Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*

ANDREA PILASTRO*, STEFANO BENETTON* & ANGELO BISAZZA†

*Department of Biology and †Department of General Psychology, University of Padova

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Male mosquitofish allocate a large proportion of their time budget to attempting to inseminate unreceptive females. Because females invest considerable time and energy in avoiding unwanted copulations, sexual harassment is expected to conflict with other activities such as foraging. We found that sexual harassment more than halved the foraging efficiency of the female in a task requiring the retrieval of food items scattered on the water surface. The presence of shoalmates relieved the female from harassment and foraging efficiency increased with the number of females in the group. Small males attempted to mate significantly more than large males, causing a greater reduction in the female's foraging efficiency. When several males competed for the same female, the larger, dominant male prevented all the other males from attempting to mate and so had fewer opportunities to attempt copulation. Harassment from solitary males appeared to be more costly, as females foraged more efficiently when chased by a group of males. Because male sexual activity has a large impact on a female's feeding efficiency and possibly on her survival and reproduction, sexual conflict is expected to be important in shaping association patterns of female mosquitofish with conspecifics of both sexes.

Characteristics that are beneficial to the reproduction of one sex may sometimes be detrimental to the other, giving rise to sexual conflict (Parker 1979). A commonly observed conflict relates to the optimal number of matings for each sex (Parker 1979; Arnqvist 1992; Smuts & Smuts 1993). In most species the optimal number of matings for the female is well below that for the male and females typically refuse to mate with most soliciting males. Sexual harassment and coercive mating can sometimes evolve as male strategies to overcome female reluctance to mate, forcing the female to accept matings that are potentially detrimental to their fitness, or engage in costly strategies to avoid them (Clutton-Brock & Parker 1995). The evolutionary dynamics of these conflicts are often complex because male sexual activity negatively affects a number of other aspects related to female fitness, including fecundity, energy expenditure, probability of disease transmission, foraging efficiency, vigilance and conspicuousness to predators (Daly 1978; Martens & Rehfell 1989; Magnhagen 1991; Magurran & Seghers 1994a; Watson et al. 1998; Blanckenhorn et al. 2002; McLain & Pratt 2002).

Correspondence: A. Pilastro, Department of Biology, University of Padova, via U. Bassi 38/B, I-35131 Padova, Italy (email: andrea.pilastro@unipd.it). A. Bisazza is at the Department of General Psychology, University of Padova, via Venezia 8, I-35131 Padova, Italy.

Among the Poeciliidae, a group of livebearing fish, the sexual activity of males is intense and in some species it reaches one sexual act/male per min throughout the breeding season (Houde 1997). Such intense sexual activity is likely to be costly to the female. For example, in the guppy, *Poecilia reticulata*, male sexual activity attracts predators (Magurran & Seghers 1994a; Houde 1997) which preferentially capture females because they are larger, and therefore more profitable as prey items (Pocklington & Dill 1995). Sexual harassment also reduces guppy females' foraging efficiency (Magurran & Seghers 1994a; Griffiths 1996). Reduced feeding efficiency in the presence of males has also been reported for another poeciliid, the sailfin molly, *Poecilia latipinna* (Schlupp et al. 2001). In both species, males achieve mating primarily by courting females and obtaining their cooperation; forced copulation (‘gonopodial thrusting’) is a secondary mating tactic used more often when females are not responsive to courtship, or by small males which are not attractive to females (Farr et al. 1986; Ptacek & Travis 1996; Houde 1997). Nevertheless in more than half of all poeciliid species, including the eastern mosquitofish, *Gambusia holbrooki*, males do not court females and all copulations are achieved through gonopodial thrusting (Farr 1989; Bisazza 1993). In these species females may suffer even greater costs than in species that rely mainly on female cooperation for successful copulation.
If sexual activity were costly for females, one might expect them to evolve strategies to minimize these costs. For example, females may aggregate in shoals if schooling reduces harassment through a dilution effect, or they may choose to associate preferentially with certain males if males differ in the extent to which they harass females. In the eastern mosquitofish, gravid females approach large, dominant males when harassed by a group of males (McPeek 1992; Bisazza & Marin 1995). This may reduce sexual harassment because guarding by a large dominant male reduces the frequency of mating attempts from subordinate males by nearly 90% (Bisazza & Marin 1995). Furthermore, while guarding a female, (large) dominant males significantly reduce their own sexual activity and therefore the extent to which they harass females.

