Using video playback to study the effect of an audience on male mating behavior in the Sailfin molly (Poecilia latipinna)

A.M. Makowicz a,*, M. Plath b, I. Schlupp a

a Department of Zoology, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019, USA
b Department of Ecology and Evolution, J.W. Goethe University Frankfurt, Siesmayerstrasse 70-72, D-60054 Frankfurt am Main, Germany

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**A B S T R A C T**

Sexual conflict in poeciliid fishes is well-documented, particularly male sexual harassment and its effects on females. For instance, male attempts to force copulations influence female feeding, energy allocation, and preference for shoaling partners. However, there has been little research conducted to determine how the social environment shapes the occurrence and intensity of sexual harassment. In this study we ask whether an audience male influences the sexual behaviors of a focal male, the correlated feeding time reduction of female Poecilia latipinna, and if the size of the audience male (larger or smaller than the focal male) influences these behaviors. We presented a video of a male, either smaller or larger than the focal male, or an empty tank (control) to a female interacting with a male or female partner and measured feeding times and sexual behaviors. We found that male sexual behaviors increased in the presence of an audience male, especially if the audience male was larger than the focal male. Females fed more in the presence of a partner female than in the presence of a male, which was independent of the audience (i.e., video treatment). Focal female aggression towards the partner female increased with the size of the audience male. The present study shows that an audience male has multiple interacting influences on both male and female behavior.

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1. Introduction

Sexual conflict affects various aspects of female life histories (Arnqvist and Rowe, 2005). For example, male sexual harassment can lead females to alter their time budgets because they need to monitor and flee surrounding males (e.g., Dadda and Bisazza, 2006; Plath et al., 2007) and thus pay opportunity costs in terms of reduced food uptake (e.g., insects: Rowe et al., 1994; Stone, 1995; catshark, Scyliorhinus canicula: Kimber et al., 2009; livebearing fishes, Poecilia spp.: Magurran and Seghers, 1994; Schlupp et al., 2001; Plath et al., 2003; Gambusia spp.: Dadda et al., 2008; Plath et al., 2007; Arrington et al., 2009). Male sexual harassment can also increase female predation risk and energy expenditure, reduce female lifespan, or cause physical or genital damage, and increase parasite or toxin transmission (Chlvers et al., 2005; Eady et al., 2007; Gay et al., 2009; Kimber et al., 2009; Magurran and Nowak, 1991; Rowe et al., 1994). The present study investigates the intensity of male pursuit of females, and female responses to this sexual harassment, in the context of the multiple ways in which female–female interactions are embedded in social (or communication) networks.

Communication between two individuals usually occurs in the presence of an audience, i.e. non-interacting individual(s). This audience can be conspecific, heterospecific, and the same or opposite sex (Leaver et al., 2007) and may influence the behaviors of the interacting pair (Matos and McGregor, 2002; Matos and Schlupp, 2005). With respect to male mate preferences, male Atlantic mollies (Poecilia mexicana) reduce and even switch their preference to deceive their audience when it is another male (Plath et al., 2008b). In fighting fish (Betta splendens), the sex of the audience influences male–male aggressive displays. Focal males tended to bite more, with a lower latency period, when in the presence of a male versus that of a female (Matos and McGregor, 2002). This social environment can play an important role in sexual conflict, often resulting in an increase or decrease of sexual or aggressive behaviors (Dzwecznzka et al., 2005; Matos and McGregor, 2002; Plath et al., 2008b; Padur et al., 2009).

In many livebearing fishes (Poeciliidae), males attempt to mate as often as once a minute (Magurran and Seghers, 1994; Plath et al., 2005), while females are mostly non-responsive to male approaches (Liley, 1983; Houde, 1997; Brewster and Houde, 2003; Deaton, 2008). Most of these mating attempts are coercive, with males continually harassing and trying to forcefully copulate with females (Schlupp et al., 2001; Agrillo et al., 2006; Plath et al., 2003).
This type of sexual harassment results in increased shoaling behavior in female eastern mosquitofish (*Gambusia holbrooki*). In this case, females prefer larger female shoals containing larger individuals when being harassed by a male since shoaling leads to a reduction of the average time each female is being harassed (Dadda et al., 2004; Agrillo et al., 2006) and allowing increased foraging (Pilastro et al., 2003).

