Male guppies (Poecilia reticulata) adjust their mate choice behaviour to the presence of an audience

Amber M. Makowicz\,1,3), Martin Plath\,2) & Ingo Schlupp\,1)

(1 Department of Zoology, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019, USA; 2 Department of Ecology and Evolution, J.W. Goethe University of Frankfurt, Frankfurt am Main, Germany)

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Summary
In recent years analyzing animal behaviour in light of the social environment has become widely accepted. Especially many mating interactions do not happen in privacy, but in a public arena, raising the question of how this affects the behaviour of both the focal individual and the observing audience individual. We studied in feral guppies whether male preferences for female body size, a correlate of fecundity, are influenced by the presence of another male, the audience. We also studied whether the audience was influenced by the observed interactions. These two aspects are not normally studied together. Furthermore, we were also interested in the question of how long changes in the behaviour of the audience male might last. We found that male preferences measured as nipping/approaches decreased in the presence of an audience. Furthermore, the audience males showed no preference for larger females when tested right after the interaction with the focal male, but returned to the typical preference for larger females after 24 h. Our study highlights the relevance of the social conditions under which mating decisions are being made.

Keywords: mate preference, communication networks, sperm competition, mate competition, mate choice copying.

Introduction
An increasing body of literature considers the question of how environmental constraints (like resource availability, presence of predators, or other ecological factors) determine the strength of sexual selection by affecting the

3) Corresponding author’s e-mail address: Amber.M.Makowicz-1@ou.edu
expression of mating preferences (Johnson & Basolo, 2003; Cotton et al., 2006; Fisher & Rosenthal, 2006a; Kim et al., 2009). On the other hand, mate choice is obviously influenced by the social environment of the signaling and choosing individual (non-independent mate choice: Pruett-Jones, 1992; Westneat et al., 2000; Johnstone, 2001; White, 2004; Earley & Dugatkin, 2005; Bonnie & Earley, 2007; Druen & Dugatkin, 2011). Animals can observe social interactions and make use of the extracted information (Oliveira et al., 1998, 2001; Doutrelant et al., 2001; McGregor et al., 2001; Brown & Laland, 2003; Bonnie & Earley, 2007), such as in assessing the physical strength of prospective opponents (Earley & Dugatkin, 2002, 2005; Earley et al., 2005). Individuals can also use this public information during mate choice to assess the quality of potential mates (Dugatkin, 1992; Nordell & Valone, 1998; Otter et al., 1999; Doutrelant & McGregor, 2000; Mennill et al., 2003; Ophir & Galef, 2003). For example, females can eavesdrop on male contests and prefer winners to losers (Bisazza et al., 1989a; Aquiloni et al., 2008; Doutrelant & McGregor, 2000, 2001; McGregor et al., 2001; Brown & Laland, 2003; Bonnie & Earley, 2007), such as in assessing the physical strength of prospective opponents (Earley & Dugatkin, 2002, 2005; Earley et al., 2005). Individuals can also use this public information during mate choice to assess the quality of potential mates (Dugatkin, 1992; Nordell & Valone, 1998; Otter et al., 1999; Doutrelant & McGregor, 2000, 2001; McGregor et al., 2001; Brown & Laland, 2003; Bonnie & Earley, 2007), such as in assessing the physical strength of prospective opponents (Earley & Dugatkin, 2002, 2005; Earley et al., 2005). 

