].
60 Indeed, free (drinking) water can be limited across temporal (e.g., season) and spatial
61 (e.g., rivers, ponds) scales in the environment [12,13]. Restricted freshwater
62 availability (or water salinity, see below) causes physiological dehydration [14],
63 which can affect a wide array of physiological and behavioural individual
64 characteristics [15]. For instance, dehydration is related to an increased plasma
65 osmolality, decreased plasma volume, increased protein content [16-18] and has
66 been shown to induce increased stress levels [19], increased oxidative damages [20]
67 all of which can led to impaired fitness or mortality [15,21].

68

69 In addition to these physiological mechanisms, dehydrated individuals often develop
70 specific responses that aim to decrease additional water loss and to promote water
71 acquisition, such as drinking behavior in freshwater [22,23]. Typical examples of
72 these responses involve major changes in activity [14,24,25], reproduction [26,27], or
73 locomotor performances [28]. In order to evade the detrimental effects of water
74 restriction, individuals can actively move in the environment to seek for and acquire
75 water to hydroregulate [15] and such process can affect habitat selection. Indeed,
76 drinking water availability has been shown to influence population distribution [12],
77 and trades-off with predation risks or food quality [29–31]. Ultimately, access to
78 freshwater and/or osmoregulation has been suggested to influence evolutionary
79 mechanisms at a global scale [32,33].

80

81 These hydroregulatory mechanisms are particularly important in coastal
82 environments where organisms are subject to complex interactions between marine
83 and terrestrial influences [34,35]. In brackish or salt water, dehydration occurs
84 through salt gain and water loss, and most organisms have to regulate the osmolality
85 of their body fluids in order to survive [36]. Coastal wetlands are characterized by a
86 high diversity of habitats ranging from fresh to salt water [34,37]. As a consequence,
87 individuals occurring in such complex environments can dynamically adjust their
88 habitat use according to their osmotic status [23,38]. Indeed, dehydrated individuals
89 are expected to select - and to move to - habitat that will allow to restore their
90 osmotic balance [39,40]. Yet, behavioural responses to limit exposure to dehydration

91 and/or osmotic disequilibrium of species occurring in the complex habitat matrix of
92 coastal wetlands have been overlooked to date (but see [23,40]).

93

94 Amphibians are particularly well-suited to investigate this question for several
95 reasons. First, amphibian species are abundant in coastal ecosystems [41]. Second,
96 these taxa are particularly dependent on fresh water availability because of their
97 complex life cycle which requires both aquatic (i.e., eggs and larvae) and terrestrial
98 environments [42]. Third, their highly vascularized and permeable skin makes them
99 particularly susceptible to water salinity [43–45]. Indeed, detrimental effects of
100 relatively high salinity have been highlighted across life-history stages (e.g., eggs,
101 larvae and adult individuals, [46]). Fourth, their comparatively low dispersal ability
102 prevents large scale movements to evade detrimental conditions, and amphibians
103 display strong habitat selection at a short spatial scale [47]. Finally, although some
104 species have been shown to be locally adapted to relatively high salinity [39,48,49],
105 their habitat preference remain markedly oriented toward lower salinity when
106 available [50]; presumably because of the fitness costs associated to dehydration [25].
107 In addition, there are evidences that reproductive females may select oviposition
108 sites based on salinity [46,51,52] because low salinity is necessary for successful
109 embryonic and larval development [46,53]. Yet, whether such habitat selection
110 occurs independently from the constraints associated to embryonic and larval
111 development remains an open question. Similarly, whether individual's osmotic
112 status influences habitat selection in amphibians has not been investigated to date.

113

114 In this study, we experimentally tested whether individuals from an amphibian
115 species (*Pelophylax* sp.) occurring in both fresh- and brackish water in coastal
116 wetlands were able to discriminate and select between different salinity
117 concentrations (0, 4, 8 and 12 g.l⁻¹). We further assessed whether hydric status
118 (hydrated *versus* dehydrated) influenced salinity-dependent water discrimination
119 and selection by experimentally manipulating individual's hydration state after an
120 acclimation to freshwater (0 g.l⁻¹) or brackish water (12g.l⁻¹). We evaluated behavioral
121 preference for and avoidance of salinity through quantification of the time spent in
122 the four salinity treatments, and made the following predictions:

- 123 - Because of the physiological costs associated to elevated salinity, individuals
124 should avoid elevated salinity.
- 125 - Dehydrated individuals should select for salinities that should improve their
126 hydric status.

