1 **Behavioural responses of feral and domestic guppies (*Poecilia***

2 ***reticulata*) to predators and their cues**

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Abstract

Predation is an important factor during adaptation to novel environments and the feralisation

of introduced domestic species often involves responding appropriately to allopatric

predators despite a background of domestication and inbreeding. Twenty years ago domestic

guppies were introduced to a semi-natural environment at Burgers' Zoo in the Netherlands,

where they have since been exposed to avian predation. We compared predation-linked

behaviours in this feral population and in domestic guppies akin to the original founders. We

found that both populations responded to a novel predator and to conspecific alarm cues.

However shoaling, an important anti-predator behaviour, was higher among feral guppies

both at baseline and when exposed to the novel predator. We did not observe a linked suite of

anti-predator behaviours across shoaling, predator inspection, alarm substance sensitivity and

boldness, suggesting that these responses may be decoupled from one another depending on

local predation regimes. As we compared two populations, we cannot identify the causal

factors determining population differences, however, our results do suggest that shoaling is

either a particularly consequential anti-predator adaptation or the most labile of the

behaviours we tested. Finally, the behavioural adaptability of domestic guppies may help to

explain their success as an invasive species.

1. Introduction

Predation is a major driver of variation across taxa, influencing behaviours that may lessen predation risk such as social grouping (Hass and Valenzuela, 2002; Magurran, 1990a; Quinn and Cresswell, 2006) and vigilance (Forslund, 1993; Lung and Childress, 2007). However, these behaviours may also carry costs. For example, grouping may increase competition for resources or exposure to parasites (Krause and Ruxton, 2002). Thus anti-predator behaviours are expected to vary across populations experiencing different levels of predation.

Studies of wild guppies (*Poecilia reticulata*) on the Caribbean island of Trinidad have shown that populations under high levels of predation form tighter shoals (Magurran and Seghers, 1991; Seghers, 1974); engage in less risky predator inspection (Magurran and Seghers, 1990, 1994) and exhibit changes in sensitivity to conspecific alarm cues (Brown et al., 2009). While extensive study of wild guppies has provided strong evidence for evolved adaptation to predation in Trinidad, the guppy is also an important invasive species affecting local ecology, with feral populations identified in 60 countries across six continents (Froese and Pauly, 2012). These feral populations are often derived from inbred ornamental guppies (Lindholm et al., 2005), artificially selected for traits that may not be adaptive in the wild (Huntingford, 2004). Their success in novel habitats with allopatric predators suggests that domestic guppies retain traits that are adaptive in new environments (Deacon et al., 2011) or have sufficient genetic diversity to adapt to new environmental challenges.

We examined the influence of feralization and predation on the behaviour of guppies from Burgers' Zoo in Arnhem, the Netherlands. The zoo contains a 120 m^3 manatee (*Trichechus manatus*) pool which also houses a feral population of guppies, derived from an initial

65 introduction of mixed-strain domestic guppies released by a zoo visitor in 1989, and

66 subsequently left untended. The release was observed by zoo curators, who estimate that it

67 consisted of approximately 100 individuals (Max Janse, Burgers' Zoo curator, personal

68 communication, 2015). The population has subsequently expanded considerably and is

69 estimated to number well over 100,000 guppies (Max Janse, personal communication;

70 Albers, 2000). These now-feralized guppies forage on plentiful manatee food and faeces, but

71 are subject to predation from three resident African darters (*Anhinga rufa*) which dive

72 regularly each day for guppies (personal observation) and have been reported to eat up to

73 1000 guppies each per day (Albers, 2000). The feral guppies resemble wild guppies in

74 appearance and have lost the bright colours and extravagant tails typical of domestic guppies

75 (fig. 1A). A previous study examined body colouration in these fish, and anecdotally reported

76 dense shoaling among these feral guppies (Albers, 2000). We compared this feral population

77 with mixed-strain domestic guppies akin to the ancestral founders and measured predation-

78 linked behaviours: shoaling, predator inspection behaviour, alarm substance sensitivity, and

79 boldness/exploration. We predicted the feral guppies would shoal more, inspect predators

80 more cautiously, be more sensitive to alarm substance and be less bold and exploratory as a

81 consequence of feralization and adaptation to predation.

