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# The effects of familiarity on escape responses in the Trinidadian guppy (*Poecilia reticulata*)

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Predation is the main driver of mortality during early life stages. The ability to avoid and evade potential threats is, therefore, favoured to evolve during the early stages of life. It is also during these early stages that the process of familiarization occurs. It has long been recognized that associating with familiar individuals confers anti predator benefits. Less, however, is known about how predator evasion is affected by social experience during early stages. In this study we test the hypothesis that familiarization acquired during early life stages improves anti predator escape responses. Using the Trinidadian guppy we examine the effect of different early social conditions in the three main components of predator evasion. Using high-speed motion analysis we compared the responsiveness, reactive distance and magnitude of the response (maximum speed, maximum acceleration and distance) of the response to a visual stimulus in groups composed either of familiar or non-familiar individuals. Surprisingly, groups composed by familiar individuals were less responsive than groups of unfamiliar individuals. It is plausible that familiarity equips individuals with better skills to accurately assess the threat avoiding false alarms. Reactive distance and magnitude of response were more dependent on individual size than on familiarity. Larger individuals reached higher maximum speeds and total distances in their escape response. Our approach allowed us to tease apart which aspects of an escape response are more likely to be influenced by early social conditions.

1 The effects of familiarity on escape responses in the Trinidadian guppy

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18 ABSTRACT

19 Predation is the main driver of mortality during early life stages. The ability to avoid and evade  
20 potential threats is, therefore, favoured to evolve during the early stages of life. It is also during  
21 these early stages that the process of familiarization occurs. It has long been recognized that  
22 associating with familiar individuals confers antipredator benefits. Less, however, is known  
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33 individual size than on familiarity. Larger individuals reached higher maximum speeds and total  
34 distances in their escape response. Our approach allowed us to tease apart which aspects of an  
35 escape response are more likely to be influenced by early social conditions.

36

37

38 INTRODUCTION

39           Predation is a powerful agent of mortality, particularly during early life stages when  
40 organisms are at heightened risk due to their smaller size (Cushing 1974). Natural selection is  
41 therefore expected to favour the development of antipredator behaviours early in life  
42 (Braithwaite & Salvanes 2005; Vilhunen & Hirvonen 2003). Antipredator behaviours are  
43 generally divided into two major types; 1) avoidance and 2) evasion (Fuiman & Magurran 1994;  
44 Weihs & Webb 1984). Avoidance includes any pre-emptive behaviours in which the individual  
45 reduces the likelihood of encountering a predator and consequently of its attack (Fuiman &  
46 Magurran 1994). Evasion, on the other hand, occurs once the predator initiates the attack. As  
47 predator avoidance is not always possible, successful predator evasion tactics are essential for  
48 survival. The behaviour and frequency at which each evasion tactic is employed is context-  
49 dependent; individuals adopt behaviours that improve their evasive response and, thus, enhance  
50 survival (Domenici 2010).

51           One way in which organisms may reduce the risk of predation is by associating with  
52 others, either by schooling or just by joining a group (Ruxton & Johnsen 2016; Ward & Webster  
53 2016). Though groups might be more conspicuous for a predator, each individual within the  
54 group has a smaller probability of being predated than if alone. Among the group antipredator  
55 benefits of enhanced vigilance, dilution of risk, predator confusion and coordinated antipredator  
56 maneuverers (Krause & Ruxton 2002; Ward & Webster 2016), there is strong evidence showing  
57 that familiarity within individuals in a group enhances antipredator behaviours (Griffiths et al.  
58 2004). Familiarity can be broadly defined as the ability to discriminate between individuals  
59 based on previous interactions (Griffiths 2003). The process of familiarization is based on visual,  
60 auditory and olfactory cues (Coffin et al. 2011; Reby et al. 2001; Zajitschek & Brooks 2008).  
61 Repeated interactions and resource-sharing leads to the familiarization. Joining a group

62 composed of familiar conspecifics brings greater fitness benefits than joining a group composed  
63 of unfamiliar individuals (Barber & Wright 2001; Griffiths & Magurran 1997b).

64         The benefits in associating with familiar individuals for the development and acquisition  
65 of successful antipredator behaviours and responses are acknowledged (Ward & Hart 2003).  
66 There is evidence that groups composed by familiar individuals are more cohesive and have  
67 reduced neighbour distance (Chivers et al. 1995; Höjesjö et al. 1998), characteristics which  
68 enhance predator confusion and dilute individual risk. Further, familiar groups have been found  
69 to have reduced within-group aggression and evolve more stable social hierarchies (Griffiths et  
70 al. 2004; Höjesjö et al. 1998; Johnsson 1997; Tanner & Keller 2012). Reduced aggression within  
71 familiar groups allows more time for predator vigilance, which may improve escape latency  
72 (Griffiths et al. 2004; Strodl & Schausberger 2012). Additionally, individuals are more likely to  
73 perform cooperative antipredator behaviours when in familiar groups, as they will have an idea  
74 of whether the others have behaved cooperatively in the past (Dugatkin & Alfieri 1991). For  
75 example, individuals in familiar groups may be more likely to perform more risky antipredator  
76 manoeuvres (Chivers et al. 1995), join predator mobbing (Grabowska-Zhang et al. 2012), or  
77 perform predator inspection (Dugatkin & Godin 1992). Such antipredator behaviours put  
78 individuals at higher risk, but improve group antipredator response.

79         While the effect and importance of familiarity on predator avoidance is well recognised,  
80 how familiarity shapes predator evasion, particularly the escape response, remains fairly  
81 unexplored. Furthermore, studies to date only focus on the effect of familiarity on the latency of  
82 the escape response (Griffiths et al. 2004; Strodl & Schausberger 2012). Successful escape  
83 responses depend on various components, such as latency, velocity and distance travelled in the  
84 response (Domenici & Blake 1997). For instance, latency considered as the time between the

85 onset of the predator attack and the start of the response is crucial for the outcome of the  
86 interaction (Fuiman et al. 2006). Also, an effective response requires moving away from the  
87 attack trajectory fast enough so the predator can not adjust it (Fuiman & Cowan 2003). Given the  
88 context-dependent nature of escape responses, it is possible that familiarity may aid predator  
89 escape by improving certain aspects of the escape response. The aim of this study was to address  
90 the role of familiarity acquired during early life stages in shaping the different components of the  
91 antipredator escape responses in the Trinidadian guppy (*Poecilia reticulata*).

92 Guppies shoal immediately after birth, and it is during these early stages that, by  
93 interacting with other individuals within the group, that the ability to discriminate between  
94 familiar and unfamiliar starts (Laland et al. 2003; Magurran et al. 1994). The importance of early  
95 conditions for the establishment and reinforcement of individual discrimination in guppies has  
96 been extensively studied (Barbosa et al. 2016; Barbosa et al. 2013; Chapman et al. 2008a;  
97 Chapman et al. 2008b). Guppies respond to a predator attack by performing a “fast-start” escape  
98 response, characteristic to most fish species (Dial et al. 2015). This evasion tactic consists of an  
99 unambiguous quick and sudden burst of swimming activity usually of only tenths of a second  
100 that propels the fish away from an oncoming predator (Domenici & Blake 1997; Webb 1978;  
101 Weihs 1973). Fast-start escape responses integrate a combination of behavioural and kinematic  
102 components (Marras et al. 2011), both of which were examined in this study.

