

1 **Prenatal exposure to predation affects predator recognition**
2 **learning via lateralization plasticity**

3

4 **Abbreviated Title**

5 Lateralization and predation recognition.

6

7 **Abstract**

8 Prey with cerebral lateralization often show a bias in escape direction and asymmetrical
9 use of eyes for scanning. Such asymmetries are likely to cause ecological disadvantages
10 when, for example, predators attack from the side in which the prey is more susceptible.
11 However, lateralized individuals are diffuse in many species and, paradoxically, their
12 frequency increases via developmental plasticity in environments with high predation
13 risk. Using wood frog tadpoles, *Lithobates sylvaticus*, we tested the hypothesis that
14 cerebral lateralization enhances predator recognition learning and thus overcomes the
15 costs of behavioral asymmetries in high-predation risk environments. In the first
16 experiment, we found tadpoles exposed to risk as embryos developed more intense
17 lateralization in a rotational test compared to predator-naive controls. Risk exposure led
18 to the more frequent development of clockwise swimming preference. In the second
19 experiment, we found that tadpoles exhibiting no behavioral lateralization and tadpoles
20 with marked clockwise swimming preference learned to recognize the predator, with the
21 latter showing a better performance as predicted. Tadpoles with anticlockwise
22 swimming preference did not learn to associate the predator with risk. Exposure to a
23 high-risk environment during early ontogeny appears to favor the development of either

24 a lateralization phenotype with refined predator recognition learning skills, or, to a
25 lesser extent, a lateralization phenotype with poor predator recognition learning skills.
26 Such individuals likely cope with predation using mechanisms other than learning.

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31 **Key-words**

32 behavioral asymmetries; cerebral lateralization; cognitive abilities; predator learning;
33 predation risk; tadpoles.

34

35 **Lay Summary**

36 Many prey species show behavioral lateralization, such as bias in escape direction and
37 asymmetrical use of eyes for scanning, that can cause ecological disadvantages. We
38 showed that tadpoles raised as embryos in a high predation risk environment mainly
39 develop a lateralization phenotype that confers enhanced predator recognition learning.
40 Learning ability can help lateralized tadpoles to survive in high-predation risk
41 environments despite behavioral asymmetries.

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47 **Introduction**

48 Cerebral lateralization has been extensively investigated in animals over the last
49 two decades, but its origin, evolution and maintenance remain unclear. A major concern
50 is the fact that a diversity of motor and sensory asymmetries often arise from cerebral
51 lateralization (Vallortigara and Rogers 2005). Since biologically relevant stimuli (e.g.,
52 predators) can be detected on either side of an animal, such asymmetries are expected to
53 cause ecological disadvantages and, consequently, negative selection on cerebral
54 lateralization (Rogers 2002; Vallortigara and Rogers 2005; Dadda et al. 2009).
55 Counterintuitively, lateralization seems to be a ubiquitous feature of animal brains
56 (Bisazza et al. 1998).

57 In many vertebrates, cognitive functions underlying defense from predators are
58 lateralized, and so are the resulting behaviors. Birds and reptiles show eye preference to
59 look at predators (Koboroff et al. 2008; Martín et al. 2010). Monkey, horses, dogs and
60 dunnarts show better recognition and escape performance when the predator appears in
61 the left visual field (Lippolis et al. 2005; Austin and Rogers 2007; Siniscalchi et al.
62 2010; Shibasaki et al. 2014). Lizards, anurans and fish show turning bias in escape
63 responses (Cantalupo et al. 1995; Yamashita et al. 2000; Lippolis et al. 2009; Bonati et
64 al. 2010; see also Bisazza and Brown 2011 for a review in fish). Given the unforgiving
65 nature of predation, the costs of sensory and motor lateralization, such as failure to
66 detect a predator with one eye or escape in the wrong direction, are paramount
67 (Vallortigara and Rogers 2005). Lateralized individuals are therefore expected to
68 undergo negative selection, especially in populations that suffer heavy predation
69 pressure. In sharp contrast to this prediction, wild poeciliid fish collected from high
70 predation risk rivers show greater lateralization (Brown et al. 2004; Brown &

71 Braithwaite 2005; Brown et al. 2007) and four recent studies found that cuttlefish,
72 poeciliid fish and damselfish reared in environments with simulated high predation risk
73 developed more intense lateralization (Jozet-Alves and Hébert 2013; Broder and
74 Angeloni 2014; Ferrari et al. 2015a; Ferrari et al. 2015b). Ferrari and colleagues (2015a)
75 also reported that, despite the intense lateralization, damselfish developed in high
76 predation risk environment had higher survival in mesocosms containing relevant
77 predators. To date, this apparent paradox remains to be explained.

