

A comparison of two methods to assess audience-induced changes in male mate choice

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Abstract Multidirectional communicative interactions in social networks can have a profound effect on mate choice behavior. Male Atlantic molly *Poecilia mexicana* exhibit weaker mating preferences when an audience male is presented. This could be a male strategy to reduce sperm competition risk: interacting more equally with different females may be advantageous because rivals might copy mate choice decisions. In line with this hypothesis, a previous study found males to show a strong audience effect when being observed while exercising mate choice, but not when the rival was presented only before the choice tests. Audience effects on mate choice decisions have been quantified in poeciliid fishes using association preference designs, but it remains unknown if patterns found from measuring association times translate into actual mating behavior. Thus, we created five audience treatments simulating different forms of perceived sperm competition risk and determined focal males' mating preferences by scoring pre-mating (nipping) and mating behavior (gonopodial thrusting). Nipping did not reflect the pattern that was found when association preferences were measured, while a very similar pattern was uncovered in thrusting behavior. The strongest response was observed when the audience could eavesdrop on the focal male's behavior. A reduction in the strength of focal males' preferences was also seen after the rival male had an opportunity to mate with the focal male's preferred mate. In comparison, the reduction of mating preferences in response to an audience was greater when measuring association times than actual mating behavior. While measuring direct sexual interactions between the focal male and both stimulus females not only the male's motivational state is reflected but also females' behavior such as avoidance of male sexual harassment [*Current Zoology* 58 (1): 84–94, 2012].

Keywords Communication networks, Male mate choice, Non-independent mate choice, Sexual selection, Sperm competition risk, Audience effect

Within social units, interacting individuals may form communication networks (Oliveira et al., 1998; Peake et al., 2002), where most attempts to communicate take place in front of one or more observers, i.e., in a public domain (Danchin et al., 2004; Druen and Dugatkin, 2011). Those 'by-standing' conspecifics have been shown to gather information by observing surrounding individuals (Brown and Laland, 2003; Valone, 2007) and use the obtained information in various contexts (Oliveira et al., 1998; Doutrelant et al., 2001). For example, females may use public information on the outcome of male aggressive interactions to assess the quality of potential mates, as has been reported for female crayfish *Procambarus clarkia* (Aquilini et al., 2008) or Japanese quail *Coturnix japonica* (Ophir and Galef, 2003). Furthermore, females may copy the mate choice

decision of other conspecific females, known as 'mate choice copying' (Dugatkin, 1992).

As females are typically assumed to be the choosier sex (Emlen and Oring, 1977; Andersson, 1994), the majority of studies on mate choice have traditionally focused on female choice (for livebearing fishes of the family Poeciliidae see Houde, 1997; Magurran, 2005). Nonetheless, males, too, can be choosy when females vary in their reproductive quality (Parker, 1983; Plath et al., 2006), when sex ratios are female-biased (Emlen and Oring, 1977), or if mating is costly for males (Wong and Jennions, 2003). The latter aspect is especially important when sperm production is limited by environmental constraints (Nakatsuru and Kramer, 1982; Verrell, 1982). There is increasing evidence that mate choice copying is not restricted to females, and males

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copy other males' mate choice as well (Witte and Ryan, 2002; Widemo, 2006; Bierbach et al., 2011b); this effect is also known from humans as the 'wedding ring effect' (Uller and Johansson 2003; Place et al., 2010). Especially for males of internally fertilizing species — such as livebearing fish from the family Poeciliidae — male mate choice copying remains a conundrum as males incur an increased risk of sperm competition when choosing another male's previous mate. In this context, Schlupp and Ryan (1997) proposed that the adaptive significance of male mate choice copying in poeciliids could be reduced costs for searching for receptive females as poeciliid females are most receptive as virgins or for a few days postpartum (Rosenthal, 1952; Constanz, 1984) and, accordingly, only a small proportion of females in a population is receptive at a time (Magurran, 2005).

Moreover, recent studies show that a visually presented male competitor affects the strength of male mating preferences in Atlantic molly *Poecilia mexicana*, with weaker preference expression when the 'by-standing' rival observed the focal male (Plath et al., 2008a, b; Ziege et al., 2009). This is termed an 'audience effect' since the presence of the by-standing rival leads to behavioral changes in the observed individual (Marler et al., 1986; Plath et al., 2008b). It has been argued that this behavior is best interpreted as an adaptation to reduce sperm competition risk (Ziege et al., 2009; Plath and Bierbach, 2011) arising from male mate choice copying (Bierbach et al., 2011b for *P. mexicana*). Focal males that cease expressing mating preferences in front of a rival make it impossible for the rival to copy their mate choice (Plath et al., 2008a; Plath and Bierbach, 2011). An alternative explanation for audience-induced changes in male mating behavior might be that focal males strategically avoid aggressive encounters with rivals, as both males may share similar intrinsic preferences for certain female phenotypes (Plath and Schlupp, 2008a).