82

83 **2. Materials and Methods**

84 *2.1. Subjects and housing*

85 Nineteen domestic and 19 feral male guppies were used as experimental subjects, with a

86 further 14 domestic and 14 feral males used either as shoaling companions or for alarm

87 substance extraction. Domestic guppies were from a mixed strain population of

88 approximately 1400 fish, first established in 2003 within the Utrecht University Biology

89   aquarium and based on a founder population of approximately 480 domestic guppies

90   purchased from two commercial suppliers (Ruinemans, Montfoort and Ruisbroek, Maassluis,

91   both The Netherlands). Feral guppies were caught with dip nets from the manatee pool at

92   Burgers' Zoo. Feral guppies were sampled in November 2009, four months before the study,

93   and rehoused in our aquarium. Thus the feral guppies had both an evolutionary history and

94   direct experience of predation. Given an estimate of two generations per year (Magurran,

95   2005; Reznick et al., 1997), the domestic guppies are estimated to have bred for 10-15

96   generations in the Utrecht aquarium and the feral guppies for 35-45 generations at Burgers'

97   Zoo. Feral guppies were treated to remove ectoparasites after capture by 15 minute

98   immersion in 2.5% salt solution as a precautionary measure. Domestic guppies had brighter

99   and more varied colouration patterns than feral guppies, but subjects of each population were

100   similarly sized. Two male red rainbowfish (*Glossolepsis incisus*) from our aquarium were

101   used as potential predators as they represented a novel, allopatric threat to both domestic and

102   feral guppies. Two weeks before the study, all experimental animals were moved to our

103   experimental laboratory.

104

105   Laboratory lights were on from 08:00 to 20:00. Fish were housed separately by

106   population/species in 80 × 50 × 40 cm tanks except for shoaling companion guppies which

107   were housed in a 90 × 50 × 25 cm tank, with domestic and feral guppies separated by an

108   opaque partition. Tanks contained gravel, plastic plants and terracotta pots, and were filtered

109   and heated to 26±1°C. Fish were fed TetraMin flake food (Tetra, Germany) daily and fresh

110   bloodworm (*Chironomidae* larvae) three times a week.

111

112   *2.2. Experimental procedures*

113   Subjects were tested for: i) boldness and exploration, ii) responses to alarm substance, and iii)

114   shoaling and predator inspection, with 3-7 days between tests. Test order was consistent so

115   any carry-over effects from each test were the same across individuals. Housing tanks were

116   divided with plastic partitions to create separate areas for subjects after testing. On test days,

117   fish were fed at the conclusion of testing. Some fish were excluded from tests due to illness

118   or for methodological reasons: 19 guppies per group were used in boldness/exploration tests,

119   17 guppies per group in alarm substance response tests, and 17 domestic and 15 feral guppies

120   in shoaling and predator inspection tests.

121

122   *2.2.1. Boldness and exploration*

123   The test tank (fig. 1B) consisted of a "sheltered" area with gravel, plastic plants and a

124   terracotta pot, and a bare, brightly lit "exposed" area, which also contained a suspended

125   opaque partition creating a novel "hidden" area not visible from the sheltered area. Notional

126   boundaries of these areas and the upper and lower halves of the tank were marked on the

127   front of the tank. At test, individual subjects were released into the sheltered area and

128   behaviour recorded for 10 minutes.

129

130   Latencies to enter exposed and hidden areas were analysed by Wilcoxon-Gehan survival test

131   (subjects that did not enter were assigned the maximum latency of 600 seconds). Time in the

132   exposed area data were log transformed and analysed by independent t test, time in the

133   hidden area was analysed by Mann-Whitney U-test. Activity was assessed by analysing

134   number of transitions between tank quadrants by independent t test.

