

Eukaryon

Volume 6 *Evolving Connections*

Article 44

3-1-2010

Playing Your Cards Right: How Guppies, *Poecilia reticulata*, Alter their Courtship Tactics in Varying Social Environments

Melissa Schramm
Lake Forest College

Follow this and additional works at: <https://publications.lakeforest.edu/eukaryon>

Disclaimer:

Eukaryon is published by students at Lake Forest College, who are solely responsible for its content. The views expressed in Eukaryon do not necessarily reflect those of the College. Articles published within Eukaryon should not be cited in bibliographies. Material contained herein should be treated as personal communication and should be cited as such only with the consent of the author.

This Senior Thesis is brought to you for free and open access by the Student Publications at Lake Forest College Publications. It has been accepted for inclusion in Eukaryon by an authorized editor of Lake Forest College Publications. For more information, please contact levinson@lakeforest.edu.

Playing Your Cards Right: How Guppies, *Poecilia reticulata*, Alter Their Courtship Tactics in Varying Social Environments

Melissa N. Schramm*

Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Abstract

This study examined how guppies, *Poecilia reticulata*, vary courtship behavior upon encountering social environments differing in sex ratio. I observed males and virgin females in social environments of varying sex ratios and noted the males' display and sneaky copulation attempt frequencies, the time a male pursued the focal female, and female responsiveness. I also examined a focal pair's courtship behavior over a 24-hr period and when in the presence of an audience male at different proximities to them. Males increased their display rates in a social environment with other individuals, significantly so in an all-male environment. Males also increased sneaky copulation attempt rates in the all-male and all-female environments. Females were more responsive to males with other individuals present than when alone with a focal male. Males did not adjust their courtship behavior in response to a single audience male, but they decreased their courtship behavior over 24-hrs. These results suggest guppies may adjust their courtship tactics in different social environments; in so doing they may maximize their mating success.

Introduction

An animal's social environment can affect it in various ways. Individuals commonly gain information from conspecifics and may use this information to adjust their behavior in an advantageous way. Thus, they may strategically alter their behaviors based on potential payoffs of using different tactics in certain social environments. Individuals' social environments can affect their physical development, biochemical hormone levels, learning, and behavior. In their native streams of Trinidad, guppies, *Poecilia reticulata*, frequently encounter varying social environments in which the sex ratio differs on short or long timescales. This study sought to investigate guppies' short-term responses to a changing social environment in terms of their courtship behavior.

Social Environment Influences Development

The social environment in which an animal matures can influence the course of its physical development. For instance, male guppies reared in a social environment where they can interact visually and physically with other conspecifics, and which thus allows for courtship and copulation, mature significantly more slowly than males reared in isolation (Miller & Brooks, 2005). Interestingly, the individuals reared in this environment have a significantly shorter lifespan than those in an environment where only courtship is possible (physical contact was restricted by a Plexiglas partition in the tanks). Following the same trend, individuals reared in the courtship-only environment live significantly shorter than those reared in isolation. This

suggests that males may be able to adjust their developmental rate to better compete with conspecifics in their social environments. The size a guppy reaches by sexual maturity is also influenced by the social environment in which it matures, specifically the density of its social environment; males reared in a higher density social environment are larger at sexual maturity (Rodd et al., 1997). There may, however, be some adverse effects of maturing at a faster rate; males who mature at a faster rate also have a significantly shorter lifespan (Miller & Brooks, 2005).

Social environment may also influence an individual's hormone production, specifically those that in turn influence physical development or behavior. This has been noted particularly in insects: honey bees, *Apis mellifera*, and bumble bees, *Bombus terrestris* (Huang et al., 1998; Bloch et al., 2000). Honey bees require physical contact with conspecifics for inhibition of reproductive development, so that they can become worker bees; individuals with restricted physical contact with conspecifics express significantly higher rates of juvenile hormone (JH) biosynthesis than individuals of a similar age, and had improved foraging ability (Huang et al., 1998). Similarly, young queenless *B. terrestris* have increased JH biosynthesis compared to those with a queen, and they are more reproductively developed (Bloch et al., 2000). Thus, the social environment in which these insects mature may somehow indicate to them that the colony (and thus themselves) will best survive if they do (or do not) invest energy in developing a reproductive system. They can then use this information strategically to alter their sexual behavior.

Animals Learn Through Their Social Environment

Several studies have observed that social environment can also facilitate learning about antipredator behavior, foraging behavior, mate choice, and aggressive behavior. Webb (1980) observed that information about approaching predators may pass through shoals of teleost fish via the diffusion of an alarm substance. Fish may also learn from individuals in their social environment how to orient themselves around their physical environment such that they can find feeding or mating grounds efficiently (Helfman & Shultz, 1984; Laland & Williams, 1997, 1998). When in isolation, individuals take varying paths to food or mating grounds, but after observation of other individuals in their environment, they tend to follow the same path they have observed. So, fish appear to use their conspecifics to obtain information about their environment that will benefit them in their foraging, mating, and general survival.

Thomas's langurs, *Presbytis thomasi*, also forage differently in groups than when alone; Wich & Sterck (2003) observed that male *P. thomasi* give a predator warning call in response to a tiger when in a group but not when alone. When foraging alone, fork-tailed drongos, *Dicrurus adsimilis*, typically give alarm calls in response to aerial predators, but rarely in response to terrestrial predators. Yet, when they follow terrestrially foraging pied babblers for kleptoparasitic opportunities, they give alarm calls in response to aerial and terrestrial predators (Ridley et al., 2007). The pied babblers

*This author wrote the paper as a senior thesis under the direction of Dr. Anne Houde.

seek shelter in response to the alarm call, which provides opportunities for *D. adsimilis* to steal food if they were to give a false alarm call. This demonstrates that social environment is not simply restricted to conspecifics, but it extends to other species with which animals interact, and the response of the social environment can influence behavior as well.

Many animals also learn about mate choice through imitation of observed social interactions (mollies (*Poecilia latipinna*): Schlupp et al., 1994; Schlupp & Ryan, 1997; Witte & Ryan, 1998, 2002; guppies (*Poecilia reticulata*): Dugatkin, 1992; Dugatkin & Godin, 1992, 1993; Briggs et al., 1996; Gobies (*Pomatoschistus microps*): Reynolds & Jones, 1999; Japanese rice fish (*Oryzias latipes*): Grant and Green, 1996). These studies suggest that females may copy the mate choice of others. In other words, seeing another female mate (or interact) with a given male may make the focal female more likely to mate with that same male or a male similar in appearance to him in the future. Focal females may also seek out mating interactions in the same place that they observed a successful courtship (Dugatkin, 1992). Still, some animals, such as feral guppies, appear to avoid locations where they have observed mating interactions rather than copying the choice of others (Brooks & Caithness, 1999). In both cases, the social environment provided information to the individual that was then used in mate selection.

An animal's observation of social interactions within its environment may also affect the degree to which it uses a behavior. For instance, after observing aggressive interactions between conspecifics, Siamese fighting fish, *Betta splendens*, and rainbow trout, *Oncorhynchus mykiss*, can quickly and correctly assess the relative fighting ability of the individuals, which they then use in subsequent interactions with those individuals such that they may avoid unfavorably matched aggressive interactions (Oliveira et al., 1998; Höjesjö et al., 2007). Oliveira et al. (2001) reports a comparable ability in Cichlid fish, *Oreochromis mossambicus*, and looked more closely at the hormones involved during this "eavesdropping." They determined that bystanders' androgen levels are significantly higher 30 mins after observing a fight. This physiological response to aggressive displays could be a method through which *O. mossambicus* acquires information about possible future competitors. These species illustrate that individuals need not learn from their own experiences with competitors if they possess the ability to ascertain the information about said competitors through simple observation; this allows them to avoid entering into direct physical competition with a superior competitor, which could result in injury or death.

The Audience Effect

Individuals can also be influenced simply by the presence of another individual in their environment, commonly referred to as an "Audience Effect." *B. splendens* alter their aggressive displays in the presence of female onlookers (Doutrelant et al., 2001; Matos and McGregor, 2002; Dzieweczynski et al., 2005). Doutrelant et al. (2001) reported that *B. splendens* increase their use of displays that communicate with both males and females and decrease their use of aggressive behaviors, such as bites, in the presence of a female. However, Matos and McGregor (2002) observed an increase in aggressive behaviors in such a situation. In addition, there seems to be an interaction between the sex of the audience and the presence of a nest, which results in differing degrees of aggression (Dzieweczynski et al., 2005). Despite some conflicting observations, it is clear that the presence of an onlooker influences *B. splendens*' behavior. The Audience Effect also influences mate choice decisions in some species, such as the Atlantic Molly, *Poecilia*

mexicana (Plath et al., 2008). In the presence of another individual, male mollies switch their initial mate preference; they spend more time pursuing the female they initially had not preferred when in the presence of a conspecific or heterospecific, *Poecilia ferrosa*, onlooker.

Similarly to *B. splendens*, male ground crickets, *Allonemobius socius*, alter their courtship tactics based on social environment (Sadowski et al., 2001). In a solitary environment, males of this species are more likely to skip certain courtship songs and alter others compared to when they are in an environment with other individuals. Males also respond to an increase in the density of individuals in their social environment by increasing courtship behavior and are more likely to perform all of their songs in courtship interactions.

A great deal of research has shown that the social environment in which a male animal has been raised, or spends most of his adult life can dramatically affect behavior. Animals use their social environment to gain information about their surroundings and often appear to alter their behavior such that they increase their fitness. For instance, the acquisition of courtship songs, though partially innate, is largely learned. Thus, when juvenile male cowbirds, *Molothrus ater ater*, mature without adult males present, they develop different courtship and copulation behaviors and display less intraspecific aggression (White et al., 2002). Accordingly, young male cowbirds that are exposed to adult males are more successful in their courtship efforts than those deprived of young male-adult male interactions (West et al., 1996).