Audience effects occur when the presence of an observing (or by-stand-ing) individual leads to changes in the behaviour of the observed individual(s) (Marler et al., 1986; Matos & McGregor, 2002; Matos et al., 2003; Vigual et al., 2004; Dzieweczynski et al., 2005, 2006; Plath & Schlupp, 2008; Townsend & Zuberbühler, 2009). Recent studies using a live-bearing fish (Poecilia mexicana, Poeciliidae) found males to show weaker expression of mating preferences (i.e., to interact more evenly with two stimulus females) when an audience male was present (Plath et al., 2008a,b, 2009; Ziege et al., 2009). It was argued that this effect is most likely driven by the need for males to conceal their mate choice from eavesdropping rivals (Ziege et al., 2009; see discussion), which could copy their mate choice and mate with the same female later (Schlupp & Ryan, 1997; Witte & Ryan, 2002). Ultimately, this behavioural adjustment, thus, seems to represent a response to sperm competition risk (as suggested by Plath & Schlupp (2008) and Ziege et al. (2009)). Indeed, sperm competition is intense in most poeciliid fishes examined so far (e.g., Evans et al., 2003a,b; Aspbury 2007; Wong & McCarthy, 2009; Evans & Pilastro, 2011), including the Trinidadian guppy (Poecilia reticulata; Pilastro et al., 2002; Dosen & Montgomerie, 2004; Evans & Pilastro, 2011).
It seems straightforward to assume that male audience effects occur in various poeciliid fishes, but our current knowledge is restricted to only few species (*P. mexicana*: Plath et al., 2008a,b,c, 2009; Ziege et al., 2009; sailfin molly, *P. latipinna*: Makowicz et al., 2010; I. Schlupp, A. Rioux, K.U. Heubel & M.J. Ryan, unpubl. data; Grijalva mosquitofish, *Heterophallus milleri*: Ziege et al., 2008). Our present study, therefore, examines the role of an audience on male mate choice in a feral population of guppies. Guppies are prime models for a variety of questions in behavioural and evolutionary ecology, especially on sexual selection and mate choice (e.g., Houde, 1997; Evans et al., 2003a,b, 2004; Magurran, 2005; Breden, 2006; Pilastro et al., 2008; Brooks & Postma, 2011; Grether & Kolluru, 2011). Here we test whether a male audience influences the focal male’s (M1) mate preference for large females in the guppy. In addition to this ‘standard’ experimental design (Plath et al., 2008a), we also investigated whether the audience male’s (M2) behaviour is influenced when allowed to exercise mate choice directly after viewing the focal male. This is different from most studies of effects of the social environment that typically take into account effects either on the focal individual (Plath et al., 2008c; Ziege et al., 2009) or the eavesdropping individual (e.g., mate choice copying: Schlupp & Ryan, 1997; Witte & Ryan, 2002), but not both. In our study we gave M2 an opportunity to exercise mate choice directly after the tests (i.e., directly after M2 had observed M1) and 24 h later, after enough time had passed for M2 to again show ‘baseline’ behaviour that would most probably not be affected by prior exposure to M1. This allowed testing whether both M1 and M2 would respond to the presence of one another and show weaker mating preferences in the presence of (or soon after presentation of) a rival male. M1 may perceive M2 as a rival that could visually eavesdrop on his mate choice because M2 was presented throughout the choice tests, while the tests on the mate choice behaviour of M2 were conducted only after M1 had resided in the test tank. Hence, we could examine whether audience effects would be seen not only during presentation of an audience, but also soon thereafter (or potentially even 24 h later).

In summary, we looked at three different aspects of guppy mate choice in one experiment: (1) effects of an audience male (M2) on the focal male (M1), (2) effects of M1 on M2 and (3) the change of M2’s preference over time. We hypothesized that there will be a reduction in strength of M1’s preference for larger bodied females when being watched by M2, and that also M2 will show weaker expression of this preference after viewing M1 interact with the
females. In addition, we predict that this initial preference reduction in M2 is only temporary; M2 should show an increase in strength of preference for the larger females (representing their baseline preference) 24 h after viewing M1.

**Methods**

*Test fish and housing conditions*

Test specimens were descendants of individuals collected in 2005 from a feral guppy population inhabiting the San Antonio River in Texas, USA (29.42449°N, 98.49462°W; Hubbs et al., 1978; Hubbs, 1982). Guppies were maintained as a randomly outbred mixed-sex stock in several large (1000 l) tanks in a greenhouse of the Aquatic Research Facility of the University of Oklahoma in Norman, OK, USA. Experiments were conducted between March and May 2009. Two weeks before the tests, individuals were transferred to the laboratory, isolated by sex and placed in groups of 25–30 fish in 38-l tanks. Test fish were maintained at a temperature of 27°C, under a 12/12 h light/dark cycle, and fed daily ad libitum with commercial flake food. All fish were non-virgins, however, all females were in similar reproductive states (i.e., pregnant) during this experiment. Focal and audience males were housed separately in two tanks without any visual contact, and randomly assigned to treatments. All test fish were used only once in a mate choice test.

