
Risk-taking behaviour variation over time in sea bass *Dicentrarchus labrax*: effects of day–night alternation, fish phenotypic characteristics and selection for growth

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Abstract:

Differences in bold and shy personality on sea bass *Dicentrarchus labrax* were investigated between a population (wild) produced from wild-brood fish and a population (selected) produced from selected-brood fish. During the experiment (112 days), fish were reared under self-feeding condition to characterize the feeding behaviour of each individual fish. Three risk-taking tests (T1, T2 and T3 of 24 h with day–night alternation) were carried out at > 1 month intervals on 180 fish of each strain in order to monitor *D. labrax* behaviour over time and in relation to the light:dark period. A risk-taking score was evaluated via a preference choice between a safe zone (without food) and a risky zone (potentially with food) by recording the number and the duration of individual passages through an opening in an opaque divider. Results showed that fish performed passages preferentially during the night period and that wild fish were generally bolder than selected fish during T1 and T2 but showed a decrease in risk taking during T3, contrary to selected fish which showed a constant increase in their risk-taking behaviour. The phenotypic characteristics of the bold fish were different in the two strains: wild bold fish were the smallest within the wild strain and selected bold fish presented the higher growth rate within the selected strain. For both strains, these bold fish were also generally characterized by a high feed-demand activity. Fish hunger state thus seemed to be the highest motivation for risk-taking behaviour under the present conditions. Furthermore, behavioural variations over tests such as higher risk taking (number of passages) and faster exploratory responses (higher score emergence) could be interpreted as relevant indicators of the learning process and habituation. According to the results, however, no real difference in coping strategy between strains could be observed at this first stage of domestication and selection.

51

Keywords

52 Boldness, Choice test, Domestication, Habituation, Learning.

53

54

Introduction

55 The study of individual variation in animal behaviour has increased over the last decade
56 (Wilson, 1998). The potential effect of consistent “personality” traits, such as the bold and
57 shy behaviour or differences in coping strategies was shown to be central in the understanding
58 of such variability (Benus *et al.*, 1991; Wilson *et al.*, 1994; Coleman & Wilson, 1998). The
59 propensity to take risks has implications in survival, reproduction and many other life history
60 and behavioural traits (Budaev, 1997 a, b). Among others, boldness is considered as a
61 personality trait and is generally defined as the propensity to take risks (Wilson *et al.*, 1993,
62 1994; Fraser *et al.*, 2001). Previous studies in fish have relied on a variety of tests to score
63 boldness (*e.g.* showing a new object: Wright *et al.*, 2003; showing a new type of food:
64 Coleman & Wilson, 1998; showing a threatening stimulus: Magnhagen & Staffan, 2005; or
65 placing fish in a totally new environment: Brown & Braithwaite, 2004). Some studies also
66 showed a relationship between boldness and other traits. For example, Ward *et al.* (2004)
67 found that bold threespine sticklebacks (*Gasterosteus aculeatus*, L.) tended to be at the front
68 of fish shoal, Sneddon (2003) showed that bold rainbow trout (*Oncorhynchus mykiss*,
69 Walbaum) were able to learn a task more rapidly than shy individuals, Godin & Dugatkin
70 (1996) observed that bolder male guppies (*Poecilia reticulata*, Peters) were more attractive to
71 females and Sundström *et al.* (2004) reported that bold brown trout (*Salmo trutta*, L.) tended
72 to become dominant. The propensity to take risks and other behavioural traits are also known
73 to be heavily influenced by hunger and demographic variables such as age and sex (Wilson *et*
74 *al.*, 1994; Krause *et al.*, 1998 a). Yet no study has been performed on Moronidae fish family
75 such as sea bass (*Dicentrarchus labrax*, L.) which is an important species in Mediterranean

76 and Atlantic aquaculture and was recently domesticated. Domestication is defined as a
77 process by which an animal population becomes adapted to the captive environment by
78 genetic changes occurring over generations and environmentally-induced developmental
79 events reoccurring at each generation (Price, 1984). Thus, to characterize fish personality in
80 order to evaluate the potential abilities in learning, stress tolerance or adaptation, appears
81 essential to assess if the welfare of domesticated fish is threatened. Further, selective breeding
82 is an unavoidable practice in the whole animal production but in marine fish, selection has
83 been applied only recently (one or two generations) and growth is the major trait of interest.
84 One commonly used approach in studying the effect of domestication is to compare wild and
85 domestic stocks of a given species (Desforges & Wood-Gush, 1976; Boice, 1980; Price,
86 1980). For that reason, our study investigated personality traits on fish produced from wild
87 (*Wild strain*) or from brood fish selected for growth (*Selected strain*).

88 The aims of this study were to characterize *D. labrax* personality traits (bold *versus*
89 shy) by offering them the choice between a safe zone (shadowed and without food) and a
90 risky zone (bright open and potentially with food) and to measure how this behaviour changed
91 over time and was influenced by day-night alternation. Therefore, we determined how the fish
92 from the two strains differed in their responses and we characterized bolder individuals
93 through the level of correlations existing between individual risk-taking behaviour and (i)
94 individual phenotypic traits (mass, length, body condition factor, and specific growth rate) or
95 (ii) individual feed demand.

96

97 **Material and methods**

98 *Experimental set up*

99 The two tested strains were hatched and reared at the experimental research station of
100 Ifremer in Palavas-les-Flots (France; Vandeputte *et al.*, 2009). They were produced from a

101 full factorial crossing (each female was crossed with each male) of 13 wild Mediterranean
102 females with (i) 20 Atlantic wild males (*Wild* strain) and (ii) 19 Atlantic selected males
103 (*Selected* strain) respectively. The *Wild* parental males were chosen among an Atlantic wild
104 population kept in captivity for a least one year. The selected males were obtained by
105 selecting the 5% longest fish at the same age (20 months, 400 g) in a population reared for
106 two years according to *D. labrax* rearing standards (Chatain, 1994). Thus all fish tested in this
107 experiment never experienced the natural environment, had the same life history except that
108 their parents presented different levels of domestication and selection. To summarize, *Wild*
109 strain was characterized by fish produced from wild parents with at least one year in captivity
110 and *Selected* strain by parents with one generation of captivity (*i.e.* domestication) and one
111 generation of selection for growth.

112 The experiment was carried out testing each condition with a triplicate per strain. The
113 6 tanks (400 l each, size: 1m long x 1m wide x 0.5 m deep) were supplied with recirculated
114 seawater. For each tank, flow rate was $4 \text{ m}^3 \text{ h}^{-1}$ and water renewal 10 % per day. Water
115 temperature was maintained at $20.2 \pm 1.5^\circ\text{C}$, oxygenation above 80 % of saturation in the
116 water-outlet, and salinity was 22.3 ± 3.3 . Tanks were sheltered by black curtains and
117 individually lighted by a 120 W lamp placed at 90 cm above the water surface. Light regime
118 was 16:8 LD (light onset at 06:00) with twilight transition periods of 30 min. Fish were fed by
119 self-feeders (Milot *et al.*, 2008) with a commercial diet for *D. labrax* (Neo Grower Extra
120 Marin 4.0, France) containing 45 % of crude protein and 20 % of lipid according to the
121 manufacturer. The experiment was performed over 112 days, with 360 fish (60 fish per tank,
122 180 fish per strain) 14 months-old at the beginning of the study.