103 In view of the evidence of the antipredator benefits of familiarity, we predicted that  
104 juvenile guppies are also more responsive and perform more successful escape responses when  
105 in groups of familiar conspecifics. To test this hypothesis, we exposed familiar and unfamiliar  
106 groups of juvenile guppies to a digital display of a looming object and quantified the difference  
107 in responsiveness (number of fish responding), reactive distance (based on the size of the

108 stimulus when the response started) and magnitude of the escape response (maximum speed and  
109 acceleration achieved during the response, and distance covered by the escaping fish). This  
110 approach allows us to identify the role of familiarity in a behaviour closely related to survival  
111 during early life stages and to pinpoint which components of an escape response are more likely  
112 to be affected by social experience.

113

## 114 METHODS

115 All guppies used were descendants of individuals collected from the Lower sections of  
116 the Tacarigua River in Trinidad. Several species of fish predators have been reported in this  
117 locality including the pike cichlid (*Crenicichla alta*), the blue acara (*Aequidens pulcher*) and the  
118 wolf fish (*Hoplias malabaricus*), which also prey intensively on juvenile guppies (Magurran &  
119 Seghers 1994). Experimental fish were housed, and all observations recorded, at the aquarium  
120 facility at the Sir Harold Mitchell Building, University of St Andrews, UK. The aquarium has an  
121 air temperature control system, which kept the tank temperatures at a mean ( $\pm$ SD) temperature of  
122 24.5°C ( $\pm$  0.3 °C). All stock tanks contained similar numbers of males, females and juveniles.  
123 Lighting conditions followed a 12-hour light/dark cycle. All fish were fed daily with TetraMin®  
124 flake food.

125

### 126 Test Fish Collection and Rearing

127 Prior to the experiment, we collected three juveniles from three different stock tanks that  
128 contained a mix of males, females and juveniles using a dip net. This ensured that the test groups  
129 were composed neither of familiar conspecifics nor of close kin. Juveniles were allocated to a 20



130 x 22 x 30 cm holding tank to create a test group. A total of 42 holding tanks were used. Black  
131 plastic sheets were placed between each tank to ensure each test group was visually isolated from  
132 adjacent groups. Fish were of similar size and randomly distributed between holding tanks (mean  
133 ( $\pm$ SD) 10.8 ( $\pm$  1.7) mm). Nevertheless, in order to be able to identify each individual during  
134 tracking, test groups were carefully constituted of different sized individuals. This size disparity,  
135 however, was not different to the one observed in groups of juvenile fish shoaling in Trinidadian  
136 rivers (personal observation). Each test group remained in its holding tank for two weeks to  
137 ensure the establishment of familiarity between tank mates (Griffiths & Magurran 1997a).

### 138 Escape Response Trials

139 Six groups were tested each day, split into three ‘familiar’ and three ‘unfamiliar’ groups.  
140 In familiar groups, individuals were tested with those they shared the holding tank with for two  
141 weeks. Unfamiliar groups were treated as a control. For unfamiliar groups, we took one fish,  
142 each from a different holding tank, and put them together in the observation chamber for testing  
143 (Figure 1).

144 All tests occurred between 9:00 and 11:00 am and at least an hour after being fed. These  
145 measures were taken to avoid differences in satiation rate and time of day that might affect the  
146 behaviour of the individuals. The experimental setup used to assess escape response was based  
147 on an established protocol (Fuiman et al. 2010), but modified for this experiment (Figure 2).  
148 Each trial involved presenting a digital display of a looming object to a test group. The digital  
149 display consists 1.8-second sequence showing black oval in the middle of a white background  
150 that increases its size to simulate an approaching object (Supplementary Information). The same  
151 stimulus has been shown to elicit a startle response in larval fish of similar size (Fuiman et al.  
152 2006; Ojanguren & Fuiman 2010). The video was presented using a LCD screen (Braun 1210)

153 located 0.23 cm from a 10x10x10 cm glass test chamber. Water depth within the observation  
154 chamber was kept at 225 ml to minimise vertical movement in escape responses. This depth was  
155 within the range which juveniles would be likely to experience in the wild (Magurran 2005). For  
156 each trial, a test group was transported to the observation chamber and given at least 10 minutes  
157 of acclimatisation to their new surroundings before testing began.

158 Individual response to the visual stimulus was recorded at 240 frames s<sup>-1</sup> using a high-  
159 speed video camera (Casio EX-FH25 EXILM) through a 45°-angled mirror to obtain an overhead  
160 view of the observation chamber. The observation chamber sat on top of a black surface and was  
161 illuminated by lamps positioned left and right of the chamber so that the response could be  
162 clearly observed. All individuals tested were gently transferred to a small petri dish with a small  
163 amount of water (so no anaesthesia was required) and photographed from above. Individual  
164 standard length was measured to nearest millimetre using ImageJ analysis software (Abràmoff  
165 et al. 2004). All tested individuals resumed normal routine swimming activity immediately after  
166 the escape responses. No fish died during the tests and after the picture was taken. After the  
167 terminus of the study, all individuals returned to stock tanks.

168

### 169 Data Analysis

170 Video recordings were analysed frame by frame to determine responsiveness (the number  
171 of fish that responded to the stimulus in each test group) and the reactive distance (the virtual  
172 distance between the looming object and the first individual that responded, calculated from the  
173 size of the oval on the screen at the moment of the start of the response and the distance of the  
174 fish from the screen) (see (Fuiman et al. 2010) for details). This method allowed us to know the  
175 exact position of the fish and determine its speed and therefore calculate maximum speed,

176 maximum acceleration and total distance covered during the escape response (magnitude of the  
177 response).

178 Videos of the individual responses were imported to ImageJ and analysed frame-by-  
179 frame to determine the reactive distance and track the fish position during the response. Reactive  
180 distance was calculated by combining perceived distance of the looming object (displayed in the  
181 top left screen of digital display) at the frame of the start of the response with the distance of the  
182 head of the individual from the screen. The position of the fish in 2-dimensional coordinates for  
183 the overhead view was obtained using the manual tracking plugin in ImageJ (Cordelières 2005),  
184 this allowed us to calculate maximum speed, maximum acceleration and total distance covered in  
185 the response (see (Fuiman et al. 2010; Fuiman et al. 2006)).

186 The responsiveness of each test group was ranked according to the number of individuals  
187 within the group that responded (either 0, 1, 2 or 3). We considered that the response was over  
188 when the distance travelled between three consecutive frames (12.5 milliseconds) was 1 mm or  
189 less. Reactive distance, maximum speed, maximum acceleration and distance travelled during a  
190 response were measured on the first fish that responded. On the only trial that two fish responded  
191 in the same frame, the fish that had the larger reactive distance was considered the first  
192 responder. The first author performed sampling and motion analysis. Blind data collection was,  
193 therefore, not possible. Nevertheless, the strict criterion for defining escape characteristics  
194 minimizes any observation bias.

