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ORIGINAL ARTICLE

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Association patterns of sailfin mollies (*Poecilia latipinna*): alternative hypotheses

Abstract Individuals may associate with each other due to a variety of selective forces, such as intra- and intersexual selection, and conspecific recognition. Previous studies have concluded that mate choice governs association behavior in polygynous species of fish. I examined whether mate choice underlies the preference for larger individuals by examining preference for association (time spent in proximity to a fish) not only between opposite-sex individuals but also between same-sex individuals of the live-bearing sailfin molly (*Poecilia latipinna*). Males and females from three size classes were tested with a large and a small object fish of the same and opposite sex. Females preferred to associate with larger over smaller males. Males also preferred to associate with larger over smaller females, as expected. The same female and male test fish also preferred to associate with larger over smaller fish of the same sex. Moreover, females demonstrated no significant difference in their strength of preference (large–small) when offered males or females. The same held true for males. When males and females were subsequently tested with one large male and one large female, females tended to prefer large males while males showed no significant preference for association based on sex. In another experiment, females were tested with a large female and a small male, and significantly preferred the former. These findings suggest that association patterns may have arisen under a variety of conditions, such as predation pressures, shoaling behavior, and associative preference behavior. The assumption that association behavior is a uniformly sufficient predictor of mate choice in fish needs to be re-examined for *P. latipinna* and other species.

Key words *Poecilia latipinna* · Association behavior · Mate choice · Preference test · Shoaling behavior

Introduction

In some species individuals associate with conspecifics, and their association preferences may be due to a variety of selective forces, such as intra- and intersexual selection, conspecific recognition, predation pressures, and shoaling behavior. These factors may work together or independently to create association patterns.

The preference for association has often been tested on species of fish, and these tests usually focus on hypotheses of mate choice using polygynous, non-resource-based mating systems, as is common in studies of live bearing, poeciliid fish (guppies, *Poecilia reticulata*: Bischoff et al. 1985; Kodric-Brown 1985; Houde and Torio 1992; Reynolds and Gross 1992; swordtails, *Xiphophorus* spp.: Basolo 1990a, 1990b; Ryan et al. 1990; Morris et al. 1996; sailfin mollies, *P. latipinna*: Schlupp et al. 1994; Marler and Ryan 1997; Ptacek and Travis 1997; Witte and Ryan 1998; mosquitofish, *Gambusia holbrooki*: McPeek 1992). Many of these experiments have focused on the preference of females to associate with larger or smaller males (or larger or smaller dimorphic traits on males) and some studies also examined male preference between large and small females. The tendency of females to associate with particular males has usually been accepted as an indication of a mating preference, a relationship which has been established directly in a few studies. Studies have examined free-field mate choice of females (guppies: Bischoff et al. 1985; Houde 1987; Kodric-Brown 1993; Reynolds 1993; mosquitofish: McPeek 1992), the mating preference of receptive versus unreceptive females

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(mosquitofish: McPeek 1992; sailfin mollies: Ptacek and Travis 1997), or the fraction of male displays eliciting a response by females (guppies: Houde and Torio 1992; Reynolds and Gross 1992).

Marler and Ryan (1997) examined the origin of female mating preferences in three species of mollies (*P. latipinna*, *P. mexicana*, and *P. formosa*) and concluded that good genes, runaway selection, or direct selection were not important in maintaining female preference for larger fish but that the preference was maintained by pleiotropic effects from a sensory bias. Sensory biases may also explain the prevalent preference of males and females for larger conspecifics of the opposite sex in numerous animal taxa (Ryan and Keddy-Hector 1992). Alternatively, this preference may arise in many sexually reproducing species because individuals with larger body size frequently have greater reproductive success (summarized by Ryan and Keddy-Hector 1992). Here I examined association preferences in contexts other than (or in addition to) mate choice. To gain a greater understanding of alternative elements guiding association patterns, same-sex association preferences should also be investigated. Results from this sort of experiment might further test for the presence of a sensory bias for certain general characteristics such as large size (Basolo 1990a; Ryan 1990). To my knowledge, same-sex association patterns have rarely been examined (but see McPeek 1992).

