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2 **Sex-dependent personality in two invasive species of mosquitofish**

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35 **ABSTRACT**

36 A key challenge in invasion biology is identifying characteristics that allow some species to
37 be repeatedly successful at invading novel environments. Invasions can often be
38 disproportionately driven by a single sex, with differences in behavioural mechanisms
39 between the sexes potentially underlying sex-biased invasiveness. Here, we took an animal
40 personality approach to study the behaviour of two repeatedly successful congeneric invasive
41 species, the western mosquitofish, *Gambusia affinis*, and the eastern mosquitofish, *Gambusia*
42 *holbrooki*. In each species, we investigated whether males and females shared common
43 personality traits (i.e. behavioural types and behavioural syndromes), with the aim of
44 identifying possible behavioural mechanisms that could help explain why mosquitofish
45 invasions are often characterised by sex-biased founder populations. We found sex-dependent
46 personality, although sex differences varied between species. Specifically, male *G. affinis*
47 were bolder and less social than female *G. affinis*, whereas we found no behavioural type
48 differences between the sexes in *G. holbrooki*. We also found a consistent correlation
49 between boldness and exploration in both sexes within *G. affinis*, but this correlation was
50 weak in *G. holbrooki*. Finally, exploration was also correlated with sociability in male *G.*
51 *affinis*, but not in females. Our results suggest that behavioural tendencies may diverge, both
52 among species and between the sexes, because of adaptation experienced during different
53 invasion pathways. Broadly, identifying the behavioural mechanisms that predict an
54 individual's 'invasiveness' may be difficult to tease apart between species because each
55 invasion is characterised by different abiotic and biotic interactions that likely require
56 different suites of behaviours. Future studies are needed to elucidate whether, in fact,
57 personality variation between the sexes can mediate the occurrence of sex-biased invasions.

58 **Keywords:** Individual variation, Animal personalities, Invasion syndrome, Invasion process,
59 Life-history, Sex ratio, Risk-taking, Sex differences.

60 INTRODUCTION

61 Individuals that successfully invade and establish into new areas often represent a non-
62 random subset of the population, and typically consist of individuals that possess a certain
63 suite of characteristics that differs from those of non-invaders (Blackburn & Duncan 2001,
64 Tingley et al. 2010, Renault et al. 2018). For instance, it has been proposed that these
65 individuals often have behavioural and life-history traits that increases their propensity to be
66 transported to new environments, exploit novel resources, establish viable populations, and
67 spread and colonise new habitats (Holway & Suarez 1999, Chapple et al. 2012, Chapple &
68 Wong 2016, Rehage et al. 2016). These same phenotypic traits also mediate how invaders
69 interact with the local environment and native biota, and thus play a pivotal role in
70 determining the ecological and evolutionary impacts of an invasion (Phillips & Suarez 2012).
71 Hence, a key challenge in invasion biology is identifying the characteristics that allow some
72 species to be repeatedly successful at invading and colonising novel environments (Chapple
73 et al. 2012).

74
75 Evidence is starting to accumulate that biological invasions can be often be
76 disproportionately driven by a single sex (Gutowsky & Fox 2011, Miller et al. 2013, Rebrina
77 et al. 2015). Skewed sex ratios at the leading edge of an invasion have been shown to have
78 profound consequences for population growth and persistence (Miller et al. 2013, Shaw et al.
79 2018), and can lead to greater adverse impacts on native communities than non-skewed sex
80 ratios (Fryxell et al. 2015). For instance, female-biased invasion front populations can
81 exponentially increase the pace of an invasion by speeding up population growth, resulting in
82 a higher probability of colonisation success (Miller et al. 2013), whereas male-biased
83 invasions may be more likely to competitively exclude native species, creating new
84 opportunities for habitats and resources to be exploited (Duckworth & Baydeav 2007,

85 Gutowsky & Fox 2011). However, despite the prevalence of sex-biased invasions, the
86 mechanisms that lead to biased sex ratios at the front of an invasion have rarely been studied.

87

88 Sex-biased invasions likely occur because males and females often differ considerably in life-
89 history and behavioural traits related to invasion (Shaw et al. 2018). For example, dispersal is
90 the mechanism that allows invaders to spread from the point of introduction into new areas
91 and thus is a pivotal component of the invasion process (Cote et al. 2010a), but males and
92 females often differ in their propensity to disperse (Trochet et al. 2016), and in traits related
93 to dispersal (e.g. morphology: Llewelyn et al. 2010; behaviour: Marentette et al. 2011). Such
94 sex-dependent traits (e.g. sex-biased dispersal) may enhance the invasiveness of a single sex,
95 leading to biased sex ratios (Miller & Inouye 2013, Fryxell et al. 2015, Shaw et al. 2018).
96 Alternatively, limited behavioural variation between the sexes would be less vulnerable to
97 selective filtering by the invasion process, and thus leading-edge populations would not be
98 expected to be disproportionately skewed towards a particular sex (Michelangeli et al. 2016a,
99 Gruber et al. 2017).

