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SEXUAL CONFLICT AND DENSITY DEPENDENCE IN THE WESTERN MOSQUITOFISH, GAMBUSIA AFFINIS (POECILIIDAE)

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ABSTRACT OF THESIS

SEXUAL CONFLICT AND DENSITY DEPENDENCE IN THE WESTERN MOSQUITOFISH, *GAMBUSIA AFFINIS* (POECILIIDAE)

Sexual conflict occurs when individuals of one sex express traits that reduce the fitness of their mates. Males of many species harass females to gain copulations, which benefits males by increasing the number of offspring they sire but imposes energetic and opportunity costs on the females they harass. This thesis examined the fitness costs of sexual harassment to females, the energetic costs of mating to males, and the factors influencing the intensity of male competition for mates in the western mosquitofish *Gambusia affinis*. I quantified male and female behavior, four female fitness components (number of offspring per female, embryo number, growth, and survival), and an index of male body condition in response to changes in operational sex ratio (experiment 1) and male and female density (experiment 2). I found that a strong, negative effect of female density on female fitness overwhelmed any potential costs of male harassment, suggesting that ecological interactions between females may play a larger role in determining female fitness than conflict between the sexes. Agonistic chases and displays between males increased as the operational sex ratio increased (became male-biased), while the number of copulations males attempted decreased. This inverse relationship suggests a tradeoff between interfering with other males and attempting additional copulations with females. Increases in chases between males were largely due to changes in female density, but not male density, suggesting that the availability of females determines whether males escalate contests with other males. In contrast, the number of displays between males depended varied with male density but did not female density. This difference between chases and displays is likely due to their difference in function; chases are performed to prevent other males from mating while displays are used to assess male competitors. I did not detect any energetic cost of mating to males.

KEYWORDS: Harassment, sexual selection, fitness, competition, operational sex ratio

Chad Smith
May 5th, 2005

SEXUAL CONFLICT AND DENSITY DEPENDENCE IN THE WESTERN MOSQUITOFISH,
GAMBUSIA AFFINIS (POECILIIDAE)

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2005

SEXUAL CONFLICT AND DENSITY DEPENDENCE IN THE WESTERN MOSQUITOFISH,
GAMBUSIA AFFINIS (POECILIIDAE)

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Arts and Sciences
at the University of Kentucky

By

Chad Christopher Smith

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Lexington, Kentucky

2005

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
LIST OF TABLES.....	v
LIST OF FIGURES	vi
CHAPTER 1 : EFFECTS OF OPERATIONAL SEX RATIO ON SEXUAL HARASSMENT, FEMALE FITNESS, AND THE ENERGETIC COSTS OF MATING TO MALES	1
SUMMARY	1
INTRODUCTION.....	2
METHODS	4
Behavioral observations	5
Female fitness	5
Cost of mating to males	6
Statistical analysis	6
RESULTS.....	6
Harassment and female aggression	6
Female fitness	7
Male competition for mates.....	7
Costs of mating to males	8
DISCUSSION	8
CHAPTER 2 : EFFECTS OF MALE AND FEMALE DENSITY ON SEXUAL HARASSMENT, FEMALE FITNESS, AND MALE COMPETITION FOR MATES	13
SUMMARY	13
INTRODUCTION.....	14
METHODS	16
Behavioral observations	17
Female fitness	17
Statistical analysis	18
RESULTS.....	19
Sexual harassment towards females.....	19
Female fitness	19
Male competition for mates.....	19
DISCUSSION	20
CHAPTER 3 : CONCLUSIONS	27
REFERENCES	30
VITA.....	36

LIST OF TABLES

Table 2.1. Treatment structure of the experiment.....	24
Table 2.2. Effects of female and male density on harassment toward focal females and female fitness.	24
Table 2.3. Effects of female and male density on the frequency of focal male copulation attempts and aggressive interactions with other males (chases and displays).	24

LIST OF FIGURES

Figure 1.1. Sexual harassment towards focal females, average number of offspring collected per female, and female growth in male-biased, even, and female-biased treatments..	11
Figure 1.2. Number of copulation attempts and male-male aggressive interactions (chases and displays) for focal males in male-biased, even, and female-biased treatments.....	12
Figure 2.1. Sexual harassment toward focal females and the average number of offspring collected per female for each combination of male and female densities.....	25
Figure 2.2. Frequency of copulation attempts and male-male aggressive interactions (chases and displays) for focal males for each combination of male and female densities..	26

CHAPTER 1 : EFFECTS OF OPERATIONAL SEX RATIO ON SEXUAL HARASSMENT, FEMALE FITNESS, AND THE ENERGETIC COSTS OF MATING TO MALES

SUMMARY

In many poeciliid fishes 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. Because the intensity of intrasexual and intersexual interactions is likely to vary with operational sex ratio, I examined the effects of sex ratio on male behavior, female fitness, and male body condition in the Western mosquitofish *Gambusia affinis* during a 10 week experiment in mesocosms. I found that female growth and reproductive success decreased as the proportion of females increased despite a significant reduction in male harassment, suggesting that variation in female density had a stronger effect on female fitness than male-female interactions. Operational sex ratio had significant effects on the frequency of aggressive interactions between males and the number of copulations males attempted; however, I did not detect a difference in male body condition between treatments. This suggests that either mating and aggression do not have an energetic cost, mating behavior and aggression require equal amounts of energy, or that the cost was eliminated by the ample supply of food provided during the experiment.

INTRODUCTION

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 they can obtain, while female fitness peaks at an intermediate optimum (Arnqvist & Nilsson, 2000; Bateman, 1948). Above this intermediate optimum, the potential benefits females gain from additional copulations from one or more males (reviewed in Zeh & Zeh, 2003; Hosken & Stockley, 2003; Jennions & Petrie, 2000) decrease but they 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 Stockley, 1997; Daly, 1978). Males may also directly increase the costs of mating to females by transferring ejaculates with toxic accessory proteins that result in decreased female longevity and reproductive success (reviewed in Chapman, 2001) or inflict 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 co-evolutionary “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 intrasexual interactions that influence reproduction and survival (Magurran, 2001). Poeciliids, a family of live-bearing fishes, 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 fishes has focused on the costs of male harassment to females. In 50% of poeciliid species, males do not exhibit sexually selected displays but attempt to copulate using a sneaky tactic thought to circumvent female choice, while others use a mixture of courtship and the sneaky tactic (Bisazza, 1993). Males attempt to mate once per minute, resulting in a 30-50% reduction in female foraging efficiency (Magurran & Seghers, 1994a; Pilastro et al., 2003; Schlupp et al., 2001). This 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 expose females to virulent pathogens (Lockhart et al., 1996; Skorping & Jensen, 2004), increase predation risk by

making females more conspicuous (Pocklington & Dill, 1995), and increase female energetic expenditure when evading males.

