

1 **Spatial learning based on visual landmarks in the**  
2 **freshwater blenny *Salaria fluviatilis* (Asso, 1801).**

3

4 **Noëlle Fabre<sup>\*</sup>, Eduardo García-Galea<sup>\*</sup> & Dolors Vinyoles<sup>\*,1</sup>**

5 <sup>\*</sup>Department of Animal Biology (Vertebrates), Faculty of Biology,

6 University of Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain.

7

8

9

10

11

12

13

14

15

16

17

---

<sup>1</sup> Corresponding author: Tel.: +34 (0) 934039808; fax: +34 (0) 934035740; email: [d.vinyoles@ub.edu](mailto:d.vinyoles@ub.edu)

18 **Abstract**

19 The use and learning of visual landmarks seems to be strongly influenced by ecological  
20 demands and vary according to habitat, sex and, presumably, dominance status. In  
21 blennies, sexes differ in their home-range extension and this could predispose them to  
22 use different mechanisms to navigate. The main aim of this study was to investigate if  
23 *Salaria fluviatilis* (a benthonic and polygynic freshwater blenny) was able to solve a  
24 learning maze using direct visual landmarks. Performances between sexes were  
25 compared and the possible relationship between the males' secondary sexual  
26 characteristics (SSCs) development and their spatial ability was considered. In this  
27 species the SSCs consist of a cephalic crest and a pair of anal glands. Fish were  
28 subjected to 10 sessions (each one consisting of 11 consecutive trials) in a specially  
29 designed apparatus where they had to find the correct exit using direct visual  
30 landmarks. The proportion of males that learned the task (80%) was higher than that  
31 of females (30%). Cephalic crest development of the male was associated with a higher  
32 readiness in solving the spatial task. These results support previous works stating the  
33 importance of ecological demands on shaping the species spatial abilities. They also  
34 provide a comprehensive perspective that would link dominance (through cephalic  
35 crest development), learning and sexual selection.

36

37 *Keywords:* dominance, fish navigation, river blenny, secondary sexual characteristics,  
38 spatial ability

39

## 40 **1. Introduction**

41 Fish rely on their capacity of navigation to perform a series of vital activities such as  
42 foraging (Hughes & Blight, 1999), locating spawning sites (Mazeroll & Montgomery,  
43 1998), or eluding predators (Aronson, 1971; Markel, 1994). Their navigation skills are  
44 based on different external stimulus such as water flow direction (Braithwaite &  
45 Girvan, 2003), electromagnetism (Walker et al., 1997), polarized light (Hawryshyn,  
46 Arnold, Bowering, & Cole, 1990) and odours (Stabell, 1984). While the most complex  
47 navigation (true navigation) consists in a map-and-compass mechanism, other  
48 navigation phenomena such as pilotage are simpler. The latter allows animals to steer  
49 home on the basis of a topographic map without resorting to a compass (Papi, 2006).  
50 In this context, pilotage depends mostly on spatial learning and memory (Salas et al.,  
51 2006). The ability to include previous experiences within spatial behaviour is essential  
52 for certain fish to survive and to adapt to environmental changes (Dodson, 1988). This  
53 permits them to perform behaviours such as homing behaviour (Thyssen, 2010; Jorge,  
54 Almada, Gonçalves, Duarte-Coelho, & Almada, 2012) and to remember and use visual  
55 landmarks (Burt & Macias García, 2003).

56 An extensive bibliography on spatial learning based on visual landmarks in fish is  
57 available. It has been described that they can use visual cues as direct and indirect  
58 reference points (Warburton, 1990; López, Broglio, Rodríguez, Thinus-Blanc, & Salas,  
59 1999) and as sequences (Mazeroll & Montgomery, 1998). They are able to gather  
60 information from different directions (Rodríguez, Duran, Vargas, Torres, & Salas, 1994)  
61 and, finally, they can establish complex geometric relationships between them (e.g.  
62 Braithwaite & De Perera, 2006; Sovrano, Bisazza, & Vallortigara, 2007; Lee,

63 Vallortigara, Ruga, & Sovrano, 2012)). The understanding of such mechanisms is  
64 growing steadily and some studies reveal that certain spatial abilities of fishes are  
65 comparable to those of adult humans and higher than those found in children and rats  
66 (Sovrano, Bisazza, & Vallortigara, 2002). This suggests that spatial information use  
67 could be more influenced by the species ecological demands than by its phylogeny.

68 The habitat where a fish population occurs shapes the importance of visual landmarks  
69 in their navigation. In experiments with sticklebacks *Gasterosteus aculeatus* it was  
70 observed that visual landmarks were used more by individuals from 1) a lake  
71 population as compared to a river population (Odling-Smee & Braithwaite, 2003), 2) a  
72 population dwelling in clear and static waters as compared to a population living in  
73 eutrophic waters of poor visibility (Girvan & Braithwaite, 1998) and 3) a population  
74 living under a high-predation pressure as compared to a low-predation risk population  
75 (Huntingford & Wright, 1989). Moreover, it has been suggested that the benthonic  
76 species of *Gasterosteus* would be more prone to use visual landmarks than the  
77 limnetic ones (Odling-Smee, Boughman, & Braithwaite, 2008).