123

124 *Test material and procedure*

125 To monitor the risk taking behaviour, each tank was separated in two unequal zones by
126 an opaque divider. The safe zone was shadowed, represented 2/3 of the space and gathered all
127 fish at the beginning of the experiment. The other zone, the risky zone was lit, represented 1/3
128 of the space and included the self-feeder and feeding area. The opaque divider had a circular
129 (12 cm Ø) opening in its centre that was equipped with a PIT-tag detection antenna connected
130 to a control device. Each fish carried a PIT-tag inserted horizontally just behind the head to
131 prevent any position change subsequent to its implantation. Such a set up allowed monitoring
132 the individual passages through the opaque divider, and the associated time stamp. The study
133 was completed by visual observations and video recording (Mini color CMOS camera
134 (Velleman) and hard disk recorder).

135 The three tests were done on the same fish groups, under stable environmental
136 conditions, and according to the same procedure, each test lasting 24h. The divider was
137 installed in each tank at 10:00 and the opening was blocked for 30 min before the test started.
138 The tests were operated at Day 1, the beginning of experiment (D1, T1), at Day 48 (D48, T2)
139 and at Day 85 (D85, T3).

140 The device to operate the feeders comprised a screened type sensor (a metal rod
141 protected by a PVC cylinder surrounded by the PIT tag detection antenna; Covès *et al.*, 2006;
142 Millot *et al.*, 2008) and a control box. During all the experiment, fish were placed under self-
143 feeding conditions (Covès *et al.*, 2006; Millot *et al.*, 2008) and food access was possible 24 h
144 a day, except during the risk-taking test. After each activation, fish were rewarded with 50
145 pellets and feed dispensers were regulated to distribute a mean of 0.5 g kg⁻¹ and 0.3 g kg⁻¹ of
146 fish at the beginning and at the end of the experiment respectively. Triggering activity
147 recordings were done continuously for 112 days. Such a set up allowed us to monitor the
148 number, the date and the hour of feed demand in each tank.

149

150 *Data analysis*

151 The traits of interest and the variables chosen to measure them were the following:

152 As group behaviour, proportion of the fish population entering in the risky zone was
153 calculated.

154 Individual risk-taking behaviour was evaluated by analyzing the total time spent in the risky
155 zone, the number of passages per hour through the opening, the time spent in the risky zone at
156 each visit and the latency before the first entry in the risky zone. The comparison of the data
157 between each test gave us an indication on the fish habituation and learning.

158 The individual score emergence (Se) was also calculated as: [test duration (min) – emergence
159 time (min)] x [test duration (min)]⁻¹, where total test duration was equal to 1440 min and
160 emergence time corresponded to the time necessary to realize the first entry in the risky zone.

161 Score emergence close to 0 therefore corresponded to a very late or no entry in the risky zone
162 while close to 1, it corresponded to a very fast entry. Correlation between successive
163 individual score emergence was evaluated (Pearson correlation between test T1 and T2; or T2
164 and T3) as criteria of fish bold or shy personality consistency over time.

165 Bold individuals were characterized by using the correlation level between individual score
166 emergence or number of passages through the opening and phenotypic traits (mass, length,
167 specific growth rate, body condition factor) and feed demand.

168 Fish individual mass was recorded at Day 1, 27, 53, 77, and 112 under light anaesthesia with
169 0.08% of clove oil.

170 The specific growth rate was calculated as:

171 G (% body mass per day) = $100 (\ln M_f - \ln M_i) \times t^{-1}$, where M_f and M_i are the final and the
172 initial body mass (g) respectively and t the total number of days.

173 The body condition factor was calculated as: K (g cm^{-3}) = $100 \times M \times L^{-3}$ where M is body
174 mass (g) and L is the standard body length (cm).

175 The number of individual feed demand (F) was recorded between each test: F_{T1} (Day 1 to Day
176 48), F_{T2} (Day 49 to Day 85) and F_{T3} (Day 86 to Day 112).

177 The mean fish mass, length and body condition factor considered for the correlation with
178 individual risk-taking behaviour were those measured at D1 for Test 1, at D53 for Test 2 and
179 at D77 for Test 3 (Table I). Three periods of growth were considered for the same correlation:
180 G_{T1} (Day 1 to Day 27), G_{T2} (Day 28 to Day 53) and G_{T3} (Day 77 to Day 112).

181 All mean values were expressed with the standard error (S.E.).

182 During the experiment, some fish died for different reasons *i.e.* some jumped out of
183 the tank or for unidentified causes: it concerned 7 *Wild* and 9 *Selected* fish during all the
184 experiment duration. These fish were excluded from the data analysis from the beginning of
185 the experiment to keep the same number and identity of fish studied during the three tests.

186 Data were analyzed for normality with a Shapiro-Wilk test and for homoscedacity
187 with a Bartlett's test. The variables "total time spent by a fish in the risky zone (%)" and
188 "individual score emergence" have undergone an arcsine transformation to normalize data
189 (Sokal & Rohlf, 1995). Then, for all variables except latency, a repeated ANOVA was used to
190 analyse the average differences between strain (fixed factor), day and night period (fixed
191 factor repeated within test), tests (fixed factor) and tanks (random factor nested within strain).
192 For latency, a repeated ANOVA was used to analyse the average differences between strains
193 (fixed factor), tests (fixed factor) and tanks (random factor nested within strain).
194 Homogeneous groups were determined with the *a posteriori* Newman and Keuls test
195 (Dagnélie, 1975). Canonical correlation analysis were performed between the following
196 variables: score emergence and number of individual passages per hour through the opening
197 (dependent variables) and fish individual mass, length, specific growth rate, body condition
198 factor and feed demand (independent variables). Since there were some likely correlations
199 between the independent variables, testing some underlying factor(s) might have occurred and

200 interpretation should be precautionary. For all tests, significant threshold was $p < 0.05$ and
201 analyses were performed using Minitab 15, Systat 11 and Statistica softwares.

202

203

Results

204 *Behavioural responses to the set up*

205 During T1, the first fish entering in the risky zone appeared hyperactive. It swam very
206 fast in all directions, banging into the tank divider and walls. After 30 s to 1 min of this type
207 of behaviour, it stayed in a fix position in a tank corner. When a second fish was entering in
208 the risky zone, its behaviour was the same than the first fish, which became again very active.
209 On the contrary, during T2 and even more during T3, fish entries in the risky zone were slow,
210 even for the first fish passage. During T2 and T3, a lot of fish remained in the risky zone, and
211 were passing in and out of the risky zone continuously and slowly.