Most studies on audience-induced changes of male mating preferences in poeciliids investigate association preferences in dichotomous choice tests. Dichotomous choice tests may have some advantages over full contact designs as confounding behavioral interactions between the choosing focal individual and the stimulus individuals are prevented and transmitted cues are under control (see Houde, 1997). Dichotomous tests are widely used not only for evaluating poeciliid mating preferences but also in species such as zebra finches *Taeniopygia guttata* (ten Cate, 1985; Clayton, 1990) and sand gobys

Pomatoschistus minutus (Lehtonen and Lindström, 2008).

Nevertheless, there might be discrepancies between the mere association with a potential mate and the motivation to show actual mating behavior towards that mate when direct interactions are allowed and, therefore, multiple cues become available. For example, in a recent study (Jeswiet and Godin, 2011), male guppies were consistent in their choice for a particular female when comparing association preferences and sexual behavior, but only when they showed a strong preference in the association tests. In the current study we therefore aimed to test whether association preferences translate into comparable patterns in male mating behavior in a full contact design. We re-analyzed data from a previous study on *P. mexicana* (Ziege et al., 2009) that addressed the question of how different forms of perceived sperm competition risk affect male mating preferences. Ziege et al. (2009) established mating preferences through association times focal males spent in proximity of two different-sized females. Measurements were repeated while the focal male experienced different forms of sperm competition risk so it was possible to compare the strength of male preferences between the two parts of the tests. The current study uses a similar experimental design but allowed the focal male and both stimulus females to interact freely. Thus, we were able to score actual mating behavior in order to determine male mating preferences.

With this approach we tried to answer two questions: (1) do *P. mexicana* males respond to various forms of perceived sperm competition risk by adjusting their mating behavior strategically as found when scoring association preferences? (2) is the pattern observed when scoring actual mating behavior comparable to the one reported for association preferences (Ziege et al., 2009)? In other words: is this method also suitable to assess audience-induced changes in mating preferences? Answering both questions will extend our knowledge of how male mating behavior is affected by the risk of sperm competition and will assess two methods that are commonly used to establish male mating preferences for congruence.

1 Materials and Methods

1.1 Study organism

The Atlantic molly is widespread in various streams, lakes and coastal lagoons along the Central American Atlantic coast. Males typically form dominance hierarchies where dominant males (typically the largest) ag-

gressively defend shoals of females (Parzefall, 1969). Poeciliid fishes are livebearers and males use their transformed anal fin, the gonopodium, to transfer sperm. As females can store sperm to fertilize several consecutive monthly broods (Schmidt, 1920), sperm competition (sensu Parker, 1970) is intense (for poeciliids see Becher and Magurran, 2004; Evans and Pilastro, 2011). Small males use an alternative, sneak-like mating tactic characterized by hiding near a group of females and relying on forced copulations when dominant males are absent (Parzefall, 1969). Neither large nor small males perform any courtship behavior (Parzefall, 1969). Sexual behavior is almost constantly exhibited in the form of pre-copulatory nipping and copulation attempts (thrusting; Parzefall, 1973). In order to obtain chemical information about her receptivity a male approaches a female from behind and touches her genital region (so-called 'nipping' behavior; Parzefall, 1973). Gonopodial thrusting typically, but not always, follows nipping (Parzefall, 1969), whereby males attempt to insert their gonopodium into the female's genital opening (Parzefall, 1973).

Several studies found *P. mexicana* males of any size to preferentially mate with larger females (Plath et al., 2006, 2008a; Ziege et al., 2009) which demonstrates that even in systems where males form dominance hierarchies and dominant males monopolize groups of females (as seen in *P. mexicana*), male mate choice can play an important role (Plath et al., 2006). This is most likely due to the fact that female fecundity is strongly correlated to body size (Riesch et al., 2010a,b). Sex-ratios in natural *P. mexicana* populations are typically female-biased (Parzefall, 1969), and males may not be able to fertilize every female in their environment (including subordinate sneaker males). So, even if large dominant males have greater mating opportunities due to increased access to more females, showing preferences for large female body size will still be advantageous as males that mate with larger, more fecund females can increase their reproductive success.

