ORIGINAL PAPER

Sex-dependent personality in two invasive species of mosquitofish

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

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Introduction

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

Evidence is accumulating that biological invasions can be often be disproportionately driven by a single sex (Gutowsky and Fox 2011; Miller and Inouye 2013; Rebrina et al. 2015). Skewed sex ratios at the leading edge of an invasion can have profound consequences for population growth and persistence (Miller and Inouye 2013; Shaw et al. 2018), and can lead to greater adverse impacts on native communities than non-skewed sex ratios (Fryxell et al. 2015). For instance, female-biased invasion front populations can exponentially increase the pace of an invasion by speeding up population growth, resulting in a higher probability of colonisation success (Miller and Inouye 2013). Alternatively, male-biased invasions may be more likely to competitively exclude native species, creating new opportunities for habitats and resources to be exploited (Duckworth and Badyaev 2007; Gutowsky and Fox 2011). However, despite the prevalence of sex-biased invasions, the mechanisms that lead to biased sex ratios at the front of an invasion have rarely been studied.

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

One relatively new approach to investigating the role of behaviour in invasions is through the study of animal personalities (see reviews: Cote et al. 2010a; Chapple et al. 2012; Sih et al. 2012; Juette et al. 2014). Animal personality refers to the concept that individuals within populations often show consistent differences in a range of behaviours (i.e. behavioural types: Sih et al. 2004), and these behaviours can covary across time/and or context (i.e. behavioural syndromes; Sih et al. 2004). Personality traits are often linked to life-history (reproduction and growth rates: Biro and Stamps 2008), ecological processes (habitat specialisation: Michelangeli et al. 2018a), and social roles within populations and communities (e.g. innovation and cultural transmission: Aplin et al. 2015). Given its direct bearing on fitness, an individual's personality should also influence its probability of transitioning through the invasion process, with different behavioural types being advantageous at different stages of invasion (Cote et al. 2010a; Fogarty et al. 2011; Chapple et al. 2012; Chapple and Wong 2016). Indeed, mounting evidence suggests that invasive individuals may exhibit combinations of behaviours that are beneficial in outcompeting native species (Pintor et al. 2009), dispersing into new habitats (Michelangeli et al. 2017), and avoiding novel predators (Mennen and Laskowski 2018). In this regard, personality differences between the sexes could underlie differences in sex-biased dispersal and sex-biased invasiveness (Mishra et al. 2018). Sex differences in the direction and magnitude of behavioural syndromes could arise due to divergent selection pressures and life-histories after maturation. This may be particularly true for sexually dimorphic

species, as marked differences in morphology (e.g. body size) can induce variance in behaviour (Shine 1989; Fairbairn et al. 2007). For instance, larger body size requires higher energetic input and, thus, personality traits that are associated with an increase in feeding rate (Biro and Stamps 2008). If personality influences an individual's level of "invasiveness" and, hence, their potential impact on the environment, it is important to consider how the sexes might differ in personality to better understand the behavioural mechanisms involved in successful invasions.

In this study, we compared the personality traits of males and females in congeneric invasive species, the western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853) and the eastern mosquitofish, *Gambusia holbrooki* (Girard, 1859). These species provide an ideal opportunity to explore sex differences in personality traits related to invasion for several reasons. First, *Gambusia* are small live-bearing freshwater fish that show pronounced sexual size dimorphism, whereby females are commonly much larger than males (Pyke 2005). Second, both species have undergone numerous deliberate (i.e. introduced as a biocontrol tool for mosquito larvae) and unintentional introductions, and have now spread and become invasive globally, placing them within the top 100 of the world's most invasive species (Lowe et al. 2000; Pyke 2008). Third, invasive populations are often characterised by demographic differences in sex ratios that can either be skewed towards males or females (Fryxell et al. 2015). Fourth, mosquitofish are having tremendous adverse impacts on native insect, amphibian and fish communities worldwide (Pyke 2008; Shulse et al. 2013). Importantly, some studies suggest that the magnitude of these impacts are dependent upon both the sex ratio (Fryxell et al. 2015), and the personality composition of invading populations (Cote et al. 2017). Thus, understanding the behavioural mechanisms driving *Gambusia* invasions is an issue of immediate importance.