78 In certain species, males and females differ in home-range size (Wittenberger, 1981)  
79 and this entails differences in ecological demands and spatial ability (Gaulin &  
80 FitzGerald, 1986, 1989). In promiscuous or polygynous mating systems, males are  
81 frequently the most mobile sex (Brown, 1966; Lockie, 1966; Ewer, 1968; Trivers, 1972)  
82 and they present more complex spatial abilities than females (Gray & Buffery, 1971;  
83 Vallortigara, 1996). However, in blennies, this circumstance is reversed; while the  
84 females have a broad home-range (Costa et al., 2011), males are restricted to their  
85 nest (Neat & Lengkeek, 2009) and they only make short excursions away from it in

86 order to feed and to defend their territory (Almada, Gonçalves, De Oliveira, & Barata,  
87 1992; Gonçalves & Almada, 1998). Distinct preference predispositions between the  
88 sexes concerning the use of landmarks for orientation could have evolved as a  
89 consequence of such home range differences. Previous works support the hypothesis  
90 that males might rely upon local landmarks more than females as has been already  
91 suggested for chicks (Tommasi & Vallortigara, 2004). In at least two species of  
92 blennies, females have a bigger telencephalon than males and, thus, they can rely  
93 more in geometric features than in local beacons (Costa et al., 2011).

94 Animal personality may also influence spatial learning. Boldness (defined as the  
95 propensity to take risks) has been associated to a higher learning ability (Dugatkin &  
96 Alfieri, 2003) as well as to a dominant status acquisition (Dahlbom, Lagman, Lundstedt-  
97 Enkel, Sundström, & Winberg, 2011). These associations may have a physiological  
98 basis; fish that grow faster and have a better response to stress are more prone to  
99 explore their surroundings (Biro & Stamps, 2010; Archard, Earley, Hanninen, &  
100 Braithwaite, 2012) and to become dominant (Hofmann, Benson, & Fernald, 1999;  
101 Pottinger & Garrick, 2001). Putting these facts together, it seems reasonable to expect  
102 that dominance and spatial learning might be related and, furthermore, that dominant  
103 males (in territorial species) might be more motivated to use visual landmarks than  
104 non-dominant males (without a territory). Among fish, visual landmarks are frequently  
105 used to define territorial limits (Heap, Byrne, & Stuart-Fox, 2012). In species with  
106 alternative reproductive tactics (ARTs) the dominant status is associated to a higher  
107 development of the secondary sexual characteristics (SSCs) (Fleming & Gross, 1994;  
108 Oliveira & Almada, 1998; Oliveira, Carneiro, Gonçalves, Canario, & Grober, 2001).

109 *Salaria fluviatilis* is one of the few blenny species that inhabits freshwaters and occurs  
110 in rivers and lakes of the Mediterranean basin. It is a benthic and speleophil-nest  
111 spawner fish (Balon, 1975) that has been described as endangered in many  
112 Mediterranean countries where it occurs (see Vinyoles & Sostoa, 2007). During the  
113 reproductive period, which extends from May to the end of July (Vinyoles & Sostoa,  
114 2007), males excavate a cavity under a stone and several females lay monolayer  
115 clutches on the underside of the stone. A good topographic knowledge of the familiar  
116 area, a good memory and a particular curiosity to explore has been attributed to this  
117 species by Wickler (1957), but the role of visual landmarks was not studied. Repeated  
118 incursions to the nest probably enable visual recognition of neighboring objects by  
119 males. In a species with similar habits, the shanny (*Liphophyrus pholis*), the use of visual  
120 landmarks has already been described (Burt de Perera & Guilford, 2008). *S. fluviatilis*  
121 represents also an appropriate model to study the relationship between spatial ability  
122 and SSCs development. During the reproduction period, males develop a cephalic crest  
123 and a pair of anal glands. Recently, boldness has been associated with the cephalic  
124 crest in this species (Fabre, García-Galea, & Vinyoles, 2014) but it is still unknown if  
125 SSCs are related to spatial ability.

126 The main objective of this study was to define whether *S. fluviatilis* is able to use solely  
127 direct visual landmarks for navigation and if there are differences between the sexes.  
128 Then, if visual landmark use is found for males, the relationship between SSCs  
129 development and spatial ability will be explored.

## 130 **2. Material and methods**

### 131 *2.1. Fish field collection and maintenance*

132 Fish were caught in the river Segre, a tributary of the Ebro River, close to the village of  
133 Camarasa (Spain) in November 2010. 80 specimens were collected overall, consisting  
134 of 40 males and 25 females of similar sizes (ranging from 60 to 70 mm in total length,  
135 TL), and 15 large females (ranging from 80 to 90 mm in TL).

136 Animals were placed in five 260 L aquaria provided with a biological filter, air diffuser  
137 and substrata (consisting of a mixture of sand, gravel and coral in proportion 2:2:1).  
138 Each aquarium contained 10 artificial nests (distributed in two five-nest parallel rows)  
139 that function both as refuges and nests for the fish. Nests consisted of transparent  
140 plastic boxes (13.5 cm width x 7 cm high x 12 cm depth) with inner sides covered with  
141 black acetate sheets to make them opaque, an opening in the front (nest entry) and a  
142 glass surface substituting the ceiling . Previous experiments with these artificial nests  
143 confirmed that the species readily accepted them as nesting sites (unpublished data).  
144 The water in the aquaria consisted of 95% depurated freshwater and 5% seawater  
145 (with a final salinity of 2 ppm) and was partially renewed every two weeks in order to  
146 maintain adequate pH, NO<sub>2</sub> and NH<sub>4</sub> levels. Temperature (mean ± SD = 22.96 ± 1.15 °C)  
147 and light regime 12L: 12D were maintained constant throughout the experiment.

148 The proportion of fish in each aquarium was the following: eight males, five median  
149 females and three large females. Only median-sized fish were used in the experiment  
150 (all males and the five median females). Large females just acted as sexual stimulus in  
151 the aquaria (in order to simulate the female size variability found in nature, Vinyoles  
152 and Sostoa (2007)). Animals were feed “ad libitum” with red quironomidae larvae (the  
153 principal prey of the species following Vinyoles (1993)) once a day. Fresh mussels were  
154 added to the diet once a week.