The fish used in this study were descendants of animals collected in 1995 in a coastal brackish lagoon near Tampico in central Mexico. Test fish came from large, randomly out-bred stocks that were established in the laboratory in 1995 and mixed with wild-caught individuals every year to maintain genetic variability. Two stocks were maintained at the Institute of Biochemistry & Biology of the University of Potsdam, and three at the Institute of Ecology, Evolution & Diversity in Frankfurt/M. Experiments were conducted in the same

way at both universities. We reared stocks in aerated and filtered 100–200 l aquaria (comprising approximately 60–100 fish each) at 27–29°C. As males and females were reared together, stimulus females were most likely pregnant; no attempt was made to specifically use postpartum females [this was also the case in Ziege et al. (2009), where the same stocks were used]. Artificial light was provided during a 12: 12 h light:dark cycle in addition to natural daylight entering the room through several windows. Aquaria were equipped with rocks and live and artificial plants. Fish were fed twice daily with commercial flake food, fish food tablets and chironomid larvae. We isolated focal males in 25-L tanks for four days before the tests (two males per tank) to make sure that they were motivated to mate. However, these two males were not used as a focal male/audience combination during tests. Tanks were visually isolated from other isolation tanks.

We tested each focal male only once; however, due to the limited number of males available from our stocks, some males were also used as audience males after they had served as a focal male, but never on the same day. No male served as an audience more than once, and focal and audience males were not familiar, i.e. came from different stock tanks. Each focal male was exposed to a new female dyad. In total we used 159 males and 54 females in this study.

1.2 Experimental design

Association preferences In brief, Ziege et al. (2009) used a test tank divided into five sections of equal size: two lateral compartments were divided by (removable) transparent Plexiglas partitions to hold the stimulus female fish. Consequently, the focal male had no possibility to mate with stimulus females but could only swim next to the females in front of the lateral compartments.

Full contact design We used the same test tank (80 cm length × 30 cm width × 30 cm depth) that served for the measurement of association times (Ziege et al., 2009), but this time, the two Plexiglas dividers were removed from the test tank, thus focal males could interact freely with both stimulus females. The sides of the test tank were covered by black plastic foil except on the front. The tank was filled to 15 cm with aged tap water at 27–28°C and illuminated by a 40 Watt incandescent lamp 35 cm above the tank in addition to room illumination (two 100 Watt neon tubes on the ceiling of the experimental room). Water in the test tank was aerated between trials, but the air pump was turned off during the experiment.

We used female body size as a choice criterion (for *P.*

mexicana see Plath et al., 2006; for various other poeciliid species see overview provided in Ala-Honkola et al., 2010). All focal males underwent an initial test to establish their baseline preference (Fig. 1; 1st part). To wit, we introduced two stimulus females (large: 51.0 ± 0.7 mm standard length, SL; small: 36.3 ± 0.5 mm SL) into the test tank. Then, we introduced a focal male (35.5 ± 0.4 mm SL) into a transparent Plexiglas cylinder (10 cm in diameter) in the center of the tank and left the fish undisturbed for 5 min. After this habituation period, the cylinder was gently lifted by hand and the focal male could choose to interact with both females. Instead

of measuring association times, in this experiment we counted pre-mating behavior (number of nips) and mating attempts (gonopodial thrusting) directed by focal males toward the large or small stimulus female during a 10 min observation period. The category ‘thrusting’ combined successful and unsuccessful copulation attempts, as it is impossible to determine behaviorally whether or not sperm were transferred.

After this 1st part of the tests, males were randomly assigned to one of five different audience treatments consisting again of a habituation phase (5 min) and a test phase (10 min) to establish their final preference.