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

Methods

Species collection and husbandry

Gambusia holbrooki (female: $n = 25$; male: $n = 25$) were collected from the Science Centre Lake (37° 54′ 28″ S; 145° 08′ 16″ E), Monash University, Victoria, Australia on 22 January 2014. All fish were caught via seine netting to minimise potential personality-biased sampling (Michelangeli et al. 2016b). Fish were housed individually in glass holding tanks (30 cm length × 15 cm width × 20 cm height) and acclimated to laboratory conditions for 1 month prior to experimentation. We housed fish individually in order to keep track of their identity during behavioural assays. Throughout the housing period, fish were kept at a temperature of 24–26 °C, and under a 12:12 h light:dark cycle. Both during housing and throughout experimentation, fish were fed ad libitum with commercial fish food.

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

Morphological measurements

All fish were measured before and after the behavioural assays. *G. affinis* were larger than *G. holbrooki* for both sexes [mean male total body length (TBL) ± standard error (SE): *G. affinis*: 23.07 ± 0.25 mm, *G. holbrooki*: 21.45 ± 0.28 mm, Mann–Whitney test: $U = 1858$, $p < 0.001$; mean

female TBL ± SE: *G. affinis*: 29.34 ± 0.56 mm, *G. holbrooki*: 25.78 ± 0.37 mm; $U = 1890$, $p < 0.001$].

Behavioural assays

For both species, in order to characterise personality types of each sex, we ran two behavioural assays, each separated by 1 h. First, we tested sociability by quantifying the tendency of individuals to shoal. Second, we tested individual boldness and exploratory behaviours. The former was characterised by the latency of fish to exit from a refuge and enter a novel environment, and the latter was quantified by recording the movement and space use of fish after exiting the refuge. These behaviours represent an individual's reaction to a social context and to a novel environment, respectively. Both sets of behaviours are hypothesised to play an important role in colonising new environments (Chapple et al. 2012; Sih et al. 2012; Chapple and Wong 2016). Behavioural assays were repeated for *G. holbrooki* a day later. We consider both repeats of the *G. holbrooki* behavioural assays in this study because it adds more precision to the dataset given the relatively small sample size when compared to the *G. affinis* dataset. We do not calculate repeatability in this study, but these behaviours have previously been found to be repeatable in both species (*G. affinis*: Cote et al. 2010b, 2011, 2013; *G. holbrooki*: Wilson et al. 2010; Polverino et al. 2018).

Tendency to shoal (sociability)

To measure social behaviour, we recorded the amount of time an individual spent near a shoal of conspecifics (sensu Ward et al. 2004; Bertram et al. 2018). The experimental aquarium (50 cm length × 25 cm width × 30 cm height) was divided lengthwise into three compartments (two small and one large central compartment) using two transparent glass partitions 12.5 cm from each end of the tank. The partitions allowed visual, but not physical or olfactory, interaction between the shoal and the focal individual. A randomly designated stimulus shoal was introduced to one of the smaller compartments 1 h before the experiment began while the other small compartment was left empty as a control. Stimulus shoals were comprised of 14 mosquitofish (seven conspecific males and seven conspecific females) that had no previous experience with the focal individual. After 1 h, the focal fish was

introduced into an opaque cylinder in the centre of the larger, central compartment and given 10 min to acclimate. At the end of the acclimation period, the cylinder surrounding the focal fish was remotely removed to allow the fish access to the central compartment with minimal disturbance. The position of the focal fish was continuously recorded for 10 min. The large compartment was divided with vertical marks every 2 cm, and the time spent by the focal fish within the 2 cm closest to the stimulus shoal was recorded. At the conclusion of the trial, individuals were returned to their holding aquaria.

Boldness and exploration in a novel environment

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

For both species, boldness was measured as the maximum time allowed for fish to exit the refuge (2700 s) minus the latency (s) to exit from the refuge, and to stay for greater than 10 consecutive seconds out of the refuge. Shorter latency to exit the refuge indicates a higher boldness and is regularly used as a metric for boldness in studies of fish (Moran et al.

2016; Hulthén et al. 2017), including mosquitofish (Wilson et al. 2010; Bertram et al. 2018; Polverino et al. 2018). Exploratory behaviour was quantified by measuring how much of the experimental arena the focal individual covered. For *G. affinis*, the area explored incorporated both the distance an individual moved and the spatial pattern of those movements. Given x–y coordinates from each video frame, each individual's continuous path was tracked, and the area an individual explored was calculated as the percentage of the arena that fell within 5 cm of the fish's path. For *G. holbrooki*, the area explored was calculated by dividing the total number of unique grid-squares an individual entered by the total number of grid squares ($n = 72$).

