1 Spearfishing promotes a timidity syndrome and increases the safe operating distances in fish

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19 **ABSTRACT**

20 In a landscape of fear, humans are altering key behaviors expressed by wild-living animals,

21 including those related to foraging, reproduction and survival. When exposed to potentially

22 lethal human actions, such as hunting or fishing, fish and wildlife is expected to behaviorally

23 respond by becoming more timid, but proving such responses underwater in the wild has been

24 challenging. Using a rich dataset collected *in situ*, we provide evidence of spearfishing-induced

25 behavioral effects in five coastal fish species using the flight initiation distance (FID) as a proxy

26 of predator avoidance and boldness. We document that spearfishing promotes a timidity

27 syndrome (i.e., an increase of the average timidity of harvested populations) and that the prey's

28 wariness is influenced by individual size, level of protection offered through marine protected

29 areas and the ability to recognize the risk posed by underwater human predators. In particular,

30 we show that changes in the appearance of the observer (spearfisher *vs.* snorkeler) modulate the

31   risk perception among the exploited species, and these differences are more evident outside

32   marine protected areas where spearfishing is allowed. We also detected a positive correlation

33   between FID and fish size, with larger specimens (that are more likely targets of spearfishers)

34   revealing larger FID. The behavioral effects were most clearly expressed in the most heavily

35   exploited species and declined towards the less desired and less targeted ones, which may be a

36   result of learning mechanisms and plasticity and/or fisheries-induced evolution of timidity. Our

37   study reveals a trade-off where intensive spearfishing negatively affects future spearfishing

38   success through behavior-based alteration of catchability. Either rotating harvest or

39   implementation of mosaics of protected and exploited areas might be needed to manage

40   spearfishing-induced timidity in exploited stocks.

41

42   **Keywords:** spearfishing, timidity syndrome, behavior, fish, human predation

43

INTRODUCTION

Human activities, such as hunting and fishing, are affecting wild populations by altering abundance, biomass, size-structure, population dynamics as well as life-histories and behavioral traits and associated demographic and evolutionary processes (Sullivan et al., 2017). A large body of literature has studied ecological (e.g. Worm et al., 2009, Arlinghaus et al., 2017) and evolutionary effects of fishing (e.g. Jørgensen et al., 2007, Laugen et al., 2014). Fishing is typically selective with respect to different traits such as size and behavior (Law, 2007, Kuparinen and Festa-Bianchet, 2017, Uusi-Heikkilä et al., 2008, Lennox et al., 2017), and elevated mortality as well as trait-selective harvesting is expected to affect size and age structure (e.g. Arlinghaus et al., 2010) as well as life-history and behavioral traits (Allendorf and Hard, 2009). For instance, many fishing methods selectively remove large and bold individuals (e.g. Klefoth et al., *in press*), altering traits of populations by favoring fast life-histories (Laugen et al., 2014, Heino et al., 2013) and more cautious behavioral types (Arlinghaus et al., 2017). These changes can be caused both by plastic responses as well as evolutionary changes (Laugen et al., 2014, Arlinghaus et al., 2017).

While fisheries-induced changes in life-histories have seen abundant attention in the literature, the possibility of fisheries altering the composition of fish behavioral types has been less explored (Heino et al., 2015). Recently, it been proposed that behavior is a sensitive indicator of exploitation in the marine environment (Goetze et al., 2017). The exploitation-induced behavioral effect has recently been defined as a "timidity syndrome", which may have a range of ecological, social and economic consequences, most notably by altering the catchability of exploited populations (Arlinghaus et al., 2017). However, despite considerable conceptual appeal, there are surprisingly few field studies that have provided clear documentation that exploited fish stocks are indeed more timid (Januchowski-Hartley et al., 2011, Alós et al., 2015, Alós et al., 2014, Tsuboi et al., 2016, Alós et al., 2016, Bergseth et al., 2016, Colefax et al., 2016), motivating the present work.

We investigated the behavioral changes induced by recreational spearfishing in several coastal sites in two Mediterranean countries. Spearfishing constitutes a globally popular

72 recreational activity with important historical and social roots, especially in the Mediterranean

73 Sea (Coll et al., 2004, Sbragaglia et al., 2016, Lloret et al., 2008). Spearfishing constitutes an

74 active fishing mode sharing similarities to hunting because spearfishers are able to visually

75 target and select their prey (Dalzell, 1996). The size-selectivity of spearfishing depends on the

76 hunting technique (shelter seeking, ambush, sit-and-wait or direct approach) and is related to the

77 fisher's experience (Diogo et al., 2017). Similar to trophy hunting (e.g. Coltman et al., 2003),

78 spearfishers select the large individuals, and as the capture depends on physical proximity of the

79 fish and the spearfisher there is also the potential for selection on behavioral phenotypes, such

80 as boldness, exploration or aggression (e.g. Januchowski-Hartley et al., 2011). Therefore, in

81 heavily exploited areas, the traits linked to the ability of fishes to hide or escape from the human

82 predator can be under a strong selective pressure (Ydenberg and Dill, 1986). Fish behavior has a

83 large plastic component and can thus rapidly change through learning and development. As a

84 consequence, levels of wariness can substantially increase due to increased spearfishing

85 pressure within few days after opening a fishing zone (Goetze et al., 2017).

86 One of the most commonly used metric to assess a prey's wariness in the presence of

87 (human or natural) predation, is the distance to which a predator can approach before the prey

88 flees. This measure, indexed as the flight initiation distance (FID; Ydenberg and Dill, 1986), has

89 been used in previous studies to estimate fish behavioral responses to spearfishers

90 (Januchowski-Hartley et al., 2011, Januchowski-Hartley et al., 2012, Tran et al., 2016,

91 Benevides et al., 2016, Feary et al., 2011, Gotanda et al., 2009, Nunes et al., 2015, Guidetti et

92 al., 2008, Cole, 1994) and may constitute a sensitive indicator of exploitation to inform

93 management (Januchowski-Hartley et al., 2014, Goetze et al., 2017). Generally, individuals

94 with shorter FID are considered bolder (Januchowski-Hartley et al., 2011, Feary et al., 2011).