To investigate the basis of the preference for larger stimuli by both males and females, I compared same-sex and opposite-sex association patterns of the live-bearing sailfin molly (*P. latipinna*). Males and females are sexually dimorphic with male secondary sexual characteristics consisting of a large, colorful dorsal fin and enhanced secondary sexual coloration. Female *P. latipinna* are not known to gain any material resources from their partners (i.e., they do not need nesting sites or foraging areas). Females prefer larger males over smaller males (Schlupp et al. 1994; Marler and Ryan 1997; Ptacek and Travis 1997; Witte and Ryan 1998). Larger

males exhibit higher rates of courtship displays than smaller males when isolated from other males, with intermediate-sized individuals displaying intermediate levels of behavioral traits (Travis and Woodward 1989). Males prefer to associate with larger females (Ptacek and Travis 1997), and smaller males tend to be excluded from mating with these females (Travis et al. 1990). Larger females have larger broods (Travis and Trexler 1987).

The primary goal of this study was to examine whether mate choice underlies the preference for larger individuals. To do this I examined the preference for association in three size classes (small, intermediate, and large) of male and female sailfin mollies when each was tested with a large and a small male (LM and SM) and a large and a small female (LF and SF; Table 1). This experiment also examined the effect of size on sailfin molly preference for association (time spent in proximity to a fish). If mate choice underlies the preference for large individuals, then I would expect females to prefer larger males but show no preference for large or small females. The same holds true in reverse for males. If natural selection underlies the preference for large individuals, then I would expect males and females to also exhibit a preference for individuals of the same sex. I found that both sexes of sailfin mollies showed a universal preference for larger fish over smaller ones regardless of sex. I subsequently investigated the association patterns of males and females when they were tested simultaneously with a large male and a large female to determine whether the universal preference for large size is context-dependent (Table 1). If mate choice underlies the female preference for larger males, then females would prefer larger males over larger females, and likewise males would prefer larger females over larger males. Finally, I examined the association patterns of females when they were tested with a large female and a small male to investigate further the influence of the context dependence of the female preference for large size (Table 1). Here I would predict that females would prefer to associate with small males

Table 1 Experimental designs (1–3) for testing preference of association using male and female sailfin mollies, *Poecilia latipinna* (*M* Male, *F* Female, *S* Small, *L* Large). The trials described for test one of each experiment were repeated for all tests performed in that experiment

(TEST FISH)				(OBJECT FISH)	
Test	N	Sex	Size (Standard length)	Treatment	
Size preference for each sex				*Large M/Small M*	*Large F/Small F*
1	20	Male	Small	Trials SM/LM & LM/SM	Trials SF/LF & LF/SF
2	20	Female	Small		
3	20	Male	Intermediate		
4	20	Female	Intermediate		
5	20	Male	Large		
6	20	Female	Large		
M and F preference for LM vs LF				*Large M/Large F*	
1	20	Male	Intermediate–large	Trials LM/LF & LF/LM	
2	20	Female	Large		
F preference for LF vs SF				*Large F/Small M*	
1	18	Female	Intermediate	Trials LF/SM & SM/LF	

over large females if mate choice underlies their basis for association.

Methods

Sailfin mollies were wild-caught in Lafayette, Louisiana, in November 1995 (experiments 1 and 2) and in September 1997 (experiment 3). Fish were maintained in the laboratory on a 14:10 h light:dark cycle using fluorescent lights during daylight hours. Stock and observation aquaria were filled with water at 6 ppt salinity and kept at 25–30 °C. Males and females were maintained together in 37-l and 53-l tanks at a female to male ratio of 3:2. I placed males of varying sizes in each stock tank with females so that males of one size were not together and females were not exposed only to males of one size. The fish were fed daily on TetraMin conditioning food. The standard length (SL) of all fish was measured from the tip of the snout to the base of the caudal fin. Male dorsal fin height was not determined as it is difficult to measure, but it is highly correlated with male SL (Farr et al. 1986). Individual body size of mature individuals varies continuously from 21–75 mm but males exhibit three peaks in this distribution; small (about 30 mm), intermediate (about 45 mm), and large (55 mm in the Louisiana population; but see Travis and Woodward 1989). Male body size variation is based primarily on a series of Y-linked alleles that control the timing of maturation and, thus, the final size at maturity (Travis et al. 1990). Time to maturity of small males is less than a third that of large males and rates of courtship increase with size while forced insemination attempts decrease with size (Farr et al. 1986).

Experiments 1–3: experimental protocol

All males used were considered mature based on fusion of the anal fin into the gonopodium. I used mature, non-receptive, and not visibly gravid females. Female sailfin mollies have a 30-day ovarian cycle and are receptive for only 2–3 days immediately after producing a brood, and they signal this receptivity to males (Farr et al. 1986; Ptacek and Travis 1997). Males still display to non-receptive females but at a consistently lower rate than towards receptive females (Sumner et al. 1994). No test fish was used in more than one test. I chose object fish from stock tanks other than those in which the test fish were maintained, so the test fish had no immediate prior experience with the object fish. I removed test fish and object fish from their stock tanks at least 15 h before testing and placed them in 53-l holding tanks that were divided into six separate darkened compartments. Separating male fish before testing increases the amount of courtship behavior exhibited by these fish (Travis 1994).