78 Several hypotheses looking at the evolution and maintenance of lateralization
79 have suggested the presence of advantages resulting from lateralization that should
80 counterbalance the costs resulting from behavioral asymmetries. These advantages
81 include superior cognitive abilities, greater capacity to deal with simultaneous tasks and
82 enhanced behavioral coordination with social companions (Levy 1977; Ghirlanda et al.
83 2004; Ghirlanda et al. 2009; MacNeilage et al. 2009; Bisazza and Brown 2011). To date
84 most of the evidence seems to support the general idea of cognitive advantages of
85 lateralization (but see Dadda et al. 2009). Individuals with more intense lateralization
86 (independently of the right-left directionality) are better in complex motor activities
87 (Magat and Brown 2009), multitasking (Rogers et al. 2004; Dadda and Bisazza 2006),
88 spatial learning (Brown and Braithwaite 2005; Sovrano et al. 2005) and numerical
89 discrimination (Dadda et al. 2015).

90 Predator-prey interactions provide an example where the costs of behavioral
91 asymmetries might be the most severe, that is resulting in death. Consequently, we
92 might expect the benefits of lateralization on cognitive performance might be present in
93 this context. One aforementioned study showed that fish with different lateralization
94 patterns from populations exposed to different predation risk had altered performance in

95 a spatial learning task (Brown and Braithwaite 2005), but this effect on spatial abilities
96 might be the result of selective pressures concomitant to predation, such as foraging. To
97 date, there are no investigations of the hypothesis on cognitive advantages for abilities
98 directly involved in the defense against predators, such learn to recognize novel
99 predators.

100 We tested the hypothesis that in high predation risk environments individuals
101 will develop intense lateralization because lateralization confers an advantage in
102 cognitive abilities directly associated with predator defense, that is novel predator
103 recognition learning. If our hypothesis is correct, this could explain the apparent
104 paradox of the maintenance of lateralization in high predation risk environments. We
105 tested our hypothesis in *Lithobates sylvaticus* tadpoles.

106 In response to high predation risk during early life history, tadpoles develop both
107 morphological and behavioral defenses, such as increased tail fin depth and increased
108 refuge use (Semlitsch and Reyer 1992; Lardner 2000; Relyea 2001; Kishida and
109 Nishimura 2004). In experiment 1, we tested whether tadpoles exposed to a high
110 predation risk environment during early ontogenesis develop a more intense
111 lateralization compared to tadpoles exposed to low predation risk as observed for
112 cuttlefish, guppies and damselfish (Jozet-Alves and Hébert 2013; Broder and Angeloni
113 2014; Ferrari et al. 2015a; Ferrari et al. 2015b). This was the prerequisite to study our
114 main hypothesis.

115 In experiment 2, we then tested our main hypothesis by focusing on predator
116 recognition learning. Many prey species have sophisticated abilities to learn to identify
117 novel predators and to develop threat-sensitive, context-dependent responses for future
118 encounters (Ferrari et al. 2010a; Brown et al. 2011). The importance of learning to

119 recognize predation threats is possibly greatest during early development as prey need
120 not only to start cataloguing predators and non-predators but to learn the danger level
121 associated with different predator types (Ferrari and Chivers 2006; Chivers et al. 2014).
122 Many aquatic prey acquire information via the simultaneous pairing of a novel predator
123 cue (odor, sight, sound) with cues from injured conspecifics (Ferrari et al. 2010a). This
124 learning process occurs after a single pairing of predator odor and injured tadpole cues
125 (hereafter “alarm cues”; Ferrari et al. 2010b; Ferrari and Chivers 2011). In experiment
126 2, we compared how tadpoles with different intensity of lateralization learn to recognize
127 the odor of a predator. According to our main hypothesis, we predicted that individuals
128 with a higher degree of lateralization should show greater predator learning ability.

129

130 **Materials and methods**

131 Ethical notes

132 University of Saskatchewan Animal Ethics Committee approved all procedures
133 (protocol n. 20060014). Experiments took place in June 2015 in Strathcona County,
134 Alberta, Canada, and were performed outdoors. Tadpoles experienced natural
135 conditions of light, temperature, and precipitation. We used water from a 1900-L tub
136 filled with well water four weeks before the beginning of the experiments. By adding
137 plankton and aquatic plants to the tub, we ensured this water had natural ponds odors,
138 but no predator cues. Tadpoles were returned to their natal ponds following the
139 completion of the experiment.

140

141 Experiment 1

142 In this experiment, we compared the lateralization of tadpoles raised in a
143 simulated high predation risk environment with those raised in a low predation risk
144 environment.