Male guppies may also use the environment in which they mature to gauge what level of aggression and courtship are necessary to maximize mating success (Miller & Brooks, 2005). Males reared in an environment where they were allowed to court and copulate had higher rates of sexual behaviors compared to males that were held in environments that restricted physical contact with females or placed the male in isolation. Thus, it appears that male guppies learn the appropriate level of courtship from the social environment in which they were raised.

Habituation and Familiarity

Habituation and familiarity also influence the use of courtship and aggressive behaviors. When a female is introduced, pairs of male guppies familiar with one another appear to engage in fewer aggressive displays with one another than unfamiliar pairs; males also pay more attention to familiar females, which suggests that familiarity with the social environment affects the level of courtship and aggressive behavior, these responses likely result in a higher mating success (Price & Rodd, 2006). Male guppies that have become habituated to a female also alter their courtship behavior in that they direct significantly more courtship attention to unfamiliar females (Kelley et al., 1999).

Guppies, Courtship and Mating

Guppies are a livebearing species with internal fertilization (Houde, 1997). They are native to the streams and rivers of Trinidad and Tobago, but they have been introduced around the world. Guppies' native streams, most of which are found in mountain forest areas, are generally clear, swiftly flowing, and relatively sterile. Along with their flashy color patterns, guppies are perhaps most well known for their conspicuous courtship of females by males. It is one of the many things that have drawn them into the spotlight for studies of sexual selection and mate choice. Though it may be limited by food in the wet season, guppies are generally reproductively active year round (Houde, 1997).

Guppies may copulate in two ways: a "true" copulation (this occurs with the cooperation of the female

and is preceded by courtship) or a “sneaky” copulation (this occurs without the cooperation of the female) (Houde, 1997). In a “true” copulation, a male will follow a female closely, waiting for an opportune moment to display; this usually occurs as the female slows her swimming. In this moment, the male performs a “sigmoid” display; he forms a rigid S-shape with his body and, quivering, he shows off his coloration. These displays are most frequently met with no response from females, and males continue to persistently pursue that or another female in a similar fashion. However, if a female is responsive, she will orient herself toward the male and glide toward him with her body bent in a C-shape. The male (still displaying) will then circle around the female as she herself turns. With this indication of receptiveness, the male will then attempt to quickly insert his gonopodium, a modified anal fin used for sperm transfer, into the female. Successful contact is followed by rapid circling with the two adjoined, and the male pulls away almost violently. If there was a successful transfer of sperm, the male jerks his entire body up and down for up to a few minutes; though the functionality of this jerking behavior remains unknown, it commonly used to identify a successful copulation. After a successful copulation, the male tends to follow a female closely for several minutes in what is referred to as “mate guarding.”

Male guppies possess an alternative courtship tactic in their repertoire, termed a “sneaky copulation attempt.” This tactic does not employ a sigmoid display nor is it done with the cooperation of the female. Instead, the male follows a female closely and waits for an opportune moment, perhaps when she is distracted, to attempt to insert his gonopodium. These attempts are rarely successful because females usually quickly dart away when they detect the male’s presence (Houde, 1997).

Male guppies adjust their use of these courtship behaviors based on the level of predation (Endler, 1987; Farr, 1975). To minimize their risk, guppies use their sneaky copulation tactic more frequently under higher predation levels, and they use their more conspicuous sigmoid display tactic at times of the day with the least amount of visible predation (Endler, 1987; Magurran & Seghers, 1990). Farr (1975) noted that guppies also decreased their overall rate of courtship in areas with higher predation.

Female guppies also adjust their mate preference in varying levels of predation (Stoner & Breden, 1988). Females typically prefer males that have unique coloration, larger amounts of orange, and a higher display frequency (Eakley & Houde, 2004; Houde, 1987; Kodric-Brown & Nicoletto, 2001). Under increased predation levels, however, they are less likely to copulate with males that are flashy and display frequently, likely because flashy, conspicuous males have a lower survival rate in such environments (Stoner & Breden, 1988).

Social Environment and Courtship Behaviors

Although it has been well established that the social environment in which an animal lives influences its behavior, relatively little research has investigated the immediate plasticity of courtship behavior in response to different social environments. Farr et al. (1974) looked at the influence of the density of conspecifics on the courtship behavior of male guppies. They found that the presence of a single male in the focal pair’s social environment resulted in a marked increase of courtship activities between them relative to the pair is alone; with increased densities there was more variability in these rates of these behaviors. Farr (1976) assessed the influence of adding a male conspecific(s) on male guppy courtship behaviors, but his study did not account for effects such as the order that each individual was exposed to each environment, and it mainly focused on

the differences between strains of *P. reticulata*. The goals of this study were to further investigate how guppies’ courtship behavior is influenced in the short term by their immediate social environment. The study modified and extended Farr’s (1976) design by physically, but not visually, isolating a focal pair of guppies from their social environment. It looked at a wider range of social environments, included measures of female response in the social environments, and considered the effect of time on the courtship interactions of a focal pair. In my experimental design, I isolated the focal pair of guppies from their social environment by placing them in a glass box within the tank; this enabled me to create any sex ratio, and to restrict the amount of harassment of females by males, as well as to easily identify the focal pair.

Specifically this study asked four questions:

- 1) Do male guppies adjust their courtship strategies in social environments with varying sex ratios?
- 2) Do receptive virgin female guppies vary their sexual responsiveness to a male in the presence of other individuals?
- 3) Do males change their courtship behavior in response to a single audience individual in their environment; is this behavior affected by the distance of the audience male?
- 4) Will the level of sexual behavior between a focal pair of guppies remain constant over the time following introduction to one another?

I investigated these questions by manipulating the social environment that focal pairs of guppies were exposed to and measuring courtship behavior. I predicted that:

- 1) Focal males will have the highest rate of sneaky copulation attempts in environments with other males and the lowest rate of sneaky copulation attempts in the environment with only females because sneaky copulations are known to be used more frequently in situations where there is more competition for mates or higher risk of predation (Rodd et al., 1995).
- 2) Males will have the lowest level of courtship behaviors in a social environment where they are alone with a focal female and the highest level of courtship behaviors in a social environment with other individuals because there are more mating possibilities and more competition for mates.
- 3) Males will spend the least amount of time courting the focal female in a social environment with other conspecifics.
- 4) Virgin female guppies will be more responsive to focal males’ courtship efforts in the social environment with other guppies present because they may perceive more opportunities for mating (Evans and Magurran, 2000; Becher and Magurran, 2004).
- 5) Males will display less frequently after 1 hr when isolated with a focal female, and will continue to decrease their courtship behavior over 24 hrs due to habituation.
- 6) Males will increase their courtship behavior in response to an audience male because he perceives increased competition for the local female.

Results

Experiment 1: Male and Female Response to Different Social Environments

To determine if male and female guppies adjust their courtship behavior in response to different social environments, I observed focal pairs in social environments that had varying sex ratios. Though the design of the experiment aimed to limit the amount of interaction between the focal individuals and their social environment, there were still some remnant courtship interactions between them. I

observed courtship behavior between the focal male and female, as well as between the individuals in the social environment and the focal male and female. Male-male interactions were infrequent between the focal male and the males in the social environment; similarly, female-female interactions were even more infrequent than male-male interactions.

Statistical analysis revealed that male display rate to a focal female differed among the social environments ($F_{3, 66} = 3.4555$, $p = 0.0213$). Post hoc pairwise comparisons indicated that males displayed to a non-virgin focal female significantly more frequently in an all-male environment than when alone with the focal female (Tukey Kramer HSD, $p < 0.05$, Fig. 4). A similar increase in display frequency was seen in the male-female environment, but this was not found to be significant. Males' overall display rate (which included displays to non-focal females) also differed between social environments ($F_{3, 66} = 15.5389$, $p < 0.0001$). Specifically, males displayed significantly more frequently overall when in the all-female environment than when in the all-male environment or when alone with the focal female (Fig. 5) and when in the male-female environment than when alone with the focal female (Tukey Kramer HSD, $p < 0.05$, Fig. 5). This was likely a simple response to the increased number of females in the social environment. Males also spent significantly less time courting the non-virgin focal female in the all-female environment than when alone with her ($F_{3, 66} = 3.9856$, $p = 0.0114$, Tukey-Kramer HSD $p < 0.05$, Fig. 6).

Male sneaky copulation attempt rate also differed between social environments ($F_{3, 66} = 5.9893$, $p = 0.0011$). Males made significantly more sneaky copulation attempts directed towards the non-virgin focal female when in the all-male environment than when in the all-female environment or when alone with the focal female. Male guppies also made significantly more sneaky copulation attempts in the male-female environment than when alone with the focal female (Tukey Kramer HSD $p < 0.05$, Fig. 7).

Contrary to the males' behavior when courting non-virgin focal females, males courting virgin focal females made significantly more sneaky copulation attempts when the pair was alone than when in a male-female social environment ($F_{1, 28} = 4.9503$, $p = 0.0343$, Fig. 8). To test the hypothesis that this difference may be due to an effect of female responsiveness on male behavior, I investigated whether female responsiveness and male sneaky copulation attempt frequency were correlated. Linear regression analysis indicated that they are negatively correlated in both the alone environment and the male-female environment ($F_{1, 13} = 4.987$, $p = 0.04375$, Fig. 12A; $F_{1, 13} = 5.121$, $p = 0.04140$, Fig. 12B, respectively). While males' sneaky copulation behavior varied, males did not spend a significantly different amount of time pursuing the virgin focal female ($F_{1, 28} = 0.1603$, $p = 0.6919$) nor was their display frequency significantly different when alone with her or in the male-female environment ($F_{1, 28} = 0.2389$, $p = 0.6288$).