*Experimental design*

We used a full-contact design to examine male mating preferences, while using female body size as a choice criterion (Abrahams, 1993; Herdman et al., 2004; Ojanguren & Magurran, 2004). The general testing procedure is outlined in Figure 1. Individual focal males (M1) could choose between two females without another male present. The focal males were then retested while an audience male (M2) was presented in half of the trials (while the other half of trials were repeated without an audience, as a control), so that any changes in the behaviour of M1 between the first and second part of the tests could be attributed to the presence of an audience (Figure 1A). Upon removal of M1, M2 was also given a choice between the same two females, and this test was repeated after 24 h (Figure 1B).
Audience affects male mate choice in guppies

Figure 1. Schematic overview of the experimental procedure and predictions regarding the strength of male preference (SOP). (A) Focal males (M1) could choose between two females (first part of the test), followed by a sequential test either with or without an audience male (M2) (second part of the tests). (B) Directly after testing M1, M2 were also tested for mating preferences.

The test tank (61 × 39 × 30 cm height) was filled to 27 cm with aged tap water of 26–28°C. White opaque Plexiglas covered all sides except the front. Illumination was provided by overhead fluorescent lighting. Prior to a test, we introduced two stimulus females (large, mean ± SE standard length: 19 ± 2 mm, and small: 14 ± 1 mm) into the test tank. Then, we introduced a focal male (M1; size-matched, 15 ± 1 mm) into a transparent Plexiglas cylinder (8 cm diameter) in the centre of the tank and left the fish undisturbed for 5 min. After the habituation period, we gently lifted the cylinder. During a 5-min observation period, male sexual behaviours (numbers of approaches/nipping at the female gonopore, copulation attempts = gonopodial thrusts, and sigmoid courtship displays) were scored.

Directly after the first part, we repeated measurement of male mating preferences, but in one half of the trials (N = 17), an audience male (M2; 15 ± 1 mm) was present, while the other half of the trials (N = 16) were repeated without an audience (control). To initiate this second part of a trial, we transferred M1 back into its habituation cylinder. M2 was placed in another transparent cylinder in the central back of the tank (approx. 5 cm from the other cylinder), or only an empty cylinder was presented (control). M2 was confined to his cylinder throughout the experiment. After another 5 min for ha-
bituation, M1 was released again and measurement of male preferences was carried out as described above.

Afterwards, M1 was removed from the test tank and played no further role in the experiment. Immediately after removal of M1, we released M2 and scored its mate choice behaviour. After this 5-min test phase, all three test fish were transferred and housed into individual aquaria (28 × 17 × 16 cm) with visual isolation for 24 h, after which time preferences of M2 were observed with the same two stimulus females as described before (5 min for acclimation followed by another 5 min of testing). Once a test was completed, the standard lengths of all fish involved were measured to the nearest mm.

Statistical analyses

We tested for a preference for the larger of the two stimulus females (Abrahams, 1993; Herdman et al., 2004; Ojanguren & Magurran, 2004) for each behaviour pattern and within each part of the test and treatment separately using paired $t$-tests (Figures 2, 3).

Our major question regarding the behaviour of M1 was whether the strength of male preferences would decrease during the treatment involving an audience, but remain constant during the control treatment without audience. Therefore, we calculated the fraction of sexual behaviours with the larger stimulus female during the first part and second part of the tests (for each behaviour category separately). Percentages were arcsine (square root)-transformed for the statistical analyses. Data for the two test parts (repeated measures) were analyzed in a repeated measures analysis of variance (rmANOVA) using ‘treatment’ (with or without audience) as a between-subjects factor. If the decline in male choosiness differed between the two treatments (with and without audience), one would predict a significant interaction effect of ‘repeated measurement by audience treatment’.

To test for a potential increase in the strength of preference of M2 during the two test parts, we compared the fraction of sexual behaviours directed toward the large female between the first and second part of the tests using paired $t$-tests.
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Figure 2. Sexual behaviour of the focal male (M1). The mean (±SE) numbers of (a) approaches/nipping at the female’s genital pore, (b) gonopodial thrusts and (c) sigmoid courtship displays toward the larger (light grey) and smaller stimulus female (dark grey). Data are presented for the first part of the tests (i.e., before an audience was presented, left), and for the second part, where half of the males were presented with an audience male (middle), while another half was retested without an audience present (right). Paired $t$-tests.
Figure 3. Sexual behaviour of the audience male (M$_2$) directly after the test involving M$_1$ (left) and 24 h later (right). The mean (±SE) numbers of (a) approaches/nipping at the female’s genital pore, (b) gonopodial thrusts and (c) courtship displays toward the larger (light grey) and smaller stimulus female (dark grey). Paired $t$-tests.
Results

Behaviour of the focal male ($M_1$)

Preference for the larger female

During the first part of the tests, $M_1$ interacted significantly more often with the larger of the two stimulus females in terms of approaches/nipping, gonopodial thrusting and sigmoidal courtship displays (Figure 2, left side). A very similar pattern was found when no audience was presented during the second part of the tests (‘control’; Figure 2, right); only in the case of sigmoidal courtship displays no significant difference was detected ($p = 0.19$). When an audience male was presented during the second part, $M_1$ retained a significant preference for the larger female in terms of approaches/nipping and courtship displays. However, $M_1$ directed comparatively fewer approaches/nipping and gonopodial thrusts toward the larger, but relatively more such behaviours toward the smaller female (Figure 2, middle).

