



Female fitness declines with increasing female density but not male harassment in the western mosquitofish, *Gambusia affinis*

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Sexual conflict occurs when individuals of one sex express traits that reduce the fitness of individuals of the other sex. In many poeciliid fish, males harass females for copulations, which is thought to reduce female fitness by lowering foraging efficiency and increasing predation risk, energetic expenditure and the likelihood of disease transmission. Mating may also be costly for males, who often engage in aggressive interactions with other males in addition to expending energy pursuing females. We examined the effects of three operational sex ratios on male behaviour, female fitness and male body condition in the western mosquitofish, *Gambusia affinis*, during a 10-week experiment. Despite a significant reduction in male harassment, female growth and reproductive success decreased as the proportion of females increased. Results suggest that increases in female density had a strong, negative effect on female fitness, overwhelming any potential costs of male harassment. Aggressive behaviour between males increased and male copulation rate decreased as the proportion of males increased, suggesting that operational sex ratio influences the number of copulations that a male attempts by altering the frequency of agonistic interactions with other males and the number of females available to mate. We did not detect a difference in male body condition between treatments. Sources of female density dependence and consequences of variation in operational sex ratio on male fitness are discussed.

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Sexual conflict over mating arises when the optimal number of copulations for males and females differs (Parker 1979). This discrepancy results because male fitness often increases with the number of copulations that they can obtain, while female fitness peaks at an intermediate optimum (Bateman 1948; Arnqvist & Nilsson 2000). Above this intermediate optimum, the potential benefits that females gain from additional copulations from one or more males (reviewed in Jennions & Petrie 2000; Hosken & Stockley 2003; Zeh & Zeh 2003) decrease, but females continue to incur the costs of mating. These costs include reduced foraging efficiency, increased predation risk, increased likelihood of disease transmission and other energetic or opportunity costs (reviewed in Daly 1978; Stockley 1997). Males may also directly increase the costs of mating to females by transferring ejaculates with toxic

accessory proteins that results in decreased female longevity and reproductive success (reviewed in Chapman 2001) or inflicting physical harm as a form of coercion to gain copulations (Clutton-Brock & Parker 1995). Sexual conflict is now becoming recognized as a key factor in the evolution of physiological, morphological and behavioural traits in a coevolutionary 'arms race' between the sexes (reviewed in Chapman et al. 2003).

One challenge for researchers is identifying sexual conflict in nature and measuring the magnitude of its effects on male and female fitness. The importance of sexual conflict, however, must be considered in the context of other factors that simultaneously affect fitness, such as food availability, predation pressure and intra-sexual interactions that influence reproduction and survival (Magurran 2001). Poeciliids, a family of live-bearing fish, are ideal candidates for these investigations because the potential for sexual conflict has been identified and their ecology has been well studied. Research exploring sexual conflict in these fish has focused on the costs of male harassment to females. Male poeciliids often bypass courtship in favour of a sneaky tactic in which the male

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approaches the female from behind and preemptively thrusts the gonopodium (the male intromittent organ) into the female gonopore (Meffe & Snelson 1989). Males attempt to mate once per minute, resulting in a 30–50% reduction in female foraging efficiency (Magurran & Seghers 1994; Schlupp et al. 2001; Pilastro et al. 2003). This reduction is thought to be costly because females have indeterminate growth and fecundity is closely related to food intake and body size (Reznick 1983). Male harassment may also increase female energetic expenditure (Watson et al. 1998; Jormalainen et al. 2001), expose females to virulent pathogens (Lockhart et al. 1996) and increase predation risk by making females more conspicuous (Pocklington & Dill 1995).

Males may also incur significant energy and opportunity costs harassing females and competing with other males for access to mates. Emlen & Oring (1977) proposed that the intensity of competition for mates is determined by operational sex ratio, the ratio of breeding males to breeding females. Operational sex ratio will directly affect the amount of harassment that females receive because of its influence on the payoff of harassment for males and the cost of resisting harassment for females (Clutton-Brock & Parker 1995). Research has shown that harassment and male competition for mates increases when the operational sex ratio is male biased and decreases when the operational sex ratio is female biased (Sih & Krupa 1995; Kvarnemo & Ahnesjö 1996; Jirotkul 1999), but few studies of sexual conflict have attempted to directly measure the fitness consequences of these interactions (but see Wigby & Chapman 2004; Crudgington et al. 2005).

