

Male Mate Choice in Mosquitofish: Personality Outweighs Body Size

Chunlin Li

Anhui University <https://orcid.org/0000-0003-4161-9013>

Xinyu Zhang

Anhui University

Peng Cui

Nanjing Institute of Environmental Sciences

Feng Zhang (✉ fzhang188@163.com)

Anhui University

Baowei Zhang

Anhui University

Research

Keywords: activity, animal personality, male mate choice, mosquitofish, shyness

Posted Date: June 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-549415/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Despite its important implications in behavioural and evolutionary ecology, male mate choice has been little studied, and the relative contribution of personality and morphological traits remains largely unknown. Using standard two-choice mating trials, we studied whether personality traits (i.e. shyness and activity) and body size of both sexes affect mate choice in male mosquitofish *Gambusia affinis*.

Results

Both shyness and activity in males were significantly repeatable and constituted a behavioural syndrome. No overall directional preference for large (or small) females with the same activity levels was detected because larger males preferred larger females and smaller males chose smaller females. However, males spent more time associating with active females regardless of their body lengths and had an enhanced preference for inactive females when they increased activity levels. We also found that more proactive (bolder and more active) males had stronger preferences for more active females.

Conclusions

Our study supports the importance of body size in male mate choice but highlights that personality traits may outweigh body size preferences when males choose mating partners.

Background

As females generally invest more than males in their offspring, they are usually considered to be highly selective when choosing their mates. Therefore, traditional studies on mate choice have mostly focused on female choice of male mates and its implications for the evolution of male ornaments [1, 2]. However, increasing evidence suggests that male mate choice is also widespread in many taxa, and possible explanations include paternal investment by males [3, 4], males' greater mating effort [5], differences in female quality [6], female-biased operational sex ratio [7], and sperm production limits [8]. Studies have highlighted that, like female mate choice, male mate choice has ecological and evolutionary significance, such as increasing population adaptation to new environments [9, 10] and accelerating sympatric speciation through reproductive isolation [11, 12].

Compared with female mate choice, less is known about the way in which males choose their mates, or the female traits that are targeted by male choice although there has been an increasing interest in male mate choice in the last few decades [9, 13]. From an evolutionary point of view, mate choice should be based on traits that can maximize the fitness of decision makers [14]. Previous studies on female mate choice have investigated a variety of male traits that may be used by females in their choices, such as

ornaments, social dominance, body size, cognition, and chemical odors [15–17]. Males may also use these traits of females to choose their mates; however comparatively, this field is much less studied [9]. Body size is one of the widely studied traits that is used in mate choice by both females and males in many species, with larger mating partners being preferred [18–20]. Larger size often confers males with advantages in resource defending, intrasexual competition, and social dominance, and there may be genetic and ecological advantages for females to mate with larger males [18, 21, 22]. Female body size is often positively correlated with their fecundity [17, 23], and highly selective males can increase reproductive success by preferentially mating with larger females, which has been empirically found in some live-bearing fish [17, 19, 24]. In addition, both males and females in some species exhibit assortative mating by body size [22, 25].

During the last few decades, evidence of animal personality has been widely found throughout the animal kingdom, and its ecological and evolutionary significance has rapidly become a hot topic [26, 27]. As proposed by Réale et al. [28], researchers often measured one or some of five personality traits, i.e. shyness-boldness, exploration, activity, aggressiveness and sociability. Some studies have suggested that personality may be viewed as a behavioral criterion influencing mate choice decisions in females and males [29–31]. Personality traits can be linked with fitness and thus are potential targets of natural and sexual selection [26]. For example, survival probability is positively correlated with activity level in the wild brown trout *Salmo trutta* [32]. Activity level has also been found to be positively correlated with rates of resource intake, and thus positively correlated with growth or reproduction [33]. To maximize reproductive success, males are predicted to choose females with personality traits that can result in higher reproductive success, for example, higher activity level. Choosiness regarding personality may also depend on the choosers' own personality type [34]. In addition, personality may covary with body size [35, 36], and thus these two phenotypic traits may interact to influence mate choice in both sexes. For example, more active females in *Poecilia mexicana* exhibit stronger mating preferences for larger males [31]. To date, the effect of personality on mate choice is still understudied [30, 37]. While some studies have focused on female's mating preferences in regards to personality, few studies have investigated males [29]. Furthermore, the relative importance of personality and body size in mate choice remains largely unknown.

In this study, we used mosquitofish *Gambusia affinis* to evaluate whether personality and body size affect male mate choice. We first characterized personality traits in male mosquitofish, including shyness and activity. These males were subsequently tested in dichotomous mate choice experiments in which females with various combinations of activity levels and body sizes were used as stimulus fish. As observed in many other poeciliid fish species [38–41], we expected male fish to prefer larger females with higher fecundity. Because females often resist rutting males due to sexual harassment [42], we further expected that the preference for larger females would be stronger in larger males that can better overcome the stronger resistance of larger females. Activity level is a positive proxy of female quality which is related to females' reproduction success [32, 33], and thus we expected that males would preferentially select females with higher activity levels. Because more proactive males have higher

competition ability and can better overcome the stronger resistance of active females [42], we further expected that more proactive males would have stronger preferences for more active females.

Methods

Study animals

Mosquitofish is a poeciliid fish species native to North America, and it has been intentionally introduced in many countries with the aim of controlling mosquitos [42]. Poeciliid fish are characterized by promiscuity, internal fertilization, ovoviviparity, and sexual dimorphism, with males being smaller than females [43]. At sexual maturity, female mosquitofish possess two gravid spots on the posterior of their abdomens, while males have a gonopodium modified from the anal fin. The mating system in this species is non-resource-based and promiscuous, in which males do not court, but instead, sneakily approach females from behind and attempt coerced copulations [42]. After internal fertilization, the fertilized ova hatch within the female ovary often during a period of 22–25 days [42]. The brood size of a female depends on its body size, with larger ones giving birth to more new-borns, which are approximately 6–8 mm in length [42]. The time for fry to reach sexual maturity varies from one month to several months, depending on the water temperature. Standard body length (SL) at sexual maturity is usually larger than 15 mm in males and 17 mm in females [42].

A total of 1500 new-borns generated from 150 wild-caught females were uniformly reared in 30 net tanks (80 × 80 × 80 cm, mesh size: 0.177 mm) in an outdoor artificial pond on the campus of Anhui University from May to July 2018 [44]. They were fed brine shrimp nauplii until two weeks old and thereafter fine-grained commercial food (TIDDLER, Weifang YEE Pet Products Co., Ltd., China; 42% crude protein, 5% crude fat, 5% crude fiber, and 11% ash). The water temperature during the rearing period ranged from 20°C to 32°C, and the pH ranged from 7.4 to 7.6. Apart from the additional food, the rearing conditions were the same as those of their conspecifics already living outside the tanks in the pond for several years, avoiding behavioral abnormalities that might arise in laboratory conditions [45].

