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ORIGINAL PAPER

Independent effects of male and female density on sexual harassment, female fitness, and male competition for mates in the western mosquitofish *Gambusia affinis*

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Abstract Operational sex ratio (the ratio of sexually active males to fertilizable females) has a major influence on male competition for mates and male–female interactions. The contributions of male and female density per se to mating system dynamics, however, are rarely examined, and the fitness consequences are often inferred rather than quantified. Male mosquitofish (*Gambusia affinis*) compete aggressively and frequently harass females for copulations, a behavior thought to reduce female fitness. Female fitness can also be reduced by increases in female density, which may affect food availability, cannibalism rates, and chemical interactions between females. I manipulated male and female densities of *G. affinis* to measure their effects on male–male aggression, male harassment toward females, and female fitness. I found that males chased rivals more often and attempted fewer copulations when female density decreased, but surprisingly male density had no significant effect on the frequency of these male behaviors. In contrast, males' agonistic displays toward other males increased with male density, but display behavior was unaffected by female density. These results suggest that male and female density do not always contribute equally or at all to the patterns of behavior we observe. Female fitness declined as female density increased, the opposite pattern expected if male harassment is costly to females. This suggests that a strong, negative effect of female density overwhelmed any potential costs of male harassment. Sources of female density dependence and the consequences of changes in male and female density to patterns of male behavior are discussed.

Keywords Operational sex ratio · Sexual selection · Sexual conflict · Poeciliidae

Introduction

Operational sex ratio (OSR: the ratio of sexually active males to fertilizable females) is a critical factor shaping male and female behavior because of its influence on the strength of sexual selection (Emlen and Oring 1977; Clutton-Brock and Parker 1992; but see Kokko and Monaghan 2001). Theory predicts that increases in OSR (more males per female) will result in an intensification of male competition for females and increase the opportunity for females to exercise mate choice (Emlen and Oring 1977). The result is greater variance in male reproductive success because some males will successfully exclude others from obtaining fertilizations by dominating male–male interactions or be chosen more often by discriminating females. Studies examining the relationship between OSR and sexual selection on males across taxa have largely supported these predictions (reviewed in Kvarnemo and Ahnesjo 1996, 2002).

Operational sex ratio can have important consequences for female fitness as well. Although a male-biased OSR may allow females to be more "choosy" in mate choice decisions (Jirotkul 2000; Ros et al. 2003), intense sexual selection can result in the evolution of male tactics that circumvent female

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preferences (Clutton-Brock and Parker 1995). Males of many species harass females for copulations, a behavior that can increase the reproductive success of harassing males while imposing energetic and opportunity costs on the females with which they mate (reviewed in Rowe et al. 1994; Stockley 1997; Arnqvist and Nilsson 2000). Male-biased operational sex ratios often increase the amount of harassment females receive by elevating male competition for fertilizations and female encounter rates with harassing males (Sih and Krupa 1995; Jirotkul 1999; Wigby and Chapman 2004; Smith and Sargent 2006). In contrast, a female-biased OSR typically relaxes the amount of male harassment toward females but can intensify female competition for mates, nesting areas, or other resources necessary for reproduction (Berglund et al. 1993; Kvarnemo et al. 1995; Lutnesky and Kosaki 1995; Grant and Foam 2002; Valero et al. 2005).

Operational sex ratio is a useful predictor of mating system dynamics, but suffers from the same statistical limitations of all ratios: It is composed of two independent variables, male density (the numerator) and female density (the denominator). Most studies have manipulated operational sex ratio by changing the density of both sexes simultaneously or changing the density of one sex while holding the other constant, an approach which obscures how the density of each sex per se contributes to patterns of male and female behavior. Studies examining how the density of each sex influences behavior are rare, and their results diverse. Alonso-Pimentel and Papaj (1996) found that male density had a stronger effect than female density on copulation duration in the walnut fly *Rhagoletis juglandis*, whereas Verrell (1982) found that male courtship rate in the red spotted newt *Notophthalmus viridescens* increased with male density, but was insensitive to changes in female density. Sexual harassment toward female water striders (*Aquarius regimis*) increased with male density and decreased with female density, but there was an interaction between male and female density on male mating success (Sih and Krupa 1995). These results illustrate that the density of each sex can have interesting and sometimes complex effects on mating system dynamics.