155 The experiment was performed from August to September 2011 (coinciding with the  
156 end of the reproductive period). The long period of time that fish were maintained in  
157 the aquaria before the experiment allowed them to habituate to captivity and  
158 manipulation (manipulation consisted in capturing them once a month with small  
159 hand-nets). Fish individual recognition was possible through the identification of  
160 certain traits such as the cephalic crest shape, mucous pore location and body  
161 pigmentation pattern. After the experiments fish were released into the river.

## 162 *2.2. Apparatus*

163 The experimental apparatus used to perform the learning task was similar to the  
164 design of the set described in Ingle and Saharian (1973) and used later on by other  
165 authors (Salas et al., 1996; López, Broglio, Rodríguez, Thinus-Blanc, & Salas, 1999,  
166 2000). This structure consisted of a rhomboidal box (hereafter referred to as decision-  
167 box) with an area of  $196 \text{ cm}^2$  and 20 cm in height, constructed with PVC pieces (0.5 cm  
168 thick) and whose vertices were opened with 5 cm openings (Fig. 1). Two of these  
169 openings (opposite to each other) gave access to a cylindrical compartment that  
170 consisted of a vertical PVC tube (11 cm in diameter x 20 cm in height) uncovered at the  
171 top. A grey plastic sliding door (intended for opening and closing the compartment)  
172 was placed between each cylindrical compartment and the decision-box. During the  
173 trials, one of the cylindrical compartments remained closed (the non-used one) while  
174 the other received the individual that had to start the trial. The other two openings  
175 from the decision-box acted as exits of the apparatus and the fish had to decide  
176 throughout the trial which one to use. These exits had sliding doors as well, but in this  
177 occasion they were made with transparent plastic (to avoid fish visual detection of the



178 closed door). During the trials one of the exits was opened (correct exit) while the  
179 other was blocked by the transparent sliding door (wrong exit). The visual landmarks  
180 used to indicate the correct exit were two similar flattered stones (6.6 x 4.5 cm and 5.4  
181 x 4.5 cm respectively). They were placed on the floor of the decision-box, one at each  
182 side of the correct exit (at a distance of 4.5 cm from the exit and at 2.5 cm from the  
183 closest apparatus wall). When the fish that was performing the trial succeed to exit the  
184 apparatus, it found two small PVC tubes outside, one at each side (4 cm diameter x 13  
185 cm length) that acted as refuges. Although these tubes were invisible to the fish until it  
186 exit the apparatus, they represented a positive stimulus.

187 The experimental apparatus plus the refuges were placed in a rectangular opaque  
188 plastic tank (47 x 56 cm base x 40.5 cm height, hereafter referred to as experimental  
189 tank) covered with the same substrata as the 260 L aquaria. Illumination was provided  
190 by two neon lights (98 x 12 cm, 60 W) placed 25 cm above each cylindrical  
191 compartment. Direct illumination of the cylindrical compartments prevented the fish  
192 from adopting the compartment as a refuge and stimulated them to seek safer places.  
193 The experimental tank was surrounded by curtains (to avoid interactions with the  
194 researcher). In order to record fish movements, a video camera (Sony Handycam HDR-  
195 SR1E) was placed above the apparatus (at a height of 140 cm). During the trials the  
196 experimental tank was filled with water (same composition and temperature as that in  
197 the aquaria) to a level of 15 cm and the lab lights were switched off.

### 198 *2.3. Learning task*

199 All fish (other than the large females) underwent the experiment. They were  
200 separated randomly from the rest in groups of two males and two females during the

201 period they were subjected to the experimental sessions (in a separate zone of 30 x 40  
202 cm surface created by placing a divider in the aquarium). This procedure was done in  
203 order to facilitate their daily capture and to prevent unnecessary manipulation of  
204 individuals that were not going to be tested. After two habituation days in the  
205 separate zone, the learning task started. Each fish performed a maximum of 10  
206 sessions (consisting of 11 consecutive trials each) during three days. Every trial  
207 consisted of placing the fish into one of the cylindrical compartments (see the  
208 assignation sequence below) and waiting until the fish chose one of the two exits.  
209 When the first decision of the fish was to exit through the “correct exit” the choice was  
210 considered “correct” while in the opposite circumstance (when it hit the closed  
211 transparent door) the choice was considered “wrong”. Following López, Broglio,  
212 Rodríguez, Thinus-Blanc, & Salas (2000) recommendations, if a fish did not emerge  
213 from the cylindrical compartment within 5 minutes it was lightly prodded with a glass  
214 rod to motivate an exit. Fish that did not emerge their heads out of the cylindrical  
215 compartment and that were immobile during the first 15 minutes of the first session  
216 were discarded from the experiment. This decision was based on previous  
217 observations that indicate that fish that presented these behaviors did not leave the  
218 cylindrical compartment during the following hour. Fish that were not discarded had  
219 no time limitation to perform the trials. Each trial finished when the fish had left the  
220 apparatus through the correct exit and hid itself in one of the PVC refuges. After 30  
221 seconds, the fish was captured and a new trial started until the session (11 trials) was  
222 completed.

223 The apparatus configuration changed between trials; the cylindrical compartments (A  
224 or B) and the correct exits (1 or 2) were switched in a pseudorandom sequence. Thus,

225 correct direction and correct turn were in a random order but with the imperative that  
226 50% of turns were to the right and the other half to the left to avoid the possibility of  
227 fish conditioning.

228 The two stones were moved to always indicate the correct exit and were the only  
229 reliable landmark to learn the task. During the 30 seconds between trials, the  
230 correspondent configuration was settled and the substratum in front of the apparatus  
231 exits was also stirred to avoid the use of any of its elements to orientate. At the end of  
232 the session, the fish was left in a 10 L bucket provided with an air-diffuser and a PVC  
233 refuge to rest. Each fish was submitted to 4 sessions during the first and second days  
234 and to two sessions during the third day, with an interval of 2-3 hours between  
235 sessions during the same day. After the sessions of the day, fish were released into  
236 their respective aquaria and fed.