Few studies to date have considered the question of how behavioral interactions among males affect male harassment of females and the correlated reduction of female feeding efficiency. Notably, Pilastro et al. (2003) demonstrated that male–male competition leads to a relaxation of male sexual harassment, and female mosquitofish (*G. holbrooki*) had more time available for feeding when males engaged in aggressive interactions (whereby large, dominant males were monopolizing females and were mostly engaged in fending off smaller, inferior males). Is the mere visual presence of a competitor sufficient to keep males from harassing females? In the present study we focused on the well-examined case where harassment reduces feeding time due to male harassment in female poeciliids (Maguran and Seghers, 1994; Plath et al., 2007) using sailfin mollies (*Poecilia latipinna*) as a model organism (see Schlupp et al., 2001). We asked whether a visually presented (“audience”) male influences male mating behavior and thus, female feeding times. The audience male was either larger or smaller than the focal male, allowing us to evaluate whether larger (superior) males keep smaller males from harassing females, or if smaller, physically weaker (and thus, inferior) competitors would distract males from harassing females (e.g., because they evoke more aggressive behavior).

We used video-animations to present the audience males to the females and focal males, which offered some advantages over using living audience fish. The focal and audience males could not interact directly, so any effect observed in the focal fish must be due to the mere visual presence of the audience male (e.g., Fisher and Rosenthal, 2007). Specifically, we gave focal females an opportunity to forage with another female or male, and interpreted any difference in feeding times to male harassment (Maguran and Seghers, 1994; Heubel and Plath, 2008; Plath, 2008; Padur et al., 2009). During the tests, we presented videos of no audience (an empty tank as a control), a smaller, or a larger audience male on a screen near the test tank. We predicted a decrease in male sexual activity for both smaller and larger male videos, with a stronger effect on focal males in the presence of the smaller male.

Regarding female feeding behavior, we predicted that female sailfin mollies forage more in the presence of a female partner than a male partner (Plath et al., 2007; Plath, 2008; Padur et al., 2009). However, female feeding time should increase in the presence of videos with audience males, because the virtual audience male should distract the focal male.

2. Material and methods

2.1. Fish maintenance

Sailfin mollies (*P. latipinna*) occur along the Atlantic coast of southeastern USA and northeastern Mexico. The mollies used in this study were first to third generation descendents of fish collected at Lincoln Park (Brownsville, TX) and maintained as randomly out-bred stocks in large (1000 L) tanks in a greenhouse of the University of Oklahoma Aquatic Research Facility in Norman. We haphazardly collected test fish from several stock tanks and acclimated them to laboratory conditions for at least 14 days prior to the tests. Focal females, partner females, partner males (hereafter referred to as focal males) and the males used to produce the videos were maintained separately in small groups in 30 cm × 30 cm × 25 cm aquaria (length × width × height) and fed commercially available flake food. Visual contact between the different tanks was not allowed. We isolated focal females individually in small tanks (28 cm × 17 cm × 16 cm) and did not feed them for 24 h before testing to ensure motivation to forage. Focal males and partner females were fed 30 min before testing, so the partner fish would not compete for food with the focal female during the tests.

During the behavioral tests, we allowed individual focal females to forage in the presence of a female and a male partner successively, and the difference in feeding times was interpreted as an effect caused by sexual harassment (Plath et al., 2003). To examine how an audience influences male harassment and the associated feeding time reductions, we conducted the tests while an audience male was presented using video playback.

2.2. Video recording

Videos showing a male sailfin molly were recorded on Panasonic DVC tapes using a Canon digital video camcorder (XL1 3CCD) at 30 frames/second (NTSC). We placed each male (*n* = 18 replicates) in a tank (61 cm × 39 cm × 30 cm) with a neutral gray background, and further restrained the fish by a three-sided clear chamber (30 cm × 7 cm × 25 cm) so that the male could be filmed in the front portion of the tank (this also helped to minimize the variation in depth, thus keeping the male at an average size throughout the video). We placed the camera 3 m away from the recording tank with two floor lamps (60 W each) placed 2 m away from the recording tank to provide additional lighting. Each male was given 30 min for acclimation and was then recorded for 1 h. In addition, we prepared videos without a playback male, showing the blank recording tank.