I conducted the preference tests in two, 53-l aquarium (61.0 × 30.5 × 30.5 cm) each of which was divided into five equal-size sections. The two end compartments were separated from the central compartment by transparent Plexiglas dividers with small holes for transmission of potential visual and chemical cues during testing. No matings could occur. The central compartment was visually divided into three sections by two lines drawn on the outside of the aquarium. The aquaria contained tan gravel and 18 cm of water that was aerated and filtered, except during testing. A soft, 60-W, cool-white light bulb was suspended 45 cm above each tank. The tanks were surrounded on three sides with cardboard to prevent the test fish from being distracted by fish in other tanks. I covered the front of the tanks with one-way film to minimize disturbing the fish with my presence. All test animals were fed prior to testing. I conducted trials from 0830–1530 h, 2 December 1995 to 14 January 1996 for experiment 1, from 16 to 20 February 1996 for experiment 2, and from 9 to 13 October 1997 for experiment 3.

I placed a test fish in the center compartment of the test tank under a rectangular net box (17 × 12 × 13 cm) for a 10-min habituation period, exposing it to potential visual and chemical cues from the two object fish in the end compartments. After habituation, I carefully removed the box by hand (the test fish were not obviously stressed by this) and observed the test fish for 10 min. I recorded time spent by the test fish within one body length of the Plexiglas divider and when the test fish was following the object fish, because this suggested that behavioral interactions were occurring. This process was repeated a second time but with the sides of the object fish switched. Switching sides of object fish controlled for potential side biases. I conducted a total of 480 trials in experiment 1, 80 trials in experiment 2, and 36 trials in experiment 3.

Experiment 1: size preference for each sex

I examined the association patterns of male and female fish in three size classes with large and small object fish of the same and opposite sex. Large and small object fish of the same sex were ≥15 mm different in SL. Using the same data, I also examined an individual's "strength of preference" for larger size (L–S) when the individual was paired with two fish of the same sex and two fish of the opposite sex. I tested male and female test fish in three size classes (small 30–39 mm SL, intermediate 41–49 mm SL, and large 51–65 mm SL) in two treatments with two trials within each treatment (Table 1). Treatment 1 consisted of a test fish tested with a large and small object male (trials SM/LM and LM/SM). The large object male was on the right and the small object male was on the left in one trial and the sides were reversed in the other trial. Treatment 2 consisted of a test fish with a large and small object female (trials SF/LF and LF/SF). Large and small object fish were haphazardly selected from individuals that fell in the same size classes as used for the test fish. Treatment orders and the trials within treatments were randomized.

On a given day, six fish were tested, one from each size class for both sexes, in both treatments. Half of the test fish saw the same two pairs of object fish for each treatment and the other half of the test fish saw another two pairs of object fish on a given day. The object fish pairs were changed daily. Testing orders for a given day were randomized with the limitation that all three size classes of test fish saw the same pairs of object fish before switching to a second pair of object fish of the opposite sex (between treatments). Each test fish was returned to the holding tank for 40 min between treatments.

Experiment 2: male and female preference for large male versus large female

I investigated the preference of both sexes of fish for a large (51–65 mm SL in all cases) fish of the same and opposite sex. I tested male or female test fish with a large male and a large female object fish (Table 1; trials LM/LF and LF/LM). Paired object fish were ≤1 mm different in SL. Small test fish were not used because I found in experiment 1 that small test fish did not show a significant preference for large fish. The testing order was randomized.

Experiment 3: female preference for large female versus small male

I examined the strength of sex versus size as a factor in female association preference. I tested female test fish (31–44 mm SL) with a large female (39–41 mm SL) and a small male (28–29 mm SL) object fish (Table 1; trials LF/SM and SM/LF). Object fish were ≥10 mm different in SL.