212

213 *Proportion of the fish population entering in the risky zone*

214 The proportion of *Wild* and *Selected* population entering in the risky zone was similar
215 ($F_{1,12}=0.03$, $p>0.05$), but changed within time: it was much lower at T1 ($23\pm 7\%$) than during
216 T2 ($89\pm 3\%$) and T3 ($85\pm 8\%$; $F_{2,12}=38.71$; $p<0.001$). The proportion of fish entering in the
217 risky zone during T1 and entering again during T2 was $98\pm 2\%$ for *Wild* and *Selected* fish.
218 Between T2 and T3, it was $81\pm 21\%$ for *Wild* fish and $98\pm 1\%$ for *Selected* fish.

219

220 *Total time spent by a fish in the risky zone, influence of day-night alternation*

221 As a general feature, both strains spent less time in the risky zone than in the safe zone
222 (Fig. 1). Whatever the strain, fish spent more time in the risky zone during the night period
223 than during the day period (Table II). Strains behaviour only differed within time: *Wild* fish
224 spent more time in the risky zone than *Selected* fish during T1 and T2, and less during T3.

225 *Wild* fish showed a significant increase of time spent in the risky zone between T1 and T2,
226 and a decrease between T2 and T3, while *Selected* strain showed a constant increase between
227 T1 and T3.

228

229 *Number of fish passages per hour through the opening, influence of day-night alternation*

230 Whatever strain, the number of fish passages per hour through the opening was higher
231 during the night period than during the day period (Fig.2, Table II). Both strains performed
232 the same number of passage through the opening during the first test. The *Wild* fish performed
233 more passages through the opening than *Selected* fish during the second test. During the third
234 test, *Selected* fish performed more passages than *Wild* fish. The number of fish passages
235 through the opening increased significantly between T1 and T2 for both strains; however it
236 decreased for *Wild* strain at T3 while it increased significantly for *Selected* strain.

237 For the *Wild* strain, this variable was positively correlated to individual feed demand (F) and
238 negatively correlated with fish mass (M) at T1 (Table III). For the *Selected* strain, it was
239 positively correlated to fish growth (G) at T1 and to F at T2 and T3.

240

241 *Time spent by a fish in the risky zone at each visit, influence of day-night alternation*

242 For both strains, the time spent by a fish in the risky zone at each visit was longer during
243 the day than during the night (Fig.3, Table II). It was identical for the two strains during T1
244 and T3 but during T2, *Selected* fish spent almost twice the time in the risky zone than *Wild*
245 fish did. The time spent by a fish in the risky zone at each passage decreased significantly
246 between T1 and T2 for both strains; however it stayed at the same level at T3 for *Wild* strain,
247 while it decreased significantly for *Selected* strain.

248

249 *Latency before the first entry of a fish in the risky zone*

250 During T1, both strains showed a strong latency before the first entry of a fish in the
251 risky zone (Fig. 4, Table II) that occurred principally after the night period. During T2, the
252 first entry was generally done before the night and *Wild* fish entered in the risky zone earlier
253 than *Selected* fish. During T3, for both strains, the first entry was also generally done before
254 the night period and *Selected* fish entered in the risky zone earlier than *Wild* fish. Both strains
255 showed a significant decrease of the latency before the first entry between the two first tests,
256 however, *Wild* fish were characterized by an increase of this latency during the third test
257 while *Selected* fish presented a decrease.

258

259 *Score emergence*

260 For the *Wild* strain, there was no correlation between individual score emergence and
261 other variables (Table III). For the 3 tests, score emergence was positively correlated to the
262 number of fish passages per hour through the opening (T1: $r = 0.487$, $p < 0.001$, $n = 173$;
263 T2: $r = 0.439$, $p < 0.001$, $n = 173$; T3: $r = 0.626$, $p < 0.001$, $n = 173$).

264 For the *Selected* strain, individual score emergence was positively correlated to fish growth at
265 T1 and to feed demand at T3 (Table III). This variable was also positively correlated to the
266 number of fish passages per hour through the opening during the 3 tests (T1: $r = 0.620$,
267 $p < 0.001$, $n = 171$; T2: $r = 0.360$, $p < 0.001$, $n = 171$; T3: $r = 0.528$, $p < 0.001$, $n = 171$).

268 No relationship was found between successive individual score for the *Selected* strain, while it
269 occurred between each test for the *Wild* strain (T1 - T2: $r = 0.164$, $p < 0.05$, $n = 173$;
270 T2 - T3: $r = 0.444$, $p < 0.001$, $n = 173$).

271

272

272 **Discussion**

273 In the present study, *D. labrax* changes in risk-taking behaviour over time were
274 revealed by the simultaneous analysis of group and individual variables which highlighted for

275 the first time how this species behaved in a trade-off between the day-night alternations and
276 how fish domestication and selection levels influenced behavioural responses.

277

278 *How did D. labrax behave in the set up?*

279 During the first test only 23% of the population entered in the risky zone. They were
280 very agitated, banging into the divider and holding position in a tank corner, sometimes until
281 the end of the test thus generating a long stay duration by a fish in the risky zone at each visit.
282 Moreover, during this test, very few passages through the opening were performed, and the
283 first passage generally occurred a long time after the experiment started. These behaviours
284 could be interpreted as an expression of fear or anxiety which generally generates a stress
285 state in individuals (Yue *et al.*, 2004) and could be classified in two patterns: active avoidance
286 reactions (flight, hiding, escape) and movement inhibition (immobility; Boissy, 1998).

287 Fish behaviour varied over time and indeed, during the second and even more during the
288 third test, fish presented an entirely different behaviour. The percentage of the population
289 entering in the risky zone increased considerably, and reached 80 to 98%. Fish swam in the
290 risky zone very slowly, finding the opening in the divider without difficulty to pass from one
291 zone to the other, as shown by the high increase in the number of passages and by the
292 decrease in the time spent in the risky zone at each passage during these two tests. Other
293 studies related to a variety of species, have also shown that intensity of fear decreases as the
294 animal masters the correct response (Solomon & Wynne, 1953; Kamin *et al.*, 1963; Starr &
295 Mineka, 1977) and might be relevant indicators of habituation which is a primitive kind of
296 learning (Humphrey, 1933; Thorpe, 1963; Hinde, 1970; Peeke & Petrinovich, 1984).
297 Generally, the learning term refers to a change in behaviour with experience (Dill, 1983), but
298 different types of learning exist: i) the individual learning which involves only a direct
299 interaction between the fish and the situation (*i.e.* stimulation or environment change) and

300 subsequent acquisition of a novel behaviour (Giraldeau *et al.*, 1994); ii) the social learning
301 which refers to learning that is influenced by observation of (or interaction with) other
302 individuals (Galef & Giraldeau, 2001); and iii) the leadership which can be defined in animal
303 groups as the initiation of a movement or a change of direction during a movement, made by
304 one or some individual(s) and followed by the rest of the group (Krause *et al.*, 2000). In the
305 present study, the majority of fish passed in the risky zone during test 1 passed again during
306 test 2 and 3 and the fish that entered first in the risky zone were also the fish that performed
307 the highest number of passages per hour through the opening. Thus, according to these results
308 it is probable that fish learned individually how to cope with the environmental change, but as
309 shown by the high increase in the percentage of the population entering in the risky zone
310 during the second test, it is also likely that social learning played an important part in this
311 change of behaviour. Learning by leadership seemed only present in the *Wild* strain. Indeed,
312 we showed that fish which presented the highest score emergence were the same over time.
313 According to this result, we could hypothesize that fish which have been produced from wild
314 parents expressed higher schooling behaviour (with leader fish) than fish produced by parents
315 with one generation of captivity and one generation of selection for growth.