127

128

129 **2. Material and methods**

130

131 **2.1. Study site, species and sampling**

132 The study was carried out on the « Réserve Naturelle Nationale de Moëze-Oléron »
133 (45°53'33.36"N, 1°04'59.16"W), situated in the Atlantic coast of France (Département
134 de la Charente-Maritime). In our study area, *Pelophylax* sp. are mainly composed of
135 viable and fertile hybrids (Graf's hybrid frog, *P. kl. grafi*) of the Marsh frog (*P.*
136 *ridibundus*) and the Perez's frog (*P. perezii*) [54].

137 Individuals were captured at night ca. 11pm during 34 sampling events, which
138 occurred, from 03/06/2021 to 13/07/2021, on fresh (<1 g.l⁻¹) and brackish (>1 g.l⁻¹)
139 ponds, with salinity ranging from 0.11 g.l⁻¹ to 8.41 g.l⁻¹ (mean= 3.50 g.l⁻¹ ± 2.41 SE,
140 measured with a conductimeter YSI Professional Plus). A total of 222 individuals
141 were captured and tested (40 from freshwater ponds, and 182 from brackish ponds).

142

143 **2.2. Manipulation of the hydric state**

144 Individuals were weighted (with a portable electronic balance ±0.1 g), measured
145 (Snout-vent length [SVL] ±0.1 cm), and sexed (131 females and 88 males). To
146 manipulate the osmotic status of individuals, we subjected frogs to an acclimation
147 period (10h) either in freshwater (0.36 g.l⁻¹ ± 0.01, N=120) or in brackish water (12.13
148 g.l⁻¹ ± 0.10, N=122). Frogs were individually placed in small containers (14*16*9 cm)
149 containing one of the treatments and the water level was adjusted to individual size
150 to allow continuous contact with water.

151 Body size and body mass between these two groups were similar at the onset of the
152 acclimation period (body mass: 32.03g ± 1.60 at 0 g.l⁻¹ and 32.67g ± 1.65 at 12 g.l⁻¹, lm

153 test: Estimate= 0.646, SE= 2.297, t-value= 0.281, p-value= 0.779; body size: 70.53 mm ±
154 1.00 at 0 g.l⁻¹ and 70.89 mm ± 1.01 at 12 g.l⁻¹, lm test: Estimate= 0.371, SE= 1.423, t-
155 value= 0.257, p-value= 0.797).

156 After 10h of acclimation, individuals were weighted (±0.1 g) to verify that water loss
157 (and thus dehydration) occurred in the 12g.l⁻¹ group. To further assess that our
158 procedure actually affected osmotic status, we further assessed plasma osmolality in
159 a subsample of individuals (N=5 in each treatment). Blood samples (50µl) were
160 obtained through cardiocentesis, centrifuged for 7 min at 3000G and plasma was
161 separated from blood and stored at -20°C. Plasma osmolality (mOsmol.kg⁻¹) was
162 measured from 10 µl aliquots on a Vapro2 osmometer (Elitech group).

163

164 **2.3. Activity and selection of salinity**

165 After the 10h acclimation period, individuals were placed out of water for 2h before
166 the actual behavioural tests.

167 To test for water selection, we constructed an arena in a large container (50 * 33 * 27
168 cm) containing 4 water compartments (14*16*9 cm) at each corner and a central area
169 (levelled to upper part of the compartments) providing a substrate without water.

170 Each compartment contained water with different salinities: 0 g.l⁻¹ (0.36 g.l⁻¹ ± 0.01
171 SE), 4 g.l⁻¹ (4.12 g.l⁻¹ ± 0.10 SE), 8 g.l⁻¹ (8.07 g.l⁻¹ ± 0.12 SE) and 12 g.l⁻¹ (12.13 g.l⁻¹ ± 0.10
172 SE) (Figure 1). These values were selected to mimic the salinity of ponds where frogs
173 were present at our study site (mean: 2.86±2.17 g.l⁻¹, min: 0.14g.l⁻¹, max: 16.19g.l⁻¹).

174 The location of each treatment in the experimental arena was randomly set for each
175 trial.

176 The test begins with 30 minutes of acclimation, during which individuals were
177 placed in the central area (out of water) in a mesh cage that prevented the frog to
178 actually reach the water compartments (Fig 1a). After 30 minutes, the cage was
179 removed (Fig 1b), and the behaviour of each individual was recorded for 2h with a
180 GoPro Hero 8.