Poeciliid (live-bearing) fish provide an ideal opportunity to study relationships between sex ratio and density because both variables vary widely in space and time in nature (Britton and Moser 1982; Capone and Kushlan 1991; Chapman et al. 1991; Haynes and Cashner 1995; Zulian et al. 1995; Jordan et al. 1998; Pettersson et al. 2004). Poeciliids are characterized by a non-resource based mating system in which females are fertilized internally by a male intromittent organ, the gonopodium (Constantz 1989). Males of many species compete vigorously for fertilizations by frequently copulating and chasing other males away from females who often sire broods from multiple males (Trexler et al. 1997; Zane et al. 1999; Becher and Magurran 2004). Male–male competition is likely to have an important influence on male fitness, as larger males can reduce access to females (Morris et al. 1992; Bisazza and Marin 1995), and the last male to mate typically sires the majority of offspring (Evans and Magurran 2001).

These patterns of male behavior have important consequences for females. Males of many species persistently harass females for copulations using a “gonopodial thrusting” tactic in which they swim up to females and rapidly thrust their gonopodium into the female gonopore (Liley 1966; Bisazza 1993). This behavior is thought to circumvent female choice because it occurs in the absence of male courtship and stereotypical female behaviors that facilitate copulation (Liley 1966; Houde 1997). Males of some species attempt to copulate once per minute, resulting in a 30–50% decrease in female foraging efficiency (Magurran and Seghers 1994a; Schlupp et al. 2001; Pilastro et al. 2003). This could result in a significant reduction in female fitness because food intake is closely correlated with growth and fecundity (Reznick 1983). Harassment may also increase predation risk (Pocklington and Dill 1995; Magurran 2001), susceptibility to disease transmission (Lockhart et al. 1996), and female energetic expenditure resisting or evading males (Stockley 1997; Watson et al. 1998; Jormalainen et al. 2001).

Female fitness can also be affected by female interactions dependent upon female density. Adults of many poeciliids cannibalize juveniles (Meffe and Snelson 1989), and because females spend significantly more time foraging than males (Magurran and Seghers 1994b), populations which contain more females are likely to experience higher rates of cannibalism. Competition for food among females may also be stronger than competition between the sexes, as field studies have shown sex differences in diet composition (Mansfield and McArdle 1998; Garcia-Berthou and Moreno-Amich 2000) and habitat use (Specziar 2004). Finally, contact with water containing other females reduces female growth, survival, and egg maturation (Lutnesky and Adkins 2003; Borg et al. 2006), suggesting that chemical interactions between females may influence some components of female fitness.

The aim of this study was to (1) evaluate how changes in the density of males and females influence intersexual and intrasexual interactions and (2) measure the fitness consequences of those interactions to females. In the western mosquitofish *Gambusia affinis*, male–male aggression and sexual harassment increase with operational sex ratio (Smith and Sargent 2006) as predicted by theory (Emlen and Oring 1977; Clutton-Brock and Parker 1995); however, the contribution of male and female density may not be equal (see references above and “Discussion”). If male harassment is harmful to females, I expect female fitness will decrease as harassment increases. A different result is predicted, however, if female intrasexual interactions have a stronger effect on female fitness, as has been previously suggested (Smith and Sargent 2006). In this case, female fitness will decrease with female density independent of the level of male harassment.

Materials and methods

Study system

The western mosquitofish *Gambusia affinis* is a freshwater poeciliid native to the southern United States but subsequently introduced throughout the world for mosquito control. Like many other poeciliids, *G. affinis* inhabit small streams and ponds with spatial and temporal variation in hydrology, predation pressure, and other ecological factors that affect demographics (Pyke 2005). A study in one population reported a 20-fold fluctuation in population size and sex ratios ranging from 1M:14F to 1M:1F (Hughes 1985a,b). The ecologically similar eastern mosquitofish *Gambusia holbrooki* has been more thoroughly studied and has reported densities of 5–44 fish/m^2 and sex ratios ranging from 38M:1F to 1M:4F (Britton and Moser 1982; Zulian et al. 1995; Jordan et al. 1998). Variable demography is a common feature in other poeciliid species as well (Capone and Kushlan 1991; Chapman et al. 1991; Jordan et al. 1998; Pettersson et al. 2004).