We edited the videos with Adobe Premiere 10 for Windows. A 3 min video clip was compiled from each male, showing the male swimming calmly from side to side with the least amount of depth variation, for a total of 32 male videos. The maximum resolution of the monitor screen, and therefore the resolution of the videos, was 1024 × 768 pixels. We determined the virtual standard length [mm] of each playback male as the maximum size on the screen; we then used these new lengths to assign the videos. For each focal male, we assigned one video showing a playback male that was larger than the focal male and one video showing a smaller playback male; size difference was 5 ± 1 mm in both cases. We made an attempt to use a maximum number of different video sequences and a maximum of different combinations.

2.3. Experimental set-up

The test tank (61 cm × 39 cm × 30 cm) was furnished with white Plexiglas on the bottom and maintained at a constant water temperature of 26.0 ± 1.0 °C. During all tests, we placed a TetraMin tropical food tablet in the center of the test tank as a food source for the focal female, and potentially also for the partner individuals. However, 30 min before the trials began, we fed the partner individuals *ad libitum* to reduce their interest in the food tablet; see above.

During the feeding tests, we placed a Sony LCD monitor (30 cm × 23 cm) 2.5 cm away from the larger side of the tank (centered) to show the playback male. Each test consisted of three treatments the order of which was randomized: (1) the playback male was larger than the focal male (2) the playback male was smaller, and (3) a video showing no male but only the empty recording tank was presented. Each focal female (*n* = 28) was given 24 h after between tests, during which time they were fed *ad libitum*, followed by another 24 h of starvation, re-tested in another video treatment and so forth.
2.4. Behavioral measurements

During each of the three treatments, each focal female was tested with a male and a partner female successively, with the order of presentation (male or female partner first) being balanced and alternated for the three test treatments. During the acclimation phase prior to a test, we placed the focal female and the partner female or focal male in two separate transparent Plexiglas boxes (30 cm × 8 cm × 8 cm, open at the top and base) in the rear center of the tank. We started the video presentation and gave the fish 5 min for acclimation. Afterwards, we lifted the two cylinders, and once both fish started to swim calmly, the 5-min observation period began. We recorded the time the focal female spent feeding, i.e., feeding on the food tablet, off the sides of the tank, on floating matter in the water column, or on particles in the gravel. Whenever a male was present, we also scored occurrences (frequencies) of pre-mating behaviors (nipping at the female genital pore), numbers of copulation attempts (gonopodial thrusting; Woodhead and Armstrong, 1985; Ptacek and Travis, 1997), and courtship duration (time spent displaying in front of the female).

We also considered female–female competition (see Heubel and Plath, 2008 for a discussion). When a female partner was present, we scored the duration of aggressive interactions between the two females (where the focal female was chasing or biting the partner female), which served as a measure of the focal female’s readiness to defend to food source. Generally, we expected starved females to become more aggressive towards others as the relative resource value of the food source increases.

After the first test unit, we transferred the focal female into the acclimation box, removed the first partner fish, and introduced a partner fish of the opposite sex into the other acclimation cylinder. After another 5 min for acclimation, we released the two fish and repeated measurement. When we completed a test series (seven days after the initial starvation day), we measured all fish involved for standard length (SL) to the closest millimeter, after which we transferred them into a stock tank.

2.5. Statistical analysis

We analyzed male sexual activity (sum of nipping and thrusting behavior), courtship duration, and the duration of aggressive interactions between females using repeated measures general linear models (rmGLM), including body sizes of focal female and male as covariates, and treating the three types of video treatment as the withinsubjects factor (repeated measurement). To analyze feeding time reductions, we first compared females’ feeding times with and without male: females spent less time feeding in the presence of a focal male than in the presence of a partner female. For a comparison among treatments (rmGLM), we calculated a score expressing the feeding time reduction by male sexual harassment as the percentage time spent feeding with the focal female less the percentage time spent feeding with the male partner. All relative data were arcsine (square root)-transformed for the statistical analyses.

3. Results

3.1. Male sexual behavior

3.1.1. Sexual activity

In the GLM, neither the covariates themselves (male and focal female body size) nor any interaction term had a significant effect ($F_{3,431} = 3.431, p ≥ 0.076$), so the covariates were removed from the model. The repeated measurement (video type) had a significant effect ($F_{3,27} = 4.108, p = 0.001$). A post hoc Pearson correlation using residuals from a GLM on mean courtship duration across video treatments revealed that males courted larger females for a longer time ($r_{77} = 0.446, p = 0.017, n = 28$; Fig. 2). The repeated measurement (video type) and the interaction of repeated measurement by focal female body size had no significant effects ($F ≤ 1.679, p ≥ 0.206$).