145

146 *Subjects*

147 We collected three wood frog egg clutches from a pond (53°29' N 113°06' W) within 36
148 h of them being laid. Each clutch was split in half and incubated in a 7.4-L plastic
149 container. Half of the embryos from each clutch were exposed to a high-risk
150 environment while the other half were exposed to a low-risk environment as described
151 below. After hatching, each of the half clutches was split into two 20-L tubs and
152 maintained for three weeks prior to testing (Gosner stage 25). After hatching, all
153 tadpoles were fed alfalfa pellets and Tetramin flakes to supplement the algae already
154 present in the pools.

155

156 *Experimental treatments of embryos*

157 The high-risk environment consisted of exposing the clutch to an alarm cue
158 solution twice per day for the entire embryonic period following the methodology of
159 Mathis et al. (2008). We obtained alarm cue by sacrificing donor tadpoles with a blow
160 to the head. The tadpole was then finely ground in a mortar and suspended in water. We
161 injected the equivalent of three tadpoles per container (20 mL of alarm cue). The low-
162 risk environment consisted of exposing the embryos to 20 mL of pond water on the
163 same injection schedule. Injection of alarm cues (or pond water) ceased immediately

164 prior to hatching to ensure that the risk treatment was restricted to the embryonic period
165 (Mathis et al. 2008; Ferrari and Chivers 2010).

166

167 *Lateralization test*

168 Thirty-six tadpoles of each treatment ($N = 12$ from each clutch) underwent the
169 rotational preference test. Rotational preference is a measure of lateralization adopted in
170 a diversity of species, such as dolphins, rats, fish, and tadpoles (Glick and Ross 1981;
171 Sobel et al. 1994; Bisazza and Vallortigara 1997; Blackiston and Levin 2013). Subjects
172 were randomly selected and placed in 0.5-L white cups filled with water. The test began
173 after a 15-min acclimation period. We recorded the test in the field (outdoors) and
174 subsequently analyzed the recordings in the lab. For each subject, we measured time
175 spent swimming in a clockwise direction and time swimming in an anticlockwise
176 direction across a 12-min observation (Blackiston and Levin 2013).

177

178 *Statistical analysis*

179 We computed the two commonly adopted lateralization indices (Cantalupo et al.
180 1995) that allowed analyzing independently the intensity (L_A index = (clockwise
181 swimming time – anticlockwise swimming time) / (clockwise swimming time +
182 anticlockwise swimming time) x 100) and directionality of lateralization (L_R index =
183 $|L_A|$). We compared the lateralization indices between the two risk treatments (high or
184 low) with two independent-samples t tests. We then compared within treatment the
185 frequency of tadpoles with clockwise and anticlockwise swimming preference with a
186 chi-square test.

187

188 Experiment 2

189 In experiment 2, we compared the ability of tadpoles, with different intensity of
190 lateralization, to learn to recognize the odor of a predator. Following previous studies
191 (Sovrano et al. 2005; Dadda and Bisazza 2006; Bibost and Brown 2014), we used a
192 lateralization test to select non-lateralized tadpoles and tadpoles highly lateralized in
193 both left and right directions. We then conditioned the subjects of each of the three
194 groups with the odor of the predator and tested their learning abilities after 48 h.

195

196 *Subjects*

197 We collected 15 wood frog egg clutches from the same pond and placed three
198 egg clutches into each of five, 370-L plastic pools filled with well water. Exposure to
199 predation has many effects on predator recognition learning of tadpoles (e.g., Ferrari
200 and Chivers 2010; Ferrari et al. 2010b; Ferrari et al. 2016). To avoid confounding
201 results and to focus on differences in lateralization, these eggs were not exposed to
202 predation risk. Again, the algae diet of the tadpoles was supplemented with alfalfa
203 pellets and Tetramin flakes. Experiments began approximately three weeks later when
204 the tadpoles were at Gosner stage 25.

205

206 *Lateralization test and subject selection*

207 We screened 400 tadpoles for their rotational preference. Subjects were
208 randomly selected from the five pools and tested in the cups as described for the
209 previous experiment. To allow subject selection, the scoring of this experiment was
210 performed in the field. After a 15-min acclimation period, we noted the swimming
211 direction of the subject (clockwise or anticlockwise). We observed each subject 10

212 times at 2-min intervals. Observations in which the subject was not swimming were
213 repeated later. Tadpoles failing to swim after three consecutive observations were
214 removed from the experiment ($< 5\%$ of subjects). We observed simultaneously 20
215 tadpoles using scan sampling. Tadpoles scoring $> 80\%$ observations with the same
216 swimming direction were assigned to the corresponding group of lateralized subjects
217 (clockwise $N = 35$; anticlockwise $N = 40$); tadpoles scoring 50% observations in the
218 same direction were assigned to the group of non-lateralized subjects ($N = 37$);
219 remaining tadpoles were not used in the following phases of experiment. After
220 selection, subjects were kept in six plastic housing tanks filled with 12 L of water for
221 the following 24 h.

