Aggressiveness-related behavioural types in the pearly razorfish

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Behavioural types (i.e., personalities or temperament) are defined as among individual differences in behavioural traits that are consistent over time and ecological contexts. Behavioural types are widespread in nature and play a relevant role in many ecological and evolutionary processes. In this work, we studied for the first time the consistency of individual aggressiveness in the pearly razorfish, *Xyrichtys novacula,* using a mirror test: a classic method to define aggressive behavioural types. The experiments were carried out in semi-natural behavioural arenas and monitored through a novel Raspberry Pi-based recording system. The experimental set up allowed us to obtain repeated measures of individual aggressivity scores during four consecutive days. The decomposition of the phenotypic variance revealed a significant repeatability score (*R)* of 0.57 [0.44 - 0.60], suggesting high predictability of individual behavioural variation and the existence of different behavioural types. Aggressive behavioural types emerged irrespective of body size, sex and the internal condition of the individual. Razorfishes are a ubiquitous group of fish species that occupy sedimentary habitats in most shallow waters of temperate and tropical seas. These species are known for forming strong social structures and playing a relevant role in ecosystem functioning. Therefore, our work provides novel insight into an individual behavioural component that may play a role in poorly known ecological and evolutionary processes occurring in this species.

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13 **Abstract:**

14 Behavioural types (i.e., personalities or temperament) are defined as among individual

15 differences in behavioural traits that are consistent over time and ecological contexts.

16 Behavioural types are widespread in nature and play a relevant role in many ecological and

17 evolutionary processes. In this work, we studied for the first time the consistency of individual

18 aggressiveness in the pearly razorfish, *Xyrichtys novacula,* using a mirror test: a classic method

19 to define aggressive behavioural types. The experiments were carried out in semi-natural

20 behavioural arenas and monitored through a novel Raspberry Pi-based recording system. The

21 experimental set up allowed us to obtain repeated measures of individual aggressivity scores

22 during four consecutive days. The decomposition of the phenotypic variance revealed a

23 significant repeatability score (*R)* of 0.57 [0.44 - 0.60], suggesting high predictability of

24 individual behavioural variation and the existence of different behavioural types. Aggressive

25 behavioural types emerged irrespective of body size, sex and the internal condition of the

26 individual. Razorfishes are a ubiquitous group of fish species that occupy sedimentary habitats in

27 most shallow waters of temperate and tropical seas. These species are known for forming strong

28 social structures and playing a relevant role in ecosystem functioning. Therefore, our work

29 provides novel insight into an individual behavioural component that may play a role in poorly

30 known ecological and evolutionary processes occurring in this species.

32 **Key-words:** Consistent individual differences, repeatability, mirror test, *Xyrichtys novacula,*

33 Mediterranean, animal personality.

1. INTRODUCTION

Like humans, animals often behave in a way that distinguishes them from other members of their species of the same sex and age (Gosling, 2001). When such behavioural differences are consistent over time and ecological contexts, they can be referred to as behavioural types, also known as personalities or temperament (Réale et al., 2007). Behavioural types often co-vary, resulting in behavioural syndromes that are defined as correlations in two or more behavioural traits (Sih, Bell & Johnson, 2004). Behavioural types and syndromes are widespread across taxa (Réale et al., 2007). In fishes, behavioural types are described along five major axes: boldness-shyness, exploration-avoidance, aggressiveness, sociability and activity (reviewed in Réale et al., 2007 and Conrad et al., 2011). The aggressiveness axis has received major attention due to its relationship with a multitude of ecological processes such as growth or dominance in a social hierarchy. However, the study of aggression *per se* is a complex trait to measure. Since aggression is a way of socialising, individual characterisation of both behavioural axes can be challenging. Strictly, aggressiveness has been defined as a hostile behaviour towards conspecifics (McGhee & Travis, 2010), and is usually related to agonistic interactions such as territorial defence or competition for resources (Réale et al., 2007; Conrad et al., 2011). Under controlled (laboratory) conditions, different tests have been used to measure aggressiveness, the mirror test being the most common and accepted practice.

The mirror test consists of placing a mirror in front of the focal individual and measuring its behavioural response (Francis, 1990; Budaev, Zworykin & Mochek, 1999). Although the mirror test has been widely used to estimate aggressive behaviour across animal taxa, considerable controversy still exists about its validity in fish aggressiveness studies (Baenninger, 1968;

on

58 Earley, Hsu & Wolf, 2000; Balzarini et al. 2014). In this taxon, the mirror test has been used to

59 measure sociability (Cattelan et al. 2017); however, aggressive displays are a particular kind of

60 social interactions.

61

62   Another way to measure aggressiveness is by the opponent test, which consists of putting

63 an intruder in the experimental arena and measuring the response of the focal individual. Some

64 authors consider the opponent test as the only valid way to evaluate the aggressive response

65 (Balzarini et al., 2014). In the opponent test, the reaction of the focal individual responds to the

66 actions of the opponent in a manner that the focal intrinsic aggressiveness is not directly studied

67 (Earley, Hsu & Wolf, 2000). When the dominance status between focal and opponent is

68 established, the opponent's position in the dominance hierarchy can be a source of potential

69 problems. The dominance status will influence the response of the focal individual in an

70 unknown way, making it difficult to obtain aggression levels that can be compared across trials

71 and between individuals (Earley, Hsu & Wolf, 2000). In contrast, the mirror test, by showing an

72 "intruder" displaying exactly the same behaviours than the focal individual, provides with a

73 constant reinforcement of aggressiveness and an unmodified image of a conspecific (Gallup,

74 1968; Rowland, 1999). Furthermore, studies in the past have shown that the individual

75 aggressiveness estimated with the mirror test can predict the aggressiveness in competitive

76 situations (Baenninger, 1968; Dore, Lefebvre & Ducharme, 1978; Meliska, Meliska & Peeke,

77 1980; Holtby, Swain & Allan, 1993). These studies support the mirror test as a proper method to

78 measure individual aggressiveness and provide empirical evidence showing that the results from

79 this test are comparable to the behavioural responses in free-living conditions. However, this test

80 needs to be adapted to the characteristics of the studied species. The most important aspects to

N

81 consider are whether the focal individual responds to the stimulus (Rowland, 1999) and if it is

82 capable of self-recognition in front of a mirror (Balzarini et al., 2014). Most animal taxa,

83 including fishes, are suspected to treat their mirror reflection as a conspecific (Andrews, 1966;

84 Gallup, 1968; Kohda et al. 2019).

85

86 Aggressiveness-related behavioural types have important implications from the individual level

87 to the whole population dynamics (Sih, Bell & Johnson, 2004). Aggressiveness is positively

88 related to growth (Huntingford, 1998; Lahti et al., 2001) and metabolic rate (Cutts, Metcalfe &

89 Taylor, 2002), which depend on the internal state of the individual. The relative condition index

90 (RCI) represents the fish internal state condition, and it is based on predicted body weight from a

91 length/weight relationship regardless of size. The RCI has been related to maturation and

92 reproduction of individuals (Morgan, 2004). Individual behavioural responses may modulate

93 internal energetic fluxes. For example, high energy requirements can imply high levels of

94 hunger, which can lead to aggressive displays to compete for food resources (Careau et al.,

95 2008). Furthermore, testosterone regulates agonistic behaviours in males (Hirschenhauser et al.,

96 2008), suggesting that aggressiveness and dominance in a social structure play an important role

97 in sexual selection and reproductive success (McGhee & Travis, 2010). In zebrafish (*Danio*

98 *rerio*) and crayfish (*Paranephrops planifrons*), dominant individuals are the most aggressive

99 (Roy & Bhat, 2018;May & Mercier, 2007). This dominant position is sometimes related to size

100 or sex. For example, in green swordtail (*Xiphophorus helleri*), larger individuals are more

101 aggressive than smaller individuals (Wilson et al., 2011), while in the Midas cichlid

102 (*Amphilophus citrinellus*) males are more aggressive than females (Francis, 1990). Besides,

103 behavioural types are of most importance to understand the colonisation process by invasive

104   species. Aggressive non-native fish are more likely to disperse from the original introductory

105   group and to better compete with native species, thereby improving the success of the invasion

106   (Rehage & Sih, 2004). These individuals are also the most likely to leave their native population

107   to explore new environments and compete for new territories (Cote et al., 2010). Behavioural

108   types are also essential to understand the responses to intrusions among individuals of the same

109   population because the most aggressive ones will defend their territory more efficiently

110   (O'Connor et al., 2015). Therefore, aggressiveness is an important axis of behaviour significantly

111   affecting the ecology and evolution of many species (Taylor, 1990; Vøllestad & Quinn, 2003).

112

113   In marine species, the study of aggressiveness has received less attention. Aggressiveness plays a

114   fundamental role in the mating behaviour of Two-spotted goby (*Gobiusculus flavescens*) (de

115   Jong et al., 2009). In Atlantic cod (*Gadus morhua*), there is a relationship between dominance

116   and fertilisation success, with dominance being established through agonistic interactions

117   between males, bigger individuals are the most aggressive, and therefore dominant over smaller

118   individuals (Hutchings, Bishop & McGregor-Shaw, 1999). Furthermore, the existence of

119   aggressiveness-related behavioural types has been confirmed in European seabass

120   (*Dicentrarchus labrax*), (Millot et al., 2014).

121

122   Razorfishes are a ubiquitous group of fish species that inhabit most of the shallow sedimentary

123   habitats of tropical and temperate seas (Nemtzov & Clark, 1994; Battaglia et al., 2010). Socially,

124   they are characterised by a complex haremic structure formed by a male and several females (4-6

125   females per male). The male defends his territory and the females that live in it from other males

126   (Marconato, And & Marin, 1995; Cardinale, Colloca & Ardizzone, 1998; Shen & Clark, 2016).

127   However, nothing is known on how behavioural types could be playing a role in the formation of

128   harems and other aspects of the social structure of this marine species. The pearly razorfish

129   (*Xyrichtys novacula*), is the only species of the genus *Xyrichtys* found in the Mediterranean Sea

130   (Candi et al., 2004). This species is a small-bodied wrasse that inhabits clear shallow waters with

131   sandy bottoms and sometimes areas covered by the seagrasses *Cymodocea nodosa* and *Zostera*

132   *marina* (Castriota et al., 2005; Battaglia et al., 2010). This species is distributed at depths

133   between 0 and 50 m (Fischer, Schneider & Bauchot, 1987). It is a diurnal species and it buries

134   itself in the sand to rest and to avoid predators during the night-time (Alós, Cabanellas-Reboredo

135   & Lowerre-Barbieri, 2012; Alós, Martorell-Barceló & Campos-Candela, 2017). The pearly

136   razorfish has a carnivorous diet based on small benthic invertebrates, which differs depending on

137   the area but basically consists of molluscs and crustaceans and occasionally of echinoderms and

138   polychaetes (Cardinale, Colloca & Ardizzone, 1997; Castriota et al., 2005; Battaglia et al., 2010).