Mating may also be costly for males through time and energy expended harassing females and competing with other males for access to mates. Emlen and 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 females receive because of its influence on encounter rates between the sexes, the payoff of harassment for males, and the cost of resisting harassment for females (Clutton-Brock & Parker, 1995). Previous studies have 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 (Jirotkul, 1999; Sih & Krupa, 1995; Kvarnemo & Ahnesjö, 1996), 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). Altering operational sex ratio can have additional consequences because changing the sex ratio varies the ratio of males to females and the density of one or both sexes. 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).

Due to the dynamic nature of the small streams and ponds poeciliids inhabit, both sex ratio and density often vary in space and time. For example, sex specific predation on females (who are usually larger than males) can result in sex ratios that are strongly male biased (Britton & Moser, 1982), while higher rates of male mortality can generate sex ratios that are female biased (Haynes & Cashner, 1995; Rodd & Reznick, 1997; Zulian et al., 1995). Population density often depends on food availability and predation pressure, both of which are subject to nutrient flow and the physical characteristics of the habitat (Winemiller, 1993; Chapman et al., 1991; Capone & Kushlan, 1991; Meffe & Snelson, 1989). Because of this, the relative importance of a given factor in determining male and female fitness is likely to vary as ecological conditions change. For example, sexual harassment may have a relatively strong effect on female fitness when sex ratios are male biased but a weak effect when sex ratios are female biased and other factors such as female-female competition for food are more important. Conversely, male fitness is likely determined by the number of fertilizations they can secure, which will be in part determined by the energy they expend obtaining mates. Therefore, increased male aggression and sperm competition when sex ratios are male biased may limit

how many successful fertilizations they can obtain but be negligible when there are many females and few male competitors to contend with.

I examined the effects of intersexual and intrasexual interactions on male and female fitness in the Western mosquitofish *Gambusia affinis*, a small poeciliid that inhabits creeks and ponds in the southern United States. I manipulated operational sex ratio and measured the frequency of male harassment toward females and four components of female fitness. I 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 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) is within their natural range (Smith, personal obs.) and that of other poeciliid fishes (Zulian et al., 1995; Chapman et al., 1991; Capone & Kushlan, 1991; Jordan et al., 1998). Each treatment was replicated 10 times, and the experiment ran for a total of 10 weeks.

Members of each sex were size matched within pools (females within 4 mm, males within 2 mm) and across treatments to minimize any 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 five 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 five 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, 1/8" 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.

Behavioral observations

In female observations, a focal female was selected and observed for 10 minutes after a five minute period to allow fish to acclimate to the experimenter. I recorded the frequency of chases towards males and other females as a measure of aggression and the number of copulation attempts directed towards the focal female. A copulation attempt was defined as a male orienting within 10 cm of the female's posterior and swimming rapidly towards her.

In male observations, a focal male was selected and observed for five minutes after a five minute acclimation period. I recorded the number of copulations attempted and aggressive interactions (displays and chases) received or initiated by the focal male. Displays only occurred 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.

Behavioral data for females and males were collected between 0900 and 1430 during weeks four and five of the experiment. Each pool was observed twice for both male and female observations during a two week period and the results from the two observations were averaged. Pools were never observed twice on the same day.

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 five weeks of the experiment. Fry present in the pools in the first five weeks of the experiment were not included because they were conceived in the wild before the treatments could have an effect. Females produce clutches continuously throughout the breeding season in approximately 30 day intervals (Koya et al., 1998; Krumholz, 1948), 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 surviving fry 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, as 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 length³. 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 were performed with SAS[®] v8.2 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 if there was a relationship 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). Non-significant terms in the model were removed in a stepwise fashion; interaction terms were removed first followed by main effects. In a separate analysis, I 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 $\alpha=0.05$.

RESULTS

Harassment and female aggression

The number of copulation attempts received by focal females increased significantly as the proportion of females in the pools increased (Spearman rank correlation: $r_s = -0.76$, $P < 0.001$, $n=30$, Fig. 1.1a). Female aggression was uncommon in all treatments (mean chases per minute \pm SE, 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: $H_2=0.003$, $P=0.99$) or other females (Kruskal-Wallis: $H_2=0.96$, $P=0.62$).

Female fitness

In the multiple regression analysis examining the relationship between sex ratio treatment, female growth, and female survival on the average number of offspring collected per female (the dependent variable), all interactions and main effects were non-significant except sex ratio treatment and consecutively removed from the analysis except sex ratio treatment. 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. 1.1b).

Female growth also decreased significantly as the proportion of females in the pools increased ($F_{2, 27}=4.91$, $P=0.02$; Fig. 1.1b). Although there was a significant relationship, 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 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 Satterwaithe method), although the average mortality in female biased pools was higher than that of the other treatments (% mortality \pm SE: male biased: $25 \pm 6\%$; even: $21 \pm 6\%$; female biased: $40 \pm 7\%$).

The number of embryos females produced after controlling for female length was not significantly different between sex ratio treatments (log transformed; overall model: $F_{3,26}=13.88$, $P<0.001$; treatment: $F=0.76$, $P=0.48$; length: $F=37.28$, $P<0.001$). There was also no significant difference between treatments in the proportion of females who did not have a matured clutch at the end of the experiment (Kruskal-Wallis: $H_2=2.70$, $P=0.26$).