Statistical analysis

Data were analysed in R version 3.3.2 (R Core Development Team 2016). Residuals were checked for normality (Shapiro–Wilk test: Royston 1995) and homogeneity of variance (Fligner-Killeen test: Conover et al. 1981). Prior to analysis, time spent in the 2 cm social zone (i.e. sociability) was rank-transformed, and latency to exit the refuge was log-transformed, to approximate Gaussian error distributions. Because each species was reared under different conditions and there were slight differences in the design of behavioural assays, we ran separate statistical tests for each species. Thus, any species-level comparison is based upon a comparison of two separate models and not statistically computed. Statistical significance was assigned at $\alpha = 0.05$.

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

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

Results

Behavioural types

Gambusia holbrooki

We found no effect of sex, trial or body length on *G. holbrooki* shoaling behaviour or time taken to re-emerge from the refuge ($p < 0.05$, Table 1; Fig. 1). Regardless of sex, fish explored more of the novel environment in trial 2 compared to trial 1, suggesting habituation to the experimental arena ($t_{1,49} = 2.77$, $p = 0.008$; Table 1). However, there was no effect of sex or body length on the exploratory behaviour of *G. holbrooki* ($p < 0.05$, Table 1, Fig. 1).

Gambusia affinis

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

Behavioural syndromes

Gambusia holbrooki

We found weak evidence of a behavioural syndrome in *G. holbrooki*. There was a marginal positive

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) percentage of novel environment explored, in *Gambusia holbrooki* (female: $n = 25$; male: $n = 25$). Models were first compared with and without the interaction terms using likelihood ratio tests (G^2). Interaction terms were removed from the final models if were non-significant. Results 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 × trial	1.392	–	–	0.238
	Sex × 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 × trial	3.368	–	–	0.066
	Sex × 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 × trial	0.031	–	–	0.860
	Sex × body length	0.281	–	–	0.596

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

correlation between time to re-emerge from the refuge (boldness) and tendency to explore the novel environment (exploration) in females, but this correlation was negative in males (Table 3; Fig. 2).

Gambusia affinis

We found a significant positive correlation between the time taken to exit the refuge (boldness) and tendency to explore the novel environment (exploration) in both male and female *G. affinis* (Table 3; Fig. 2). There was also evidence that time spent shoaling with conspecifics (sociability) and tendency to explore the novel environment (exploration) were positively correlated in male *G. affinis* (Table 3). This correlation was not present in females, but the correlation coefficients did not significantly differ between the sexes (Table 3).

Discussion

We found evidence for sex-specific personality in invasive mosquitofish, but these sex differences varied depending on species. Specifically, male *G. affinis* were bolder and less social than female *G. affinis*, but we found no behavioural type differences between the sexes in *G. holbrooki*. There was also a positive correlation between boldness and exploration within *G. affinis*, which was consistent in magnitude and direction in both males and females. Notably, however, we also found that sociability was correlated with exploration in male *G. affinis*, but not in females. There was only a weak positive correlation between boldness and exploration in female *G. holbrooki*, but this correlation was negative in males. The absence of a common behavioural syndrome between *Gambusia* species is inconsistent with the hypothesis that there is a specific suite of behaviours that might help to explain both species' successful invasion history. Instead, our results suggest that behavioural tendencies may
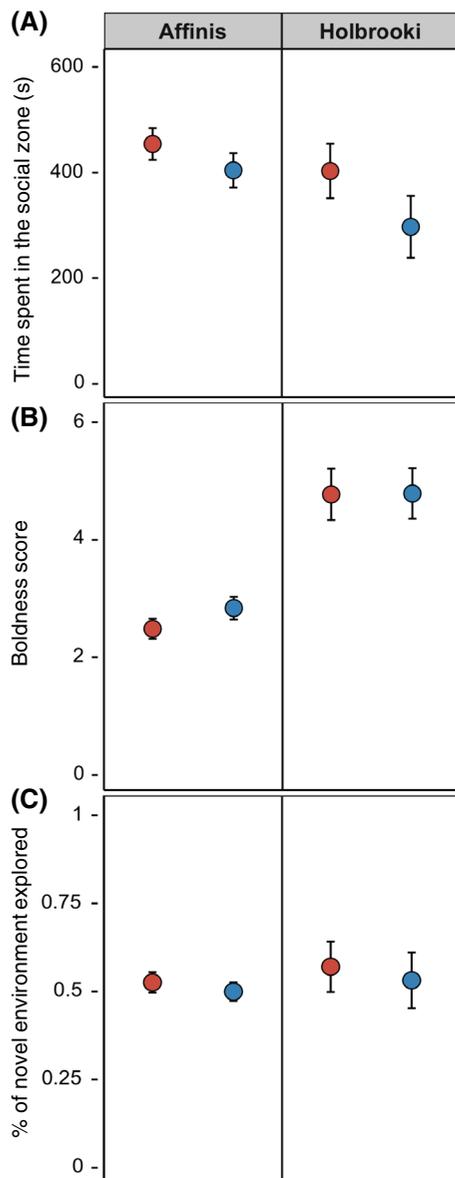
Fig. 1 Mean (± 95% CI) trait-level differences of females (red) and males (blue) across two *Gambusia* species (*G. affinis* and *G. holbrooki*) in **a** tendency to shoal (i.e. time spent within the 2 cm social zone; sociability), **b** boldness score (i.e. log maximum time allowed for fish to exit the refuge (2700 s) minus the log latency (s) to exit from the refuge; boldness) and **c** tendency to explore a novel environment (% of novel environment explored; exploration). Sample sizes differed between species; *G. affinis* (female: n = 112, male: n = 111), *G. holbrooki* (female: n = 25, male: n = 25)