135

2.2.2. Alarm substance responses

137 Mixed-population alarm substance was prepared each test day following established

138 protocols (Brown and Godin, 1999; Brown et al., 2009): a feral and a domestic male guppy

139 were euthanized in ice water and skin and skeletal muscle homogenized with 50 μl ddH$_2$O,

140 then filtered through glass wool with ddH$_2$O to a final volume of 100 ml, which was kept on

141 ice.

142

143 Tests were conducted in a $40 \times 25 \times 25$ cm tank containing 20 cm depth of water and covered

144 on three sides with white plastic. After 2 minutes for the subject to habituate, 4 ml of ddH$_2$O

145 was added with a pipette to start the 20 minute test. Ten minutes later, 4 ml of alarm

146 substance solution was added. Tested subjects were placed in a holding tank to prevent

147 interaction with untested subjects, then moved to the 'tested' division of their home tanks at

148 the end of each day. The test tank was cleaned and refilled before each test. Time immobile

149 ("freezing") and distance swum were analysed by repeated measures ANOVA, with

150 experimental phase (before and after addition of alarm substance) as the repeated factor and

151 population as the independent factor. Freezing data were log transformed before analysis.

152

2.2.3. Shoaling and predator inspection behaviour

154 The test tank (fig. 1C) was lined with gravel and divided with plastic partitions into left

155 (shoal), central (subject) and right (predator) sections. The transparent left partition was "one-

156 way glass" so the shoal could not see the predator or subject (Mathis et al., 1996). The right

157 partition consisted of an impermeable silicone-sealed transparent partition and a removable

158   opaque partition. The shoal section was lit with a 3W LED spotlight to ensure the

159   effectiveness of the one-way glass. Two interlocked plastic cups with matching 3 cm

160   diameter holes were suspended in the subject section, and a shoaling zone adjacent to the

161   companion shoal was marked on the front of the tank, 6 cm (2 body lengths) from the left

162   partition.

163

164   On each test day, 5 same-population companion fish were placed in the shoal section and a

165   red rainbowfish was placed in the predator section. A subject was placed in the suspended

166   cups, with the holes misaligned. After 2 minutes' habituation, the holes were aligned and

167   when the subject exited, the 20 minute test began. After 10 minutes, the opaque partition was

168   removed to reveal the red rainbowfish. We recorded time spent in the shoaling zone and

169   number of predator inspection bouts by each subject over the whole test. At the end of each

170   day, the red rainbowfish and shoaling companion fish were returned to their home tanks.

171   Populations were tested on alternate days to balance test order. Shoaling time data were

172   square root transformed and analysed by repeated measures ANOVA, with experimental

173   phase (before and after the predator was revealed) as the repeated factor and population as the

174   independent factor. Frequency of predator inspection was analysed by independent samples t

175   test.

176

177   *2.2.4. Correlations between anti-predator behaviours*

178   Relationships between responses to predation cues (the potential predator or alarm cues) were

179   analysed by correlation of behavioural difference scores. A single measure was calculated for

180   the behaviours modulated by exposure to predation cues (shoaling duration, number of

181   predator inspection bouts and freezing duration) by subtracting pre-exposure performance

182   from post-exposure performance. These three difference scores were then analysed by

183   Pearson's correlations. Shoaling data difference scores were log transformed prior to analysis.

184

185   *2.3. Analysis*

186   Tests were videoed using a Logitech Pro 9000 webcam and VirtualDub video capture

187   software. Boldness and exploration tests, and shoaling and predator inspection tests were

188   scored with JWatcher 1.0. Alarm substance tests were analysed with Ethovision XT (Noldus

189   Information Technology, Netherlands). Statistical analyses were conducted with IBM SPSS

190   Statistics 20.

191

192   *2.4. Ethics statement*

193   All procedures were carried out in accordance with Dutch law and approved by the Utrecht

194   animal experimentation commission ("Dierexperimentencommissie Utrecht") under licence

195   2010.I.03.036.