195

196 Statistical Analysis

197 Differences in responsiveness between familiar and unfamiliar groups were tested with a  
198 Wilcoxon rank sum test to account for the fact that responsiveness was a discrete variable. In  
199 order to investigate the effect of familiarity on reactive distance and in the magnitude of the  
200 response (maximum speed, maximum acceleration and distance covered in a response) we used  
201 General Linear Models (GLMs). Each full model included familiarity as main effect treatment  
202 and standard length as a covariate, as well as their interaction. Diagnostic plots revealed  
203 significant departures from normality of the residuals for both responses variables reactive  
204 distance and total distance. Normal distribution of residuals was achieved by log-transformation.  
205 We tested if all factors were needed in the minimal adequate model using Akaike's Information  
206 Criterion (Burnham & Anderson 2002). Specifically, we calculated  $\Delta AIC$ , the difference  
207 between the AIC of each model and that of the estimated best model (the model with the lowest  
208 AIC) (Supplementary Information). We also calculated Akaike weights, which are estimates of  
209 the probability that each model is the best in the model set, to assess uncertainty about which  
210 model is best (reflected in multiple models having similar Akaike weights). All analyses were  
211 performed in using R (Team 2015).

212

213 **RESULTS**

214

215 Responsiveness

216 A total of 42 groups composed by three different sized individuals were tested. Of the 30  
217 groups in which one or more individuals responded, 17 groups were familiar and 13 groups were

218 unfamiliar. There was a significant effect of familiarity on responsiveness (Wilcoxon rank sum:  
219  $W=1197, p < 0.005$ ) (Figure 3), where responsiveness was higher in unfamiliar groups. In the  
220 majority of familiar groups only one individual in the group responded, whereas the unfamiliar  
221 groups showed more instances where two or more individuals reacted to the stimulus.

222

### 223 Reactive distance

224 The best explanatory model for the effect of familiarity on reactive distance did include  
225 the main effects and interaction between standard length and treatment (Table 1, Figure 4A,  
226 Supplementary Information). We failed to detect an effect of familiarity on reactive distance  
227 ( $F_{1,28} = 0.194, p = 0.663$ ) (Figure 4A).

228

### 229 Magnitude of the response

230 The best selected GLM for explaining the effect of familiarity on maximum speed,  
231 maximum acceleration and distance did not include the interaction between standard length and  
232 treatment, but length was important as a covariate (Table 1, Figure 4B, C, Supplementary  
233 Information). There was no significant effect of familiarity on maximum speed ( $F_{1,27} = 2.53, p =$   
234  $0.123$ ), maximum acceleration ( $F_{1,27} = 3.47, p = 0.07$ ) or total distance ( $F_{1,27} = 2.34, p = 0.138$ ).  
235 Individual length, however, had a significant effect on maximum speed ( $F_{1,27} = 15.59, p = 0.004$ ),  
236 maximum acceleration ( $F_{1,27} = 6.42, p = 0.017$ ), and total distance ( $F_{1,27} = 12.17, p = 0.001$ )  
237 (Table 1, Figure 4, Supplementary Information).

238

## 239 DISCUSSION

240           A novel contribution of this study is that it examines the consequences of familiarity  
241 during early stages in the performance of escape responses separating the multiple aspects of the  
242 response to determine which parts depend on the social environment. Through high-speed  
243 analysis of the escape responses in familiar and unfamiliar groups of guppies, we were able to  
244 unambiguously demonstrate that familiarity plays a significant role in shaping how groups of  
245 fish respond to a stimulus. Unfamiliar groups had more individuals perform an escape response  
246 than those in familiar groups. A plausible explanation is that familiarity could allow individuals  
247 to be better able perceiving a lower threat from the stimulus. Rather unexpectedly, other  
248 components of the escape response, namely latency and magnitude, were not affected by  
249 familiarity. Furthermore, the speed and distance covered in the response were correlated with  
250 individual size rather than with level of familiarity within the group. In combination, our study  
251 suggests that, while familiarity affects how groups respond to a visual stimulus, it plays a less  
252 meaningful role in determining the quality of the escape response.

253           Our results are clear in demonstrating that familiarity affects group responsiveness. There  
254 were a greater number of individuals responding within each group among unfamiliar groups  
255 than among familiar groups. While most fish species rely on the escape response to avoid a  
256 potential predator (Domenici 2010; Fuiman & Magurran 1994), escaping may not always be the  
257 best strategy (Lima & Dill 1990; Ward & Webster 2016; Ydenberg & Dill 1986). If there is  
258 enough information to accurately predict the level of threat in a given environment, then it is  
259 advantageous for a prey to only flee when it is necessary for survival avoiding false alarms that  
260 could in turn attract the attention of nearby predators (Ward et al. 2011). For example, minnows  
261 performed antipredator behaviours in response to a realistic pike model, whereas an unrealistic

262 stimulus elicited no response (Magurran & Girling 1986). The lower responsiveness in familiar  
263 groups may be a result of improved vigilance. According to the theory of limited attention,  
264 performance is reduced when attention must be divided among different tasks (Dukas 2002).  
265 Therefore, if individuals are not spending time inspecting or acting aggressively toward group  
266 mates, as is often found among unfamiliar individuals (Griffiths et al. 2004; Johnsson 1997;  
267 Tanner & Keller 2012), then they are likely to have more time to dedicate to other tasks, such as  
268 predator vigilance (Strodl & Schausberger 2012; Strodl & Schausberger 2013; Zach et al. 2012).  
269 Guppies from familiar groups may have been able to accurately assess the non-threatening nature  
270 of the stimulus. Contrastingly, unfamiliar groups may have been more skittish and, thus more  
271 likely to be startled by the stimulus. Interacting with unfamiliar individuals can be stressful  
272 (Choleris et al. 1998), particularly if such interactions are associated with increased aggression  
273 (Galef et al. 1984). Individuals may perceive higher risk when shoaling with unfamiliar  
274 conspecifics, as was found in fathead minnows who had a higher production of epidermal alarm  
275 substance cells when in unfamiliar shoals than familiar shoals (Wisenden & Smith 1998).  
276 Furthermore, escape responses from the digital display may be misinterpreted as an attack by the  
277 other group mates. Aggression is common among guppies, in both natural as well as laboratory  
278 conditions (Magurran 2005; Thibault 1974). Therefore, it is plausible that an individual guppy  
279 would flee from an unfamiliar group mate that is performing a fast-start response, as this could  
280 be misinterpreted as an attack.

281           We failed to detect an effect of familiarity on the reactive distance of an escape response.  
282 Comparable studies have found that familiarity reduces the latency of an escape response. For  
283 example group-living mites *Phytoseiulus persimilis* reacted more quickly to an attack of a  
284 predator when they were in a familiar pair (Strodl & Schausberger 2012). Similarly, familiar

285 juvenile brown trout responded 14% faster than unfamiliar ones when exposed to a simulated  
286 predator attack (Griffiths et al. 2004). In both studies reduction in reaction time has been  
287 attributed to the associated benefits of the theory of limited attention. Our results therefore  
288 indicate that familiarity is more important in antipredator behaviours earlier in a predator  
289 sequence. A predator must successfully encounter, attack and capture a prey, where a prey's  
290 strategy is to interrupt this sequence. It has been suggested that avoiding the encounter and attack  
291 are a prey's best strategy (Fuiman & Magurran 1994). Previous experiments included an entire  
292 predator interaction, such as a model heron swinging forward and plunging its beak into the  
293 water (Griffiths et al. 2004) or a live predator (Strodl & Schausberger 2012), and could, therefore  
294 elicit such behaviours. In contrast, our experiment only elicited behaviours seen in the last few  
295 milliseconds of the attack.