Male competition for mates

The number of copulations attempted by focal males increased as the proportion of females increased (Spearman rank correlation: $r_s=0.76$, $P<0.001$, $n=30$; Fig. 1.2a). This was accompanied by a decrease in male-male aggression. The number of chases ($F_{2, 27}=20.41$, $P<0.001$; Fig. 1.2b) and displays ($F_{2, 27}=5.30$, $P=0.01$; Fig. 1.2b) between males decreased as the proportion of females increased. Hence, mating activity and the frequency of aggressive interactions had an inverse relationship: 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 behavior 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 Satterwaithe method).

DISCUSSION

I found that the average number of offspring collected per female and female growth decreased as the proportion of females increased, despite a fourfold reduction in male harassment. It is important to note that while the total number of fish put into the pools at the start of the experiment was the same, manipulating the sex ratio not only changes the relative numbers of males to females but also the density of each sex within the pools. These results suggest that increasing female density resulted in a strong, negative effect on female fitness, and that this effect masked any costs imposed on females by males due to harassment.

There are three possible explanations for a reduction in female fitness with increasing female density: chemical or behavioural suppression, fry cannibalism, and competition for food. Suppression of reproduction among females has been documented in birds, mammals, and insects (Breihagen & Slagsvold, 1988; Cuvillier-Hot et al., 2002; Faulkes & Abbott, 1997; Solomon & French, 1997), and can be mediated chemically (via pheromones or metabolites) or behaviorally (through aggressive interactions between females). The low level of aggressive behavior 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* was reduced when females were raised 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 (Weeks & Gaggiotti, 1993; Rodd et al., 1997; Dahlgren, 1979; Reznick & Braun, 1987) if it increases lifetime reproductive success (Williams, 1966). Alternatively, accumulations of toxic metabolic waste (Beebee & Wong, 1992) or other metabolites (Goser & Ratte, 1994; Thorp & Barthalmus, 1975; 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 2-3 times larger than males.

A second possibility is that competition for food is stronger among females than between the sexes, resulting in an intensification of competition as female density in the pools increased. Males and females are known to eat different prey types (Mansfield & McArdle, 1998), most likely because of differences in gape size (Taylor et al., 2001) and nutritional requirements. Competition between females may be further intensified because acquiring food is an important determinant of female fitness (Reznick, 1983). This explanation is unlikely in the context of our experiment, however, as 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 experienced higher rates of cannibalism despite our attempts to prevent it. Cannibalism alone, however, does not account for the reduction in female growth, indicating that while cannibalism may be an important factor influencing offspring survival, other interactions between females appear to be influencing female fitness.

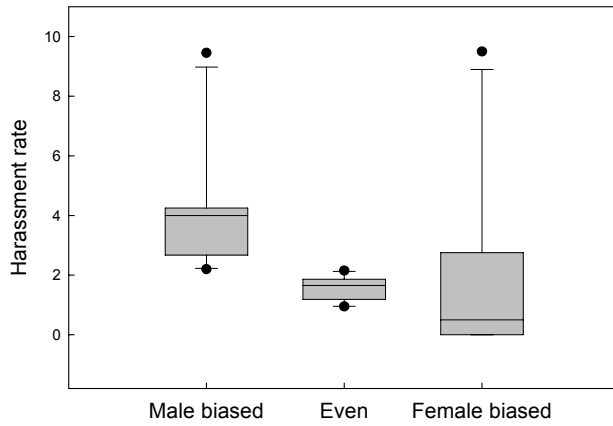
Effects of sex ratio on male behavior and body condition

Altering the sex ratio had marked effects on male behavior. In agreement with Emlen and Oring (1977) and other empirical studies (Kvarnemo & Ahnesjo, 1996), agonistic chases and displays between males increased as the proportion of males increased, most likely because of increased encounter rates between males and the decrease of available females in the pools. This was accompanied by a 32% reduction in the number of copulations focal males attempted, suggesting that the number of females, the frequency of male-male interactions, or both influenced male sexual activity. These results have clear implications because as operational sex ratio varies in nature (Zulian et al., 1995; Pettersson et al., 2004; Snelson, 1989), male poeciliids will be confronted with tradeoffs 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 tradeoffs will presumably also have energetic costs as males allocate resources to different behaviors.

Despite differences in intrasexual aggression and mating activity, there was no difference in male condition between treatments. One explanation is that these behaviors might not carry energetic costs to males. This is unlikely because aggression is known to have metabolic costs (Briffa & Elwood, 2001; Haller, 1996) 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 caloric expenditure in the different treatments. Marler and 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 indices across treatments. Because the metabolic costs of these behaviors are unknown, I cannot distinguish between these hypotheses.

TABLES AND FIGURES

a)



b)

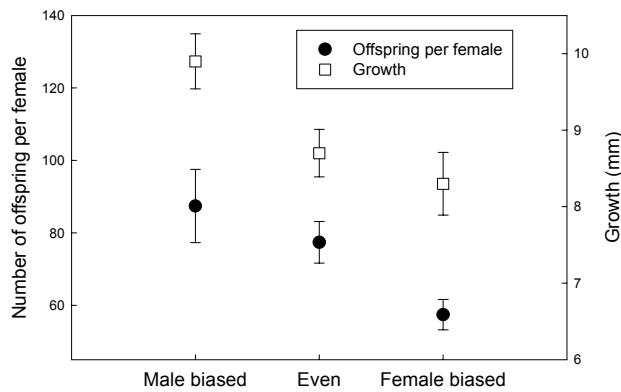
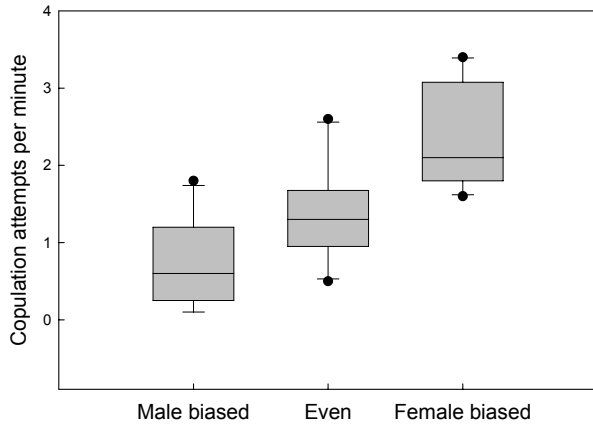