196

197   **3. Results**

198   *3.1. Boldness and exploration*

199   Feral and domestic guppies did not significantly differ in their latency to enter the exposed

200   area (Wilcoxon-Gehan statistic = 0.10, df = 1, P = 0.92) or total time in the exposed area (t

201   test, t_{36} = 0.70, P = 0.49). There was a non-significant trend for feral guppies to enter the

202   hidden area faster (Wilcoxon-Gehan statistic = 3.30, df = 1, P = 0.069, fig. 2A) and to spend

203   more time in the hidden area (Mann-Whitney U test, U = 240, N =19 per group, P = 0.085;

204   fig. 2B). Populations did not significantly differ in number of transitions between quadrants (t

205   test, $t_{36} = 0.43$, $P = 0.67$).

206

207   Subjects responded differently to the sheltered and exposed areas, spending significantly less

208   time in the exposed area than would be expected by chance (one-sample t test, $t_{37} = -2.63$, P

209    $= 0.012$). Similarly, subjects spent significantly less time in the hidden area after entering the

210   exposed area than would be expected by chance (one-sample Wilcoxon signed rank test, $W =$

211   180, $N = 38$, $P = 0.005$; chance expectations were based on the relative volume of each area).

212   This avoidance of the exposed and hidden areas supports the use of time spent in these areas

213   as combined measures of boldness and exploration (sensu Réale et al., 2007).

214

215   *3.2. Alarm substance responses*

216   Alarm substance significantly increased freezing (repeated measures ANOVA, $F_{1,32} = 44.98$,

217    $P < 0.001$, fig. 2C), and decreased distance swum ($F_{1,32} = 7.79$, $P = 0.009$), but these

218   responses did not differ significantly between populations, nor were there significant

219   interactions between population and alarm substance exposure (all: $F_{1,32} \leq 0.74$, $P \geq 0.40$).

220

221   *3.3. Shoaling and predator inspection behaviour*

222   Exposure to the red rainbowfish significantly increased shoaling (repeated measures

223   ANOVA, $F_{1,30} = 14.00$, $P = 0.001$), and feral guppies spent longer shoaling than domestic

224   guppies ($F_{1,30} = 10.71$, $P = 0.003$). There was no significant interaction between population

225   and predator exposure ($F_{1,30} = 0.86$, $P = 0.36$). Both populations only engaged in predator

226   inspection once the red rainbowfish was visible (mean rate: 4.2 inspections/trial), however

10

227 predator inspection frequency did not significantly differ between populations (t test, $t_{30} =$

228 1.52, $P = 0.14$).

229

230 *3.4. Correlations between anti-predator behaviours*

231 We found no significant correlations between the difference scores for shoaling, predator

232 inspection and freezing in either domestic or feral guppies (Pearson's correlations, $-0.39 \leq r \leq$

233 0.19, $N = 14 - 17$, all $P \geq 0.17$).

234

4. Discussion

236 Both feral and domestic guppies increased their shoaling behaviour and engaged in predator

237 inspection when a novel predator was revealed, and both were sensitive to alarm substance.

238 Feral guppies shoaled more than domestic guppies, both before and after exposure to a novel

239 predator, but the populations did not significantly differ in predator inspection, responses to

240 alarm substance or in exploration and boldness. There was no evidence that the anti-predator

241 responses formed a suite of coupled behaviours.

242

243 Predation levels have been strongly implicated in population differences in shoaling among

244 wild guppies (Magurran, 2005), and adaptation to predation at Burgers' Zoo may have driven

245 the shoaling phenotype in the feral guppies. Predators have been shown to target individuals

246 within a group that exhibit less cohesive grouping behaviour (Ioannou et al., 2012; Quinn and

247 Cresswell, 2006) and thus prey selection by predators would be expected to increase the

248 population-level shoaling phenotype over time. We did not find evidence for covariance of

249 the different anti-predator behaviours we measured, a result that suggests anti-predator

11

250   behaviours were uncoupled, and that shoaling was the most labile of the behaviours we

251   tested, perhaps because it carries the most significant costs and benefits. The different anti-

252   predator behaviours may be independently responsive, reflecting differences in the adaptive

253   value of each behaviour depending on the specific predator threat. Alternatively, the

254   uncoupling may reflect limits on the variation that can emerge, for example as a result of

255   differences in allelic diversity at loci which regulate each behaviour. Larger sample sizes may

256   have helped reveal differences in the other behaviours measured, however predation effects

257   on guppy behaviours are not always consistent (Brown et al., 2009; Seghers and Magurran,

258   1995) indicating that other factors can have an influence.