3.2. Female behavior

3.2.1. Feeding time reduction

Females spent less time feeding in the presence of a focal male compared with the tests involving a partner female (paired t-tests: video without male: $t_{27} = 4.108, p = 0.0001$; video with smaller more sexual behaviors when the large male video was presented (Fig. 1).
cant effect (the model. The repeated measurement (video type) had a signifi-
in the final model (presentation (repeated measurement) did not affect feeding time
so they were removed from the final model. Still, the type of video
during the three video treatments (no male, smaller male or larger male videos).

3.2.2. Female–female aggressive behavior

No statistically significant effects of focal or partner female
body size or the interaction terms involving female body size were
detected (F ≤ 0.517, p ≥ 0.479), so we removed the covariates from the
model. The repeated measurement (video type) had a significant
effect (F1,27 = 9.551, p = 0.005), and aggression increased from
the empty tank treatment over the smaller male treatment to the
treatment showing a larger audience male (Fig. 4).

4. Discussion

4.1. Male sexual behavior

Male P. latipinna increased their mean sexual activity when an
audience was present. This was more pronounced with a larger
audience male than with a smaller one. This result is not compat-
ible with the prediction that males should exhibit less sexual
behavior when distracted by another (audience) male. We propose
that sperm competition may play a central role here, namely (see
Evans et al., 2003; Dosen and Montgomery, 2004; Aspbury, 2007;
Wong and McCarthy, 2009; Ziege et al., 2009). The social organization
in poeciliid fishes can be highly dynamic, and males enter or
leave shoals more often than females (Gambusia holbrooki, Zulian et al.,
1995; Poecilia reticulata, Griffiths and Magurran, 1998; Russell et al.,
2004). This creates varying levels of mate competition risk
and intensity. Several recent studies demonstrated that male fish
respond to the visual presence of a rival and adjust their mating
behavior and sperm expenditure strategically in the context of per-
ceived sperm competition risk (Evans et al., 2003; Zbinden et al.,
2004; Plath and Schlupp, 2008; Wong and McCarthy, 2009; Ziege
et al., 2009). In G. holbrooki, for example, Evans et al. (2003) reported
on males exhibiting increased rates of thrusting and higher sperm
expenditure when at a higher risk of sperm competition.

Changes in the focal male’s mating behavior could be explained
by the proximity of the audience male to the female. Padur et al.
(2009) found that in P. mexicana, males increased their sexual activ-
ity when an audience was presented outside of the main test arena
(increased sperm competition risk), but not when presented inside
the test tank (when the audience was within their immediate
communication range). An audience male inside the tank even leads
to decreased male sexual activity (Plath et al., 2008a,b; Plath and
Schlupp, 2008). It is possible that focal males conceal their interest
in a particular female when an audience is within immediate com-
unication range due to male mate choice copying (Schlupp and
Ryan, 1997; Padur et al., 2009; Ziege et al., 2009). In our current
study the audience male was presented outside of the main test
arena, which concurs with the findings of Padur et al. (2009), as
the focal male increased his overall sexual behaviors in the presence of
an audience.

How can the effect of audience male body size on male sexual
activity be explained? Poeciliid females typically prefer larger
males to smaller ones (e.g., Pracek and Travis, 1997; MacLaren et al.,
2004; Plath et al., 2008a,b), so the increase in focal male sexual
behavior could be in response to female choice: smaller males
might benefit from adjusting their behaviors in order to compete
with the preferred larger males.

In one species of swordtail (Xiphophorus birchmani), males court
females more often when a rival male is presented on a screen near
the test tank (Fisher and Rosenthal, 2007). This was interpreted as
courtsship behavior serving the dual function of attracting mates
and to deter rivals (Fisher and Rosenthal, 2007). Although male
saillfin mollies also use fin displays in the context of male–male
aggression (Parzefall, 1969), in our study we found no such effect.
Again, the distance between the focal and audience males may
play an important role also in this context: unless the audience male is
in the same physical domain as the focal male, it could be ener-
gytically more efficient if the male focuses on the female rather
that on the other male in the distance. Hence, when focusing on
the female, a male under sperm competition risk increases actual
sexual behaviors leading to more sperm transfer (nipping and espec-
ially copulation attempts) rather than time spent courting.