95 Several studies have investigated how FID can be affected by natural and human factors, such

96 as fishing pressure (key finding is an increased FID under fishing pressure), protection status of

97 a given site (shorter FID in marine protected areas) and body size (increased FID with size)

98 (Januchowski-Hartley et al., 2011, Januchowski-Hartley et al., 2012, Feary et al., 2011, Gotanda

99 et al., 2009). Changes in the FID can be caused by a combination of both selection and

100 evolution and plasticity within the realm of learned predator avoidance, predator recognition

101 and inspection behaviors (Kelley and Magurran, 2003). No matter which mechanism, a

102 spearfishing-induced increase in the FID has been repeatedly reported and will negatively affect

103 future catchability and exposure of fishes to spearfishers (Feary et al., 2011).

104 The rapid increase of spearfishing in the last 60 years in the Mediterranean area (Coll et

105 al., 2004) represents a new pressure for wild coastal fish populations but also an opportunity to

106 understand how fish cope with the sudden arrival of a new predator, the underwater human

107 predator. Fishes can became aware of the presence of predators by olfactory (Chivers and

108 Smith, 1998), tactile or auditory stimuli (Kelley and Magurran, 2003), but in clear water

109 conditions typical of many areas of the Mediterranean Sea, visual cues are likely the primary

110 source of information that allows fishes to recognize predators from long distances (Aksnes and

111 Giske, 1993). Fishes quickly learn how to reduce predation risk, thereby moderating the

112 fundamental growth-mortality trade-off (Werner and Hall, 1974, Ydenberg and Dill, 1986,

113 Stamps, 2007). Learning may be both an individual or social process, contributing to the large

114 plasticity in behavioral repertoire that is typical of many fish species (Odling-Smee and

115 Braithwaite, 2003, Kieffer and Colgan, 1992, Mangel and Stamps, 2001). However, a large

116 body of literature has also revealed a high individual variability in behavioral traits, typically

117 referred to as behavioral types or personalities (Carere and Maestripieri, 2013). Although

118 personality can emerge from clonal fishes reared in apparently identical conditions (e.g.

119 Bierbach et al., 2017), consistent variation in behavioral traits has been found to carry a large

120 genetic (i.e., heritable) component (Dochtermann et al., 2015). Thus, spearfishing-induced

121 selection on behavior could also induce evolutionary change towards timidity (Andersen et al.,

122 2017).

123 We investigated the FID expressed across several species in the wild, in different

124 geographical areas and under different levels of protection offered by marine protected areas

125 where spearfishing is not allowed. To mechanistically understand how fishes respond to human-

126 induced predation threat, we included a 'spearfishing' treatment, which allowed testing the

127 ability of fishes to recognize the threat represented by humans. Our predictions were: (*i*) FID

128 increases according to the threat level associated by fishes to the encounter with spearfishers

129 (higher FID in the presence of a diver with a speargun; higher FID outside the marine protected

130 areas); (*ii*) FID increases in proportion to the historic spearfishing pressure on a given species

131 (the higher the historic exploitation on a given species, the higher is the FID); and (*iii*) FID

132 increases with fish size because larger fishes are preferentially targeted by spearfishers (the

133 larger the fish, the higher the FID).

134

135 **MATERIALS AND METHODS**

136 *Study species*

137 We targeted five common coastal fish taxa subjected to different levels of spearfishing

138 harvesting in the following order (from low to high historic exploitation intensity): (1)

139 *Symphodus tinca*; (2) *Sarpa salpa*; (3) mugilidae (considering the following species: *Chelon*

140 *labrosus*, *Mugil cephalus*, *Liza aurata* and *Liza ramada*); (iv) *Diplodus vulgaris*; and (v)

141 *Diplodus sargus*. This gradient was compiled on the basis of literature (Harmelin et al., 1995,

142 Coll et al., 2004, Lloret et al., 2008, Rocklin et al., 2011) and reflecting the authors' knowledge

143 of spearfishers' habits in the studied areas. FID was measured in a 2×2 factorial design in two

144 areas with different level of harvesting protection status (protected/unprotected sites) and in two

145 diver treatments (potentially threatening spearfishing and non-threatening snorkeling). These

146 treatments and sites represented a gradient of threatening situations for the fishes: strong threat

147 (unprotected/spearfishing: hereafter Spear-NP), medium threat (unprotected/snorkeling:

148 hereafter Snork-NP), weak threat (protected/spearfishing: hereafter Spear-P) and very weak

149 threat (protected/snorkeling: hereafter Snork-P).

150

151 *Study sites and experimental design*

152 The FID of the five target species was surveyed between May and October 2016 in 3

153 different marine protected areas (MPAs) of the North-West Mediterranean Sea. The study was

154 conducted in the MPA of Bonifacio Straits, Corsica, France (protected zone: 41° 35.28' N, 9°

155 21.76'E; non-protected reference zone: 41° 36.99'N, 9° 21.19' E), the MPA of Cerbère-

156   Banyuls, France (protected zone: 42° 28.58 N, 3° 9.40'E; non-protected reference zone: 42°

157   29.31'N, 3°7.79' E) and the MPA of Medes Islands, Spain (protected zone: 42° 2.67'N, 3°

158   13.40'E; non-protected zone: 42° 6.20°N, 3° 10.50'E). We chose three MPAs and two countries

159   to generalize our findings and reduce the impact of specific uncontrolled contextual factors

160   within any given site/country. Hereafter, we will refer to the three different areas as Banyuls,

161   Bonifacio and Medes.

162   The FID was surveyed inside and outside each MPA. All observations were conducted

163   at a minimum distance of 750 m from the MPA borders, to avoid potential spillover of naive

164   fish from the MPA (Alós et al., 2015, Januchowski-Hartley et al., 2013). All sites were surveyed

165   by the same operator (LM). The spearfisher was equipped with a speargun (100 cm long,

166   rubbers were removed and substituted with PVC tubes to not violate regulations of the MPA)

167   and with the typical spearfishing equipment (black wetsuit, long black fins and a little black

168   mask). The snorkeler was equipped with a shorty bluish wetsuit, short bluish fins and a large

169   colored mask. The different diver configurations (spearfisher/snorkeler) were applied inside and

170   outside each MPA in a random order over 4-7 consecutive days.