100

101 One relatively new approach to investigating the role of behaviour in invasions is through the
102 study of animal personalities (see reviews: Cote et al. 2010a, Chapple et al. 2012, Sih et al.
103 2012, Juelle et al. 2014). Animal personality refers to the concept that individuals within
104 populations often show consistent differences in a range of behaviours (i.e. behavioural
105 types: Sih et al. 2004), and these behaviours can covary across time/and or context (i.e.
106 behavioural syndrome; Sih et al. 2004). Personality traits are often linked to life-history
107 (reproduction and growth rates: Biro & Stamps 2008), ecological processes (habitat
108 specialisation: Michelangeli et al. 2018a), and social roles within populations and
109 communities (e.g. innovation & cultural transmission: Aplin et al. 2015). Given its direct

110 bearing on fitness, an individual's personality should also influence its probability of
111 transitioning through the invasion process, with different behavioural types being
112 advantageous at different stages of invasion (Cote et al. 2010a, Fogarty et al. 2011, Chapple
113 et al. 2012, Chapple & Wong 2016). Indeed, mounting evidence suggests that invasive
114 individuals may exhibit combinations of behaviours that are beneficial in outcompeting
115 native species (Pintor et al. 2009), dispersing into new habitats (Michelangeli et al. 2017),
116 and avoiding novel predators (Mennen & Laskowski 2018). In this regard, personality
117 differences between the sexes could underlie differences in sex-biased dispersal and sex-
118 biased invasiveness (Michelangeli et al. 2016a, Mishra et al. 2018). Sex differences in the
119 direction and magnitude of behavioural syndromes could arise due to divergent selection
120 pressures and life-histories after maturation. This may be particularly true for sexually
121 dimorphic species, as marked differences in morphology (e.g. body size) can induce variance
122 in behaviour (Shine 1989, Fairbairn et al. 2007). For instance, larger body size requires
123 higher energetic input and, thus, personality traits that are associated with an increase in
124 feeding rate (Biro & Stamps 2008). If personality influences an individual's level of
125 'invasiveness' and, hence, their potential impact on the environment, it is important to
126 consider how the sexes might differ in personality to better understand the behavioural
127 mechanisms involved in successful invasions.

128

129 In this study, we compared the personality traits of males and females in congeneric invasive
130 species, the western mosquitofish, *Gambusia affinis* (Baird & Girard 1853) and the eastern
131 mosquitofish, *Gambusia holbrooki* (Girard 1859). These species provide an ideal opportunity
132 to explore sex differences in personality traits related to invasion for several reasons. First,
133 *Gambusia* are small live-bearing freshwater fish that show pronounced sexual size
134 dimorphism, whereby females are commonly much larger than males (Pyke 2005). Second,

135 both species have undergone numerous deliberate (i.e. introduced as a biocontrol tool for
136 mosquitoes) and accidental introductions, and have now spread and become invasive
137 globally, placing them within the top 100 of the world's most invasive species (Pyke 2008).
138 Third, invasive populations are often characterised by demographic differences in sex ratios
139 that can either be skewed towards males or females (Fryxell et al. 2015). Fourth,
140 mosquitofish are having tremendous adverse impacts on native insect, amphibian and fish
141 communities worldwide (Pyke 2008, Schluse et al. 2013). Importantly, some studies suggest
142 that the magnitude of these impacts are dependent upon both the sex ratio (Fryxell et al.
143 2015), and the personality composition of invading populations (Cote et al. 2017). Thus,
144 understanding the behavioural mechanisms driving *Gambusia* invasions is an issue of
145 immediate importance.

146

147 The approach used in this study allowed us to determine if each species and sex share
148 common behavioural syndromes and, in so doing, provides insights into the behavioural traits
149 that might contribute to invasiveness. We hypothesised that males and females would differ
150 in a range of behaviours related to invasion, but that these differences would vary among
151 species due to the divergent introduction pathways and local environmental conditions
152 experienced by each species.

153

154 **METHODS**

155 **Species collection and husbandry**

156 *Gambusia holbrooki* (female: $n = 25$; male: $n = 25$) were collected from the Science Centre
157 Lake ($37^{\circ} 54' 28''$ S, $145^{\circ} 08' 16''$ E; 10:14 h light:dark), Monash University, Victoria,
158 Australia on 22 January 2014. All fish were caught via seine netting to minimise potential
159 personality-biased sampling (Michelangeli et al. 2016b). Fish were housed individually in

160 glass holding tanks (30 cm length × 15 cm width × 20 cm height) and acclimated to
161 laboratory conditions for 1 month prior to experimentation. We housed fish individually in
162 order to keep track of their identity during behavioural assays. Throughout the housing
163 period, fish were kept at a temperature of 24–26 °C, and under a 12:12 h light:dark cycle.
164 Both during housing and throughout experimentation, fish were fed *ad libitum* with
165 commercial fish food.