Virgin females also had different levels of responsiveness to male displays in the different social environments ($F_{1, 28} = 11.1730$, $p = 0.0024$). They responded significantly more to the focal males' displays when in the male-female environment than when alone with the focal male (Tukey Kramer HSD $p < 0.05$, Fig. 8). In fact, all but one of the successful copulations that occurred during observations took place in the male-female social environment. The one virgin that copulated when alone with a male did so shortly after copulating in the male-female social environment.

Controlling for a "Box" Effect

To determine whether my experimental setup of a tank within a tank affected the level of male courtship behavior I compared male courtship behavior in the experimental setup to their behavior when there was no physical barrier between the focal pair and social environment. The experiment confirmed that males did not display at a significantly different frequency when they were allowed physical contact with fish in their social environment versus when they were not ($F_{1, 21} = 0.7489$, $p = 0.3966$). Not surprisingly, the frequency of sneaky copulation attempts was significantly greater when physical contact was allowed with fish in the social environment ($F_{1, 21} = 5.5451$, $p = 0.0283$). This is likely due to the reduced number of females physically available to the male when the focal pair is separated from the fish in his social environment by a smaller tank, thereby reducing the frequency that the focal male is in position to sneak. Time spent courting a focal female could not be compared because the females were indistinguishable from one another.

Audience Effect

I also examined the effect of a single audience male on the courtship behavior of a focal male. I found no significant difference in male display frequency ($F_{2, 40} = 0.0334$, $p = 0.9672$), frequency of sneaky copulation attempts ($F_{2, 40} = 0.4668$, $p = 0.6304$), or the time males spent courting a focal female ($F_{2, 40} = 1.3300$, $p = 0.2759$) between environments where the focal male was alone with the focal female, where an audience male was present within 15 cm, and where the audience male was kept 15cm away (Fig. 12).

Habituation Effect

To test whether guppy courtship changed with habituation with a focal female, I allowed the males to spend 25 hrs with the female and recorded the frequency of courtship behavior (displays, sneaks, and time) upon meeting, 1 hr later, 24 hrs later, and 25 hrs later. There was a significant effect of time since meeting on courtship behavior, with the highest display rates and sneaky copulation rates upon meeting and lower rates subsequently (significantly so for 1 hr and 25 hrs but not significantly so for 24 hrs) (Displays: $F_{3, 69} = 2.2682$, $p = 0.0883$, Tukey Kramer HSD < 0.05 ; Sneaks: $F_{3, 69} = 2.8861$, $p = 0.0418$, Tukey Kramer HSD < 0.05 , Fig. 10). There was no significant change in the time a focal male spent courting the focal female throughout the habituation period. Figure 11 shows that displays decrease, sneaks increase, and time spent pursuing a female does not change during habituation.

Discussion

Males' and Virgin Females' responses to different social environments

I found that male and female guppies adjust their courtship behavior in different social environments. As hypothesized, as the number of males increased in the social environment, I observed that focal males increased their sneaky copulation attempt rate (Fig. 7) and display rate (Fig. 4) toward non-virgin focal females. The overall display rate (displays to both the focal females and those in the social environment) increased in an all-female environment (Fig. 5). Males also spent less time courting the focal female in the all-female environment (Fig. 6). Concerning virgin females, males made more sneaky copulation attempts toward a virgin when alone with her than when in the presence of other conspecifics (Fig. 9), but virgin females responded more to males in an environment with conspecifics than when alone with a focal male (Fig. 8).

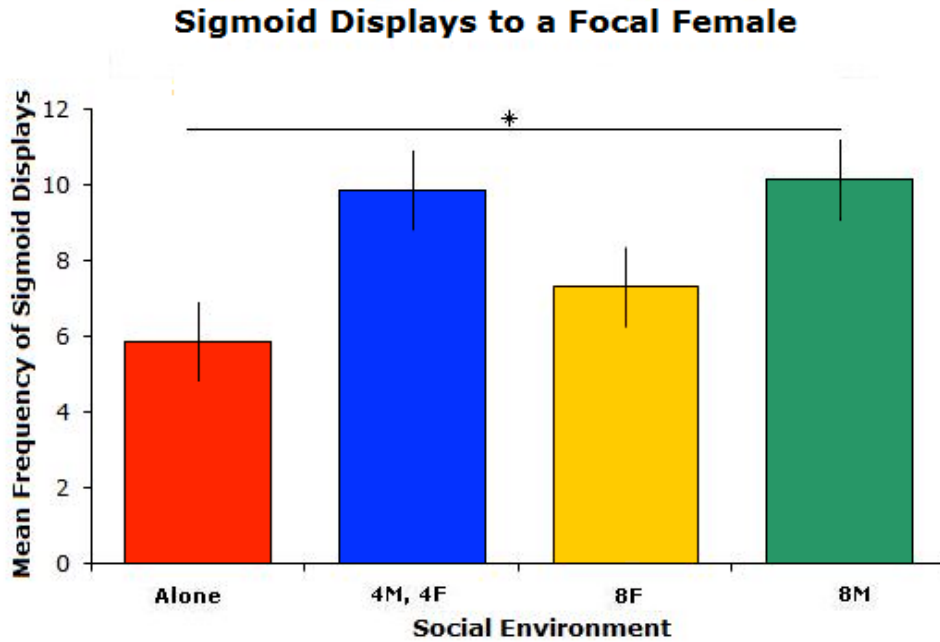


Figure 4. Sigmoid Displays to a Focal Female in Different Social Environments. Males displayed to a non-virgin focal female significantly more frequently in an all-male environment than when alone with the focal female ($F_{3, 66} = 3.4555$, $p = 0.0213$, Tukey < 0.05).

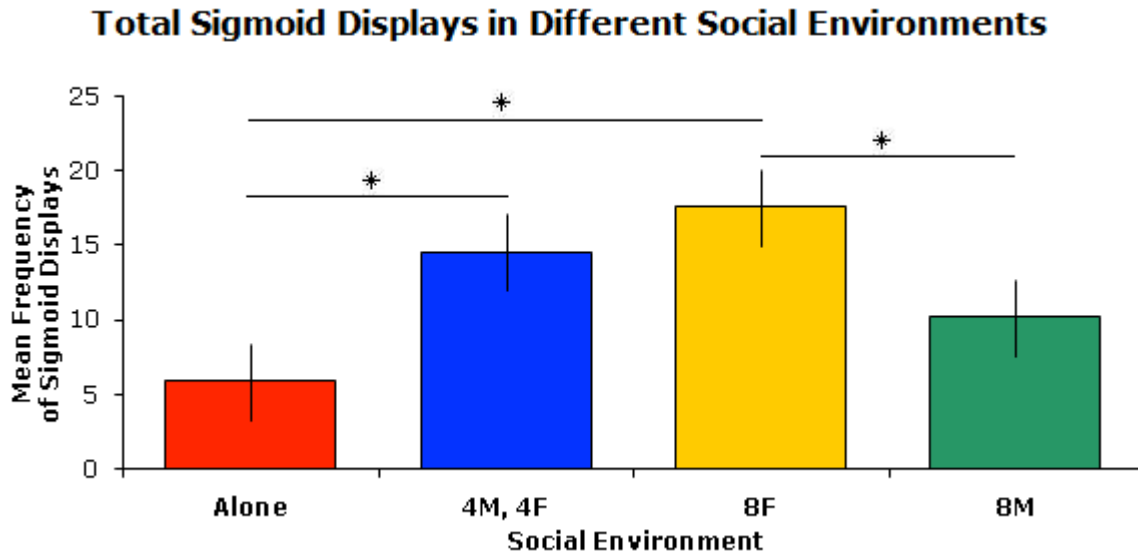


Figure 5. Total Sigmoid Displays in Different Social Environments. Males displayed more frequently overall when in the all-female environment than when alone with the focal female or in the all-male environment. Males also displayed more in the male-female environment than when alone with the focal female. ($F_{3, 66} = 15.5389$, $p < 0.0001$, Tukey < 0.05).

Time Spent Pursuing a Focal Female

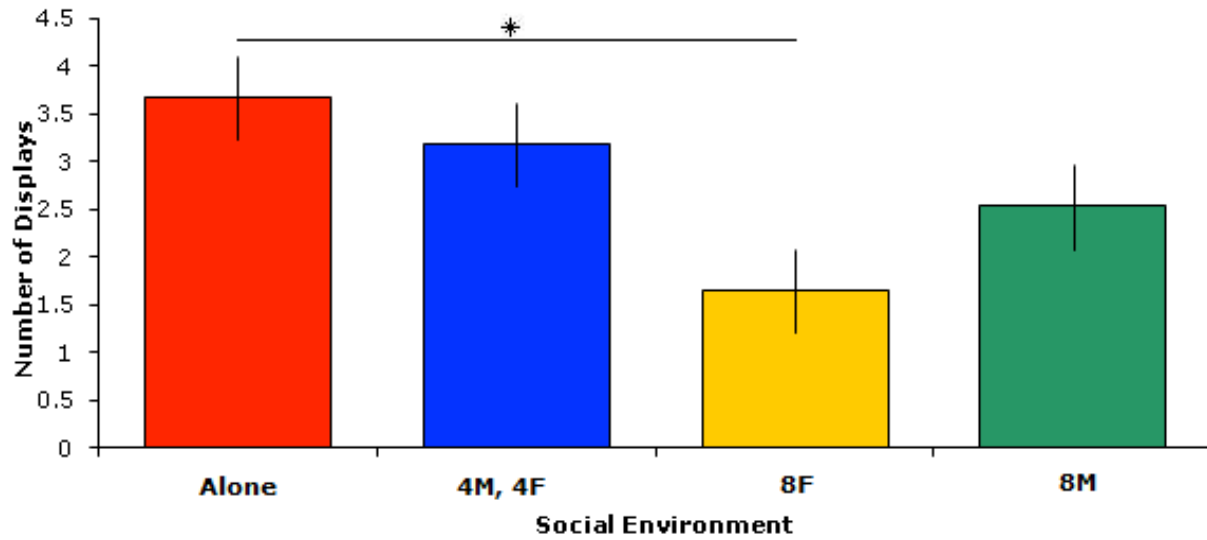


Figure 6. Time Focal Males Spent Pursuing a Focal Female. Males spent significantly less time courting the non-virgin focal female in the all-female environment than when alone with her ($p=0.0114$, $F=3.9856$, $DF=3$, Tukey=0.3874).