139

140   The pearly razorfish is a protogynous, monandric hermaphrodite. It has an appreciable sexual

141   dimorphism showing differences in head shape, length of pelvic fin and colouration patterns,

142   with females presenting a pearly spot on the abdomen (Cardinale, Colloca & Ardizzone, 1998;

143   Candi et al., 2004). In the pearly razorfish, aggressiveness plays a fundamental role in the

144   establishment and maintenance of harems. Males are highly territorial and defend their harem

145   from other males in contiguous territories; male attacks against females have not been detected

146   (Shen & Clark, 2016). The reproductive period of this species takes place during the summer

147   (July to September), and only dominant males participate in spawning (Marconato, And &

148   Marin, 1995; Cardinale, Colloca & Ardizzone, 1998). Despite the importance of this behavioural

149   trait, the individual repeatability for aggressiveness has never been explored in this species.

150 Therefore, the pearly razorfish offers a unique opportunity to understand how an individual's

151 aggressiveness can determine its position in a complex social system.

152

153 The main objective of this study is to quantify for the first-time the aggressiveness in the pearly

154 razorfish and to determine the existence of behavioural types in this species. We determined the

155 repeatability of aggressiveness using metrics obtained under laboratory conditions applying a

156 standardised mirror test. We explored the effects of body size, sex, individual condition and the

157 effect of captivity and isolation in the expression of aggressiveness using generalised linear

158 mixed models and the decomposition of the phenotypic variance into between- and within-

159 individual components.

160

161 Our working hypotheses were i) individual aggressiveness is predictable – aggressiveness-related

162 behavioural types exist, ii) aggressiveness levels are higher in males and larger individuals (as a

163 response to an increased territorial defence), and iii) aggressiveness is negatively correlated with

164 the individual condition, as aggressive individuals spend more energy in antagonistic interactions

165 to protect their territory. Our work represents the first study on behavioural types in razorfishes,

166 providing the first step to understand the causes and consequences of individual behaviour in this

167 marine fish group.

168

169 **2. MATERIAL AND METHODS**

170   2.1. Origin of the experimental fish

171 From April 30th to July 12th 2019, a total of 6 weekly sessions of experimental fishing were

172 carried out in shallow waters (10-15 m deep) of the Marine Protected Area in the Palma Bay,

173   Mallorca (39º27'56" N 02º43'37" E). Twelve fish were caught during each experimental fishing

174   session (this number was limited by the available experimental arenas, see below). Each fishing

175   session was carried out during the morning, from 10:00 to 14:00, using standardised hook-and-

176   line gear and live shrimp as bait (a classical capture method used by local recreational anglers).

177   Fish with deep-hooking (i.e., hooks reaching the internal/vital parts of the body) were not used

178   for this behavioural study to ensure that all individuals presented the least disturbance associated

179   with the capture method. Once 12 individuals were captured (after up to 1 hour of sampling), fish

180   were transported in an aerated 80 l container with a constant oxygen flux from the capture site to

181   the Marine Research and Aquaculture Laboratory (LIMIA) in Andratx, Mallorca (39º32'39" N

182   02º22'42" E). At the LIMIA, fish were individually housed in the experimental behavioural

183   arenas for posterior behavioural testing (Figure 1). Neither mortalities nor stress (assessed

184   through visual inspection of the body) due to captivity and transport was observed.

185

186   2.2.   Characteristics of the behavioural arenas

187   The present study constitutes the first attempt to maintain the pearly razorfish in social isolation.

188   The study of behavioural types in the pearly razorfish constitutes a new challenge because this

189   species requires specific attributes of the experimental arenas to simulate its natural environment.

190   In natural conditions, the pearly razorfish buries in the sand at night to avoid predators (Alós,

191   Cabanellas-Reboredo & Lowerre-Barbieri, 2012; Alós, Martorell-Barceló & Campos-Candela,

192   2017), and thus it is necessary to include the appropriate substrate in the experimental arenas to

193   provide this particular type of shelter. We developed several prototypes of behavioural arenas

194   before carrying out the experimental trials. The optimal behavioural arena was composed by an

195   aquarium of 120 l with closed UV-purified seawater re-circulation that allowed for complete

196 control of light and temperature (see Figure 1). Each aquarium had its sump station where a

197 protein skimmer, bio-media for biological filtration and aeration maintained the optimal

198 conditions during the week (Figure 1). Each behavioural arena had the same quantity of sand (20

199 kg), which generated a ~5 cm high accumulation of sand at the bottom with a precise

200 granulometry (0.5 – 12 mm) simulating the pearly razorfish habitat, allowing the fish to use the

201 sand as refuge.

202

203 The closed water system facilitated the control of environmental variables (such as temperature)

204 and facilitated the maintenance of the aquarium throughout the experimental period. The sand,

205 filters and the aquarium walls were cleaned and the water was changed before a new individual

206 was introduced in the behavioural arena. The temperature of each behavioural arena was

207 controlled by a water heater located in the sump of each aquarium, and it was programmed to

208 remain constant at 21°C (mean and s.d. of the water temperature for all experiments was 21.1 ±

209 1.8°C). We used a light screen EHEIM powerLED + installed at the top of each behavioural

210 arena to reproduce the natural conditions of light/dark cycle, which was automatically controlled

211 with an EHEM LEDcontrol +. We reproduced a natural photoperiod of 12 hours of light and 12

212 hours of dark in which the sunrise was set at 07:00 and the sunset at 19:00. In this way, we

213 maximised the same environmental conditions to all individuals, an essential requirement to

214 study behavioural repeatability (Réale et al., 2007).

215

216 From all individuals captured, we only considered the ones from which we had at least two days

217 of experimental testing recordings. Due to some initial problems with the video generation and

218 storage of the novel tracking system (see below), we had to discard data from some individuals.

219   For this study, we used the data from 49 wild pearly razorfish (37 females and 12 males) with an

220   average total length (TL) of 15.2 ± 2.4 cm and total weight (TW) of 42.80 ± 20.8 g (Figure 2).

221   Pearly razorfish were visually sexed as this species shows sexual dimorphism. Significant

222   differences in both TL and TW were observed between sexes as expected for a protogynous

223   hermaphrodite. Each individual was isolated in an experimental arena for one week (three days

224   for acclimation and four days for behavioural testing) and was assigned an individual identifier

225   number (ID). During this period, the individuals were fed 1 g of fresh shrimps per day. We were

226   able to observe that the behaviours recorded in the aquarium were the same as those recorded in

227   the sea, slow swimming, they buried themselves at night, or when scared, and they usually ate

228   every day, suggesting that we succeeded in providing suitable captive habitat for this species.

229

230   2.3.   Video recording for data collection

231   During the week in captivity, all individuals were continuously monitored (video recorded in 2-

232   dimensions) using a camera set-up attached in front of each behavioural arena (Figure 1). The

233   camera set-up was based on a Raspberry Pi system equipped with an 8MP Raspberry Pi Camera

234   Module V2 with a Sony image sensor (Figure 1). The small camera allowed to record videos at

235   1080 pixels with a framerate of 30 frames per second. A 128GB USB stick was connected to the

236   Raspberry Pi to store all the generated videos. A screen was attached to the Raspberry Pi where

237   the recordings could be played confirming the correct functioning of the system. This recording

238   system has proven useful for behavioural experiments (Jolles et al., 2019). Overall, we provide a

239   new experimental protocol that allows for the monitoring of individual razorfish under laboratory

240   conditions without disturbances (cameras were permanently mounted and recording) that can be

241   used for behavioural quantification in other species.

242

243   During the experimental period, approximately 6900 h of recording were obtained. The

244   advantages of using the continuous recording for behavioural scoring are, i) raw data is

245   generated and adequately stored for reproducibility analysis, ii) fish are not unnecessarily

246   disturbed as the camera remains installed during the entire captive period, iii) it provides with the

247   opportunity to measure behaviour in real-time and, iv) it provides with the raw data for further

248   automatization of behavioural scoring through machine learning algorithms.

249

250   2.4.   Standardised aggressiveness test

251   After the acclimation period (first three days), the individual behavioural assessments using the

252   conventional mirror test started (Balzarini et al., 2014; Way et al., 2015). Each fish was subject

253   to one test per day for four consecutive days. During testing, a mirror of the same size as the

254   lateral side of the behavioural arena was externally attached to each aquarium for one hour

255   during the daytime. Three different metrics were visually evaluated from the videos to estimate

256   individual aggressiveness: i) the number of bites, defined as the number of times the individual

257   bit the mirror, it was considered a bite when fish approached the mirror with their mouth open

258   (Francis, 1990; Budaev, Zworykin & Mochek, 1999; Bell & Stamps, 2004; Bell, 2005; Scotti &

259   Foster, 2007; Snekser et al., 2009), ii) the number of rams, described as a fast approach with

260   physical contact to the mirror but with the mouth closed (Balzarini et al., 2014) and, iii) the

261   number of charges, described as a fast swim towards the mirror but without direct contact with it

262   (Scotti & Foster, 2007; Bell, Henderson & Huntingford, 2010; Ryan White & White, 2015). We

263   were not able to obtain the four tests for all individuals due to problems with the storing of

264   audio-visual material, but for 49 individuals a minimum of two measurements were obtained (see

265   sample size in Figure 2).

266

267   2.5.   Data analysis

268   The degree of consistency in individual behaviour is usually quantified with the repeatability (R)

269   score (Nomakuchi, Park & Bell, 2009). The R score describes the proportion of phenotypic

270   variance that is explained by among-individual differences by decomposing between- and

271   within-individual variance thus defining behavioural types (Dingemanse & Dochtermann, 2013).

272   The raw R score was computed as the quotient between the between-individual variance ($Vind_0$,

273   the variance across random intercepts of individuals) and the within-individual variance (Ve_0,

274   residual variance, the variance associated with measurement error and phenotypic flexibility) for

275   a given behavioural score (Nakagawa & Schielzeth, 2010; Dingemanse & Dochtermann, 2013).

276   We used a Generalised Linear Mixed Model (GLMM) to decompose variances to estimate R and

277   explore contextual effects using a Bayesian approach (Hadfield, 2010). To use a single

278   aggressiveness score in the model, we carried out a Principal Component Analysis (PCA) with

279   the three behavioural metrics (number of bites, number of rams and number of charges) at their

280   original scale (counts).We considered the values of the Principal Component 1 (PC1) as

281   aggressiveness score for fitting the GLMM (Figure 3).