171

172   *Data collection*

173   The operator swam on the surface along a linear transect over rocky reef bottom

174   between 1 and 3 m depth, identifying the target fish from the surface, and then swimming

175   towards it at a steady speed (Januchowski-Hartley et al., 2014). When the fish reacted to the

176   presence of the operator (usually flight away but in few cases, in the marine protected areas,

177   positive interactions also occurred) the position was marked on the bottom with a small weight.

178   Positive interactions were not considered in this study. The distance between the location of

179   escape and the marked position was then measured with a tape and recorded as FID (Ydenberg

180   and Dill, 1986). Flight was considered to have occurred when the fish increased its swimming

181   speed or changed its swimming direction in response to the operator. For each target fish (<10

182   cm), the species was determined and total length was estimated to the nearest cm. To establish

183   the accuracy of the size estimation, the length of known size static objects (length range 10-70

184   cm; n = 30) was estimated with an error of 8.4 ± 6.4% (average percent error ± standard

185   deviation). Larger mobile species and individuals were counted first to minimize the chance of

186   approaching a target fish that had been disturbed by a previous trial, consecutive trials were

187   conducted a minimum of 10 m apart, and in the opposite direction to which a disturbed fish fled,

188   assuming independence of samples within sites.

189

190   *Statistical analysis*

191   We implemented a linear mixed model with random intercepts using FID as response

192   variable, fish length as covariate, treatment (four levels: Spear-NP; Snork-NP; Spear-P; Snork-

193   P) and taxa (five levels *D. sargus, D. vulgaris, Mugilidae, S. salpa* and *S. tinca*) as fixed effects,

194   and the three areas (Bonifacio, Banyuls and Medes) as random effect. Model fitting was

195   examined by checking normality of residuals and plotting theoretical quantiles versus

196   standardized residuals. We used the "lme4" R package (Bates et al., 2014) to implement the

197   linear mixed models, and "lsmeans" R package (Lenth, 2016) to run multicomparisons among

198   slopes or means. All analyses were conducted in R 3.3.1 (R Core Team 2016) with a 95%

199   confidence interval.

200

201   **RESULTS**

202   The FID of N = 1,328 fishes (345 *S. tinca,* 177 *S. salpa*, 130 mugilidae, 286 *D. vulgaris*

203   and 390 *D. sargus*) distributed among areas and treatments was scored (Table 1). All the fixed

204   terms revealed significant effects on FID (Table 2). Most importantly, a significant three-way

205   interaction effect among size, treatment and species was detected ($\chi^2_{12,1328}$ = 37.47, p < 0.0001;

206   Table 2), indicating that the treatments exerted differential effects depending on the size of the

207   fish and the species.

208   The mean FID, while accounting for the mean effect of size, increased outside the MPA

209   both in the presence of the spearfisher and snorkeler for the historically most targeted species

210   (*D. sargus*, *D. vulgaris* and *S. salpa;* Table 3, Figs. 2, 3a), while for the historically less targeted

211   mugilidae species and *S. tinca* the FID increase was recorded only in the presence of the

212   spearfisher (Table 3, Figs. 2, 3a). Outside the MPA, the mean FIDs were always larger than 4 m

213   (except for *S. tinca*; see Table 3). Inside the MPA, however, the FID was not significantly

214   different in the presence of either spearfisher or a non-threatening snorkeler, with the exception

215   of the reaction of *S. tinca* (Table 1, Figs. 2, 3a). By contrast, the presence of a spearfisher

216   outside the MPA increased the mean FID in all the species except for the less targeted *S. salpa*

217   and *S. tinca* (Table 3, Figs. 2, 3a).

218   The presence of a snorkeler inside the MPA triggered a larger FID in *D. sargus* than in

219   *S. tinca* (Table 3, Figs. 2, 3b). Inside the MPA, in presence of a spearfisher, the mean FIDs were

220   significantly larger in *D. sargus* and *D. vulgaris* than in *S. tinca* (Table 3, Figs. 2, 3b). Outside

221   MPA, in presence of a snorkeler, the FID was significantly shorter for *S. tinca* compared to the

222   other four species/species groups (Table 3, Figs. 2, 3b). Finally, outside the MPA in the

223   presence of a spearfisher, *D. sargus* and *D. vulgaris* showed significantly elevated FID, while

224   *mugilidae* and *S. salpa* revealed intermediate FID, and *S. tinca* the shortest FIE (Table 3, Figs.

225   2, 3b).

226   The positive linear correlation between size and FID indicated that in almost all

227   circumstances larger fish were more timid (Table 2, Fig. 2). The comparison within species

228   indicated that the size effect increased outside the MPA only in two taxa (Table 3, Figs. 2, 4a):

229   *mugilidae* (stronger effect of size in the presence of a snorkeler), and *D sargus* (stronger effect

230   of size on FID both in the presence of a snorkeler and a spearfisher). The comparison within

231   treatment also indicated that the size effect was significantly more pronounced in *D. sargus* than

232   in *S. tinca* in the presence of a spearfisher inside the MPA and in the presence of a snorkeler

233   outside the MPA (Table 3, Figs. 2, 4b). In the presence of a spearfisher outside the MPA, the

234   effect of size was highly variable among species: stronger effects were recorded in the most

235   targeted *D. sargus* and *D. vulgaris*, intermediate effects in the intermediately targeted *mugilidae*

236   and *S. salpa* and the weakest effect on FID in the least targeted *S. tinca* (Table 3, Figs. 2, 4b).