Statistical analyses

Results were analyzed to examine the effects of size and sex of the test fish on time spent near large and small object fish. For statistical analyses, I arbitrarily compared the time that a given test fish spent on the right side of the chamber within trials and between

treatments. This method was used to provide statistical independence of data for individual test fish. This process of analysis differs from other similarly designed experiments (Basolo 1990a, 1990b; Ryan et al. 1990; Morris et al. 1996); in those experiments, the time spent with a given object fish was added together (between trials) within a treatment (after sides were reversed), which can result in a lack of statistically independent data based on the behavior of the test fish (Houde 1997). My method of analysis is a different approach for ensuring statistical independence than that suggested by Houde (1997), and is also slightly more conservative. Houde (1997) suggested that preference be measured as the difference in time spent with each male, thus resulting in one, not two, data points per pair of males. In experiment 1, I measured an individual's preference to associate with one fish over the other (time spent near each fish compared). I also examined an individual's "strength of preference" (SOP) for larger size of one sex versus the other sex by calculating the time a test fish spent with the larger fish minus time spent with the smaller fish (L–S). I used non-parametric statistics (Kruskal-Wallis one-way analysis of variance by ranks, Wilcoxon-Mann-Whitney test, and Wilcoxon signed-ranks test; Siegel and Castellan 1988). All tests were two-tailed because I had no a priori prediction as to association behavior. For experiment 1, based on Bonferroni's inequality (Snedecor and Cochran 1980), I reduced alpha because each data set was analyzed four times: $\alpha = 0.05/4 = 0.013$. For experiments 2 and 3, I set $\alpha = 0.05$.

Results

Size preference for each sex

Females spent significantly more time near large males than near small males ($n = 60$, $Z = -6.06$, $P < 0.0001$, Wilcoxon signed-ranks test; Fig. 1A). Male test fish also spent significantly more time near large than near small females ($n = 60$, $Z = -5.83$, $P < 0.0001$; Fig. 1B). When I examined the time spent near object fish of the same sex, I found that the same test females also spent significantly more time near large object females than near small object females ($n = 60$, $Z = -4.83$, $P < 0.0001$; Fig. 1A). Male test fish also spent significantly more time near large object males than near small object males ($n = 60$, $Z = -5.36$, $P < 0.0001$; Fig. 1B). Thus, sailfin mollies demonstrated a universal preference to associate with larger over smaller conspecifics.

There was a tendency for female test fish to show a greater SOP for larger male as compared to larger female size ($n = 60$, $Z = -2.25$, $P = 0.025$; $\alpha = 0.013$, Wilcoxon signed-ranks test; Fig. 2A). Male test fish, on the other hand, did not demonstrate a significant difference in their SOP for larger female size compared with larger male size ($n = 60$, $Z = -1.09$, $P = 0.272$; Fig. 2B). This suggests that the preference for larger size is not necessarily context-dependent.

The three size classes of female test fish did not demonstrate significantly different SOPs for large over small object males ($n = 60$, $H = 7.85$, $P = 0.019$; $\alpha = 0.013$, Kruskal-Wallis test; Fig. 3A). The result, however, suggests that smaller test females spent less time near large males than did intermediate and large test females. The three size classes of female test fish also did not demonstrate a significantly different SOP for large over small object females ($n = 60$, $H = 6.60$,
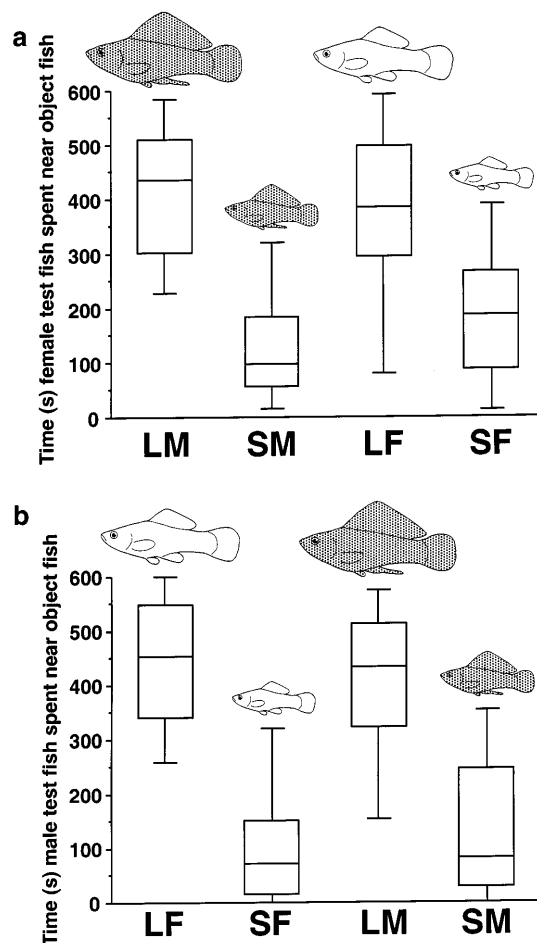
Fig. 1 Experiment 1. Time (s) during the 10-min observation period that *Poecilia latipinna* female fish spent near large males and small males, and large females and small females (**A**), and male fish spent near large females and small females, and large males and small males (**B**) (*M* male, *dark fish*; *F* female, *light fish*; *L* large, *S* small). Here and throughout all data pertain to test fish preferences for object fish. The *upper and lower horizontal lines* of the box represent the first and third quartiles and the *middle horizontal line* represents the median. The *whiskers* represent the range