282

283   Regarding the contextual variance and the structure of the fixed part of the GLMM, we

284   considered three variables: body size (related to sex), RCI and experimental day. Considering the

285   effects of these variables, we estimated the adjusted-R scores −adjusted R after controlling for

286   the confounding fixed effects (Dingemanse & Dochtermann, 2013). The sequential

287 hermaphroditism of the pearly razorfish inevitably implies that body size and sex are correlated,

288 creating a co-linearity conflict in our GLMM. We selected the fish body size (in cm) as a

289 continuous variable to adjust the GLMM, but the results are discussed as the body size / sexual

290 effect. We computed the RCI, a measure of the internal state of the individual, as the ratio

291 between the observed fresh weight (in g) and the predicted weight from an independently

292 estimated length-weight relationship for the species (Battaglia et al., 2010) following the

293 protocol in Morgan, (2004). The RCI was preferred because, in contrast to Fulton's condition

294 index, it is independent of body size (Morgan, 2004). Finally, the experimental day (an integer

295 number from 1 to 4, assigned depending on the actual experimentation date) was included in the

296 model to control for potential effects of captivity (e.g., acclimation effect). The three variables

297 were scaled and mean-centred as they represented factors in very different units. The fixed

298 structure of the full model (considering all variables) was reduced using bidirectional elimination

299 (i.e. 'step-by-step' backward reduction, which is a combination of the backward and forward

300 stepwise selection) until the lowest Deviance Information Criteria (DIC) was attained (we

301 assumed a cut-off of two points in the process).

302

303 The full GLMM considered a random structure based on a nested variable attributable to the

304 individual nested in experimental weeks (as described in the experimental protocol). The

305 reduction of the model was based on bidirectional elimination, the same strategy used for the

306 fixed structure of the GLMM. The parameters and the Bayesian Credibility Intervals (BCI, 2.5%

307 and 97.5%) of the LMMs were estimated using a Bayesian approach with the default settings

308 from the library MCMCglmm (Hadfield, 2010). In all cases, convergence of the chains was

309 attained and checked by plotting the distribution of the residuals. A transformation (natural

310   logarithm) was applied to the cases where the normality of the residuals was not fulfilled. We

311   used the reduced GLMM to compute adjusted-R scores for each fish. The BCI of the adjusted-R

312   scores was interpreted to detect the presence of behavioural types. A likelihood ratio test (LRT)

313   was used to calculate the significance of the adjusted-R. According to the LRT, the reduction in

314   the DIC (ΔDIC) provided by the GLMM where $Vind_0$ was constrained to 0 was used to detect

315   significant $Vind_0$. Adjusted-R scores were considered significant when comparing the

316   unconstrained GLMM to the constrained GLMM resulted in DIC reductions larger than 2. To

317   check if there was an effect of TL within the same sex, we separately analysed the data

318   corresponding to the females (n = 37) following the same protocol explained above. We could

319   not do this for the males due to the small sample size (n = 12).

320

321   2.6.   Ethical notes

322   This study was positively evaluated by the Ethical Committee for Animal Experimentation of the

323   University of the Balearic Islands (references for the protocols CEEA 97/0718 and CEEA

324   107/01/19), and authorised by the Animal Research Ethical Committee of the Conselleria

325   d'Agricultura, Pesca i Alimentació and the Direcció General de Pesca i Medi Marí of the

326   Government of the Balearic Islands (reference for the authorisations ES070050000502 and

327   2019/20/AEXP).

328

329   **3. RESULTS**

330   **3.1.   Sample size and general results**

331   Aggressiveness was successfully scored (at least twice) for all 49 individuals. In all tests, the

332   three metrics for aggressiveness (number of bites, number of rams and number of charges) were

333   successfully quantified. The first two components of the PCA applied to the three

334   aggressiveness' metrics are shown in Figure 3. The PC1 explained 87.6% of the total variance,

335   showing a correlation betwenn the three variables (Figure 3). We therefore selected the PC1

336   scores as a unique measure for aggressiveness. The aggressiveness score defined by the PC1 was

337   normalised to a natural number by multiplying by a factor of -1, so the higher scores described

338   the more aggressive individuals with larger number of bites, number of rams and number of

339   charges (Figure 3), and the lower scores described lower levels of antagonistic behaviours in

340   front of the mirror (Figure 3).

341

342   3.2.   Effect of contextual variables in the aggressiveness score

343   The estimates and confidence intervals (CI) for the effects of the contextual variance on the

344   aggressiveness score of the pearly razorfish are shown in Table 1. The GLMM suggests that

345   neither the body size nor the sex has a significant effect on the aggressiveness of the pearly

346   razorfish (Table 1 and Figure 4). Results were consistent in the female-only data set being the

347   scores of small and large females similar (Table 2 and Figure 5). The individuals presented a

348   mean ± s.d. RCI of 1.28 ± 3.65 (range of -9.53 to 13.07). The results regarding the RCI were also

349   non-significant in both GLMMs, suggesting that the condition of the fish was not related to its

350   aggressiveness score (Table 1). Finally, the effect of the experimental day was also non-

351   significant in both models, rejecting any effect of the days under laboratory conditions on

352   aggressiveness levels (Table 1). In both fitted GLMMs (complete and female-only data set), only

353   the ID of the individual (between-individual variance) was retained in the random structure of

354   the model according to the DIC optimisation, rejecting an among-week effect.

355

356   3.3.   Adjusted Repeatability and Bayesian Credibility Intervals

357   The aggressiveness scores for complete data set averaged 33.38 ± 91.15 (Figure 4), and their

358   adjusted-*R* score was 0.57 with a BCI of [0.44 - 0.60] (Table 1). For the female-only data set, the

359   average aggressiveness was 28.98 ± 85.38 (Figure 5), and their adjusted-*R* score 0.36 with a BCI

360   of [0.27 - 0.48] (Table 2). The fit and the DIC of the constrained (DICc) GLMM suggested that

361   this adjusted-*R* score could be considered significant in both cases, evidencing the existence of

362   aggressive and non-aggressive behavioural types (Table 1 and 2). The model shows that the *R*

363   was significant: DIC = 558.22 and DICc = 641.32 (for the complete data set. Table 1) and DIC =

364   481.33 and DICc = 446.62 (for the female-only data set, Table 2). This was reflected by a large

365   phenotypic variation ranging from individuals who bit on average 350 times per test (for

366   example, XN0035, Figure 4) and individuals that did not interact with the mirror (for example,

367   XN0047, Figure 4), independently of their sex and size.

368

369   **4. DISCUSSION**

370   This work is the first behavioural study considering captive razorfishes. Aggressiveness was

371   tested on 49 pearly razorfish and our results showed a *R* score for aggressiveness of 0.54 [0.48 -

372   0.61]. The *R* score was statistically significant, demonstrating that consistent differences among

373   individuals exist and corroborating the existence of aggressiveness-related behavioural types in

374   the pearly razorfish. Most importantly, assuming a *R* score of 0.54 (average of our values), 49

375   individuals (with an average of three experimental trials per individual) provided an accuracy

376   (root mean square error) of ~0.1 for estimating phenotypic behavioural variation, and a power

377   greater than ~0.8 for estimating behavioural syndromes following Dingemanse and Dochtermann

378   (2013). The variables considered in this study (body size and sex, RCI and experimental day) did

379   not affect on the aggressiveness score of the pearly razorfish. Therefore, we rejected our initial

380   hypotheses that males were more aggressive than females and that the most aggressive

381   individuals were those with the lowest RCI. These results suggest that aggressiveness is intrinsic

382   to the individual's life history, and that aggressiveness may be involved in different aspects of

383   the ecology and biology of the pearly razorfish. Moreover, the fast acclimatisation, the low-stress

384   levels observed, and the fact that the individuals responded to the mirror, confirms that our

385   system is adequate to measure aggressiveness in pearly razorfish, making this species a

386   successful candidate for behavioural ecology studies in marine fish.

387

388   Our results suggest that the mirror test is an excellent methodology to measure aggressiveness in

389   the pearly razorfish, providing real measures of agonistic interactions in front of a conspecific

390   individual. The mirror test has been widely used in fish, with excellent results, which show the

391   optimisation of this test to measure aggressive behaviours (Francis, 1990; Grantner & Taborsky,

392   1998; Rowland, 1999; Budaev, Zworykin & Mochek, 1999; Vøllestad & Quinn, 2003; Pham et

393   al., 2012). Since fish, like many other taxa, do not recognise their reflection in the mirror

394   (Gallup, 1968) –at least if they are exposed to such stimulus for short periods of time as when

395   performing a behavioural test (Kohda et al., 2019)– the mirror test entails reinforced

396   aggressiveness given that it provides the continuous stimulation by an opponent (Gallup, 1968;

397   Rowland, 1999). Furthermore, the mirror test is also used in territoriality studies to determine

398   which male is in a better position to feed and reproduce. In our species, behaviours such as

399   territoriality and dominance of some males over others have been described (Scotti & Foster,

400   2007; Snekser et al., 2009; McGhee & Travis, 2010; Blowes, Pratchett & Connolly, 2013).

401   Overall, the mirror test has some advantages compared to other methodologies such as the

402   opponent test (Rhoad, Kalat & Klopfer, 1975). The number of experimental individuals used is

403   reduced to the focal individuals. This is particularly relevant in our study species because

404   individuals bury in the sand when they feel threatened, thus making the capture process within

405   the tank challenge. Placing the "opponent" and the focal individual side by side during the trials

406   would significantly increase the individuals' stress and anxiety, affecting on the results obtained

407   during the behavioural testing. Moreover, the opponent test requires an accurate selection of

408   opponent fish because size, sex and behaviour may influence the focal individual's response

409   (Earley, Hsu & Wolf, 2000; Wilson et al., 2011). If different opponents are used, it becomes

410   challenging to compare the results. Obtaining aggressiveness scores for the focal individuals

411   regardless of the opponent's characteristics would require a vast sample size to repeat the

412   experiment with multitude of different opponents.