181 After the experiments, individuals were shortly released at their site of capture.

182

183 At the end of the 30min acclimation period (before actual access to water
184 compartments), we assessed the orientation of the individuals toward one of the
185 water compartments using the orientation of the antero-posterior axis of the
186 individuals toward one of the water treatments. This variable can reflect whether
187 individuals can discern water salinity without actual contact (prevented by the mesh
188 cage).

189 Once the mesh cage was removed, from the 2h footages for each individual we
190 extracted the following variables:

191 - Latency before moving in one of the water compartments.

192 - Time spent out of the water and time spent in each water compartment.

193 - Total number of movements (between compartments including the central area out
194 of water).

195

196 **2.4. Statistical analyses**

197 *2.4.1. Manipulation of the hydric state*

198 We compared osmolality and body mass after acclimation at 0 g.l⁻¹ or 12 g.l⁻¹) with
199 lmer tests (Linear Mixed-Effects models) with individual identity as a random effect,
200 and Tukey post-hoc tests.

201

202 2.4.2. *Orientation without access to water*

203 We analyzed orientation toward water compartments with Fisher's chi square tests,
204 and Fisher's post-hoc tests.

205

206 2.4.3. *Activity and selection of salinity*

207 2.4.3.1. Activity

208 We used lms (linear models) with acclimation to salinity (0 g.l⁻¹ or 12 g.l⁻¹) as a
209 covariate to investigate the influence of hydric status on the latency before reaching a
210 water compartment and on the time spent out of water; while the total number of
211 movements was analyzed using poisson glms with acclimation to salinity as a
212 covariate.

213

214 2.4.3.2. Water selection

215 We used Fisher's chi square tests, and Fisher's post-hoc tests to test whether hydric
216 status influenced the salinity of the first visited compartment.

217 For total time spent in each salinity, we used binomial glmer with the salinity
218 treatment as a covariate, and individual identity as a random effect. Because of the
219 large difference of time spent out of *versus* in water between hydric status (see
220 results), this analysis was performed with total spent in each treatment transformed
221 as a proportion of the total time spent in water.

222 The influence of individual morphology (body size) on the proportion of time spent
223 in each treatment between hydric status was investigated using glmer (interaction
224 between treatment and body size [SVL]) with individual identity set as a random
225 effect.

226

227 For all analyses, we included interaction with salinity of the pond of capture as a
228 covariate, and interaction with sex as a covariate. We never found any significant
229 effect of these two variables (salinity of the pond of capture, all $p > 0.062$; sex, all $p >$
230 0.094), and these variables were excluded from our final models.

231

232

233 All data analysis were performed using R 3.6.3 [55] and Rstudio v1.1.419.

234 3. Results

235 3.1. Manipulation of the hydric state

236 Acclimation at 12 g.l⁻¹ significantly affected hydration state of individuals as shown
237 by the higher loss of body mass (i.e., water effluxes) in this group. Frogs acclimated
238 to 12 g.l⁻¹ lost on average 4.98±0.27g (~15.6% of their initial body mass, Estimate=-
239 4.98, SE=0.272, t-value=-18.280, p-value<0.001) while individuals from the 0g.l⁻¹
240 group lost less mass, with an average of 0.53±0.19g (~1.2% of their initial body mass,
241 Estimate=-0.54, SE=0.189, t-value=-2.817, p-value=0.006). Body mass loss differed
242 significantly between groups (lm test: estimate=-14.435, SE= 0.73, t=-19.83, p<0.001).
243 In addition to water loss illustrated by change in body mass, acclimation at 12 g.l⁻¹
244 significantly affected plasma osmolality (which reflects both water loss and salt gain)
245 of individuals that increased from 245.4 mOsm.kg⁻¹ ± 4.26 SE to 364.4 mOsm.kg⁻¹ ±
246 7.33 SE (Estimate=119.00, SE=5.848, t-value=20.35, p-value<0.001), while it slightly,
247 but not significantly decreased in individuals maintained at 0g.l⁻¹ (from 249.0
248 mOsm.kg⁻¹ ± 3.36 SE to 242.6 mOsm.kg⁻¹ ± 5.09 SE, Estimate=-6.40, SE=5.492, t-
249 value=-1.165, p-value=0.244). Final osmolality differed significantly between groups
250 (lm test: estimate=-121.80, SE= 7.38, t=16.50, p<0.001).