Male *G. affinis* lack ornamentation and elaborate courtship displays characteristic of many other poeciliids, relying solely on the gonopodial thrusting tactic (see "Introduction") to obtain copulations. Females birth clutches of live young continuously throughout spring and summer in approximately 30-day intervals (Krumholz 1948; Koya et al. 1998), after which, the offspring swim away without further parental investment. Cannibalism is commonly observed, with females cannibalizing more than males (Hubbs 1991). Water-borne chemicals from females (but not male) conspecifics have also been shown to reduce ovary size, growth, and survival of other females (Lutnesky and Adkins 2003), although it is unknown if female density influences the magnitude of this effect.

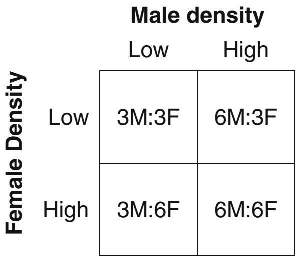
Experimental design

This study was conducted at the University of Kentucky Ecological Research Facility in Lexington, Kentucky from June to August 2004. Wading pools (1.4 m in diameter, filled 18 cm deep with conditioned tap water) were randomly assigned to one of four treatments in a 2×2 factorial design, with male and female density as the two factors. Each factor was comprised of two levels of density, low (three fish) and high (six fish). This resulted in four treatments, each of which was replicated eight times: low male/low female, low male/high female, high male/low female, and high male/high female (Fig. 1). This design permits an examination of the overall effects of male and female density as well as a pairwise comparison between two operational sex ratios at the same population density (6M:3F vs 3M:6F). The densities (4–8 fish/m^2) and sex ratios (1:2, 1:1, 2:1) in these treatments are well within the natural range of poeciliid fish in the field (see "Study system"). One pool in the high male/high female treatment suffered high mortality 2 weeks into the experiment and was removed from the analysis.

G. affinis were collected locally from Spindletop Creek, measured (total length, the tip of the snout to the end of the tail), and allocated to a treatment. Members of each sex were size-matched within pools and across treatments to minimize any confounding effects of male preference for larger females and size-related dominance interactions within the sexes (Bisazza and Marin 1995). Pools were covered with shade cloth to exclude predators and reduce colonization by insects and diurnal fluctuations in water temperature. Nutrafin© Livebearer fish food was added to each pool daily (5% of fish biomass) for the duration of the study (10 weeks). For the first 5 weeks of the experiment, newly born fry were removed from the pools three times a week with a hand-net to maintain the original densities of fish in the pools. These offspring were not included in any of the analysis because they were conceived in the wild before the treatments could have an effect. For the last 5 weeks of the experiment, fry were removed daily to estimate the number of offspring produced by females (see "Female fitness" below). To reduce cannibalism by adults and facilitate fry collection, a refuge for fry (30 cm in diameter, 1/8″ nylon mesh) was placed into each pool the day before the collections began. Algae were also allowed to grow in the pools to provide additional cover for fry and a more natural environment for adults.

Behavioral observations

Behavioral data were collected between 0900 and 1430 hours during weeks 2 and 3 of the experiment. Females and males were observed consecutively, the order of which was



Female Density	Male density: Low	Male density: High
Low	3M:3F	6M:3F
High	3M:6F	6M:6F

Fig. 1 Treatment structure of the experiment. Each *box* represents a treatment (n=8) consisting of low or high densities of males (M) and females (F)

alternated for every set of observations. Each pool was sampled twice over the 2-week period and the results from the two trials averaged for analysis. No pool was sampled more than once on the same day.

To estimate the amount of male harassment females received, the first female sighted in the pool under observation was designated as the focal female for that observation period. The number of copulation attempts directed toward her was recorded for 10 min after a 5-min acclimation period to allow fish to habituate to my presence. A copulation attempt was defined as a male orienting within 10 cm of the female's posterior and swimming rapidly towards her.