Sneaky Copulation Attempts on Focal Females

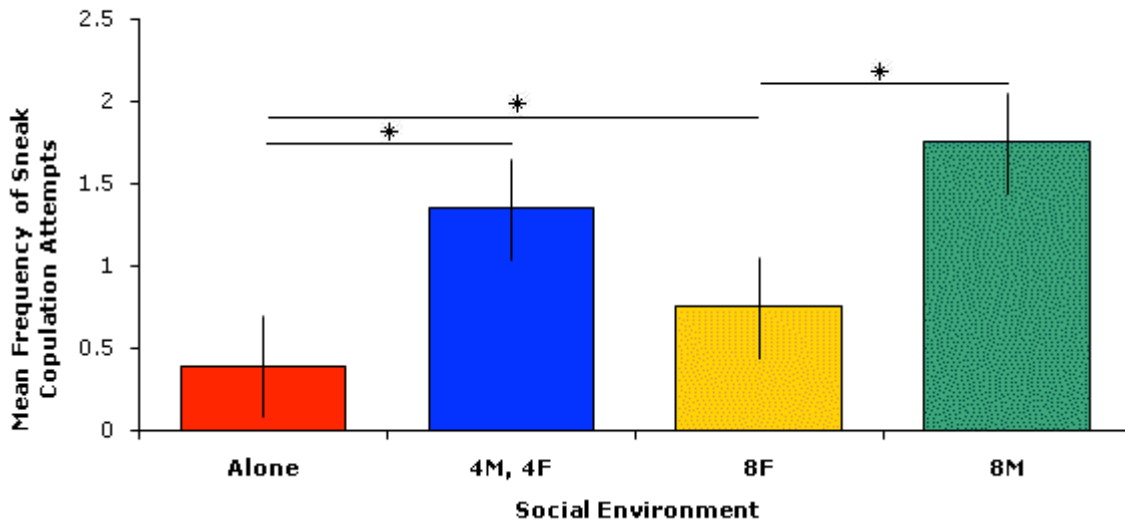


Figure 7. Mean Sneaky Copulation Attempt Rate on Focal Females. Males made significantly more sneaky copulation attempts towards the focal female when in the male environment and the male-female environment than when alone with the focal female or in the all-female environment ($F_{3,66}=5.9893$, $p=0.0011$, Tukey < 0.05).

Female Responses 2 or Greater: Total Displays

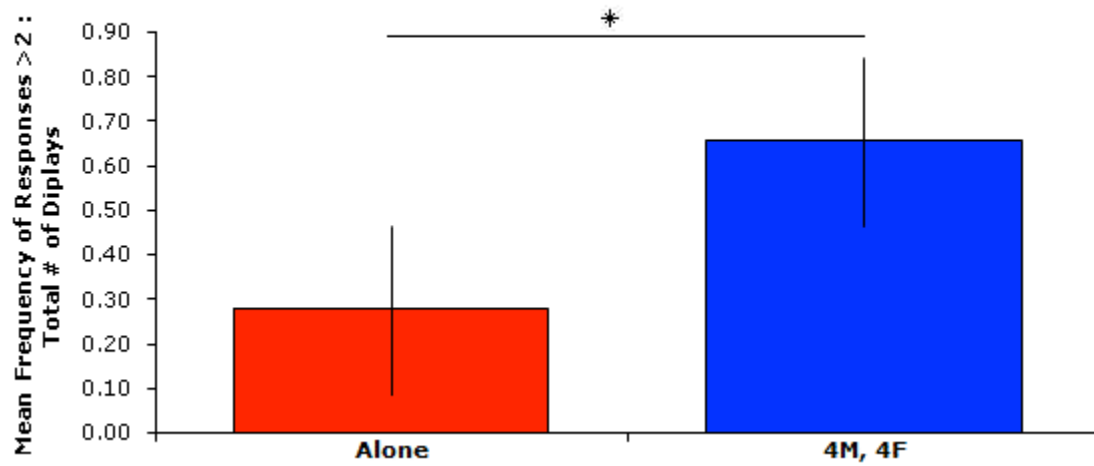


Figure 8. Virgin Female Responsiveness to Male Sigmoid Displays. Virgin female guppies were more responsive to the focal male's sigmoid displays when in the male-female environment than when alone ($F_{1,28}=11.1730$, $p=0.0024$, Tukey <0.05).

Sneaky Copulation Attempts on Virgin Focal Females

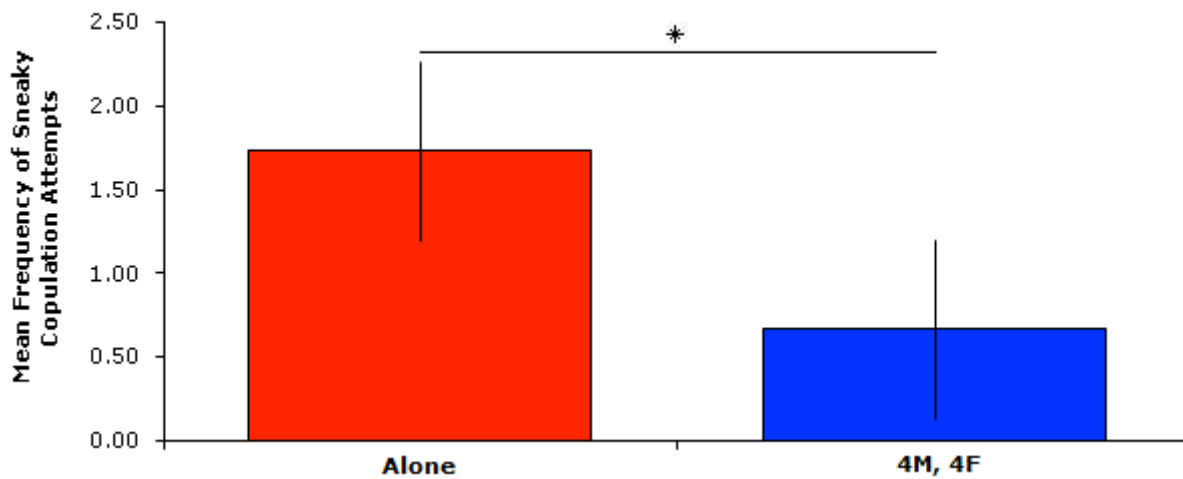


Figure 9. Sneaky Copulation Attempt on Virgin Focal Females. Males made significantly more sneaky copulation attempts on a virgin focal female when alone with her versus when in the male-female environment ($F_{1,28}=4.9503$, $p=0.0343$, Tukey <0.05).

Habituation of a Focal Pair

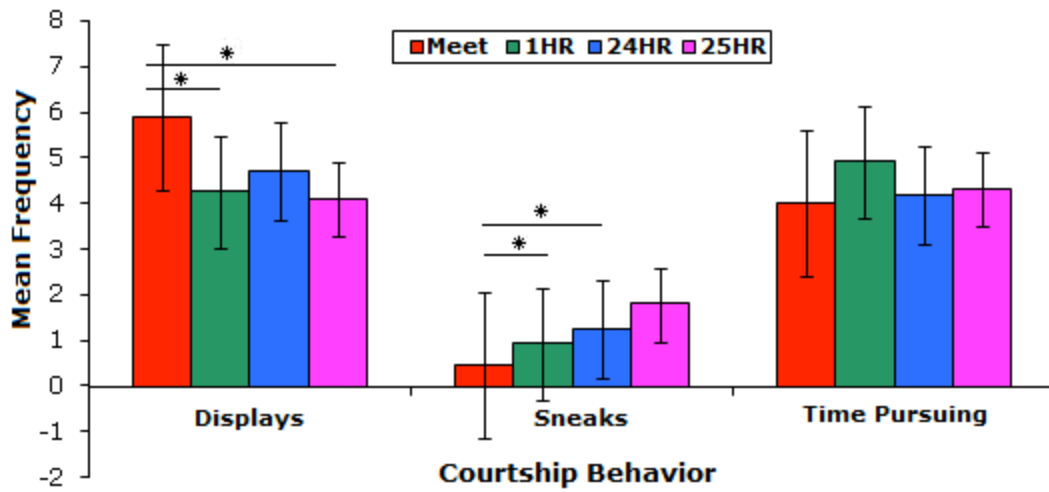


Figure 10. Male Courtship Behavior Over a 25-hr Period. Male guppies displayed and attempted to sneak copulate more frequently after 1 hr and 25 hrs than when they had just met (Displays: $F_{3,69} = 2.2682$, $pd = 0.0883$, Tukey < 0.05 ; Sneaks: $F_{3,69} = 2.8861$, $ps = 0.0418$, Tukey < 0.05). There was no significant difference in the time a focal male spent courting the focal female throughout the habituation period.