$P = 0.037$; $\alpha = 0.013$; Fig. 3B). But, again the result suggests that smaller test fish spent less time near larger object females. The three size classes of male test fish also did not demonstrate a significantly different SOP for large over small object males ($n = 60$, $H = 8.37$, $P = 0.015$; $\alpha = 0.013$; Fig. 4A). The result here also suggests that smaller test males spent less time near large object males than did intermediate and large test males. The three size classes of male object fish showed no significant difference in their SOP for large over small object females ($n = 60$, $H = 0.70$, $P = 0.700$; Fig. 4B). Thus, test fish of all sizes showed a similar level of SOP for association with large over small object fish of the same or opposite sex, but smaller test fish of both sexes showed a tendency to exhibit a lower SOP for large over small object fish than did intermediate and large test fish.
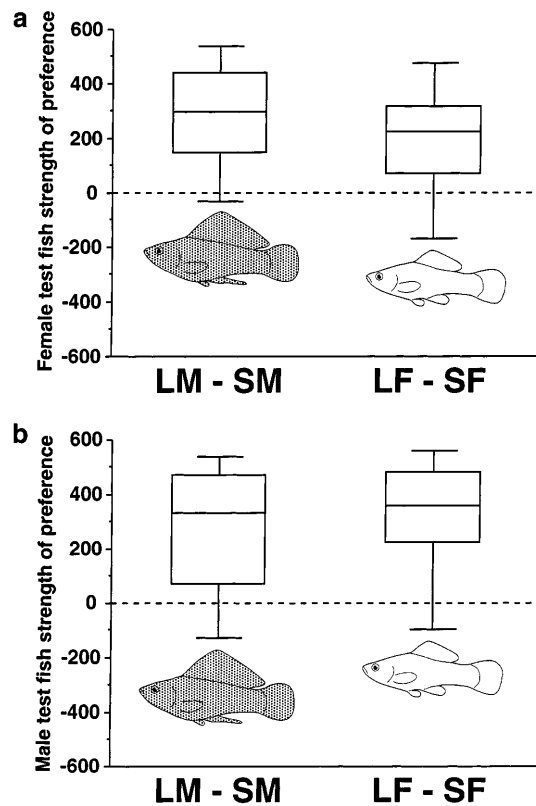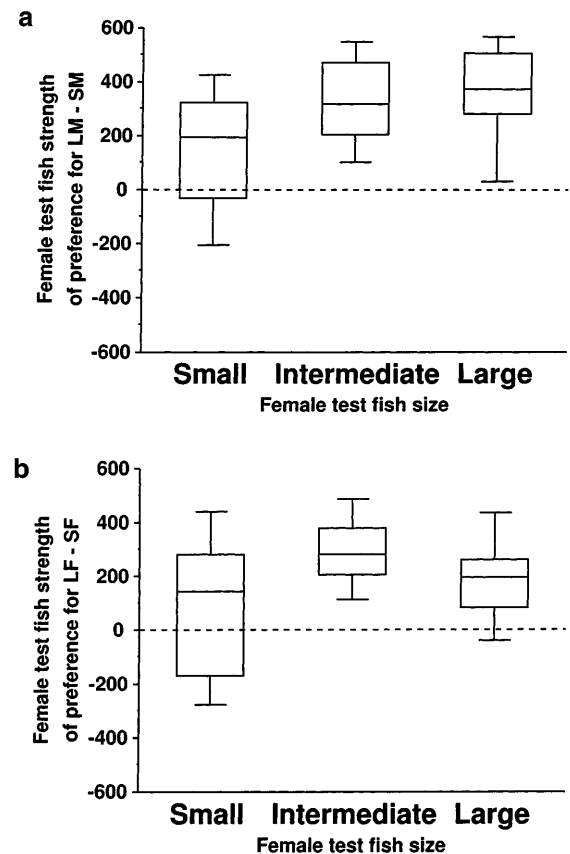
Fig. 2 Experiment 1. Strength of preference (time test fish spent near LM–SM versus LF–SF) exhibited by *P. latipinna* females for large males and large females (**A**), and males for larger males and large females (**B**) (*M* male, *dark fish*; *F* female, *light fish*; *L* large, *S* small). See legend to Fig. 1 for explanation of box plot