A focal male was then selected and observed for 5 min. Five-minute observations were sufficient to estimate treatment effects on male behavior because males interact much more frequently with other fish than females. I recorded the frequency of copulation attempts and aggressive interactions (displays and chases) between males. Displays only occurred between males and consisted of one male approaching another and arching his body into a sigmoid shape, often accompanied by the two individuals circling or swimming parallel to one another.

Female fitness

I quantified four measures of female fitness: number of offspring per female, embryo number, female growth, and female survival. I assessed the number of offspring per female by removing all visible juveniles from the pools daily for the last 5 weeks of the experiment, 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 offspring produced per female per pool minus any mortality that occurred before collection.

At the end of the experiment, females were measured, euthanized according to IACUC protocol with tricaine methane sulfonate (MS-222), fixed in 10% formalin, and preserved in 70% isopropyl alcohol. Embryo number was determined by dissecting females and counting the number of fertilized ova, which develop in a single clutch before parturition. Female growth for each pool was calculated as the difference between the average initial and final total length of females. Female survival for each pool was recorded as the number of females in a pool that survived to the end of the experiment.

Statistical analysis

Statistical analyses were performed using SAS© v8.2 (Cary, NC, USA). Assumptions of normality and homogeneity 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 and Rohlf 1981). Effects of male and female density on the dependent variables were assessed using a two-way analysis of variance (ANOVA), and treatment means were compared using Fisher's least significant difference (LSD) tests. Effect sizes were calculated using omega-squared (ω^2), the sum of squares of the independent variable divided by the total sum of squares (Kirk 1982). Effects of male and female density on female survival were assessed by fitting the data to a generalized mixed model with a logit link function and a binomial error distribution (GLIMMIX macro), with individual pools entered as a random effect. Odds ratios are presented as effect sizes for these data (Sokal and Rohlf 1981). All significance tests were two-tailed.

Residual plots of male display rates revealed an outlier in the low male/high female treatment. No transformations could remedy the strong effect this value had on the distribution of the data. Following Sokal and Rohlf (1981), Dixon's test was performed to confirm it as an outlier ($r=0.73$, $p<0.01$), and the data point was winsorized (replaced with the value of the next largest datum in the sample). This normalized the distribution for the two-way ANOVA.

Results

Male competition for mates

Focal males attempted more copulations as the density of females increased, whereas male density had no effect (Fig. 2a, Table 1). Although male density did not have a significant effect, the average number of copulation attempts was always lower in pools with more males (Fig. 2a). Focal males attempted more copulations in the female-biased compared to the male-biased treatment (Fisher's LSD: 6M:3F vs 3M:6F, $P=0.04$).

Focal males chased other males less in pools with more females, but there was no significant effect of male density on chases (Fig. 2b, Table 1). The number of copulations males attempted and interference between males through chasing, then, were inversely related: when female density increased, the frequency of chases between males decreased, and the frequency of copulation attempts toward females increased (Fig. 2a,b). Focal males chased other males more often in the male-biased compared to the female-biased treatment (Fisher's LSD: 6M:3F vs 3M:6F, $P=0.04$), a difference largely due to changes in female rather than male density (Table 1).

In contrast, focal males engaged in male–male agonistic displays more frequently when male density increased, but