413

414   From assessing aggressiveness in our population, we have obtained a relatively high R score

415   (0.54) compared with other studies. The average R across 98 species is 0.37 (Bell, Hankison &

416   Laskowski, 2009). Other studies, however, have reported higher R scores. In the blue catfish

417   (*Luciana godei*), an R score of 0.72 was obtained for aggressive behaviours (McGhee & Travis,

418   2010). In the zebrafish, specific R scores were recorded for different metrics of aggressiveness:

419   an R score of 0.39 was obtained for overall aggressive behaviours (Roy & Bhat, 2018), an R

420   score of 0.56 for the number of bites (Way et al., 2015), and a lack of repeatability was reported

421   for mirror tests experiments in zebrafish (Sbragaglia et al., 2019). These differences suggest that

422   the R score is sensitive to the selected aggressiveness metric. In our study, a PCA was performed

423   to obtain an aggressiveness score (PC1) that was the result of reducing the dimensions of our

424   measured variables. The PC1 explained 87.9% of the variability of the data set, and it was

425   mostly related to the number of bites (Figure 3), which seems to be the most relevant metric to

426   quantify aggressiveness at the individual level according to previous work (Way et al., 2015) and

427   our findings. Our results demonstrated that aggressiveness is a constant behaviour maintained

428   between individuals and overtime in the pearly razorfish (Table 1 and Figure 4 for the complete

429   data set; Table 2 and Figure 5 for female-only data set)

430

431   Differences in aggressiveness were not related to body size. Since the pearly razorfish is a

432   sequential hermaphrodite, size and sex are correlated, and thus the lack of effect should be

433   jointly interpreted. Other studies revealed a positive relationship between aggressiveness and size

434   in different species such as the lion-headed cichlid, (*Steatocranus casuarius*), (Budaev,

435   Zworykin & Mochek, 1999); the green swordtails, (Wilson et al., 2011) and the Midas cichlid,

436   (Francis, 1990). In general, it has been found that aggressiveness plays an important role in

437   social structure, where the most aggressive males have a higher position in the mating hierarchy

438   (McGhee & Travis, 2010). This could be the case of the pearly razorfish, a species that lives in

439   harems formed by a male and several females (Espino et al., 2015) and where there is intra-

440   sexual but not between-sexes competition. Male and female territories usually overlap,

441   suggesting that the shared space is used for reproduction (Shen & Clark, 2016). Likely, male

442   aggression is probably related to their position in the social structure and their resource holding

443   potential (RHP), defined as the ability to win a contest and maintain earned resources (Parker,

444   1974; Hurd, 2006). RHP depends on the size of the opponents, and the motivation, physiological

445   state and behaviour of the focal individual (Parker, 1974; Nijman & Heuts, 2000). Thus,

446   aggressiveness on pearly razorfish might be playing an essential role in the structure of the

447   population.

448

449   Our initial hypothesis that the RCI was negatively correlated with aggression was also rejected.

450   Individual behavioural responses may modulate internal energetic fluxes. Since aggressive

451   behaviours are energetically very demanding (Katano & Iguchi, 1996), highly aggressive

452   individuals may have a poor condition because they invest a lot of time and energy in

453   antagonistic behaviours (Grantner & Taborsky, 1998).–Most studies agree that aggressive

454   behaviours are energetically costly, and different internal growth rates, maturity stages and

455   metabolic rates are negatively related to aggressiveness (Cutts, 1998; Vøllestad & Quinn, 2003).

456   In the present study, we did not find a relationship between the RCI and the aggressiveness

457   score. This could be since metabolic levels may vary between seasons due to food availability,

458   while behaviour remains more constant over time (Vøllestad & Quinn, 2003; Bouwhuis et al.,

459   2014). We also considered the effect of the experimental day in our GLMM to control for

460   potential effects of captivity. Visually, individuals displayed normal behaviour after the three

461   days of acclimation. In the same way, fishes could have habituated to the mirror during the

462   experiments. Studies have shown that continuous exposure to stimuli over a series of consecutive

463   days can decrease the level of behavioural reactivity due to habituation. Long acclimatisation

464   could reduce fish activity levels (O'Neill et al., 2018), decrease exploration rates and increase

465   individual's boldness over time (Adriaenssens & Johnsson, 2011). The effect of acclimatisation

466   has also been reported on aggressiveness; in fact, the number of aggressive interactions can

467   decrease significantly across trials (Roy & Bhat, 2018). In the pearly razorfish, however, the

468   correlation between experimental day and aggressiveness score was not statistically significant,

469   rejecting any acclimation or habituation effect.

470

471   Our results suggest that aggressiveness is a significant individual intrinsic factor, and we can

472   derive some potential physiological and ecological consequences from the existence of

473   aggressiveness behavioural types in the pearly razorfish. Aggressiveness has been closely related

474   to growth and metabolic rates (Cutts, 1998; Vøllestad & Quinn, 2003), reproduction

475   (Hirschenhauser et al., 2008; Snekser et al., 2009; McGhee & Travis, 2010) and dominance in

476   social structures (Grantner & Taborsky, 1998; Earley, Hsu & Wolf, 2000; May & Mercier, 2007;

477   Pham et al., 2012; Blowes, Pratchett & Connolly, 2013). Therefore, aggressiveness could be

478   linked to the life history of the pearly razorfish affecting the individual fitness and its position

479   within its social group. Instead of expecting a difference in aggressiveness related to sex, size or

480   condition, we could expect differences in aggressiveness linked to the social structure of this

481   species. We could also expect aggressiveness to be related to the formation and maintenance of

482   individual territories. Following this idea, the most aggressive males would be the ones obtaining

483   the best feeding patches and the highest quality mates. Similarly, females could compete with

484   each other for the best males. These hypotheses deserve more attention and require the

485   combination of measurements of aggressiveness scores in the laboratory alongside with the

486   measurement of territory sizes in the field.

487

488   The relationship between dominance and aggressiveness extends across taxa (May & Mercier,

489   2007; Snekser et al., 2009). For instance, in salmonid species, the most aggressive individuals

490   have better competitive capabilities, making them dominant and monopolize high-quality

491   territories (Huntingford, 1998). Aggressive individuals defend high-quality territories since the

492   benefit obtained from this territory is greater than the cost of defending it (Bacon, Ball &

493   Blackwell, 1991; Stamps & Krishnan, 1999). Aggressiveness plays a crucial role in obtaining

494   and maintaining territories. Similarly, the attack of an intruder to obtain a territory will cease if

495   the benefit obtained is not enough (Stamps & Buechner, 1985; Stamps & Krishnan, 1999).

496   Animals' ability to achieve and defend their territories depends mostly on their behaviour

497   (Stamps, 1994; Dingemanse & Réale, 2005). Therefore, our work demonstrating the existence of

498   aggressive behavioural types provides a novel perspective on understanding the territorial

499   societies of the pearly razorfish.

500

501   Furthermore, it is necessary to understand how disturbances affect fish behaviour and

502   populations (Biro, Post & Booth, 2007; Biro, Beckmann & Stamps, 2010). The study of

503   behavioural types is essential to understand ecological patterns and population dynamics (Conrad

504   et al., 2011; Sih et al., 2012). Intraspecific variability in fish behaviour is closely related to the

505   resilience of populations to environmental disturbances (e.g. climate change) (Wolf et al., 2007).

506   It has been shown that anthropic disturbances such as fishing have important effects on

507   behavioural types (Biro et al., 2007, Biro et al., 2010). Fishing is one of the biggest drivers of

508   evolution in fish species (Sullivan, Bird & Perry, 2017). Fish vulnerability to fishing is a

509   complex phenomenon in which many processes are involved, and in which behaviour plays a

510   significant role (Arlinghaus et al., 2017). It has been demonstrated that fishers select for certain

511   behaviours (activity, awakening times) in wild pearly razorfish populations (Alós et al., 2016;

512   Martorell-Barceló, Campos-Candela & Alós, 2018). In other species, fishing selects against bold

513   and aggressive individuals (Arlinghaus et al., 2017). This could also be the case in the pearly

514   razorfish populations. Since the most aggressive individuals tend to take more risks in dangerous

515   situations, their removal would likely reduce the likelihood of them being killed by natural

516   predators, (Sih et al., 2012; Arlinghaus et al., 2017). In this way, the intraspecific variability of

517   aggressiveness in the pearly razorfish can be reduced in exploited populations, affecting a

518   multitude of ecosystem processes with significant ecological and economic consequences. For all

519   the reasons mentioned above, this research deserves further attention and the development of

520   experimental protocols involving laboratory scoring and wild assessment of behaviour in real

521   exploited populations.

522

523   In this study, we provide for the first time a behavioural type scoring for the pearly razorfish.

524   However, our research has some limitations. First, we obtained the experimental fish via

525   experimental fishing, and we provide evidence that fishing selects a type of behaviour, skewing

526   the sampled population. Despite this, our individuals were obtained from a population located in

527   a Marine Protected Area where fishing is not allowed, and therefore the entire repertoire of

528   behaviours should be vulnerable. Furthermore, the sampling equipment used was based on

529   natural baits. Generally, the link between hook and line vulnerability and aggressiveness has

530   been found in fisheries using artificial lures (Lennox et al. 2017). Therefore, in our opinion, our

531   sample was representative of the studied population. Second, we tested a small sample of males

532   (N = 12). It is possible that increasing male's sample size would translate into significant

533   differences in aggressiveness associated with the size. In the present study we only considered

534   aggressiveness. However, in future works, it would be interesting to study behavioural

535   syndromes between aggressiveness and other behavioural types, including chronotypes.

536

537   **5.** CONCLUSIONS

538   Our work shows, for the first time, the presence of behavioural types in the pearly razorfish,

539   provided by a significant and high repeatability score. Body size (correlated with sex), RCI and

540 the experimental day did not have a significant effect on the aggressive behaviours of the pearly

541 razorfish. Aquariums with a closed water system and the mirror test have proven successful as a

542 methodology for studying aggressive behaviour in the pearly razorfish. The results obtained from

543 the mirror test revealed consistent differences in aggressiveness levels among individuals. This is

544 the first behavioural study on the pearly razorfish under controlled laboratory conditions, its fast

545 acclimatisation and low-stress levels observed make this species a successful candidate for

546 behavioural studies of marine fish. This study also provides novel information on fish

547 aggressiveness. We believe this is the first step for a better understanding of the pearly razorfish

548 personality and its implications in ecological and evolutionary processes.

549

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565 **Literature cited:**

566 Adriaenssens B, Johnsson JI. 2011. Learning and context-specific exploration behaviour in

567   hatchery and wild brown trout. *Applied Animal Behaviour Science* 132:90–99. DOI:

568   10.1016/j.applanim.2011.03.005.

569 Alós J, Cabanellas-Reboredo M, Lowerre-Barbieri S. 2012. Diel behaviour and habitat utilisation

570   by the pearly razorfish during the spawning season. *Marine Ecology Progress Series*

571   460:207–220. DOI: 10.3354/meps09755.

572 Alós J, Martorell-Barceló M, Campos-Candela A. 2017. Repeatability of circadian behavioural

573   variation revealed in free-ranging marine fish. *Royal Society Open Science* 4:160791. DOI:

574   10.1098/rsos.160791.

575 Alós J, Palmer M, Rosselló R, Arlinghaus R. 2016. Fast and behavior-selective exploitation of a

576   marine fish targeted by anglers. *Scientific Reports* 6:38093. DOI: 10.1038/srep38093.

577 Andrews E. 1966. Slate-Colored Junco Response to Mirror. *Bird-Banding* 37:206. DOI:

578   10.2307/4511292.