259

260   We used an allopatric fish predator to allow us to test the responses of both populations to a

261   novel predator, and sensitivity to predators was evident in both feral and domestic guppies'

262   shoaling, predator inspection behaviour and alarm substance responses. Many generations of

263   domestication and artificial selection has thus not eliminated predator sensitivity in domestic

264   guppies and this may help to explain the success of introduced guppies despite novel local

265   allopatric predators (Deacon et al., 2011). Predation-linked behaviours have previously been

266   implicated in the success or failure of species introductions (Holway and Suarez, 1999) and

267   other invasive poeciliid species show elevated anti-predator behaviour compared to non-

268   invasive species (Rehage et al., 2005). Our finding that anti-predator responses are

269   maintained despite domestication has implications for captive breeding and reintroduction

270   programmes for endangered fish (see e.g. Brown and Day (2002)).

271

272   We cannot discount the possibility that aspects other than predation may have shaped the

273   feral phenotype as our study compared only two populations, and additional feral populations

274	would clearly be essential to eliminate alternative explanations for our results (Dingemanse et

275	al., 2009; MacLean et al., 2012; Reader and Hrotic, 2012). While both the feral and the

276	domestic populations were originally derived from mixed domestic strains, making it

277	unlikely that phenotypic differences were due to differential levels of inbreeding, they are not

278	derived from the same source populations and so founder effects cannot be discounted. We

279	also considered other environmental influences, however differences in food availability

280	between domestic and feral guppies are unlikely to have been a factor as feral guppies are

281	able to feed ad libitum due to the manatees' feeding regime. While the manatee pool is

282	constantly filtered and tested and water quality resembles that of an aquarium, guppy

283	ectoparasites such as *Gyrodactylus* spp., are present at low levels. However, the feral guppies

284	were treated to remove parasites after capture, four months prior to the study. Moreover, any

285	direct effects of surviving parasites would be predicted to result in reduced shoaling, as

286	infected individuals are avoided within shoals (Croft et al., 2011), and *Gyrodactylus* infection

287	reduces shoal cohesion (Hockley et al., 2014). Residual developmental or evolutionary

288	effects of *Gyrodactylus* presence are also unlikely to explain the observed shoaling

289	phenotype, because these ectoparasites are transmitted socially. Consequently, a population

290	history of *Gyrodactylus* infection would be predicted to lead to reduced grouping as an

291	adaptation to avoid infection.

292

293	Caution should be taken when drawing conclusions about adaptation from two-population

294	studies such as our own, since any two populations are likely to differ on numerous

295	characteristics, not just the factor proposed to favour adaptation. However, comparisons of

296	two populations or species is a commonly used approach, and one that has made many useful

297	contributions (Dingemanse et al., 2009; Reader, 2014). Such comparisons can provide a

298 starting point, demonstrating population differences that suggest plausible hypotheses for

299 further work. They are particularly valuable when experimental manipulation of the proposed

300 causal variable presents ethical or logistical problems. The specific circumstances of the

301 Burgers' zoo population provides an independent and rare opportunity to examine 20 years of

302 guppy evolution in well-characterised and semi-controlled feral conditions. The general

303 background of the introduced fish is known, and the zoo environment affords a relatively

304 stable environment with minimal variation in factors such as water quality, temperature, or

305 food availability that can vary extensively in most feral or wild environments, often varying

306 together with predation regime (Magurran 2005). The above considerations, in combination

307 with previous work on predation and shoaling tendencies (Huizinga et al., 2009; Magurran,

308 1990b; Magurran and Seghers, 1991), suggest that 20 years of predation in a feral

309 environment is the most likely explanation for the observed increase in shoaling behaviour in

310 these guppies. Despite years of artificial selection, ornamental fish such as domestic guppies

311 retain both behavioural sensitivity to predator threats and the capacity to adapt to these.

