

## Audience effect alters mate choice in male *Heterophallus milleri* (Poeciliidae)

Publikumseffekt verändert die Partnerwahl der Männchen von  
*Heterophallus milleri* (Poeciliidae)

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**Zusammenfassung:** Man spricht von Publikumseffekten, wenn die Gegenwart eines beobachtenden Tieres das Verhalten des beobachteten Individuums beeinflusst. Wir stellten uns die Frage, ob Publikumseffekte die Partnerwahl der Männchen beim süd mexikanischen Grijalva-Moskitofisch (*Heterophallus milleri*) beeinflussen. In simultanen Wahlversuchen reduzierten *H. milleri* Männchen ihre sexuelle Aktivität in Gegenwart eines als Publikum präsentierten Männchens, jedoch verstärkte sich die Ausprägung ihrer Paarungspräferenzen. Dieses Ergebnis deutet auf eine Monopolisierung des bevorzugten Weibchens durch das wählende Männchen hin.

An increasing number of studies provides evidence that mate choice decisions can be influenced by the social environment (i.e., non-independent) (reviewed in BONNIE & EARLEY 2007, VALONE 2007). For example, females may learn to evaluate the quality of males while eavesdropping on male-male interactions (DOUTRELANT & MCGREGOR 2000, AQUILONI et al. 2008). Females may also choose their mating partner after they had seen another female sexually interact with a potential mate (mate choice copying: DUGATKIN 1992, DUGATKIN & GODIN 1992, 1993, WITTE & UEDING 2003). Just like females, males of the livebearing fish *Poecilia latipinna* also copy the mate choice of other males (SCHLUPP & RYAN 1997). SCHLUPP & RYAN (1997) argued that in livebearing fishes (family Poeciliidae) sexual attention by other males probably serves as an indicator that the female is in the receptive stage of her approximately monthly sexual cycle, during which time copulations are more likely to fertilize the female's oocytes.

Recent studies provided evidence that even the mere presence of an observing (by-standing) same-sex individual (i.e., an audience male) can have a profound effect on males' mate choice decisions (PLATH et al. 2008a, b, c, 2009). In dichotomous choice tests Atlantic molly (*P. mexicana*) males spent less time near the initially preferred female, and spent more time near the initially non-preferred female when a conspecific audience male was presented during the second part of the trials (PLATH et al. 2008a, b). Male preferences were highly consistent when we presented no audience male instead (control).

It is currently not known if audience effects are common among livebearing fishes or restricted to mollies. In the present study we examined the effect of an audience on male mate choice in Grijalva mosquitofish, *Heterophallus milleri* Radda, 1987, a small poeciliid species endemic to the Río Grijalva/Usumacinta drainage in southern Mexico (MILLER 2005) and so far having received little scientific attention. For

this study, *Heterophallus milleri* ( $N = 55$  individuals) were caught from two different sites: the Río Amatan (near the confluence of Río Amatan and Río Oxolotán) and the Arroyo Tacubaya (both Río Grijalva/ Usumacinta drainage) near Tapijulapa (Estado do Tabasco), in September 2008. After the tests all fish were returned to their site of origin. We intended to compare the behavioral data obtained from *H. milleri* with the results from *P. mexicana*. Therefore, we re-analyzed previously published data (PLATH et al. 2008a) and collected  $N = 32$  additional *P. mexicana* from Arroyo Bonita (an affluent to the Río Oxolotán) to increase the sample size. Males and females were kept isolated for at least 15 h (i.e., overnight) prior to the tests in plastic tanks, filled with aerated river water. Fish were fed once a day with commercially available fish food tablets.

The choice tests followed PLATH et al. (2008a, b, in press). We used a portable test tank (42.6 x 30 x 16.5 cm), which was divided into three equal zones: the central one was the neutral zone; the two outer zones were defined as preference zones. The stimuli were presented in two smaller tanks (19.5 x 30 x 14.5 cm) on either side of the test tank. All tanks were built with UV transparent Plexiglas, filled to 16 cm height with river water, and were aerated between trials. Prior to a test, we chose two different-sized stimulus females and introduced them into one of the two stimulus compartments each. Then, we introduced a focal male into a transparent plastic cylinder (8.5 cm diameter) in the center of the neutral zone and left the fish undisturbed for 5 min. After the habituation period, we gently lifted the cylinder and we initiated measurement of male preferences. In our set-up, we measured male mating preferences as association times, because a previous study has shown that differences in association times in this experimental situation directly translate into different numbers of actual mating attempts at least in *P. mexicana* (PLATH et al. 2006).