251

252 3.2. Orientation without access to water

253 During the 30 min period in the mesh cage, hydrated individuals did not
254 preferentially orient to specific compartments (water compartments with different
255 salinities or the dry area, p=0.08, Figure 2). In strong contrast, dehydrated
256 individuals significantly avoided to orient toward the compartment containing water
257 with elevated salinity (12g.l⁻¹, all p<0.012, Figure 2), significantly oriented more often

258 toward the dry area (all $p < 0.037$, Figure 2) but were equally oriented toward other
259 compartments (all $p > 0.768$, Figure 2). Such diverging orientation pattern is
260 exemplified by the significant difference of the number of individuals that oriented
261 toward the elevated salinity compartment (12g.l^{-1}) between hydrated and dehydrated
262 individuals ($p = 0.025$, all other $p > 0.195$, Figure 2).

263

264 3.3. Activity and selection of salinity

265 Once the mesh cage was removed, patterns of activity and compartment use were
266 strongly different between hydrated and dehydrated individuals.

267

268 3.3.1. Activity

269 The latency before reaching a water compartment was longer in dehydrated
270 individuals than in hydrated ones ($15.04\text{min} \pm 2.072\text{ SE}$ and $2.04\text{min} \pm 0.642\text{ SE}$
271 respectively, lm test: Estimate= -13.001, SE= 2.169, t-value= -5.994, p-value<0.001).

272 Similarly, hydrated individuals moved more than dehydrated ones ($18.68 \pm 1.531\text{ SE}$
273 *versus* $2.23 \pm 0.240\text{ SE}$ movements respectively, glm test: Estimate= 2.124, SE= 0.192, t-
274 value= 11.05, p-value<0.001).

275 During the whole duration of the experiment, hydrated individuals spent more time
276 out of water than dehydrated ones ($66.82\% \pm 3.25\text{ SE}$ and $4.32\% \pm 1.25\text{ SE}$, lm test:
277 Estimate= -62.494, SE= 3.482, t-value= -17.950, p-value<0.001, Figure 3).

278

279 3.3.2. Water selection

280 Focusing on the first visited water compartment, hydrated individuals selected
281 equally between salinities ($p = 0.125$). In contrast, dehydrated individuals

282 significantly avoided the water compartment containing water with elevated salinity
283 as the number of individuals choosing the water at 12g.l⁻¹ for their first visit was
284 significantly lower than those choosing either 0g.l⁻¹ or 4g.l⁻¹ (all p<0.024) but not
285 different from those choosing 8 g.l⁻¹ (p=0.116). This diverging choice in first visited
286 water compartment is exemplified by the significant difference of the number of
287 individuals that first visited the elevated salinity (12g.l⁻¹) compartment between
288 hydrated and dehydrated individuals (p=0.040, all other p>0.323).

289

290 A similar result was found across the whole duration of the experiment. Overall, if
291 both hydrated and dehydrated individuals spent a different amount of time in each
292 compartment (hydrated individuals: Sum sq= 16404, F-value=5.9673, p-value<0.001;
293 dehydrated individuals: Sum sq=66833, F-value=13.73, p-value<0.001), the
294 proportion of time spent in each treatment was different for the two groups (Figure
295 4, Table 1). Hydrated individuals spent more time in 0g.l⁻¹ than in other
296 compartments (all p<0.033) but used equally these other compartments (all p>0.555)
297 (Figure 4, Table 1). In contrast, dehydrated individuals spent significantly less time in
298 12g.l⁻¹ than in other compartments (all p<0.001) but used equally these other
299 compartments (all p>0.636).

300 The amount of time spent in each compartment differed between hydrated and
301 dehydrated individuals (all p-value <0.04), except for the freshwater compartment
302 (0g.l⁻¹) in which hydrated and dehydrated individuals spent a similar amount of time
303 (lm: Estimate=0.323, SE=5.654, t-value=0.057, p-value=0.955).

304

305 Interestingly, we found an effect of the size of the tested individuals on the water
306 selection in dehydrated individuals solely (Figure 5). Indeed, in this group, larger
307 individuals tended to select less often the freshwater treatment (Estimate=-0.783,
308 SE=0.412, t-value=-1.900, p-value=0.06, Figure 5) but significantly selected more often
309 the 8g.l⁻¹ salinity treatment (Estimate=1.017, SE=0.385, t-value=2.639, p-value=0.009,
310 Figure 5). For hydrated individuals, there was no differences according to size in the
311 observed responses (all p-value>0.09).