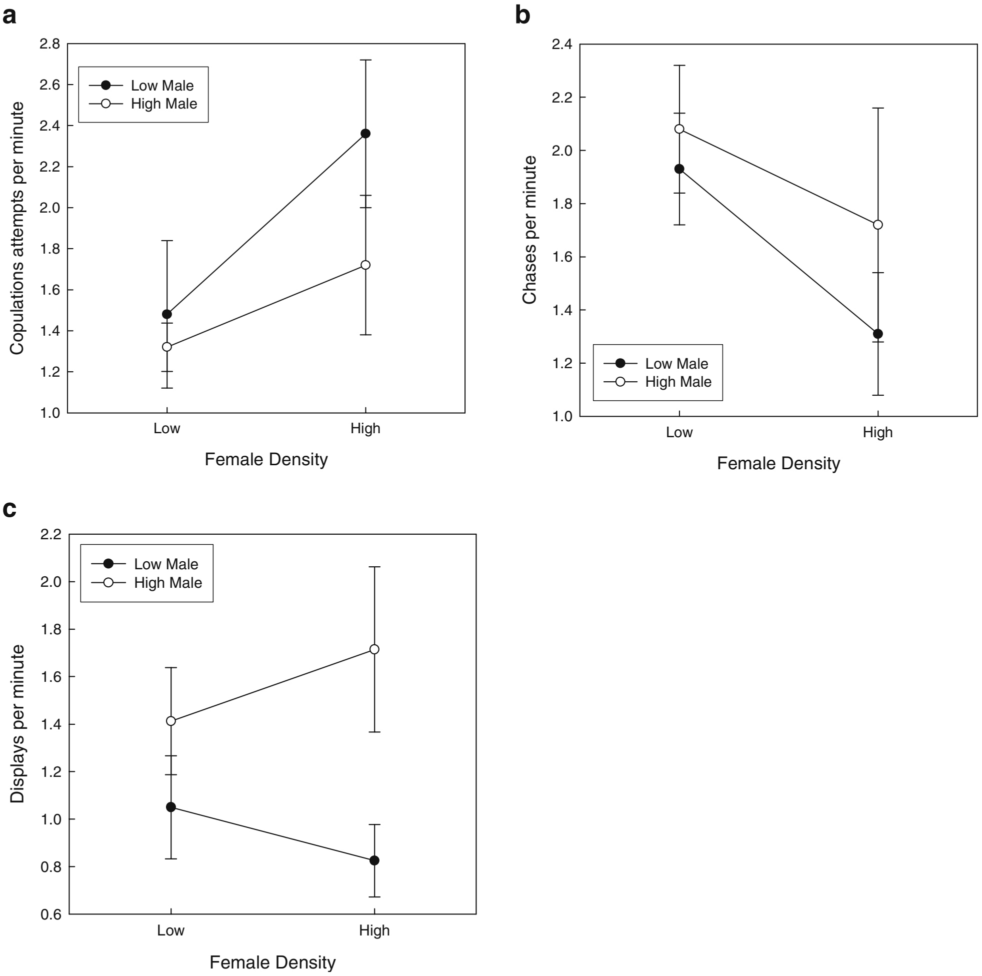


Fig. 2 **a** Copulation attempts, **b** male–male chases, and **c** male–male displays by focal males for each combination of male and female densities. *Circles* are treatment means±standard errors

female density had no effect (Fig. 2c, Table 1). Although focal males displayed 59% more often to other males in the male-biased compared to the female-biased treatment, this difference was not statistically significant (Fisher's LSD: 6M:3F vs 3M:6F, P=0.08, Fig. 2c).

Table 1 Effects of male and female density on the frequency of focal male copulation attempts and aggressive behaviors toward other males

	Copulation attempts		Chases[a]		Displays	
Factor	$F(\omega^2)$	P	$F(\omega^2)$	P	$F(\omega^2)$	P
Male density	1.56(0.00)	0.36	0.48(0.00)	0.49	7.77(0.15)	0.02
Female density	4.10(0.10)	0.05	4.82(0.10)	0.04	0.97(0.00)	0.81
Male × female	0.61(0.00)	0.55	0.19(0.00)	0.67	0.21(0.01)	0.25

Values for dependent variables are F-ratios (effect size) and two-tailed probability values.

df=1,27 for all terms

[a] Log-transformed

Sexual harassment towards females

Focal females were harassed less often as female density increased, but there was no effect of male density on the amount of harassment females received (Fig. 3a, Table 2). Focal females were harassed more in the male biased compared to the female biased treatment (Fisher's LSD: 6M:3F vs 3M:6F, P=0.02).