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322 **References**

323 Albers, P.C.H., 2000. Evidence for evolution of guppies in a semi-natural environment. Neth.

324   J. Zool. 50, 425-433.

325 Brown, C., Day, R.L., 2002. The future of stock enhancements: lessons for hatchery practice

326   from conservation biology. Fish Fish. 3, 79-94.

327 Brown, G.E., Godin, J.-G.J., 1999. Chemical alarm signals in wild Trinidadian guppies

328   (*Poecilia reticulata*). Can. J. Zool. 77, 562-570.

329 Brown, G.E., Macnaughton, C.J., Elvidge, C.K., Ramnarine, I., Godin, J.-G.J., 2009.

330   Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian

331   guppies. Behav. Ecol. Sociobiol. 63, 699-706.

332 Croft, D.P., Edenbrow, M., Darden, S.K., Ramnarine, I.W., van Oosterhout, C., Cable, J.,

333   2011. Effect of gyrodactylid ectoparasites on host behaviour and social network structure

334   in guppies *Poecilia reticulata*. Behav. Ecol. Sociobiol. 65, 2219-2227.

335 Deacon, A.E., Ramnarine, I.W., Magurran, A.E., 2011. How reproductive ecology

336   contributes to the spread of a globally invasive fish. PLoS ONE 6, e24416.

337 Dingemanse, N.J., Van der Plas, F., Wright, J., Réale, D., Schrama, M., Roff, D.A., Van der

338   Zee, E., Barber, I., 2009. Individual experience and evolutionary history of predation

339   affect expression of heritable variation in fish personality and morphology. Proc. R. Soc.

340   Lond. B Biol. Sci. 276, 1285-1293.

341 Forslund, P., 1993. Vigilance in relation to brood size and predator abundance in the barnacle

342   goose, *Branta leucopsis*. Anim. Behav. 45, 965-973.

343 Froese, R., Pauly, D. 2012. Fishbase. World Wide Web electronic publication.,

344   http://www.fishbase.org, version (10/2012).

345 Hass, C., Valenzuela, D., 2002. Anti-predator benefits of group living in white-nosed coatis

346   (*Nasua narica*). Behav. Ecol. Sociobiol. 51, 570-578.

15

347 Hockley, F.A., Wilson, C.A.M.E., Graham, N., Cable, J., 2014. Combined effects of flow

348   condition and parasitism on shoaling behaviour of female guppies *Poecilia reticulata*.

349   Behav. Ecol. Sociobiol. 68, 1513-1520.

350 Holway, D.A., Suarez, A.V., 1999. Animal behavior: an essential component of invasion

351   biology. Trends Ecol. Evol. 14, 328-330.

352 Huizinga, M., Ghalambor, C.K., Reznick, D.N., 2009. The genetic and environmental basis

353   of adaptive differences in shoaling behaviour among populations of Trinidadian guppies,

354   *Poecilia reticulata*. J. Evol. Biol. 22, 1860-1866.

355 Huntingford, F.A., 2004. Implications of domestication and rearing conditions for the

356   behaviour of cultivated fishes. J. Fish Biol. 65 (Supplement A), 122-142.

357 Ioannou, C.C., Guttal, V., Couzin, I.D., 2012. Predatory fish select for coordinated collective

358   motion in virtual prey. Science 337, 1212-5.

359 Krause, J., Ruxton, G.D. 2002. *Living in Groups*. Oxford University Press, Oxford, UK.

360 Lindholm, A.K., Breden, F., Alexander, H.J., Chan, W.-K., Thakurta, S.G., Brooks, R., 2005.

361   Invasion success and genetic diversity of introduced populations of guppies *Poecilia*

362   *reticulata* in Australia. Mol. Ecol. 14, 3671-3682.

363 Lung, M.A., Childress, M.J., 2007. The influence of conspecifics and predation risk on the

364   vigilance of elk (*Cervus elaphus*) in Yellowstone National Park. Behav. Ecol. 18, 12-20.