166

167 *Gambusia affinis* (female: $n = 110$; male: $n = 112$) were supplied by the Sacramento-Yolo
168 Mosquito and Vector Control District. These fish represent a mix of hatchery-reared and
169 field-collected fish. Fish were transported to the Centre for Aquatic Biology and Aquaculture
170 (CABA), University of California Davis on 18 March 2008, and housed in groups of ~60 in
171 80 L flow-through fibreglass tanks, and acclimated to laboratory conditions for 1 month prior
172 to experimentation. All individuals were marked with a minimally invasive elastomer tag
173 (northwest Marine Technologies, Shaw Island, WA, USA) under a low dose (5 mg L^{-1}) of
174 anaesthetic (MS-222). Each individual received a randomly assigned unique identifier by
175 injecting one of four colours subcutaneously into four locations on the caudal peduncle (two
176 on each side). Throughout the housing period, fish were kept at a constant temperature (22–
177 23 °C) on a natural photoperiod (14:10 h light:dark), and were fed commercial fish food *ad*
178 *libitum*.

179

180 **Behavioural assays**

181 For both species, in order to characterise personality types of each sex, we ran two
182 behavioural assays, each separated by 1 h. First, we tested sociability by quantifying the
183 tendency of individuals to shoal. Second, we tested individual boldness and exploratory
184 behaviours. The former was characterised by the latency of fish to exit from a refuge and

185 enter a novel environment, and the latter was quantified by recording the movement and
186 space use of fish after exiting the refuge. These behaviours represent an individual's reaction
187 to a social context and to a novel environment, respectively. Both sets of behaviours are
188 hypothesised to play an important role in colonising new environments (Chapple et al. 2012,
189 Sih et al. 2012, Chapple & Wong 2016). Behavioural assays were repeated for *G. holbrooki* a
190 day later. We consider both repeats of the *G. holbrooki* behavioural assays in this study
191 because it adds more precision to the dataset given the relatively small sample size when
192 compared to the *G. affinis* dataset. We do not calculate repeatability in this study, but these
193 behaviours have previously been found to be repeatable in both species (*G. affinis*: Cote et al.
194 2010, 2011, 2013; *G. holbrooki*: Wilson et al. 2010, Polverino et al. 2018).

195

196 ***Tendency to shoal (sociability)***

197 To measure social behaviour, we recorded the amount of time an individual spent near a
198 shoal of conspecifics (*sensu* Ward et al. 2004, Bertram et al. 2018). The experimental
199 aquarium (50 cm length × 25 cm width × 30 cm height) was divided lengthwise into three
200 compartments (two small and one large central compartment) using two transparent glass
201 partitions 12.5 cm from each end of the tank. The partitions allowed visual, but not physical
202 or olfactory, interaction between the shoal and the focal individual. A randomly designated
203 stimulus shoal was introduced to one of the smaller compartments 1 h before the experiment
204 began, while the other small compartment was left empty as a control. Stimulus shoals were
205 comprised of 14 mosquitofish (seven conspecific males and seven conspecific females) that
206 had no previous experience with the focal individual. After 1 h, the focal fish was introduced
207 into an opaque cylinder in the centre of the larger, central compartment and given 10 min to
208 acclimate. At the end of the acclimation period, the cylinder surrounding the focal fish was
209 remotely removed to allow the fish access to the central compartment with minimal

210 disturbance. The position of the focal fish was continuously recorded for 10 min. The large
211 compartment was divided with vertical marks every 2 cm, and the time spent by the focal fish
212 within the 2 cm closest to the stimulus shoal was recorded. At the conclusion of the trial,
213 individuals were returned to their holding aquaria.

214

215 ***Boldness and exploration in a novel environment***

216 One hour after the sociability assay, boldness and exploration were assessed by recording
217 behaviour in a novel environment. The experimental arenas differed slightly for data
218 collected on each species. For *G. affinis*, the experimental arena was an opaque, white plastic
219 tank (80 cm length × 80 cm width × 20 cm height) filled with 10 cm of water, and furnished
220 with half flower pots in two corners, which served as additional refuges. For *G. holbrooki*,
221 the experimental arena consisted of a glass aquarium (60 cm length × 30 cm width × 30 cm
222 height), filled with 15 cm of water, with 72 equal grid squares marked on its base. For both
223 species, focal fish were added gently to an upright, cylindrical (9–10 cm diameter) opaque
224 PVC pipe refuge on one side of the experimental arena. After 10 min, a 4 cm wide door to the
225 refuge chamber was remotely opened, allowing fish access to the experimental arena. We
226 then allowed the fish 45 min to leave the refuge, recording the time to exit. After the fish left
227 the refuge, we then allowed an additional 5 min to explore the novel environment. Because
228 we gave fish an additional 5 min to explore the novel environment after it left the refuge, we
229 treated both behavioural measures as independent behavioural traits. Trials ended either 5
230 min after fish left the refuge or after 45 min (2700 s) if animals did not leave the refuge.