579 Arlinghaus R, Laskowski KL, Alós J, Klefoth T, Monk CT, Nakayama S, Schröder A. 2017.

580   Passive gear-induced timidity syndrome in wild fish populations and its potential ecological

581   and managerial implications. *Fish and Fisheries* 18:360–373. DOI: 10.1111/faf.12176.

582 Bacon PJ, Ball F, Blackwell P. 1991. A model for territory and group formation in a

583   heterogeneous habitat. *Journal of Theoretical Biology* 148:445–468. DOI: 10.1016/S0022-

584   5193(05)80231-0.

585 Baenninger R. 1968. Catechol amines and social relations in siamese fighting fish. *Animal*

586   *Behaviour* 16:442–447. DOI: 10.1016/0003-3472(68)90038-9.

587 Balzarini V, Taborsky M, Wanner S, Koch F, Frommen JG. 2014. Mirror, mirror on the wall:

588   The predictive value of mirror tests for measuring aggression in fish. *Behavioral Ecology*

589   *and Sociobiology* 68:871–878. DOI: 10.1007/s00265-014-1698-7.

590   Battaglia P, Castriota L, Consoli P, Falautano M, Romeo T, Andaloro F. 2010. Age and growth

591   of pearly razorfish, *Xyrichtys novacula* (Linnaeus 1758), in the central Mediterranean Sea.

592   *Journal of Applied Ichthyology* 26:410–415. DOI: 10.1111/j.1439-0426.2009.01383.x.

593   Bell AM. 2005. Behavioural differences between individuals and two populations of stickleback

594   Behavioural differences between individuals and two populations of stickleback

595   (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology* 18:464–473. DOI:

596   10.1111/j.1420-9101.2004.00817.x.

597   Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis.

598   *Animal Behaviour* 77:771–783. DOI: 10.1016/j.anbehav.2008.12.022.

599   Bell AM, Henderson L, Huntingford FA. 2010. Behavioral and respiratory responses to stressors

600   in multiple populations of three-spined sticklebacks that differ in predation pressure.

601   *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental*

602   *Physiology* 180:211–220. DOI: 10.1007/s00360-009-0395-8.

603   Bell AM, Stamps JA. 2004. Development of behavioural differences between individuals and

604   populations of sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour* 68:1339–1348.

605   DOI: 10.1016/j.anbehav.2004.05.007.

606   Biro PA, Beckmann C, Stamps JA. 2010. Small within-day increases in temperature affects

607   boldness and alters personality in coral reef fish. *Proceedings of the Royal Society B:*

608   *Biological Sciences* 277:71–77. DOI: 10.1098/rspb.2009.1346.

609   Biro PA, Post JR, Booth DJ. 2007. Mechanisms for climate-induced mortality of fish populations

610   in whole-lake experiments. *Proceedings of the National Academy of Sciences* 104:9715–

611   9719. DOI: 10.1073/pnas.0701638104.

612   Blowes SA, Pratchett MS, Connolly SR. 2013. Heterospecific aggression and dominance in a

613   guild of coral-feeding fishes: The roles of dietary ecology and phylogeny. *The American*

614   *Naturalist* 182:157–168. DOI: 10.1086/670821.

615   Bouwhuis S, Quinn JL, Sheldon BC, Verhulst S. 2014. Personality and basal metabolic rate in a

616   wild bird population. *Oikos* 123:56–62. DOI: 10.1111/j.1600-0706.2013.00654.x.

617   Budaev S V, Zworykin DD, Mochek AD. 1999. Consistency of individual differences in

618   behaviour of the lion-headed cichlid, *Steatocarnus casuarius*. *Behavioural Processes*

619   48:49–55. DOI: 10.1016/S0376-6357(99)00068-6.

620   Candi G, Castriota L, Andaloro F, Finoia MG, Marino G. 2004. Reproductive cycle and sex

621   inversion in razor fish, a protogynous labrid in the southern Mediterranean Sea. *Journal of*

622   *Fish Biology* 64:1498–1513. DOI: 10.1111/j.0022-1112.2004.0404.x.

623   Cardinale M, Colloca F, Ardizzone GD. 1997. Feeding ecology of Mediterranean razorfish

624   *Xyrichtyhys novacula* in the Tyrrenian Sea (Central Mediterranean Sea). *Journal of Applied*

625   *Ichthyology* 13:105–111. DOI: 10.1111/j.1439-0426.1997.tb00109.x.

626   Cardinale M, Colloca F, Ardizzone GD. 1998. Growth and reproduction of *Xyrichtys novacula*

627   (Pisceas: Labridae) in the Mediterranean Sea. *Scientia Marina* 62:193–201. DOI:

628   10.3989/scimar.1998.62n3193.

629   Careau A V, Thomas D, Humphries MM, Réale D, Oikos S, May N. 2008. Energy metabolism

630   and animal personality *Oikos* 117:641–653. DOI: 10.1111/j.2008.0030-1299.16513.x.

631   Castriota L, Scarabello MP, Finoia MG, Sinopoli M, Andaloro F. 2005. Food and feeding habits

632   of pearly razorfish, *Xyrichtys novacula* (Linnaeus, 1758), in the southern Tyrrhenian Sea:

633   variation by sex and size. *Environmental Biology of Fishes* 72:123–133. DOI:

634   10.1007/s10641-004-6576-0.

635   Cattelan S, Lucon-Xiccato T, Pilastro A, Griggio M. 2017. Is the mirror test a valid measure of

636   fish sociability? *Animal Behaviour*. DOI: 10.1016/j.anbehav.2017.03.009.

637   Conrad JL, Weinersmith KL, Brodin T, Saltz JB, Sih A. 2011. Behavioural syndromes in fishes:

638   a review with implications for ecology and fisheries management. *Journal of Fish Biology*

639   78:395–435. DOI: 10.1111/j.1095-8649.2010.02874.x.

640   Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A. 2010. Personality traits and dispersal

641   tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society*

642   *B: Biological Sciences* 277:1571–1579. DOI: 10.1098/rspb.2009.2128.

643   Cutts C. 1998. Aggression and growth depression in juvenile Atlantic salmon: the consequences

644   of individual variation in standard metabolic rate. *Journal of Fish Biology* 52:1026–1037.

645   DOI: 10.1006/jfbi.1998.0647.

646   Cutts CJ, Metcalfe NB, Taylor AC. 2002. Juvenile Atlantic Salmon (*Salmo salar*) with relatively

647   high standard metabolic rates have small metabolic scopes. *Functional Ecology* 16:73–78.

648   DOI: 10.1046/j.0269-8463.2001.00603.x.

649   Dingemanse NJ, Dochtermann NA. 2013. Quantifying individual variation in behaviour: mixed-

650   effect modelling approaches. *Journal of Animal Ecology* 82:39–54. DOI: 10.1111/1365-

651   2656.12013.

652   Dingemanse NJ, Réale D. 2005. Natural selection and animal personality. *Behaviour* 142:1159–

653   1184. DOI: 10.1163/156853905774539445.

654   Dore F, Lefebvre L, Ducharme R. 1978. Threat display in *Betta splendens*: Effects of water

655   condition and type of agonistic stimulation. *Animal Behaviour* 26:738–745. DOI:

656   10.1016/0003-3472(78)90140-9.

657 Earley RL, Hsu Y, Wolf LL. 2000. The use of standard aggression testing methods to predict

658   combat behaviour and contest outcome in *Rivulus marmoratus* dyads (Teleostei:

659   Cyprinodontidae). *Ethology* 106:743–761. DOI: 10.1046/j.1439-0310.2000.00586.x.

660 Espino F, Triay-Portella R, González JA, Haroun R, Tuya F. 2015. Population structure of the

661   pearly razorfish, *Xyrichtys novacula* (Actinopterygii: Labridae), in sand-seagrass mosaics:

662   spatial variation according to habitat features and sampling techniques. *Scientia Marina*

663   79:179–188. DOI: 10.3989/scimar.04219.05A.

664 Fischer W, Schneider M, Bauchot M-L. 1987. Fiches FAO d'identification des espèces pour les

665   besoins de la pêche : Mediterranée et Mer Noir / Volume II / Vertèbrés : Rajidae. In: *Fiches*

666   *FAO d'identification des espèces pour les besoins de la pêche : Mediterranée et Mer Noir /*

667   *Volume II / Vertèbrés*.

668 Francis RC. 1990. Temperament in a fish: A longitudinal study of the development of individual

669   differences in aggression and social rank in the midas cichlid. *Ethology* 86:311–325. DOI:

670   10.1111/j.1439-0310.1990.tb00439.x.

671 Gallup GG. 1968. Mirror-image stimulation. *Psychological Bulletin* 70:782–793. DOI:

672   10.1037/h0026777.

673 Gosling SD. 2001. From mice to men: What can we learn about personality from animal

674   research? *Psychological Bulletin* 127:45–86. DOI: 10.1037/0033-2909.127.1.45.

675 Grantner A, Taborsky M. 1998. The metabolic rates associated with resting, and with the

676   performance of agonistic, submissive and digging behaviours in the cichlid fish

677   *Neolamprologus pulcher* (Pisces: Cichlidae). *Journal of Comparative Physiology B:*

678   *Biochemical, Systemic, and Environmental Physiology* 168:427–433. DOI:

679   10.1007/s003600050162.

680   Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: The

681   MCMCglmm R package. *Journal of Statistical Software* 33. DOI: 10.18637/jss.v033.i02.

682   Hirschenhauser K, Wittek M, Johnston P, Möstl E. 2008. Social context rather than behavioral

683   output or winning modulates post-conflict testosterone responses in Japanese quail

684   (*Coturnix japonica*). *Physiology & Behavior* 95:457–463. DOI:

685   10.1016/j.physbeh.2008.07.013.

686   Holtby LB, Swain DP, Allan GM. 1993. Mirror-elicited agonistic behaviour and body

687   morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus*

688   *kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* 50:676–684. DOI:

689   10.1139/f93-078.

690   Huntingford F. 1998. Social status and growth rates as determinants of site attachment in

691   juvenile Atlantic salmon. *Journal of Fish Biology* 53:314–321. DOI:

692   10.1006/jfbi.1998.0704.

693   Hurd PL. 2006. Resource holding potential, subjective resource value, and game theoretical

694   models of aggressiveness signalling. *Journal of Theoretical Biology*. DOI:

695   10.1016/j.jtbi.2006.01.001.

696   Hutchings JA, Bishop TD, McGregor-Shaw CR. 1999. Spawning behaviour of Atlantic cod,

697   *Gadus morhua*: evidence of mate competition and mate choice in a broadcast spawner.

698   *Canadian Journal of Fisheries and Aquatic Sciences* 56:97–104. DOI: 10.1139/f98-216.

699   Jolles JW, Briggs HD, Araya-Ajoy YG, Boogert NJ. 2019. Personality, plasticity and

700   predictability in sticklebacks: bold fish are less plastic and more predictable than shy fish.