365 MacLean, E.L., Matthews, L.J., Hare, B.A., Nunn, C.L., Anderson, R.C., Aureli, F., Brannon,

366   E.M., Call, J., Drea, C.M., Emery, N.J., Haun, D.B., Herrmann, E., Jacobs, L.F., Platt,

367   M.L., Rosati, A.G., Sandel, A.A., Schroepfer, K.K., Seed, A.M., Tan, J., van Schaik, C.P.,

368   Wobber, V., 2012. How does cognition evolve? Phylogenetic comparative psychology.

369   Anim. Cogn. 15, 223-38.

370 Magurran, A.E., 1990a. The adaptive significance of schooling as an antipredator defense in

371   fish. Ann. Zool. Fenn. 27, 51-66.

Magurran, A.E., 1990b. The inheritance and development of minnow antipredator behavior. Anim. Behav. 39, 834-842.

Magurran, A.E. 2005. *Evolutionary Ecology: The Trinidadian Guppy*. Oxford University Press, Oxford.

Magurran, A.E., Seghers, B.H., 1990. Population differences in predator recognition and attack cone avoidance in the guppy *Poecilia reticulata*. Anim. Behav. 40, 443-452.

Magurran, A.E., Seghers, B.H., 1991. Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. Behaviour 118, 214-234.

Magurran, A.E., Seghers, B.H., 1994. Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. Behaviour 128, 121-134.

Mathis, A., Chivers, D.P., Smith, R.J.F., 1996. Cultural transmission of predator recognition in fishes: intraspecific and interspecific learning. Anim. Behav. 51, 185-201.

Quinn, J.L., Cresswell, W., 2006. Testing domains of danger in the selfish herd: sparrowhawks target widely spaced redshanks in flocks. Proc. R. Soc. Lond. B Biol. Sci. 273, 2521-2526.

Reader, S.M. 2014. Evolution of Cognition. In: J.B. Losos (Editor), Oxford Bibliographies in Evolutionary Biology, Oxford University Press, New York.

Reader, S.M., Hrotic, S.M., 2012. The limits of chimpanzee-human comparisons for understanding human cognition. Behavioral Brain Sciences 35, 238-9.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biol. Rev. 82, 291-318.

Rehage, J.S., Barnett, B.K., Sih, A., 2005. Behavioral responses to a novel predator and competitor of invasive mosquitofish and their non-invasive relatives (*Gambusia* sp.). Behav. Ecol. Sociobiol. 57, 256-266.

397   Reznick, D.N., Shaw, F.H., Rodd, F.H., Shaw, R.G., 1997. Evaluation of the rate of evolution

398   in natural populations of guppies (*Poecilia reticulata*). Science 275, 1934-1937.

399   Seghers, B.H., 1974. Schooling behavior in the guppy (*Poecilia reticulata*): an evolutionary
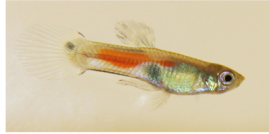
400   response to predation. Evolution 28, 486-489.