Female fitness

The number of offspring collected per female decreased sharply as the density of females, but not males, increased (Fig. 3b, Table 2). This reduction in female reproductive success does not appear to be the result of a reduction in fecundity. Female density did not affect the average number of embryos females were carrying at the end of the experiment (Table 2), including analysis where only gravid females were considered (F_{male}=0.31, P=0.58; F_{female}= 0.06, P=0.80; $F_{\mathrm{interaction}}$=0.33, P=0.57). Gravid females were also producing the same number of embryos for their size regardless of male or female density (F_{male}=0.56, P=

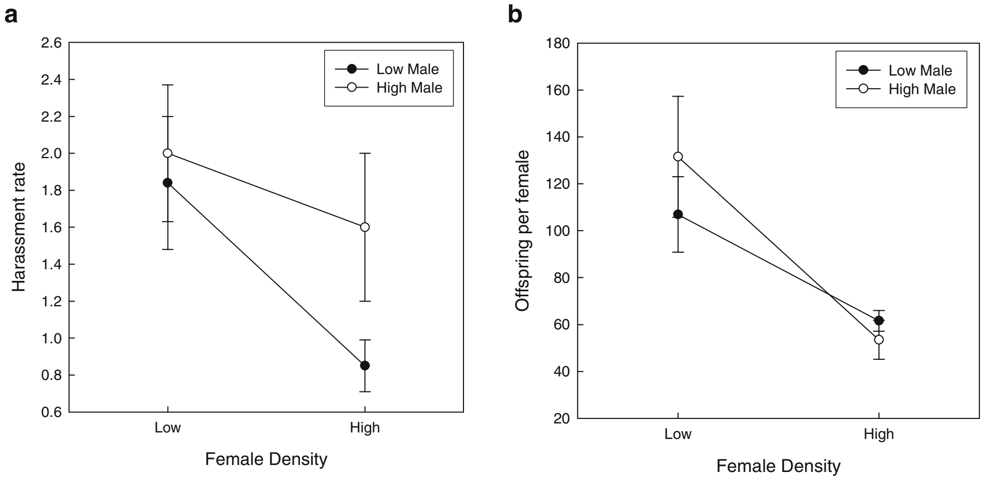


Fig. 3 **a** Sexual harassment toward focal females and **b** the average number of offspring collected per female for each combination of male and female densities. *Circles* are treatment means±standard errors

0.46; $F_{\text{female}}=0.28$, $P=0.60$; $F_{\text{interaction}}=0.47$, $P=0.55$, $F_{\text{length}}=16.6$, $P<0.001$). Female growth and survival were unaffected by the density of each sex (Table 2).

Discussion

The patterns of agonistic chases and displays observed between males in this study were in agreement with the prediction of Emlen and Oring (1977) that a male-biased sex ratio increases male competition for mates. My analysis suggests, however, that male and female density played unequal roles in determining the frequency of these behaviors. Chases between *G. affinis* males were largely dependent on female density, whereas the effect of male density was in the predicted direction but not significant. In contrast, the frequency of male–male displays was dependent on male density and unrelated to female density. These results suggest that operational sex ratio alone provides an incomplete picture of the factors affecting mating system dynamics, as male and female density can each have unique effects on behavior.

In many poeciliid fishes, males vigorously compete for fertilizations by aggressively displacing rival males and copulating frequently with females who often produce broods sired by multiple males (Trexler et al. 1997; Zane et al. 1999; Becher and Magurran 2004). As female density varies, males will face a tradeoff between devoting time and energy preventing other males from mating and attempting copulations with additional females. Utilizing both of these tactics is likely to be important for male reproductive success because males cannot completely restrict access to females (Smith, personal observation, Bisazza and Marin 1995), and the last male to mate typically sires most of the offspring (guppies, Evans and Magurran 2001). Male density, theoretically, should also affect this tradeoff because increasing the density of competitors should affect intrasexual encounter rates and the intensity of male–male competition for mates. High male densities did reduce the number of copulations males attempted and increase male–male interference through chasing; however, only changes in female density (and not male density) were statistically significant in the analysis. This suggests that female density has a predominant role influencing the frequency of these male behaviors.

Males may respond to low female densities by preventing other males from mating through chasing, rather than attempting redundant copulations with the small number of

Table 2 Effects of male and female density on harassment toward focal females and components of female fitness

	Harassment		Offspring per female		Survival		Growth		Embryo number	
Factor	$F(\omega^2)$	p	$F(\omega^2)$	p	F(odds)	p	$F(\omega^2)$	p	$F(\omega^2)$	p
Male density	1.90(0.03)	0.18	0.82(0.00)	0.61	0.37(0.88)	0.46	0.00(0.00)	0.98	0.39(0.01)	0.55
Female density	4.40(0.10)	0.05	16.91(0.30)	<0.001	0.61(0.40)	0.68	1.69(0.02)	0.22	3.28(0.11)	0.08
Male × female	0.79(0.00)	0.38	0.35(0.00)	0.33	0.41(3.56)	0.36	2.23(0.04)	0.15	0.04(0.00)	0.84