316 Finally, our results suggest that the behavioural response changes over tests could be
317 related first to habituation and both individual learning (with strengthening over time) and
318 social learning (based on the congener's behaviour observation) and second, for fish
319 presenting less than one generation in captivity, to a possible leadership learning.

320

321 *How did the day-night alternation influence risk taking behaviour?*

322 As a general feature, *D. labrax* spent more time in the risky zone and performed the
323 majority of passages through the opening during the night period. This explained that the time
324 spent by a fish in the risky zone at each visit was higher during the day than during the night

325 period. They were thus more actively moving during the night period. In natural environment,
326 it has been shown that fish reduce their individual risk of predation by entering refuges
327 wherein they are less susceptible to predation than in open habitat (Godin, 1997; Persson *et*
328 *al.*, 1997). According to these observations and to our results, we could hypothesize that fish
329 considered the safe zone as a refuge, and performed the majority of passages when the risk
330 had decreased, that is during the night period, when there was no more light difference
331 between risk and safe zone. However, the high decrease over time of the latency before the
332 first entry of a fish in the risky zone seemed to show that the fish perception of the light
333 difference between the two zones and the day-night alternation had less importance, and thus
334 the dangerous character of the risky zone had decreased over time. Such behavioural changes,
335 could be, one more time, explained by habituation, but also by learning process.

336

337 *What are the effects of fish domestication and selection levels on risk taking behaviour?*

338 General behaviour was quite similar for both *D. labrax* strains, but some differences
339 appeared during the successive tests. Indeed, during the first test, the *Wild* strain was
340 characterized by a longer total time spent in the risky zone than the *Selected* strain. During the
341 second test even if both strains increased the number of passages through the opening and
342 decreased the latency before the first entry and the time spent at each passage in the risky
343 zone, these behavioural changes were more marked for *Wild* strain than for *Selected* strain.
344 Indeed, *Selected* fish were characterized by a higher latency before the first entry in the risky
345 zone, by a lower number of passages through the opening and by a longer stay duration in the
346 risky zone at each visit, than *Wild* fish. This might indicate that *Selected* fish took less risk
347 than *Wild* fish at this date. Finally, during the third test, *Selected* strain showed either a
348 decrease of time spent at each visit and of latency before the first entry in the risky zone, an
349 increase of total time spent and of number of passages in the risky zone. While *Wild* strain

350 showed a decrease of total time spent in the risky zone and an increase of 51% in latency
351 before the first entry. Moreover, during this test, *Wild* fish were also characterized by a lower
352 number of passages through the opening than *Selected* fish. In summary, even if *Selected* fish
353 were characterized by a lower risk taking behaviour than *Wild* fish during the first two tests,
354 they were also characterized by progressive adaptation to the environmental changes, while
355 *Wild* fish seemed more variable in their responses over time. In *Selected* fish, this low degree
356 of variability in risk taking behaviour and consequently in the group coping strategy over time
357 might be a first consequence of fish domestication and selection.

358 Strains differed also by their phenotypic characteristics associated to boldness. Indeed,
359 *Selected* bold fish had a higher growth rate during the first test and a higher feed demand
360 activity during the second and the third tests. Such correlations have already been found in
361 salmonids selected for growth, for which in addition to that, an increased willingness to
362 accept risk to access food was showed (Johnsson & Abrahms, 1991; Johnsson *et al.*, 1996;
363 Fernö & Järvi, 1998; Biro *et al.*, 2004; Huntingford & Adams, 2005). Thus, in *Selected* strain,
364 increased boldness might be due to their higher food needs, since bold fish during the first test
365 presented a higher specific growth rate, and during the second and the third test fish were
366 characterized by a higher feeding motivation than shy individuals. *Wild* bold fish, as for them,
367 were characterized by a higher feed demand activity during the period following the first test
368 but also by a smaller mass than shy individuals. These results seemed to be in opposition to
369 the previous conclusions done on *Selected* fish, but Brown & Braithwaite (2004) have
370 demonstrated that wild populations of poeciliidae (*Brachyrhaphis episcopa*, Steindachner)
371 showed a positive relation between body size and time to emerge from a shelter, with larger
372 fish taking longer to emerge; Dowling & Godin (2002) found the same phenomenon in
373 Banded killifish (*Fundulus diaphanous*, Lesueur). In general, large wild individuals are
374 predicted to favour lower risk behavioural options than small individuals, currently explained

375 by their nutritional state (Krause *et al.*, 1998 b; Grand, 1999; Reinhardt & Healey, 1999;
376 Brown *et al.*, 2005). Thus, if *Wild* bold fish were smaller in mass, it is perhaps due to a
377 depleted nutritional state and they might be more disposed to take risk in order to compensate
378 such depletion. If this correlation appeared only during the first test, it might be, once again,
379 because it was during this first test that the risky zone presented the most dangerous character
380 for fish.

381 The risk-taking behaviour is usually the result of a trade-off between risk aversion and other
382 motivations such as hunger, curiosity or need to maintain inter-individual distances (Leblond
383 & Reeb, 2006). In this study, fish hunger state seemed to be the highest motivation for *D.*
384 *labrax* risk-taking behaviour.

385 In conclusion, the present study has demonstrated that, in *D. labrax*, i) the time spent in
386 a risky zone (in total and at each visit), the number of passages through an opening and the
387 score emergence compared over time and between day and night period, were relevant
388 indicators of fish learning process and habituation and that ii) those indicators could be used
389 as standardized measures of cultured fish “personality”. It also showed that risk-taking
390 behaviour seemed to be correlated with fish mass, growth and feed demands which seemed to
391 highlight the important effect of fish hunger state on this behaviour. According to the results,
392 however, no real difference in coping strategy between strains could be observed at this first
393 stage of domestication and selection. To better understand domestication and/or selection
394 effects on *D. labrax* behaviour and adaptability, it would be therefore necessary to perform
395 measurement on fish produced from at least a second generation of domestication or
396 selection.