237

238   **DISCUSSION**

239   We found that spearfishing promotes a timidity syndrome in exploited fishes and we

240   confirmed all the three predictions we initially formulated. We found the risk perception of

241   fishes changed with the appearance of the diver (spearfisher *vs* snorkeler) in a way

242   commensurate with the level of threat posed by each of these two types of divers. Such

243   differences were most evident outside the MPA, where fishes have historically experienced

244   more encounters with threatening spearfishers and where certain behavioral types and

245   morphotypes were selectively harvested, supporting the hypothesis of a spearfishing-induced

246   timidity syndrome (Arlinghaus et al., 2017, Arlinghaus et al., 2016). Our finding that the

247   timidity response was more pronounced not only in the historically most targeted species (e.g.,

248   *D. sargus* compared to *S. tinca*) but also in the largest fish further underscored that spearfishing

249   has promoted increased shyness in the most targeted species and sizes. The positive correlation

250   between FID and individual size can most plausibly be explained by experiential effects, but

251   might also carry an evolutionary component, similar to findings reported from a freshwater

252   salmonid targeted by recreational anglers in Japan (Tsuboi et al., 2016).

253

254   *Effect of spatial protection status against harvesting on FID*

255   Protection of aquatic areas against exploitation has well known effects on fish behavior

256   and typically has resulted in a decrease of fish timidity in a range of case studies ranging from

257   spearfishing (Gotanda et al., 2009, Januchowski-Hartley et al., 2012, Januchowski-Hartley et

258   al., 2011, Tran et al., 2016) to angling (Alós et al., 2014, Alós et al., 2015, Colefax et al., 2016,

259   Cooke et al., 2017, Twardek et al., 2017). Agreeing with our results, previous studies have also

260   shown that this response is species-specific and mainly shown by the most intensively targeted

261   species (e.g. Colefax et al., 2016, Alós et al., 2015). We found that outside the MPA, the mean

262   FID value were always larger than 4 m (except for *S. tinca*), which constitutes the maximum

263   shooting range of the most commonly used spearguns (personal observations: VS, LM, EA) and

264   strongly reduces the effectiveness of underwater human predation and elevates fish survival and

265   hence fitness in the harvested areas. This finding means that common metrics used to assess fish

266   abundance, such as catch rates or visual census, might strongly bias the perception about true

267   fish abundance and negatively affect fisher satisfaction (Alós et al., 2015, Arlinghaus et al.,

268   2017). Our findings also underscore the proposition that fish wariness assessed either visually or

269   by underwater cameras can be used as a sensitive indicator of changes in fishing pressure

270   (Goetze et al., 2017). Our findings in spearfishing can probably be extended to other

271   recreational fishing gears (Tsuboi et al., 2016, Alós et al., 2015) and may well apply to many

272   commercial gears as well (Arlinghaus et al., 2017).

273

274   *Risk recognition of human-induced predation threat*

275   Changes in the appearance of the observer (spearfisher *vs* snorkeler) were expected to

276   generate different responses among the targeted species. Our results demonstrate that fishes

277   were able to discriminate between different level of threats (spearfisher vs snorkeler), and this

278   ability varied among species according to their historical exposure to harvesting, body size and

279   level of protection against harvesting. Fishes are therefore able to differentially sense human-

280   induced predation risk and fine tune their behavioral response (Frid and Dill, 2002). In the case

281   of spearfishing, the optimal FID navigates the fundamental trade-off among energy acquisition

282   (e.g., time spent foraging) and the risk of being captured or attacked by natural predators

283   (Ydenberg and Dill, 1986), which has strong repercussions for individual fitness (Frid and Dill,

284   2002). Although fishes are generally known to accomplish this trade-off when being exposed to

285   natural predators (e.g. Werner and Hall, 1974), our work shows that the same is also valid for

286   underwater human predators. For example, among the studied species, the historically most

287   exploited ones, *D. sargus* and *D. vulgaris* (Lloret et al., 2008, Rocklin et al., 2011), showed a

288   steep increase of FID along the gradient of human-induced predation risk. The recognition of

289   spearfishers as a potential predator adds important information to the generally small and partly

290   contradictory body of literature on this topic. For example, the presence of a speargun was not

291   associated with a specific response in exploited parrotfishes in a previous study (Januchowski-

292   Hartley et al., 2012), whilst the unexploited lined bristletooth (*Ctenochaetus striatus*) was found

293   to be able to discriminate between a snorkeler with or without a speargun (Tran et al., 2016).

294   Our results support the hypothesis that the response to spearfishers is species-specific and

295   probably harvest-dependent, and that the most heavily exploited species and fish sizes within a

296   species are able to use visual cues to assess human-predation risk and properly allocate

297   resources to avoid the risk to being harmed or captured, similar to the case in natural predation

298   (Kelley and Magurran, 2003, McCormick and Manassa, 2008). Also, a sizable literature on

299   catch-and-release recreational angling has shown that fishes are able to develop rapid hook

300   avoidance (Klefoth et al., 2013) and that previous catch-and-release results in behavioral

301   alteration and the seeking of refugees (Klefoth et al., 2008).