Fig. 3 Experiment 1. Strength of preference (time test fish spent near LM–SM or LF–SF) exhibited by three sizes of *P. latipinna* females (small, intermediate, and large) for large males (**A**) and large females (**B**) (*M* male, *F* female, *L* large, *S* small). See legend to Fig. 1 for explanation of box plot

Male and female preference for large male versus large female

Based on the universal preference of male and female object fish for large object fish of the same and opposite sex and the different SOPs exhibited by female and male object fish for large male size versus large female size (experiment 1), I examined the preference for association by male and female test fish when paired with a large male object fish and a large female object fish. Female test fish seemed to spend more time near large object males than large object females ($n = 20$, $Z = -1.867$, $P = 0.062$; $\alpha = 0.05$, two-tailed; Fig. 5A). On the other hand, there was no significant difference in the time that male test fish spent near large object males over large object females ($n = 20$, $Z = -0.485$, $P = 0.627$, two-tailed; Fig. 5B). Thus, females may be exhibiting mate choice, but it is unclear if this is the only factor affecting their behavior.

Female preference for large female versus small male

To investigate further the basis for female preference, I also tested female test fish with a large object female and small object male. Female test fish spent significantly more time near large object females than near small object males ($n = 18$, $Z = -3.245$, $P = 0.001$; two-tailed; Fig. 6). This finding suggests that mate choice was not necessarily the most important factor influencing female association behavior.

Discussion

This study draws attention to the need to validate the connection between association behavior and mating behavior by appropriately designed experiments. By studying association patterns between opposite- and same-sex sailfin mollies, I found that the preference for larger conspecifics is universal, and not limited to opposite-sex individuals. This suggests that the preference for larger fish may not necessarily have arisen through sexual selection as might have been predicted if tests with same-sex fish had not been performed. Instead, natural selection (e.g., predation pressures, shoaling responses) may also play an important role in creating the association patterns observed in *P. latipinna*, and this may have been the basis for the origin of a general
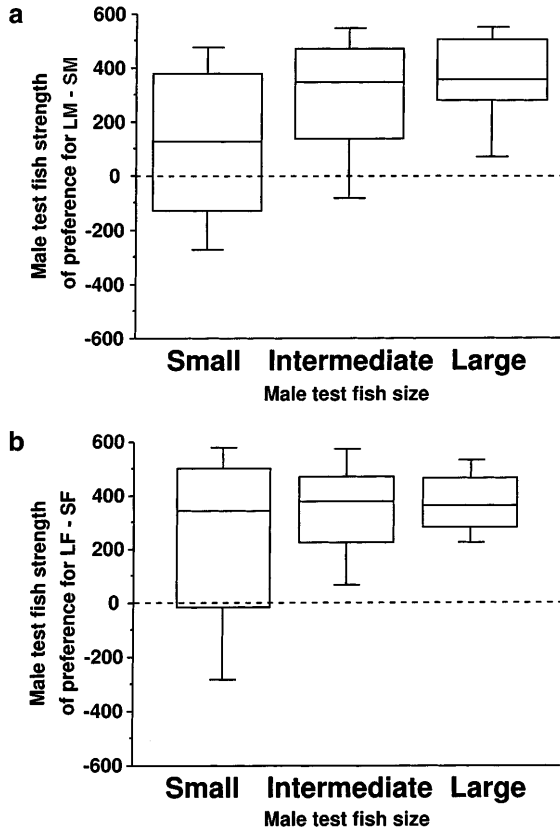
Fig. 4 Experiment 1. Strength of preference (time test fish spent near LM–SM or LF–SF) exhibited by three sizes of *P. latipinna* males (small, intermediate, and large) for large males (**A**) and large females (**B**) (*M* male, *F* female, *L* large, *S* small). See legend to Fig. 1 for explanation of box plot

sensory bias (Basolo 1990a; Ryan 1990) for larger individuals. Alternatively, the preference for large size evolved through sexual selection and is expressed in both sexes.