231

232 For both species, boldness was measured as the maximum time allowed for fish to exit the
233 refuge (2700 s) minus the latency (s) to exit from the refuge, and to stay for greater than 10
234 consecutive seconds out of the refuge. Shorter latency to exit the refuge indicates a higher

235 boldness and is regularly used as a metric for boldness in studies of fish (Moran et al. 2016,
236 Hulthén et al. 2017), including mosquitofish (Wilson et al. 2010, Bertram et al. 2018,
237 Polverino et al. 2018). Exploratory behaviour was quantified by measuring how much of the
238 experimental arena the focal individual covered. For *G. affinis*, the area explored
239 incorporated both the distance an individual moved and the spatial pattern of those
240 movements. Given x – y coordinates from each video frame, each individual’s continuous
241 path was tracked, and the area an individual explored was calculated as the percentage of the
242 arena that fell within 5 cm of the fish’s path. For *G. holbrooki*, the area explored was
243 calculated by dividing the total number of unique grid-squares an individual entered by the
244 total number of grid squares ($n = 72$).

245

246 **Morphological measurements**

247 All fish were weighed and measured before and after the behavioural assays. *G. affinis* were
248 larger than *G. holbrooki* for both sexes (mean male total body length (TBL) \pm standard error
249 (SE): *G. affinis*: 23.07 ± 0.25 mm, *G. holbrooki*: 21.45 ± 0.28 mm, Mann-Whitney test: $U =$
250 1858 , $p < 0.001$; mean female TBL \pm SE: *G. affinis*: 29.34 ± 0.56 mm, *G. holbrooki*: $25.78 \pm$
251 0.37 mm; $U = 1890$, $p < 0.001$).

252

253 **Statistical analysis**

254 Data were analysed in R version 3.3.2 (R Core Development Team 2016). Residuals were
255 checked for normality (Shapiro-Wilk test: Royston 1995) and homogeneity of variance
256 (Fligner-Killeen test: Conover et al. 1981). Prior to analysis, time spent in the 2 cm social
257 zone (i.e. sociability) was rank-transformed, and latency to exit the refuge was log-
258 transformed, to approximate Gaussian error distributions. Because each species was reared
259 under different conditions and there were slight differences in the design of behavioural

260 assays, we ran separate statistical tests for each species. Thus, any species-level comparison
261 is based upon a comparison of two separate models and not statistically computed. Statistical
262 significance was assigned at $\alpha = 0.05$.

263

264 We first tested whether the sexes differed in the individual behavioural traits studied using
265 linear models for the *G. affinis* dataset, and linear mixed-effects models (LMM; package
266 *lme4*, Bates et al. 2015) for the *G. holbrooki* dataset. Models contained the fixed effects of
267 sex, body length and a sex \times body length interaction. We also included trial number and sex
268 \times trial number interaction as fixed factors, and individual ID as a random factor within the
269 mixed-effects models in order to consider the repeated measures design of the *G. holbrooki*
270 dataset. P-values of interaction terms were calculated using likelihood ratio tests (G^2) for
271 LMM's (Bolker et al. 2009) and Wald's F-tests were used for linear models. If interaction
272 terms were non-significant they were removed from the final models.

273

274 We assessed trait correlations within species and sex to determine the presence of
275 behavioural syndromes. To do this, we estimated the magnitude of pairwise relationships
276 between behavioural traits using spearman-rank correlations and compared the correlation
277 coefficients using the Fisher z -transformations.

278

279 **RESULTS**

280 **1. Behavioural types**

281 *1a) Gambusia holbrooki*

282 We found no effect of sex, trial or body length on *G. holbrooki* shoaling behaviour or time
283 taken to re-emerge from the refuge ($p < 0.05$, Table 1; Figure 1). Regardless of sex, fish
284 explored more of the novel environment in trial 2 compared to trial 1, suggesting habituation

285 to the experimental arena ($t_{1,49} = 2.77, p = 0.008$; Table 1). However, there was no effect of
286 sex or body length on the exploratory behaviour of *G. holbrooki* ($p < 0.05$, Table 1, Figure 1).

287

288 *1b) Gambusia affinis*

289 Female *G. affinis* spent more time shoaling than male *G. affinis* ($t_{1,219} = 2.632, p = 0.009$;
290 Table 2, Figure 1). Regardless of sex, larger fish spent less time shoaling with conspecifics
291 than smaller fish, although the effect of body length was marginally non-significant ($t_{1,219} = -$
292 $1.917, p = 0.057$; Table 2). Males re-emerged from the refuge faster than females ($t_{1,219} =$
293 $2.483, p = 0.014$; Table 2, Figure 1), but this effect was dependent on body length (sex \times
294 body length interaction: $F_{1,218} = 5.394, p = 0.021$; Table 2), with smaller males re-emerging
295 faster from the refuge than larger males ($t_{1,110} = -2.326, p = 0.022$). We found no effect of sex
296 or body length on the tendency to explore the novel environment ($p < 0.05$, Table 2).

297

298 **2. Behavioural syndromes**

299 *2a) Gambusia holbrooki*

300 We found weak evidence of a behavioural syndrome in *G. holbrooki*. There was a marginal
301 positive correlation between time to re-emerge from the refuge (boldness) and tendency to
302 explore the novel environment (exploration) in females, but this correlation was negative in
303 males (Table 3; Figure 2).