Habituation of A Focal Pair

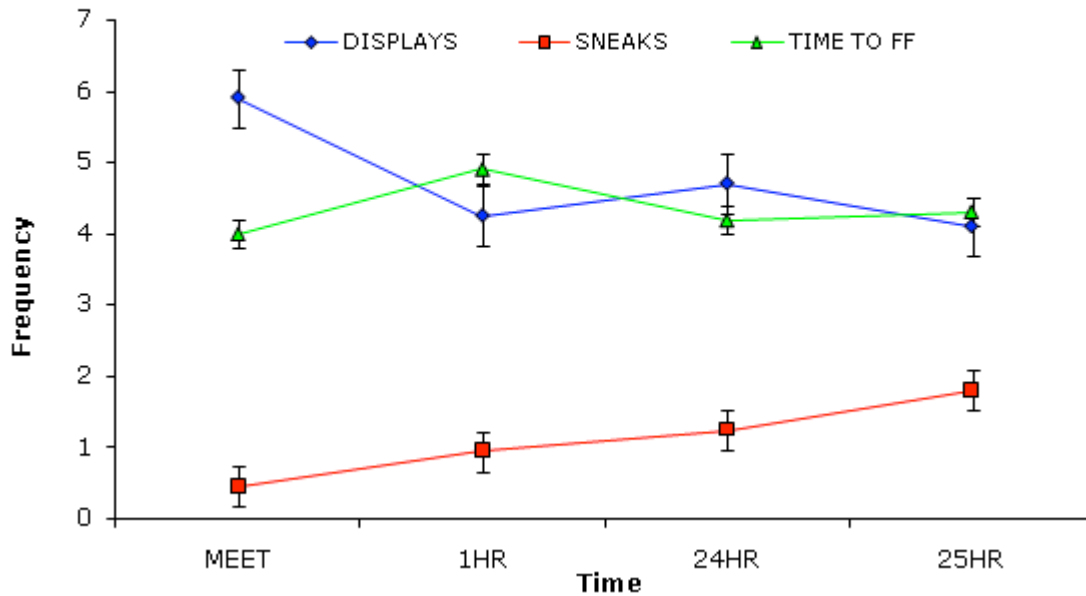


Figure 11. Male Courtship Behavior Over a 25-hr Period. Habituation of a focal pair showed an increase in sneaky copulation attempts and an inverse relationship between display frequency and time spent pursuing the focal female (Displays: $F_{3,69} = 2.2682$, $pd = 0.0883$, Tukey < 0.05 ; sneaks: $F_{3,69} = 2.8861$, $ps = 0.0418$, Tukey < 0.05).

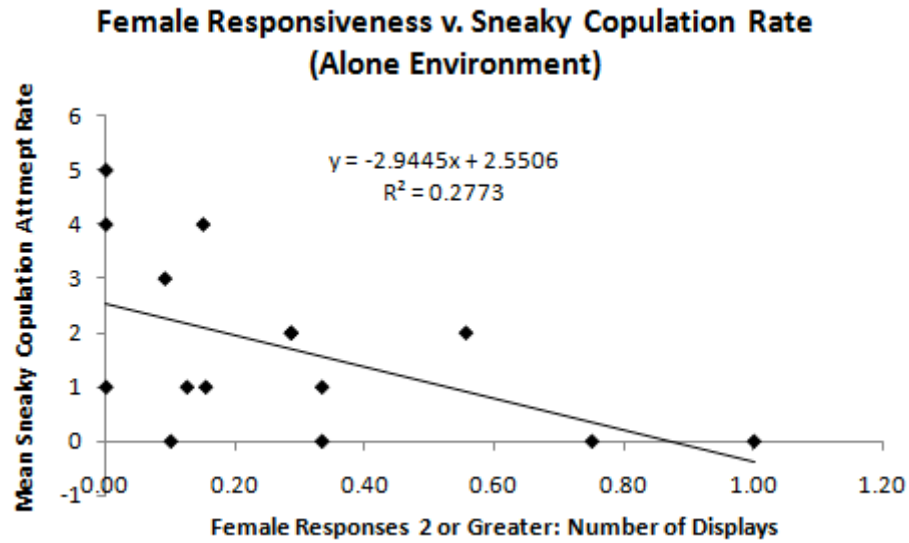


Figure 12A. Correlation of Sneaky Copulation Attempt Rate and Female Responsiveness in Alone Environment. Frequency of sneaky copulation attempts negatively correlated with female responsiveness ($F_{1,13}=4.987$, $p=0.04375$).

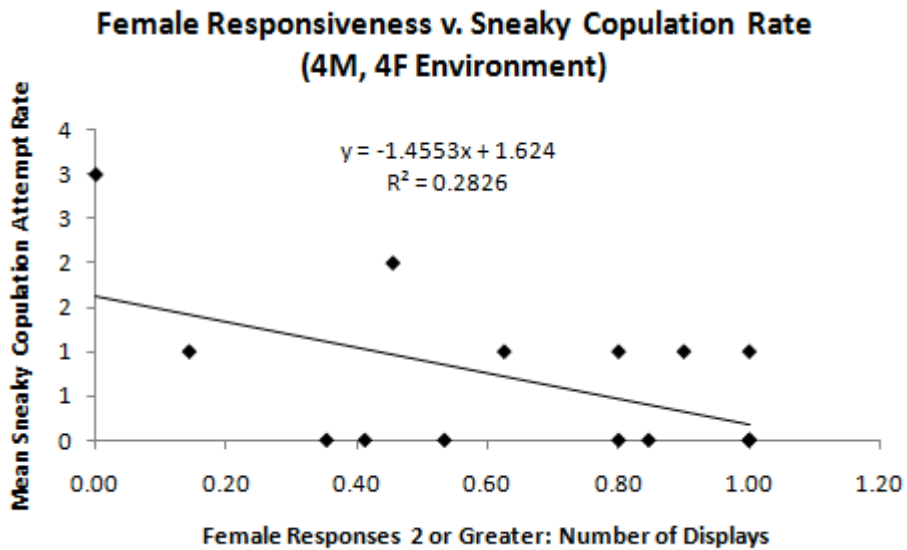


Figure 12B. Correlation of Sneaky Copulation Attempt Rate and Female Responsiveness in Male/Female Environment. Frequency of sneaky copulations attempts was negatively correlated with female responsiveness ($F_{1,13}=5.121$, $p=0.04140$).

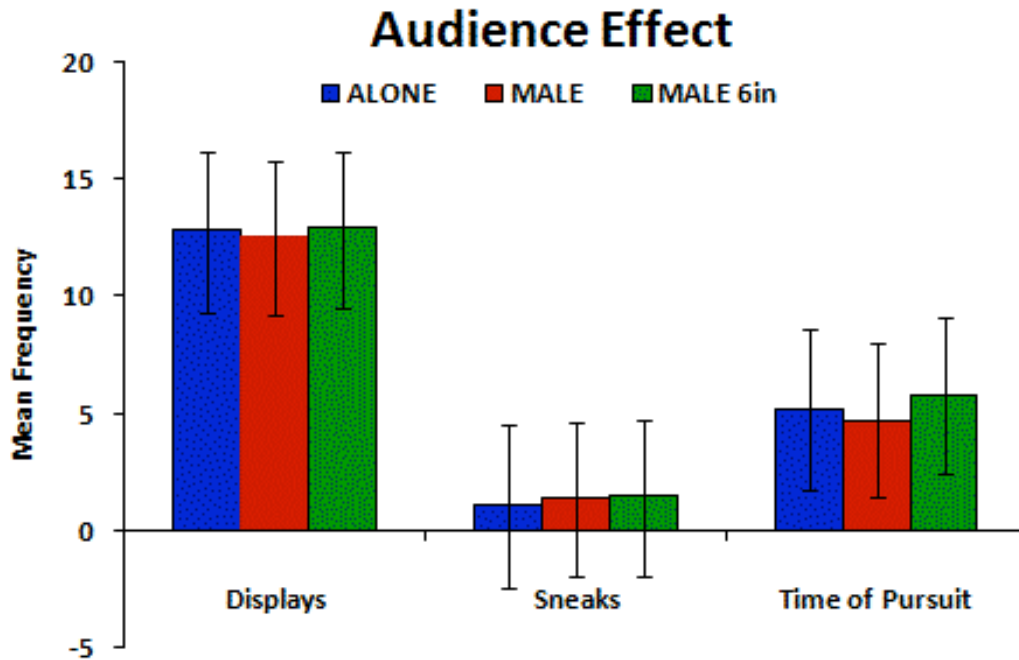


Figure 13. Effect of an Audience Male on the Level of a Focal Male's Courtship Behavior. The presence of a single rival male conspecific had no significant effect on the focal male's display rate, sneaky copulation attempt rate, or time he spent courting the focal female.

The increase in male courtship activity, sigmoid displays and sneaky copulation attempts, toward a focal female in social environments with other males is likely due to a perception of increased sexual competition. This type of behavioral change has been noted elsewhere: Farr (1976) demonstrated that focal male guppies increase their display rate to the focal females in the presence of females and a single rival male, compared to that when he is alone with female, and Evans & Magurran (1999) showed that males raised in a male-biased social environment made sneaky copulation attempts more frequently than they displayed. Thus, it appears the presence of males encourages higher courtship levels in male guppies. Magellan & Magurran (2007) suggest that male guppies have an established 'mating profile' that is determined by the social environment in which they were raised; they characterized males as having a high, medium, or courtship rate. However, while males characterized as "high" had consistently higher levels than those characterized as medium or low, all three groups varied their levels of courtship behavior between the male-biased, female-biased, and environments of an equal operational sex ratio (OSR). Sneaky copulation rate increased and display rate decreased in a male-biased OSR. Miller & Brooks (2005) also recognized that a male's mating behavior was strongly influenced by the social environment in which he spends most of his time, but they noted that males' levels of sexual behavior increased as the rearing environment allowed more sexual activity, in terms of the amount of physical contact allowed between the focal male and individuals in his social environment. It was highest when physical contact (and thus copulation) was possible, and decreased in the courtship-only environment (visual

contact but physical contact was possible) and solitary environment.

While these studies considered the social environment in which males spend extended periods of time, my study looked at short-term adjustments to social environment. The increase in courtship behavior when in the presence of males suggests that a male guppy can assess his social environment and adjust his courtship efforts accordingly. Males may perceive that there is more sexual competition and increase their courtship efforts accordingly to contend with this competition/improve the probability of success.