My results were similar to another study with *P. latipinna* (Ptacek and Travis 1997); both studies demonstrated male and female preference to associate with larger over smaller fish of the opposite sex (Fig. 1A,B). However, in the Ptacek and Travis (1997) study, only receptive females demonstrated a significant preference for large over small males; non-receptive females showed no preference. My findings are more similar to those of other studies of poeciliid fish in which females have shown choice behavior even when receptive state was not controlled (e.g., guppies: Stoner and Breden 1988; Dugatkin and Godin 1992; Dugatkin 1996; mollies: Marler and Ryan 1997; swordtails: Basolo 1990a; mosquitofish: McPeek 1992). For males, a preference for larger females could be explained as a preference for more fecund females. This is especially true in those taxa where female fecundity is positively correlated with body size (van den Berghe and Gross 1989; Travis et al. 1990).

The sailfin mollies in my experiment also preferred to associate with larger fish over smaller fish of the same sex
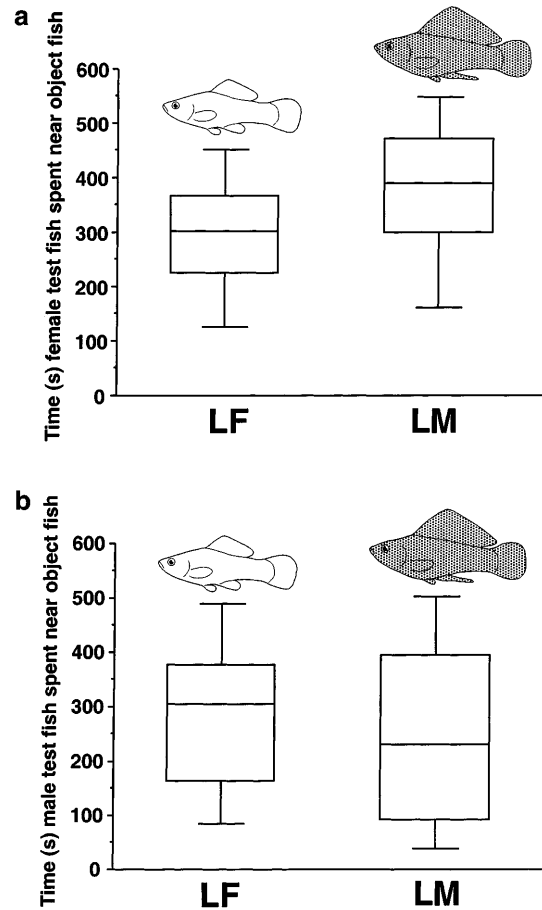
Fig. 5 Experiment 2. Time (s) that *P. latipinna* females spent near large females and large males (**A**), and males spent near large females and large males (**B**) (*M* male, *dark fish*; *F* female, *light fish*; *L* large.). See legend to Fig. 1 for explanation of box plot
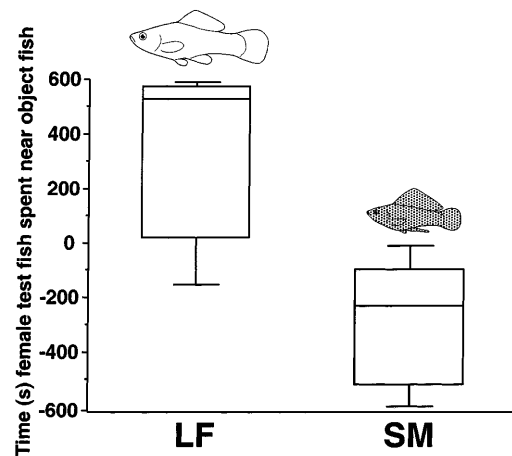
Fig. 6 Experiment 3. Time (s) that female *P. latipinna* spent near large females and small males (*M* male, *dark fish*; *F* female, *light fish*; *L* large; *S* small). See legend to Fig. 1 for explanation of box plot

(Fig. 1A,B). In the only other study of same-sex association preference, McPeek (1992) found that gravid female mosquitofish did not spend more time near larger gravid

females than smaller ones. He proposed that these results indicate that female choice for larger males in his previous experiments represents mate choice and not simply choice to associate with larger conspecifics per se. Following these conclusions, one would predict that in my experiment, males and females would demonstrate a greater SOP (L–S) for larger-size than for smaller-size fish of the opposite sex. However, I found no significant difference in the SOP exhibited by male test fish for larger male size over larger female size (Fig. 2B). Females, on the other hand, seemed to prefer larger males over larger females (Fig. 2A). These findings suggest that, in sailfin mollies, mate choice may be one factor that affects the preference for large fish but that other factors are also involved. Thus, my results leave the association behavior of these fish open to numerous alternative interpretations.