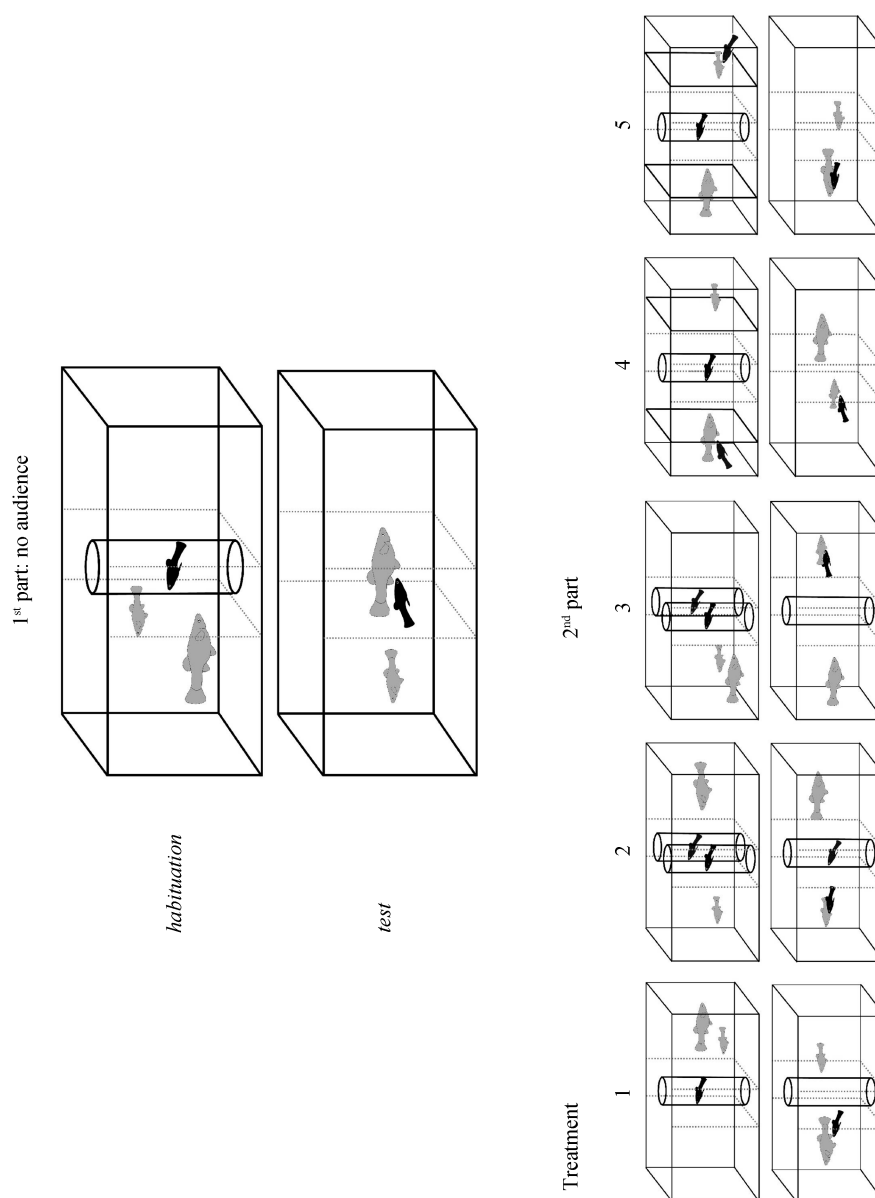


Fig. 1 Experimental set-up

A focal male (*dark-gray*) was given a choice between two different-sized females (*light-gray*). During the five different audience treatments, an audience/rival male (*gray*) was presented during or before the 2nd part of the experiment. Each part consisted of a 5-min habituation and a 10-min testing phase. For details see main text.

This episode is henceforth called the 2nd part (see Fig. 1). The 2nd part was conducted directly after the 1st test part (i.e. after another 5 min habituation period). During Treatment 1, we presented an empty transparent Plexiglas cylinder without an audience male in the central back of the neutral zone. This control treatment was conducted to test whether any changes in the expression of male preferences in the course of the experiment were truly due to the audience or whether the focal male's motivation to choose would generally decrease over time. Only if the decrease of male preferences during the audience/sperm competition risk treatments (2–5) was stronger than in the control Treatment 1 would this difference be interpretable as an audience effect.

In Treatment 2 we presented a conspecific audience male inside the transparent cylinder throughout the 2nd part. The audience male was confined in its cylinder, so any direct physical interaction was ruled out (Plath et al., 2008a). In Treatment 3 the audience male (36.4 ± 0.4 mm SL) was presented inside the transparent Plexiglas cylinder during the habituation phase, but was gently removed before the focal male was released from his cylinder to exercise mate choice (the focal male could see the removal of the audience male). Doing so, we created another experimental situation in which the focal male also perceived non-specific sperm competition risk, but could not see the audience male during the actual mate choice trial. Treatments (2) and (3) belong to the category of “non-specific sperm competition risk” treatments because audience males in these experimental treatments represent potential rivals that could mate with one (or both) stimulus female(s) at a later point in time. Specific sperm competition risk was simulated in treatments (4) and (5), where the focal male could observe the rival male interact sexually with one of the two stimulus females [see Wong and McCarthy (2009) for a similar design using eastern mosquitofish *Gambusia holbrooki*]. To realize Treatment 4 and (5), it was necessary to reinsert the two Plexiglas dividers at both ends of the test tank in order to hold the stimulus females. The rival male was placed into one of the females' stimulus compartments during the habituation phase of the 2nd part of the experiment. In half of the trials, the rival male could interact with the previously preferred female [Treatment 4], while in another half of trials the rival male was placed within the stimulus compartment of the previously non-preferred female and thus, had the possibility to interact with it [Treatment 5]. We scored sexual behaviors shown by the rival