302

303   *The ability of different sized fishes to respond to human-induced predation risk*

304   The positive relationship between individual size and FID was a common pattern in all

305   the five studied species. This result might be explained by a lower risk-taking tendency

306   associated with the higher reproductive value of large individuals (Gotanda et al., 2009) or

307   simply be a natural outcome of learning processes through development. The finding can also

308   be explained by fisheries-induced evolution of increased timidity that is most expressed in the

309   most vulnerable morphological or ontogenetic stage. Our field methods were not intended to

310   differentiate among behavioral plasticity and evolutionary responses. Nevertheless, size effects

311   were significantly stronger in highly exploited fish species (specifically *D. sargus),* and this

312   could support a plastic hypothesis. Therefore, the observed changes in FID should be mainly

313   caused by previous experience with spearfishers across ontogeny, even if evolutionary

314   explanations cannot be ruled out. In the MPAs the size effect in *D. sargus* was stronger in

315   presence of a spearfisher than when confronting a non-threatening snorkeler. This may be

316   explained by experiences acquired beyond the boundaries of protected areas, because some *D.*

317   *sargus* individuals are known to visit areas outside the MPAs (e.g. Di Lorenzo et al., 2014)

318   where they may experience spearfishing and learn. Otherwise, larger individuals could have

319   experienced spearfishing predation risk inside the MPA due to poaching activities. Beside the

320   relative contribution of these two not mutually exclusive explanations, such sharp differences in

321   the size effect within and outside MPA confirm the fish wariness as a suitable indicator of

322   human-induced predation risk in selected species (Goetze et al., 2017).

323

324 *Mechanisms underlying the FID responses*

325   The timidity syndrome caused by exploitation has been explained by two main

326 mechanisms (Arlinghaus et al., 2017): the first is related to a genetic basis for those behaviors

327 that contribute to fishing vulnerability, as demonstrated in recreational angling (Klefoth et al.,

328 2013, Philipp et al., 2009). This means that selective forces induced by human predation are

329 operating on genetic components of those traits controlling FID, leading to fisheries-induced

330 evolution of behavior (Heino et al., 2015, Uusi-Heikkilä et al., 2008). The second mechanism

331 relates to a purely plastic behavioral response of fish that is mediated by learning processes to

332 avoid exposure to fishing. This can be achieved by both individual (private, e.g., catch-and-

333 release or spearfishing-induced injury) and social experiences (e.g., seeing conspecifics being

334 injured or captured). It is highly likely that, similar to the case in life-history traits, behavioral

335 plasticity overrules evolutionary effects, but previous common-garden-experiments using both

336 laboratory models as well as wild-living populations raised in laboratories have shown that size-

337 selective harvesting can indeed evolutionarily lead to timidity (Uusi-Heikkilä et al., 2015,

338 Tsuboi et al., 2016).

339   On different timescales, both plastic and evolutionary responses may contribute to the

340 modulation of FID. However, considering the broad connectivity of marine systems and the

341 often-observed absence of genetic structuring for many marine fish populations (see Sahyoun et

342 al., 2016, Pujolar et al., 2013), the observed increase of FID outside the MPA seems more likely

343 explained by learning processes than by genetic causes. A stronger role of learning mechanisms

344 is also supported by the fact that periodically harvested fishing closures resulted in a decrease of

345 FID (Januchowski-Hartley et al., 2014). Nevertheless, the hypothesis of an ongoing

346 spearfishing-induced selection of FID cannot be excluded and should be tested by common

347 garden experiments or by reciprocal transplants in the wild (Diaz Pauli and Heino, 2014).

348

349 **CONCLUSIONS**

350   Our study provides a comprehensive assessment of how spearfishing modulates fish

351   anti-human-predator behavior in the wild commensurate with the level and intensity of

352   harvesting pressure directed at given species, sizes and sites. The minimum distance to which a

353   fish can be approached by a human predator can be related conceptually to the safe operating

354   space concept developed by Rockstrom et al. (2009). We suggest that spearfishing

355   systematically increases the safe operating distance of fishes, which benefits individual fitness

356   and conservation and harms spearfisher`s catch rates. The behavior adopted by the observer in

357   this study (chasing the fishes from the surface) constitutes an active harvesting technique where

358   the ultimate decision to catch a fish is taken by the spearfisher. This demonstrates that a timidity

359   syndrome is also possible by means of active fishing gears and not only by passive fishing gear

360   types as previously argued (Diaz Pauli and Sih, 2017, Arlinghaus et al., 2017). If spearfishers

361   want to maintain high catch rates, either implementation of rotating closures, careful constraints

362   on local spearfishing pressure and implementation of a network of marine protected areas with

363   spill-over of naïve fishes to unprotected sites (Januchowski-Hartley et al., 2013) can be

364   recommended. These means should recover boldness of previously exploited fishes and

365   maintain catch rates high. However, if spearfishing systems remain unmanaged or poorly

366   managed, constant erosion of catchability is likely to occur, which reduces the index-value of

367   spearfishing data to index abundance and in extreme cases may also harm non-threatening

368   snorkelers by reducing visual contacts with fish. Such changes could fuel competition among

369   spearfishers and lead to social and political conflict.

370

371   **Acknowledgments**

376

377   **Authors' contribution**

378   EA conceived and designed the study with the contribution of LM; LM acquired data;

379   VS, LB, BW supported during acquisition of data; VS and RA statistically analysed the data,

380   VS, EA and RA interpreted the data, and VS led the writing of the manuscript with inputs by all

381   other co-authors. All authors gave final approval for publication.