#### 237 *2.4. Measured variables*

238 Fish were considered to have learnt (i.e. they reached the acquisition criterion) when  
239 they attained 9 correct choices in a session. In previous essays, it was seen that fish  
240 that reached this punctuation, maintained or increased it in successive sessions;  
241 therefore, when a fish attained the acquisition criterion it was not subjected to more  
242 sessions (This entailed that not all fish required to perform the 10 sessions). Each fish  
243 was assigned a learning category (yes, no) depending on whether they had reached the  
244 acquisition criterion within a maximum of the 10 sessions or not. For the fish in the  
245 learning category “yes” learning rapidity was evaluated through the number of  
246 sessions performed to attain the acquisition criterion. The time each fish needed to  
247 complete a session was recorded in order to calculate the average time per session (s)

248 for each sex and learning category. The average time per session refers to the sum of  
249 times for all the sessions (each one covering from the moment we introduced a fish  
250 inside the cylinder for the first time until it finished the 11<sup>th</sup> trial), divided by the total  
251 number of sessions it required to learn. Except for discarded fish, individuals emerged  
252 almost immediately from the cylinder (once the fish emerged, even if still inside the  
253 cylinder, they started to explore the decision-box from this position). Time was kept  
254 also constant between trials and between fish. Therefore, the observed time  
255 differences are a good estimator of fish exploration and they are inversely associated  
256 to impulsivity.

257 At the end of the experiment, individuals were measured in a small transparent  
258 container with a graph paper at the back. The variables considered were: TL for both  
259 sexes and SSC development for males (cephalic crest height and gland mean  
260 diameter). Cephalic crest height (Cr) was measured as the distance from the middle of  
261 the cranium to the top of the crest (mm) and the gland mean diameter (GI) was  
262 obtained as the average of the first and second anal gland diameter. Both SSCs  
263 development variables were divided by TL, thus obtaining ratioCr (Cr/TL) and ratioGI  
264 (GI/TL). All variables were  $\log(x + 1)$  transformed before statistical analyses.

## 265 *2.5. Statistical analyses*

266 In order to analyze whether the development of SSCs was related to the number of  
267 sessions required attaining the acquisition criterion, a generalized linear model (GLM)  
268 with a Poisson distribution of errors (with the link function log) was used. In this  
269 model, “number of sessions until acquisition” was the dependent variable (ranging  
270 from 1 to 10, where the fish that did not learn were assigned a 10). Each model

271 included initially as covariates:  $\log(\text{TL})$ ,  $\log(\text{ratioCr})$  and  $\log(\text{ratioGl})$ , with all the paired  
272 interactions between them. The best model was found by extracting parameters using  
273 the AIC criterion corrected for small samples sizes (AICc) (Burnham & Anderson, 2002).  
274 Error adjustment was verified by visual inspection of the normal probability plots.  
275 Statistical analyses were performed with the `glm` function in the package `stats` from  
276 the free software R (R Core Team, 2012). The best model was found by using  
277 sequentially the `drop1` function.

278

### 279 **3. Results**

280 There were no significant differences in TL (mean  $\pm$  SD, mm) between males ( $91.6 \pm$   
281  $7.9$ ) and females ( $90.5 \pm 3.2$ ) when the experiment was conducted  
282 ( $t=0.41, d.f.=18, P=0.69$ ). Only 10 individuals of each sex out of the 40 males and 25  
283 females subjected to the learning task were able to achieve it. The remaining  
284 individuals were discarded because they did not emerge from the cylindrical  
285 compartment (Fig. 1) during the first session. There were no significant differences in  
286 the proportion of discarded males versus females ( $G\text{-test}=2.34, d.f.=1, P=0.13$ ). Before  
287 the experiment, six fish (3 males and 3 females) were submitted to 10 sessions without  
288 landmarks. None of them attained the learning criterion and they maintained a  
289 random error tax. In addition, all the fish used in the experiment (including those that  
290 did learn), when committing an error, they pumped against the transparent door as in  
291 the first session. Therefore, these facts altogether confirm that fish could not detect  
292 the door.

293 The number of correct choices in the first session followed a random pattern for all  
294 individuals (binomial distribution  $P > 0.20$ ). Approximately more than half of the fish  
295 that were able to perform the experiment attained the acquisition criterion (55%).  
296 Among the fish that solved the task within 10 sessions there were 8 males (80% of  
297 males) and 3 females (30% of females), implying that males showed a major capacity  
298 than females in finding the exit of the apparatus by learning the position of the visual  
299 landmarks (G-test= 5.3, d.f.=1,  $P=0.021$ ). The three females that reached the  
300 acquisition criterion required 7, 7 and 9 sessions respectively. The number of sessions  
301 that each of the eight males needed to learn the task can be found in table 1. There  
302 were no significant differences in the average time per session (s) between sexes (Fig  
303 2a; Mann–Whitney  $U$ -test,  $n = 10$ ,  $P > 0.05$ ) and between fish depending on their  
304 category of learning (yes, no) (Fig 2b; Mann–Whitney  $U$ -test,  $n = 10$ ,  $P = 0.07$ ), although  
305 in this case the result is marginally significant. There were no differences in length  
306 between fish that learn and fish that did not ( $t = -0.49$ , d.f.=18,  $P = 0.63$ ).