701   *Animal Behaviour* 154:193–202. DOI: 10.1016/j.anbehav.2019.06.022.

702   de Jong K, Wacker S, Amundsen T, Forsgren E. 2009. Do operational sex ratio and density

703 affect mating behaviour? An experiment on the two-spotted goby. *Animal Behaviour*

704 78:1229–1238. DOI: 10.1016/j.anbehav.2009.08.006.

705 Katano O, Iguchi K. 1996. Individual differences in territory and growth of ayu, *Plecoglossus*

706 *altivelis* (Osmeridae). *Canadian Journal of Zoology* 74:2170–2177. DOI: 10.1139/z96-245.

707 Kohda M, Hotta T, Takeyama T, Awata S, Tanaka H, Asai J, Jordan AL. 2019. If a fish can pass

708 the mark test, what are the implications for consciousness and self-awareness testing in

709 animals? *PLOS Biology* 17:e3000021. DOI: 10.1371/journal.pbio.3000021.

710 Lahti K, Laurila A, Enberg K, Piironen J. 2001. Variation in aggressive behaviour and growth

711 rate between populations and migratory forms in the brown trout, *Salmo trutta*. *Animal*

712 *Behaviour* 62:935–944. DOI: 10.1006/anbe.2001.1821.

713 Lennox RJ, Alós J, Arlinghaus R, Horodysky A, Klefoth T, Monk CT, Cooke SJ. 2017. What

714 makes fish vulnerable to capture by hooks? A conceptual framework and a review of key

715 determinants. Fish and Fisheries 18:986–1010. DOI: 10.1111/faf.12219.

716 Marconato A, And VT, Marin G. 1995. The mating system of *Xyrichthys novacula*: sperm

717 economy and fertilization success. *Journal of Fish Biology* 47:292–301. DOI:

718 10.1111/j.1095-8649.1995.tb01896.x.

719 Martorell-Barceló M, Campos-Candela A, Alós J. 2018. Fitness consequences of fish circadian

720 behavioural variation in exploited marine environments. *PeerJ* 6:e4814. DOI:

721 10.7717/peerj.4814.

722 May HY, Mercier AJ. 2007. Duration of socialization influences responses to a mirror: responses

723 of dominant and subordinate crayfish diverge with time of pairing. *Journal of Experimental*

724 *Biology* 210:4428–4436. DOI: 10.1242/jeb.011288.

725 McGhee KE, Travis J. 2010. Repeatable behavioural type and stable dominance rank in the

726   bluefin killifish. *Animal Behaviour* 79:497–507. DOI: 10.1016/j.anbehav.2009.11.037.

727   Meliska CJ, Meliska JA, Peeke HVS. 1980. The relationship of mirror-elicited display to combat

728   behaviors in *Betta splendens*. *Behavioral and Neural Biology* 30:207–217. DOI:

729   10.1016/S0163-1047(80)91089-4.

730   Millot S, Cerqueira M, Castanheira M-F, Øverli Ø, Oliveira RF, Martins CIM. 2014.

731   Behavioural stress responses predict environmental perception in european sea bass

732   (*Dicentrarchus labrax*). *PLoS ONE* 9:e108800. DOI: 10.1371/journal.pone.0108800.

733   Morgan MJ. 2004. The relationship between fish condition and the probability of being mature in

734   American plaice (*Hippoglossoides platessoides*). *ICES Journal of Marine Science* 61:64–

735   70. DOI: 10.1016/j.icesjms.2003.09.001.

736   Nakagawa S, Schielzeth H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical

737   guide for biologists. *Biological Reviews* 85.4: 935-956. DOI: 10.1111/j.1469-

738   185X.2010.00141.x.

739   Nemtzov SC, Clark E. 1994. Intraspecific egg predation by male razorfishes (Labridae) during

740   broadcast spawning: Filial cannibalism or intra-pair parasitism? *Bulletin of marine science*

741   55.1 (1994): 133-141. DOI: https://www.researchgate.net/publication/233665638.

742   Nijman V, Heuts BA. 2000. Effect of environmental enrichment upon resource holding power in

743   fish in prior residence situations. *Behavioural Processes*. 49.2: 77-83. DOI: 10.1016/S0376-

744   6357(00)00078-4.

745   Nomakuchi S, Park PJ, Bell MA. 2009. Correlation between exploration activity and use of

746   social information in three-spined sticklebacks. *Behavioral Ecology* 20:340–345. DOI:

747   10.1093/beheco/arp001.

748   O'Connor CM, Reddon AR, Ligocki IY, Hellmann JK, Garvy KA, Marsh-Rollo SE, Hamilton

749   IM, Balshine S. 2015. Motivation but not body size influences territorial contest dynamics

750   in a wild cichlid fish. *Animal Behaviour* 107:19–29. DOI: 10.1016/j.anbehav.2015.06.001.

751   O'Neill SJ, Williamson JE, Tosetto L, Brown C. 2018. Effects of acclimatisation on behavioural

752   repeatability in two behaviour assays of the guppy *Poecilia reticulata*. *Behavioral Ecology*

753   *and Sociobiology* 72:166. DOI: 10.1007/s00265-018-2582-7.

754   Parker GA. 1974. Assessment strategy and the evolution of fighting behaviour. *Journal of*

755   *Theoretical Biology*. 47.1: 223-243 DOI: 10.1016/0022-5193(74)90111-8.

756   Pham M, Raymond J, Hester J, Kyzar E, Gaikwad S, Bruce I, Fryar C, Chanin S, Enriquez J,

757   Bagawandoss S, Zapolsky I, Green J, Michael Stewart A, Robison BD, Kalueff A V. 2012.

758   Assessing social behavior phenotypes in adult zebrafish: shoaling, social preference, and

759   mirror biting test. *Zebrafish Protocols for Neurobehavioral Research*. Totowa, NJ: Humana

760   Press. DOI: 10.1007/978-1-61779-597-8_17.

761   Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal

762   temperament within ecology and evolution. *Biological Reviews* 82:291–318. DOI:

763   10.1111/j.1469-185X.2007.00010.x.

764   Rehage JS, Sih A. 2004. Dispersal behavior, boldness, and the link to invasiveness: A

765   comparison of four gambusia species. *Biological Invasions* 6:379–391. DOI:

766   10.1023/B:BINV.0000034618.93140.a5.

767   Rhoad KD, Kalat JW, Klopfer PH. 1975. Aggression and avoidance by *Betta splendens* toward

768   natural and artificial stimuli. *Animal Learning & Behavior* 3:271–276. DOI:

769   10.3758/BF03213443.

770   Rowland WJ. 1999. Studying visual cues in fish behavior: A review of ethological techniques.

771   *Environmental Biology of Fishes* 56:285–305. DOI: 10.1023/A:1007517720723.

772 Roy T, Bhat A. 2018. Repeatability in boldness and aggression among wild zebrafish (*Danio*

773   *rerio*) from two differing predation and flow regimes. *Journal of Comparative Psychology*

774   132:349–360. DOI: 10.1037/com0000150.

775 Ryan White J. 2015. The role of boldness and other personality traits in the ecology of juvenile

776   marine fishes. Doctoral dissertation, James Cook University.

777 Sbragaglia V, Alós J, Fromm K, Monk CT, Díaz-Gil C, Uusi-Heikkilä S, Honsey AE, Wilson

778   ADM, Arlinghaus R. 2019. Experimental size-selective harvesting affects behavioral types

779   of a social fish. *Transactions of the American Fisheries Society*. 148.3: 552-568. DOI:

780   10.1002/tafs.10160.

781 Scotti M-AL, Foster SA. 2007. Phenotypic plasticity and the ecotypic differentiation of

782   aggressive behavior in Threespine stickleback. *Ethology* 113:190–198. DOI:

783   10.1111/j.1439-0310.2006.01311.x.

784 Shen D, Clark E. 2016. Territorial and reproductive behavior of the three Caribbean Razorfishes

785   of the genus *Xyrichtys* (Labridae) at Bonaire. *International Journal of Ichthyology* 22:33–

786   59.

787 Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary

788   overview. *Trends in Ecology & Evolution* 19:372–378. DOI: 10.1016/j.tree.2004.04.009.

789 Sih A, Cote J, Evans M, Fogarty S, Pruitt J. 2012. Ecological implications of behavioural

790   syndromes. *Ecology Letters* 15:278–289. DOI: 10.1111/j.1461-0248.2011.01731.x.

791 Snekser JL, Leese J, Ganim A, Itzkowitz M. 2009. Caribbean damselfish with varying territory

792   quality: correlated behaviors but not a syndrome. *Behavioral Ecology* 20:124–130. DOI:

793   10.1093/beheco/arn123.

794 Stamps J. 1994. Territorial behavior: testing the assumptions. In: *Advances in the Study of*

795   *Behavior*. 173–232. DOI: 10.1016/S0065-3454(08)60354-X.

796   Stamps JA, Buechner M. 1985. The territorial defense hypothesis and the ecology of insular

797   vertebrates. *The Quarterly Review of Biology* 60:155–181. DOI: 10.1086/414314.

798   Stamps JA, Krishnan V V. 1999. A learning-based model of territory establishment. *The*

799   *Quarterly Review of Biology* 74:291–318. DOI: 10.1086/393163.

800   Sullivan AP, Bird DW, Perry GH. 2017. Human behaviour as a long-term ecological driver of

801   non-human evolution. *Nature Ecology & Evolution* 1:0065. DOI: 10.1038/s41559-016-

802   0065.

803   Taylor EB. 1990. Variability in agonistic behaviour and salinity tolerance between and within

804   two populations of juvenile chinook salmon, *Oncorhynchus tshawytscha* , with contrasting

805   life histories. *Canadian Journal of Fisheries and Aquatic Sciences* 47:2172–2180. DOI:

806   10.1139/f90-242.

807   Vøllestad LA, Quinn TP. 2003. Trade-off between growth rate and aggression in juvenile coho

808   salmon, *Oncorhynchus kisutch*. *Animal Behaviour* 66:561–568. DOI:

809   10.1006/anbe.2003.2237.

810   Way GP, Ruhl N, Snekser JL, Kiesel AL, McRobert SP. 2015. A comparison of methodologies

811   to test aggression in zebrafish. *Zebrafish* 12:144–151. DOI: 10.1089/zeb.2014.1025.

812   Wilson AJ, de Boer M, Arnott G, Grimmer A. 2011. Integrating personality research and animal

813   contest theory: Aggressiveness in the green swordtail *Xiphophorus helleri*. *PLoS ONE*

814   6:e28024. DOI: 10.1371/journal.pone.0028024.

815   Wolf M, van Doorn GS, Leimar O, Weissing FJ. 2007. Life-history trade-offs favour the

816   evolution of animal personalities. *Nature* 447:581–584. DOI: 10.1038/nature05835.