In addition to the costs of intersexual selection, intrasexual interactions between females may also have important fitness consequences. Female-biased operational sex ratios can result in competition over males, nesting sites, food and other resources necessary for reproduction due to a reduction in the number of mates available per female (Kvarnemo et al. 1995; Grant & Foam 2002) or increases in female density per se (Berglund et al. 1993). Increases in female density, for example, can have a negative effect on female fitness when females compete for food separately from males (Clutton-Brock 1989) or defend territories from other females (Wauters et al. 2004).

We examined the effects of intersexual and intrasexual interactions on male and female fitness in the western mosquitofish, *Gambusia affinis*, a small poeciliid that inhabits streams and ponds in the southern United States. We manipulated operational sex ratio and measured the frequency of male harassment towards females, female–female aggression and four components of female fitness. We also measured the effect of operational sex ratio on the frequency of male agonistic interactions and the energetic costs of mating to males.

METHODS

This study was conducted at the University of Kentucky Ecological Research Facility in Lexington, Kentucky, U.S.A., from June to August 2003. Mosquitofish were collected locally, measured (standard length: tip of the snout to the base of the caudal fin), and assigned to

a wading pool (1.4 m in diameter, filled 15 cm high with conditioned tap water) with one of three sex ratio treatments: male biased (12M:4F), even (8M:8F) or female biased (4M:12F). The density of fish in the pools (10 fish/m²) was within their natural range (C. C. Smith, personal observation) and that of other poeciliid fish (Capone & Kushlan 1991; Chapman et al. 1991; Zulian et al. 1995; Jordan et al. 1998). Each treatment was replicated 10 times, and the experiment ran for 10 weeks.

Members of each sex were size-matched within pools (females within 4 mm, males within 2 mm) and across treatments to minimize confounding effects of male preference for larger females and dominance interactions within the sexes (Bisazza & Marin 1995). Tetramin fish flakes (5% of pool biomass) and a protein supplement (9:1 mixture of blended liver and collared greens, 2.5% of pool biomass) were added to each pool daily to provide food for the fish. For the first 5 weeks of the experiment, newly born fry were removed three times a week with a hand net to maintain the original densities of fish in the pools. For the last 5 weeks of the experiment, fry were collected daily to estimate the number of offspring females produced (see *Female Fitness*, below). Because adults are known to cannibalize juveniles, a refuge permeable to fry but not to adults (30 cm in diameter, 3.2-mm mesh) was placed into each pool the day before the fry collections began. Algae were also allowed to grow in the pools to provide additional cover for fry and a more natural environment for adults.

Behavioural Observations

For observations of females, we selected a focal female, allowed a 5-min period for the fish to acclimate to the experimenter, and observed her for 10 min. We recorded the frequency of chases towards males and other females as a measure of female aggression and the number of copulation attempts directed towards the focal female as a measure of male sexual harassment. A copulation attempt was defined as a male orienting within 10 cm of the female's posterior and swimming rapidly towards her.

For observations of males, we selected a focal male, allowed a 5-min acclimation period, and observed him for 5 min. We recorded the number of copulations attempted and aggressive male–male interactions (displays and chases) received or initiated by the focal male. Displays occurred only between males and consisted of one male approaching the other and arching his body into a sigmoid shape, often accompanied by the two individuals circling or swimming parallel to each other.

Behavioural data for females and males were collected between 0900 and 1430 hours during weeks 4 and 5 of the experiment. Each pool was observed twice for both male and female observations during a 2-week period, and the results from the two observations were averaged. Pools were never observed twice on the same day.

Female Fitness

We quantified four measures of female fitness: number of offspring per female, embryo number, growth and

survival. We assessed the number of offspring per female by removing all visible juveniles from the pools daily for the last 5 weeks of the experiment. Fry present in the pools in the first 5 weeks of the experiment were not included because they were conceived in the wild before the treatments could have had an effect. Females produce clutches continuously throughout the breeding season in approximately 30-day intervals (Krumholz 1948; Koya et al. 1998), allowing all females the opportunity to give birth at least once during the collection interval. The total number of fry collected in each pool was divided by the number of females placed into the pool at the start of the experiment to obtain the average number of offspring per female for each pool. This value is the number of fry birthed per female minus any mortality that occurred before collection.

At the end of the experiment, males and females were measured, weighed, euthanized according to IACUC protocol with tricaine methane sulfonate (MS-222), fixed in 10% formalin and preserved in 90% isopropyl alcohol. Embryo number was determined by dissecting females and counting the number of ova, which mature together as a clutch before parturition. Females without matured ova were not included in the analysis, because it was not possible to determine how many oocytes would be fully yolked in these cases. Female growth was calculated as the difference between the average initial and final standard lengths of female fish in each pool. Female survival was recorded as the proportion of females in a pool that survived to the end of the experiment.