male (nipping at the female gonopore, and gonopodial thrusting) to make sure that the focal male would actually be faced with the risk of sperm competition. Mean \pm SE numbers of sexual behaviors shown by rival males in Treatment 4 were determined as 27 ± 8 (nipping) and 13 ± 4 (thrusting), and as 24 ± 7 (nipping) and 11 ± 4 (thrusting) in Treatment 5. In 20 cases (out of 59 trials), rival males in treatments (4) and (5) exhibited no nipping or thrusting behavior towards the stimulus female. We, therefore, re-ran all analyses reported below while leaving out those trials in which rival males did not show any sexual behavior, but the direction of the results was not affected (results not shown).

1.3 Statistical analysis

In order to compare association preferences with direct sexual interactions we included data from Ziege et al. (2009) in our analyses, where we scored $n = 145$ trials (Treatment 1, control – no sperm competition risk, $n = 30$; Treatment 2, audience present during experiment – non-specific sperm competition risk, $n = 39$; Treatment 3, audience only present during habituation period – non-specific sperm competition risk, $n = 27$; Treatment 4, rival male with preferred female – specific sperm competition risk, $n = 25$; Treatment 5, rival male with non-preferred female – specific sperm competition risk”, $n = 24$). In the second (full contact) experiment, we scored a total of $n = 151$ trials (Treatment 1, $n = 30$; Treatment 2, $n = 30$; Treatment 3, $n = 32$; Treatment 4, $n = 30$; Treatment 5, $n = 29$). In eight cases, focal males exhibited no mating behavior during the 1st part of a trial, so we excluded those data from analyses. All statistical analyses were conducted using SPSS 12.0 (SPSS Inc., Chicago, USA). Data are generally presented as means \pm SE and were tested with Kolmogorov-Smirnov-tests for normality. Relative data were arcsine (square root)-transformed prior to statistical analysis.

To investigate the overall direction of male preferences we first compared the number of sexual behaviors towards (or times spent in association with) the large and small stimulus female using paired *t*-tests. Our major question was whether focal males would alter their individual choice decisions between the two parts of a trial, i.e. before and after presentation of an audience, and especially whether the pattern found for actual mating behaviors resembles the one reported for association preferences (see Ziege et al., 2009). To do so, we calculated a score indicating the change in male preferences between the 1st and 2nd part of the tests, and we compared the score derived from the association preference experiment (Ziege et al., 2009) with the data

stemming from our full contact mate choice experiment (this study). We analyzed pre-mating (nipping) behavior and mating attempts (gonopodial thrusting) separately. The strength of male preferences was calculated for the 1st and 2nd part of each trial as the proportion of sexual behaviors (nipping or thrusting), or relative association times near the initially (during the 1st part) preferred female, respectively. We calculated a score as the difference between the proportion of sexual behaviors directed toward the initially preferred female during the 2nd and 1st part, such that no change in male preferences would lead to a score of zero, and negative values would indicate that the focal males exhibited less nipping/thrusting with the initially preferred female during the 2nd part of a trial. A univariate General Linear Model (GLM) was run, with preference scores as the dependent variable, and ‘audience treatment’ (five levels) and ‘experimental design’ (two levels: association preference tests and full-contact design) as fixed factors. Body size of the focal and audience males (standard lengths) were initially included as covariates, but were non-significant and thus, excluded from the final model. Fisher's LSD tests were employed for *post hoc* multiple comparisons.

Nonparametric Spearman rank correlations were

conducted to test for correlations between the rival male's sexual activity (number of nippings and gonopodial thrusts) during Treatments 4 and 5 and the strength of decline in mating preferences of the focal male (preference score).

2 Results

2.1 Direction of male preferences

During the 1st part of the tests, males showed a strong preference for the larger stimulus female as measured by association times (time near stimulus female; large: 324 ± 12 s; small: 206 ± 11 s, paired *t*-test: $t_{144} = 5.25$, $P < 0.001$), pre-copulatory behavior (# nipping; large: 40 ± 3 ; small: 23 ± 2 ; $t_{150} = 11.15$, $P < 0.001$) and copulation attempts (# thrusting; large: 12 ± 1 ; small: 7 ± 1 ; $t_{150} = 7.59$, $P < 0.001$).