312

313

314

315

316

317 4. Discussion

318 Our experiment allows to demonstrate that frogs select water bodies based on their
319 salinity and that such selection differ between hydrated and dehydrated individuals.
320 Experimental manipulation of the hydric status (acclimation to 0g.l⁻¹ or 12g.l⁻¹) prior
321 to behavioural tests influenced the orientation of individuals toward specific water
322 compartments, but also activity patterns and the amount of time spent in each
323 salinity.

324

325 One of the most salient results from our study is the ability of frogs to orient to (and
326 thus select) lower salinity before actually having a direct access to water. Indeed,
327 detection of water (or soil) salinity has been usually shown to require contact
328 between water and chemosensory organs [56–58]. In our context, the mesh cage
329 prevented individuals to have direct access to water, suggesting that frogs can assess
330 water salinity without contact. Such result further suggests that indirect
331 environmental cues can be used by frogs to assess water salinity. Two different but
332 not mutually exclusive mechanisms can be hypothesized in our context. First,
333 olfaction may be used to assess water salinity (based on both sodium and chloride
334 ions) as it has been recently shown in fish [59,60]. Yet, as stated above, such example
335 involved actual contact between water and olfactory organs. Volatile compounds
336 have been shown to be used by shore insects to select for the salinity of their habitat
337 [61]. However, these volatile compounds seems to be produced by specific bacteria
338 [62,63] and such salinity-dependent bacterial activity seems unlikely in our
339 experimental context where treatment water was freshly prepared before each
340 behavioural trial. Alternatively, it is also plausible that frogs can visually assess

341 water salinity based on differing reflection of light between salt- and fresh water [64].
342 Moreover, fresh and saltwater differ in their depolarization ratio [65]. Amphibians
343 have been shown to detect polarized light [66–68], and are able to use polarization
344 patterns for orientation [66]. Although our experimental setup do not allow to tease
345 apart these different but not mutually exclusive mechanisms, our result highlight
346 that frogs can assess water salinity without direct contact. Such ability may reveal
347 critical for individuals occurring in coastal wetlands where the salinity of water
348 bodies can dynamically vary through space and time and where selection of
349 adequate water salinity can ultimately influence reproductive success and individual
350 survival. Future experimental studies are required to assess whether frogs rely on
351 olfactory or visual cues (or a combination thereof) to orient toward low salinity water
352 bodies.

353

354 Activity patterns were highly divergent between hydrated and dehydrated
355 individuals. Overall, hydrated individuals took less time to explore water
356 compartments and moved more during the behavioral trials. Such differences
357 dovetail relatively well with the expected consequences of dehydration on activity
358 levels and locomotor performances [24,25]. Interestingly, hydrated individuals spent
359 more time emerged than their dehydrated counterparts, indicating that dehydrated
360 individuals actively seek contact with water in order to allow cutaneous water
361 absorption (“cutaneous drinking”, [69]) to equilibrate their hydromineral balance
362 [50]. Conversely and as expected, such need to osmoregulate did not occur in
363 hydrated individuals which thus remained out of water for longer period of time.

364 Future studies should usefully explore whether similar contrasts can occur following
365 dehydration in air rather than in brackish water.

366

367 Importantly, water selection differed between hydrated and dehydrated individuals.
368 Overall, hydrated individuals spent most of their immersed time in freshwater. Yet,
369 when using the other water compartments, they spent similar amount of time in each
370 of the salinity treatment irrespective of salt concentration. Such result indicates that
371 although hydrated individuals preferred to be in contact with fresh water, they did
372 not actively avoid the highest salinity compartment as compared to the two lower
373 salinities. In strong contrast, dehydrated individuals actively selected for water
374 salinities below the one to which they were acclimated. They spent similar amount
375 of time in freshwater and in the two intermediate salinities, all of which would allow
376 individuals to correct, at least in part, for both dehydration and elevated osmolality.
377 As shown in other species [40,70], dehydrated individuals actively avoided the
378 highest salinity presumably to avoid additional water loss and salt gain, both of
379 which are likely to jeopardize activity, locomotion [71–73] and ultimately survival
380 [74]. Interestingly, we found a small but significant effect of body size on water
381 selection, as expected from the larger surface area to volume ratio of smaller
382 individuals making them more susceptible to water loss due to high salinity [75]. In
383 dehydrated individuals, smaller frogs spent more time in freshwater and less time in
384 brackish water (8 g.l^{-1}), while no effect of body size was apparent in hydrated
385 individuals.