Figure 1.1. Sexual harassment towards focal females (a, copulation attempts per minute), average number of offspring collected per female (b, circles), and female growth (b, squares) in male-biased, even, and female-biased treatments. Symbols and bars for offspring per female and growth represent means and standard errors.

a)



b)

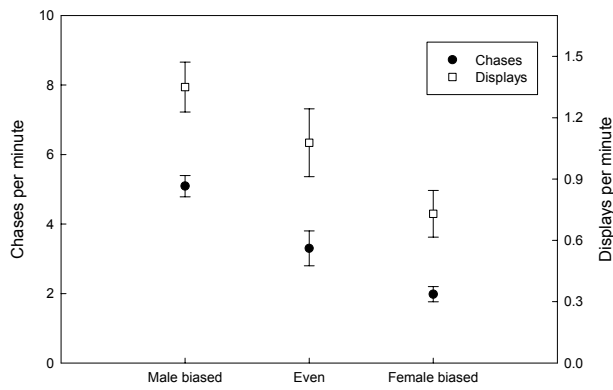


Figure 1.2. Number of copulation attempts (a) and male-male aggressive interactions (b, chases and displays) for focal males in male-biased, even, and female-biased treatments. Symbols and bars for chases and displays represent means and standard errors.

CHAPTER 2 : EFFECTS OF MALE AND FEMALE DENSITY ON SEXUAL HARASSMENT, FEMALE FITNESS, AND MALE COMPETITION FOR MATES

SUMMARY

Sexual conflict is thought to have significant consequences for male and female reproductive success, but the costs of conflict are rarely examined in the context of other processes that simultaneously affect fitness. In poeciliid fishes, male harassment toward females is thought to reduce female fitness by lowering foraging efficiency and increasing predation risk, energetic expenditure, and susceptibility to disease transmission. These costs, however, may be negligible compared to ecological interactions between females that are strongly influenced by changes in female density, such as cannibalism, competition for food, and behavioral and chemical suppression of growth or reproduction. I manipulated male and female densities of the western mosquitofish *Gambusia affinis* in mesocosms to examine their effects on male harassment, female fitness, and male competition for mates. Female reproductive success decreased significantly when female density increased, despite lower levels of harassment. This suggests that a strong, negative effect of increased female density overwhelmed any potential costs of male harassment. Changes in the density of each sex also influenced male competition for mates, with increases in female density decreasing the frequency of agonistic male-male chases and increasing the number of copulations attempted per male. Male density had no significant effect on these behaviors. In contrast, the number of displays between males increased with male density but was unrelated to female density. This suggests that male and female density do not always equally contribute to the frequency of harassment toward females and the intensity of male competition for mates.

INTRODUCTION

Sexual conflict arises when individuals of one sex express traits that reduce the fitness of individuals of the other sex (Parker, 1979). For example, mate guarding, prolonging copulation, and harassing females for copulations can benefit males by increasing their probability of securing paternity but can result in an array of energetic and opportunity costs for females (Stockley, 1997). While studies have demonstrated that male behavior and seminal products can result in significant fitness costs for females (Chapman et al., 1995; Jormalainen et al., 2001; Kolodziejczyk & Radwan, 2003; Rice, 1996; Rowe, 1994; Arnqvist & Nilsson, 2000), the costs of sexual conflict are rarely examined in the context of other ecological processes that simultaneously affect fitness (Sih & Krupa, 1996; but see Sih & Krupa, 1995; Maklakov et al., 2005). One challenge to researchers is to identify the relative strength of sexual conflict in the context of these other processes.

Males of many poeciliid (live-bearing) fishes persistently harass females for copulations using a “gonopodial thrusting” tactic in which they swim up to females and preemptively thrust their gonopodium (the intromittent organ) into the female gonopore (Meffe & Snelson, 1989). Males attempt to mate up to three times per minute, resulting in a 30-50% reduction in female foraging efficiency (see Chapter 1, Magurran & Seghers, 1994a; Pilastro et al., 2003; Schlupp et al., 2001). This may 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 & Dill, 1995; Magurran, 2001), susceptibility to disease transmission (Lockhart et al., 1996), and female energetic expenditure evading males (Stockley, 1997; Jormalainen et al., 2001).

Previously, I suggested that ecological interactions between females may play a larger role in determining female fitness than male harassment in the Western mosquitofish *Gambusia affinis* (see Chapter 1). Adults of many poeciliids cannibalize juveniles (Meffe & Snelson, 1989), and because females spend significantly more time foraging than males (Magurran & Seghers, 1994b), populations which contain more females are likely to experience higher rates of cannibalism. Competition for food among females may also have greater fitness consequences than harassment because of the importance of food intake in determining female growth and fecundity. Field studies have noted sex differences in diet composition (Mansfield & McArdle, 1998; Garcia-Berthou & Moreno-Amich, 2000) and habitat use (Specziar, 2004), suggesting that competition for food within the sexes may be stronger than competition between them. Finally,

contact with water containing other females reduces female growth and survival (Lutnesky & Adkins, 2003), suggesting that chemical interactions between females may influence some components of female fitness.

The relative importance of intrasexual interactions and sexual conflict will be determined by the factors that influence the strength of those interactions. While interactions between females are likely mediated by female density, the amount of harassment females receive will depend on encounter rates with males and the intensity of male competition for mates, which influences the costs and benefits of harassment (Clutton-Brock & Parker, 1995). Emlen and Oring (1977) proposed that the intensity of competition for mates is determined by operational sex ratio, the ratio of breeding males to breeding females. Experiments manipulating operational sex ratio have found that male competition for mates increases when operational sex ratio is male-biased, and decreases when operational sex ratio is female-biased as Emlen and Oring (1977) predict (reviewed in Kvarnemo & Ahnesjo, 1996; Reichard et al., 2004; Grant et al., 2000). In poeciliid fishes, males compete vigorously for fertilizations by frequently copulating and chasing other males away from females, who can store sperm for months (Constantz, 1989) and often produce broods from multiple sires (Zane et al., 1999; Evans & Magurran, 2001). Agonistic interactions between males increase in frequency as the proportion of males in the population increases (see Chapter 1, Jirotkul, 1999), as predicted by Emlen and Oring (1977). However, the number of copulations a male attempts decreases as the proportion of males increases because of increased agonistic interference (see Chapter 1, Jirotkul, 1999). Of empirical interest is determining how changes in OSR influence patterns of male behavior and their fitness consequences for each sex.