We measured the times the male spent in each of the two preference zones, i.e., near both types of females during a 5 min observation period.

To account for potential side-biases, we placed the male into the cylinder again after the first observation period. We then interchanged the two stimulus females, and after another habituation phase of 5 min we repeated measurement of male preferences for another 5 min. We summed the times spent near both kinds of females during the two tests units. These two test units are henceforth called the 1<sup>st</sup> part of a trial.

Directly thereafter, we repeated measurement of male association times, but we presented an audience male (2<sup>nd</sup> part). To initiate the 2<sup>nd</sup> part of a trial, we introduced the focal male into his cylinder again. We then placed an audience male in another transparent cylinder in the central back of the neutral zone, equidistant to the two females. The audience male was confined in his cylinder throughout the experiment. Thus, chemical and physical interactions were ruled out. We carried out habituation, measurement of male association preferences and switching of side-assignments of the stimulus females between the two measurements as described above. Again, we summed association times near either kind of females during the two test units.

The focal males experienced one of the following two treatments: (1) in approximately half of the tests we presented a conspecific audience male during the 2<sup>nd</sup> part of the trials. (2) We used a control treatment to determine if any effect detected was due to motivational changes over the time of an experiment. For this control we used only an empty cylinder and no audience male during the 2<sup>nd</sup> part. In total we conducted  $N = 41$  tests with *P. mexicana* [15 of these without audience ('control')] and  $N = 34$  with *H. milleri* (18 without audience). All fish involved in a trial were measured for standard length to the nearest millimeter after the test was completed (*P. mexicana*, focal males:  $35.4 \pm 1.1$  mm, audience males:  $35.1 \pm 1.5$ , large stimulus females:  $42.1 \pm 1.2$  mm, small females:  $30.0 \pm 0.8$  mm, *H. milleri*, focal males:  $20.1 \pm 0.7$  mm, audience males:  $19.9 \pm 1.1$  mm, large females:  $20.6 \pm 0.5$  mm, small females:  $13.9 \pm 0.4$  mm).

To test for a male preference for large female body size, association times near the two types of females were compared using paired *t*-tests. Our main question was whether the focal males altered their individual association preferences when we presented an audience male. The statistical analyses were therefore based on a comparison of individual association times between the 1<sup>st</sup> and 2<sup>nd</sup> part of a trial (before and after presentation of an audience male). Because we predicted that the focal males would spend more time in the neutral zone and thus, less time in the preference zones when we presented an audience (2<sup>nd</sup> part), we did not use absolute association times for the statistical analysis. Instead we calculated relative association times as [time spent near female A/(time spent near female A + time spent near female B)], where A is the female the male had preferred during the 1<sup>st</sup> part of a test (henceforth called the “initially preferred female”). In our first analysis, we compared the relative times males spent near the initially preferred female during the 1<sup>st</sup> and 2<sup>nd</sup> part of a trial within each test series using paired *t*-tests. Moreover, we compared the relative time near the initially preferred female using a repeated measures General Linear Model (GLM), in which we included ‘species’ and ‘treatment’ (with or without audience during the 2<sup>nd</sup> part) as

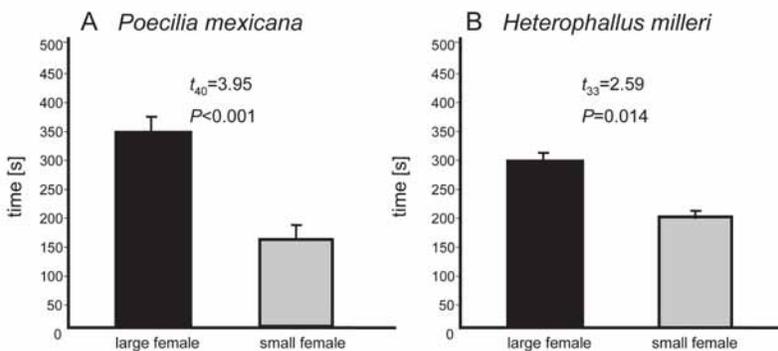
between-subjects factors. We arcsine-transformed all relative data prior to statistical analysis and tested for normality using Kolmogorov-Smirnov-tests. Data are generally given as mean ± standard error.