386

387 Finally, we did not found any influence of the salinity of the pond on which
388 individuals were captured. Such result is likely to be linked to the spatial and
389 temporal dynamics of salinity in our environmental context [34,35]. Indeed, our
390 study species is relatively mobile [47] and it is plausible that individuals move
391 between ponds depending on environmental characteristics (including salinity) and
392 may have experienced a relatively large panel of salinity across this costal habitat
393 [34,35], which may lessen putative local acclimation to the salinity condition of each
394 pond.

395

396 **5. Conclusions**

397 Our study shows that coastal frogs can assess water salinity and select water bodies
398 based on both their salinity and the individuals' hydric and/or osmotic status, which
399 indicate behavioural omoregulation. In coastal wetlands where salinity of water
400 bodies can dynamically vary through space and time [34], such ability is potentially a
401 key factor affecting individual movements, habitat choice and thus species
402 distribution. We emphasize that knowledge of factors affecting habitat choice can
403 have direct implications for the management of natural habitats and may greatly
404 influence conservation actions [1]. In this respect, our study highlights the
405 importance of salinity-dependent habitat heterogeneity and especially the presence
406 of freshwater environments as structuring factors for the amphibian community.

407

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420

421 *Data Availability Statement*

422 Data will be made available by the corresponding author upon acceptance.

423

424 *Competing interests statement*

425 The authors declare that they have no conflict of interest.

426

427 *Ethics statement*

428 This work was approved by the French authorities under permits R-

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648 **Caption to figures**

649 Figure 1: Experimental set up (a) with the mesh cage and (b) after the mesh cage has
650 been removed

651

652 Figure 2: Number of individuals that oriented toward each compartment while in the
653 mesh cage (no access to water) according to their hydric state

654

655 Figure 3: Proportion of time in water (irrespective of salinity) and out of water for
656 hydrated and dehydrated individuals.

657

658 Figure 4: Proportion of time spent in each treatment (as a proportion of the total time
659 in water) according to the hydric state of individuals

660

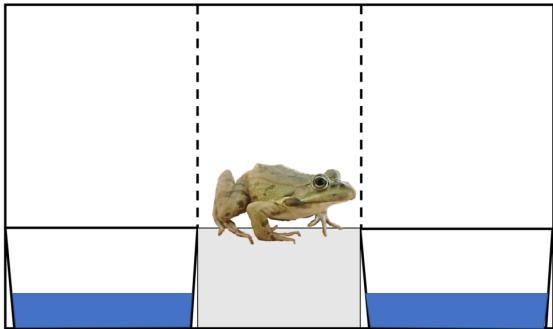
661 Figure 5: Effect of individual morphology (body size) on the proportion of time spent
662 in each treatment (as a proportion of the total time in water), for hydrated
663 individuals (left column, abcd) or dehydrated individuals (right column, efgh)

664

(a)



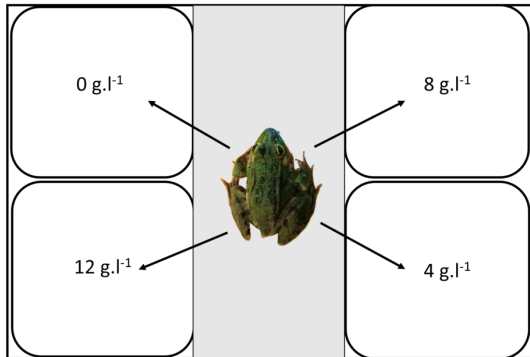
30 minutes



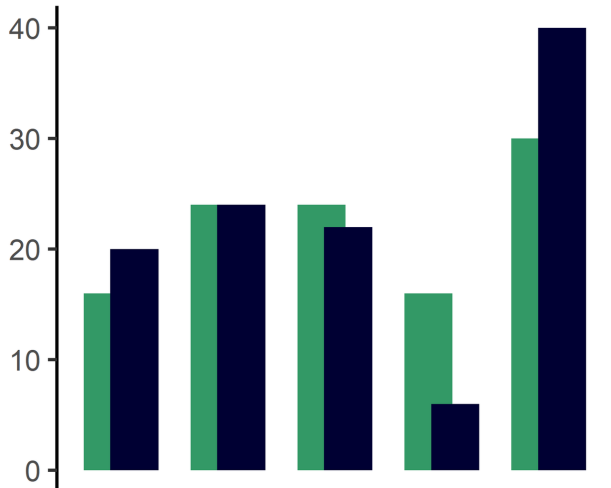
(b)