On the other hand, female standard length had an influence on
the time the focal male spent courting (Fig. 2). Larger females have
larger broods, which could greatly increase the male’s overall fit-
ness. In addition, virgin female Trinidadian guppies (P. reticulata)
copy the mate choice of other mature (i.e., large) females (Amfacher
and Dugatkin, 2005); consequently, males would benefit even more
from courting large females. It is possible that males tend to vary
their sexual behaviors with the increased size of female body size
(as suggested in Fig. 2), however, this has yet to be investigated.

4.2. Female behavior

Females spent less time feeding when interacting with a male
compared with a partner female. This effect was not dependent on
the audience treatment. In addition, there was no additive effect of females suffering even more when an audience male, either smaller or larger, was present (in contrast to guppies, Brewer and Houde, 2003). This raises the question of why females invariably reduced foraging in the presence of males, even though male sexual activity increased in audience treatments. It is possible that the presence of a third (audience) fish leads to increased perceived “safety-in-numbers”, a possible counter-effect to the negative effect of male harassment (Dadda et al., 2004; Agrillo et al., 2006). Shoaling provides protection from predators (Godin, 1986; Magurran, 1990; Magurran et al., 1995) and females probably fed more calmly when a third fish (especially a large one) was present because of increased perceived safety (e.g., Caraco, 1979; Ward and Krause, 2001). “Safety-in-numbers” or the “dilution effect” is also shown in other species, including social pinnipeds, where the females reduce the amount of sexual harassment each individual receives with increased numbers (Cappozzo et al., 2008).

Focal females increased their aggression towards the partner female when there was an audience male present, even more so with a larger audience male. At first sight, one might be tempted to argue that females display this aggression to attain the best males possible, namely, the larger audience male. Males that are able to survive and grow to a larger body size before becoming sexually reproductive may indicate good genes, and females that mate first with an attractive male may receive larger amounts of sperm than females that mate with him later. However, it seems rather contradictory that females are being harassed by males and flee from the magnitude of copulation attempts (Plath et al., 2007; this study) but would still actively compete for access to males. Hence, it seems more likely that the focal females became more aggressive because of hunger. Aggressive chasing may keep the other female away from the food source. For instance, in house crickets, both males and females increase aggressiveness, and therefore success in attaining the food source, when hunger levels are higher than that of the competitor (Nosil, 2002). One hypothesis is that when an audience male is added to the interacting females, increased perceived safety-in-numbers (see above) may lead to generally increased activity levels, which also brings about that females may increase aggressive behavior. Boldness of such behaviors has been shown to increase among individuals when in a group setting when compared to an individual setting (Webster et al., 2007). It is possible that there is a social setting that influences female–female aggression, which when two females are together with no audience, is not implicated. The context of such a social setting requires further research, if such does in fact occur.

On the other hand, it is possible that females increase aggression towards other individuals when the number of competitors increases. Aquatic environments fluctuate in availability of food resources through time and space, and thus, can also vary in the predictability of said resources (Dennemsoer and Thiel, 2007). Therefore, when increasing the number of conspecifics in the presence of a starved female, competitiveness for the food source results in aggression and monopolization of that resource (interference competition; Amasarekare, 2002). Under this hypothesis, one would assume that the female would be non-discriminate in aggression towards other conspecifics when she, herself, is starved. Nevertheless, this is less likely to be the case: females in this study did increase aggressiveness when an audience male, either smaller or larger, was around, but only with a partner female. In the presence of two males, a partner and an audience, females showed no aggressive behavior at all. Therefore, it is more likely that an increase in numbers of aggressive behaviors specifically increases the boldness of females towards other females. Further testing is required to fully understand the aggressive interactions between the focal and partner female, and whether this is indeed a result of hunger.

Our results and the complexity of (post hoc) interpretations support the general notion that studying behavior in the context of social interaction networks introduces a completely new level of complexity in results and interpretation (Matos and Schlupp, 2005). This study addresses how sexual harassment adjusts with the presence of a male audience. The fact that some of the trends found in this study were not predicted (e.g., increased female aggression) clearly highlights that far more research is needed to understand behavioral adjustment of male and female mating behavior in social networks, and poeciliid fishes are a promising model system for this.

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