Males of both species showed a clear preference for the larger of the two females during the 1<sup>st</sup> part of the tests (i.e., in the absence of an audience male, figs. 1 A, B).

In both species, males spent less time interacting with the two females when an audience male was presented (*P. mexicana*, without audience: 527 ± 14 s, with audience: 383 ± 22 s, *H. milleri*, without audience: 507 ± 14 s, with audience: 412 ± 12 s).

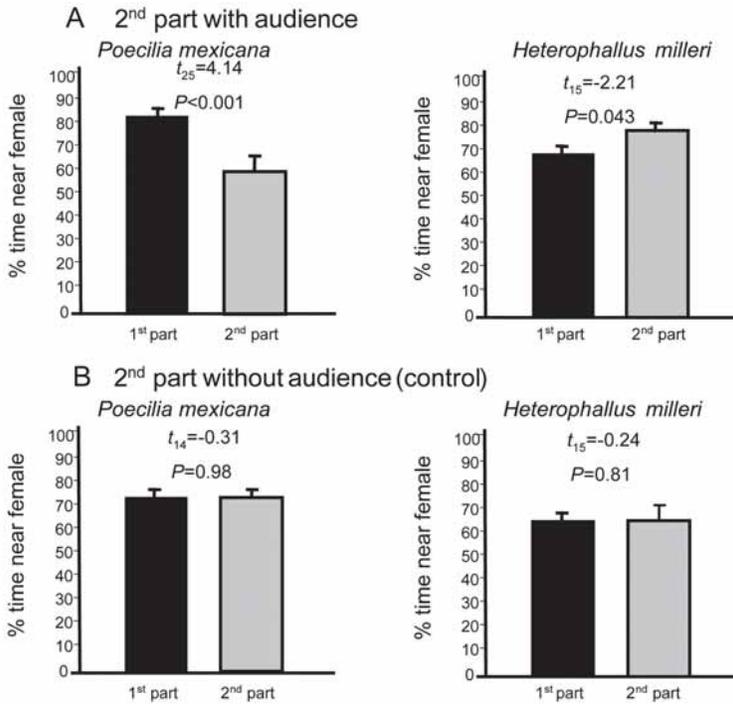
Initial male preferences remained stable when no audience male was presented during the 2<sup>nd</sup> part of the tests (‘control’, fig. 2 B). When an audience male was presented, males of the two species altered their preferences, but in completely different ways: while *P. mexicana* males showed weaker expression of mating preferences, *H. milleri* males even increased their preference for the initially preferred female (fig. 2 A). This difference between species in their response to an audience male was reflected by a significant interaction effect of ‘repeated measurement x species x treatment’ (tab. 1).

Two major hypotheses might explain the observed audience effect in *P. mexicana* (PLATH



**Figs. 1 A and B:** Male *Poecilia mexicana* (A) as well as *Heterophallus milleri* (B) spent more time associating with the larger (black) than with the smaller of the two stimulus females (gray) during the first part of the choice tests (paired *t*-tests).

**Abb. 1 A und B:** Männchen von *Poecilia mexicana* (A) und *Heterophallus milleri* (B) verbrachten im ersten Teil der Wahlversuche mehr Zeit mit dem größeren (schwarz) als mit dem kleineren (grau) der beiden Stimulusweibchen (gepaarte *t*-Tests).



**Figs. 2 A and B:** The relative time *Poecilia mexicana* (left) and *Heterophallus milleri* males (right) spent with the initially preferred female during the first (black) and second part of the tests (gray), i.e., before and after presentation of an audience (A). No audience was presented during the second part in the control treatment (B). Paired *t*-tests.