307 The best GLM model for the number of sessions depending on TL and SSC  
308 development included only the variable  $\log(\text{ratioCr})$ , which had a negative effect on  
309 the number of sessions (table 2). The males that had a major cephalic crest  
310 development during the session were those who learned faster (i.e. they needed fewer  
311 sessions to attain the acquisition criterion). It should be noted, in addition, that the  
312 three males that learned faster (they only needed the 4 first sessions) tended to have,  
313 apart from a large cephalic crest, larger glands than the rest (Table 1).

#### 314 **4. Discussion**

315 The present study is the first experimental approximation to the use and learning of  
316 direct visual landmarks in *S. fluviatilis*. The first finding was that the species used visual  
317 landmarks to navigate and that males and females appear to differ in the learning task  
318 resolution. These differences might be attributed to distinct spatial abilities in the  
319 sexes adjusted to their distinct ecological demands (Odling Smee & Braithwaite, 2003).  
320 This result supports the initial hypothesis of *S. fluviatilis* males being more prone than  
321 females to use visual landmarks, because males need to return to their nests after the  
322 short excursions they make in search for food or to defend their territories. However it  
323 is not clear whether there are other mechanisms at work, their relative importance or  
324 the interaction between them. Females seemed to use visual landmarks less than  
325 males. In some blennies, it has been found that females have a bigger telencephalon  
326 than males (Carneiro, Andrade, Oliveira, & Kotrschal, 2001; Costa et al., 2011).  
327 Telencephalon is related to memory and spatial learning in fish (Overmier & Hollis,  
328 1990; Broglio, Rodríguez, & Salas, 2003; Rodríguez et al., 2002) and it is important in  
329 the construction of the spatial map (Salas et al. 1996; López, Broglio, Rodríguez,  
330 Thinus-Blanc, & Salas, 1999, 2000). Spatial maps could be constituted by a series of  
331 submaps obtained through different sensory systems-(Sovrano, Bisazza, & Vallortigara,  
332 2003; Quinn, 2005). The task presented in this experiment involved the use of only a  
333 single direct visual landmark (position of two stones indicating the correct exit of the  
334 maze). Females could have had a more global perception of the surroundings and they  
335 could have relied more in other stimulus that were not fixed throughout the  
336 experiment (owing to the apparatus change of configuration). Female blennies have  
337 larger home ranges than males (Costa et al., 2011) and they need to travel long  
338 distances in order to visit and reproduce with several males (Kraak, 1996; Fagundes,

339 Gonçalves, & Oliveira, 2007). In this context, it is likely that they prefer a wider signal  
340 repertory in order to navigate in large areas. Future studies are needed in order to  
341 ascertain if these hypotheses are correct by more strictly adjusting the learning task to  
342 the females' ecological demands.

343 An association between learning and dominance on the one hand (Zhuikov, 1993) and  
344 between learning and boldness on the other (Dugatkin & Alfieri, 2003) has been  
345 established. Cephalic crest in blennies has been related both to dominance (Oliveira,  
346 Carneiro, Gonçalves, Canario, & Grober, 2001) and boldness (Fabre, García-Galea, &  
347 Vinyoles, 2014). Based on this premise, the positive relationship between cephalic  
348 crest development and learning ability found in this experiment seems coherent. The  
349 relationship we found between cephalic crest size and speed of learning cannot be  
350 attributed to age differences. All males had the same length and age at the beginning  
351 of the experiment. Age determination (through the observation of opercular bones  
352 and back-calculations in length following Vinyoles & Sostoa (2007)) permits us to  
353 ascertain that these fish were one year old. Differences in crest development could  
354 otherwise be influenced by other variables such as androgen concentration (Oliveira,  
355 Carneiro, Gonçalves, Canario, & Grober, 2001). Androgen concentration has already  
356 been related to spatial memory in rats (Benice & Raber, 2009).

357 In a study performed with guppies (*Poecilia reticulata*) it was found that females  
358 preferred to reproduce with males that solved a spatial task easier (Shohet & Watt,  
359 2009). Some studies with blennies found that the cephalic crest is a sexual  
360 characteristic preferred by females (Gonçalves & Oliveira 2003; Fagundes, Gonçalves,  
361 & Oliveira, 2007) and this could give support to the crest as a cue used by females to



362 select bold and fast-learning males. However, other studies suggest that the cephalic  
363 crest plays an important role in intrasexual competition (Oliveira, Almada, Forsgren, &  
364 Gonçalves, 1999; Fabre, García-Galea, & Vinyoles, 2014).

365 Previous works studying visual landmark learning in fish through resolution of mazes  
366 show that some species such as the goldfish (*Carassius auratus*) adapt suitably to  
367 methodologies similar to that used in the present study (Salas et al., 1996; López,  
368 Broglio, Rodríguez, Thinus-Blanc, & Salas 1999, 2000). Contrarily, *S. fluviatilis*  
369 presented difficulties in adapting to the learning task. Despite the fact that the fish  
370 used in this experiment were specifically habituated to captivity and manipulation,  
371 only a 30.8 % of them were able to perform the task. This produced a small sample  
372 size, so that obtained results must be interpreted with caution. It must be highlighted  
373 that in different animal species the adaptability to learning tasks and experimental  
374 conditions presents a high variability (Shettleworth, 1993). An exhaustive training, such  
375 as the one described here, has never been performed before to a speleophil-nest  
376 spawner fish and it is likely that fishes with this reproductive behaviour would be more  
377 sensitive to manipulation. A difference in emotional responsiveness between sexes as  
378 an explanation of the observed differences in this experiment is unlikely, as the  
379 proportion of discarded fish was not different between sexes. According to this,  
380 preliminary data from a previous experiment (Fabre et al., unpublished data) suggest  
381 that there are not differences in boldness (propensity to take risks) between males and  
382 females. The present results apply to those males and females that accepted the  
383 experimental design (probably, bold individuals) but future studies are required to  
384 investigate if these results still hold with shyer individuals. They also provide relevant  
385 information for the conservation of this endangered species (e.g. habitat

386 requirements) and make a good start point for future research on spatial learning in  
387 fish. Increasing the habituation period to the apparatus or using other similar fish  
388 models (whith a conservation status allowing the use of a higher number of  
389 individuals) should be taken into account.