While the Emlen and Oring (1977) hypothesis predicts that the ratio of males to females should determine the intensity of male-male competition, an increase in male density may not produce the same effect as an equivalent decrease in female density (Alonso-Pimentel & Papaj, 1996). Mating strategies may also vary with the density of one sex but be insensitive to changes in the density of the other sex. For example, Verrel (1982) found that the rate of male courtship in red spotted newts (*Notophthalmus viridescens*) increases with male density, but not female density. Sexual harassment in the water strider *Aquarius regimens* (Sih & Krupa, 1995) and copulation duration in the walnut fly *Rhagoletis juglandis* (Alonso-Pimentel & Papaj, 1996) varies as Emlen and Oring (1977) would predict, but are both dependent on male and female density. These examples illustrate that the density of each sex may not be equally important in determining the patterns of male and female behavior. Operational sex ratio and density vary

widely over space and time in natural populations of poeciliids (Haynes & Cashner, 1995; Pettersson et al., 2004; Zulian et al., 1995; Britton & Moser, 1982; Chapman et al., 1991; Capone & Kushlan, 1991; Jordan et al., 1998), providing a relevant ecological context in which to study these relationships.

The goal of this study was to determine the relative effect of sexual harassment and female density on female fitness in the Western mosquitofish *Gambusia affinis*, a small poeciliid native to creeks and ponds in the southern United States. I also examined how changes in male and female density influence the intensity of male competition for mates.

METHODS

This study was conducted at the University of Kentucky Ecological Research Facility in Lexington, Kentucky from June to August 2004. Wading pools (1.4m in diameter, filled 15 cm deep with conditioned tap water) were randomly assigned to one of four treatments in a 2 x 2 factorial design with male and female density as the two factors (Table 2.1). Each factor comprised of two levels of density, low (3 fish) and high (6 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. One pool in the high male/high female treatment suffered high mortality two weeks into the experiment and was removed from the analysis.

Western mosquitofish (*Gambusia affinis*) were collected locally, 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 (less than 4 mm) 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 & Marin, 1995). Pools were covered with shade cloth to exclude predators and reduce 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 (ten weeks). For the first five 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. For the last five weeks of the experiment, fry were collected 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 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.

Behavioral observations

To estimate the amount of sexual harassment females received, a focal female was selected. The number of copulation attempts directed toward her was observed for 10 minutes after a five minute 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 five minutes. 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.

Behavioral data were collected between 0900 and 1430 during weeks two and three of the experiment. Females and males were observed consecutively, the order of which was alternated for every set of observations. Each pool was sampled twice over the two week period and the results from the two trials averaged for analysis. No pool was sampled more than once on the same day.

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 five weeks of the experiment. Fry present in the pools in the first five weeks of the experiment were not included because they were conceived in the wild before the treatments could have an effect. Females produce clutches continuously throughout the breeding season in approximately 30 day intervals (Koya et al., 1998; Krumholz, 1948), 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 average number of offspring birthed per female 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 matured ova, which mature together as a clutch before parturition. Females without matured ova were not included in the analysis, as it was not possible to determine how many oocytes would have been fully yolked in those cases. 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 proportion of females in a pool that survived to the end of the experiment.

Statistical analysis

Statistical analyses were performed using SAS[®] v8.2. 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 & Rohlf, 1981). Effects of male and female density on the dependent variables were assessed using a two-way ANOVA and treatment means were compared using Fisher's LSD tests (PROC GLM). For embryo number, total length was included as a covariate to control for the relationship between female size and fecundity. Effect sizes were calculated using omega-squared (ω^2), the proportion of the population variance attributed to the effect (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. The same procedure was used to test for differences in the proportion of females with matured ova at the end of the experiment. Odds ratios are presented as effect sizes for female survival (Sokal & Rohlf, 1981). All significance tests were two-tailed.

Residual plots of male display rates revealed an extreme 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 ($r=0.73$, $p<0.01$) the data point was then winsorized (replaced it with the value of the next largest datum in the sample). This normalized the distribution for the two-way ANOVA. I am unsure if this particular data point was due to an unusually aggressive male in the pool or a recording error. The point is clearly unrepresentative of the population of samples from the low male/high female treatment: the display rate for this pool (4.2 displays per minute) was 2.5 standard deviations from the mean and 2.8 times larger than the next highest value recorded for the low male/high female treatment (1.5 displays per minute). It was also the largest value of all the measurements of male display rate in the experiment.

RESULTS

Sexual harassment towards females

The frequency of harassment towards focal females decreased significantly as female density increased, but there was no effect of male density on the amount of harassment focal females received (Two-way ANOVA: $F_{male}=1.90$, $df=1$, $P=0.18$; $F_{female}=4.40$, $df=1$, $P=0.05$; $F_{interaction}=0.79$, $df=1$, $P=0.38$; Fig. 2.1a, Table 2.2). The lack of a significant effect of male density appears due to relatively low levels of harassment in the high male/low female treatment (Fig. 2.1a). Although the data suggest that harassment at the two male densities differ depending on female density, the interaction term was not significant (see above).

Female fitness

The number of offspring collected per female decreased sharply as the density of females increased (Two-way ANOVA: $F_{male}=0.25$, $df=1$, $P=0.61$; $F_{female}=14.23$, $df=1$, $P<0.001$; $F_{interaction}=1.0$, $df=1$, $P=0.33$; Fig. 2.1b, Table 2.2). Embryo number (Two-way ANOVA: $F_{male}=0.01$, $df=1$, $P=0.93$; $F_{female}=0.02$, $df=1$, $P=0.88$; $F_{interaction}=0.08$, $df=1$, $P=0.78$; $F_{length}=31.89$, $P<0.001$), female growth (Two-way ANOVA: $F_{male}=0.00$, $df=1$, $P=0.98$; $F_{female}=1.58$, $df=1$, $P=0.22$; $F_{interaction}=2.23$, $df=1$, $P=0.15$), and female survival (Mixed model: $F_{male}=0.55$, $P=0.46$; $F_{female}=0.17$, $P=0.68$; $F_{interaction}=0.85$, $P=0.36$; degrees of freedom calculated using the Satterwaithe method) were unaffected by changes in male and female density (Table 2.2). There was no significant effect of male or female density on the number of females with matured ova at the end of the experiment (Mixed model: $F_{male}=0.00$, $P=0.97$; $F_{female}=1.82$, $P=0.19$; $F_{interaction}=0.08$, $P=0.78$; degrees of freedom calculated using the Satterwaithe method).