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409

References

- 410 Benus, R., Bohus, B., Koolhas, J. & van Oortmerssen, G. (1991). Heritable variation for
411 aggression as a reflection of individual coping strategies. *Experientia* **47**, 1008-1019.
- 412 Biro, P.A., Abrahams, M.V., Post, J.R. & Parkinson, E.A. (2004). Predators select against
413 high growth rates and risk-taking behaviour in domestic trout populations. *Proceeding of*
414 *the Royal Society B* **271**, 2233-2237.
- 415 Boice, R. (1980). Domestication and degeneracy. In *Comparative Psychology. An*
416 *Evolutionary Analysis of Animal Behavior* (Denney, M.R., ed.), pp. 84-99. New York:
417 Wiley.
- 418 Boissy, A. (1998). Fear and fearfulness in determining behavior. In *Genetics and the*
419 *behaviour of domestic animals* (Temple Grandin, ed.), pp. 67-111. Colorado.
- 420 Brown, C. & Braithwaite, V.A. (2004). Size matters: a test of boldness in eight populations of
421 the poeciliid *Brachyrhaphis episcopi*. *Animal Behaviour* **68**, 1325-1329.
- 422 Brown, C., Jones, F. & Braithwaite, V.A. (2005). In situ examination of boldness-shyness
423 traits in the tropical poeciliid, *Brachyrhaphis episcopi*. *Animal Behaviour* **70**, 1003-1009.

424 Budaev, S.V. (1997a). Alternative styles in the European wrasse, *Symphodus ocellatus*:
425 boldness-related schooling tendency. *Environmental Biology of Fishes* **49**, 71-78.

426 Budaev, S.V. (1997b). ‚Personality’ in the guppy (*Poecilia reticulata*): a correlation study of
427 exploratory behaviour and social tendency. *Journal of Comparative Psychology* **111**, 399-
428 411.

429 Chatain, B. (1994). Estimation et amélioration des performances zootechniques de l’élevage
430 larvaire de *Dicentrarchus labrax* et de *Sparus auratus*. Thèse de Doctorat d’Etat, Univ.
431 d’Aix-Marseille II 199 pp.

432 Coleman, K. & Wilson, D.S. (1998). Shyness and boldness in pumpkinseed sunfish:
433 individual differences are context-specific. *Animal Behaviour* **56**, 927-936.

434 Covès, D., Beauchaud, M., Attia, J., Dutto, G., Bouchut, C. & Bégout Anras, M.L. (2006).
435 Long-term monitoring of individual fish triggering activity on a self-feeding system: An
436 example using European sea bass (*Dicentrarchus labrax*). *Aquaculture* **253**, 385-392.

437 Dagnélie, P. (1975). Théorie et méthodes statistiques, Applications agronomiques. Gembloux:
438 Presses Agronomiques de Gembloux vol. 2, 463 pp.

439 Desforges, M.F. & Woog-Gush, D.G.M. (1976). Behavioral comparisons of Aylesbury and
440 Mallard ducks: Sexual behaviour. *Animal Behaviour* **24**, 391-397.

441 Dill, L.M. (1983). Adaptive flexibility in the foraging behavior of fishes. *Canadian Journal of*
442 *Fisheries and Aquatic Science* **40**, 398-408.

443 Dowling, L.M. & Godin, J.-G.J. (2002). Refuge use in a killifish: influence of body size and
444 nutritional state. *Canadian Journal of Zoology* **80**, 782-788.

445 Fernö, A. & Järvi, T. (1998). Domestication genetically alters the anti-predator behaviour of
446 Anadromous brown trout (*Salmo trutta*) - a dummy predator experiment. *Nordic Journal*
447 *of Freshwater Research* **74**, 95-100.

448 Fraser, D.F., Gilliam, J.F., Daley, M.J., Le, A.N. & Skalsk, G.T. (2001). Explaining
449 leptokurtic movement distributions: intrapopulation variation in boldness and exploration.
450 *The American Naturalist* **158**, 124-135.

451 Galef Jr, B.G. & Giraldeau, L.A. (2001). Social influences on foraging in vertebrates: causal
452 mechanisms and adaptive functions. *Animal Behaviour* **61**, 3-15.

453 Giraldeau, L.A., Caraco, T. & Valone, T.J. (1994). Social foraging: individual learning and
454 cultural transmission of innovations. *Behavioural Ecology* **5**, 35-43

455 Godin, J.-G.J. (1997). Evading predators. In *Behavioural ecology of teleost fishes* (J.-G.J.
456 Godin, ed) Pp. 191-236. Oxford University Press, Oxford.

457 Godin, J.G. & Dugatkin, L.A. (1996). Female mating preference for bold males in the guppy,
458 *Poecilia reticulata*. *Proceeding of the National Academy of Sciences* **93**, 10262-10267.

459 Grand, T.C. (1999). Risk-taking behaviour and the timing of life history events: consequences
460 of body size and season. *Oikos* **85**, 467-480.

461 Hinde, R.A. (1970). *Animal behaviour: a synthesis of ethology and comparative psychology*.
462 2nd edn, (MacGraw-Hill, ed). New York.

463 Huntingford, F.A. & Adams, C. (2005). Behavioural syndromes in farmed fish: implications
464 for production and welfare. *Behaviour* **142**, 1207-1221.

465 Humphrey, B. (1933). *The nature of learning*. (Kegan Paul, Trench & Trubner, ed). London.

466 Johnsson, J.I. & Abrahams, M.V. (1991). Interbreeding with domestic strain increases
467 foraging under threat in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental
468 study. *Canadian Journal of Fisheries and Aquatic Science* **48**, 237-247.

469 Johnsson, J.I., Petersson, E., Jonsson, E., Bjornsson, B.T. & Jarvi, T. (1996). Domestication
470 and growth hormone alter antipredator behaviour and growth patterns in juvenile brown
471 trout, *Salmo trutta*. *Canadian Journal of Fisheries and Aquatic Science* **53**, 1546-1554.

472 Kamin, L.J., Brimer, C.J. & Black, A.H. (1963). Conditioned suppression as a monitor of fear
473 of the CS in the course of avoidance training. *Journal of Comparative Physiology and*
474 *Psychology* **56**, 497-501.

475 Krause, J., Hoare, D., Krause, S., Hemelrijk, C.K. & Rubenstein, D. (2000). Leadership in
476 fish shoals. *Fish and Fisheries* **1**, 82-89.

477 Krause, J., Loader, S.P., McDermott, J. & Ruxton, G.D. (1998 a). Refuge use by fish as a
478 function of body length-related metabolic expenditure and predation risks. *Proceeding of*
479 *the Royal Society B* **265**, 2373-2379.

480 Krause, J., Reeves & P., Hoare, D. (1998 b). Positioning behaviour in roach shoals: the role of
481 body length and nutritional state. *Behaviour* **135**, 1031-1039.

482 Leblond, C. & Reeb, S.G. (2006). Individual leadership and boldness in shoals of golden
483 shiners (*Notemigonus crysoleucas*). *Behaviour* **143**, 1263-1280.

484 Magnhagen, C. & Staffan, F. (2005). Is boldness affected by group composition in young-of-
485 the-year perch (*Perca fluviatilis*)? *Behavioural Ecology and Sociobiology* **57**, 295-303.

486 Millot, S., Bégout, M.L., Person-Le Ruyet, J., Breuil, G., Di-Poï, C., Pineau, P., Roué, M. &
487 Sévère, A. (2008). Feed demand behavior in sea bass juveniles: effects on individual
488 specific growth rate variation and health (inter-individual and inter-group variation).
489 *Aquaculture* **274**, 87-95.