222

223 *Predator recognition learning test*

224 The test for predator learning followed a well-established methodology (Ferrari
225 and Chivers 2009; Ferrari et al. 2010b). For the conditioning, subjects were placed in
226 0.5-L cups filled with water and, after one hour of acclimation, 5 mL of salamander
227 odor paired with 5 mL of either alarm cue (clockwise $N = 22$; anticlockwise $N = 26$;
228 non-lateralized $N = 24$) or water (clockwise $N = 13$; anticlockwise $N = 14$; non-
229 lateralized $N = 13$) were added to the cups. We obtained predator odor from four wild-
230 caught tiger salamanders (*Ambystoma tigrinum*) that were fed a diet of earthworms and
231 housed individually in 1.5 L of water for 24 h prior to the collection of stimulus water.
232 Tadpoles from this location do not innately recognize salamander odor (Ferrari et al.
233 2010b). We prepared alarm cue as described for experiment 1, and we suspended it in
234 20 mL of water for each donor tadpole. We expected that subjects conditioned with
235 alarm cue would learn to recognize salamander odor as a predator; false-conditioning

236 with water served as control for neophobia toward salamander odor. One hour following
237 conditioning, subjects were placed in pails according to experimental groups.

238 Testing took place 48 h following the conditioning. We moved the subjects
239 individually in the test cups and left them to acclimate for 30 min. We measured the
240 baseline activity (pre-injection observation phase) of each subject by counting the
241 number of times the tadpole crossed the median line of the cup in 4 min. Activity was
242 again measured for 4 min after injection of 5 mL of salamander odor (post-injection
243 observation phase). A marked reduction in activity after predator odor injection in the
244 group conditioned with alarm cue is considered evidence of learning (Ferrari et al.
245 2010a; Brown et al. 2011). We did not test seven tadpoles because of reduced baseline
246 activity (< 6 crossing during the 4 minute pre-injection observation phase).

247

248 *Statistical analysis*

249 To exclude that lateralized individuals were non-lateralized individuals with an
250 extreme lateralization score that occurred by chance, we compared the relative
251 abundance of lateralized individuals observed with those expected by chance. We
252 simulated 10000 random binomial distributions each from 10 observations in 400
253 individuals. We used frequency distribution of lateralized individuals from the
254 simulations to compute P -values that describe whether our observed data differed from
255 the random binomial distribution. For example, the P -value for the clockwise tadpoles
256 was: $P = (N \text{ of simulated populations with } N \text{ of clockwise individuals} \geq N \text{ of observed}$
257 $\text{clockwise tadpoles}) / 10000$. We then compared the frequency of tadpoles with
258 clockwise and anticlockwise swimming preference with a chi-square test.

259 We analyzed the activity scores of the predator recognition learning test with
260 repeated measures ANOVAs. In the first repeated measures ANOVA, we fitted
261 conditioning type (alarm cue or water) and lateralization group (clockwise,
262 anticlockwise, or non-lateralized) as between-subjects factors, and observation phase
263 (pre- or post-stimulus period) as within-subjects factor. Since we studied learning
264 ability, we focused on the slopes of activity change between pre- and post-stimulus
265 period rather than on the absolute value of the activity. To study the three-way
266 interaction in the previous model, we built three independent repeated measures
267 ANOVAs by splitting the data according the three lateralization groups. The last
268 repeated measures ANOVA (between-subjects factor: lateralization group; within-
269 subjects factor: observation phase) compared the change in activity score of groups that
270 learned predator odor using only the data of the subjects conditioned with alarm cue.

271

272 **Results**

273 Experiment 1

274 The L_R index did not differ between the two predation risk treatment ($t_{70} =$
275 $0.887, P = 0.378$). The analyses on the L_A index revealed that tadpoles of the high
276 predation risk treatment were more lateralized than tadpoles of the low predation risk
277 environment (high predation risk: 57.26 ± 33.13 , *mean* \pm *SD*; low predation risk: 39.05
278 ± 57.26 ; $t_{70} = 2.529, P = 0.014$; Figure 1).

279 In the high predation risk environment, we observed more tadpoles showing
280 clockwise ($N = 24$) than tadpoles showing anticlockwise ($N = 12$) swimming preference
281 ($\chi^2_1 = 4.000, P = 0.046$; Figure 1). In the low predation risk environment, we found an

282 equal number of tadpoles showing clockwise ($N = 17$) and anticlockwise ($N = 19$)
283 swimming preference ($\chi^2_1 = 0.111$, $P = 0.739$; Figure 1).

284

285 Experiment 2

286 *Lateralization test*

287 The comparison with the simulated binomial distributions showed that both
288 tadpoles with clockwise and anticlockwise swimming preference occurred more often
289 than expected by chance ($P = 0.005$ and $P < 0.001$, respectively). Tadpoles with
290 clockwise swimming preference and anticlockwise swimming preference occurred with
291 the same frequency ($\chi^2_1 = 0.333$, $P = 0.564$).