Male competition for mates

The number of copulations attempted by focal males increased as the density of females increased (Two-way ANOVA: $F_{male}=0.88$, $df=1$, $P=0.36$; $F_{female}=4.37$, $df=1$, $P=0.05$; $F_{interaction}=0.36$, $df=1$, $P=0.55$; Fig. 2.2a, Table 2.3). There was no significant effect of male density on the number of copulations focal males attempted, although the average number of copulation attempts was always lower in pools with more males (Fig. 2.2a).

The number of chases between males was significantly lower in pools with more females, but there was no significant effect of male density on the number of chases involving focal males (Two-way ANOVA: $F_{male}=0.48$, $df=1$, $P=0.49$; $F_{female}=4.82$, $df=1$, $P=0.40$;

$F_{interaction}=0.19$, $df=1$, $P=0.67$; Fig. 2.2b, Table 2.3). Interference between males through chasing, then, was inversely related to the number of copulations focal males attempted: when female density increased, the frequency of chases between males decreased and the frequency of copulation attempts toward females increased (Fig. 2.2a, 2.2b). The number of chases between males was higher in the male-biased compared to the female-biased treatment (Fisher's LSD: high male/low female vs. low male/high female, $P=0.04$). This difference is largely due to changes in female rather than male density (see two-way ANOVA above; Table 2.3).

In contrast, the number of displays between males increased significantly when male density increased but there was no significant effect of female density on the frequency of displays (Two-way ANOVA: $F_{male}=6.53$, $df=1$, $P=0.02$; $F_{female}=0.06$, $df=1$, $P=0.81$; $F_{interaction}=1.41$, $df=1$, $P=0.25$; Fig. 2.2c, Table 2.3). The mean number of displays was 59% higher in the male-biased compared to the female-biased treatment, although this difference was not statistically significant (Fisher's LSD: high male/low female vs. low male/high female, $P=0.08$, Fig. 2.2c).

DISCUSSION

Sexual harassment is thought to result in a conflict between the sexes because the costs for males and females are asymmetric (Clutton-Brock & Parker, 1995). Males gain a fitness payoff from harassing females because of the additional copulations they obtain, while females benefit little because additional copulations are superfluous after an intermediate number has been reached (Arnqvist & Nilsson, 2000) but they still suffer the costs of harassment (Clutton-Brock & Parker, 1995; Stockley, 1997). I previously suggested that female density-dependent interactions could be a more important determinant of female fitness than harassment in *Gambusia affinis*, although the experimental design confounded sex ratio with the density of each sex (see Chapter 1). This is resolved in the current study by manipulating the density of each sex independently.

I found that increasing female density reduced the number of offspring collected by 50% despite a significant reduction in harassment in those pools. 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 effect of female density, particularly with respect to reproductive success.

Most studies examining density dependence in poeciliids (Weeks, 1993; Rose, 1959; Dahlgren, 1979) and other taxa (Harrison & Cappuccino, 1995; Hixon, 1991; Gosler & Ratte, 1994; Sillett et al., 2004) have found that increases in population density are associated with reductions in growth, reproductive success, and survival. These studies, however, rarely consider the effects of each sex separately. An exception is in mammals, where sex differences in habitat use and intrasexual agonistic interactions are thought to result in differential effects of male and female density on fitness. In red deer (*Cervus elaphus*), females and juveniles feed together in grassland while 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, 1989; Clutton-Brock et al., 1985). 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 females in *G. affinis*, however, is rare (see Chapter 1). There is evidence that male and female poeciliids differ in habitat use (Specziar, 2004) and diet (Mansfield & McArdle, 1998; Garcia-Berthou & Moreno-Amich, 2000), perhaps due to differences in gape size (Taylor et al., 2001) or nutritional requirements, which could result in fiercer competition for food within the sexes than between them. This is unlikely to have affected the outcome of this experiment, however, since food availability and size was controlled for by distributing a homogenous food source according to the biomass of the fish in each pool.

A more likely explanation for the observed reduction in female reproductive success at high female densities is that females cannibalize juveniles at a higher rate than males. Female time budgets in poeciliids are dominated by foraging (Magurran & Seghers, 1994b; Meffe & Snelson, 1989), 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 & Seghers, 1994b). Females are also often two to three times larger than males (Meffe & Snelson, 1989), and thus females are likely to require more food for somatic maintenance in addition to the energy required for reproduction. Although refuges for fry were provided in the pools, cannibalism may still have occurred because fry were not always within the enclosures. An experiment manipulating the density of each sex with known quantities of juveniles is required to explicitly test this hypothesis.

Female density, male density, and male competition for mates

The intensity of harassment toward females is intimately linked to male competition for mates. In 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 (Becher & Magurran, 2004; Zane et al., 1999; Trexler et al., 1997). The patterns of agonistic chases and displays observed between males were in agreement with Emlen and Oring's (1977) prediction that a male-biased sex ratio increases male-male competition for mates, although the analysis suggests that female and male density played unequal roles in determining the frequency of these behaviors. The frequency of chases between males was largely dependent on female density (the effect of male density was in the predicted direction but not significant), while the frequency of displays was dependent on male density and unrelated to female density.

As female density varies, males will face a tradeoff between devoting time and energy preventing other males from mating and attempting copulations with 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 obs., Bisazza & Marin, 1995) and the last male to mate typically sires more offspring (guppies, Evans & Magurran, 2001). When female density is low, males may achieve higher fitness by preventing other males from mating through chasing rather than attempting redundant copulations with the small number of 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. When female density is high, the potential to fertilize multiple females is also high, and therefore the fitness payoff may be greater if males allocate more time and energy copulating with multiple females rather than chasing males. Females may also not be economically defendable at high densities (Brown, 1964; Warner & Hoffman, 1980; Shuster & 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 poeciliids, male 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.