296       Familiarity has been found to enhance avoidance tactics. For example, predator confusion  
297 was enhanced in shoals of familiar fathead minnows that had reduced neighbour distance and  
298 more shoal cohesion in response to predator stimuli compared to unfamiliar shoals (Chivers et al.  
299 1995). Tighter shoal cohesion reduces the probability of being captured by a predator (Mathis &  
300 Smith 1993). In addition, familiar shoals exhibited a greater number of predator inspections with  
301 more inspectors per inspection when faced with a model pike (Chivers et al. 1995). Predator  
302 inspection, where an individual or small group of individuals approach a predator, pause and  
303 swim away (Pitcher 1992), enables prey to gain valuable information on the threat of a predator.  
304 This behaviour, though risky to inspectors, is associated with improved avoidance of a predator  
305 attack (Godin & Davis 1995; Magurran 1990; Magurran & Pitcher 1987). Therefore, it is likely  
306 that familiarity is more crucial in antipredator behaviour associated with predator avoidance than  
307 predator evasion.



308           The effect of familiarity on the magnitude of the response was not significant. It is  
309 recognized that there is a crucial need to implement an integrative approach that accounts for all  
310 aspects of an escape response in order to obtain a clear understanding of the mechanisms of  
311 response to a predator (Domenici 2010). While other behavioural variables may affect the  
312 magnitude of an escape response, our study provides strong evidence that familiarity is not one  
313 of them. Our results showed that size had a far greater effect in the magnitude of the response  
314 than familiarity. This result is consistent with previous studies that have shown that the  
315 magnitude of the fast-start response in young fish increases with body length (Dial et al. 2015).  
316 While behavioural effects on the locomotive performance cannot be ruled out (Domenici 2010),  
317 our study and others (Gibb et al. 2006; Ojanguren & Braña 2003) lend strong support that the  
318 magnitude of a fast-start response is largely determined by morphology, rather than by social  
319 conditions.

320           In this study we provided a strong test for the relative effect of familiarity in modulating  
321 predator avoidance behaviour measuring several aspects of the escape responses using high  
322 speed video analysis. The familiarity effect on group responsiveness may be an adaptive  
323 response in which familiar groups have improved antipredator performance, as individuals  
324 conserve energy and are less conspicuous by not fleeing in a non-threatening situation.  
325 Nevertheless, further studies are necessary to elucidate this. Our results also suggest that the  
326 effects of familiarity on the response are perhaps unlikely to play a role on escape performance  
327 in the last few milliseconds of a predator attack. Instead, we believe that familiarity is more  
328 likely to affect behaviour earlier in a predator-prey interaction, which then affects the quality of  
329 the response. Taken together our study further contributes to previous ones, by distinguishing  
330 which components of an escape response are modulated by familiarity.

331

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336

337 REFERENCES

- 338 Abràmoff MD, Magalhães PJ, and Ram SJ. 2004. Image processing with ImageJ. *Biophotonics*  
339 *International* 11:36-43.
- 340 Barber I, and Wright HA. 2001. How strong are familiarity preferences in shoaling fish?  
341 *Animal Behaviour* 61:975-979. 10.1006/anbe.2000.1665
- 342 Barbosa M, Camacho-Cervantes M, and Ojanguren AF. 2016. Phenotype matching and early  
343 social conditions affect shoaling and exploration decisions *Ethology* 122:171-179.  
344 10.1111/eth.12455
- 345 Barbosa M, Ojanguren AF, and Magurran AE. 2013. Courtship display persists despite early  
346 social deprivation. *Ethology* 16:496-502. 10.1111/eth.12087
- 347 Braithwaite VA, and Salvanes AGV. 2005. Environmental variability in the early rearing  
348 environment generates behaviourally flexible cod: implications for rehabilitating  
349 wild populations. *Proceedings of the Royal Society B-Biological Sciences* 272:1107-  
350 1113. 10.1098/rspb.2005.3062
- 351 Burnham KP, and Anderson DR. 2002. *Model selection and multi-model inference: A*  
352 *practical information- Theoretic approach*. New York: Springer-Verlag.
- 353 Chapman BB, Morrell LJ, Benton TG, and Krause J. 2008a. Early interactions with adults  
354 mediate the development of predator defenses in guppies. *Behavioral Ecology*  
355 19:87-93. 10.1093/beheco/arm111
- 356 Chapman BB, Ward AJW, and Krause J. 2008b. Schooling and learning: early social  
357 environment predicts social learning ability in the guppy, *Poecilia reticulata*. *Animal*  
358 *Behaviour* 76:923-929. 10.1016/j.anbehav.2008.03.022
- 359 Chivers DP, Brown GE, and Smith RJF. 1995. Familiarity and shoal cohesion in fathead  
360 minnows (*Pimephales promelas*): implications for antipredator behaviour. *Canadian*  
361 *Journal of Zoology* 73:955-960. 10.1139/z95-111
- 362 Choleris E, Valsecchi P, Wang Y, Ferrari P, Kavaliers M, and Mainardi M. 1998. Social  
363 Learning of a Food Preference in Male and Female Mongolian Gerbils is Facilitated  
364 by the Anxiolytic, Chlordiazepoxide. *Pharmacology Biochemistry and Behavior*  
365 60:575-584. 10.1016/S0091-3057(98)00005-7