We investigated whether sexual harassment imposes a cost on female mosquitofish via a reduction in foraging efficiency, and whether patterns of association with conspecifics can alter this cost. Specifically, we predicted that (1) the presence of sexually active males reduces the feeding efficiency of gravid females, (2) the presence of shoalmates dilutes sexual harassment by males thus lowering its costs for individual females, (3) females pay larger costs in the presence of small males because such males try to mate more often and are more efficient at insemination (Pilastro et al. 1997), and (4) females pay lower costs when more males are present, because male–male competition reduces male sexual activity (Bisazza & Marin 1995).

**METHODS**

**Study Species**

Eastern mosquitofish were introduced to Europe nearly a century ago and are now widely distributed throughout the Mediterranean countries. In this internally fertilizing livebearing species, sperm survive for several months in the ovary and the genital tract of the female and mating can occur at any time in the female’s breeding cycle (Constantz 1989). Males lack precopulatory displays and achieve copulations only through gonopodial thrusting, a coercive mating tactic (McPeek 1992; Pilastro et al. 1997). Such thrusts involve a male approaching a female from behind and trying to remain within the blind portion of her visual field. Once under her belly, he rotates the gonopodium forwards and tries to insert it into her genital pore. The female can resist mating attempts by fleeing, changing orientation or lying against an object. In natural populations each male makes about one mating attempt/min (Bisazza & Marin 1995). The rate of success of these attempts is less than 1%, and small males are more successful than large ones because they are less conspicuous and more manoeuvrable when they attempt to insert the gonopodium (Hughes 1985; Bisazza & Marin 1995; Pilastro et al. 1997).

Female eastern mosquitofish never appear to cooperate overtly with males during copulation and try to resist male mating attempts at all stages of their reproductive cycle (Bisazza & Marin 1995). However, when virgin, for 1 or 2 days after parturition or when they have been deprived of males for more than a month, females tend to associate with males. In particular, they prefer large males and groups of males over single males (Bisazza et al. 2001). This behaviour has been interpreted as a way to bias mating success in favour of certain male phenotypes (Bisazza & Pilastro 2000; Bisazza et al. 2001). Because mosquitofish have a resource-free mating system, this behaviour may be linked to indirect, genetic benefits similar to those observed in the closely related guppy (Reznick & Gross 1992).

**Maintenance and Experimental Conditions**

Adult eastern mosquitofish were collected with a hand net from a stream near Padova, Italy (Idrovia Padova-Venezia, Camin) and immediately transferred to the laboratory where they were acclimated for at least a month before the experiments. We collected about 400 adult fish. Groups of 20–25 individuals at a sex ratio of about 1:1 were maintained in 150-litre aquaria provided with gravel and live plants (Ceratophyllum). Mortality during captivity was low (<1%) and limited to the acclimation period. Aquaria were provided with a heater and artificial light (24–26 °C; 13:11 h light:dark regime). Fish received commercial food flakes twice daily, in the morning and in the afternoon, except on weekends when they were fed once per day. The research was authorized by the Istituto Superiore di Sanità and the fish were caught under a fishing permit from the Regione Veneto.

Tests were done in an aquarium (55 × 35 cm and 40 cm high with gravel on the bottom), illuminated by two 15-W fluorescent lights and maintained at 25 °C. Five equally spaced plastic barriers (11 × 40 cm) simulated the plants in the natural environment. Each barrier was composed of a series of elongated bars 1 cm wide and 1 cm apart. The apparatus was placed in a quiet darkened room. A green nylon net was placed against the front glass, which acted as a one-way screen to prevent the fish from seeing the observer.

In each experiment we measured the feeding activity of one focal female. We used gravid females, avoiding those close to parturition because they are often aggressive; we also excluded virgin and recently postpartum females (2–3 days), which tolerate males nearby (Bisazza et al. 2001). The day before the test the focal female was isolated in an aquarium that was a replica of the test apparatus and maintained without food until the start of the experiment (mean deprivation time ± SD=17.2 ± 1.6 h). This food deprivation time was similar to that normally experienced by fish in captivity (see above). Males and females other than the focal female were fed to satiation with food flakes and frozen bloodworm (Chironomus) larvae shortly before the test to ensure that food flakes did not attract them during the test.