490 Peeke, H.V.S. & Petrinovich, L. (1984). Habituation, sensitization and behavior. Academic
491 Press, New York.

492 Persson, L., Diehl, S., Eklöv, P. & Christensen, B. (1997). Flexibility in fish behaviour:
493 consequences at the population and community levels. In *Behavioural ecology of teleost*
494 *fishes*. (J.-G.J. Godin, ed). Pp. 316-343. Oxford University Press, Oxford.

495 Price, E.O. (1980). Sexual behaviour and reproductive competition in male wild and domestic
496 Norway rats. *Animal Behaviour* **28**, 657-667.

497 Price, E.O. (1984). Behavioural aspects of animal domestication. *Review of Biology* **59**, 1-32.

498 Reinhardt, U.G. & Healey, M.C. (1999). Season- and size-dependent risk taking in juvenile
499 Coho salmon: experimental evaluation of asset protection. *Animal Behaviour* **57**, 923-933.

500 Sneddon, L.U. (2003). The bold and the shy: individual differences in rainbow trout. *Journal*
501 *of Fish Biology* **62**, 971-975.

502 Sokal, R.R. & Rohlf, F.J. (1995). Biometry. The principles and practice of statistics in
503 biological research. (W.H. Freeman & Company ed). Pp. 887. New York.

504 Solomon, R.L. & Wynne, L.C. (1953). Traumatic avoidance learning: acquisition in normal
505 dogs. *Genetic Psychology Monographs* **67**, 1-19.

506 Starr, M.D., Mineka, S., 1977. Determinants of fear over the course of avoidance learning.
507 *Learning and Motivation* **8**, 332-350.

508 Sundström, L.F., Petersson, E., Höjesjö, J., Johnsson, J.I. & Järvi, T. (2004). Hatchery
509 selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for
510 dominance. *Behavioural Ecology* **15**, 192-198.

511 Thorpe, W.H. (1963). Learning and instinct in animals. 2nd edn. London: Methuen.

512 Vandeputte, M., Dupont-Nivet, M., Haffray, P., Chavanne, H., Cenadelli, S., Parati, K., Vidal,
513 M.-O., Vergent, A., Chatain, B. (2009). Response to domestication and selection for
514 growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks.
515 *Aquaculture* **286**, 20-27.

516 Ward, A.W., Thomas, P., Hart, P.B. & Krause, J. (2004). Correlates of boldness in three-
517 spined sticklebacks (*Gasterosteus aculeatus*). *Behavioural Ecology and Sociobiology* **55**,
518 561-568.

519 Wilson, D.S. (1998). Adaptive individual differences within single populations. *Philosophical*
520 *Transaction of the Royal Society of London B* **353**, 199-205.

- 521 Wilson, D.S., Clark, A.B. & Biederman, L. (1993). Shy bold continuum in pumpkinseed
522 sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. *Journal of*
523 *Comparative Psychology* **107**, 250-260.
- 524 Wilson, D.S., Clark, A.B., Coleman, K. & Dearstyne, T. (1994). Shyness and Boldness in
525 humans and other animals. *Trends in Ecology and Evolution* **9**, 442-446.
- 526 Wright, D., Rimmer, L.B., Pritchard, V.L., Krause, J. & Butlin, R.K. (2003). Inter and intra-
527 population variation in shoaling and boldness in the zebrafish (*Danio rerio*). *Naturwiss*
528 **90**, 374-377.
- 529 Yue, S., Moccia, R.D. & Duncan, I.J.H. (2004). Investigating fear in domestic rainbow trout,
530 *Oncorhynchus mykiss*, using an avoidance learning task. *Applied Animal Behaviour*
531 *Sciences* **87**, 343-354.

- 1 **Table I.** Mean (\pm SE) fish mass (M), length (L), specific growth rate (G) and body condition factor (K) for each strain and for each test and
 2 results of one way ANOVA used to analyse the mean differences between strains for each test.

Variables	Test 1			Test 2			Test 3		
	Selected (n=171)	Wild (n=173)		Selected (n=171)	Wild (n=173)		Selected (n=171)	Wild (n=173)	
M (g)	126.83 \pm 3.35	104.35 \pm 2.45	***	135.84 \pm 3.39	111.67 \pm 2.44	***	153.38 \pm 3.75	125.84 \pm 2.80	***
L (cm)	21.71 \pm 0.17	21.24 \pm 0.59	ns	22.21 \pm 0.17	21.13 \pm 0.14	***	23.08 \pm 0.17	21.94 \pm 0.14	***
G (% day ⁻¹)	-0.07 \pm 0.02	0.07 \pm 0.05	*	0.34 \pm 0.02	0.23 \pm 0.02	***	0.33 \pm 0.04	0.43 \pm 0.01	*
K (g cm ⁻³)	1.19 \pm 0.01	1.15 \pm 0.01	***	1.20 \pm 0.01	1.16 \pm 0.01	***	1.21 \pm 0.09	1.16 \pm 0.01	***

- 3
 4 Level of significance: * p < 0.05, ** p < 0.01, *** p < 0.001 and ns means no significant value.

5 **Table II.** Results of repeated ANOVAs and Newman and Keuls tests used to analyse the average differences between strain (fixed factor), day
6 and night period (fixed factor repeated within test), tests (fixed factor) and tanks (random factor nested within strain) for each variable.
7 Abbreviations are as follows: W: *Wild*; S: *Selected*; T1: Test 1; T2: Test 2; T3: Test 3; N: Night; D: Day.
8

<i>Source</i>	<i>Total time spent by a fish in the risk zone</i>				<i>Number of fish passages per hour through the opening</i>			
	df	F	P > F	Newman and Keuls	df	F	P > F	Newman and Keuls
Strain	1 & 2028	0.08	>0.05	ns	1 & 2028	2.58	>0.05	ns
Period (test)	3 & 2028	34.96	<0.001	*	3 & 2028	51.65	<0.001	*
Test	2 & 2028	676.5	<0.001	*	2 & 12028	427.31	<0.001	*
Tank (strain)	4 & 2028	15.93	<0.001	Tank differences	4 & 2028	71.03	<0.001	Tank differences
Strain x Period (test)	3 & 2028	0.22	>0.05	ns	3 & 2028	0.19	>0.05	ns
Strain x Test	2 & 2028	31.55	<0.01	W > S at T1 & T2 W < S at T3 T1 < T3 < T2 for W T1 < T2 < T3 for S	2 & 2028	49.12	<0.001	W = S at T1 W > S at T2 W < S at T3 T1 < T2 = T3 for W T1 < T2 < T3 for S
Test x Period	2 & 2028	4.37	<0.05	N = D at T1 N > D at T2 & T3	2 & 2028	12.71	<0.001	N = D at T1 N > D at T2 & T3

<i>Source</i>	<i>Time spent by a fish in the risk zone at each visit</i>				<i>Latency before the first entry by a fish in the risk zone</i>			
	df	F	P > F	Newman and Keuls	df	F	P > F	Newman and Keuls
Strain	1 & 1270	0.0002	>0.05	ns	1 & 1014	0.23	>0.05	ns
Period (test)	3 & 1270	10.27	<0.01	D > N				
Test	2 & 1270	18.86	<0.001	*	2 & 1014	822.34	<0.001	*
Tank (strain)	4 & 1270	12.08	<0.001	Tank differences	4 & 1014	71.27	<0.001	Tank differences
Strain x Period (test)	3 & 1270	0.79	>0.05	ns				
Strain x Test	2 & 1270	7.89	<0.01	W = S at T1 & T3 S > W at T2 T1 > T2 = T3 for W T1 > T2 > T3 for S	2 & 1014	31.56	<0.001	W = S at T1 W < S at T2 W > S at T3 T1 > T3 > T2 for W T1 > T2 > T3 for S
Test x Period	2 & 1270	0.33	>0.05	ns				

9 The * means these tests are not valid as interaction are significant. For all tests, significant threshold was p< 0.05.