366 Coffin HR, Watters JV, and Mateo JM. 2011. Odor-Based Recognition of Familiar and Related  
367 Conspecifics: A First Test Conducted on Captive Humboldt Penguins (*Spheniscus*  
368 *humboldti*). *PLoS One* 6:e25002. 10.1371/journal.pone.0025002  
369 Cordelières FP. 2005. Manual Tracking. <http://rsbweb.nih.gov/ij/plugins/track/Manual>  
370 [Tracking plugin.pdf](http://rsbweb.nih.gov/ij/plugins/track/Manual).  
371 Cushing DH. 1974. The possible density-dependence of larval mortality and adult mortality  
372 in fishes. In: Blaxter JHS, editor. *The Early Life History of Fish*. New York: Springer-  
373 Verlag. p 103-111.  
374 Dial TR, Reznick DN, and Brainerd EL. 2015. Effects of neonatal size on maturity and escape  
375 performance in the Trinidadian guppy. *Functional Ecology* 30:943-952.  
376 10.1111/1365-2435.12565  
377 Domenici P. 2010. Context-dependent variability in the components of fish escape  
378 response: integrating locomotor performance and behavior. *Journal of Experimental*  
379 *Biology* 313:59-79.  
380 Domenici P, and Blake RW. 1997. The kinematics and performance of fish fast-start  
381 swimming. *The Journal of Experimental Biology* 200:1165-1178.  
382 Dugatkin LA, and Alfieri M. 1991. Guppies and the TIT FOR TAT Strategy: Preference Based  
383 on Past Interaction. *Behavioral Ecology and Sociobiology* 28:243-246.  
384 Dugatkin LA, and Godin J-GJ. 1992. Prey approaching predators: a cost-benefit perspective.  
385 *Annales Zoologici Fennici* 29:233-252.  
386 Dukas R. 2002. Behavioural and ecological consequences of limited attention. *Philosophical*  
387 *Transactions of the Royal Society B: Biological Sciences* 357:1539-1547.  
388 10.1098/rstb.2002.1063  
389 Fuiman LA, and Cowan JH. 2003. Behaviour and Recruitment Success in Fish Larvae:  
390 Repairability and Covariation of Survival Skills. *Ecology* 84:53-67. 10.1890/0012-  
391 9658(2003)084[0053:BARSIF]2.0.CO;2  
392 Fuiman LA, and Magurran AE. 1994. Development of predator defences in fishes. *Reviews in*  
393 *Fish Biology and Fisheries* 4:145-183. 10.1007/bf00044127  
394 Fuiman LA, Meekan MG, and McCormick MI. 2010. Maladaptive behavior reinforces a  
395 recruitment bottleneck in newly settled fishes. *Oecologia* 164:99-108.  
396 10.1007/s00442-010-1712-3  
397 Fuiman LA, Rose KA, Cowan Jr JH, and Smith EP. 2006. Survival skills required for predator  
398 evasion by fish larvae and their relation to laboratory measures of performance.  
399 *Animal Behaviour* 71:1389-1399. 10.1016/j.anbehav.2005.11.013  
400 Galef BG, Kennett DJ, and Wigmore SW. 1984. Transfer of information concerning distant  
401 foods in rats: A robust phenomenon. *Animal Learning & Behavior* 12:292-296.  
402 10.3758/bf03199970  
403 Gibb A, C., Swanson B, O., Wesp H, Landels C, and Liu C. 2006. Development of the Escape  
404 Response in Teleost Fishes: Do Ontogenetic Changes Enable Improved  
405 Performance? *Physiological and Biochemical Zoology* 79:7-19. doi:10.1086/498192  
406 Godin J-GJ, and Davis SA. 1995. Who Dares, Benefits: Predator Approach Behaviour in the  
407 Guppy (*Poecilia reticulata*) Deters Predator Pursuit. *Proceedings of the Royal Society*  
408 *of London B: Biological Sciences* 259:193-200. 10.1098/rspb.1995.0028  
409 Grabowska-Zhang AM, Sheldon BC, and Hinde CA. 2012. Long-term familiarity promotes  
410 joining in neighbour nest defence. *Biology Letters* 8:544-546.  
411 10.1098/rsbl.2012.0183

412 Griffiths SW. 2003. Learned recognition of conspecifics by fishes. *Fish and Fisheries* 4:256-  
413 268. 10.1046/j.1467-2979.2003.00129.x

414 Griffiths SW, Brockmark S, Höjesjö J, and Johnsson JI. 2004. Coping with divided attention:  
415 the advantage of familiarity. *Proceedings of the Royal Society of London B: Biological*  
416 *Sciences* 271:695-699. 10.1098/rspb.2003.2648

417 Griffiths SW, and Magurran AE. 1997a. Familiarity in schooling fish: how long does it take  
418 to acquire? *Animal Behaviour* 53:945-949. 10.1006/anbe.1996.0315

419 Griffiths SW, and Magurran AE. 1997b. Schooling Preferences for Familiar Fish Vary with  
420 Group Size in a Wild Guppy Population. *Proceedings: Biological Sciences* 264:547-  
421 551.

422 Höjesjö J, Johnsson JI, Petersson E, and Järvi T. 1998. The importance of being familiar:  
423 individual recognition and social behavior in sea trout (*Salmo trutta*). *Behavioral*  
424 *Ecology* 9:445-451. 10.1093/beheco/9.5.445

425 Johnsson JI. 1997. Individual Recognition Affects Aggression and Dominance Relations in  
426 Rainbow Trout, *Oncorhynchus mykiss*. *Ethology* 103:267-282. 10.1111/j.1439-  
427 0310.1997.tb00017.x

428 Krause J, and Ruxton GD. 2002. Living in groups. In: Evolution OSiEa, editor: New York:  
429 Oxford University Press. p 228.

430 Laland KN, Brown C, and Krause J. 2003. Learning in fishes: from three-second memory to  
431 culture. *Fish and Fisheries* 4:199-202. 10.1046/j.1467-2979.2003.00124.x

432 Lima SL, and Dill LM. 1990. Behavioural decisions made under the risk of predation: a  
433 review and prospectus. *Canadian Journal of Zoology* 68:619-640.

434 Magurran AE. 1990. The adaptive significance of schooling as an anti-predator defence in  
435 fish. *Annales Zoologici Fennici* 27:51-66.

436 Magurran AE. 2005. *Evolutionary Ecology: The Trinidadian Guppy*: Oxford University Press,  
437 New York.

438 Magurran AE, and Girling SL. 1986. Predator model recognition and response habituation  
439 in shoaling minnows. *Animal Behaviour* 34:510-518. 10.1016/S0003-  
440 3472(86)80119-1

441 Magurran AE, and Pitcher TJ. 1987. Provenance, Shoal Size and the Sociobiology of  
442 Predator-Evasion Behaviour in Minnow Shoals. *Proceedings of the Royal Society of*  
443 *London Series B Biological Sciences* 229:439-465. 10.1098/rspb.1987.0004

444 Magurran AE, and Seghers BH. 1994. Predator Inspection Behaviour Covaries with  
445 Schooling Tendency Amongst Wild Guppy, *Poecilia reticulata*, Populations in  
446 Trinidad. *Behaviour* 128:121-134.

447 Magurran AE, Seghers BH, Shaw PW, and Carvalho GR. 1994. Schooling preferences for  
448 familiar fish in the guppy, *Poecilia reticulata*. *Journal of Fish Biology* 45:401-406.  
449 10.1111/j.1095-8649.1994.tb01322.x

450 Marras S, Killen SS, Claireaux G, Domenici P, and McKenzie DJ. 2011. Behavioural and  
451 kinematic components of the fast-start escape response in fish: individual variation  
452 and temporal repeatability. *The Journal of Experimental Biology* 214:3102-3110.  
453 10.1242/jeb.056648

454 Mathis A, and Smith RJF. 1993. Chemical alarm signals increase the survival time of fathead  
455 minnows (*Pimephales promelas*) during encounters with northern pike (*Esox*  
456 *Lucius*). *Behavioral Ecology* 4:260-265. 10.1093/beheco/4.3.260

457 Ojanguren AF, and Braña F. 2003. Effects of size and morphology on swimming  
458 performance in juvenile brown trout (*Salmo trutta* L.). *Ecology of Freshwater Fish*  
459 12:241-246. 10.1046/j.1600-0633.2003.00016.x

460 Ojanguren AF, and Fuiman LA. 2010. Seasonal variability in antipredator performance of  
461 red drum larvae. *Marine Ecology Progress Series* 413:117-123. 10.3354/meps08697

462 Pitcher TE. 1992. Who dares, wins: the function and evolution of predator inspection  
463 behaviour in shoaling fish. *Netherlands Journal of Zoology* 42:371-391.