During the 2nd part of the tests this preference for large stimulus females was lost in all treatments except Treatment 1 (control) in the case of association times (Table 1). When measuring actual mating behavior, a preference for large stimulus females was retained in all treatments except Treatment 4 (rival male with preferred female – specific sperm competition risk; Table 1). In the case of thrusting behavior the *P*-value in the control Treatment 1 bordered significance ($P = 0.062$; Table 2).

Table 1 Means \pm SE for (a) absolute times (in seconds) focal males spent near large and small stimulus females, (b) thrusting, and (c) nipping behavior of the focal males towards either large or small females during the 2nd part of the trials

Experiment/ treatment	Larger female	Smaller female	df	<i>t</i>	<i>P</i>
(a) Association times (s)					
(1)	313 ± 19	221 ± 20	29	2.85	0.008**
(2)	224 ± 16	195 ± 17	38	1.05	0.30
(3)	253 ± 22	261 ± 22	26	-0.21	0.84
(4)	263 ± 19	261 ± 17	24	0.05	0.96
(5)	297 ± 20	226 ± 19	23	1.84	0.079(*)
(b) Gonopodial thrusting					
(1)	8 ± 2	4 ± 1	29	1.94	0.062(*)
(2)	9 ± 2	4 ± 1	29	2.95	0.006**
(3)	9 ± 2	1 ± 0	31	3.77	< 0.001***
(4)	6 ± 2	3 ± 2	29	0.78	0.442
(5)	10 ± 2	1 ± 1	28	3.46	0.002**
(c) Nipping					
(1)	16 ± 2	7 ± 1	29	3.29	0.003**
(2)	19 ± 3	8 ± 2	29	3.38	0.002**
(3)	21 ± 5	5 ± 1	31	3.54	< 0.001***
(4)	13 ± 4	12 ± 7	29	0.10	0.922
(5)	26 ± 6	5 ± 1	28	3.71	< 0.001***

Paired *t*-tests, * statistically significant, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Treatment 1, control – no sperm competition risk (s.c.r.); Treatment 2, audience present during experiment – non-specific s.c.r.; Treatment 3, audience only present during habituation period – non-specific s.c.r.; Treatment 4, rival male with preferred female – specific s.c.r., Treatment 5, rival male with non-preferred female – specific s.c.r.

Table 2 Results from univariate GLMs using preference scores [based on association times, data from Ziege et al. (2009) and mating behaviors in a full-contact design (Exp. 2, this study)] as dependent variable

Experiment/ treatment	df	Mean square	F	P
<i>(a) Thrusting</i>				
Experimental design	1	0.537	7.440	0.007**
Treatment 1–5	4	0.201	2.780	0.027*
Experimental design × Treatment 1–5	4	0.005	0.062	0.993
Error	286	0.072		
<i>(b) Nipping</i>				
Experimental design	1	0.884	14.387	< 0.001***
Treatment 1–5	4	0.101	1.639	0.164
Experimental design × Treatment 1–5	4	0.017	0.278	0.892
Error	286	0.061		

Experimental design and treatment were fixed factors. Sexual behaviors were (a) gonopodial thrusting and (b) nipping.

2.2 Comparison of changes in individual male preferences

Thrusting and association times Analyzing the changes in individual male preferences (preference score) based on gonopodial thrusting, overall, revealed a very similar pattern to the one reported for association preferences (Fig. 2a, b), as confirmed by a non-significant interaction term ‘treatment by experimental design’ in the GLM (Table 2). The effect of the factor ‘treatment’ was significant (Table 2) and *post hoc* pair-wise comparisons (Fisher’s LSD) revealed that Treatment 2 (audience present during experiment – non-specific sperm competition risk) differed significantly from the control Treatment 1 ($P = 0.004$), Treatment 3 (audience present only during habituation period – non-specific sperm competition risk) ($P = 0.005$) and Treatment 5 (rival male with non-preferred female – specific sperm competition risk) ($P = 0.020$), but not from Treatment 4 (rival male with preferred female – specific sperm competition risk) ($P = 0.25$). Moreover, a significant main effect of ‘experimental design’ (Table 2) indicates that the measurement of actual mating behavior generally led to a weaker decrease in the strength of male mating preferences (Fig. 2A, B).