390

## 391 **Acknowledgments**

392 We thank J.D. Rodríguez-Teijeiro for reviewing the manuscript, X. Fabre and A.  
393 Goldhoorn for lab assistance, G. Harwood and V. Bonet for English revision, the two  
394 anonymous reviewers for their recommendations, and the University of Barcelona for  
395 providing animal facilities. Research was funded by the research team “Biologia de  
396 Vertebrats” (2009 SGR 43) included in the Universities and Research Program (PIGC)  
397 from Generalitat de Catalunya.

398

## 399 **References**

400 Almada, V. C., Gonçalves, E. J., De Oliveira, R. F., & Barata, E. N. (1992). Some features  
401 of the territories in the breeding males of the intertidal blenny *Lipophrys pholis*  
402 (Pisces: Blenniidae). *Journal of the Marine Biological Association of the United*  
403 *Kingdom, 72*, 187.

404 Archard, G. A., Earley, R. L., Hanninen, A. F., & Braithwaite, V. A. (2012). Correlated  
405 behaviour and stress physiology in fish exposed to different levels of predation  
406 pressure. *Functional Ecology, 26*, 637-645.

407 Aronson, L. R. (1971). Further studies on orientation and jumping behavior in the  
408 gobiid fish, *Bathygobius saporator*. *Annals of the New York Academy of Sciences*,  
409 188, 378-392.

410 Balon, E.K. (1975). Terminology of intervals in fish development. *Journal of the*  
411 *Fisheries Board of Canada*, 32, 1663-1670. Benice, T. S., & Raber, J. (2009).  
412 Testosterone and dihydrotestosterone differentially improve cognition in aged  
413 female mice. *Learning & Memory*, 16, 479-485.

414 Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate  
415 promote consistent individual differences in behavior? *Trends in Ecology &*  
416 *Evolution*, 25, 653-659.

417 Braithwaite, V. A., & De Perera, T. B. (2006). Short-range orientation in fish: how fish  
418 map space. *Marine and Freshwater Behaviour and Physiology*, 39, 37-47.

419 Braithwaite, V. A., & Girvan, J. R. (2003). Use of waterflow to provide spatial  
420 information in a small-scale orientation task. *Journal of Fish Biology*, 63, 74-83.

421 Broglio, C., Rodríguez, F., & Salas, C. (2003). Spatial cognition and its neural basis in  
422 teleost fishes. *Fish and Fisheries*, 4, 247-255.

423 Brown, L. E. (1966). Home range and movement in small mammals. *Symposium of the*  
424 *Zoological Society of London*, 18, 111-142.

425 Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a*  
426 *practical information-theoretic approach*. New York: Springer.

- 427 Burt, T., & Macias Garcia, C. (2003). Amarillo fish (*Girardinichthys multiradiatus*) use  
428 visual landmarks to orient in space. *Ethology*, *109*, 341-350.
- 429 Burt de Perera, T., & Guilford, T. C. (2008). Rapid learning of shelter position in an  
430 intertidal fish, the shanny *Lipophrys pholis* L. *Journal of Fish Biology*, *72*, 1386-  
431 1392.
- 432 Carneiro, L. A., Andrade, R. P., Oliveira, R. F., & Kotrschal, K. (2001). Sex differences in  
433 home range and dorso-lateral telencephalon in the Azorian rock-pool blenny.  
434 *Society of Neuroscience Abstracts*, *27*.
- 435 Costa, S. S., Andrade, R., Carneiro, L. A., Gonçalves, E. J., Kotrschal, K., & Oliveira, R. F.  
436 (2011). Sex Differences in the Dorsolateral Telencephalon Correlate with Home  
437 Range Size in Blenniid Fish. *Brain, Behaviour and Evolution*, *77*, 55-64.
- 438 Dahlbom, S. J., Lagman, D., Lundstedt-Enkel, K., Sundström, L. F., & Winberg, S. (2011).  
439 Boldness predicts social status in zebrafish (*Danio rerio*). *PLoS One*, *6*, e23565.
- 440 Dodson, J. J. (1988). The nature and role of learning in the orientation and migratory  
441 behavior of fishes. *Environmental Biology of Fishes*, *23*, 161-182.
- 442 Dugatkin, L. A., & Alfieri, M. S. (2003). Boldness, behavioral inhibition and learning.  
443 *Ethology Ecology & Evolution*, *15*, 43-49.
- 444 Ewer, R.F. (1968). *Ethology of mammals*. London: Logos.

445 Fabre, N., García-Galea, E., & Vinyoles, D. (2014). Boldness is related to the  
446 development of the cephalic crest in the male of the river blenny *Salaria fluviatilis*  
447 (Asso, 1801). *Current Zoology*, *60*, 373-380.

448 Fagundes, T., Gonçalves, D. M., & Oliveira, R. F. (2007). Female mate choice and mate  
449 search tactics in a sex role reversed population of the peacock blenny *Salaria pavo*  
450 (Risso, 1810). *Journal of Fish Biology*, *71*, 77-89.

451 Fleming, I. A., & Gross, M. R. (1994). Breeding competition in a Pacific salmon (coho:  
452 *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution*, *48*,  
453 637-657.

454 Gaulin, S. J. C., & FitzGerald, R. W. (1986). Sex Differences in Spatial Ability: An  
455 Evolutionary Hypothesis and Test. *The American Naturalist*, *127*, 74-88.