464 Reby D, Hewison M, Izquierdo M, and Dominique P. 2001. Red deer (*Cervus elaphus*) hinds  
465 discriminate between the roars of their current harem-holder stag and those of  
466 neighbouring stags. *Ethology* 107:951-959. 10.1046/j.1439-0310.2001.00732.x

467 Ruxton GD, and Johnsen S. 2016. The effect of aggregation on visibility in open water.  
468 *Proceedings of the Royal Society B: Biological Sciences* 283. 10.1098/rspb.2016.1463

469 Strodl MA, and Schausberger P. 2012. Social Familiarity Reduces Reaction Times and  
470 Enhances Survival of Group-Living Predatory Mites under the Risk of Predation.  
471 *PLoS One* 7:e43590. 10.1371/journal.pone.0043590

472 Strodl MA, and Schausberger P. 2013. Social familiarity relaxes the constraints of limited  
473 attention and enhances reproduction of group-living predatory mites. *Oikos*  
474 122:1217-1226. 10.1111/j.1600-0706.2012.20833.x

475 Tanner CJ, and Keller L. 2012. Nest distribution varies with dispersal method and  
476 familiarity-mediated aggression for two sympatric ants. *Animal Behaviour* 84:1151-  
477 1158. 10.1016/j.anbehav.2012.08.018

478 Team RC. 2015. R: A Language and Environment for Statistical Computing. R Foundation  
479 for Statistical Computing. R 2.15.3 ed. Vienna, Austria: R Foundation for Statistical  
480 Computing.

481 Thibault RE. 1974. Genetics of cannibalism in a viviparous fish and its relationship to  
482 population density. *Nature* 251:138 - 140.

483 Vilhunen S, and Hirvonen H. 2003. Innate antipredator responses of Arctic charr  
484 (*Salvelinus alpinus*) depend on predator species and their diet. *Behavioral Ecology*  
485 *and Sociobiology* 55:1-10. 10.1007/s00265-003-0670-8

486 Ward A, and Webster M. 2016. *Sociality: The Behaviour of Group-Living Animals*: Springer  
487 International Publishing.

488 Ward AJW, and Hart PJB. 2003. The effects of kin and familiarity on interactions between  
489 fish. *Fish and Fisheries* 4:348-358. 10.1046/j.1467-2979.2003.00135.x

490 Ward AJW, Herbert-Read JE, Sumpter DJT, and Krause J. 2011. Fast and accurate decisions  
491 through collective vigilance in fish shoals. *Proceedings of the National Academy of*  
492 *Sciences* 108:2312-2315. 10.1073/pnas.1007102108

493 Webb PW. 1978. Fast-start Performance and Body Form in Seven Species of Teleost Fish.  
494 *The Journal of Experimental Biology* 74:211-226.

495 Weihs D. 1973. The mechanism of rapid starting of slender fish. *Biorheology* 10:343-350.

496 Weihs D, and Webb PW. 1984. Optimal avoidance and evasion tactics in predator-prey  
497 interactions. *Journal of Theoretical Biology* 106:189-206.

498 Wisenden BD, and Smith RJF. 1998. A re-evaluation of the effect of shoalmate familiarity on  
499 the proliferation of alarm substance cells in ostariophysan fishes. *Journal of Fish*  
500 *Biology* 53:841-846.

501 Ydenberg RC, and Dill LM. 1986. The Economics of Fleeing from Predators. In: Jay S.  
502 Rosenblatt CBM-CB, and Peter JBS, eds. *Advances in the Study of Behavior*: Academic  
503 Press, 229-249.

504 Zach GJ, Peneder S, Strodl MA, and Schausberger P. 2012. Social familiarity governs prey  
505 patch-exploitation, - leaving and inter-patch distribution of the group-living  
506 predatory mite *Phytoseiulus persimilis*. *PLoS One* 7:e42889.  
507 10.1371/journal.pone.0042889

508 Zajitschek Susanne RK, and Brooks Robert C. 2008. Distinguishing the Effects of Familiarity,  
509 Relatedness, and Color Pattern Rarity on Attractiveness and Measuring Their Effects  
510 on Sexual Selection in Guppies (*Poecilia reticulata*). *The American Naturalist*  
511 172:843-854. 10.1086/593001

512

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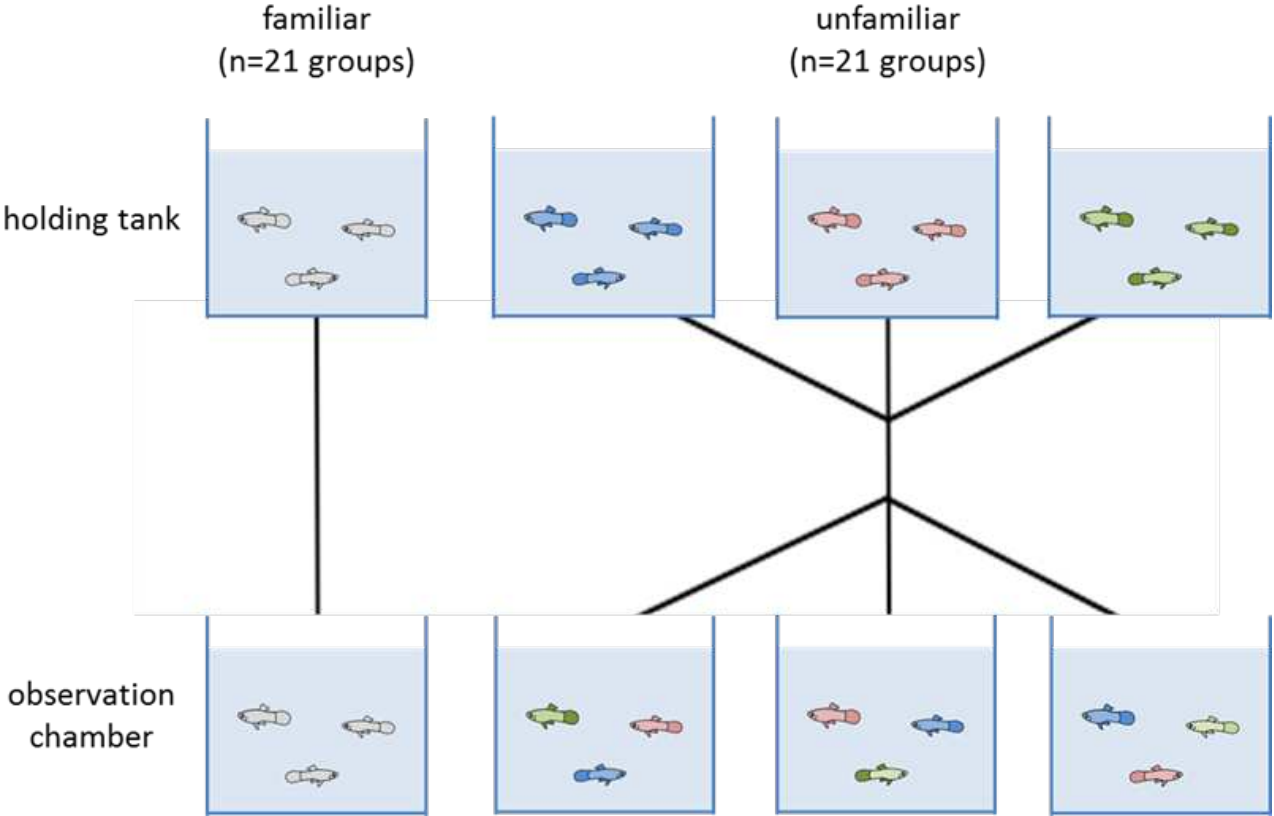
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## **Figure 1**(on next page)

### Figure 1

Figure 1 - Diagram of the two experimental treatments (familiar and unfamiliar). Individuals were allocated to a holding tank with two other conspecifics for two weeks. Each testing day, three groups were tested where fish remained with those they had been sharing a tank with (familiar treatment). The other three groups had the individuals swapped so that none of the fish had encountered each other previously (unfamiliar treatment). Forty-two groups were tested in total, 21 of each treatment.