304

305 *2b) Gambusia affinis*

306 We found a significant positive correlation between the time taken to exit the refuge
307 (boldness) and tendency to explore the novel environment (exploration) in both male and
308 female *G. affinis* (Table 3; Figure 2). There was also evidence that time spent shoaling with
309 conspecifics (sociability) and tendency to explore the novel environment (exploration) were

310 positively correlated in male *G. affinis* (Table 3). This correlation was not present in females,
311 but the correlation coefficients did not significantly differ between the sexes (Table 3).

312

313 **DISCUSSION**

314 We found evidence for sex-specific personality in invasive mosquitofish, but that these sex
315 differences varied depending on species. Specifically, male *G. affinis* were bolder and less
316 social than female *G. affinis*, but we found no behavioural type differences between the sexes
317 in *G. holbrooki*. There was also a positive correlation between boldness and exploration
318 within *G. affinis*, which was consistent in magnitude and direction in both males and females.
319 Notably, however, we also found that sociability was correlated to exploration in male *G.*
320 *affinis*, but not in females. There was only a weak positive correlation between boldness and
321 exploration in female *G. holbrooki*, but this correlation was negative in males. The absence of
322 a common behavioural syndrome between *Gambusia* species is inconsistent with the
323 hypothesis that there is a specific suite of behaviours that might help to explain both species'
324 successful invasion history. Instead, our results suggest that behavioural tendencies may
325 diverge among species and between the sexes because of adaptation experienced during
326 different invasion pathways.

327

328 Sex differences in boldness and sociability in *G. affinis* are likely a product of disparate
329 reproductive and life-history strategies. Females often bear a higher cost of reproduction than
330 males, resulting in females having life-history and behavioural traits associated with a slower
331 pace-of-life that maximises fecundity and reproductive output (e.g. longer life span, less risk-
332 taking; Debecker et al. 2016). Indeed, fecundity selection is a major evolutionary force
333 selecting for larger body size in females in sexually size-dimorphic species (Shine 1989), and
334 higher levels of risk-taking have previously been linked to lower fecundity in female

335 mosquitofish (Wilson et al. 2010). Females also tend to be more social than males and utilise
336 the anti-predator benefits of group shoaling (i.e. dilution effects: Foster & Treherne 1981,
337 increased vigilance: Hoare et al. 2000) as a risk-avoidance strategy. Shoaling has also been
338 shown to reduce the foraging and reproductive costs of sexual harassment by males of female
339 mosquitofish (Pilastro et al. 2003). In several aquatic organisms, females have also been
340 shown to be less bold than males (Harris et al. 2010, King et al. 2013, Biro et al. 2014,
341 Debecker et al. 2016). Such low risk-taking behaviour may be particularly important for
342 female mosquitofish, which may be preferentially targeted by predators as they are larger
343 than males, and consequently represent more profitable prey (Britton & Moser 1982).
344 Interestingly, we also found in the current study that larger males took longer to re-emerge
345 from the refuge than smaller males. This result corroborates with the idea that being larger
346 makes you more vulnerable to predators, thus larger individuals adopt less risky behavioural
347 strategies. On the other hand, smaller male *G. affinis* may have been faster to exit the refuge
348 because the costs associated with hiding, such as the loss of reproductive opportunities
349 (Martín et al. 2003), outweigh the benefits of such risk-avoidance behaviours. Indeed, male
350 mating success is typically highly variable, particularly for smaller males who are often
351 perceived as lower quality mates by females (Tomkins et al. 2018), which likely encourages a
352 'high risk, high reward' behavioural strategy in these smaller males (King et al. 2013).

353

354 It is somewhat surprising, then, that we did not find the same differences in boldness and
355 sociability between male and female *G. holbrooki*. A possible explanation for this lack of
356 divergence in boldness and social traits is that *G. holbrooki*, in this study, were sourced from
357 an environment with low predation pressure, and thus the risk of emerging from a refuge and
358 the benefits of shoaling in a group were perceived by females to be low, resulting in females
359 being equally likely to take 'risks' as males. An alternative reason for a lack of sex

360 differences in *G. holbrooki* more broadly is that the body range size of our study population
361 was different to the natural variation in body size observed in other wild populations (e.g.
362 McPeck 1992). The size differences between males and females in *G. holbrooki* was
363 comparatively smaller than *G. affinis*, thus the costs associated with having larger body size
364 may not be as robust in our *G. holbrooki* population, favouring selection towards similar
365 behavioural tendencies between the sexes (Fairbairn et al. 2007).