In July, 80 sexually mature males (SL > 15 mm), indicated by a clear apical hook at the gonopodium tip [42], were randomly net caught from the rearing tanks and were randomly categorized into two groups with each containing 40 individuals. One group was tested in the following Experiment 1 (Expt 1) and the other in Experiment 2 (Expt 2, Fig. 1). To avoid any external stimulus, the males were individually kept in black, opaque, cylindrical tanks (height: 9 cm; diameter: 15 cm; hereafter, holding tank) with a black, opaque, cylindrical refuge chamber (height: 5 cm; diameter: 7 cm) placed in the center. The holding tanks were filled with oxygenated tap water, and the fish were given > 24 h to acclimate to the chamber before the experiments. Then each group of the 40 males were evenly divided into 10 subgroups that were tested separately with different healthy, active, virgin females as stimuli. Specifically, for each subgroup of males used in Expt 1, one pair of mature females with different body lengths (22 mm vs. 18 mm) were used as stimuli, and for each subgroup in Expt 2, one pair of mature females with the same body size (22 mm) were used. Because the intensity and size of gravid spots are linked with developmental stages and

clutch size of female live-bearing fish and thus may influence mate choice of males [46, 47], we paired females with similar sizes and intensities of gravid spots. Because the handlings of fish during experiments were always in water, and the fish were always given enough time to acclimate, no stressful responses were observed in the stimulus females.

To mimic two distinct degrees of female activity for males to choose from, females were restricted (inactive female, IF) or not (active female, AF) by a transparent plastic cylinder (6.5 cm diameter × 8 cm high) as required during the experiments. After a period of acclimation, the IFs adapted to the trap and lowered their activity levels with no obvious abnormal behaviors. Instead of using actual activity levels that may vary significantly and not as expected during experiments, this man-made neat difference in activity levels between stimulus females can generate more robust results. Because the cylinders used to trap the females were transparent and were separated by plexiglass, males could not perceive that the IFs were constrained.

General experimental procedure

To test the effect of the personality of male mosquitofish on their mate choice, open arena assays were firstly used to measure shyness and activity twice for each male in two successive days (see the subsection of personality measurements for details). Subsequently, mate choice experiments were carried out for each of the two groups of 40 males, respectively (see the subsection of mate choice experiments for details). The first mate choice experiment (Expt 1, Fig. 1a) tested males' preferences for body sizes (22 mm vs. 18 mm) of mature females and whether these preferences were influenced by female activity levels (active vs. inactive) and male personality traits. The second one (Expt 2, Fig. 1b) tested males' preferences for females with the same body size but different activity levels (active vs. inactive) and whether their preferences changed when the inactive female increased its activity level.

The experiments were carried out in a white opaque plastic tank (37 cm long × 30 cm wide × 20 cm high, Fig. 2) in the same laboratory under enough light and constant temperature (26°C). A camera (Sony HDR-CX510, 55× extended zoom, Sony Corporation, Tokyo, Japan) was fixed above the tank to record the behaviors of the subjects throughout. To avoid potential disturbances, the experimenters were shielded from the experimental apparatus by a 1.5-m high opaque curtain during each trial. To minimize observer bias, blinded methods were used when all behavioral data were recorded and/or analyzed. At the end of all experiments, each male was gently placed against the transparent wall of a glass tank to measure its SL (accurate to 0.1 mm), and all the fish were released to the rearing pond.

Personality measurements

In the assays for shyness and activity, a black, opaque, cylindrical refuge chamber (the same refuge placed in the holding tank, hereafter, starting refuge) was fixed at the far end of the experimental tank (Fig. 2a). A sliding trapdoor (3 × 3 cm) on the side of the starting refuge facing the arena was connected to a piece of fishing line that allowed experimenters to remotely open the refuge to allow fish to emerge from the chamber and move toward the arena without disturbance. The tank was filled with oxygenated

tap water to a depth of 3 cm, and the water was exchanged after each subject was tested. Males were not fed beyond 12 hours before the personality assays.

At the beginning of the assay, a male was gently transferred from the holding tank to the closed starting refuge in the arena. The subject was allowed to acclimate for 5 min, and then the trapdoor of the refuge was remotely opened and was not closed until the end of the trial. Following previous studies [48-50], shyness was measured as the time taken by the subject to swim out of the refuge, that is, for its whole body to cross the trapdoor. All the subjects emerged from the refuge within 6 min. After the male left the refuge, it was video-tracked continuously for 10 min to record its movement trajectory. At the end of the trial, the subject was immediately transferred to its holding tank. A total of 600 image stacks were extracted from the 10-min movement videos (one frame per second); Image J (<http://rsbweb.nih.gov/ij/>) was used to delineate each subject's movement pathway, of which the total length was used to quantify its activity level.

Mate choice experiments

To carry out the mate choice trials in a dichotomous chamber, the white opaque plastic tank was divided into three compartments separated by plexiglass, only allowing visual contact between fish in different compartments. At the center of each compartment, there was a transparent plastic cylinder (6.5 cm diameter × 8 cm high) connected to a piece of fishing line, by which the experimenters could remotely pull up the cylinder to allow the trapped fish to swim freely. Two dark lines were drawn on the bottom of the tank to demarcate a neutral zone (11 cm wide) and two preference zones (5-cm wide; Fig. 2b). The tank was filled with 7-cm-depth oxygenated tap water, which was changed after each trial. One hour before the mate choice experiments, males were fed to avoid the effect of hunger.

Expt 1: In this experiment, four successive sub-experiments were designed to study whether male's personality and female's activity level influence male preference for female body size. Firstly, the two females (active large female, AL_F; active small female, AS_F) were separately placed outside the cylinders in the end compartments of our experimental setting, and a male was trapped inside the cylinder in the central compartment. After a 10-min acclimatization period, the male was remotely released to allow it to choose between the two females, and its movement behavior was video-tracked for 12 min (Expt 1a). Secondly, the male and the smaller female (18 mm, IS_F) were trapped inside their cylinders, while the larger female (22 mm, AL_F) was outside the cylinder. After 10 min for acclimatization, the male was released to swim freely and was video-tracked for 12 min (Expt 1b). Thirdly, the male and the 22-mm female (IL_F) were trapped, while the 18-mm female (AS_F) swam freely. After 10 min, the male was released and video-tracked for 12 min (Expt 1c). Fourthly, the male and the two females (IL_F, IS_F) were all trapped initially, and after 10 min, the male was released and video-tracked for 12 min (Expt 1d). To make the same experimental conditions for all males, they underwent the same order of the above treatments. The non-randomized order may have mask effect on the treatments (i.e. the order effect). However, the 10-min acclimatization between treatments may reduce the possible order effect.

Expt 2: Two sub-experiments were used to explore whether males (another 40 individuals) choose mating partners according to male's personality traits and female's activity level. Firstly, two stimulus females of the same body size (22 mm) were separately placed in the end compartments, with the IF restricted inside its cylinder while the AF freely swam outside. A male was restricted inside the cylinder in the central compartment for 10 min to acclimate and observe the activities of both females. Then, the experimenter allowed the male to swim around freely and video-tracked its movement for 12 min (Expt 2a). Secondly, the male was restricted inside the cylinder again, and the IF was released to swim freely (its label changed to 'IFtoAF' accordingly). After 10 min for acclimatization, the male was released and video-tracked for 12 min (Expt 2b). To avoid potential side-biases, the locations of AF and IF were exchanged between trials.

Statistical analyses

Following the protocol of Dingemanse and Dochtermann [51], a bivariate linear mixed-effects model was fitted using the R package *MCMCglmm* for the males used in Expt 1 and 2, respectively, to measure the repeatability of each behavior (i.e. personality) and the among-individual correlation between the two behaviors (i.e. behavioral syndrome). The two \log_e -transformed behavioral traits were concurrently included as the response variables in each model with individual ID as a random effect. The bivariate models were run for 220,000 iterations after 20,000 burn-in iterations and were thinned by 25 iterations. Model convergence was confirmed by the Gelman-Rubin diagnostic test (function *gelman.diag*).