456 Gaulin, S. J. C., & Fitzgerald, R. W. (1989). Sexual selection for spatial-learning ability.  
457 *Animal Behaviour*, *37*, 322-331.

458 Girvan, J. R., & Braithwaite, V. A. (1998). Population differences in spatial learning in  
459 three-spined sticklebacks. *Proceedings of the Royal Society of London. Series B:*  
460 *Biological Sciences*, *265*, 913-918.

461 Gonçalves, E., & Almada, V. (1998). *A comparative study of territoriality in intertidal*  
462 *and subtidal blennioids (Teleostei, Blennioidei)*. Netherlands: Springer.

463 Gonçalves, D., & Oliveira, R. (2003). *Time spent close to a sexual partner as a measure*  
464 *of female mate preference in a sex-role-reversed population of the blenny Salaria*  
465 *pavo (Risso) (Pisces: Blenniidae)*. Berlin / Heidelberg: Springer.

466 Gray, J. A., & Buffery, A. W. H. (1971). Sex differences in emotional and cognitive  
467 behavior in mammals including man: adaptive and neural bases. *Acta*  
468 *Psychologica*, 35, 89-111.

469 Hawryshyn, C. W., Arnold, M. G., Bowering, E., & Cole, R. L. (1990). Spatial orientation  
470 of rainbow trout to plane polarized light: the ontogeny of e-vector discrimination  
471 and spectral sensitivity characteristics. *Journal of Comparative Physiology*, 166,  
472 565–574.

473 Heap, S., Byrne, P., & Stuart-Fox, D. (2012). The adoption of landmarks for territorial  
474 boundaries. *Animal Behaviour*, 83, 871-878.

475 Hofmann, H. A., Benson, M. E., & Fernald, R. D. (1999). Social status regulates growth  
476 rate: consequences for life-history strategies. *Proceedings of the National*  
477 *Academy of Sciences*, 96, 14171-14176.

478 Hughes, R. N., & Blight, C. M. (1999). Algorithmic behaviour and spatial memory are  
479 used by two intertidal fish species to solve the radial maze. *Animal Behaviour*, 58,  
480 601-613.

481 Huntingford, F. A., & Wright, P. J. (1989). How sticklebacks learn to avoid dangerous  
482 feeding patches. *Behavioural processes*, 19, 181-189.

483 Ingle, D. J., & Sahagian, D. (1973). Solution of a spatial constancy problem by goldfish.  
484 *Physiological Psychology*, 1, 83-84.

485 Jorge, P. E., Almada, F., Gonçalves, A. R., Duarte-Coelho, P., & Almada, V. C. (2012).  
486 Homing in rocky intertidal fish. Are *Lipophrys pholis* L. able to perform true  
487 navigation? *Animal Cognition*, *15*, 1173-1181.

488 Kraak, S. B. M. (1996). A quantitative description of the reproductive biology of the  
489 Mediteranean blenny *Aidablennius sphinx* (Teleostei, Blenniidae) in its natural  
490 habitat. *Environmental Biology of Fishes*, *46*, 329–342.

491 Lee, S. A., Vallortigara, G., Ruga, V., & Sovrano, V. A. (2012). Independent effects of  
492 geometry and landmark in a spontaneous reorientation task: a study of two  
493 species of fish. *Animal cognition*, *15*, 861-870.

494 Lockie, J. D. (1966). Territory in small carnivores. *Symposium of the Zoological Society*  
495 *of London*, *18*, 143-165.

496 López, J. C., Broglio, C., Rodríguez, F., Thinus-Blanc, C., & Salas, C. (1999). *Multiple*  
497 *spatial learning strategies in goldfish (Carassius auratus)*. Berlin / Heidelberg:  
498 Springer.

499 López, J. C., Broglio, C., Rodríguez, F., Thinus-Blanc, C., & Salas, C. (2000). Reversal  
500 learning deficit in a spatial task but not in a cued one after telencephalic ablation  
501 in goldfish. *Behavioural Brain Research*, *109*, 91-98.

502 Markel, R. W. (1994). An adaptive value of spatial learning and memory in the blackeye  
503 goby, *Coryphopterus nicholsi*. *Animal Behaviour*, *47*, 1462-1464.

504 Mazeroll, A. I., & Montgomery, L. (1998). Daily Migrations of a Coral Reef Fish in the  
505 Read Sea (Gulf of Aqaba, Israel): Initiation and Orientation. *Copeia*, *4*, 893-905.

506 Neat, F., & Lengkeek, W. (2009). Sexual selection in blennies. In R.A. Patzner, E.J.  
507 Gonçalves, P.A. Hastings, & B.G. Kapoor (Eds.) *The Biology of Blennies* (pp. 249-  
508 278). Enfield: Science Publishers.

509 Odling-Smee, L., Boughman, J., & Braithwaite, V. (2008). *Sympatric species of*  
510 *threespine stickleback differ in their performance in a spatial learning task*. Berlin /  
511 Heidelberg: Springer.

512 Odling-Smee, L., & Braithwaite, V. A. (2003). The influence of habitat stability on  
513 landmark use during spatial learning in the three-spined stickleback. *Animal*  
514 *Behaviour*, 65, 4, 701-707.

515 Oliveira, R. F., & Almada, V. C. (1998). Androgenization of dominant males in a cichlid  
516 fish: androgens mediate the social modulation of sexually dimorphic traits.  
517 *Ethology*, 104, 841–858.

518 Oliveira, R. F., Almada, V. C., Forsgren, E., & Gonçalves, E. J. (1999). Temporal variation  
519 in male traits, nesting aggregations and mating success in the peacock blenny.  
520 *Journal of Fish Biology*, 54, 499-512.