Displays may provide information that could be used to make decisions about how time and energy is allocated between chasing rivals, mating, and searching for an alternative school of females. Large males typically initiate agonistic chases in poeciliids (Bisazza et al., 1996; Bisazza & Marin, 1991), but when opponents are evenly matched, as was the case in this experiment, displays may provide information about which individual will escalate the contest. Selection should favor display behavior in this circumstance to reduce the probability of protracted bouts of chasing between males, which are likely to be energetically costly (Haller, 1996). Theory suggests that as the contested resource becomes more valuable, individuals should be more motivated to escalate contests (reviewed in Parker, 1984). Indeed, the frequency of chases increased when the female density decreased, 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.

TABLES AND FIGURES

Table 2.1. Treatment structure of the experiment. Each box represents a treatment consisting of low or high densities of males (M) and females (F).

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

Table 2.2. Effects of female and male density on harassment toward focal females and female fitness. Values are effect sizes (ω^2) except for survival (log odds ratio).

Source	Offspring				
	Harassment	per female	Survival	Growth	Fecundity
Male density	0.03	0.00	0.88	0.00	0.00
Female density	0.10*	0.30***	0.40	0.02	0.00
Female x Male	0.00	0.00	3.56	0.04	0.00
Total length	-	-	-	-	0.53***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

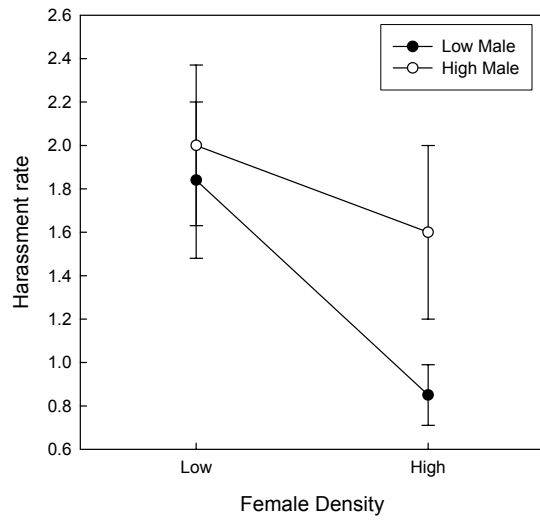
Table 2.3. Effects of female and male density on the frequency of focal male copulation attempts and aggressive interactions with other males (chases and displays). Values are effect sizes (ω^2).

Source	Copulation		
	Attempts	Chases[†]	Displays
Male density	0.00	0.00	0.15*
Female density	0.10*	0.10*	0.00
Female x Male	0.00	0.00	0.01

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[†] log transformed

a)



b)

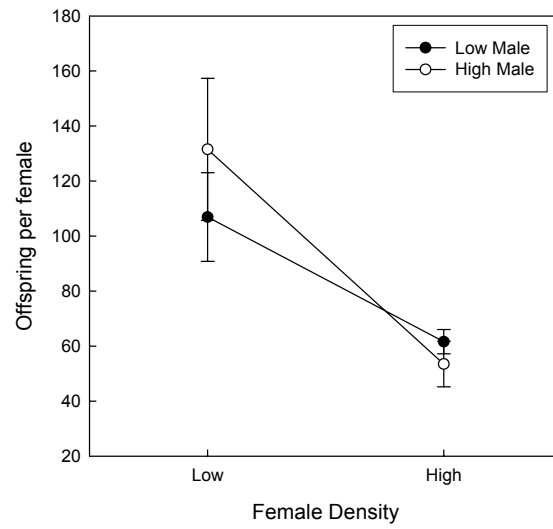
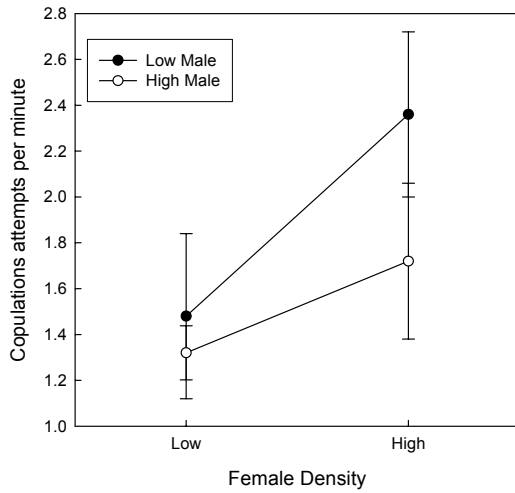
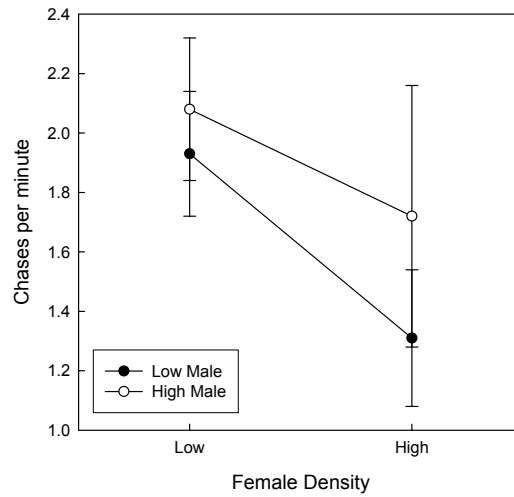


Figure 2.1. Sexual harassment (a, copulation attempts per minute) toward focal females and the average number of offspring collected per female (b) for each combination of male and female densities. Circles are treatment means \pm standard errors.

a)



b)



c)

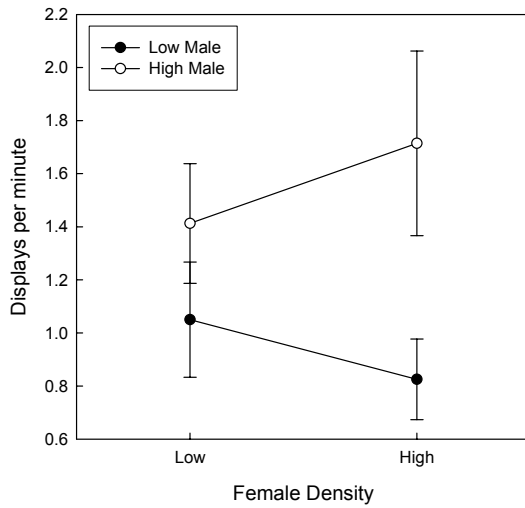


Figure 2.2. Frequency of copulation attempts (a), and male-male aggressive interactions (chases, b; displays, c) for focal males for each combination of male and female densities. Circles are treatment means \pm standard errors.