366

367 We observed markedly different personality traits between species. A consistent behavioural
368 correlation between boldness and exploration was present in male and female *G. affinis*, but
369 this correlation was weak in *G. holbrooki*. Indeed, observed behavioural differences between
370 species are limited by the fact that we only compared one population of each species with
371 unequal sample sizes. Thus, these results should be interpreted with much caution as our
372 study does not offer a robust test of species differences, which was not the main aim of this
373 research. However, past studies have found differences in behavioural traits between *G.*
374 *affinis* and *G. holbrooki* (e.g. dispersal: Rehage & Sih 2004; antipredator response: Rehage et
375 al. 2005). In this study, behavioural differences between species could be a result of
376 differences in the level of predation pressure along the introduction pathway and/or the local
377 environment. Our finding that a boldness-exploration behavioural syndrome differed between
378 species is consistent with most comparative studies on behavioural correlations, which have
379 found remarkable variation in syndromes, particularly those related to boldness (Bell & Sih
380 2007, Dingemanse et al. 2007; Michelangeli et al. in press). These studies suggest that high-
381 consistency in behavioural syndromes are often linked to high-predation sites that place
382 consistent selection on groups of behaviours, particularly behaviours linked to risk taking,
383 compared to more benign environments which favour variable behavioural strategies
384 (Heinen-Kay et al. 2016). For example, in three-spine sticklebacks, *Gasterosteus aculeatus*

385 (Linnaeus, 1758), populations raised in high predation risk environments exhibit a boldness-
386 aggression syndrome, whereas populations raised in low predation environments lost this
387 syndrome (Bell & Sih 2007). It should also be noted, however, that differences between
388 species could be a result of differences in how each species were reared in our study; as *G.*
389 *holbrooki* were housed in isolation during the experimental period rather than in groups, and
390 this could have had an influence on their behaviour (Gómez-Laplaza & Morgan 2000, Bevan
391 et al. 2018).

392

393 Interestingly, we found evidence that sociability is correlated to exploration in male *G.*
394 *affinis*, but not females. Sociability has previously been found to be linked to dispersal in *G.*
395 *affinis*, whereby asocial individuals tend to disperse further, faster and more frequently than
396 social individuals when population densities are high (Cote et al. 2010b, 2011, 2013).
397 Sociability-dependent dispersal in *G. affinis* has also been found to generate more severe
398 impacts on native aquatic insect communities compared to random dispersal (Cote et al.
399 2017). Furthermore, dispersal propensity is higher in males compared to females (Cote et al.
400 2010b, 2011). Our results, together with these earlier studies, suggest that males (i.e. the
401 more dispersive sex), that are asocial and bold, would be more likely to disperse away from
402 established populations (i.e. high-density populations) and lead the invasion front. On the
403 other hand, sociability appears to be independent of exploration and boldness in females.
404 These sex-specific differences in personality and dispersal may thus have important
405 implications for the spread and invasion of western mosquitofish, and for their impacts on
406 native ecosystems, as the behavioural composition of range-front populations may be sex-
407 dependent. Conversely, due to a lack of behavioural differences between sexes, *G. holbrooki*
408 invasions may be less prone to skewed sex ratios at the invasion front. A future study that
409 explores the interaction between sex- and behavioural-dependent dispersal, and its

410 implications for founder populations, would yield interesting insights into the spread
411 dynamics of invasive mosquitofish populations.

412

413 To conclude, our results suggest that different mosquitofish invasions have required different
414 behavioural tendencies to succeed, and that some of these behaviours are likely sex-
415 dependent. We found limited evidence of sex-specific personality in *G. holbrooki*, suggesting
416 that both sexes have an equal invasion potential. In contrast, differences in syndromes
417 between male and female *G. affinis* could be a mechanism that leads to sex-dependent
418 dispersal in this species, and thus unequal sex ratios at the leading edge, but future studies are
419 needed to test the validity of these hypotheses. Overall, identifying the behavioural
420 mechanisms that predict an individual's 'invasiveness' is difficult to tease apart between
421 species because each invasion is characterised by different abiotic and biotic interactions that
422 likely require different suites of behaviours (Felden et al. 2018, Mennan & Laskowski 2018).
423 Future studies are needed to elucidate whether, in fact, personality variation between the
424 sexes can mediate the occurrence of sex-biased invasions.

425

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433

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637 **FIGURE LEGENDS**

638 **Figure 1.** Mean (\pm standard error) trait-level differences of females (black) and males (blue)
639 across two *Gambusia* species (*G. affinis* and *G. holbrooki*) in A) tendency to shoal (i.e. time
640 spent within 2 cm social zone; sociability), B) boldness score (i.e. log maximum time allowed
641 for fish to exit the refuge (2700 s) minus the log latency (s) to exit from the refuge; boldness)
642 and C) tendency to explore a novel environment (% of novel environment explored;
643 exploration). Sample sizes differed between species; *G. affinis* (female: n = 112, male: n =
644 111), *G. holbrooki* (female: n = 25, male: n = 25).

645

646 **Figure 2.** Sex regression lines for relationship between tendency to explore a novel
647 environment (% of novel environment explored) and boldness score (i.e. log maximum time
648 allowed for fish to exit a refuge (2700 s) minus the log latency (s) to exit from the refuge)
649 within two mosquitofish species, *Gambusia affinis* (top; female: n = 112, male: n = 111) and
650 *Gambusia holbrooki* (bottom; female: n = 25, male: n = 25). Males = dashed lines, triangles,
651 females = solid line, circles.