Male mate preference for certain females was determined as the association time, that is, the time a male spent within the preference zone near each female stimulus. The association time is widely used to measure mating preferences in female and male live-bearing fish [17, 52-56]. To further avoid disturbances from experimental operations, the middle 10 min of each 12-min free-choice period was used for the analyses. Time 1 (T1) was defined as the association time of the male with the large (or active) female and T2 with the small (or inactive) female. Male's strength of preference (SOP) for large versus small (in Expt 1a and 1d) or active versus inactive females (in Expt 2a) was calculated as: $SOP = (T1 - T2) / (T1 + T2)$. The SOPs ranged from -1 to 1, with larger values indicating a stronger preference for large (or active) females. The SOPs were tested using the Shapiro-Wilk test and were found to be normally distributed (all p values > 0.25).

The mean values of shyness and activity measured for each male in the two personality trials were used in the following analyses. A t-test was used to compare the differences in body length, shyness, and activity between the males used in Expt 1 and 2. Principal component analysis (PCA) was performed for the two groups of males, separately, to summarize the two highly correlated behavioral traits into two independent principal components (PC1 and PC2). The repeated-measures ANOVA in R package *nlme* and post hoc Tukey's test with a Bonferroni correction were used to test and compare the differences in time spent by males in mate choice assays among the three different zones (i.e. neutral zone and the two preference zones). A paired t-test was used to investigate the change in males' association time with the IF in Expt 2a after it was released in Expt 2b (IFtoAF).

General linear mixed models were performed by R package *lme4* to fit the effects of male body length, PC1, and PC2 on male mate preferences for the larger (Expt 1a and 1d) or the active female (Expt 2a). The SOPs were used as the dependent variables, male body length, PC1, and PC2 as the fixed effects, and the pair ID of stimulus females as the random effect in the models. Two-way interactions between body length and PC1 and PC2 were initially included but were excluded in the final models because there were no significant effects. There were no significant correlations between body length and PC1 (Pearson correlations, Expt 1: $r = 0.23$, $p = 0.151$; Expt 2: $r = 0.12$, $p = 0.462$) and PC2 (Expt 1: $r = 0.09$, $p = 0.579$; Expt 2: $r = -0.05$, $p = 0.754$). Thus, there was no problem with multicollinearity associated with the models. All analyses were carried out using R 3.6.1 [57], and data are displayed as mean \pm standard error.

Results

Body length and personality of males

The body lengths of males tested in Expt 1 (18.2 ± 0.3 mm, range: 15–22 mm) were not different ($t = 0.28$, $df = 77.5$, $p = 0.778$) from those in Expt 2 (18.1 ± 0.3 mm, 16–23 mm). There were no significant differences in the two behavioral traits (shyness: $t = 1.30$, $df = 77.9$, $p = 0.196$; activity: $t = 1.66$, $df = 77.9$, $p = 0.100$) between the two groups of males. The two principal components (PCs) for males in Expt 1 and 2 received the same axis loadings from the two personality traits, that is, a positive loading from shyness and a negative loading from activity for PC1, and both as positive loadings for PC2 (Table 1). The repeatability of shyness was 0.243 (95% credible interval: 0.005–0.438) for the males in Expt 1 and 0.310 (0.069–0.559) for the males in Expt 2. The repeatability of activity was 0.373 (0.092–0.607) for the males in Expt 1 and 0.473 (0.225–0.691) for the males in Expt 2. Shyness and activity were not significantly correlated at the within-individual level but were negatively correlated at both phenotypic and among-individual levels, with the latter indicating a strong shyness-activity behavioral syndrome (Table 2).

Table 1 Personality trait scores (mean \pm standard error) and results of the principal components analysis of personality traits of the male mosquitofish tested in the mate choice experiments

Personality traits	Males in Experiment 1			Males in Experiment 2		
	Personality scores	PC1 loadings	PC2 loadings	Personality scores	PC1 loadings	PC2 loadings
Shyness (s)	76.6 ± 7.2	0.71	0.71	63.5 ± 7.0	0.71	0.71
Activity (cm)	1295.6 ± 55.8	-0.71	0.71	1166.9 ± 53.6	-0.71	0.71
Eigenvalues	/	1.25	0.65	/	1.19	0.77
Variance explained (%)	/	78.6%	21.4%	/	70.4%	29.6%
Cumulative variance (%)	/	78.6%	100%	/	70.4%	100%

Table 2 The partitions of phenotypic correlations between shyness and activity for males used in mate choice experiments. Significant correlations were determined by the 95% credible intervals not including zero and are displayed in bold

	Phenotypic level	Among-individual level	Within-individual level
Expt 1	-0.453 (-0.597, -0.216)	-0.958 (-0.994, -0.363)	-0.253 (-0.498, 0.040)
Expt 2	-0.241 (-0.461, -0.009)	-0.937 (-0.990, -0.440)	0.132 (-0.201, 0.385)

Mate choices

Expt 1: Although the neutral zone was larger than the preference zone in all the experiments, male mosquitofish spent more time in the preference zones than in the neutral zone. No overall directional preference of males for large (or small) females, with the same activity levels (active or inactive), was detected (Expt 1a: 250.3 ± 18.8 s for large active females and 238.1 ± 18.1 s for small active females, $z = -0.54$, $df = 78$, $p = 1$; Expt 1d: 231.0 ± 19.9 s for large inactive females and 217.2 ± 15.8 s for small inactive females, $z = -0.62$, $df = 78$, $p = 1$; Fig. 3a and 3d). Males spent more time associating with the active female (Expt 1b: 294.5 ± 24.7 s; Expt 1c: 283.1 ± 17.1 s) than the inactive one (Expt 1b: 214.0 ± 23.3 s, $z = -2.77$, $df = 78$, $p = 0.017$; Expt 1c: 201.2 ± 14.9 s, $z = 4.13$, $df = 78$, $p < 0.001$), regardless of whether it was large or small (Fig. 3b and 3c).

Expt 2: Consistent with Expt 1, males in Expt 2 spent more time in the preference zones than in the neutral zone. Males spent more time associating with the active female (284.7 ± 21.1 s) than the inactive female (206.0 ± 19.5 s; $z = 3.24$, $df = 78$, $p = 0.004$; Fig. 4a). Males increased their association time with inactive females (in Expt 2a) when they were released to swim freely in Expt 2b ($t = 2.03$, $df = 39$, $p = 0.049$). This

increase resulted in no difference in association time with the two females in Expt 1b (AF: 231.2 ± 21.7 s; IFtoAF: 246.9 ± 21.0 s; $z = -0.60$, $df = 78$, $p = 1$; Fig. 4b).

Factors influencing male strength of preference

When the paired females had the same activity level, male body length had a significant effect on its strength of preference (SOP) for the larger females, with larger males tending to select the larger female (Expt 1a: $t = 2.095$, $p = 0.036$; Expt 1d: $t = 4.213$, $p < 0.001$; Fig. 5a and 5b; Table 3). When choosing from the paired females with the same body length, the SOP for active females was negatively correlated with PC1 in males ($t = -2.298$, $p = 0.022$; Fig. 5c; Table 3). This indicated that proactive males (bolder and more active) preferred active females.