Nipping and association times The pattern uncovered by measuring pre-copulatory behavior (nipping) did not show the same trend as detected when analyzing association preferences (Fig. 2A, C). Indeed, in this analysis we found no significant difference between treatments (Table 2). The reduction in strength of mating preferences measured as nipping frequencies was significantly weaker than the reduction in preferences

determined as association preferences (effect of ‘experimental design’ in Table 2).

The number of nippings and gonopodial thrusts of the rival male during Treatment 4 (rival male with preferred female – specific sperm competition risk) and Treatment 5 (rival male with non-preferred female – specific sperm competition risk) towards the stimulus females did not significantly influence the strength of the observed audience effect (i.e., reduced strength of the focal male’s mating preference). This was true for both the measurement of association times ($r \leq 0.32$, $P \geq 0.12$) and the measurement of actual mating behavior (thrusting: $r \leq 0.29$, $P \geq 0.12$; nipping: $r \leq 0.04$, $P \geq 0.86$).

Negative preference scores in our present study indicate that focal males spent relatively more time with the non-preferred (mostly smaller) stimulus females during the 2nd part (and copulated more with those females, respectively). However, when considering absolute numbers of sexual behaviors (Table 1) focal males in general showed reduced sexual activity during the 2nd part and did not show an overall preference for the smaller female.

3 Discussion

Audience effects are found in various taxa and different contexts (Matos et al., 2003; Zelandt et al., 2008; Semple et al., 2009). In the current study we asked if *P. mexicana* males respond to various forms of perceived sperm competition risk (due to the presence of an audience male) by strategically adjusting their mating behavior. Furthermore, we asked if the pattern observed

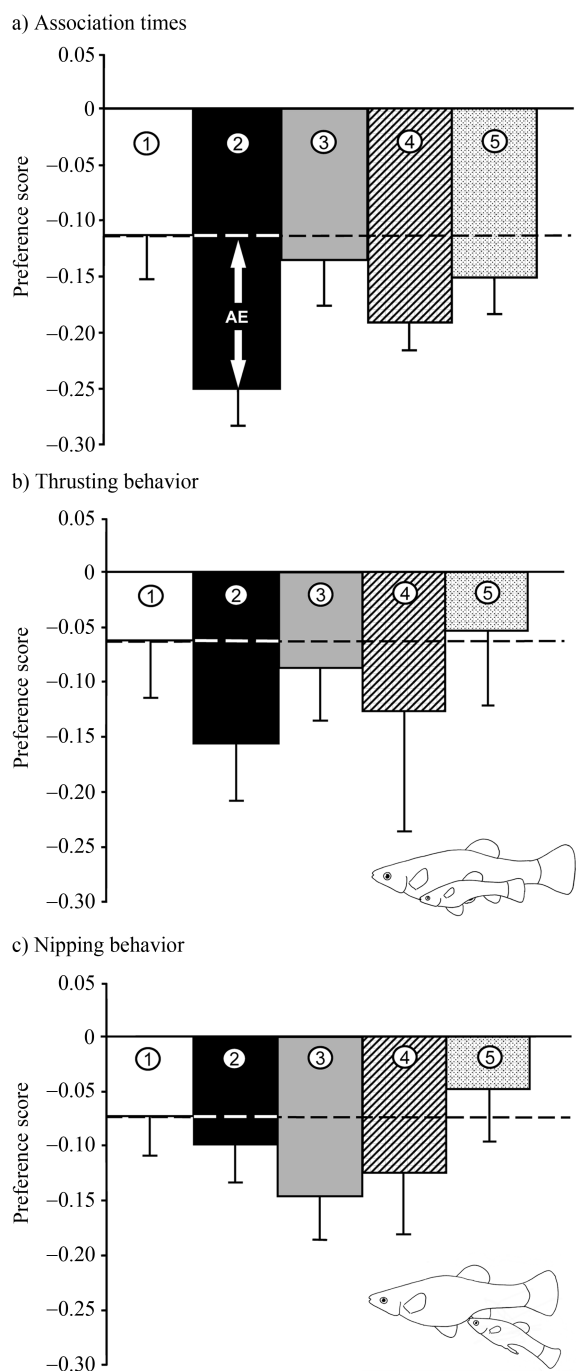


Fig. 2 Changes in strength of male mating behavior measured by (a) association times, (b) thrusting, and (c) nipping during the five audience treatments [(1) no audience (control), (2) audience male was presented throughout 2nd test period, (3) audience was presented only during habituation phase preceding the 2nd test period, (4) like (3) but audience male could interact sexually with previously preferred female, (5) like (3) but audience male could interact sexually with previously non-preferred female]

Shown are preference scores; negative values indicate that male mating behavior decreased during the 2nd part of the tests. The difference between the control Treatment 1 and Treatment 2 can be interpreted as audience effect (AE).

when scoring actual mating behavior is comparable to the one reported by Ziege et al. (2009) where association preferences were measured. Our study is the first to compare two methods used to score audience-induced changes in male mate choice.