Figure 1



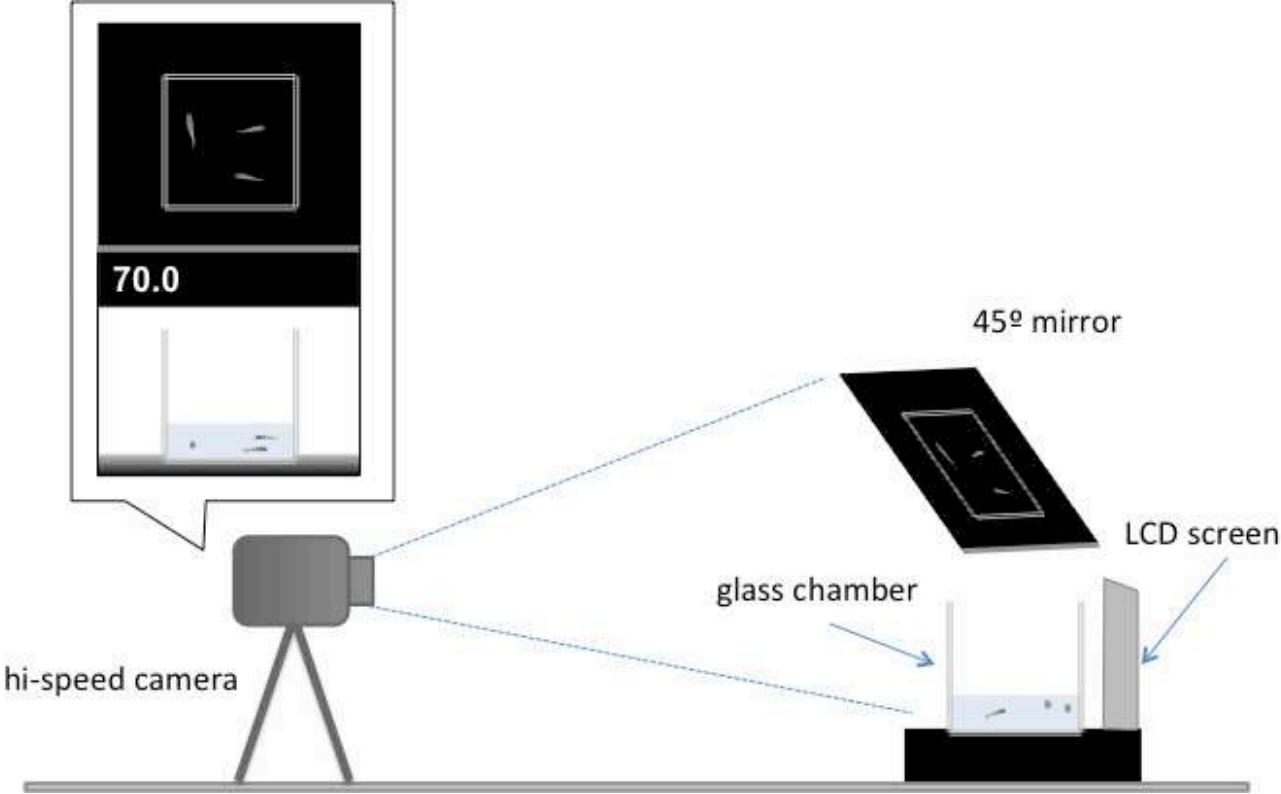


## **Figure 2**(on next page)

### Figure 2

Figure 2 - Illustration of the experimental setup. A camera was placed 1 m away from a glass tank (10 x 10 x 10 cm) positioned before the LDC screen that showed the digital display of a looming object. The front of the tank and the overhead view of the tank were recorded in high-speed video for each trial. The distance in centimetres of the digital looming object was displayed on the top left of the screen.

Figure 2

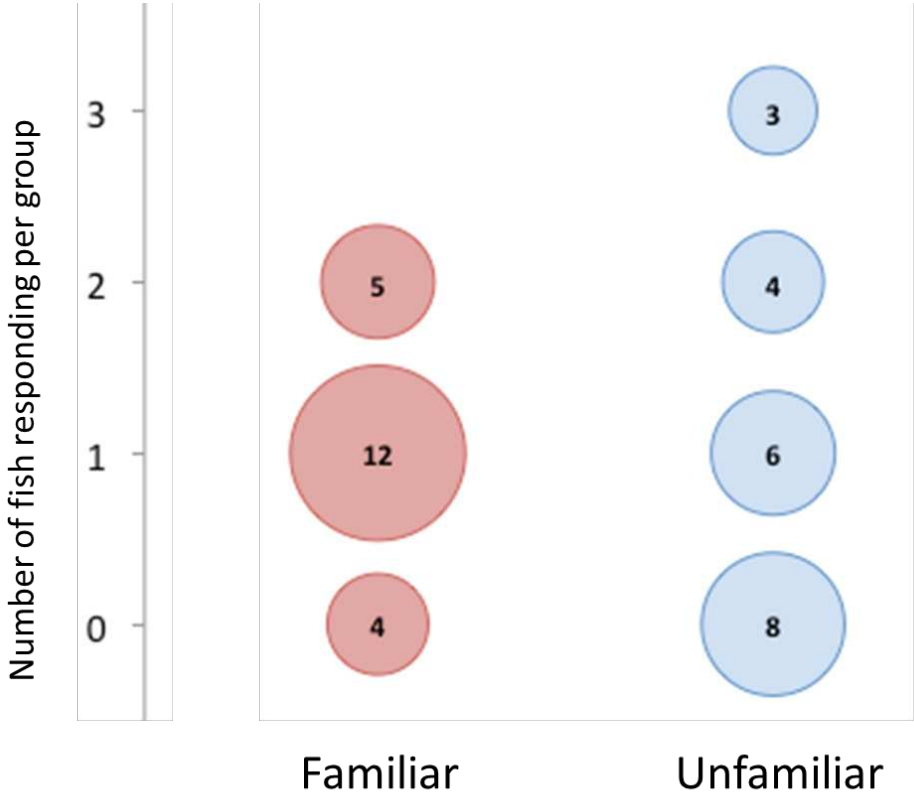


**Figure 3**(on next page)

Figure 3

Figure 3 - Responsiveness for familiar and unfamiliar groups in terms of how many individuals in a group of three responded to the stimulus. The numbers within the bubbles give the number of groups.