652 **Table 1:** Main effects of sex, body length and trial on a) time spent shoaling with conspecifics, b) time to re-emerge from a refuge, and c)
653 percentage of novel environment explored, in *Gambusia holbrooki* (female: $n = 25$; male: $n = 25$). Models were first compared with and without
654 the interaction terms using Likelihood ratio tests (G^2). Interaction terms were removed from the final models if were non-significant. Results
655 were obtained from linear mixed effects models (LMM) and contained individual ID as a random factor.

Behaviour	Fixed effects	G^2	β	t	p
a) Time spent shoaling	Sex	–	0.498	1.474	0.147
	Body length	–	0.004	-0.085	0.932
	Trial	–	0.170	1.393	0.170
	Sex x trial	1.392	–	–	0.238
	Sex x body length	0.150	–	–	0.698
b) Time to re-emerge from refuge	Sex	–	0.270	0.795	0.431
	Body length	–	0.059	1.085	0.284
	Trial	–	0.277	1.440	0.156
	Sex x trial	3.368	–	–	0.066
	Sex x body length	0.071	–	–	0.790
c) % of novel environment explored	Sex	–	0.022	0.382	0.704
	Body length	–	0.014	1.543	0.129
	Trial	–	0.093	2.772	0.008
	Sex x trial	0.031	–	–	0.860
	Sex x body length	0.281	–	–	0.596

656 G^2 = chi-squared value. β = co-efficient. Bold refers to significant terms a $P < 0.05$. Note that each species differed substantially in sample size.

657

658 **Table 2:** Main effects of sex and body length on a) time spent shoaling with conspecifics, b) time to re-emerge from a refuge, and c) percentage
 659 of novel environment explored, in *Gambusia affinis* (female: $n = 110$; male: $n = 112$). Results were obtained from linear models. Bold terms
 660 indicate significant results

Behaviour	Fixed effects	F	β	t	p
a) Time spent shoaling	Sex	–	0.612	2.632	0.009
	Body length	–	-0.052	-1.917	0.057
	Sex × body length	0.214	–	–	0.644
b) Time to re-emerge from refuge	Sex	–	3.356	2.483	0.014
	Body length	–	0.008	0.326	0.745
	Sex × body length	5.394	0.128	2.323	0.021
c) % of novel environment explored	Sex	–	0.022	0.800	0.425
	Body length	–	<0.001	0.164	0.870
	Sex × body length	1.931	–	–	0.166

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673 **Table 3:** Correlation coefficients (r) for each behavioural correlation estimated using Spearman rank correlation tests and Fisher z statistic
 674 comparing the sex-specific effect sizes. Bold scores refer to significant correlation coefficients.

Species	Correlation	♂		♀		Fisher z	Total (♂+♀)	
		r	p	r	p		r	p
<i>G. holbrooki</i>	Boldness - Exploration	-0.11	0.46	0.26	0.07	$z = -1.82, p = 0.07$	0.08	0.40
	Sociability - Exploration	-0.03	0.84	0.11	0.43	$z = -0.70, p = 0.48$	0.05	0.59
	Sociability - Boldness	0.01	0.99	0.03	0.86	$z = -0.12, p = 0.90$	0.01	0.93
<i>G. affinis</i>	Boldness - Exploration	0.28	<0.01	0.32	< 0.01	$z = -0.33, p = 0.74$	0.26	< 0.01
	Sociability - Exploration	0.21	0.02	0.05	0.61	$z = 1.19, p = 0.23$	0.14	0.04
	Sociability - Boldness	0.10	0.30	0.10	0.30	$z = 0.01, p = 0.99$	0.08	0.24