401   Seghers, B.H., Magurran, A.E., 1995. Population differences in the schooling behavior of the

402   Trinidad guppy, *Poecilia reticulata* - adaptation or constraint? Can. J. Zool. 73, 1100-

403   1105.
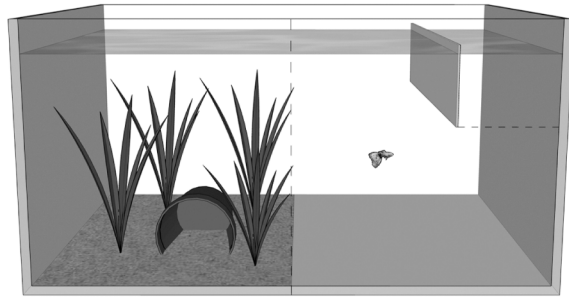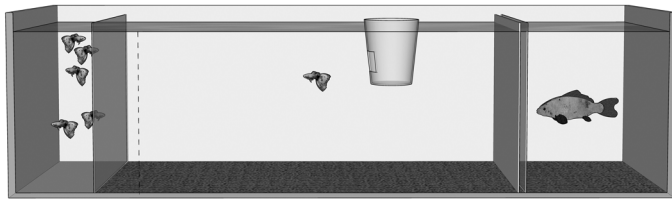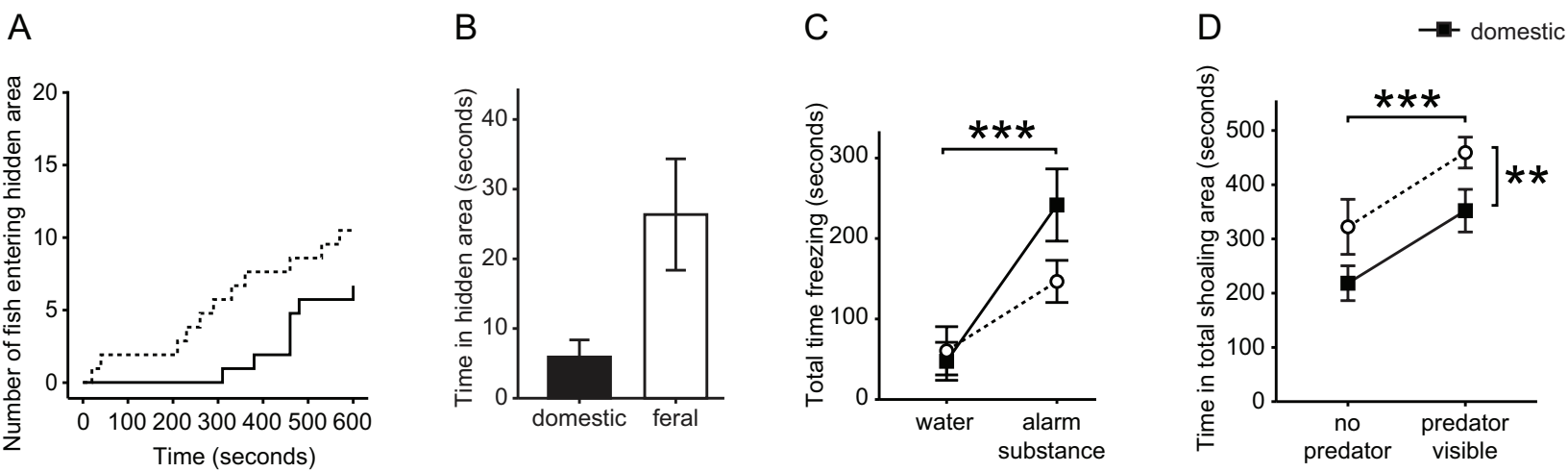
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A

B

C

Figure 2

407   Figure 1. (A) Example photos of domestic (left) and feral (right) guppies (W.T. Swaney). (B)

408   Sketch of boldness and exploration test apparatus. The 80 cm × 50 cm × 40 cm tank

409   comprised a "sheltered" area and a brightly lit "exposed" area containing the "hidden" area

410   which subjects could not see into from their starting position in the sheltered area. (C) Sketch

411   of shoaling and predator inspection test apparatus. The 90 cm × 50 cm × 25 cm tank was

412   divided into a 10 cm wide shoal section (left), a 20 cm wide predator section (right) and a

413   subject section (centre) containing the cups from which subjects started.

414

415   Figure 2. (A) Cumulative timeline of subjects' latency to reach the hidden area and (B) total

416   time in the hidden area in exploration and boldness tests (feral $N = 19$, domestic $N = 19$). (C)

417   Total time subjects spent frozen after addition of water and alarm substance in alarm

418   substance tests (feral $N = 17$, domestic $N = 17$). (D) Total time subjects spent in proximity to

419   the shoal in "no predator" and "predator visible" parts of shoaling and predator inspection

420   tests (feral $N = 15$, domestic $N = 17$). Data are means ± SEM, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

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