Figure 1

Representative diagram of the behavioural arenas.

Behavioural arenas were composed by an aquarium (container where the fish were held captive) connected to the filtering water system through a small sink. The tank contained seawater and sand that served as refuge for the fish. A filtration system was installed behind the tank, consisting of two sponges of different thickness, a biological filtration system and a skimmer responsible for removing the excess of urea. An airstone aerator, a water heater and a water pump were located in the filtration section. The recording system consisted of a Raspberry Pi computer and a Raspberry Pi camera. This system was mounted in front of the aquarium to have a frontal vision during the aggressiveness tests. On the photo, a fish can be seen swimming in an aquarium during the experimental period (diagram by Javier Sanllehi Hansson).

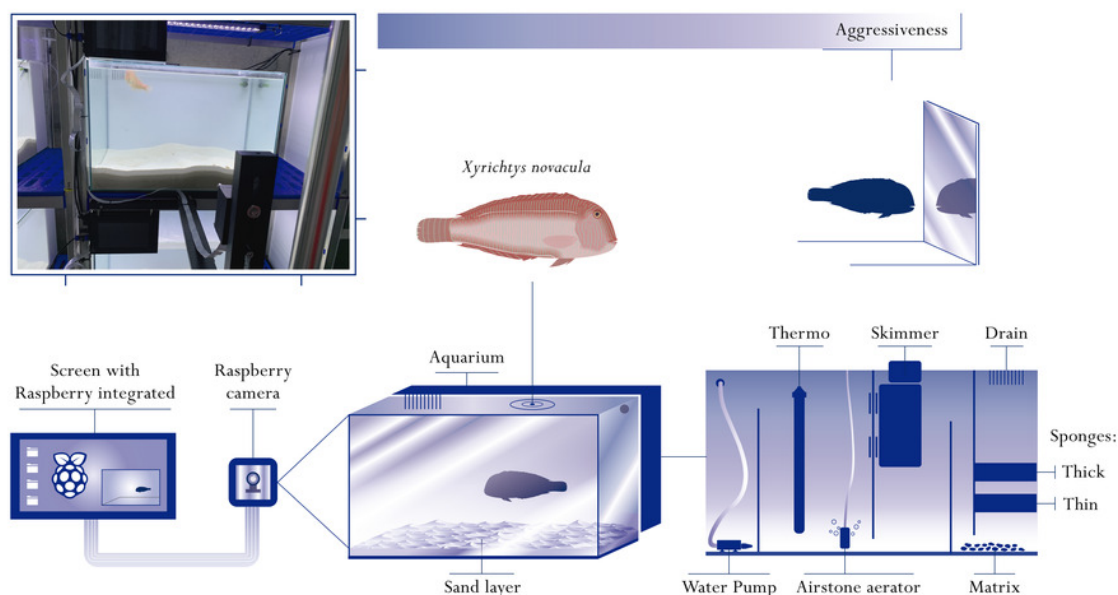



Figure 2

Graphical representation of the sample

(A) Histogram of the distribution of body sizes (in cm) for the individuals considered in this study. (B) Boxplot of the weight differences (in g) between females and males. (C) Bar graph with the number of tests (number of individual samples) performed to each individual. (D) Scatterplot of the relative condition index (RCI) for each individual. Males are represented in green and females in orange.

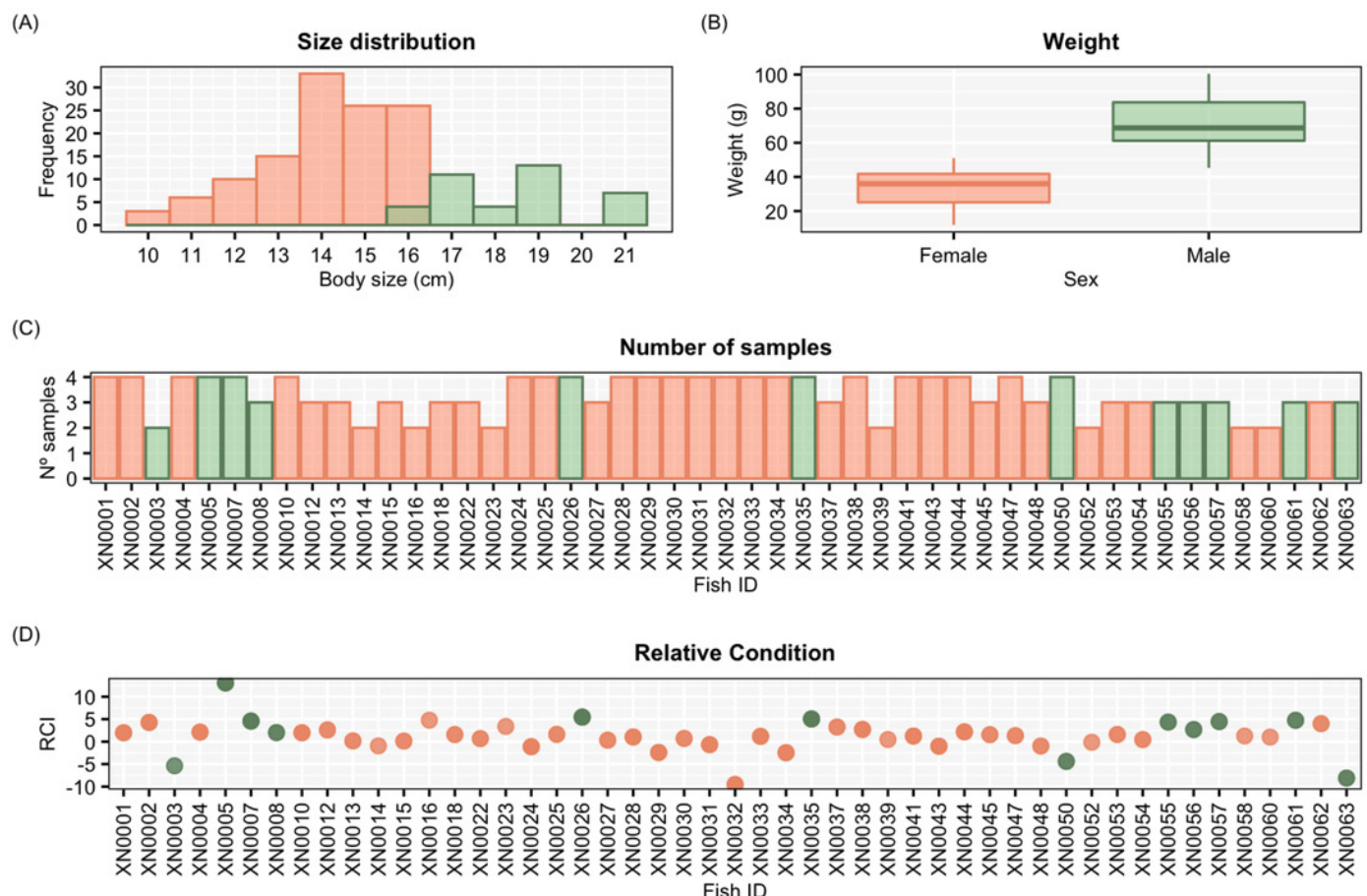



Figure 3

PCA (Principal component analysis)

Graphical representation of the two components (PC1 and PC2) from the principal component analysis (PCA) and the three correlated aggressiveness metrics (number of bites, rams and charges). Males are represented in green and females in orange.

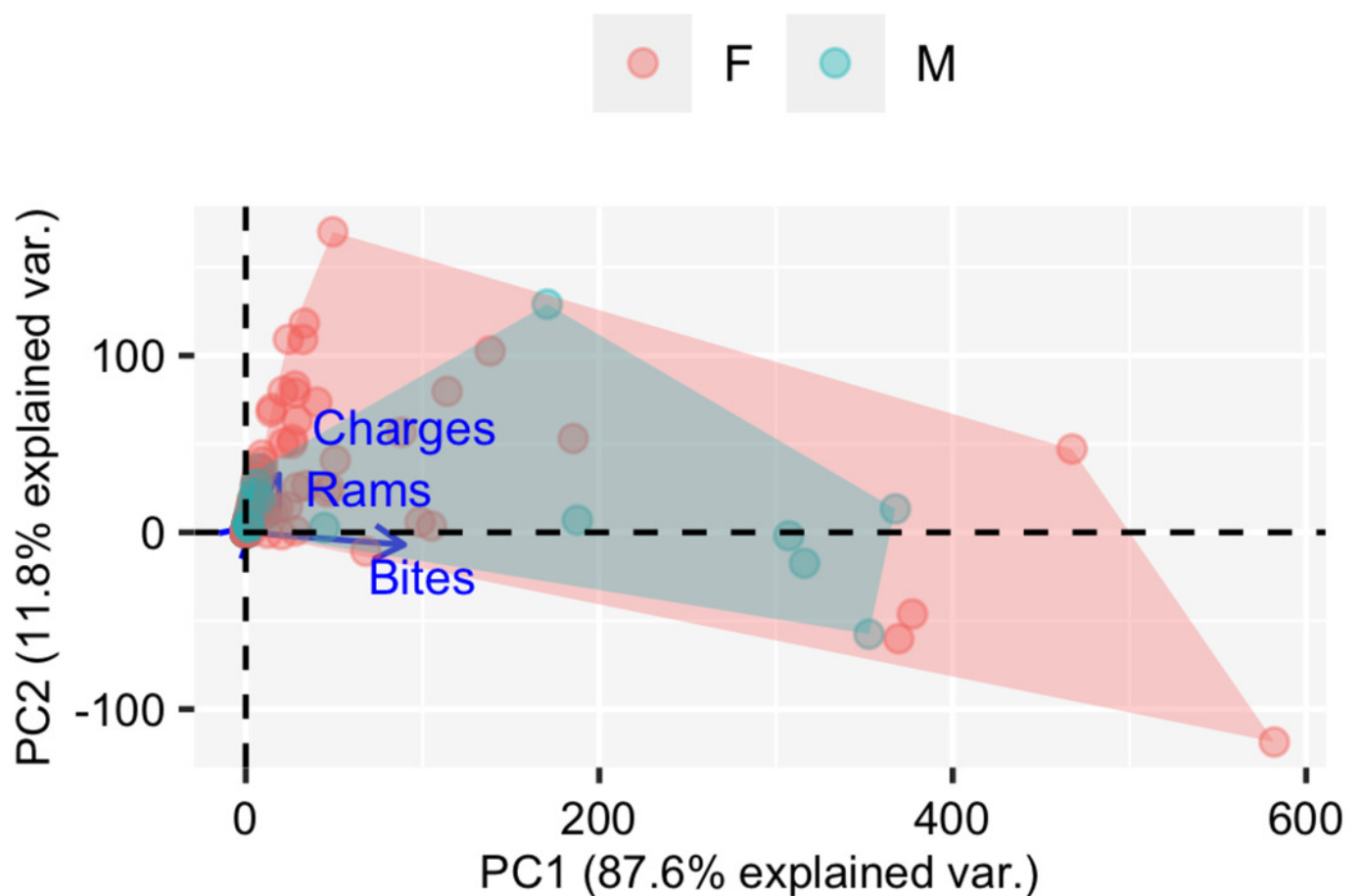



Figure 4

Results for data set

Results of aggressiveness scores obtained from the complete data set (including males and females). (A) Density population plot. (B) Individual's aggressiveness score per each experimental day, where each colour represents an individual. (C) Violin plot representing the individual's variance of aggressiveness scores, where each dot represents the mean aggressiveness score for each individual. Females are represented in orange and males in green.