**Abb. 2 A und B:** Die relative Zeit, die Männchen von *Poecilia mexicana* (links) und *Heterophallus milleri* (rechts) mit dem zuerst bevorzugten Weibchen während des ersten (schwarz) bzw. zweiten Versuchsteils (grau) verbrachten (A). Im Kontrollversuch wurde kein Publikumsmännchen während des zweiten Versuchsteils präsentiert (B). Gepaarte *t*-Tests.

**Tab. 1:** Results from a General Linear Model (GLM) using the relative time spent with the initially preferred female during the 1<sup>st</sup> and 2<sup>nd</sup> part of the tests as dependent variables (repeated measures, R. m.), and 'species' (*Poecilia mexicana* or *Heterophallus milleri*) and 'treatment' (with or without audience male during the 2<sup>nd</sup> part) as factors.

**Tab. 1:** Ergebnisse eines Generellen Linearen Modells (GLM), in dem die relative Aufenthaltszeit in der Nähe des zuerst bevorzugten Weibchens während des ersten und zweiten Versuchsteils als abhängige Variable (Messwiederholungen, 'R. m.') und die Artzugehörigkeit ('species', *Poecilia mexicana* oder *Heterophallus milleri*) sowie die Versuchssituation ('treatment') als Faktoren behandelt wurden.

	<i>d. f.</i>	Mean square	<i>F</i>	<i>P</i>
<b>Within-subjects effects</b>				
Repeated measurement (R. m.)	1	0.03	0.53	0.47
R. m. x species	1	0.52	10.86	<b>0.0016**</b>
R. m. x treatment	1	0.08	1.72	0.19
R. m. x species x treatment	1	0.37	7.66	<b>0.0072**</b>
Error	69	0.05		
<b>Between-subjects effects</b>				
Species	1	0.05	0.85	0.36
Treatment	1	0.11	1.91	0.17
Species x treatment	1	0.06	0.97	0.33
Error	69	0.06		

& SCHLUPP 2008). The “split-attention” hypothesis assumes that poeciliids may not be able to dedicate simultaneous attention to mate choice and a same-sex observer. However, a previous study using *P. mexicana* females showed that female mating preferences are not affected by a same-sex audience, so this hypothesis seems rather unlikely (PLATH et al. in press). Furthermore, avoidance of aggressive interactions could be ruled out as an explanation, since also *P. mexicana* males from a population with reduced aggression (“cave mollies” from a subterranean habitat in southern Mexico) showed this effect (PLATH et al. 2008b). Because audience effects seem to apply specifically to males but not females, it was argued that avoidance of sperm competition (see EVANS et al. 2007) plays a major role (PLATH & SCHLUPP 2008). If males cease expressing mate choice, they do not risk that the audience male will later copy his mate choice (*sensu* SCHLUPP & RYAN 1997). Reduced male sexual activity in both species examined here is indeed compatible with this explanation—males may try to avoid giving away valuable information about their preferred mate.

*Poecilia mexicana* males not only reduced their sexual activity but also concealed their mating preferences by interacting more with the previously non-preferred female. In another study (using a full-contact design), it was shown that *P. mexicana* males even mislead rivals by initially interacting almost exclusively with the previously non-preferred female when an audience was presented (PLATH et al. 2008c). This was interpreted as an adaptation to avoid male mate choice copying (PLATH & SCHLUPP 2008). Unpublished data show that *P. mexicana* males indeed copy other males’ mate choice just like *P. latipinna* males (C. KRONMARCK & M. PLATH, unpublished data).

By contrast, *H. milleri* males appear to use a different tactic. The observed increase of the initial mating preference while an audience was presented points towards a monopolization tactic. Concealment of mating preferences obviously plays no role in this species. To date *H. milleri* rank among the least researched

poeciliid fishes, so little information is available about the mating system of this species. Based on our current study, we propose that male mate choice copying plays a minor or even no role in *H. milleri*. We also propose that monopolization of females is a common tactic in this species. Hence, we propose a future study that compares audience effects and male mate choice copying among various poeciliid fishes: based on our current results, we predict that an audience will have an effect on male mate choice similar to the effect seen in *P. mexicana* only in species with pronounced male mate choice copying.

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