292

293 *Predator recognition learning test*

294 The initial repeated measures ANOVA revealed a significant effect of
295 observation phase ($F_{1,97} = 100.653$, $P < 0.001$), but no significant effect of conditioning
296 type ($F_{1,97} = 0.027$, $P = 0.869$) nor lateralization group ($F_{2,97} = 0.684$, $P = 0.507$).

297 Observation phase \times conditioning type and observation phase \times lateralization group
298 interactions were significant ($F_{1,97} = 13.524$, $P < 0.001$ and $F_{2,97} = 5.177$, $P = 0.007$,
299 respectively), but the conditioning type \times lateralization group interaction was not
300 significant ($F_{2,97} = 0.321$, $P = 0.726$). More importantly, the observation phase \times
301 conditioning type \times lateralization group was significant ($F_{2,97} = 3.726$, $P = 0.028$; Figure
302 2).

303 In the three repeated measures ANOVAs that analyzed independently the three
304 lateralization groups there was a significant effect of observation phase (non-lateralized
305 tadpoles: $F_{1,31} = 61.127$, $P < 0.001$; clockwise tadpoles: $F_{1,32} = 70.362$, $P < 0.001$;

306 anticlockwise tadpoles: $F_{1,34} = 8.753$, $P = 0.006$) but no significant effect of
307 conditioning type (non-lateralized tadpoles: $F_{1,31} = 0.317$, $P = 0.578$; clockwise
308 tadpoles: $F_{1,32} = 0.054$, $P = 0.818$; anticlockwise tadpoles: $F_{1,34} = 0.319$, $P = 0.576$). The
309 observation phase \times conditioning type interaction was significant in the non-lateralized
310 tadpoles and in the clockwise tadpoles ($F_{1,31} = 8.034$, $P = 0.008$ and $F_{1,32} = 18.774$, $P <$
311 0.001 , respectively; Figure 2) indicating decrease in activity after salamander odor
312 injection was greater for subjects conditioned with alarm cue than for subjects
313 conditioned with water. In sharp contrast, the observation phase \times conditioning type
314 interaction was not significant in the model of the anticlockwise tadpoles ($F_{1,34} = 0.045$,
315 $P = 0.833$; Figure 2) indicating no difference in activity decrease between subjects
316 conditioned with alarm cue and subjects conditioned with water.

317 The last repeated measures ANOVA that compared the changing rate in activity
318 between the non-lateralized tadpoles and clockwise tadpoles (the two groups that
319 learned to recognize the salamander odor as predator odor) indicated a significant effect
320 of observation phase ($F_{1,41} = 167.990$, $P < 0.001$) but no significant effect of
321 lateralization group ($F_{1,41} = 0.573$, $P = 0.454$). The observation phase \times lateralization
322 group interaction was significant ($F_{1,41} = 5.356$, $P = 0.026$; Figure 3), indicating that the
323 clockwise tadpoles responded more to the salamander odor than the non-lateralized
324 tadpoles.

325

326 **Discussion**

327 Under high predation risk, several prey species show intense lateralization (e.g.,
328 Brown et al. 2004; Ferrari et al. 2015a), despite the fact that behavioral asymmetries are
329 expected to be costly when dealing with predators (Vallortigara and Rogers 2005).

330 Searching for a solution to this paradox, we found partial support for the hypothesis that
331 in lateralized individuals the costs of behavioral asymmetries are balanced by enhanced
332 predator recognition learning abilities..

333 In experiment 1, *L. sylvaticus* tadpoles showed developmental plasticity of
334 lateralization similar to the plasticity reported in previous studies on fish and cuttlefish
335 (e.g., Jozet-Alves and Hébert 2013; Ferrari et al. 2015a). Tadpoles exposed to high risk
336 as embryos were more lateralized than tadpoles maintained under low risk in the
337 rotational preference test. The plastic response of lateralization to high predation risk is
338 therefore a common coping mechanism in both aquatic vertebrates and invertebrates.
339 Extending these findings, in tadpoles this response can be activated by environmental
340 cues perceived during the embryonic stage, preparing individuals to live in a risky
341 environment from the first day after hatching. Plasticity of lateralization has also been
342 detected in response to other environmental factors (Bisazza and Brown 2011),
343 including level of environmental enrichment (Bibost et al. 2013), hypoxia (Lucon-
344 Xiccato et al. 2014) and light exposure during development (Dadda and Bisazza, 2012).
345 Together with our results, these works seem to suggest that lateralization is extremely
346 plastic both in embryonic and adult anamniotes, and that between-individual variation
347 in lateralization may not be as dependent on genetic variation as previously thought.