521 Oliveira, R. F., Carneiro, L. A., Gonçalves, D. M., Canário, A. V. M., & Grober, M. S.  
522 (2001). 11-Ketotestosterone Inhibits the Alternative Mating Tactic in Sneaker  
523 Males of the Peacock Blenny, *Salaria pavo*. *Brain, Behavior and Evolution*, 58, 28-  
524 37.

525 Overmier, J. B., & Hollis, K. L. (1990). Fish in the think tank: learning, memory, and  
526 integrated behavior. *Neurobiology of Comparative Cognition*, 205-236.



527 Papi, F. (2006). Navigation of marine, freshwater and coastal animals: concepts and  
528 current problems. *Marine and Freshwater Behaviour and Physiology*, 39, 3-12.

529 Pottinger, T. G., & Carrick, T. R. (2001). Stress responsiveness affects dominant-  
530 subordinate relationships in rainbow trout. *Hormones and Behavior*, 40, 419-427.

531 Quinn, T. P. (2005). *The Behaviour and Ecology of Pacific Salmon and Trout*. Seattle:  
532 University of Washington Press.

533 R Core Team (2012). *R: A Language and Environment for Statistical Computing*. R  
534 Foundation for Statistical Computing. Vienna, Austria.

535 Rodríguez, F., Duran, E., Vargas, J. P., Torres, B., & Salas, C. (1994). Performance of  
536 goldfish trained in allocentric and egocentric maze procedures suggests the  
537 presence of a cognitive mapping system in fishes. *Animal Learning & Behavior*, 22,  
538 409-420.

539 Rodríguez, F., López, J. C., Vargas, J. P., Gómez, Y., Broglio, C., & Salas, C. (2002).  
540 Conservation of Spatial Memory Function in the Pallial Forebrain of Reptiles and  
541 Ray-Finned Fishes. *The Journal of Neuroscience*, 22, 2894-2903.

542 Salas, C., Broglio, C., Rodríguez, F., López, J. C., Portavella, M., & Torres, B. (1996).  
543 Telencephalic ablation in goldfish impairs performance in a 'spatial constancy'  
544 problem but not in a cued one. *Behavioural Brain Research*, 79, 193-200.

545 Salas, C., Broglio, C., Durán, E., Gómez, A., Ocaña, F. M., Jiménez-Moya, F., &  
546 Rodríguez, F. (2006). Neuropsychology of learning and memory in teleost fish.  
547 *Zebrafish*, 3, 157-171.

- 548 Shettleworth, S. J. (1993). Where is the comparison in comparative cognition?  
549 Alternative research programs. *Psychological Science*, *4*, 179-184.
- 550 Shohet, A. J., & Watt, P. J. (2009). Female guppies *Poecilia reticulata* prefer males that  
551 can learn fast. *Journal of Fish Biology*, *75*, 1323-1330.
- 552 Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2002). Modularity and spatial  
553 reorientation in a simple mind: encoding of geometric and nongeometric  
554 properties of a spatial environment by fish. *Cognition*, *85*, 51-59.
- 555 Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2003). Modularity as a fish (*Xenotoca*  
556 *eiseni*) views it: Conjoining geometric and nongeometric information for spatial  
557 reorientation. *Journal of Experimental Psychology: Animal Behavior Processes*, *29*,  
558 199-210.
- 559 Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2007). How fish do geometry in large and  
560 in small spaces. *Animal cognition*, *10*, 47-54.
- 561 Stabell, O. B. (1984). Homing and olfaction in salmonids: a critical review with special  
562 reference to the Atlantic salmon. *Biological Reviews*, *59*, 333-388.
- 563 Thyssen, L. (2010). Homing behaviour of *Parablennius parvicornis* (Pisces: Blenniidae).  
564 *Anales Universitarios de Etología*, *4*, 25-30.
- 565 Tommasi, L., & Vallortigara, G. (2004). Hemispheric processing of landmark and  
566 geometric information in male and female domestic chicks (*Gallus gallus*).  
567 *Behavioural brain research*, *155*, 85-96.

- 568 Trivers, R. L. (1972). Parental investment and sexual selection. In B. G. Campbell (Eds.),  
569 *Sexual selection and the descent of man* (pp. 136-179). Aldine, Chicago.
- 570 Vallortigara, G. (1996). Learning of colour and position cues in domestic chicks: males  
571 are better at position, females at colour. *Behavioural processes*, 36, 289-296.
- 572 Vinyoles, D. (1993). *Biologia i ecologia de Blennius fluviatilis (Pisces: Blenniidae) al Riu*  
573 *Matarranya*. PhD. Dissertation, Universitat de Barcelona.
- 574 Vinyoles, D., & De Sostoa, A. (2007). Life-history traits of the endangered river blenny  
575 *Salaria fluviatilis* (Asso) and their implications for conservation. *Journal of Fish*  
576 *Biology*, 70, 4, 1088-1108.
- 577 Walker, M. M., Diebel, C. E., Haugh, C. V., Pankhurst, P. M., Montgomery, J. C., &  
578 Green, C. R. (1997). Structure and function of the vertebrate magnetic sense.  
579 *Nature*, 390, 371-376.
- 580 Warburton, K. (1990). The use of local landmarks by foraging goldfish. *Animal*  
581 *Behaviour*, 40, 500-505.
- 582 Wickler, W. (1957). Vergleichende Verhaltensstudien an Grundfischen. I. Beiträge zur  
583 Biologie, besonders zur Ethologie von *Blennius fluviatilis* Asso im Vergleich zu  
584 einigen anderen Bodenfischen. *Zeitschrift für Tierpsychologie*, 14, 393-428.
- 585 Wittenberger, J. F. (1981). *Animal social behavior*. Boston: Duxbury Press.
- 586 Zhuikov, A. Y. (1993). Avoidance learning and aggression in guppies. *Animal behaviour*,  
587 45, 825-826.