Figure 3

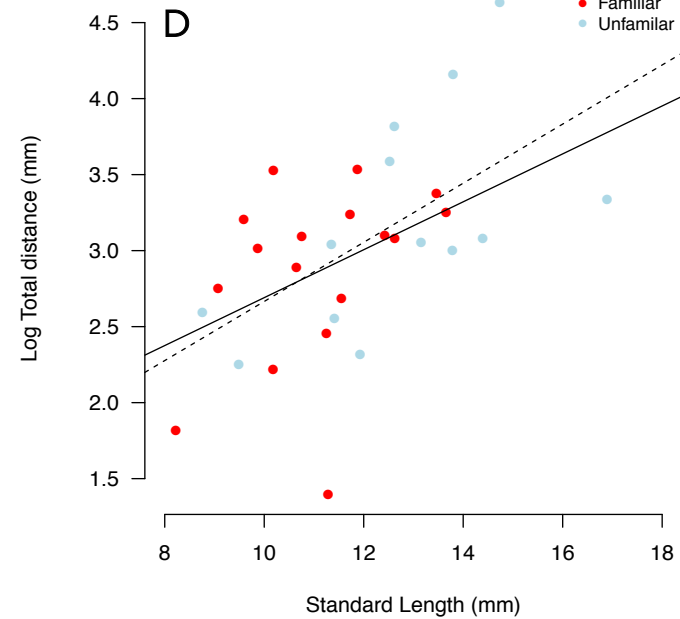
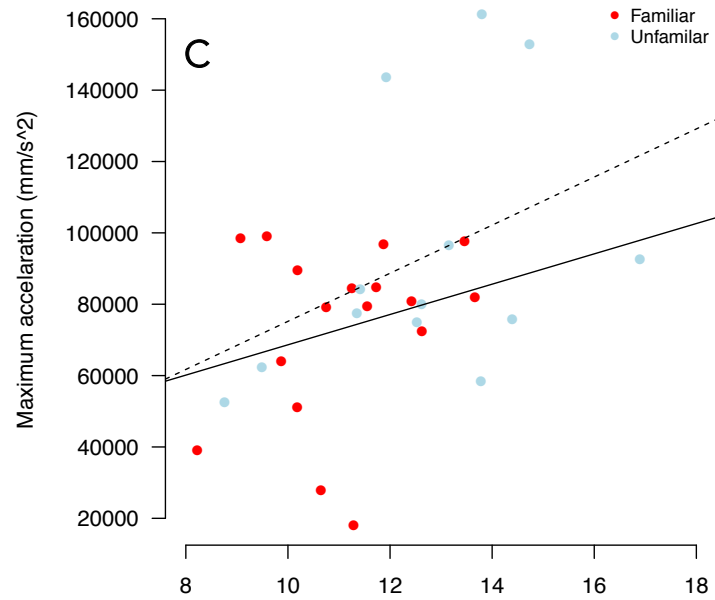
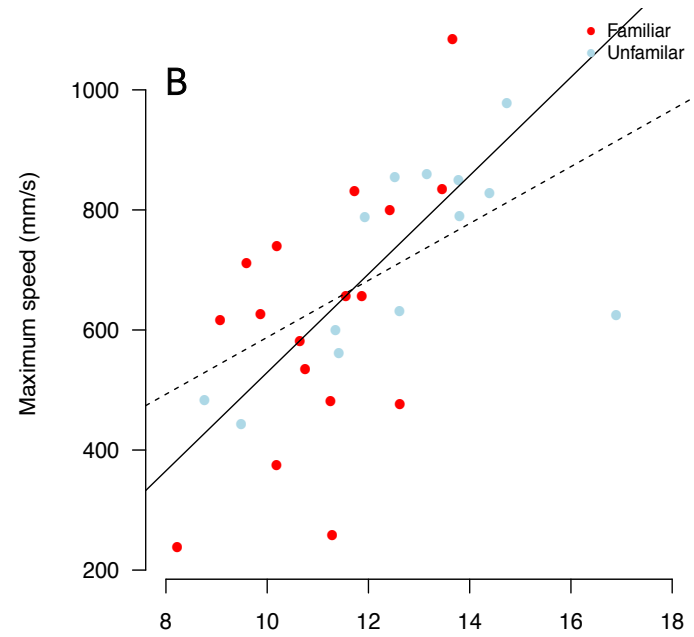
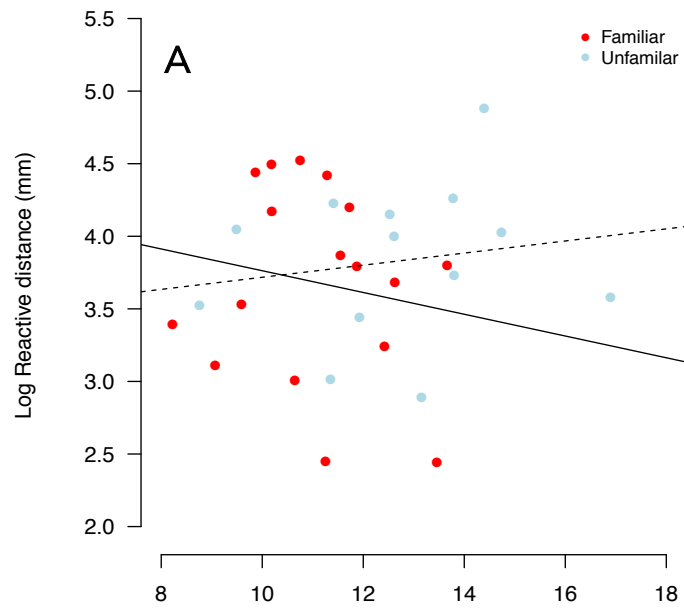


**Figure 4**(on next page)

Figure 4

Figure 4 - Variation in reactive distance (A), maximum speed (B), maximum acceleration (C) and total distance (D), in familiar (open circles) and unfamiliar (closed circles) groups. Lines were fitted using the coefficients of linear models.

Figure 4



## **Table 1** (on next page)

Table 1

Table 1 - Generalized linear models for testing the effect of familiarity on different qualitative measures of response. Model selection was performed using Akaike's information criterion (AIC). Both maximal and minimal adequate models are shown. The model with the lowest AIC was selected as being the minimum adequate model.

1  
2  
3 Table 1

4

| Response variable                    | Explanatory variable | df | Sum Sq   | F value | p-value |
|--------------------------------------|----------------------|----|----------|---------|---------|
| Reactive distance<br>AIC - 63.4      | Treatment            | 1  | 163      | 0.194   | 0.663   |
|                                      | Length               | 1  | 32       | 0.038   | 0.847   |
|                                      | Treatment + length   | 1  | 713      | 0.850   | 0.365   |
| Reactive distance<br>AIC - 58.7      | Intercept            | 29 | 10.88    |         |         |
| Maximum speed<br>AIC - 397.34        | Treatment            | 1  | 6913     | 2.527   | 0.123   |
|                                      | Length               | 1  | 3414     | 12.48   | 0.001   |
|                                      | Treatment + length   | 1  | 2623     | 0.959   | 0.336   |
| Maximum speed<br>AIC - 394.43        | Length               | 1  | 4106     | 15.59   | 0.004   |
| Maximum acceleration<br>AIC - 708.15 | Treatment            | 1  | 2.96e+09 | 3.427   | 0.075   |
|                                      | Length               | 1  | 3.10e+09 | 3.591   | 0.069   |
|                                      | Treatment + length   | 1  | 1.36e+08 | 0.158   | 0.694   |
| Maximum acceleration<br>AIC - 705.26 | Length               | 1  | 5.34e+09 | 6.425   | 0.017   |
| Total distance<br>AIC - 57.91        | Treatment            | 1  | 0.781    | 2.342   | 0.138   |
|                                      | Length               | 1  | 3.015    | 9.037   | 0.005   |
|                                      | Treatment + length   | 1  | 0.030    | 0.089   | 0.767   |
| Total distance<br>AIC - 54.04        | Length               | 1  | 3.788    | 12.17   | 0.001   |