348 Tadpoles raised under high predation risk were also more likely to develop a
349 clockwise swimming preference. Previous studies that experimentally manipulated
350 predation risk did not detect effects on the directionality of lateralization (Jozet-Alves
351 and Hébert 2013; Broder and Angeloni 2014; Ferrari et al. 2015a; Ferrari et al. 2015b),
352 with one exception. De Santi et al. (2000) found that guppies exposed to predation
353 showed a greater tendency to approach the predator with their shoal mate on their right.

354 In a study on wild fish populations, Brown et al. (2007) found that *Brachyraphis*
355 *episcopi* collected from high predation rivers showed a marked preference for looking at
356 novel objects with the left eye. This preference disappeared in laboratory reared
357 offspring, suggesting it was due to developmental plasticity as in our experiment. The
358 variation in directionality of lateralization is interesting, as having a cognitive function
359 processed in the right or in the left hemisphere should theoretically be equivalent
360 (Vallortigara and Rogers 2005). Yet, sometimes, entire populations show an average
361 bias in the directionality of lateralization, an alignment that has been associated also
362 with coordination in social group (Bisazza et al. 2000). Our result and those of previous
363 works support the idea that, at least in some species, the variability of predation
364 pressure across space and time could induce population-level biases in directionality of
365 lateralization.

366 In experiment 2, we tested our main hypothesis. We compared lateralized and
367 non-lateralized tadpoles for their ability to learn to recognize predator odors as
368 dangerous. We found an unexpected and more intricate picture, revealing the effect of
369 both the intensity and directionality of lateralization in tadpoles' predator learning
370 abilities. Tadpoles with marked anticlockwise swimming preference in the lateralization
371 test demonstrated impaired learning ability and failed to recognize the odor of the
372 predator as a threat. By contrast, both tadpoles with a clockwise swimming preference
373 and tadpoles with no swimming preference in the lateralization test were able to
374 recognize the predator, but showed different effectiveness; tadpoles from the clockwise
375 group displayed a stronger response to predator odors than the non-lateralized group in
376 this task, suggesting a better ability to learn to recognize predators.

377 Conflicting with our results, several studies have reported that more lateralized
378 individuals tended to outperform non-lateralized ones in cognitive tasks, independently
379 of the directionality of lateralization (e.g., Rogers et al. 2004; Sovrano et al. 2005;
380 Dadda and Bisazza 2006; Magat and Brown 2009). The sole exception is a study by
381 Bibost and Brown (2014) showing that rainbowfish with a left-eye preference when
382 looking at conspecifics, were faster at learning to find a food reward indicated by red
383 light than those with right-eye preference (Bibost and Brown 2014). These results
384 combined with our findings suggest that directionality of lateralization contributes to
385 shape individual cognitive performances as well, but this contribution seems to be
386 limited to specific learning tasks. The underlying mechanism driving this effect is
387 unfortunately unclear.

388 Psychological research provides a possible proximate explanation to the effect of
389 directionality of lateralization in cognitive abilities. In humans, the left hemisphere
390 usually dominates language processing whereas the right hemisphere dominates spatial
391 processing, but occasionally, both functions are associated with the same hemisphere
392 (Flöel et al. 2005). In several species of primates, dogs, horses, rodents, birds, lizards,
393 frogs and fish, cognitive functions associated with predator recognition and response to
394 risky stimuli are located in the right hemisphere (reviewed in Vallortigara and Rogers
395 2005; Rogers 2010), although in other cases this pattern is absent (Brown and Magat
396 2011). By measuring rotational preference, we possibly have looked at lateralization of
397 only one (or few) basic cognitive functions involved in our learning task, such as odor
398 perception, discrimination ability, general learning and memory, for which hemispheric
399 dominance might not be fixed. Association and dissociation of this function with the
400 ones involved in predator-related information processing can be an important source of

401 performance variation in cognitive tasks where both are implicated, especially in lower
402 vertebrates that lack consistent communication between the two hemispheres. However,
403 more data must be acquired to evaluate this possible proximate explanations. Studies on
404 personality and lateralization offer an alternative explanation to this hemispheric-based
405 hypothesis. In some fish species lateralization has been linked to personality (Brown
406 and Bibost 2014), and different personality types may be related to different predator
407 recognition learning abilities (Brown et al. 2013). Personality might, therefore, present
408 an alternative proximate explanation of the effect that we found.