We introduced the focal females to the experimental tank 30 min before the test started and the other fish 15 min after the focal female. The experiment began when we released 12 pieces of food flakes on the water surface. These were small squares (1 × 1 mm) cut from flakes of tropical fish food (TetraMin Tropical Flakes). To ensure that the 12 pieces were scattered evenly over the
water surface, we placed them at equal distances on a plastic sheet (2 × 60 cm), which was turned upside down to release the food. The flakes remained on the water surface until eaten or the trial ended. In the few cases in which the male or a nonfocal female took a food item the trial was aborted and excluded from the analyses. The test ended when the focal female ate all 12 pieces of food or 30 min from the food release.

We recorded the time in which the female ate each of the 12 food pieces. From these data we derived two measures of feeding efficiency: latency to eat the first item (the time from food release to the first food item being eaten) and the feeding rate of the focal female (the number of food items consumed/min from the start of the test). In tests with males, we also recorded the number of copulatory attempts that each male made (Bisazza & Marin 1995). After the test, we anaesthetized all fish in a water bath containing a mild dose of MS222 and measured total length (TL) to the nearest 1 mm. They were then revived in conditioned fresh water (Stress Coat, Aquarium Pharmaceuticals Inc. Chalfont, PA, U.S.A.) and returned to stock aquaria. All fish recovered after being measured and were returned to the original point of capture within a few weeks of testing.

**Experiment 1: Foraging Efficiency**

We compared the feeding efficiency of females harassed by a male with two control groups: a female tested alone and a female tested with another satiated female. We tested 45 focal females, 15 in each group. Focal females measured a mean ± SD of 41.4 ± 3.47 mm (N = 45) with no significant difference between the three groups (ANOVA: $F_{2,44} = 0.61, P = 0.55$). Males measured 31.5 ± 1.96 mm (N = 15).

**Experiment 2: Presence of Other Females**

We tested four experimental groups differing in the number of females present. In one condition, we tested a focal female with a male, using the same procedure as in experiment 1 ($\bar{x}$ ± SD TL: focal female: 42.0 ± 4.62 mm, N = 14; male: 26.9 ± 2.77 mm, N = 14). In the other conditions, one (focal female: 41.5 ± 4.58 mm, N = 17; male: 26.5 ± 1.81 mm, N = 17), two (focal female: 40.8 ± 2.56 mm, N = 12; male: 25.7 ± 1.23 mm, N = 12) or three (focal female: 39.9 ± 0.74 mm, N = 10; male: 25.5 ± 1.27 mm, N = 10) satiated females were present during the test. There was no significant difference in the length of males (Kruskal–Wallis test: $H_2 = 2.38, P = 0.51$) or focal females ($H_2 = 2.04, P = 0.57$) between groups.

**Experiment 3: Male Body Size**

We tested a focal female with a single male, using the same procedure as experiment 1, and systematically varied the size of males. The mean total length of fish ± SD was 29.9 ± 3.14 mm (range 25–33 mm, N = 16) and 42.3 ± 0.60 mm (range 41–43 mm, N = 16) for males and females, respectively.

**Experiment 4: Male–Male Competition**

We measured female foraging efficiency in two conditions. We tested a focal female with either a single male ($\bar{x}$ ± SD TL: female: 42.1 ± 0.57 mm, N = 10) or a group of three males (female: 42.2 ± 0.63 mm, N = 10, $F_{3,15} = 0.14, P = 0.71$). In each group there was a large (29.3 ± 0.48 mm), a medium (27.1 ± 0.32 mm) and a small male (25.0 ± 0.00 mm). The mean total length of males in the triplets did not differ from the length of single males (one male: 26.9 ± 0.72 mm, N = 10; three males: 27.1 ± 0.33 mm, N = 30; $t_{38} = 0.33, P = 0.74$).

The procedure of this experiment was slightly modified with respect to experiments 1–3. To increase interaction among males we halved the number of bars in each barrier so that they were 3 cm apart. The day before the experiment, we formed triplets of males and allowed single males or triplets to settle with a female in a replica of the test tank. On the next morning, we introduced the single male or the triplet of males into the test tank and allowed them to settle for 30 min to permit the hierarchy to re-establish after the introduction.

Male mosquitofish are aggressive towards other males. However, provided that the housing environment is enriched with shelters (such as the comb-like plastic barriers used in this study) where small males can hide or escape attacks by larger males, putting males together does not result in physical contact between them.