In the second experiment, male and female preference for LM versus LF, I examined further the preference for large fish. I found that when tested with large individuals of each sex, neither males nor females showed a significant preference for one sex over the other (Fig. 5A,B). From my observations, male test fish appeared to be spending equal time courting females and displaying to the object males which may explain why males showed no preference for females as might have been expected. Females seem to prefer larger males over larger females. However, these results would not have been significant had I used a less conservative route for examining association preference. In the third experiment, female preference for LF versus SM, I found that females preferred to associate with large females over small males (Fig. 6). These results support the conclusion that the association preferences of sailfin mollies are not solely explained by mate choice factors.

In experiment 1, male and female preference for LM versus SM and LF versus SF, the different size classes of test fish (small, intermediate, and large) showed some differences in their SOP for larger object fish over small ones. Small test males and small test females seemed to spend less time near larger object males than did intermediate and large test fish. These results support those of Ptacek and Travis (1997), who found the strength of male preference to increase as male size increased. These results indicate that the size of a test fish can be an important variable that needs to be considered when testing association behavior in fish.

In and of themselves, my data do not support any one hypothesis about the preference for association in sailfin mollies. The preferences I recorded may be explained by any combination of the following hypotheses: (1) associative preference behavior (sensu Dugatkin and Sargent 1994), (2) predation pressures, and (3) shoaling behavior.

Dugatkin and Sargent (1994) suggested that if males can assess their attractiveness, then they may prefer to associate with other males that they view as more attractive, i.e., potentially more attractive to the other sex, or ones that they view as less attractive, e.g., to predators. Alternatively, the preference for larger size may be indirectly due to a shoaling response that is a consequence of differential predation on different size classes.

Schlupp and Ryan (1996) found that females of *P. latipinna* school with conspecific females in the field and laboratory. In my experiment, intermediate-and large-sized *P. latipinna* showed a significant preference to associate with larger fish whereas smaller individuals did not. Instead, smaller individuals tended to avoid larger fish. It is known that two species of herons (*Casmerodius alba* and *Egretta thula*) preferentially prey on larger, mature sailfin mollies over smaller ones (Trexler et al. 1994). These results suggest that size-assortative shoaling behavior may be a response to predation levels, as Magurran and Seghers (1991) previously found for guppies.

The preference of male and female sailfin mollies to associate with large object fish may indicate that the preference for larger fish during mate choice may be a consequence of a pre-existing or sensory bias (Basolo 1990a; Ryan 1990). Marler and Ryan (1997) found that female *P. latipinna* (bisexual) and *P. formosa* (unisexual) prefer larger males. They argue that the preference of *P. formosa* was inherited from its ancestors, *P. latipinna* and *P. mexicana*. They further proposed that the preference for large males may have originated in the ancestors of the poeciliids because the preference for large males is characteristic of other species in this family (Basolo 1990a; Ryan et al. 1990; McPeek 1992). I propose that the preference by female *P. latipinna* for larger fish, per se, may have been subsequently exploited (i.e., sensory exploitation; Ryan 1990) by males through the evolution of a large dorsal fin. My results, when considered in light of these prior experiments, suggest that not only is the preference for larger males ancestral, but that the female's preference for large body size may have arisen in contexts other than mating. This is also supported by the results of Marler and Ryan (1997) showing that *P. formosa* (a gynogenetic fish) preferred to associate with larger males even though they obtain no genetic benefits from males. The preference for large males may be maintained by selection on pleiotropy or correlated traits (Ryan 1990; Kirkpatrick and Ryan 1991). For example, selection on females to shoal with certain-size fish could also select a female preference to mate with certain-size fish.

Ptacek and Travis (1997) found that receptive females demonstrated a significant preference for larger over smaller males whereas females that were not controlled for receptivity showed no preference for large or small males. Witte and Ryan (1998) found that females that were separated from males for at least 18 days before testing preferred to associate with larger males over smaller males. In my studies I found that females that were only separated from males for at least 15 h preferred to associate with larger fish of either sex. Thus, it is unclear as to why both Witte and Ryan (1998) and this study demonstrated that non-receptive females showed a preference for large over small males whereas the study of Ptacek and Travis (1997) did not. The explanation may simply be related to the type and levels of predation found in each population, as has been found for guppies (Godin

and Briggs 1996). Future studies should compare results using receptive and non-receptive females, from the same populations, in similar types of tests to examine mate choice behavior more accurately, especially in live-bearing fish.

In conclusion, my data do not permit easy interpretation of the association preferences documented. The results do, however, indicate that alternative explanations to mate choice should be considered. Experiments examining preference for association behavior, in the future, should be designed to account for hypotheses other than mate choice behavior.

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Communicated by J.D. Reynolds