2 hours



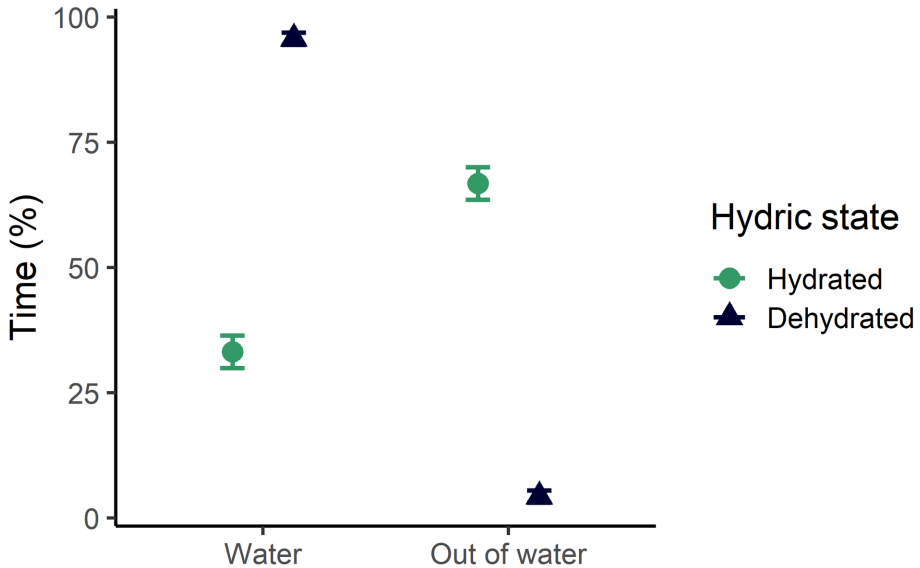
Number of individuals

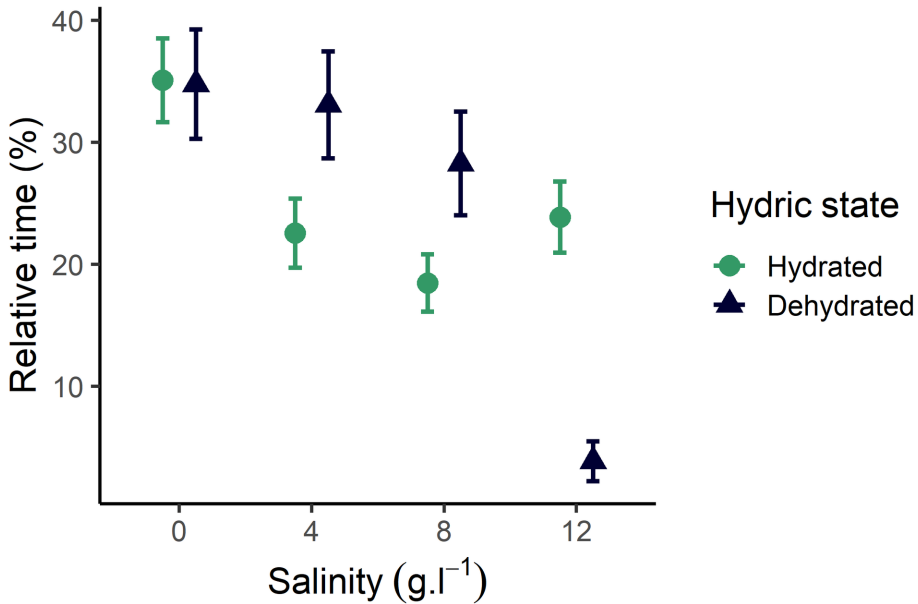


Hydric state

- Hydrated
- Dehydrated

Orientation





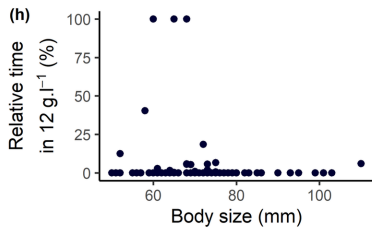
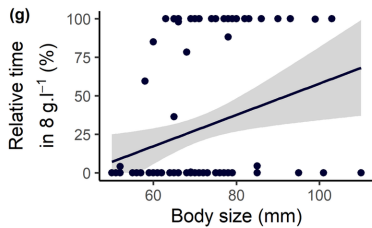
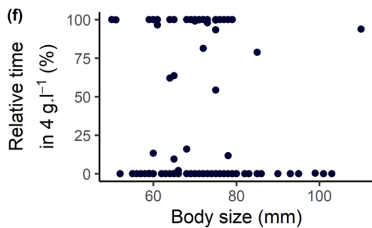
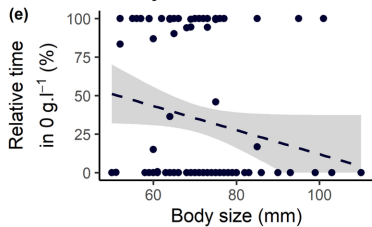
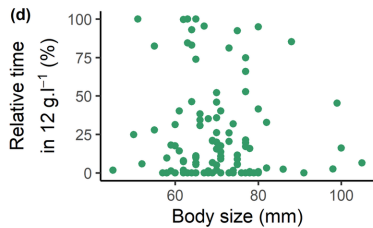
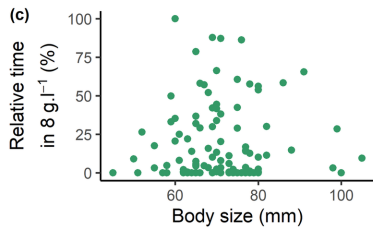
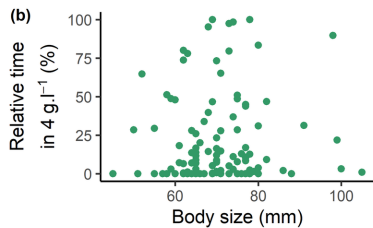
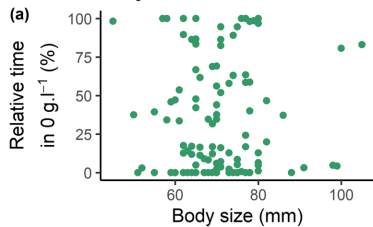
Hydrated individuals**Dehydrated individuals**

Table 1: Pair-wise comparisons of the proportion of time spent in each treatment (as a proportion of the total time in water) in hydrated and dehydrated individuals.

		Estimate	SE	t-value	p-value
Hydrated individuals N=110	0g/1-4g/L	12.543	4.119	3.045	0.013