Similarly, males adjust their courtship tactics in environments with differing levels of predation. In environments with higher levels of predation, male guppies use their conspicuous sigmoid displays while they increase their use of sneaky copulation attempts (Endler, 1987; Magurran & Seghers, 1990). Addressi & Visabergghi (2001) described social facilitation as "the increased probability of performing a class of behaviors in the presence of a conspecific performing the same class of behavior." Thus, according to the concept of social facilitation, a focal male would be more likely to perform the same courtship behaviors, which is consistent with the observations made in this study. This, however, seems only a proximate cause of the behavioral adjustments males make when encountering a different social environment. Ultimately, variation and plasticity in male courtship behavior likely reflects effects on probability of mating and the reproductive success of individuals who make these adjustments.

Males also adjusted their courtship behavior in an all-female environment. In line with my prediction, while in this environment, males displayed more overall (to focal and

nonfocal females) and spent less time courting the non-virgin focal female (Fig. 5, Fig. 6). The simplest explanation for these adjustments in courtship behavior is that the focal male increased his courtship efforts simply because there were more females available to court, and indeed, the increase was due entirely to displays performed to females outside the box (Fig. 4, Fig 5). In addition, Evans & Magurran (2007) noted that males raised in a female-biased social environment displayed to females more than they attempted sneaky copulations. Though their observation may also imply a long-term strategic decision by males rather than simply being a result of the fact that more females are present, it still appears that the presence of females in the social environment may influence male courtship behavior such that it encourages sigmoid displays. In both social environments with females in my study, males displayed significantly more than when in the all-male environment and alone environment. Focal males also spent less time courting a focal female in the all-female environment than when alone with her in my study; this is most likely because he occupied his time courting the other females in his social environment. These observations suggest that males may not display at maximum frequency when alone with an individual female, but are capable of displaying more, and they choose to do so when in the presence of other individuals, male or female. Alternatively, males may only display when they are positioned correctly near a female; thus a social environment with more females would present more opportunities for the male to display and account for the increased display rate in the all-female environment.

Initially, the observation that focal males made more sneaky copulation attempts towards a virgin female when alone with her than when in the presence of other individuals appeared to contradict the finding that males increased their sneaky copulation attempt rate toward a non-virgin female when there were more males in the environment versus when he was alone with her. The difference could be reconciled, however, if the responsiveness of the focal female is taken into consideration; virgin females were more responsive to male courtship efforts when in a social environment with other fish present than when alone with him (Fig. 8). Thus, the difference in sneaky copulation attempt rate to virgin females when alone versus with other fish may reflect a response by males to the difference in female responsiveness; males are more likely to sneak when females are less responsive. And, indeed, Figures 12A and 12B show a negative correlation between sneaky copulation rate and female responsiveness both when in the alone environment and when in the male/female environment.

As predicted, virgin females increased responsiveness in a social environment with other guppies (Fig. 8). While this observation is difficult to explain, I suggest that females might be more willing to copulate with a male if there are other males in her environment so that she could copulate again shortly after and improve the probability that her offspring have advantageous genetic information (Jennions and Petrie, 2000; Pitcher et al, 2003; Evans & Magguran, 2000). Female guppies commonly use the sperm of multiple males to sire their young; a median of two males fathered each brood in paternity studies done by Becher & Magurran (2004). Social facilitation cannot be applied in this situation because the females in the social environment were non-virgins, and therefore non-responsive to male courtship efforts. It would be worthwhile to investigate how a virgin focal female behaves when the females in her social environment are also virgins, and, thus, she is observing other responsive females and or successful courtship interactions.

Habituation of a Focal Pair

Consistent with my hypothesis, the habituation studies using an isolated focal pair of guppies showed a significant decrease in display frequency after one hr (Fig. 10). McFarland (1985) defines habituation as phenomenon in which repeated applications of stimulus often result in decreased responsiveness (p. 316). Thus, a decrease in male display frequency after he has associated with a female for an extended period of time equates to the pair becoming habituated with one another. Although there was a slight increase in displays 24-hrs after the focal pair was introduced, this difference was not significant. In fact, there was no significant adjustment in male display rate after the 1-hr mark (Fig. 10). In line with this, Kelley et al. (1999) observed that males direct significantly more of their courtship efforts toward unfamiliar females in their environments. Zajitschek et al. (2006), however, observed no difference in the courtship efforts directed toward a familiar (physically associated for 21 days) and unfamiliar female. Still, it appears that males do not display toward a focal female at a consistent rate that is comparable to the rate upon introduction after they have become habituated with one another. Once a male recognizes that the female is unresponsive, and thus displaying is less profitable, he seems to reduce his display rate once and then maintains it at that level.

Unexpectedly, and somewhat out of line with the concept of habituation, males increased their rate of sneaky copulation attempts as they spent more time with the focal female. After one hr, males significantly increased their rate of sneaky copulation attempts from that upon first meeting the focal female (Fig. 10). Following that, however, there was not a significant change in the rate of sneaky copulation attempts. Based on these observations, it appears that habituation initially results in a decrease of sigmoid displays and an increase in sneaks but only to a certain point. This initial change in courtship behavior could be due to the male's persistent efforts being unrewarded, and thus, he decreases his use of more energetically demanding sigmoid displays and increases his use of sneaks, which likely requires less of an energy investment. This change could be explained by a male's perception of probability of success; initially, sigmoid displays have a higher perceived probability of success and sneaking a very low one, but the probability of success for sigmoid displays decreases as the male determines that the female is unresponsive.

The Audience Effect

Contrary to my hypothesis, males did not significantly adjust their courtship behavior in the presence of a single audience male, regardless of proximity. An audience effect has been observed in other species, such as *Betta splendens*; the presence of a male rival encourages more bites from the focal male *Betta* and less time near the rival than when there is a female conspecific present (Matos & McGregor, 2002). Furthermore, a female audience increases a focal male's intensity of conspicuous displays that are used for communication with both males and females and decreases his use of highly aggressive displays that are used solely for communication with males (Doutrelant et al., 2001). Male guppies, however, do not seem to be influenced by the presence of a single rival male. The disparity here likely results from the fact that *B. splendens* are territorial, solitary fish, and guppies are non-territorial, social fish. While the presence of 4+ males in a male guppy's social environment promotes an adjustment in his courtship behavior, as previously discussed, the presence of a single male does not appear to significantly influence courtship tactics. The audience effect should be further investigated with increasing numbers of conspecifics to identify how many

audience conspecifics are required for a male to adjust his courtship behavior. It is possible that there are a minimum number of individuals required to be in the social environment before a male guppy changes his courtship behavior. If this number is determined, the concept of proximity could be revisited in studies of males' strategic adjustment of courtship behavior.

Limitations of Results Due to Experimental Design

The frequency of sigmoid displays and sneaky copulation attempts toward the focal female may have been elevated in my experiment due to the experimental setup because the box inside of the tank kept the focal female in the proximity of the focal male. In addition, the overall sneaky copulation attempt rate was low in my experiment because focal males did not have physical access to females in their social environment. While these limitations exist, they do not weaken the conclusion that the males' reactions to a changing social environment, only that the exact levels of courtship behavior.

Conclusions

Based on the results of this study, I conclude that both male and virgin female guppies adjust their courtship behavior as they encounter varying social environments. My study proposes that not only do young males use their rearing social environment as an indicator of mate competition to adjust their reproductive strategies and maturation rate (Schultz & Warner, 1989; Rodd et al. 1997), but mature males use their immediate social environment to decide how frequently to use their sneaky tactic vs. their display tactic; these adjustments likely maximize their reproductive fitness and immediate fitness. However, while this study suggests evolutionary causes and consequences of males adjusting courtship behavior, it did not assess whether or not this change resulted in reproductive (or overall) fitness different from that of a male who exhibits consistency in his courtship behavior rates. This would be a good direction for future studies to pursue.

Methods

Study Organisms

Fish used in this study were obtained from a stock population descended from wild guppies collected in 1999 and 2004 from the Paria River in Trinidad. Fish stocks were maintained in groups of approximately 30 adults plus offspring in 25 or more 40-L aquaria, with out-breeding maintained by transfers of fish among stock tanks every 1-2 generations. This resulted in a breeding population of more than 500 adults at any time. Fish for observations were held in 40-liter aquaria that contained filtered and conditioned tap water, gravel, and java moss, *Vesicularia dubayana*. Tanks used during observations contained only gravel, a heater, and a sponge filter (filters were removed during observations in experiment 2). All aquaria used for observations were held at approximately 22-25°C, and were exposed to a 12:12h light:dark cycle, illuminated with 15 W broad-spectrum fluorescent lights; light intensity was reduced with a layer of white paper. Fish were visually isolated from those in other tanks during observations by cardboard surrounding the tanks on three sides. Males used in the study were approximately 2.5 cm long in total length and females were chosen to be one to be approximately 1.5 times the length of the males. Non-virgin female and male test subjects were chosen arbitrarily from stock tanks. Virgin females were separated from males before they reached sexual maturity and maintained in single sex groups; they had interacted with a male once previously for 30 mins in another experiment but had not been inseminated. Fish were fed twice daily with TetraMin (Tetra, Melle, Germany) tropical flakes in the morning and brine shrimp nauplii in the afternoon. To minimize the effect of familiarity, focal males, focal females, and fish in their social environments were all taken from different holding tanks. All observations began within 30 mins of lights-on: between 9:00 and 11:00 a.m. during May-July 2008 in Lake Forest, IL. Fish were given

a small portion of flakes during the initial acclimation period to encourage courtship behavior rather than foraging (Abrahams, 1993).