Males indeed responded to various forms of sperm competition risk by adjusting their mating behavior. When measuring association preferences (data from Ziege et al., 2009) and thrusting behavior (this study), the strongest audience effect was observed in Treatment 2 (audience present during experiment – non-specific sperm competition risk), but an effect was also detected in Treatment 4 (rival with preferred female – specific sperm competition risk).

In Treatment 2 the audience was presented throughout the 2nd part of the tests, creating non-specific sperm competition risk including the opportunity for eavesdropping by the audience male, and in Treatment 4 the focal male had seen the previously preferred female sexually interact with the rival male. Notably, no audience effect was observed in Treatment 3, during which no possibility for eavesdropping was provided. The most plausible explanation for audience-induced changes in the behavior of *P. mexicana* males, as seen during Treatment 4, is the avoidance of a female that has recently been inseminated by a rival. Generally, multiple mating is common in poeciliids (Constanz, 1984; Evans and Magurran, 2000) and females can store sperm to fertilize a succession of monthly broods (Constanz, 1984), so sperm competition is expected to be high in natural populations of *P. mexicana*.

But what about Treatment 2 (audience present during experiment – non-specific sperm competition risk)? We argue that reducing preference expression in front of a potential eavesdropper allows males to reduce sperm competition risk (Ziege et al., 2009; Plath and Bierbach, 2011). *P. mexicana* males copy each other's mate choices, leading to increased sperm competition risk for the copying and the copied male (Bierbach et al., 2011b). Males that cease expressing mating preferences in front of other males may therefore prevent rivals from mating with their preferred mate at a later point in time (Plath and Schlupp, 2008b).

When comparing data from Ziege et al. (2009) (association preference tests) with this study (preferences were determined in a full-contact design) we found that mating preferences of *P. mexicana* males determined by scoring actual mating behavior were less sensitive to an audience. In other words, the strength of preference decreased less under perceived sperm competition risk. A

treatment-specific response comparable in direction to the pattern observed when measuring association times could only be found when observing thrusting but not pre-copulatory (nipping) behavior. So, why are audience-induced changes in male mating preferences stronger when measuring association times? We posit that the sexual interactions between males and females we measured reflect not only the focal male's motivation to mate with a particular female or to cease expressing this preference. It can be assumed that the multiple interactions among three freely swimming fish influenced the outcome of our study because the female's behavior also had an impact on the male's behavior. For example, sexual harassment is common in poeciliids (Plath et al., 2007; Magurran, 2011) and the direct (damage of genital tract; Constanz, 1984) and indirect costs for females (reduced foraging opportunity; Plath et al., 2003; Plath, 2008) are high. Females, therefore, may avoid the magnitude of male mating attempts by lying against an object, changing direction, fleeing (Bisazza, 1993; Condon and Wilson, 2006), or shoaling with other females (Agrillo et al., 2006). Sometimes females even bite approaching males (D.B., personal observation). These female behaviors are likely to affect the magnitude of observable male mating behaviors, but, as our analyses show, not the general direction of preferences (see also Jeswiet and Godin, 2011).

One general problem in ethological research is to find an appropriate method of measurement that will provide the most reliable data for a specific set of questions. Sexual selection studies often use dichotomous choice tests to establish mating preferences; still, measuring actual mating behavior might appear to be a more realistic proxy for male mating preferences. In this context, our results highlight that measuring association preferences (Ziege et al., 2009) may reveal stronger audience effects, as full contact choice tests may be confounded by multiple behavioral interactions including female avoidance of male harassment. Despite differences in the strength of the captured effect, both methods of measurement revealed that poeciliid males are able to gather complex social information from their environment and use this information to fine-tune their own mate choice (Bierbach et al., 2011b). In line with our previous study (Ziege et al., 2009) our present findings suggests that avoidance of sperm competition is a decisive factor influencing male mate choice decisions. However, in order to understand how communication in social networks has evolved it will be necessary to acknowledge the multidirectional nature of interactions in

communication networks, as networks in nature are even more complex than in simplified laboratory experiments.

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