Table 3 Effects of body length and principal components (PCs) of personality traits of the male mosquitofish on the strength of male mating preference for larger females (Expt 1a: 22-mm vs. 18-mm active female; Expt 1d: 22-mm vs. 18-mm inactive females) and for active females (Expt 2a: active vs. inactive female)

Experiment	Variables	Estimate	95% credible intervals	<i>t</i> value	<i>p</i> value
Expt 1a	Variance of the random effect	0.004	0, 0.294		
	PC1	0.021	-0.085, 0.126	0.410	0.682
	PC2	-0.055	-0.252, 0.145	-0.557	0.577
	Body length	0.086	0.004, 0.170	2.095	0.036
Expt 1d	Variance of the random effect	0.017	0, 0.311		
	PC1	-0.048	-0.132, 0.035	-1.158	0.247
	PC2	-0.096	-0.260, 0.061	-1.234	0.217
	Body length	0.143	0.072, 0.215	4.213	< 0.001
Expt 2a	Variance of the random effect	0.010	0, 0.351		
	PC1	-0.138	-0.263, -0.014	-2.298	0.022
	PC2	0.046	-0.134, 0.229	0.513	0.608
	Body length	0.068	-0.017, 0.154	1.655	0.098

Discussion

We found that male mosquitofish were highly selective when choosing a mate, providing further evidence for male mate choice being widespread in animals [9, 17]. In the wild, males often encounter more than one receptive female but are unable to mate with all of them at the same time. Differences in female quality and the gap between the number of available mates and the mating capacity of a male are often suggested as the factors governing male mate choice [6, 7]. As with females, an interesting question arises with male mate choice: are there certain phenotypic female traits that are driven evolutionarily by male mate choice? Compared with female mate choice, the evolutionary mechanism underlying male mate choice remains poorly understood despite some studies [29, 58]. In this study, we found that male mosquitofish can choose mating partners according to females' and males' personality traits and body size and that females' activity level may outweigh body size preference in male mate choice.

Body size is commonly highlighted in studies of mate choice because it is often positively linked with female fecundity and, thus, males choosing larger females are predicted to obtain higher reproductive fitness [17, 23]. Despite some studies reporting no preference for female body size [29], males in some live-bearing fish species have been found to preferentially mate with larger females [17, 19, 24], which was also expected in the present study. We found no overall directional preference of males for large (or small) females with the same activity levels. This finding is consistent with that of McPeck [59], who also found no overall preference for larger females. The overall non-directional pattern of preference for female body size was attributed to the fact that large females were preferred by large males while small females were selected by small males. Positive assortative mating by body size in males has been found in many species [22, 25, 60]. In poeciliid fish species characterized by promiscuity, males often sneakily follow females and attempt coerced copulations, while females often resist males because of sexual harassment [42]. Larger males can better overcome the stronger resistance of larger females to obtain the associated higher reproductive success. In the wild, larger males usually dominate the social hierarchies in populations and have more competitive advantages during mating [61, 62]. However, to have some chance of mating, smaller males must approach smaller females with weaker resistance ability [22].

We found strong evidence that male mosquitofish preferred females with higher activity levels, regardless of their body size, and that when the enclosed female was released to swim actively, males increased their association time with it. Like body size, activity level has also been suggested to be linked with the fecundity potential of females because (1) activity is generally linked to metabolic processes, and thus higher activity level partly reflects better body conditions [63]; and (2) active females are more competitive than inactive individuals in foraging and intraspecific interactions, and thus can allocate more energy to their offspring [64]. Therefore, female activity level could be a potential criterion for males to use in choosing mating partners as males can increase their reproductive success by choosing active females.

Consistent with Xu et al. [44], we found the existence of personality and behavioral syndrome in male mosquitofish from the same population. Assortative mating was not only found related to body size, but also to personality, with proactive males (bolder and more active) preferring more active females. Some studies have found negative assortative preference related to behavioral traits and speculated that dissimilarity may increase behavioral compatibility between paired mating partners [65], and thus

facilitate parental labor division, which may benefit offspring in biparental species [66]. Conversely, it has also been argued that cooperation between mating partners during the reproduction period could be promoted by behavioral synchronization (i.e. positive assortment), thus increasing reproductive success [60, 67]. In this study, more proactive males were found to have stronger preferences for more active females, probably indicating a personality-based positive assortative mate preference that has been found in other non-monogamous species (e.g. [20, 25, 68]). As discussed above, activity level can be considered a positive proxy of female quality, and proactive males are often more competitive during mating [69]. Therefore, proactive males more frequently attempt to and more successfully mate with active females that may have stronger resistance but higher fecundity. Positive assortative mating, a probable general tendency of non-random mating, could have important evolutionary implications not only in ecological adaptation and speciation [60], but also in maintaining personality diversity within populations [30], which deserves further research.

Conclusions

Our study suggests that both body size and personality traits play important roles in the mate choice of male mosquitofish. There was no overall directional preference for large (or small) females with the same activity levels because larger males preferred larger females and smaller males chose smaller females. More proactive males preferentially associated with females of higher activity levels, probably because this trait indicates higher fecundity potential. Body size is commonly highlighted in studies of mate choice; however, our study implies that personality traits may outweigh body size preferences in male mate choice.

Declarations

Ethics approval and consent to participate

Mosquitofish is an invasive species in China; thus, no ethics committee review is required to capture the fish in the wild (Ministry of Environmental Protection of the People's Republic of China 2016, Index No.000014672/2016-01463). All experiments were approved by the Institutional Animal Care and Use Committee of Anhui University (IACUC, AHU). No harm was done to the fish, and no fish died during or after the experiments.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (Grant numbers 31970500, 31770571), AHU (Grant number S020118002/101), and Biodiversity Investigation, Observation and Assessment Program of Ministry of Ecology and Environment of China.

Authors' contributions

CL, FZ and BZ conceived and designed the experiments, carried out the data analyses and wrote the manuscript. XZ and PC performed the experiments. All authors have contributed substantially to the work and gave final approval for publication.

Acknowledgements

We thank Binbin Zhao and Zheng Kong for their help during the fish rearing, and Dan Wang, Shaofei Yan and Jiaming Fan for their help with the experiments.

Author details

¹School of Resources and Environmental Engineering, Anhui University, No.111, Jiulong Road, Hefei 230601, China. ²Anhui Province Key Laboratory of Wetland Ecosystem Protection and Restoration, Anhui University, No.111, Jiulong Road, Hefei 230601, China. ³Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment, No.8, Jiangwangmiao Road, Nanjing 210042, China. ⁴School of Life Sciences, Anhui University, No.111, Jiulong Road, Hefei 230601, China

References

1. Darwin C. The origin of species. London: John Murray; 1859.
2. Lehtonen J, Parker G, Schärer L. Why anisogamy drives ancestral sex roles. *Evolution*. 2016;70:1129-1135.
3. Jennions M, Kokko H. Sexual selection. In: Westneat DF, Fox CW, editors. *Evolutionary Behavioral Ecology*. Oxford: Oxford University Press; 2010. p. 343–364.
4. Trivers R. Parental investment and sexual selection. In: Campbell BG, editors. *Sexual selection and the Descent of Man, 1871–1971*. Chicago: Aldine-Atherton; 1972. p. 136–179.
5. Servedio M, Lande R. Population genetic models of male and mutual mate choice. *Evolution*. 2006;60:674-685.
6. Parker G. Mate quality and mating decisions. In: Bateson PPG, editors. *Mate Choice*. Cambridge: Cambridge University Press; 1983. p. 141–166.