382

383   **Data Accessibility**

384   Data will be available on Dryad (http://datadryad.org/) upon acceptance of the paper.

385

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571

572 **Table 1.** The number of observations collected for each species or group of species in each Western Mediterranean areas (Banyuls, Bonifacio, Medes) and for

573 each treatment (Snork-P: snorkeling inside the marine protected area; Spear-P: spearfishing inside the marine protected area; Snork-NP: snorkeling outside the

574 marine protected area; Spear-NP: spearfishing outside the marine protected area)

Species	Banyuls				Bonifacio				Medes				Total
	Snork-P	Spear-P	Snork-NP	Spear-NP	Snork-P	Spear-P	Snork-NP	Spear-NP	Snork-P	Spear-P	Snork-NP	Spear-NP	
S. tinca	29	28	31	30	43	29	28	26	27	25	19	30	345
S. salpa	19	8	7	14	21	18	11	9	23	17	13	17	177
mugilidae	18	17	3	9	3	5	8	11	22	20	5	9	130
D. vulgaris	34	19	30	47	20	20	15	19	21	13	20	28	286
D. sargus	68	37	32	37	34	30	20	16	25	33	32	26	390

575 **Table 2.** The results of the liner mixed model implemented for Flight Initiation Distance (FID). The

576 fixed factors, the covariate and their interactions are reported together with degree of freedom (df), the

577 Chi-square value statistics (X^2) and the p value (p). The overall mean estimation of FID is reported for

578 each species together with standard error in parentheses. Letters indicate the output of the Tukey post

579 hoc test (a > b > c). See Table 3 for further results.

Predictor variables	df	X^2	p
Size	1	390.19	< 0.0001
Species	4	379.32	< 0.0001
Treatment	3	858.54	< 0.0001
Size × Treatment	3	52.96	< 0.0001
Size × Species	4	66.10	< 0.0001
Treatment × Species	12	115.27	< 0.0001
Size × Treatment × Species	12	37.47	< 0.0001

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582 **Table 3.** The results of the linear mixed model implemented for flight initiation distance (FID).

583 The estimations of FID are related to the significant three way interactions reported in Table 2

584 (Size × Treatment × Species). The estimation of FID within the same species along the

585 treatments are reported in the left part of the table (grey area), while the estimation of FID

586 within the same treatment along the species are reported in the right part (white area). Species

587 are listed according to an increasing gradient of spearfishing pressure and treatments (Snork-P:

588 snorkeling inside the marine protected area; Spear-P: spearfishing inside the marine protected

589 area; Snork-NP: snorkeling outside the marine protected area; Spear-NP: spearfishing outside

590 the marine protected area) following an increasing gradient of threat. The mean FID values

591 adjusted for the average size (22.5 cm) and the effect of size on FID (slope) are reported

592 together with the standard error in parentheses. Letters indicate the output of the Tukey post hoc

593 test (a > b > c). † Indicates a trend between groups (p<0.10).

| Within species	Mean FID (SE)	Size effect (SE)	Within treatmnet	Mean FID (SE)	Size effect (SE)
S.tinca			**Snork-P**		
Snork-P	(a) 1.51 (0.33)	0.09 (0.03)	*S.tinca*	(b) 1.51 (0.33)	0.09 (0.03)
Spear-P	(b) 1.18 (0.34)	0.05 (0.03)	*S.salpa*	(ab) 1.85 (0.36)	0.04 (0.02)
Snork-NP	(a) 2.09 (0.36)	0.08 (0.03)	*mugilidae*	(ab) 2.64 (0.50)	0.01 (0.02)
Spear-NP	(a) 2.55 (0.38)	0.06 (0.03)	*D. vulgaris*	(ab) 2.54 (0.37)	0.09 (0.04)
S.salpa			*D.sargus*	(a) 2.48 (0.32)	0.09 (0.02)
Snork-P	(b) 1.85 (0.36)	0.04 (0.02)	**Spear-P**		
Spear-P	(b) 1.95 (0.45)	0.06 (0.04)	*S.tinca*	(b) 1.18 (0.34)	(b) 0.05 (0.03)
Snork-NP	(a) 4.39 (0.40)	0.15 (0.03)	*S.salpa*	(ab) 1.95 (0.45)	(ab) 0.06 (0.04)
Spear-NP	(a) 5.65 (0.43)	0.14 (0.04)	*mugilidae*	(ab) 2.33 (0.51)	(ab) 0.10 (0.03)
mugilidae			*D. vulgaris*	(a) 2.50 (0.39)	(ab) 0.10 (0.05)
Snork-P	(bc) 2.64 (0.50)	(b) 0.01 (0.02)	*D.sargus*	(a) 2.72 (0.37)	(a) 0.18 (0.02)
Spear-P	†(c) 2.33 (0.51)	(ab) 0.10 (0.03)	**Snork-NP**		
Snork-NP	†(b) 4.31 (0.47)	(a) 0.18 (0.03)	*S.tinca*	(b) 2.09 (0.36)	(b) 0.08 (0.03)
Spear-NP	(a) 6.58 (0.46)	(ab) 0.11 (0.03)	*S.salpa*	(a) 4.39 (0.40)	(ab) 0.15 (0.03)
D. vulgaris			*mugilidae*	(a) 4.31 (0.47)	(ab) 0.18 (0.03)
Snork-P	(c) 2.54 (0.37)	0.09 (0.04)	*D. vulgaris*	(a) 4.69 (0.40)	(ab) 0.12 (0.04)
Spear-P	(c) 2.50 (0.39)	0.10 (0.05)	*D.sargus*	(a) 5.37 (0.33)	(a) 0.22 (0.02)
Snork-NP	(b) 4.69 (0.40)	0.12 (0.04)	**Spear-NP**		
Spear-NP	(a) 7.67 (0.44)	0.27 (0.04)	*S.tinca*	(c) 2.55 (0.38)	(c) 0.06 (0.03)
D.sargus			*S.salpa*	(b) 5.65 (0.43)	(b) 0.14 (0.04)
Snork-P	(c) 2.48 (0.32)	(c) 0.09 (0.02)	*mugilidae*	(ab) 6.58 (0.46)	(b) 0.11 (0.03)
Spear-P	(c) 2.72 (0.37)	(b) 0.18 (0.02)	*D. vulgaris*	(a) 7.67 (0.44)	(ab) 0.27 (0.04)
Snork-NP	(b) 5.37 (0.33)	(ab) 0.22 (0.02)	*D.sargus*	(a) 7.38 (0.33)	(a) 0.31 (0.02)
Spear-NP	(a) 7.38 (0.33)	(a) 0.31 (0.02)			