Cost of Mating to Males

The energetic cost of mating to males was estimated using an index of male body condition. Male body condition was calculated by drying males in a convection oven overnight at 60°C and dividing dry mass by standard length cubed. These values were then averaged for each pool. Male survival was recorded as the proportion of males in a pool that survived to the end of the experiment.

Statistical Analysis

Statistical analysis was performed with SAS v8.2 (Cary, North Carolina, U.S.A.) using a generalized linear model (PROC GLM). Assumptions of normality and homoscedasticity of variances were assessed using the Anderson–Darling test and Levene’s test, respectively. Data that did not meet these assumptions were transformed where appropriate (Sokal & Rohlf 1981), and nonparametric tests were used if the data remained non-normal and/or heteroscedastic. Multiple regression was used to determine whether there was a relation between the average number of offspring collected per female and sex ratio treatment, female growth and female mortality (the proportion of females that died in a pool). Nonsignificant terms in the model were removed in a stepwise fashion; interaction terms were removed first, followed by main effects. In a separate analysis, we tested for differences in male and

female survival between treatments using a generalized linear mixed model with a binomial error distribution (SAS GLIMMIX macro), with treatment entered as a fixed effect and the individual pools entered as a random effect. All significance tests were two tailed with an α of 0.05.

RESULTS

Harassment and Female Aggression

The number of copulation attempts received by focal females decreased significantly as the proportion of females in the pools increased (Spearman rank correlation: $r_s = -0.76$, $N = 30$, $P < 0.001$; Fig. 1a). Female aggression was uncommon in all treatments (mean \pm SE chases per minute: male biased: 0.1 ± 0.7 ; even: 0.2 ± 0.8 ; female biased: 0.2 ± 1.5), and there were no significant differences between treatments in female chases towards males (Kruskal–Wallis test: $H_2 = 0.003$, $P = 0.99$) or other females ($H_2 = 0.96$, $P = 0.62$).

Female Fitness

In the multiple regression analysis examining the effect of sex ratio treatment, female growth and female survival on the average number of offspring collected per female

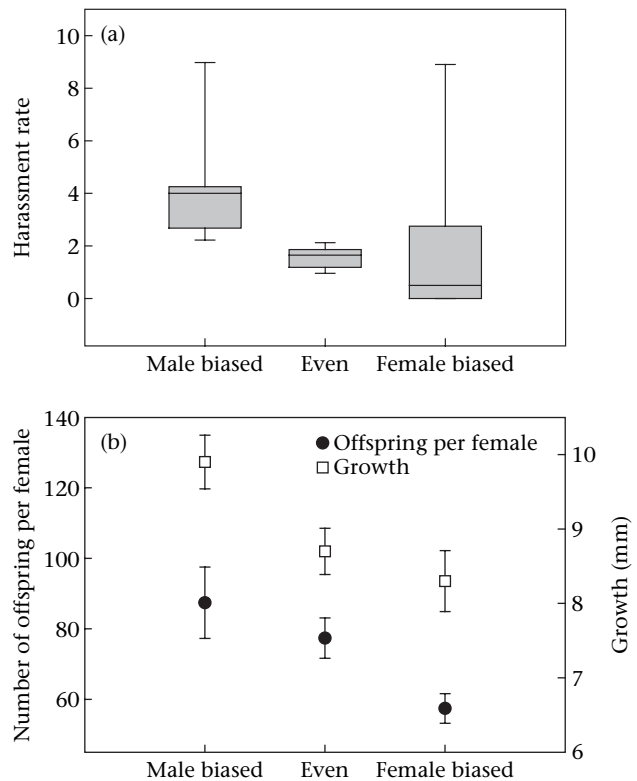


Figure 1. (a) Sexual harassment towards focal females. Shaded boxes represent the interquartile range, bars within shaded boxes are median values and whiskers indicate the 10th and 90th percentiles. (b) Female fitness. Closed circles denote reproductive success (mean \pm SE number of offspring collected per female) and open squares denote female growth (mean \pm SE difference in standard length) for each treatment.

(the dependent variable), all interactions and main effects were nonsignificant except sex ratio treatment and were consecutively removed from the analysis. The average number of offspring collected per female decreased significantly as the proportion of females in the pools increased ($F_{2,27} = 4.56$, $P = 0.02$; Fig. 1b).

Female growth also decreased significantly as the proportion of females in the pools increased ($F_{2,27} = 4.91$, $P = 0.02$; Fig. 1b). Although there was a significant relation, the multiple regression analysis (see above) suggests that differences in female growth between treatments did not contribute a significant portion of the variation in the number of offspring we collected. This analysis, however, does not account for potentially important effects of reduced growth on lifetime reproductive success.