Measures of Courtship Behavior

In this study, male courtship behavior included sigmoid displays, sneaky copulation attempts, and the time a male spent pursuing a female. I recorded the rates at which males used these behaviors in each of the environments. Sigmoid displays met the following criteria: the male positioned himself in the line-of-sight of a female, curved his body into an S-shape and maintained his display for at least a second. Sneaky copulation attempts were identified with the following indicators: they were preceded by the male following the female closely, were not preceded by a display, visible thrusting of the gonopodium toward the female's gonopodial duct, and followed by a darting separation of the two fish. Successful copulations were noted; I used male "jerking" behavior as the ultimate indicator of a successful copulation (Houde, 1997). Time of pursuit included the time a male spent displaying or attempting to sneak copulate with a female and the time a male followed a female, marked by his mimicking her directional movements. Focal female courtship behavior was ranked on a scale of 0-5 (Houde, 1997) during observations in environments five and six of Experiment 1, where 0= no response, 1= the female oriented herself toward the male, 2= the female glided in a C-shape toward the male, 3= the male circled the female as she rotates to face him, 4= the male attempts to insert his gonopodium into the female. The copulation is unsuccessful; there is no transfer of sperm, and 5= the male inserts his gonopodium into the female, successfully transferring sperm; this is marked by male "jerking" behavior (Liley, 1966).

EXPERIMENT 1: Male Courtship Behavior and Virgin Female Responsiveness in Different Social Environments

I placed a focal pair of guppies (a male and non-virgin female) in a 19-liter tank (30 cm x 15 cm x 20 cm) contained in a 57-liter tank (60 cm x 30 cm x 30 cm) with one of six social environments, consisting of 1) no other fish in the outer aquarium, 2) 4 female and 4 male guppies in the outer aquarium, 3) 8 female guppies in the outer aquarium, 4) 8 male guppies in the outer aquarium 5) a virgin focal female (10-15 wks old) and no other fish in the outer aquarium, or 6) a virgin focal female, 4 non-virgin females and 4 males in the outer aquarium (Fig. 1). The focal pair could see the fish in their social environment and interact with them through the glass, but the tank prevented direct contact and eliminated olfactory cues. In treatments 1-4, the pairs were given 10-20 mins to acclimate to their physical and social environments before I observed male courtship behavior for 10 mins. In treatments 5 and 6, the virgin female was given 10 mins to acclimate to her social and physical environments before a focal male was introduced to prevent copulation from occurring prior to the observation session. In all treatments, I recorded the frequency of sigmoid displays performed by the focal male to the focal female and those to females in the social environment, sneaky copulation attempts on the focal female, male-male displays, and the time the focal male spent pursuing the focal female. In treatments 5 and 6, the virgin focal female's response to each display from the focal male was ranked on a scale of 0-5 (Houde, 1997). Twenty-four focal males were each observed in environments 1-4; 15 focal males were observed in both environments 5 and 6. Males were observed in each environment in a randomized order so as to minimize order effects, and study subjects were returned to a stock tank after observations.

Controlling for the Effect of Experimental Design

In order to determine if the design of my experiment affected level of male courtship behavior, I conducted an additional experiment in which the focal pair was physically but not visually separated from the social environment. Males were observed 1) with a non-virgin focal female in a small tank contained in a larger tank (as previously described) with 4 males and 4 non-virgin females in it and 2) in the larger tank with 4 other males and 5 non-virgin females. The latter allowed for physical contact between the focal male and his social environment. In this experiment, I compared the overall display rate and sink rates of males between the environments.

EXPERIMENT 2: Male Courtship Behavior in the Presence of an Audience Male

I also asked the question, does a focal male adjust his courtship behavior in the presence of a single audience male and does the distance from the audience male affect this? To answer this question, I placed a focal pair of guppies, a male and a non-virgin female, in a 19-liter tank (30 cm x 15 cm x 20 cm) contained in a 57-liter tank (60

cm x 30 cm x 30 cm) with one of three social environments 1: alone (no audience male outside of the inner tank), 2: near male (audience male was kept 0-6 cm outside of the inner tank), 3: far male (the audience male was kept 15-30 cm away from the inner tank) (Fig. 2). Audience males were separated from the inner tank by a plastic barrier. To keep the number of plastic barriers between the focal pair and the audience male constant in all environments, the closest barrier was removed when the audience male was placed behind the far barrier. Previous trials indicated that the focal pair could see the fish in their social environment through the barriers, but the tank and barriers inhibited direct contact and olfactory cues. The pairs were given 10 mins to acclimate to their social environment before I observed male courtship behavior for 10 mins. I recorded the frequency of sigmoid displays, sneaky copulation attempts, male-male displays, and the time the focal male spent pursuing the focal female. Twenty males were each observed in all the three environments in a randomized sequence so as to eliminate order effects. Study subjects were returned to their experimental housing tanks after observations were completed and were not re-used.

EXPERIMENT 3: Habituation of a Focal Pair in Isolation

Since non-virgin females are consistently unresponsive to males' courtship displays, I set up an experiment to determine if males would maintain a constant frequency of courtship behaviors, or if their frequency would decline with habituation to the focal female. I tested this hypothesis by placing a male and a non-virgin female in a 19-liter tank (30 cm x 15 cm x 20 cm) contained in a 57-liter tank (60 cm x 30 cm x 30 cm) (Fig. 3) and giving them 10 mins to acclimate to their environment. During a 10-min observation period, I recorded the male's courtship behavior in terms of the sigmoid display frequency, sneaky copulation attempt frequency, and the amount of time he spent pursuing the focal female. The pair was observed an hr after the initial introduction and acclimation period and the same times the next day. Fish were given a small portion of flake food prior to observation on both days. Twenty focal pairs were observed in this study, and all fish were returned to a stock tank after observations were completed.

Statistical Analyses

All analyses were conducted using JMP 5.1. I tested for differences in sigmoid display frequency, sneaky copulation attempt frequency, time spent courting, virgin female responsiveness between social environments using ANOVA to compare means among treatments. Male identity was added as a blocking factor in each analysis. I used the Tukey-Kramer procedure to carry out post-hoc comparisons.

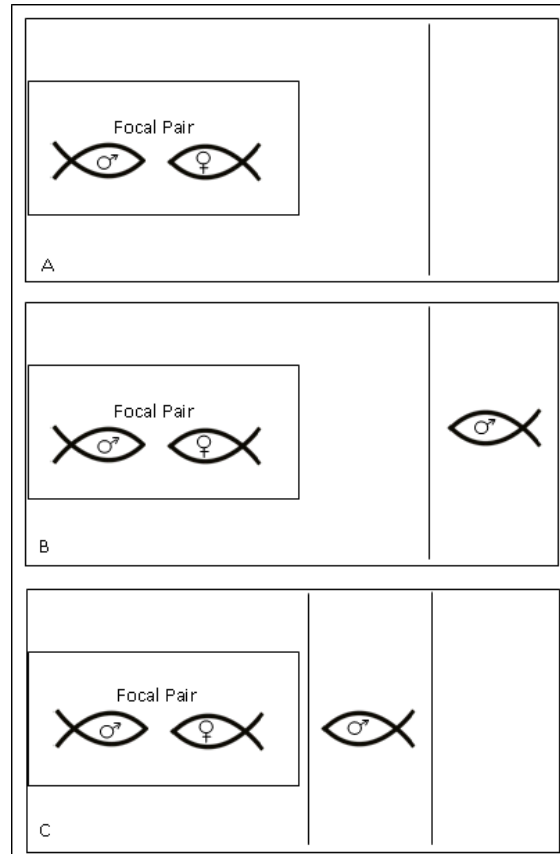


Figure 2. Experimental Treatments for Audience Effect, Experiment 2. A focal pair of guppies was observed A) alone, B) with a male separated by 15 cm, and C) with a male separated only by the tank and a clear plastic divider.

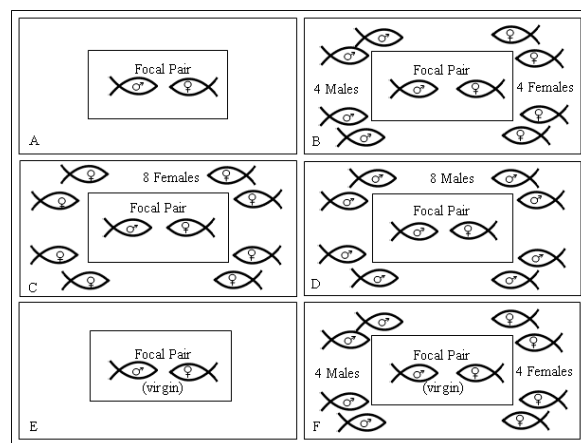


Figure 1. Social Environments for Male Courtship Behavior and Virgin Female Response Observations, Experiment 1. Courtship Behavior of focal males was observed in social environments A) with only the focal pair: a male and a non-virgin female, B) with a focal pair and an additional 4 males and 4 females, C) with a focal pair and an additional 8 females, D) with a focal pair and additional 8 males, E) with only the focal pair: a male and a virgin female, and F) with a focal pair (virgin) and an additional 4 males and a 4 non-virgin females. Female response was also measured in environments E and F.

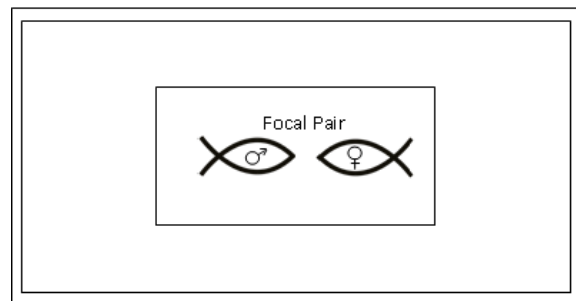


Figure 3. Setup for Habituation Experiment, Experiment 3. The courtship behavior of a focal pair of guppies was observed upon initial introduction, 1 hr later, 24 hrs later, and 1 hr later on the second day in a 19-liter tank (30 cm x 15 cm x 20 cm) contained within a 57-liter tank (60 cm x 30 cm x 30 cm).