409 Considering the two experiments together, there is partial support for our initial
410 hypothesis. The lateralized phenotype with enhanced predator recognition learning
411 ability (clockwise swimming preference) corresponds to the more abundant lateralized
412 phenotype in the population experiencing high predation risk during development;
413 however, also the frequency of the lateralized phenotype with poor predator recognition
414 learning abilities (anticlockwise swimming preference) increased in the high predation
415 environment (although less than for clockwise tadpoles). It is clear that for the former
416 group of tadpoles (clockwise swimming preference) developmental plasticity of
417 lateralization is an important mechanism to cope with predation risk of the environment:
418 by developing lateralization phenotypes with enhanced cognitive abilities in learning
419 predator identity, these tadpoles are prepared for a life under high-risk conditions. For
420 non-learning lateralized tadpoles (anticlockwise swimming preference), developmental
421 plasticity of lateralization causes the fitness costs associated with failed predator
422 recognition learning, in addition to the costs of behavioral asymmetries. Tadpoles with
423 anticlockwise swimming preference might balance the costs of impaired learning
424 performance with other defensive mechanisms, such as enhanced escaping performance

425 (Dadda et al. 2010). Other potential advantages of tadpoles with anticlockwise
426 swimming preference emerge if we consider the effects of selection on lateralization at
427 the group level (Brown 2005). For example, if most of the tadpoles in a risky
428 environment develop the same directionality of lateralization because it is associated
429 with enhanced predator recognition learning ability, predators may learn to exploit this
430 bias and attack preferentially to one side (Vallortigara and Rogers 2005). It is possible
431 that tadpoles with anticlockwise swimming preference gain an advantage by behaving
432 differently compared to the majority of tadpoles when swimming and escaping from
433 predators. Theoretical studies suggest that a minority of individuals with opposite
434 lateralization pattern compared to the majority are favored by frequency-dependent
435 selection due to the advantage of being unpredictable when escaping predators or
436 competing with conspecifics (Ghirlanda and Vallortigara 2004; Ghirlanda et al., 2009).
437 Our results seem therefore to suggest that both the cognitive advantage of lateralization
438 and the frequency-dependent selection for unpredictability concurrently might shape the
439 population-level lateralization under high predation risk. Of course, such ideas still need
440 to be tested empirically and we need to exclude that this anticlockwise-lateralized
441 phenotype is simply a non-adaptive by-product of the mechanism leading to the other
442 lateralization phenotype.

443 In conclusion, our work demonstrated the existence of prenatal plasticity for
444 lateralization following exposure to predation risk, where increased risk increases the
445 frequency of lateralized individuals when predation risk in the environment is high. This
446 results in two lateralized phenotypes: one, more abundant, that is associated with
447 enhanced predator learning abilities; a second with the opposite lateralization direction
448 which seem maladapted for high predation environments considering cognitive abilities,

449 but may gain frequency dependent advantages. Such findings suggest that the evolution
450 of lateralization in prey species might be intimately associated with selective pressures
451 arising from predation in a way more complex than previously believed.

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664 **Figure legends**

665 **Figure 1**

666 Frequency distribution of L_R index in tadpoles exposed to either high or low predation
667 risk environment as embryos (experiment 1).

668

669 **Figure 2**

670 Change in activity of tadpoles in the predator learning test (experiment 2). A greater
671 decrease in activity of tadpoles conditioned with alarm cues than those conditioned with
672 water is evidence of learning. Data points represent *mean* \pm *SEM* number of median line
673 crossing.