CHAPTER 3 : CONCLUSIONS

The role of sexual conflict in shaping the evolution of male and female traits is a topic of vigorous debate in evolutionary ecology (Hosken & Snook, 2005; Cordero & Eberhard, 2003; Eberhard, 2005). One critique of many studies measuring fitness costs of sexual conflict to females is that they are conducted in a laboratory setting in the absence of other processes that simultaneously affect fitness (Cordero & Eberhard, 2003; but see Maklakov et al., 2005). This thesis provides evidence in a semi-natural study of the poeciliid fish *Gambusia affinis* that the costs of sexual harassment to females are small compared to the large effects of female density on female reproductive success and possibly female growth. Selection for female traits that reduce harassment may be weak if the costs imposed by males are relatively small compared to selection resulting from interactions between females or other sources of natural selection. For example, females could potentially reduce harassment by hiding in algae or other refuges when they are pursued, but the fitness cost of lost foraging opportunities may be too great to permit that behavior to evolve.

There are a number of potentially important costs of sexual harassment, however, that were not addressed by this study. Male harassment may increase predation risk to females by increasing their conspicuousness and reducing vigilance (Pocklington & Dill, 1995; Magurran, 2001). Harassment may also increase the risk of disease transmission (Lockhart et al., 1996; Skorping & Jensen, 2004), particularly because males frequently move between schools of females in search of new mates while females maintain greater fidelity to their shoal mates (Croft et al., 2003; Griffiths & Magurran, 1997). Effects of harassment on foraging efficiency and the energetic costs of evading males may also be more pronounced in nature when the food source is ephemeral and is of lower nutritional quality than the commercially produced food provided in this study. While female density undoubtedly has strong effects on female fitness and the number of copulations males obtain in *G. affinis*, identifying the magnitude of the costs of sexual conflict to both sexes in the context of other ecological processes is key to our understanding of these systems.

The mechanism resulting in reduced female fitness with increasing female density also remains unresolved. Cannibalism is likely responsible for the significant decrease in offspring collected per female in both studies presented here, although this cannot be verified without an additional experiment that measures the effect of male and female density on cannibalism rate. The experimental designs also did not allow me to assess the reproductive state of females during the offspring collection period, so while it is likely that all or most of the females gave

birth during this time because of their perpetual pregnancy during the breeding season, the possibility exists that treatments varied with regard to the number of females that in the pools that produced young (although there was no significant differences in the proportion of females with matured ova at the end of both experiments).

Causes of depressed female growth (see Chapter 1) also remain unexplained. Reduced growth as a function of increased density has been noted in poeciliids (Weeks, 1993) and other aquatic organisms (Rose, 1960; Goser & Ratte, 1994; Hixon, 1991). This response is usually attributed to either accumulations of metabolites or other biological products that interfere with growth and reproduction (Beebee & Wong, 1992; Goser & Ratte, 1994; Lutnesky & Adkins, 2003) or adaptive allocation of resources into other life history traits, such as offspring size or number (Cleavers et al., 1997; Burns, 1995; Reznick, 1983; Reznick & Braun, 1987; Reznick & Yang, 1993). Although offspring number was quantified by dissecting embryos from females, I did not take measurements of offspring size. Life history theory predicts (Williams, 1966; Smith & Fretwell, 1974) females may divert energy from other processes (such as growth) into producing larger offspring if larger offspring have higher survival. If cannibalism increases with female density, larger offspring may be favored at high female densities in a manner similar to life history responses to high predation environments in the guppy *Poecilia reticulata* (Reznick & Bryga, 1987; Reznick & Endler, 1982).

Interestingly, there was no evidence of reduced female growth as a function of female density as predicted in the factorial experiment (see Chapter 2). This discrepancy is possibly due to differences in female densities between the studies. In the sex ratio experiment (see Chapter 1), female densities for each treatment were four, eight, and twelve while in the factorial study the maximum female density was six per pool. It is possible that female density was too low in the factorial experiment to detect an effect. This warrants further investigation.

Males in both studies competed vigorously for females in a manner consistent with Emlen and Oring's (1977) predictions. In addition, male and female density played unequal roles in determining the frequency of agonistic chases and displays (see Chapter 2) and both experiments suggest a relationship between agonistic interference between males and the number of copulations a male attempts. What remains unknown is how changes in male behavior influence reproductive success, which can only be determined through paternity analysis. Male eastern mosquitofish (*Gambusia holbrooki*) successfully copulate with females in only 5% of their attempts (Bisazza & Marin, 1995), suggesting that any decrease in the number of copulations a male attempts is likely to significantly reduce male fitness.

While the number of copulations a male attempts correlates with the amount of sperm recovered from females in the eastern mosquitofish (Evans et al., 2003a), double-mating experiments in guppies (*Poecilia reticulata*) suggest that time to re-mating and courtship rate are the only significant factors affecting paternity (Evans & Magurran, 2001). Furthermore, female cryptic choice (Eberhard, 1996) and post-copulatory interactions between male ejaculates also play a significant role in determining the amount of sperm retained by females and the number of offspring males sire in guppies (Evans et al., 2003b; Pilastro et al., 2004). Future studies need to integrate the effects of pre-copulatory and post-copulatory sexual selection. Although evidence for female choice in mosquitofish is limited to when females are virgins or held for extended periods of time without male contact (Bisazza & Marin, 1991; Bisazza et al., 2001), females may potentially influence male competition for mates through subtle changes in behavior (Bisazza et al., 2001) or patterns of association with other females (Pilastro et al., 2003) that have yet to be rigorously explored.

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