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597   **Figure 1.**
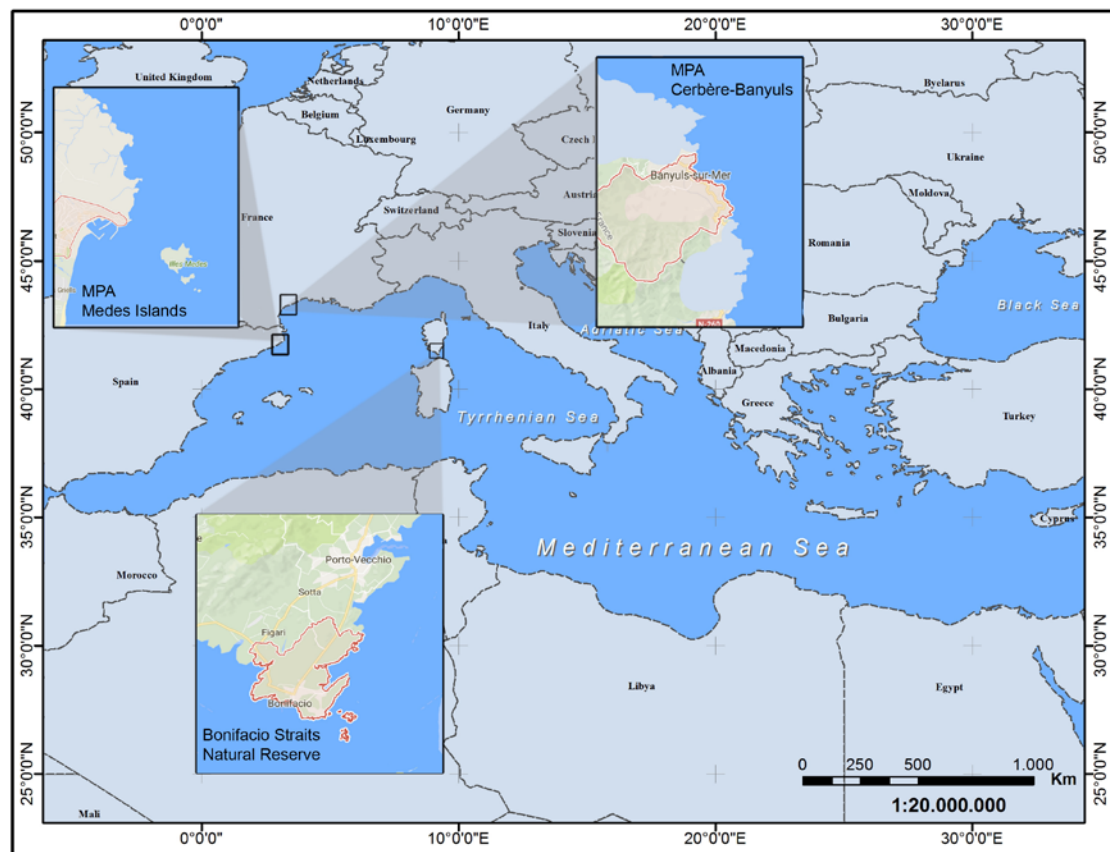
598   Areas of study. Surveys were performed in and out three protected areas: the Bonifacio Straits

599   Natural Reserve (Corsica), the Marine Protected Area of of Cerbère-Banyuls (France) and the

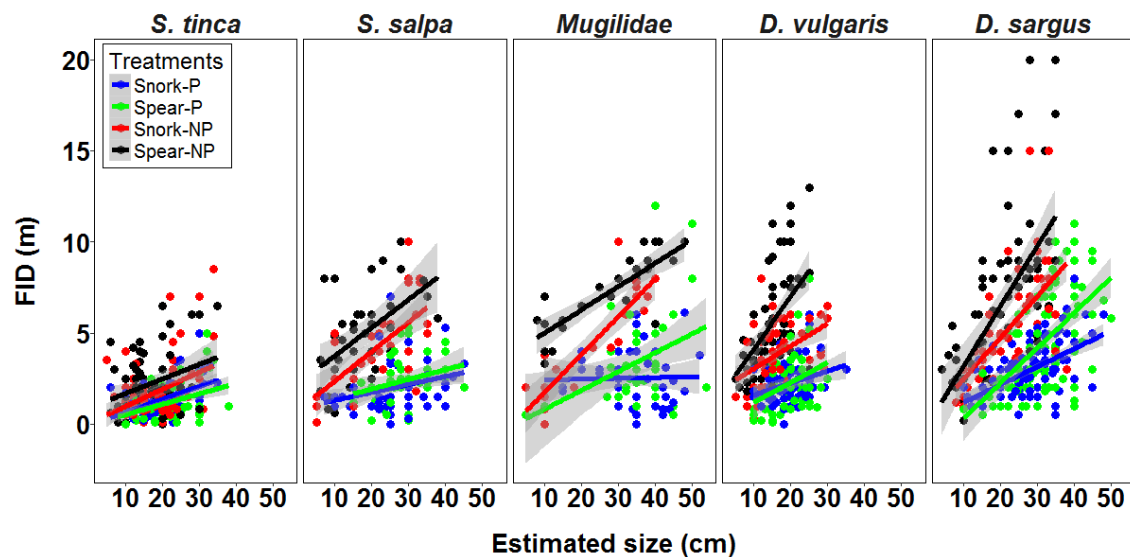600   Marine Protected Area of Medes islands (Spain) in the Mediterranean Sea.