10 **Table III.** Canonical correlation coefficients between dependent variables (*e.g.* the number of fish passages per hour through the opening (Np)
 11 and the individual score emergence (Se)) and independent variables (*e.g.* fish mass (M), length (L), specific growth rate (G), body condition
 12 factor (K), and the number of individual feed demand (F)) for each strain and for each test.

Variables	Selected						Wild					
	Test 1 (n=171)		Test 2 (n= 171)		Test 3 (n=171)		Test 1 (n=173)		Test 2 (n= 173)		Test 3 (n=173)	
	Np	Se	Np	Se	Np	Se	Np	Se	Np	Se	Np	Se
M	-0.844	-0.577	0.857	-0.288	0.258	0.002	-0.227 *	0.073	-0.523	0.159	0.236	-0.881
L	0.903	0.651	-0.580	0.374	0.023	0.132	0.090	-0.059	0.590	-0.375	-0.082	0.743
G	0.238 **	0.247 **	0.114	0.074	0.108	-0.036	0.051	0.122	0.022	0.060	0.131	0.159
K	-0.024	0.004	-0.290	-0.109	-0.015	-0.007	0.244	-0.112	0.252	0.013	-0.189	0.136
F	-0.037	-0.017	0.148 *	0.131	0.196 **	0.199 **	0.488 ***	0.075	0.088	0.091	0.046	0.000

13
 14 Canonical correlation coefficients are given with p-value and the number of individuals (n).
 15 Level of significance: * p < 0.05, ** p < 0.01, *** p < 0.001.
 16

1 **Figure captions**

2

3 **Figure 1.** Mean (\pm SE) total time spent by a fish in the risky zone (%) during day period
4 (undotted) and night period (dotted) for each strain (*Selected* in white and *Wild* in grey) and
5 for each test. Symbols (*) indicate significant differences between strains (repeated ANOVA
6 and Newman & Keuls test, ** $p < 0.01$).

7

8 **Figure 2.** Mean (\pm SE) number of fish passages per hour through the opening during day
9 period (undotted) and night period (dotted) for each strain (*Selected* in white and *Wild* in
10 grey) and for each test. Symbols (*) indicate significant differences between strains (repeated
11 ANOVA and Newman & Keuls test, *** $p < 0.001$; NS, no significant value).

12

13 **Figure 3.** Mean (\pm SE) time spent by a fish in the risky zone at each visit (min) during day
14 period (undotted) and night period (dotted) for each strain (*Selected* in white and *Wild* in
15 grey) and for each test. Symbols (*) indicate significant differences between strains (repeated
16 ANOVA and Newman & Keuls test, ** $p < 0.01$; NS, no significant value).

17

18 **Figure 4.** Mean (\pm SE) latency before the first entry of a fish in the risky zone (min) for each
19 strain (*Selected* in white and *Wild* in grey) and for each test. The white parts on the Y-axis
20 represent day period and the black one represents night period. Symbols (*) indicate
21 significant differences between strains (repeated ANOVA and Newman & Keuls test, ***
22 $p < 0.001$; NS, no significant value).

23

24

25

Figure 1
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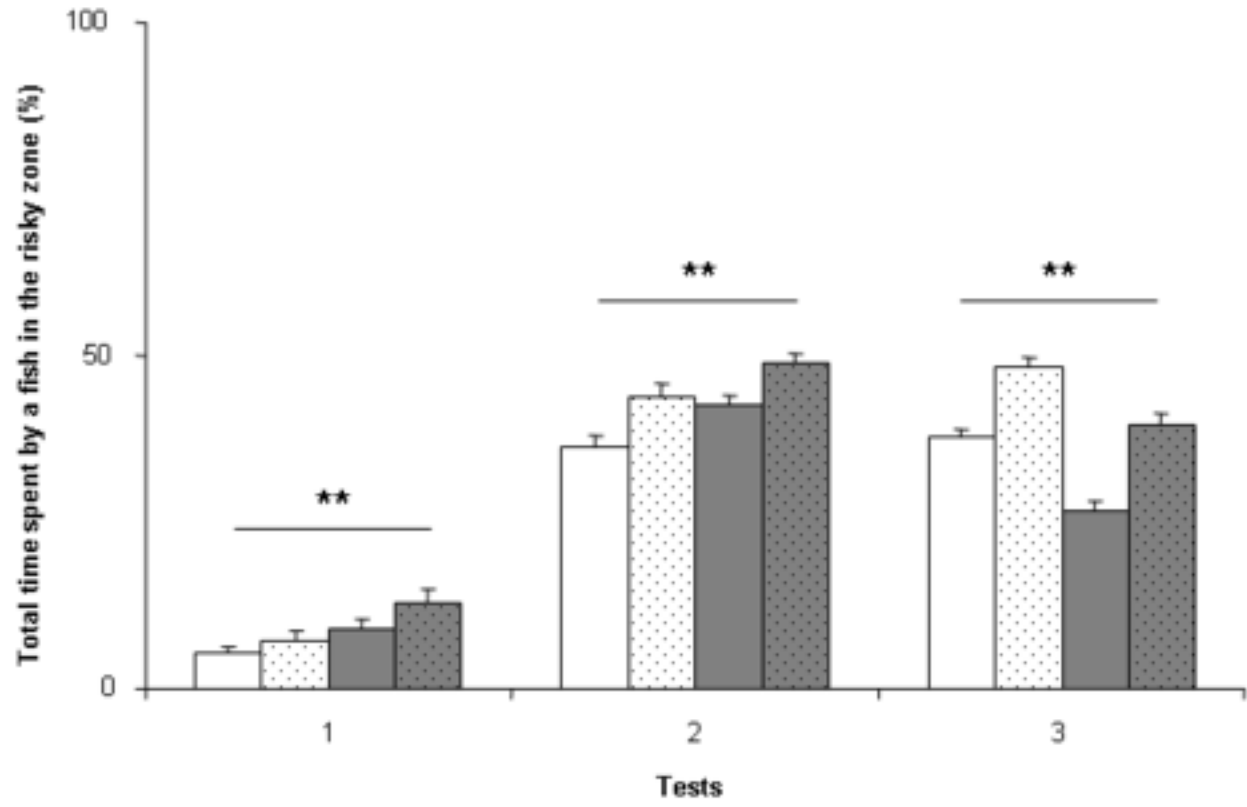