Female survival was not significantly different between sex ratio treatments ($F = 1.33$, $P = 0.28$, degrees of freedom calculated using Satterthwaite method), although the average mortality in female-biased pools was higher than that in the other treatments ($\bar{X} \pm \text{SE}$ percentage of mortality: male biased: $25 \pm 6\%$; even: $21 \pm 6\%$; female biased: $40 \pm 7\%$).

The number of embryos that females produced was not significantly different between sex ratio treatments when controlling for female length (log transformed; overall model: $F_{3,26} = 13.88$, $P < 0.001$; treatment: $F = 0.76$, $P = 0.48$; length: $F = 37.28$, $P < 0.001$) or without controlling for female length (log transformed; $F_{2,27} = 0.93$, $P = 0.48$). There was no significant difference between treatments in the proportion of females without a matured clutch at the end of the experiment (Kruskal–Wallis test: $H_2 = 2.70$, $P = 0.26$).

Male Competition for Mates

The number of copulations attempted by focal males increased as the proportion of females increased (Spearman rank correlation: $r_s = 0.76$, $N = 30$, $P < 0.001$; Fig. 2a). This was accompanied by a decrease in male–male aggression. The number of chases ($F_{2,27} = 20.41$, $P < 0.001$; Fig. 2b) and displays ($F_{2,27} = 5.30$, $P = 0.01$; Fig. 2b) between males decreased as the proportion of females increased. Hence, mating activity and the frequency of aggressive interactions had an inverse relation: copulation rates were highest in female-biased treatments, where male–male aggression was lowest, and lowest in male-biased treatments, where male–male aggression was highest.

Costs of Mating to Males

Despite differences in mating and aggressive behaviour between treatments, there were no significant differences in male condition at the end of the experiment ($F_{2,27} = 1.03$, $P = 0.37$). There were also no significant differences in male survival ($F = 0.76$, $P = 0.48$, degrees of freedom calculated using Satterthwaite method).

DISCUSSION

We found that the average number of offspring collected per female and female growth decreased as the proportion

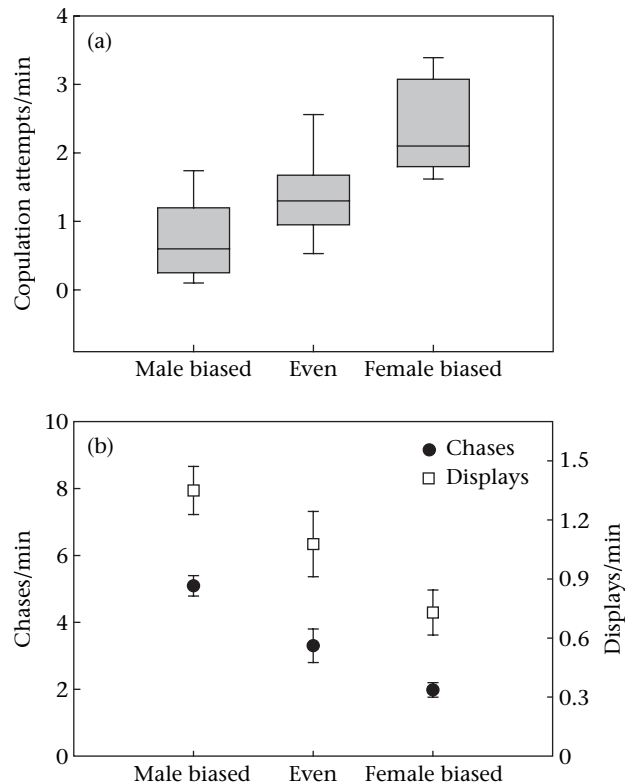


Figure 2. (a) Number of copulation attempts by focal males. Shaded boxes represent the interquartile range, bars within shaded boxes are median values and whiskers indicate the 10th and 90th percentiles. (b) Male–male aggression. Closed circles denote the number of chases (mean \pm SE) and open squares denote the number of displays (mean \pm SE) received and initiated by focal males for each treatment.

of females increased, despite a four-fold reduction in male harassment. Although the total number of fish put into the pools at the start of the experiment was the same, manipulating the sex ratio changed not only the relative numbers of males to females, but also the density of each sex within the pools. Our results suggest that increases in female density had a strong, negative effect on female fitness, and that this effect masked any costs imposed on females by males because of harassment. In a subsequent experiment that independently manipulated male and female density, increasing female density also reduced the number of offspring collected per female (C. C. Smith, unpublished data). This demonstrates that female density rather than sex ratio per se is responsible for reduced female reproductive success.