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Figure 2. The raw flight initiation distance measurements (dots) are presented for each species

listed according to an increasing gradient of spearfishing pressure for the four treatments in

function of the estimated size of fishes. The different colors represent the four treatments (blue:

snorkeling in marine protected area; green: spearfishing in marine protected area; red:

snorkeling outside marine protected area; black: spearfishing outside marine protected area)

used in this study. The solid lines represent the linear correlation between FID and size

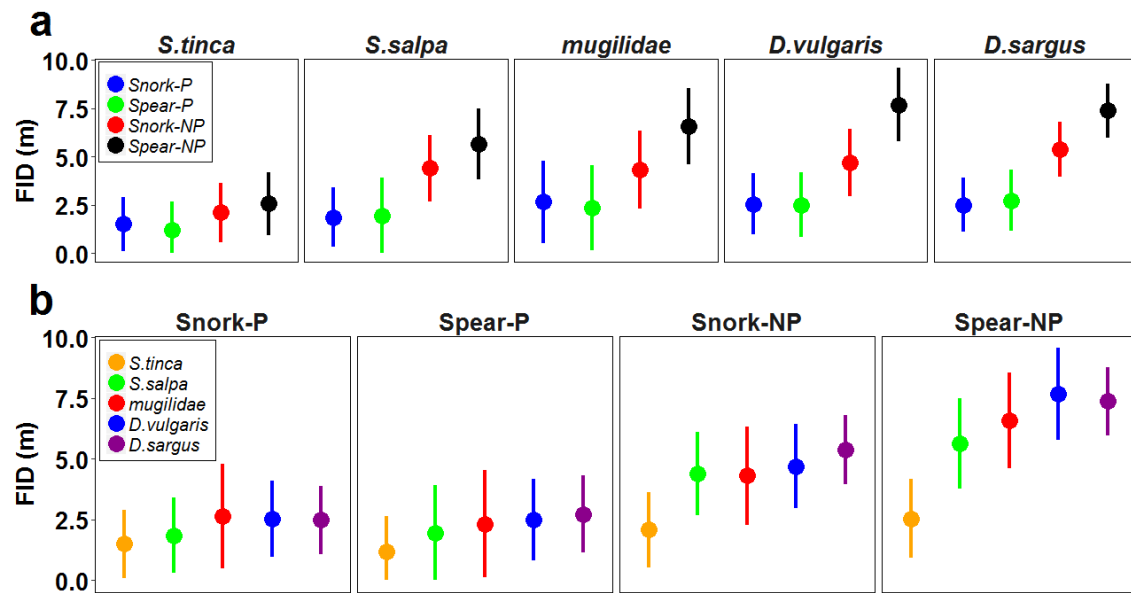expressed together with the 95% confidence interval (grey area).

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618 **Figure 3.** The results of the linear mixed model implemented for FID are reported to better

619 visualize the information shown in Table 3. The estimation of FID are related to the significant

620 three way interactions reported in Table 2 (Size × Treatment × Species). The mean estimation of

621 FID adjusted for the average (22.5 cm) size effect are reported: (a) within the same species for

622 each treatment; (b) within the same treatment for each species. In both cases, species and

623 treatments are listed according to an increasing gradient of spearfishing pressure and threat,

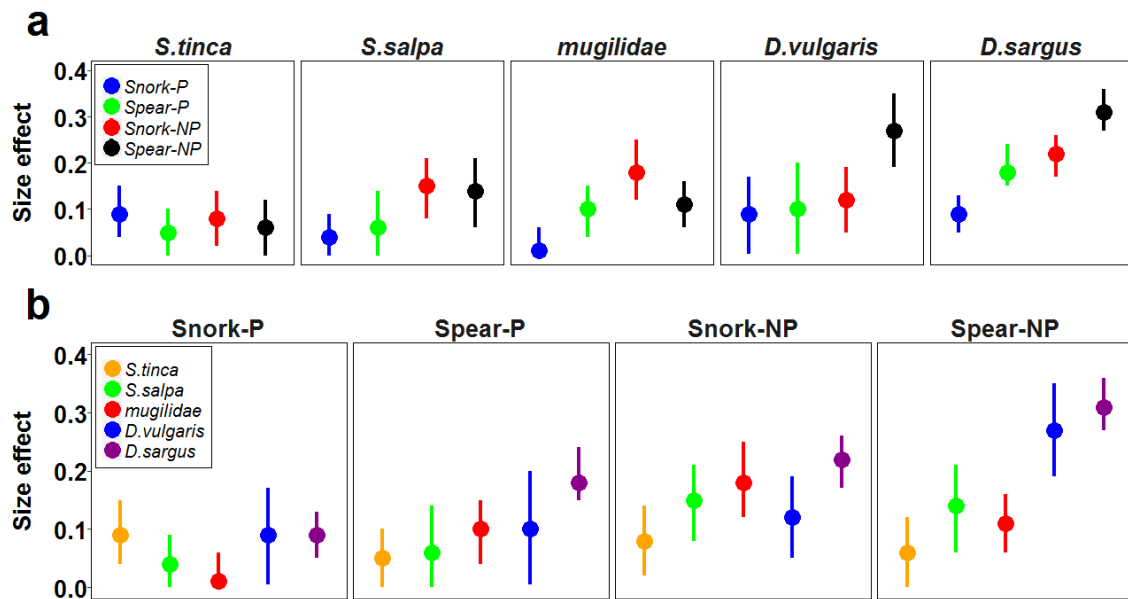624 respectively. Vertical lines indicate the 95% confidence interval.

625

Figure 4. The results of the linear mixed model implemented for FID are reported to better visualize the information present in Table 3. The estimation of the FID are related to the size effect of three way interactions reported in Table 2 (Size $\times$ Treatment $\times$ Species). The estimation of the linear correlation between size and FID (slopes) are reported: (a) within the same species for each treatment; (b) within the same treatment for each species. In both cases, species and treatment are listed according to an increasing gradient of spearfishing pressure and threat, respectively. Vertical lines indicate the 95% confidence interval.