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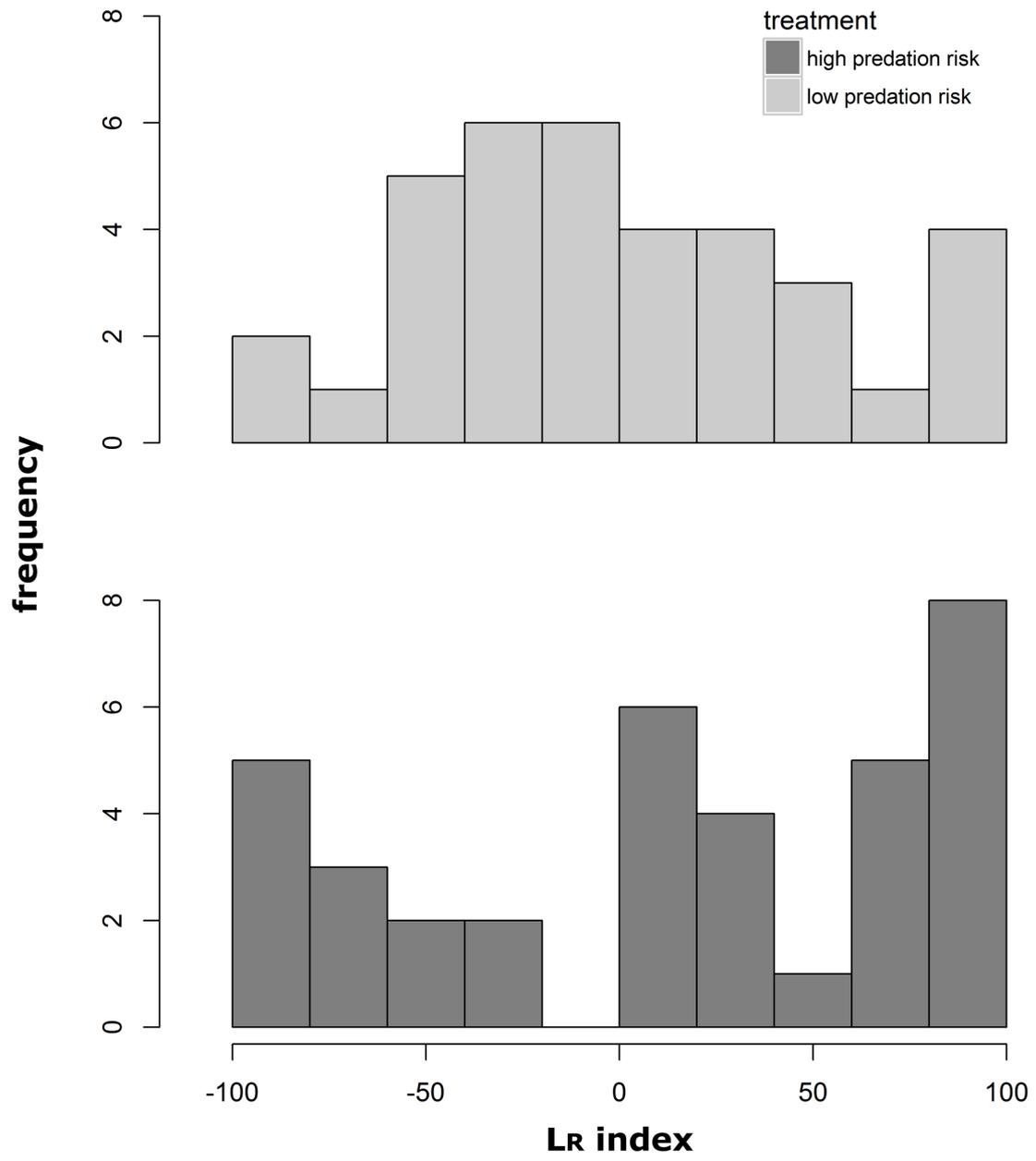
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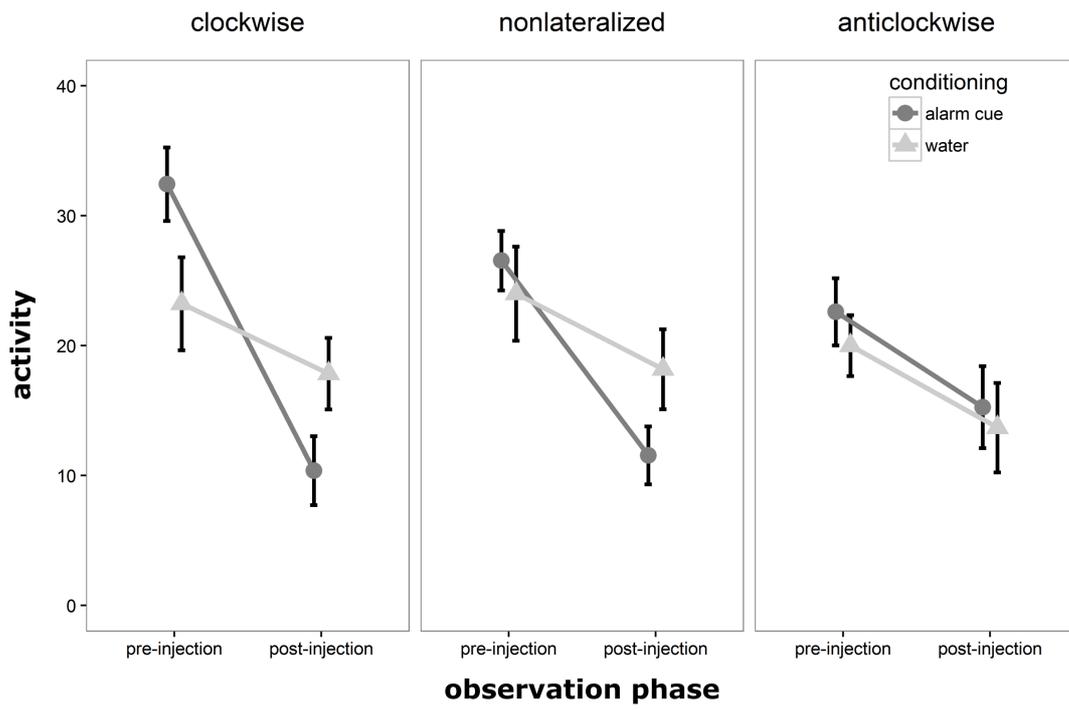


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