diverge among species and between the sexes because of selection pressures experienced during different invasion pathways.

Sex differences in boldness and sociability in *G. affinis* are likely a product of disparate reproductive and life-history strategies. Females often bear a higher cost of reproduction than males, resulting in females having life-history and behavioural traits associated with a slower pace-of-life that maximises fecundity and reproductive output (e.g. longer life span, less risk-taking; Debecker et al. 2016). Indeed, fecundity selection is a major evolutionary force selecting for larger body size in females in sexually size-dimorphic species (Shine 1989), and higher levels of risk-taking have previously been linked to lower fecundity in female mosquitofish (Wilson et al. 2010). Females also tend to be more social than males and utilise the anti-predator benefits of group shoaling (i.e. dilution effects: Foster and Treherne 1981; increased vigilance: Hoare et al. 2000) as a risk-avoidance strategy. Shoaling has also been shown to reduce the foraging and reproductive costs of sexual harassment by males of female mosquitofish (Pilastro et al. 2003). In several aquatic organisms, females have also been shown to be less bold than males (Harris et al. 2010; King et al. 2013; Biro et al. 2014; Debecker et al. 2016). Such low risk-taking behaviour may be particularly important for female mosquitofish, which may be preferentially targeted by predators as they are larger than males, and consequently represent more profitable prey (Britton and Moser 1982). Interestingly, we also found in the current study that larger males took longer to re-emerge from the refuge than smaller males. This result corroborates with the idea that being larger makes you more vulnerable to predators, thus larger individuals adopt less risky behavioural strategies. On the other hand, smaller male *G. affinis* may have been faster to exit the refuge because the costs associated with hiding, such as the loss of reproductive opportunities (Martín et al. 2003), outweigh the benefits of such risk-avoidance behaviours. Indeed, male mating success is typically highly variable, particularly for smaller males who are often perceived as lower quality mates by females (Tomkins et al. 2018), which likely encourages a "high risk, high reward" behavioural strategy in these smaller males (King et al. 2013).

It is somewhat surprising, then, that we did not find the same differences in boldness and sociability between male and female *G. holbrooki*. A possible explanation for this lack of divergence in boldness and social traits is that *G. holbrooki* in this study were sourced from an environment with low predation

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 of novel environment explored, in *Gambusia affinis* (female: $n = 110$; male: $n = 112$). Results were obtained from linear models

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

Bold terms indicate significant results

Table 3 Correlation coefficients (r) for each behavioural correlation estimated using Spearman rank correlation tests and Fisher z statistic comparing the sex-specific effect sizes

Species	Correlation	♂		♀		Fisher z	Total (♂ + ♀)	
		r	p	r	p		r	p
G. holbrooki	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
G. affinis	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

Bold scores refer to significant correlation coefficients

pressure, and thus the risk of emerging from a refuge and the benefits of shoaling in a group were perceived by females to be low, resulting in females being equally as likely to take "risks" as males. An alternative reason for a lack of sex differences in *G. holbrooki* is that the body range size of our study population was different to the natural variation in body size observed in other wild populations (e.g. McPeek 1992). The size differences between males and females in *G. holbrooki* was comparatively smaller than *G. affinis*, thus the costs associated with having larger body size may not be as robust in our *G. holbrooki* population, favouring selection towards similar behavioural tendencies between the sexes (Fairbairn et al. 2007).