Values for dependent variables are *F*-ratios (effect size) and two-tailed probability values.
df=1,27 for all terms except survival (degrees of freedom were determined by the Satterwaithe method)

available females. This would be particularly advantageous if male mosquitofish recognize females with which they have recently mated. In the guppy *Poecilia reticulata*, males increase courtship behavior towards novel females (Kelley et al. 1999), suggesting that male poeciliids may have the cognitive capacity to distinguish between familiar and unfamiliar mates. The potential to fertilize multiple females also increases with female density, and therefore, the fitness payoff may be greater if males allocate more time and energy copulating with multiple females rather than chasing other males. At high female densities, females may not be economically defendable (Brown 1964; Warner and Hoffman 1980; Shuster and Wade 2003), and as a result, males may instead opt to scramble for inseminations rather than increase pre-copulatory efforts to interfere with competitors.

In contrast, the number of displays between males increased as male density increased, but was unrelated to female density. In *G. affinis*, displays are most likely to be used to signal motivational state, body size, or other attributes directly related to competition for fertilizations because females are the main resource that males actively contest. As a result, the frequency of displays may increase with male density because encounter rates between males will be higher when there are more males in the population.

Male displays may provide information that could be used to make decisions about how time and energy are allocated between chasing rivals, mating, and searching for an alternative school of females. Large males typically initiate agonistic chases in poeciliids (Bisazza and Marin 1991; Bisazza et al. 1996), but when opponents are evenly matched, as was the case in this experiment, displays may provide information about which individual will escalate the contest (Maynard-Smith and Harper 2004). Selection should favor display behavior in this circumstance to reduce the probability of protracted bouts of chasing between males, a behavior with high metabolic costs (reviewed in Haller 1995). Theory suggests that as a contested resource (e.g., mates) becomes more valuable, individuals should be more motivated to escalate contests (reviewed in Parker 1984). Indeed, the frequency of chases increased when fewer females were available, but the frequency of displays did not. Displays are likely to be much less energetically costly than chases, and as a result, males may benefit by assessing closely matched opponents when they are encountered.

Sexual harassment, female density, and female fitness

Male harassment is thought to result in a conflict between the sexes because the costs for males and females are asymmetric (Clutton-Brock and Parker 1995). Males gain a fitness payoff from harassing females because of the additional copulations they obtain, whereas females benefit little because additional copulations are superfluous after an intermediate number has been reached (Arnqvist and Nilsson 2000), but they still suffer the costs of harassment (Clutton-Brock and Parker 1995; Stockley 1997). Harassment toward females decreased as female density increased in this experiment, most likely because harassment was diluted among greater numbers of females in the pools. Similar effects of female density on harassment rates have been observed in water striders (Sih and Krupa 1995) and may explain why females of the eastern mosquitofish *G. holbrooki* aggregate when being harassed by males (Pilastro et al. 2003; Dadda et al. 2005; Agrillo et al. 2006). Female reproductive success, however, was 50% lower at high female densities despite the reduction in male harassment. Male harassment also did not appear to reduce female growth, survival, or the number of embryos females produced. This suggests that any effects of male harassment on female fitness were masked by a strong, negative effect of female density, particularly with respect to reproductive success. In contrast to a previous study of *G. affinis* (Smith and Sargent 2006), female growth did not decrease as female density increased, although female densities were much lower in the current study compared to the previous experiment. Together, these results suggest that interactions dependent on female density can have significant effects on female fitness.