Decrease in male preferences

A significant interaction effect of ‘repeated measurement by audience treatment’ for approaches/nipping confirms that preferences of $M_1$ decreased in strength during the audience treatment, but not in the control treatment (rmANOVA; Table 1). No statistical support for such an effect was found in the case of the less frequent thrusting and courtship (‘rm by audience treatment’; Table 1).

Behaviour of the previous audience male ($M_2$)

Preference for the larger female

Directly after the previous test, the former audience male ($M_2$) was also given an opportunity to choose. No significant preference was detected in approaches/nipping and thrusting, and $M_2$ even tended to court the smaller female more often than the larger female, but this difference was not statistically significant (Figure 3). Twenty-four hours later, $M_2$ showed a strong (highly significant) preference for the large female in approaches/nipping and thrusting, and tended to prefer the larger female in numbers of courtship displays ($p = 0.06$; Figure 3).
Table 1. rmANOVA on the fraction of behaviours focal males directed toward the larger of the two stimulus females (arcsine (square root)-transformed) during the first and second parts of the tests (repeated measurements, rm).

<table>
<thead>
<tr>
<th>(a) No. of approaches/nipping</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p</th>
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<tr>
<td>Within subjects effects</td>
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<td>1</td>
<td>0.04</td>
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<td>1.49</td>
<td>8.29</td>
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<tr>
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<tr>
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<tr>
<td>Error</td>
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<tr>
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<td>1.41</td>
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<tr>
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<th>(c) No. of sigmoid courtship displays</th>
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<tr>
<td>Error</td>
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<td>0.16</td>
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Increase in male preferences

Comparing the strength of preference during the two parts of the test revealed a significant increase in the case of the very frequent approaches/nipping (paired $t$-test: $t_{16} = -2.24, p = 0.039$), but not for the far less frequent thrusting ($t_{16} = -1.06, p = 0.31$) or courtship ($t_{16} = -1.56, p = 0.14$).

Discussion

Several recent studies have highlighted the role of social context for mating decisions (Nordell & Valone, 1998; Johnstone, 2001; Earley & Dugatkin, 2005; Matos & Schlupp, 2005), thus acknowledging that information can be public and may be used by individuals other than the intended receiver (Valone & Templeton, 2002; Danchin et al., 2004; Dabelsteen, 2005; Grosenick...
et al., 2007; Valone, 2007). Here we investigated whether and how the presence of an audience affects male mate choice in the guppy, looking both at the focal male and the audience male. Although there have been few studies investigating audience effects on male mate choice (Plath et al., 2008c), audience effects were so far described primarily in other contexts; e.g., swordtail (*Xiphophorus birchmanni*, Poeciliidae) males court females more intensely in the presence of an audience male, suggesting that male courtship in that species has a dual function of attracting mates and deterring rivals (Fisher & Rosenthal, 2006b), while male sticklebacks (*Gasterosteus aculeatus*) court females less in the portion of a test tank in which an audience male is visible (Dzieweczynski & Rowland, 2004). Notably, *P. reticulata* males showed slightly (albeit not significantly) more courtship displays when the audience male was present (Figure 2C), but it is currently not clear whether or not courtship displays serve as a signal during male–male competition also in guppies.

The question of how an audience affects male mating behaviour, and especially their mate choice has been studied most intensely in Atlantic mollies (*P. mexicana*; Plath et al., 2008a,b,c, 2009; Padur et al., 2009; Ziege et al., 2009). Here we describe a similar effect in guppies (*P. reticulata*): just like *P. mexicana* males, also guppy males (M1) decreased the strength of preference for larger females in the presence of another (audience) male. This was true for the most frequent behaviour category (approaches/nipping), but not for the far less frequent thrusting and courtship. Low statistical power due to very low overall frequencies of occurrence may be responsible for a lack of statistically significant difference in behaviours like thrusting.