There are three possible explanations for a reduction in female fitness with increasing female density: chemical or behavioural suppression, fry cannibalism and female competition for food. Suppression of reproduction between females has been documented in birds, mammals and insects (Breihagen & Slagsvold 1988; Faulkes & Abbott 1997; Cuvillier-Hot et al. 2002), and can be mediated chemically (via pheromones or metabolites) or behaviourally (through aggressive interactions between females). The low level of aggressive behaviour between females in our study makes behavioural suppression unlikely in

G. affinis. However, Lutnesky & Adkins (2003) found that female growth and ovary size in *G. affinis* were reduced when females were reared in water inhabited by adult female conspecifics, suggesting that there may be chemically mediated inhibition of growth and reproduction. Females may use environmental cues about density to allocate resources between storage, growth and reproduction (Dahlgren 1979; Reznick & Braun 1987; Weeks & Gaggiotti 1993; Rodd et al. 1997) if this increases lifetime reproductive success (Williams 1966). Alternatively, accumulations of toxic metabolic waste (Beebee & Wong 1992) or other metabolites (Thorp & Barthalmus 1975; Gosler & Ratten 1994; Rodriguez-Munoz et al. 2003) in the pools may have inhibited growth and reproduction. These chemical products may be produced at a faster rate in populations with more females, who are two to three times larger than males.

A second possibility is that competition for food is stronger between females than between the sexes, resulting in an intensification of competition as female density in the pools increased. Males and females of this species eat different prey types (Mansfield & McArdle 1998), probably because of differences in gape size (Taylor et al. 2001) and nutritional requirements. Competition between females may be further intensified because fecundity is dependent upon food intake (Reznick 1983). This explanation is unlikely in the context of our experiment, however, because food size was homogeneous and distributed according to the biomass of the fish in the pools.

A third explanation for our result is that fry cannibalism was higher in pools with higher female density, resulting in the observed differences in reproductive success. Mosquitofish readily cannibalize juveniles regardless of food availability (Dionne 1985; Meffe & Crump 1987), and females may eat conspecifics at a higher rate than males because of their larger size and energetic requirements. In our experiment, refuge for fry was provided and pools were sampled daily to minimize fry cannibalism, but it is possible that treatments with higher female densities may have led to higher rates of cannibalism despite our attempts to prevent it. Cannibalism alone, however, does not account for the reduction in female growth. This suggests that additional factors (e.g. chemical or behavioural interactions) besides cannibalism related to female density appear to influence female fitness.

Effects of Sex Ratio on Male Behaviour and Body Condition

Altering the sex ratio had marked effects on male behaviour. In agreement with Emlen & Oring (1977) and other empirical studies (reviewed in Kvarnemo & Ahnesjö 1996), agonistic chases and displays between males increased as the proportion of males increased, probably because of increased encounter rates between males and the decrease in available females in the pools. This increase in aggression was accompanied by a 32% reduction in the number of copulations that focal males attempted, suggesting that the number of females, the frequency of male–male interactions or both influenced male sexual activity.

Any reduction in the number of copulations a male attempts is not trivial. Studies of other poeciliids have found that only 3% of unsolicited copulation attempts actually result in insemination (Bisazza & Marin 1995) and sperm competition is intense (Constantz 1984; Zane et al. 1999; Evans & Magurran 2001; Beecher & Magurran 2004). Operational sex ratio varies widely in nature (Snelson 1989; Zulian et al. 1995; Pettersson et al. 2004), so male poeciliids will be confronted with trade-offs in the time and energy allocated to attempting copulations, preventing other males from copulating and pursuing other activities that increase survival (e.g. foraging and avoiding predators). These trade-offs will presumably also have energetic costs as males allocate resources to different behaviours.

Despite differences in intrasexual aggression and mating activity, there was no difference in male condition between treatments. One explanation is that these behaviours might not carry energetic costs to males, but this is unlikely because aggression has metabolic costs (Haller 1995; Briffa & Elwood 2001) and is associated with stress-related elevations in hormones such as glucocorticoids and androgens that can have deleterious effects (reviewed in Buchanan 2000). A second possibility is that the food provided was sufficiently abundant and nutritious to compensate for the increased male energetic expenditure in the different treatments. Marler & Moore (1991), for example, found that food supplementation eliminated the costs of increased aggression in the mountain spiny lizard, *Sceloporus jarrovi*. Finally, the levels of intrasexual aggression and mating activity in our experiment may have been equally energetically costly, resulting in equivalent condition indexes across treatments. Because the metabolic costs of these behaviours are unknown, at this time we cannot distinguish between these hypotheses.

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