7. Barry K, Kokko H. Male mate choice: why sequential choice can make its evolution difficult. *Anim Behav.* 2010;80:163-169.
8. Kokko H, Monaghan P. Predicting the direction of sexual selection. *Ecol Lett.* 2001;4:159-165.
9. Edward D, Chapman T. The evolution and significance of male mate choice. *Trends Ecol Evol.* 2011;26:647-654.
10. Lorch P, Proulx S, Rowe L, Day T. Condition-dependent sexual selection can accelerate adaptation. *Evol Ecol Res.* 2003;5:867-881.
11. Pierotti MER *et al.* Individual variation in male mating preferences for female coloration in a polymorphic cichlid fish. *Behav Ecol.* 2008;19:483-488.
12. Ritchie M. Sexual selection and speciation. *Annu Rev Ecol Evol Syst.* 2007;38:79-102.
13. Fitzpatrick CL, Servedio MR. The evolution of male mate choice and female ornamentation: a review of mathematical models. *Curr Zool.* 2018;64:323-333.
14. Rosenthal G. Mate choice: the evolution of sexual decision making from microbes to humans. Princeton: Princeton University Press; 2017.
15. Petrazzini M, Bisazza A, Agrillo C, Lucon-Xiccato T. Sex differences in discrimination reversal learning in the guppy. *Anim Cogn.* 2017;20:1081-1091.
16. Rüschenbaum S, Schlupp I. Non-visual mate choice ability in a cavefish (*Poecilia mexicana*) is not mechanosensory. *Ethology.* 2013;119:368-376.
17. Schlupp I. Male mate choice in livebearing fishes : an overview. *Curr Zool.* 2018;64:393-403.
18. Aquiloni L, Gherardi F. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. *J Zool.* 2008;274:171-179.
19. Dosen L, Montgomerie R. Mate preferences by male guppies (*Poecilia reticulata*) in relation to the risk of sperm competition. *Behav Ecol Sociobiol.* 2004;55:266-271.
20. Ptacek M, Travis J. Mate choice in the sailfin molly, *Poecilia latipinna*. *Evolution.* 1997;51:1217-1231.
21. Guevara-Fiore P, Stapley J, Watt P. Mating effort and female receptivity: how do male guppies decide when to invest in sex? *Behav Ecol Sociobiol.* 2010;64:1665-1672.
22. Rueger T, Gardiner N, Jones G. Size matters: male and female mate choice leads to size-assortative pairing in a coral reef cardinalfish. *Behav Ecol.* 2016;27:1585-1591.
23. Helfman G, Collette B, Facey D, Bowen B. The diversity of fishes: biology, evolution, and ecology. New York, NY.: John Wiley & Sons; 2009.
24. Méndez-Janovitz M, Garcia C. Do male fish prefer them big and colourful? Non-random male courtship effort in a viviparous fish with negligible paternal investment. *Behav Ecol Sociobiol.* 2017;71:160.
25. Montiglio PO, Wey T, Chang A, Fogarty S, Sih A. Multiple mating reveals complex patterns of assortative mating by personality and body size. *J Anim Ecol.* 2016;85:125-135.
26. Dingemanse N, Réale D. Natural selection and animal personality. *Behaviour.* 2005;142:1159-1184.

27. Sih A, Bell A, Johnson J, Ziemba R. Behavioral syndromes: an integrative overview. *Q Rev Biol.* 2004;79:241-277.
28. Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. Integrating animal temperament within ecology and evolution. *Biol Rev.* 2007;82:291-318.
29. Chen B *et al.* Personality differentially affects individual mate choice decisions in female and male Western mosquitofish (*Gambusia affinis*). *PLoS ONE.* 2018;13:e0197197.
30. Schuett W, Tregenza T, Dall S. Sexual selection and animal personality. *Biol Rev.* 2010;85:217-246.
31. Sommer-Trembo C *et al.* Does personality affect premating isolation between locally-adapted populations? *BMC Evol Biol.* 2016;16:138.
32. Adriaenssens B, Johnsson J. Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. *Ecol Lett.* 2013;16:47-55.
33. Biro PA, Stamps JA. Are animal personality traits linked to life-history productivity? *Trends Ecol Evol.* 2008;23:361-368.
34. Sommer-Trembo C, Schreier M, Plath M. Different preference functions act in unison: mate choice and risk-taking behaviour in the Atlantic molly (*Poecilia mexicana*). *J Ethol.* 2020;38:215-222.
35. Brown C, Braithwaite V. Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Anim Behav.* 2004;68:1325-1329.
36. Meuthen D, Ferrari M, Lane T, Chivers D. Plasticity of boldness: high perceived risk eliminates a relationship between boldness and body size in fathead minnows. *Anim Behav.* 2019;147:25-32.
37. Munson AA, Jones C, Schraft H, Sih A. You're Just My Type: Mate Choice and Behavioral Types. *Trends Ecol Evol.* 2020;35:823-833.
38. Bisazza A, Marconato A, Marin G. Male mate preferences in the mosquitofish *Gambusia holbrooki*. *Ethology.* 1989;83:335-343.
39. Callander S, Backwell P, Jennions M. Context-dependent male mate choice: the effects of competitor presence and competitor size. *Behav Ecol.* 2011;23:355-360.
40. Hoysak D, Godin JG. Repeatability of male mate choice in the mosquitofish, *Gambusia holbrooki*. *Ethology.* 2007;113:1007-1018.
41. Mautz B, Jennions M. The effect of competitor presence and relative competitive ability on male mate choice. *Behav Ecol.* 2011;22:769-775.
42. Pyke G. A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Rev Fish Biol Fish.* 2005;15:339-365.
43. Evans J, Pilastro A, Schlupp I. Ecology and evolution of poeciliid fishes. Chicago: The University of Chicago Press; 2011.
44. Xu W *et al.* Environmental complexity during early life shapes average behavior in adulthood. *Behav Ecol.* 2021;32:105-113.
45. Carere C, Nascetti G, Carlini A, Santucci D, Alleva E. Actions for restocking of the European lobster (*Homarus gammarus*): a case study on the relevance of behaviour and welfare assessment of