Figure 2
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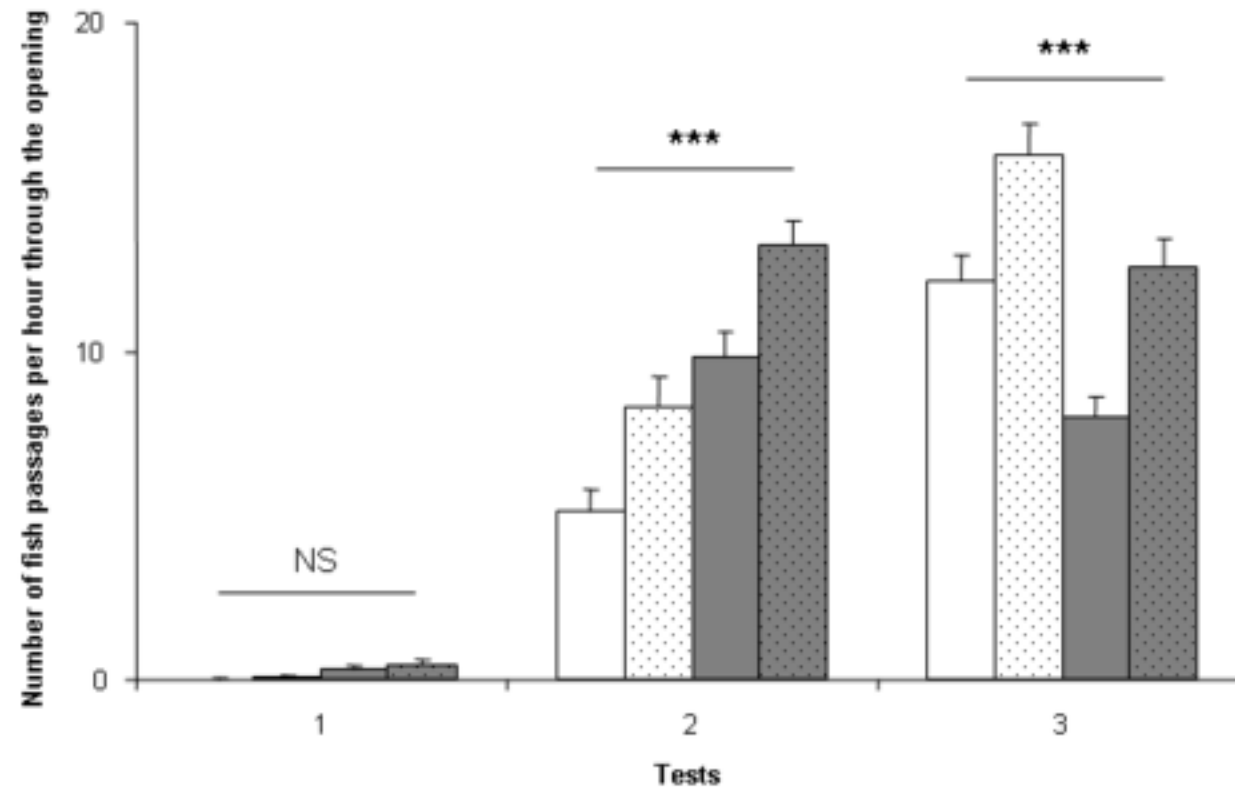


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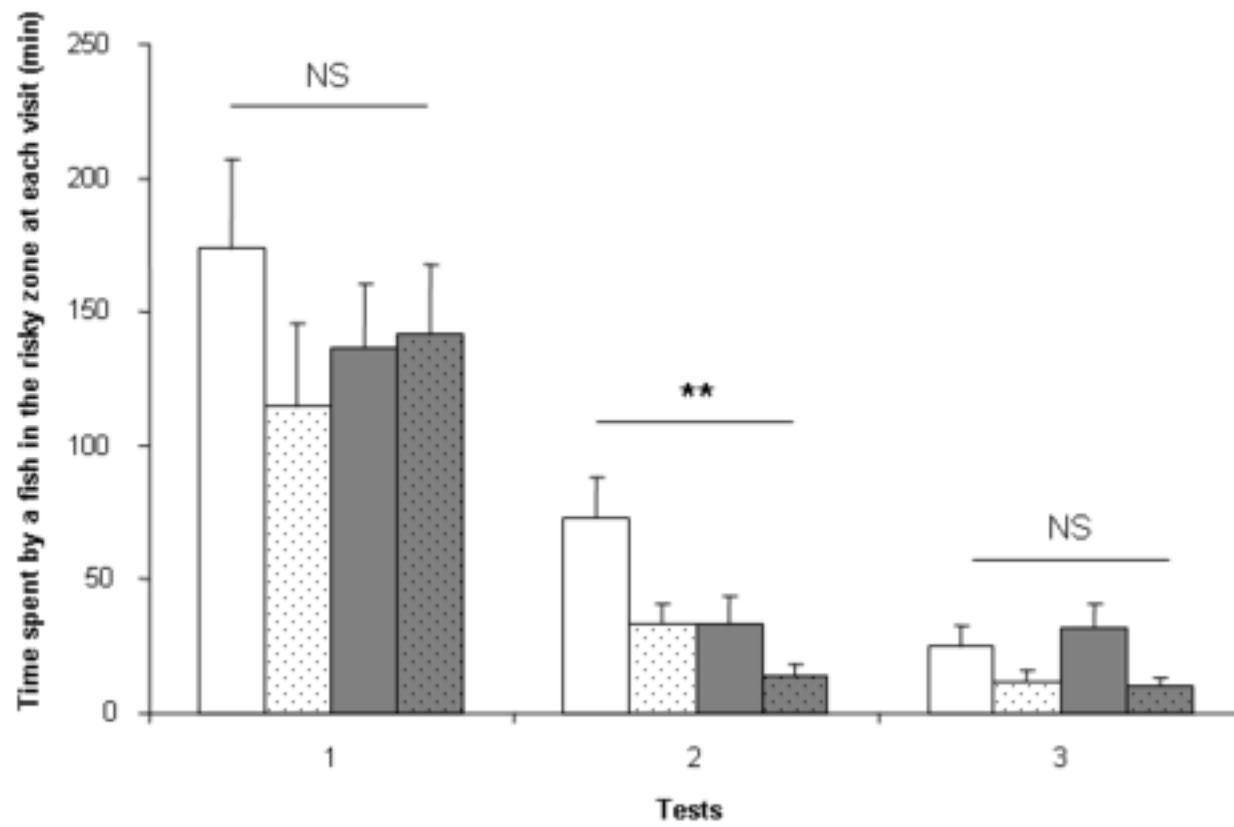


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