	0g/1-8g/1	16.63	4.119	4.037	<0.001
	0g/1-12g/1	11.225	4.119	2.725	0.033
	4g/1-8g/L	4.087	4.119	0.992	0.754
	4g/1-12g/L	-1.318	4.119	-0.32	0.989
	8g/1-12g/L	-5.405	4.119	-1.312	0.555
Dehydrated individuals N=112	0g/1-4g/L	1.69	5.482	0.308	0.99
	0g/1-8g/1	6.496	5.482	1.185	0.636
	0g/1-12g/1	30.921	5.482	5.641	<0.001
	4g/1-8g/L	4.806	5.482	0.877	0.817
	4g/1-12g/L	29.231	5.482	5.333	<0.001
	8g/1-12g/L	24.425	5.482	4.456	<0.001

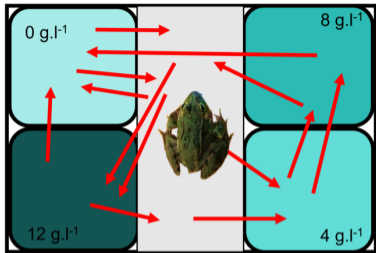
🕒 10 hours



0 g.l⁻¹ salinity

N=110

Hydrated



More time out of water

Equal time between each salinities

Exploration: More movements

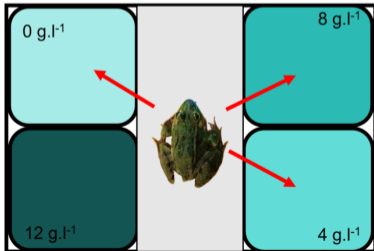
🕒 10 hours



12 g.l⁻¹ salinity

N=112

Dehydrated



Less time out of water

More time in low salinity treatments

Less movements