How can this effect be explained? Theoretically, males could try to avoid aggressive interactions by moving away from the preferred female (see Plath et al., 2008a,b for discussion), which implies that aggressive behaviour would play an important role in determining the expression of male mating preferences. This hypothesis received little support at least in *P. mexicana*, because a very similar audience effect was found also in a population with strongly reduced aggression, the Cave molly (Plath et al., 2008b). Aggression does occur among male guppies (Baerends et al., 1955), and males in the laboratory may even form dominance hierarchies (Gorlick, 1976; Kodric-Brown, 1992, 1993; Price & Rodd, 2006), but aggressive behaviour is thought to play a minor role for mate acquisition under natural conditions.
(Houde, 1988, 1997; Farr, 1989). For example, guppy males reduce aggressive interactions when a female is around (Price & Rodd, 2006).

A recent study (Plath et al., 2009) argued against another alternative interpretation, namely, the ‘split-attention hypothesis’: if split-attention played a role, then also females should alter their mate choice decisions in the presence of a same-sex audience. Even though Poecilia females (P. mexicana and P. formosa) spent considerable time interacting with the audience female, no comparable decline in the expression of mating preferences was detected. It was, therefore, argued that the adaptive significance of altered mate choice behaviour in the presence of an audience is probably linked to an increased risk of sperm competition (Plath & Schlupp, 2008; Ziege et al., 2009). First, male competitors are likely to show the same intrinsic mating preferences. For example, male mating preferences for large female body size appear to be widespread in poeciliid fishes (guppies: Abrahams, 1993; Herdman et al., 2004; Ojanguren & Magurran, 2004; mosquitofish (Gambusia holbrooki): Bisazza et al., 1989b; sailfin mollies (P. latipinna): Ptacek & Travis, 1997; Gumm & Gabor, 2005). More equal allocation of mating efforts with respect to different female phenotypes may, therefore, be adaptive under sperm competition risk. Secondly, a rival may observe the focal male and copy his choice at a later point in time (Schlupp & Ryan, 1997), so males would benefit from concealing their interest in a particular female. Male mate choice copying in poeciliids is thought to have evolved because sexual attention by a given male can be indicative of female receptivity (Schlupp & Ryan, 1997). It was recently shown that P. mexicana males respond to an audience male by showing weaker mating preferences only when the audience male can eavesdrop on the focal male’s behaviour, but not when the audience is presented only before the tests (Ziege et al., 2009). It seems, therefore, that P. mexicana males indeed attempt to conceal their mating preferences in front of a rival that might copy his mate choice (Ziege et al., 2009).

But what about guppies? Our two experimental approaches (for M1 and M2) differ in one important experimental detail: for M1, the audience male (M2) could eavesdrop on the behaviour of M1, while the first part of the choice tests for M2 were conducted only after M2 had seen M1, but M1 was no longer present during the actual choice tests. Still, the behaviour of M2 clearly differed between the two parts. It seems possible that in guppies male audience effects are just retained (at least for some minutes) also when the
audience is no longer physically present. At first sight, an alternative explanation in this context could be male mate choice copying (Schlupp & Ryan, 1997; Witte & Ryan, 2002). In this scenario M2’s initially reduced preference for the larger female might be a response to M1 preferring a particular female, and M2 may have copied his mate choice decision. This explanation would be congruent with our previous hypothesis on audience effects in *P. mexicana* stating that males (M1) reduce the expression of mating preferences to prevent rivals from copying their mate choice (Plath & Schlupp, 2008). However, M1 did not reverse their preferences but overall still preferred the larger stimulus female (Figure 2), so mate choice copying most likely cannot explain the effect seen in our study. Another hypothesis would be that M2 responded to perceived sperm competition risk: poeciliid males may avoid females they have recently seen interact sexually with another male (Dosen & Montgomerie, 2004; Wong & McCarthy, 2009; Ziege et al., 2009), as this may be an indication of increased sperm competition risk (*sensu* Parker 1970; Constanz 1984; Evans & Pilastro, 2011). Clearly, the interactive effects of male mate choice copying and avoidance of sperm competition risk, as well as the dynamics of multiple individuals interacting requires more theoretical and experimental work in the future and guppies will be an exciting model species to investigate this new set of questions.

**Acknowledgements**

The experiments reported in this paper comply with the current laws of the United States of America on animal experimentation. Experiments were approved by the University of Oklahoma IACUC (R05-001).

**References**


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