Many studies examining density dependence in poeciliids (Rose 1959; Dahlgren 1979; Weeks 1993) and other taxa (Hixon 1991; Goser and Ratte 1994; Harrison and Cappuccino 1995; Sillett et al. 2004) have found that increases in population density are associated with reductions in growth, reproductive success, and survival, but the effects of each sex separately are rarely considered. Differential effects of male and female density on female fitness have been found in a few systems where the sexes differ in habitat use or female intrasexual agonistic interactions are prevalent. For example, female and juvenile red deer *Cervus elaphus* feed together in grassland, whereas males feed in patches dominated by heather (Clutton-Brock 1989). Reductions in female fecundity and survival are thought to stem from competition for food between adult females and juveniles foraging in the same area (Clutton-Brock et al. 1985; Clutton-Brock 1989). In red squirrels (*Sciurus vulgaris*), increases in female density, but not male density, reduce female fecundity and survival, most likely because females compete aggressively for territories that provide food and nesting sites (Wauters et al. 2004).

Aggression between female mosquitofish, however, is rare (Smith and Sargent 2006). There is evidence that male and female poeciliids differ in habitat use (Specziar 2004) and diet (Mansfield and McArdle 1998; Garcia-

Berthou and Moreno-Amich 2000), perhaps due to differences in gape size (Taylor et al. 2001), predation risk (Croft et al. 2004, 2006), or nutritional requirements. This could result in fiercer competition for food within the sexes than between them, although this is unlikely to have affected the outcome of this experiment because food availability and size were controlled for by distributing a homogenous food source according to the biomass of the fish in each pool.

A more probable explanation for the observed reduction in female reproductive success at high female densities is that females cannibalize juveniles at a higher rate than males. Indeed, Hubbs (1991) found that female *G. affinis* cannibalized more juveniles than males in a laboratory experiment. Although studies estimating cannibalism in enclosures (Dionne 1985; Hubbs 1991; Benoit et al. 2000, the current study) are criticized as overestimating cannibalism's importance (Nesbit and Meffe 1993), large mesocosm experiments where enclosure effects are minimized suggest that cannibalism is an important ecological phenomenon in nature rather than an experimental artifact (Taylor et al. 2001).

Sex differences in cannibalism rates are expected because female time budgets in poeciliids are dominated by foraging (Meffe and Snelson 1989; Magurran and Seghers 1994b), most likely because fecundity and growth are largely dependent upon food consumption (Reznick 1983). In contrast, male reproductive success is limited by the number of fertilizations they can obtain (Bateman 1948; Trivers 1972), and as a consequence, males spend most of their time locating and attempting to copulate with females (Magurran and Seghers 1994b). Females are also often two to three times larger than males (Meffe and Snelson 1989), and thus, females are likely to require more food for somatic maintenance in addition to the energy required for reproduction.

The present experiment supports sex differences in cannibalism as a likely explanation. Females dissected at the end of the experiment were producing the same number of embryos regardless of male or female density, suggesting that juvenile mortality, rather than differences in reproductive output between high and low density pools, was responsible for differences in the number of offspring collected. Other explanations are possible, however, as it was not feasible to quantify the timing and size of reproductive bouts for individual females during the course of the experiment. Female density, for example, could affect the length of the gestation period or the variance in offspring production between females. Whereas cannibalism is a likely explanation for the reduction in female reproductive success, future studies should manipulate male and female density with a known number of juveniles to provide a direct test of the hypothesis.

In conclusion, this study found that male and female density differentially affects patterns of male behavior and female fitness. It is commonly assumed that the ratio of males to females is the primary determinant of mating system dynamics, when in fact, male and female density do not always each contribute equally or at all to the patterns of behavior we observe, an implicit assumption of the hypothesis of Emlen and Oring (1977; Alonso-Pimentel and Papaj 1996). Furthermore, we should not overlook how the changes in demography that increase male harassment towards females may also simultaneously affect the strength of other ecological processes that influence female fitness. Whereas this in itself does not necessarily preclude sexual conflict as a potential source of selection, it will be important if the evolution of traits that reduce the costs of sexual conflict are opposed by other selective pressures (Arnold and Wade 1984). For example, high mortality rate at the juvenile life stage can have significant effects on future episodes of selection on body size in adult water striders (Fairbairn et al. 2003), a trait important in the ecology of sexual conflict in this group (Sih and Krupa 1992; Fairbairn and Preziosi 1994; Preziosi and Fairbairn 2000). The absence of an observed cost of male harassment to females in the current study suggests that the opportunity for selection by sexual conflict may be small in *G. affinis* relative to female density-dependent interactions.

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