- cultured juveniles. *Rend. Lincei*. 2014;26:59-64.
46. Deaton R. Factors influencing male mating behaviour in *Gambusia affinis* (Baird & Girard) with a coercive mating system. *J Fish Biol*. 2008;72:1607–1622.
 47. Norazmi-Lokman N, Purser G, Patil J. Gravid spot predicts developmental progress and reproductive output in a livebearing fish, *Gambusia holbrooki*. *PLoS ONE*. 2016;11:e0147711.
 48. Brown C, Jones F, Braithwaite V. Correlation between boldness and body mass in natural populations of the poeciliid *Brachyrhaphis episcopi*. *J Fish Biol*. 2007;71:1590-1601.
 49. Cote J, Fogarty S, Brodin T, Weinersmith K, Sih A. Personality-dependent dispersal in the invasive mosquitofish: group composition matters. *P Roy Soc B-Biol Sci*. 2011;278:1670-1678.
 50. Polverino G, Cigliano C, Nakayama S, Mehner T. Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. *Behav Ecol Sociobiol*. 2016;70:2027-2037.
 51. Dingemanse NJ, Dochtermann NA. Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J Anim Ecol*. 2013;82:39-54.
 52. Jeswiet S, Godin JG. Validation of a method for quantifying male mating preferences in the guppy (*Poecilia reticulata*). *Ethology*. 2011;117:422-429.
 53. Wagner W. Measuring female mating preferences. *Anim Behav*. 1998;55:1029-1042.
 54. Plath M, Blum D, Schlupp I, Tiedemann R. Audience effect alters mating preferences in a livebearing fish, the Atlantic molly, *Poecilia mexicana*. *Anim Behav*. 2008;75:21-29.
 55. Wong B, McCarthy M. Prudent male mate choice under perceived sperm competition risk in the eastern mosquito fish. *Behav Ecol*. 2009;20:278–282.
 56. Callander S, Backwell P, Jennions M. Context-dependent male mate choice: the effects of competitor presence and competitor size. *Behav Ecol*. 2012;23:355–360.
 57. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2018.
 58. Amundsen T, Forsgren E. Male mate choice selects for female coloration in a fish. *P Natl Acad Sci USA*. 2001;98:13155-13160.
 59. McPeck M. Mechanisms of sexual selection operating on body size in the mosquitofish (*Gambusia holbrooki*). *Behav Ecol*. 1992;3:1-12.
 60. Jiang Y, Bolnick D, Kirkpatrick M. Assortative mating in animals. *Am Nat*. 2013;181:E125-E138.
 61. Basolo A. Variation between and within the sexes in body size preferences. *Anim Behav*. 2004;68:75-82.
 62. Bierbach D *et al*. Social network analysis resolves temporal dynamics of male dominance relationships. *Behav Ecol Sociobiol*. 2014;68:935-945.
 63. Metcalfe N, Van Leeuwen T, Killen S. Does individual variation in metabolic phenotype predict fish behaviour and performance? *J Fish Biol*. 2016;88:298-321.

64. Careau V, Thomas D, Humphries M, Réale D. Energy metabolism and animal personality. *Oikos*. 2008;117:641-653.
65. Scherer U, Kuhnhardt M, Schuett W. Different or alike? Female rainbow kribbs choose males of similar consistency and dissimilar level of boldness. *Anim Behav*. 2017;128:117-124.
66. Royle N, Schuett W, Dall S. Behavioral consistency and the resolution of sexual conflict over parental investment. *Behav Ecol*. 2010;21:1125-1130.
67. Schuett W, Dall S, Royle N. Pairs of zebra finches with similar 'personalities' make better parents. *Anim Behav*. 2011;81:609-618.
68. Ariyomo T, Watt P. Disassortative mating for boldness decreases reproductive success in the guppy. *Behav Ecol*. 2013;24:1320-1326.
69. Myhre L, Forsgren E, Amundsen T. Effects of habitat complexity on mating behavior and mating success in a marine fish. *Behav Ecol*. 2013;24:553-563.

Figures

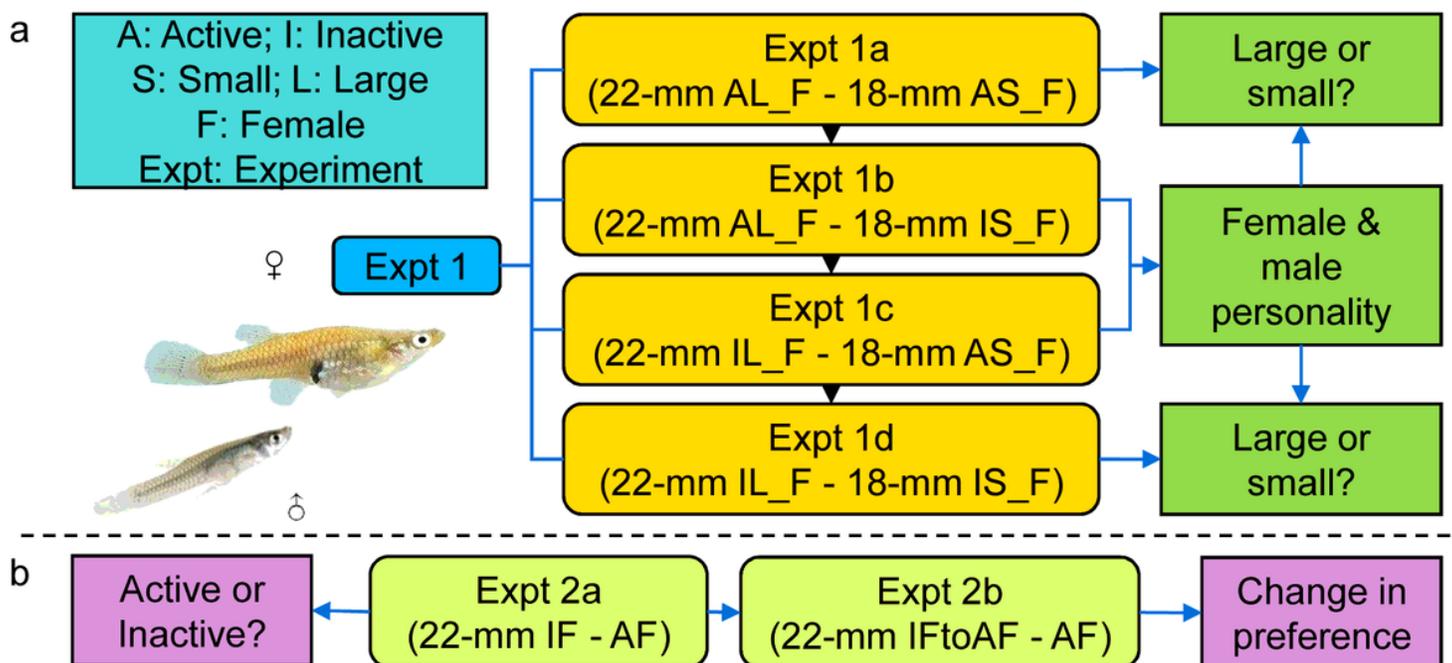


Figure 1

Overview of our experiments on male mate choice in mosquitofish. Each of 40 males underwent Expt 1a–1d in Expt 1, and each of the other 40 males underwent Expt 2a–2b in Expt 2