**Statistical Methods**

We tested data for normality and homogeneity of variance. Nonparametric tests were used when these criteria were not met even after appropriate transformation of the data. All probabilities are two tailed. Where non-parametric tests were used, we give exact probabilities. Statistics were done with SPSS 10.1.4.

**RESULTS**

**Experiment 1: Foraging Efficiency**

Both measures of foraging efficiency indicated that, when alone, the female foraged more efficiently than when harassed by a male, but the presence of another satiated female did not alter her foraging efficiency (Fig. 1). In particular, in a two-way ANOVA with number of fish (1 or 2), and presence or absence of the male as independent factors, only the latter was significantly associated with a decrease in foraging efficiency (latency to eat the first item, log transformation: model: $F_{2,42} = 20.0, P < 0.001$; number of fish: $F_{1,42} = 0.60, P = 0.44$; presence of male: $F_{1,42} = 33.9, P < 0.0001$; feeding rate, log transformation: model: $F_{2,42} = 10.82, P < 0.001$; number of fish: $F_{1,42} = 50.19, P = 0.67$; presence of male: $F_{1,42} = 17.87, P < 0.0001$).

**Experiment 2: Presence of Other Females**

Although the total number of mating attempts increased with the number of females in the tank
the number of mating attempts received by the focal female decreased ($F_{3,49}=9.48$, $P<0.001$; linear term: number of females, log transformation: $F_{1,49}=24.32$, $P<0.001$).

Foraging efficiency also tended to increase with the number of females (latency to eat the first item: $F_{2,49}=3.25$, $P=0.03$; linear term: $F_{1,49}=7.35$, $P=0.009$; feeding rate: Kruskal–Wallis ANOVA: $H_{3}=7.25$, $P=0.06$; Fig. 2). Across treatments, foraging efficiency was inversely related to the number of male mating attempts (Spearman rank correlation: latency to eat the first item: $r_{S}=0.362$, $N=53$, $P=0.008$; feeding rate: $r_{S}=-0.384$, $N=53$, $P=0.004$).

The male did not direct more mating attempts towards the focal female than expected by chance ($X_{5}±SE$; mating attempts towards focal female: $1.41±0.10$; expected: $1.38±0.08$; paired $t$ test: $t_{38}=0.30$, $P=0.77$). The expected number of mating attempts under the null hypothesis was calculated as the total number of male mating attempts divided by the number of females in the tank.

**Experiment 3: Male Body Size**

The frequency of male mating attempts was inversely correlated with male size (Pearson correlation coefficient: $r_{14}=-0.81$, $N=16$, $P<0.001$; Fig. 4a) and was significantly correlated with both measures of foraging efficiency (Spearman rank correlation: latency to eat the first item: $r_{S}=0.54$, $N=16$, $P<0.001$; feeding rate: $r_{S}=-0.75$, $N=16$, $P=0.001$). Male total length was positively correlated with feeding rate: $r_{S}=0.63$, $N=16$, $P<0.01$; Fig. 4b) but not with latency to eat the first item ($r_{S}=-0.40$, $N=16$, $P=0.12$).

**Experiment 4: Male–Male Competition**

The largest male in the triplet performed the majority of the mating attempts ($X±SD=80.6±16.4\%$, range 47.5–100%; Fig. 5). Large males made significantly more attempts than both medium-sized males (Wilcoxon test: $T=0$, $N=10$, $P=0.005$) and small males ($T=0$, $N=10$, $P=0.005$), but medium-sized and small males did
not differ ($T=11, N=10, P=0.69$; overall significance, Friedman test: $\chi^2_{\alpha}=16.27, P<0.001$). The dominant male in the triplets made fewer mating attempts than did single males ($t_{19}=3.20, P=0.005$) but overall we found no difference between the number of mating attempts made by single males and the total attempts by the triplets of males ($t_{19}=0.98, P=0.34$).

The foraging efficiency of females was greatest in the treatment with three males, considering both latency to eat the first item ($t_{19}=3.41, P=0.003$, log-transformed data) and feeding rate ($t_{19}=2.65, P=0.016$; Fig. 6).