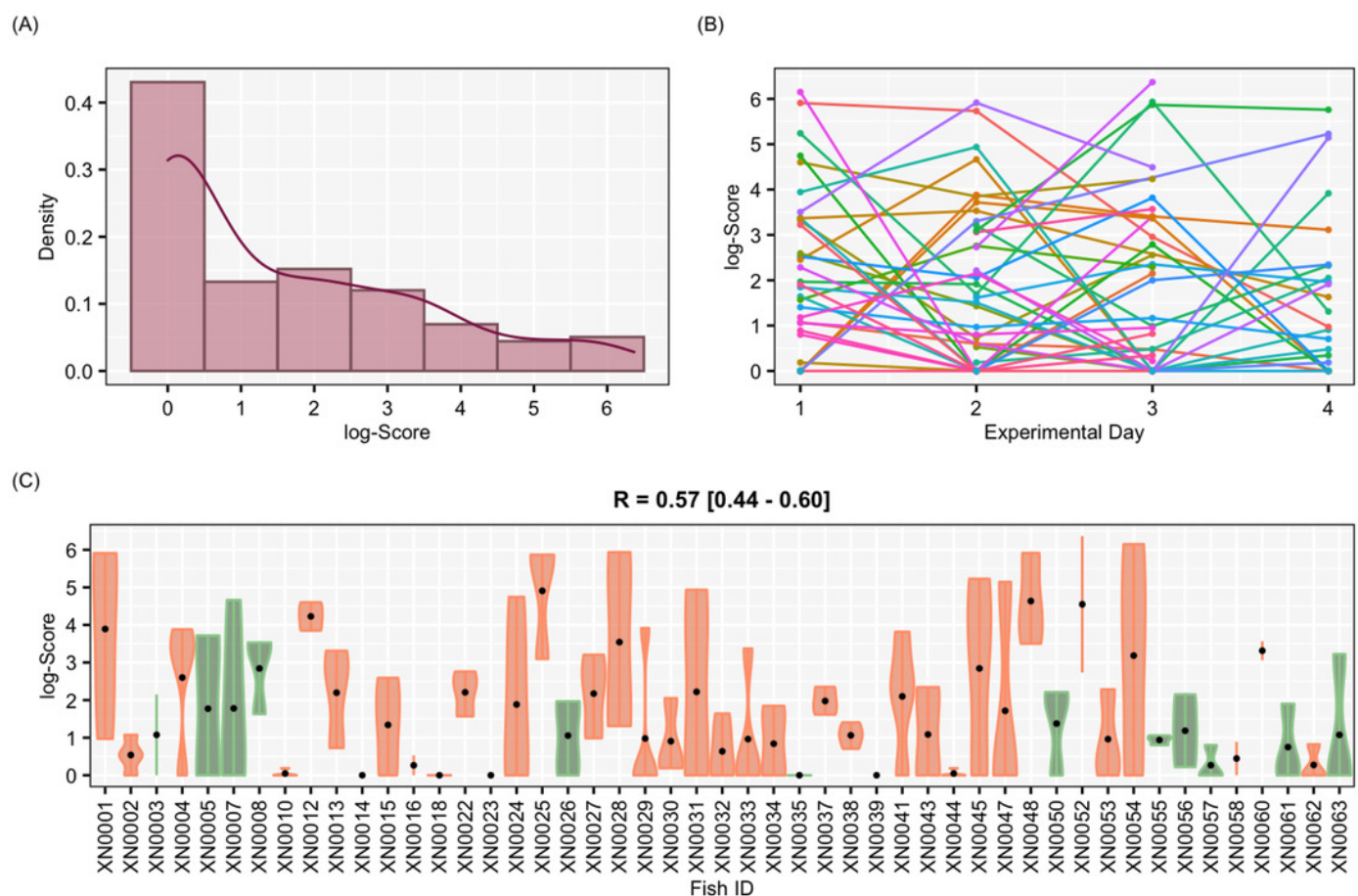
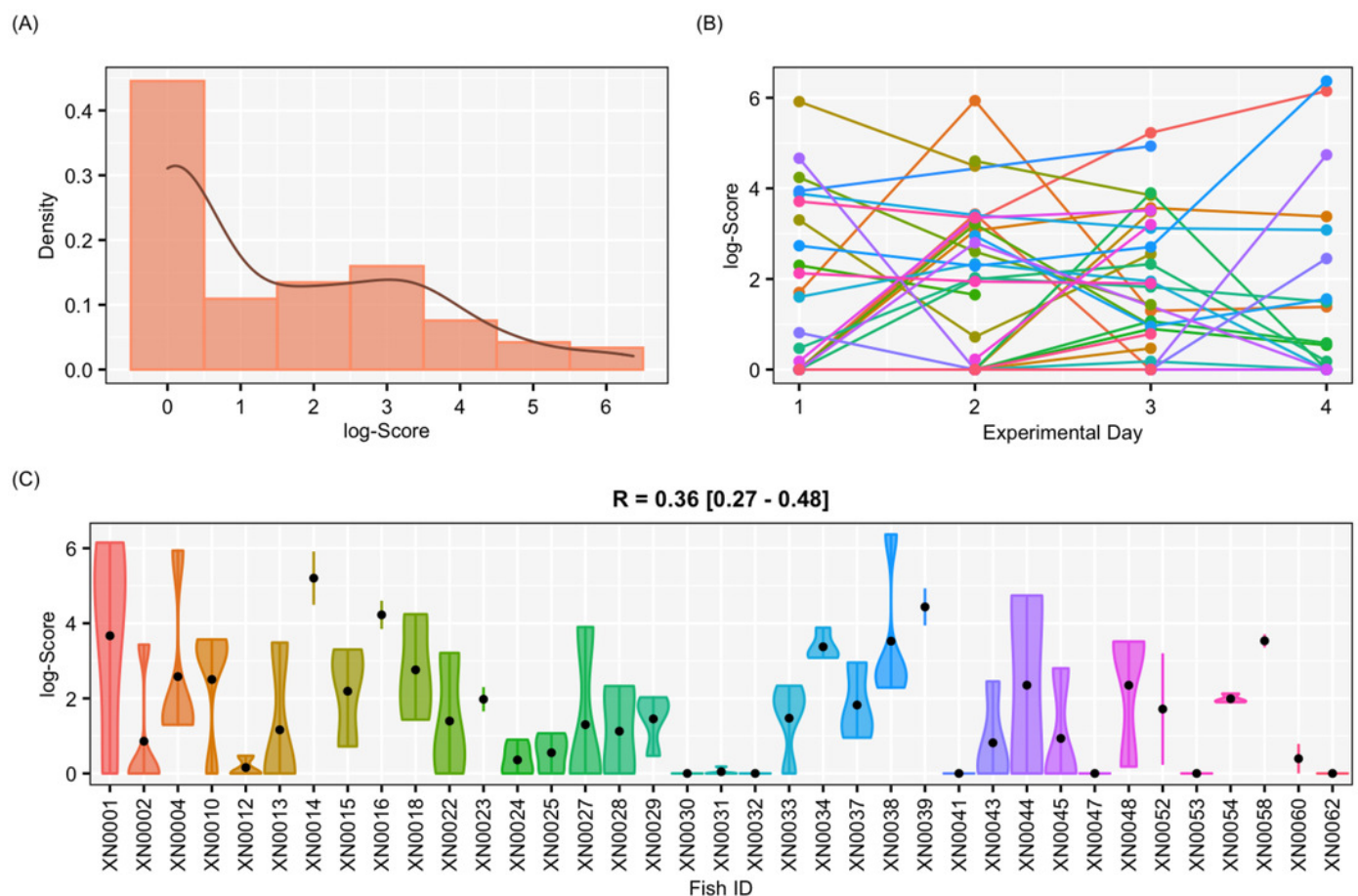



Figure 5

Results for females-only data set

Results of aggressiveness scores obtained from the female-only data set. (A) Density population plot. (B) Individual's aggressiveness score per each experimental day, where each colour represents an individual. (C) Violin plot representing the individual's variance of aggressiveness scores, where each dot represents the individual's mean.

Results of the GLMM for the complete data set

Results of the GLMM for the complete data set (including males and females). Environmental covariates with their lower and upper BCI (l-BCI and u-BCI, respectively) are shown. The model was fitted by 3 environmental variables: experimental day, body size (related with sex: the males were bigger than the females); and the relative condition index (RCI). None of these variables have an effect on aggressiveness. The table also shows the adjusted-R score, statistically significant as evidenced by the values of DIC and DICc, the between- (Vind0) and within-individuals (Ve0) variances and p-values.

	Mean	l-BCI	u-BCI	p-value
Intercept	1.68	1.27	2.12	<0.001*
Experimental day	0.17	-0.03	0.37	0.09
Body Size	0.12	-0.32	0.59	0.59
RCI	-0.08	-0.50	0.39	0.73
V_{ind0}	1.90	1.08	3.02	
V_{e0}	1.54	1.18	1.95	
Adjusted-R	0.57	0.44	0.60	
DIC = 558.22 (DICc = 641.32)				

Results of the GLMM from the female-only data set

Results of the GLMM for the females-only data set. Environmental covariates with their lower and upper BCI (l-BCI and u-BCI, respectively) are shown. The model was fitted by 3 environmental variables: experimental day, body size and the relative condition index (RCI). None of these variables have an effect on aggressiveness behaviour as in the complete data set. The table also shows the adjusted-R score, statistically significant as evidenced by the values of DIC and DICc, the between- (Vind0) and within-individuals (Ve0) variances and p-values.

	Mean	l-BCI	u-BCI	p-value
Intercept	1.65	1.20	2.13	<0.001*
Experimental day	0.14	-0.11	0.40	0.31
Body size	0.10	-0.37	0.56	0.68
RCI	0.02	-0.39	0.45	0.90
V_{ind0}	1.42	0.45	2.66	
V_{e0}	1.75	1.37	2.61	
Adjusted-R	0.36	0.27	0.48	
DIC = 481.33 (DICc = 446.62)				