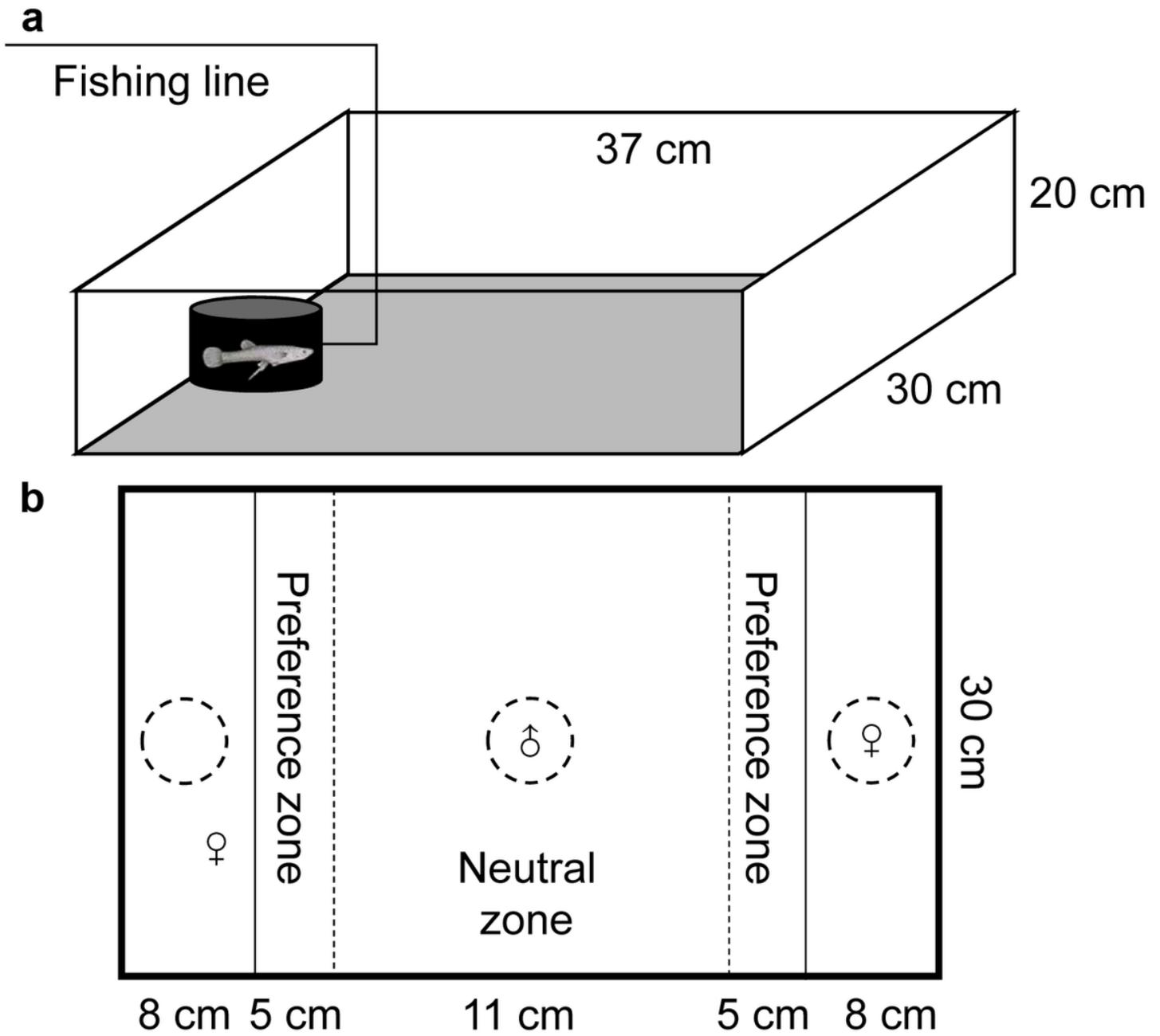


Figure 2

Experimental apparatus for measuring (a) shyness and activity, and (b) mate preference of male mosquitofish

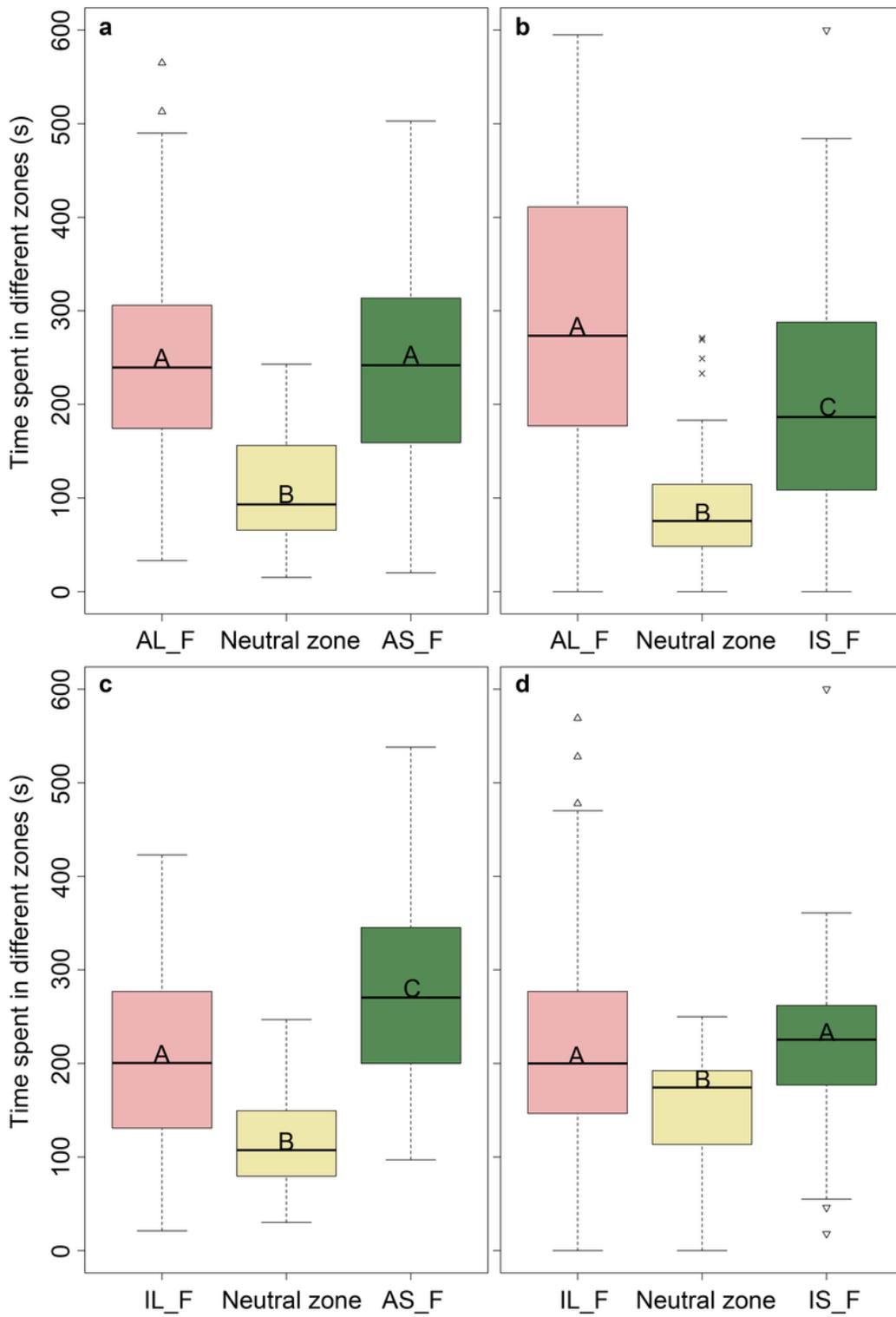


Figure 3

Time spent by male mosquitofish associated with different females: (a) Expt 1a; (b) Expt 1b; (c) Expt 1c; (d) Expt 1d. There was no significant difference between zones with the same capital letters in the boxes. AL_F represents active large female, AS_F active small female, IS_F inactive small female, and IL_F inactive large female