**DISCUSSION**

As predicted, sexual harassment markedly reduced the foraging efficiency of female mosquitofish in our laboratory conditions. Females feeding alone or with a satiated shoalmate showed feeding rates about four times greater than females chased by a male. An alternative explanation of our results is that the observed decrease in foraging efficiency was due to the focal female showing sexual interest towards the male. However, this explanation is unlikely because we excluded recently postpartum and virgin females (Bisazza et al. 2001) and we used only gravid mid-cycle females, which always actively avoid sexually active males (McPeek 1992; Bisazza et al. 2001). Although the consequences for female fitness under natural conditions are difficult to estimate, food availability does affect fecundity in poeciliid fish (Hester 1964; Reznick 1983) and it may affect other fitness components, such as immune function and survival (Demas & Nelson 1998; Byrom et al. 2000; Wilson 2001).
The reduction in feeding observed here appears to be much larger than that recorded in the two other poeciliids studied, *P. reticulata* and *P. latipinna* (Magurran & Seghers 1994a; Schlupp et al. 2001). This difference may be related to differences in male mating behaviour between the species. In *P. reticulata* and *P. latipinna*, males alternate between courtship and gonopodal thrusting, but male mosquitofish use only thrusting as a mating strategy. Avoiding copulations from courting males is probably not very costly, because females need only refuse to cooperate with the males. Conversely, avoiding forced copulatory attempts requires females to focus attention on approaching males and continuously adjust their position to avoid them. In support of this idea, Schlupp et al. (2001) showed that small sneaker male sailfin mollies imposed a much greater reduction in the foraging efficiency of females.

Experiment 2 showed that in the presence of a group of females the males slightly increased their total sexual activity. However, male mating attempts were shared equally between the available mates. As a result, harassment of each single female decreased steadily with increasing group size, with a consequent improvement in the focal female’s foraging efficiency. One advantage of being in a group could thus be a simple function of dilution of gonopodial thrusts. Avoiding gonopodial thrusts probably shares some basic mechanisms with vigilance to predators (Magurran & Nowak 1991). Another advantage of foraging in large shoals may therefore be that females can detect and monitor approaching males more easily and thus continue feeding for longer than fish alone or in smaller shoals, as hypothesized for vigilance to predators (Milinski 1993). In our experimental design shoalmates of the focal female were satiated and therefore did not compete for the same food. Under natural conditions competition for food is expected to influence foraging efficiency; therefore this factor needs to be considered in determining the precise feeding payoff of living in groups.

The characteristics of males also seem to affect the costs of sexual harassment. In both the mosquitofish and the sailfin molly, small males impose a greater cost to females (Schlupp et al. 2001; this study). Our results suggest this is because small males make more mating attempts than large males. Accordingly, females should seek to remain close to larger males.

It may also pay females to remain near large males because in many species association with certain males can reduce harassment. Among gerrids for example, females carrying males do not have to struggle to avoid copulations, but unpaired females spend considerable energy avoiding harassing males (Wilcox 1984). Orang-utan, *Pongo pygmaeus*, females associate with adult males to reduce sexual harassment from subadult males (Fox 2002). In experiment 4 in our study, large dominant males prevented the other males from attempting to mate and so had fewer mating opportunities themselves. In this experiment, females foraged more efficiently when chased by a group of three males than when they were with a single male. This result cannot be explained simply by the reduction in the rate of gonopodial thrusting that occurred when males were engaged in competition. In this study the difference in sexual harassment with one and three males was not statistically significant, but it was significant (P<0.05) in a study with a larger sample size (Bisazza & Marin 1995 reanalysed). However, remaining in groups may be advantageous even when the number of mating attempts does not depend on group size. The success rate of gonopodial thrusting rapidly decreases with increasing size of the male (Bisazza & Marin 1995; Pilastro et al. 1997) so there is a much smaller risk of the female being inseminated when, as a result of competition, most mating attempts are made by a large male.

Magurran & Nowak (1991) suggested that female guppies face a trade-off between vigilance for predators and avoidance of forced copulations. Male guppies exploited this circumstance by increasing their rate of gonopodial thrusting in the presence of a predator (reviewed by Houde 1997). If time and attention devoted to searching for food are also traded off against avoiding forced copulations, males should benefit from pursuing hungry females because they should devote less effort to avoiding forced mating. However, our results contradict this prediction. In experiment 2, at all group sizes, males directed the same number of mating attempts towards focal and satiated females.

In female poeciliids same-sex schooling is thought to be related to predator avoidance, whereas association with males is believed to be caused by epigamic selection, in particular for indirect benefits. We have shown that association patterns between males and females can significantly mediate the costs of sexual harassment. In particular, female association with large males can provide direct benefits in terms of reduced sexual harassment and increased foraging efficiency.

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