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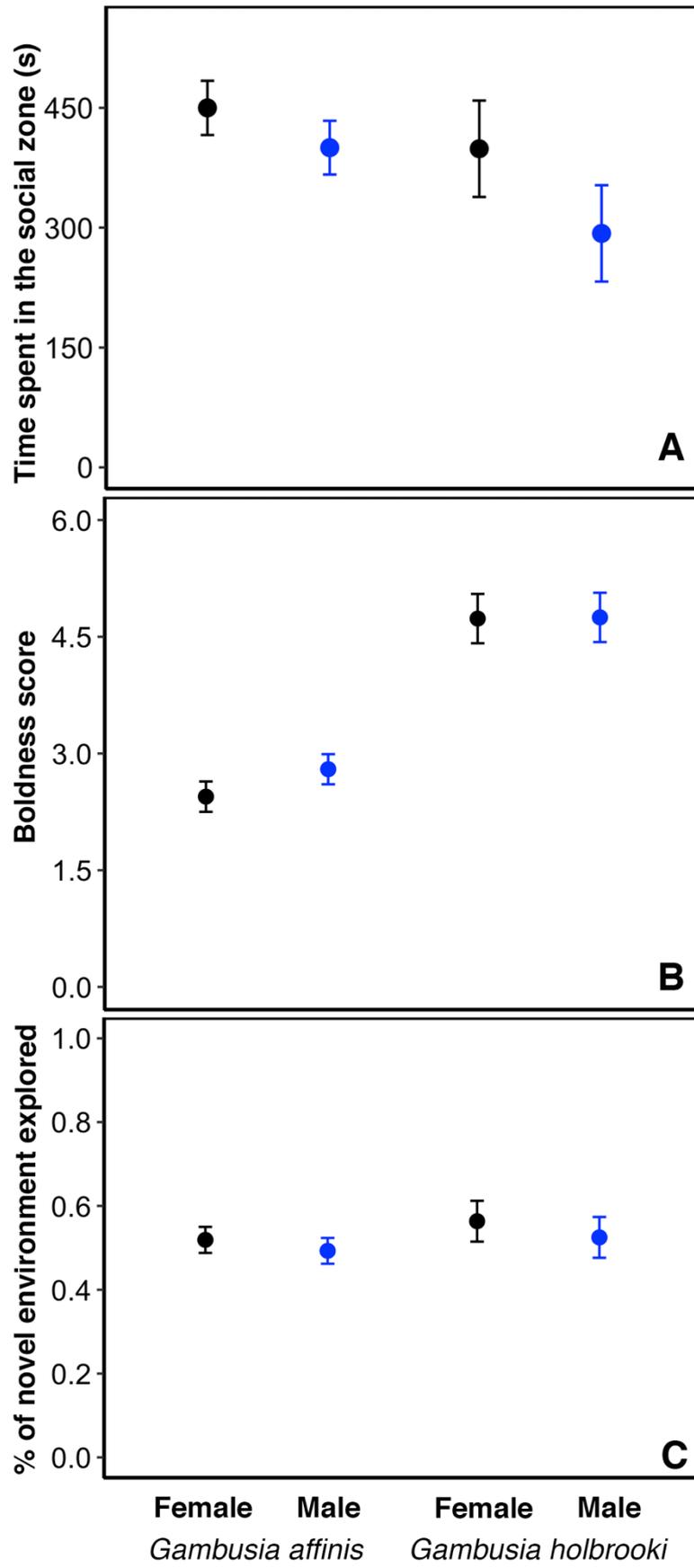


Figure 1.

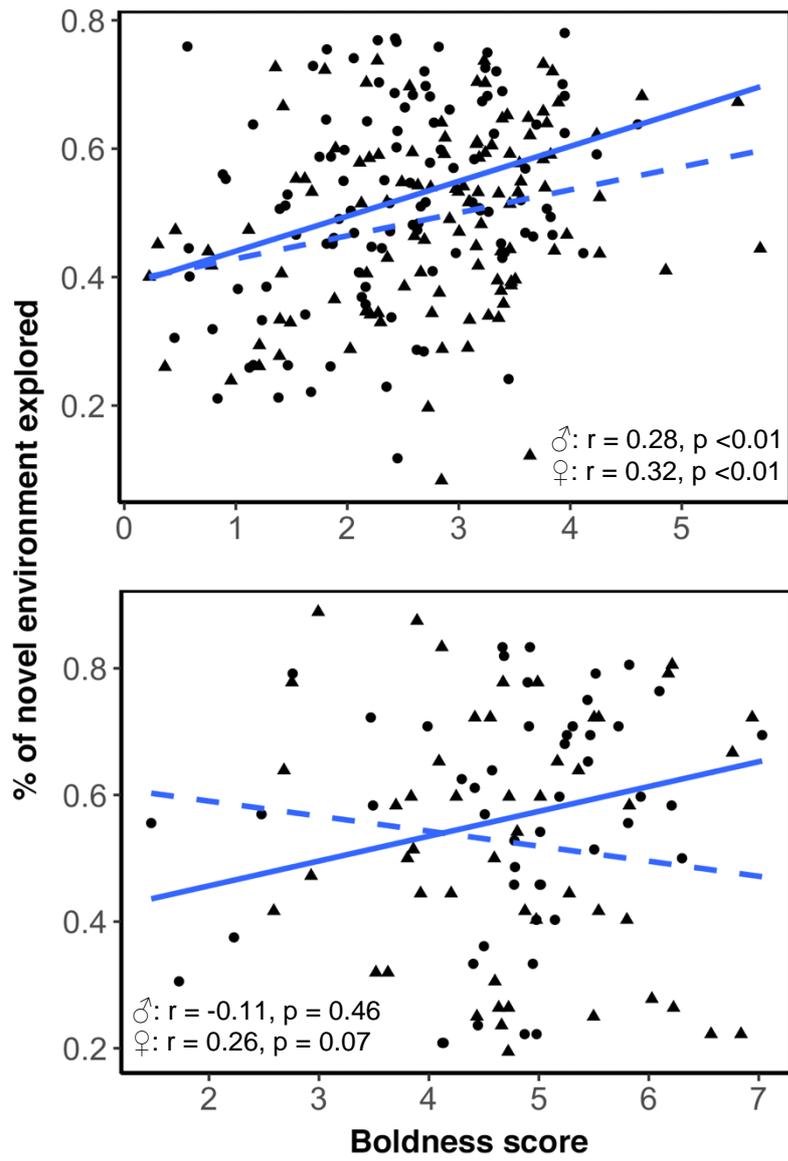


Figure 2.