We observed markedly different personality traits between species. A consistent behavioural correlation between boldness and exploration was present in male and female *G. affinis*, but this correlation was weak in *G. holbrooki*. Indeed, observed behavioural differences between species are limited by the fact that we only compared one population of each species with unequal sample sizes. Thus, these results should be interpreted with much caution as our study does not offer a robust test of species differences, which was not the main aim of this research. However, past studies have found differences in behavioural traits between *G. affinis* and *G. holbrooki* (e.g. dispersal: Rehage and Sih 2004; antipredator response: Rehage et al. 2005). In this study, behavioural differences between species could be a result of differences in the level of predation pressure along the introduction pathway and/or the local environment. Our finding that a boldness-exploration behavioural syndrome
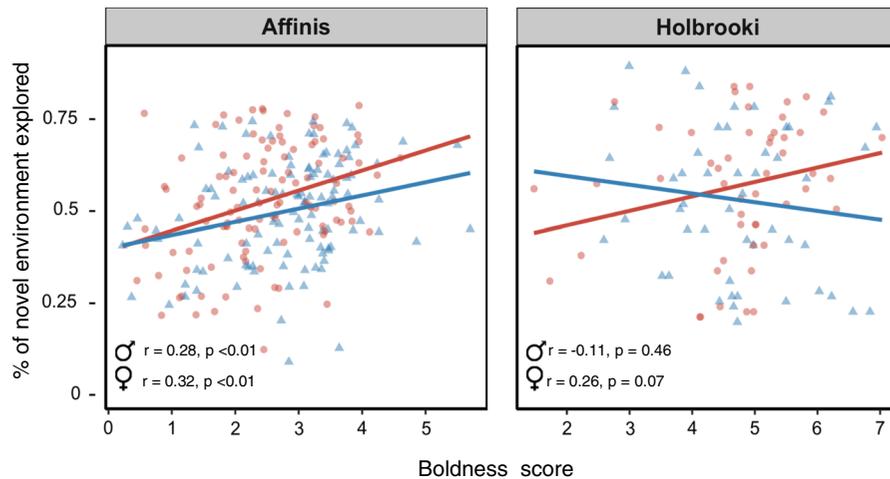
Fig. 2 Sex regression lines for relationship between tendency to explore a novel environment (% of novel environment explored) and boldness score [i.e. log maximum time allowed for fish to exit a refuge (2700 s) minus the log latency (s) to exit from the refuge] within two mosquitofish species, *Gambusia affinis* (female: n = 112, male: n = 111) and *Gambusia holbrooki* (female: n = 25, male: n = 25). Males = blue, triangles, females = red, circles

differed between species is consistent with most comparative studies on behavioural correlations, which have found remarkable variation in syndromes, particularly those related to boldness (Bell and Sih 2007; Dingemanse et al. 2007; Michelangeli et al., 2018b). These studies suggest that high-consistency in behavioural syndromes are often linked to high-predation sites that place consistent selection on groups of behaviours, particularly behaviours linked to risk taking, compared to more benign environments which favour variable behavioural strategies (Heinen-Kay et al. 2016). For example, in three-spine stickle-backs, *Gasterosteus aculeatus* (Linnaeus, 1758), pop-ulations raised in high predation risk environments exhibit a boldness-aggression syndrome, whereas populations raised in low predation environments do not (Bell and Sih 2007). It should also be noted that differences between species could be a result of differences in how each species were reared in our study; *G. holbrooki* were housed in isolation during the experimental period rather than in groups, and this could have had an influence on their behaviour (Gómez-Laplaza and Morgan 2000; Bevan et al. 2018).

Interestingly, we found evidence that sociability is correlated with exploration in male *G. affinis*, but not females. Sociability has previously been found to be linked to dispersal in *G. affinis*, whereby asocial individuals tend to disperse further, faster and more

frequently than social individuals when population densities are high (Cote et al. 2010b, 2011, 2013). Sociability-dependent dispersal in *G. affinis* has also been found to generate more severe impacts on native aquatic insect communities compared to random dispersal (Cote et al. 2017). Furthermore, dispersal propensity is higher in males compared to females (Cote et al. 2010b, 2011). Our results, together with these earlier studies, suggest that males (i.e. the more dispersive sex), that are asocial and bold, would be more likely to disperse away from established popu-lations (i.e. high-density populations) and lead the invasion front. On the other hand, sociability appears to be independent of exploration and boldness in females. These sex-specific differences in personality and dispersal may thus have important implications for the spread and invasion of western mosquitofish, and for their impacts on native ecosystems, as the behavioural composition of range-front populations may be sex-dependent. Conversely, due to a lack of behavioural differences between sexes, *G. holbrooki* invasions may be less prone to skewed sex ratios at the invasion front. A future study that explores the interaction between sex- and behavioural-dependent dispersal, and its implications for founder populations, would yield interesting insights into the spread dynamics of invasive mosquitofish populations.

To conclude, our results suggest that different mosquitofish invasions have required different

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

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