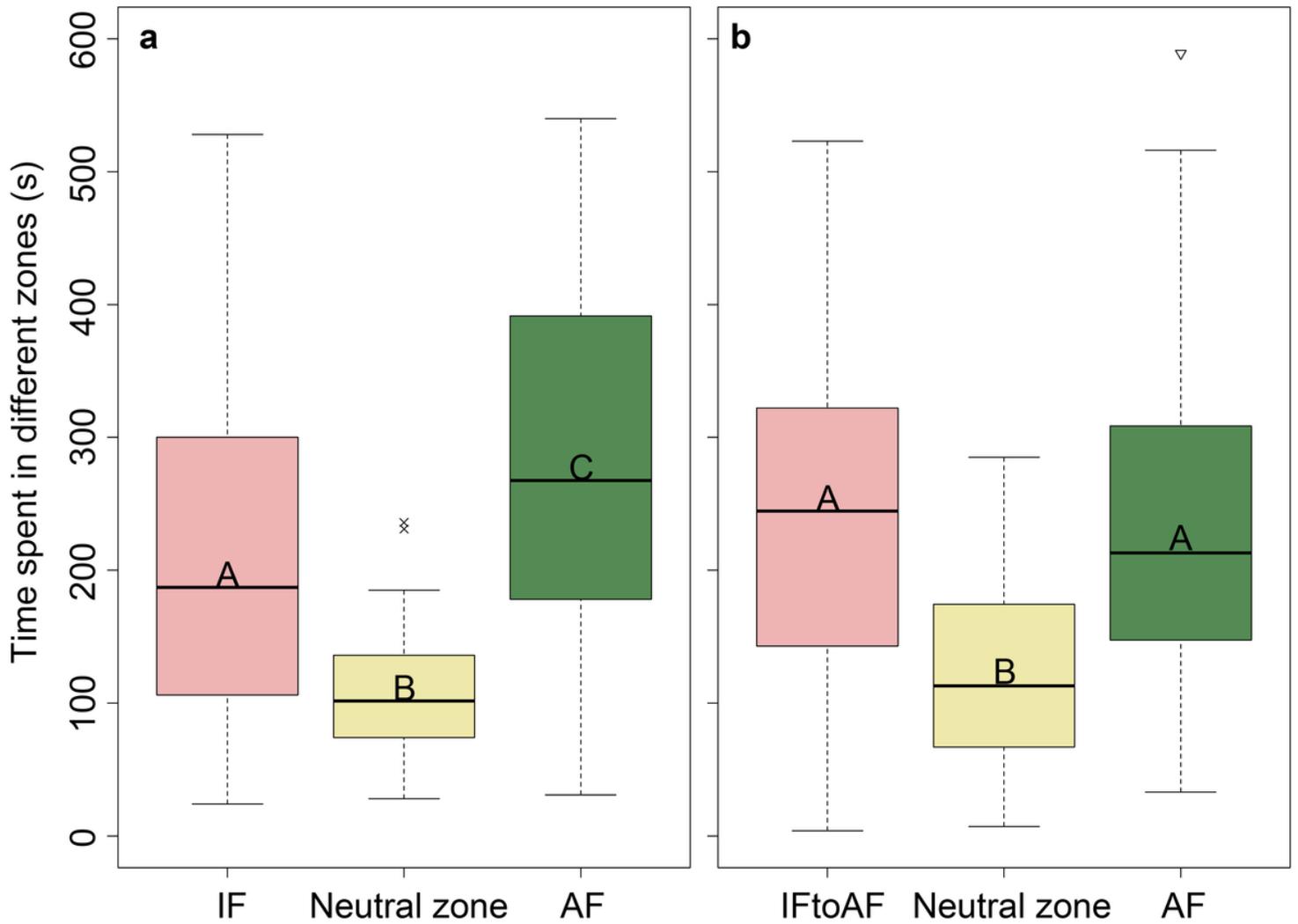


Figure 4

Time spent by male mosquitofish associated with different females: (a) Expt 2a; (b) Expt 2b. There was no significant difference between zones with the same capital letters in the boxes. IF represents inactive female, AF active female, IFtoAF the female changing from inactive to active status

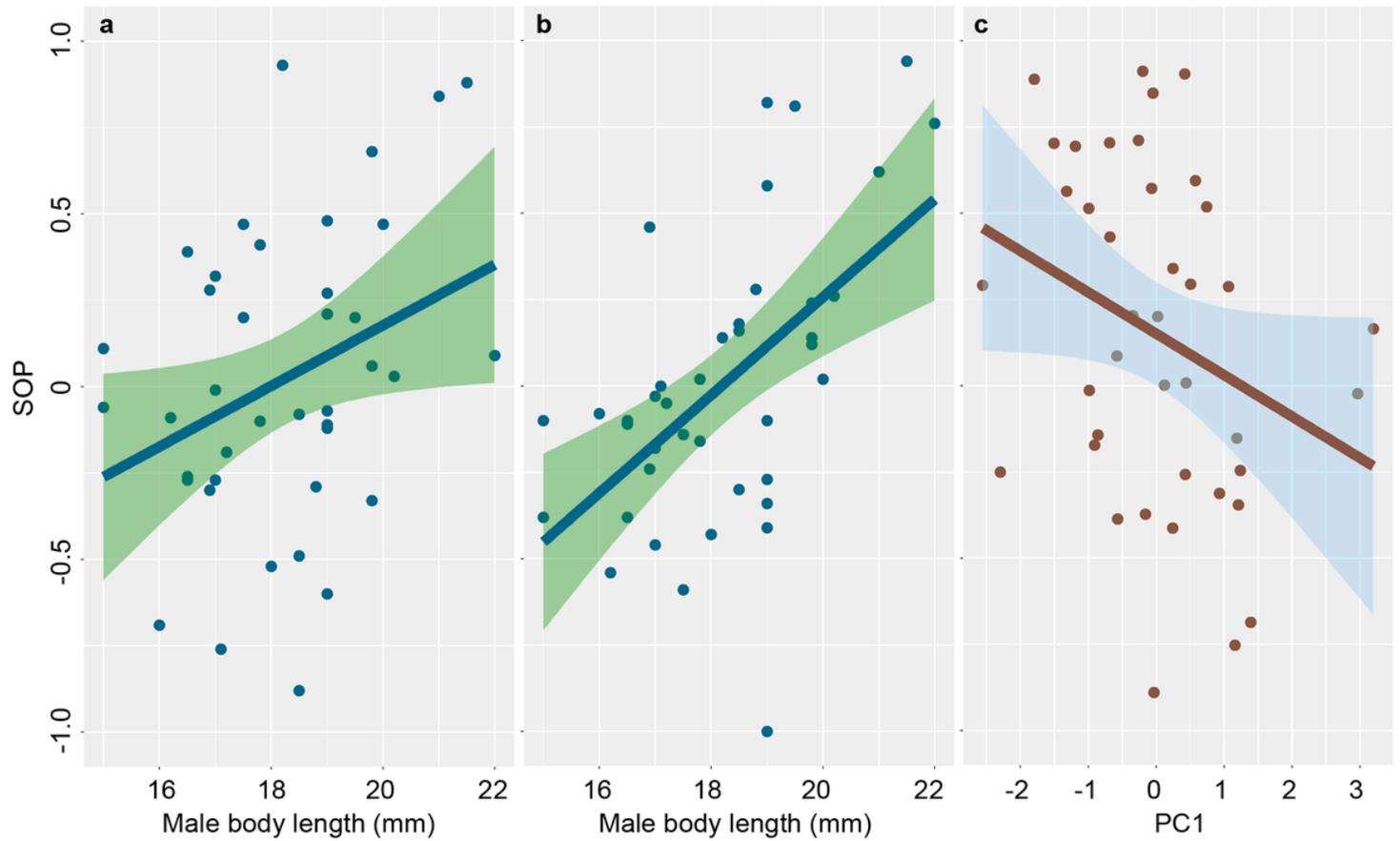


Figure 5

The strength of preference (SOP) as a function of body length and the first principal component of personality traits of males (PC1): (a) the SOP for the larger of two active females (Expt 1a), (b) the SOP for the larger of two inactive females (Expt 1d), (c) the SOP for the more active of two 22-mm females (Expt 2a)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Rawdata.xlsx](#)