Acknowledgements

I'd like to thank Anne Houde, my thesis advisor, for her guidance, encouragement, and support. My thanks also goes to Helen Rodd, Douglas Light, and Kathryn Dohrmann for their direction. Thank you to Jennilee Wallace, my coworker during our summer research, my friends and colleagues who helped me throughout this experience, and my family for their continual support and encouragement.

Note: Eukaryon is published by students at Lake Forest College, who are solely responsible for its content. The views expressed in Eukaryon do not necessarily reflect those of the College. Articles published within Eukaryon should not be cited in bibliographies. Material contained herein should be treated as personal communication and should be cited as such only with the consent of the author.

References

- Abrahams, Mark V. (1993). The trade-off between foraging and courting in male guppies. *Animal Behaviour*, 45, 673-681.
- Addressi, E. & Visabergchi, E. (2001). Social Facilitation of eating novel food in tufted capuchin monkeys (*Cebus apella*), input provided by group members and responses affected in the observer. *Animal Cognition*, 4, 297-303.
- Becher, S.A. & Magurran, A.E. (2004). Multiple mating and reproductive skew in Trinidadian guppies. *Proceedings of the Royal Society of London*, 271, 1009-1014.
- Bloch, G., Borst, D.W., Huang, Z., Robinson, G.E., Cnaani, J., & Hefetz, A. (2000). Juvenile hormone titers, juvenile hormone biosynthesis, ovarian development and social environment in *Bombus terrestris*. *Journal of Insect Physiology*, 46, 47-57.
- Briggs, S.E., Godin, J.J., & Dugatkin, L.A. (1996). Mate-choice copying under predation risk in the trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology*, 7(2), 151-157.
- Brooks, R. & Caithness, N. (1999). Intersexual selection, sneak copulations and male ornamentation in guppies (*Poecilia reticulata*). *South Africa Journal of Zoology*, 34, 48-51.
- Doutrelant, C., McGregor, P.K., Oliveira, R.F. (2001). The effect of an audience on intrasexual communication in male Siamese fighting fish, *Betta splendens*. *Behavioral Ecology*, 12(3), 283-286.
- Dugatkin, L. A. (1992). Sexual selection and imitation, females copy the mate choice of others. *American Naturalist*, 139, 1384-1389.
- Dugatkin, L.A. & Godin, J-G.J. (1992). Reversal of female mate choice by copying in the guppy. *Proc. R. Soc. Lond. Ser. B*, 249, 179-184.
- Dugatkin, L.A. & Godin, J-G.J. (1993). Female mate copying in the guppy (*Poecilia reticulata*), age-dependent effects. *Behavioral Ecology*, 4(4), 289-292.
- Dziewieczynski, T.L., Early, R.L., Green, T.M., & Rowland, W.J. (2005). Audience effect is context dependent in Siamese fighting fish, *Betta splendens*. *Behavioral Ecology*, 16, 1025-1030.
- Eakley, A.L. & Houde, A.E. (2004). Possible role of female discrimination against 'redundant' males in the evolution of colour pattern polymorphism in guppies *Proc. R. Soc. Lond. B (Suppl.)* 271, S299-S30.
- Endler, J.A. (1987). Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Animal Behavior*, 35, 1376-1385.
- Evans, J.P., & Magurran, A.E. (1999). Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. *Animal Behavior*, 58, 1001-1006.
- Evans, J.P. & Magurran, A.E. (2000). Multiple benefits of multiple mating in guppies. *Proceedings of the National Academy of Sciences*, 97(18), 10074-10076
- Farr, J.A. (1974). A quantitative analysis of social interaction of the guppy, *Poecilia reticulata* (Pisces, Poeciliidae) as a function of population density. *Animal Behavior*, 22, 582.
- Farr, J.A. (1975). The role of predation in the evolution of social behavior of natural populations of the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). *Evolution*, 29, 151-158.
- Farr, J.A. (1976). Social facilitation of male sexual behavior, intrasexual competition, and sexual selection in the guppy, *Poecilia reticulata*. *Evolution*, 30(4), 707-717.
- Grant, W.A. & Green, L.D. (1996). Mate copying versus preference for actively courting males by female Japanese medaka (*Oryzias latipes*). *Behavioral Ecology*, 7(2), 165-167.
- Helfman, G.S. & Schultz, T. (1984). Social transmission of behavioural traditions in a coral reef fish. *Animal Behavior*, 32(2), 379-384.
- Höjesjö, J., Anderson, P., Engman, A., Johnsson, J.I. (2007). Rapid bystander assessment of intrinsic fighting ability, behavioural and heart rate responses in rainbow trout. *Animal Behaviour*, 74, 1743-1751.
- Houde, A.E. (1987). Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution*, 41(1), 1-10.
- Houde, A.E. (1997). *Sex, Color, and Mate Choice in Guppies*. New Jersey, Princeton University.
- Huang, Z.Y., Plettner, E., & Robinson, G.E. (1998). Effects of social environment and worker mandibular glands on endocrine-mediated behavioral development in honey bees. *Journal of Comparative Physiology and Anatomy*, 183, 143-152.
- Kodric-Brown, A. & Nicoletto, P.F. (2001). Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. *Behav Ecol Sociobiol*, 50, 346-351.
- Kelley, J. L., Graves, J. A., & Magurran, A. E. (1999). Familiarity breeds contempt in guppies. *Nature*, 401, 661-662.
- Jennions, M.D. & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*. 75, 2-64.
- Laland, K.N. & Williams, K. (1997). Shoaling generates social learning of foraging information in guppies. *Animal Behavior*, 53, 1161-1169.
- Laland, K.N. & Williams, K. (1998). Social transmission of maladaptive information in the guppy. *Behavioural Ecology*, 9, 492-499.
- Liley, N.R. (1966). Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behav. Suppl.*, 13, 1-197.
- Matos, R.J., & McGregor, P.K. (2002). The effect of the sex of an audience on male-male displays of Siamese fighting fish (*Betta splendens*). *Behaviour*, 139, 1211-1221.
- Magurran, A.E., Seghers, B.H. (1990). Risk sensitive courtship in the guppy (*Poecilia reticulata*). *Behaviour*, 112 (3-4), 194-201(8).
- Magellan, K. & Magurran, A.E. (2007). Behavioural profiles, individual consistency in male mating behaviour under varying sex ratios, *Animal Behavior*, 74, 1545-1550.
- McFarland, D. (1985). *Animal Behavior: Psychobiology, ethology, and evolution*. California, Benjamin/Cummings.
- Miller, L.K. & Brooks, R. (2005). The effects of genotype, age and social environment on male ornamentation, mating behavior, and attractiveness. *Evolution* 59(11), 2414-2425.
- Oliveira, R.F., McGregor, P.K., & Latruffe, C. (1998). Know thine enemy, fighting fish gather information from observing conspecific interactions. *Proc R Soc Lond B*, 265, 1045-1049.
- Oliveira, R.F., Lopes, M. Carneiro, L.A., & Canario, A.V.M. (2001). Watching fights raises fish hormone levels. *Nature*, 409, 475.
- Plath, M., Blum, D. Schlupp, I., & Tiedemann, R. (2008). Audience effect alters mating preferences in a livebearing fish, the Atlantic molly, *Poecilia mexicana*. *Animal Behavior*, 75(1), 21-29.

Price, A.C. and Rodd, F.H. (2006). The effect of social environment on male-male competition in guppies (*Poecilia reticulata*). *Ethology*, 112, 22-32.

Pitcher, T.E., Neff, B.D., Rodd, H., & Rowe, L. (2003). Multiple mating and sequential mate choice in guppies, females trade up. *Proceedings of the Royal Society of London Biology*, 270, 1623-1629.

Reynolds, J.D. & Jones, J.C. (1999). Female preference for preferred males is reversed under low oxygen conditions in the common goby (*Pomatoschistus microps*). *Behavioural Ecology*, 10, 149-154.

Ridley, A.R., Child, M.F., & Bell, M.B.V. (2007). Interspecific audience effects on the alarm-calling behaviour of a kleptoparasitic bird. *Animal Behaviour*, 3(6), 589-591.

Rodd, F.H., Reznick, D.N., & Sokolowski, M.B. (1997). Phenotypic plasticity in the life history traits of guppies, responses to social environment. *Ecology*, 78(2), 419-433.

Sadowski, J.A., Grace, J.L. & Moore, A.J. (2001). Complex courtship behavior in the striped ground cricket, *Allonemobius socius* (Orthoptera, Gryllidae), Does social environment affect male and female behavior? *Journal of Insect Behavior*, 15(1), 69-84.

Schlupp, I., Marler, C., & Ryan, M.J. (1994). Benefit to male sailfin mollies of mating with heterospecific females. *Science*, 263(5145), 373-374.

Schlupp, I. & Ryan, M.J. (1997). Male Sailfin mollies (*Poecilia latipinna*) copy the mate choice of other males. *Behavioral Ecology*, 8(1), 104-107.

Schultz, E. T., & Warner, R.R. (1989). Phenotypic plasticity in life-history traits of female *Thalassoma bifasciatum* (Pisces, Labridae). 1. Manipulations of social structure in tests for adaptive shifts of life-history allocations. *Evolution* 43, 1497-1506.

Stoner, G. & Breden, F. 1988. Phenotypic differentiation in female preference related to geographic variation in male predation risk in the Trinidad guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, 22(4), 285-291.

Webb, P.W. (1980). Does schooling reduce fast-start response latencies in teleosts? *Comparative Biochemistry and Physiology*, 65(A), 231-234.

West, M.J., King, A.P., & Freeberg, T.M. (1996). Social malleability in cowbirds, new measures reveal new evidence of plasticity in the eastern subspecies (*Molothrus ater ater*). *Journal of Comparative Physiology*, 110(1), 15-26.

White, D.J., King, A.P., & West, M.J. (2002). Facultative development of courtship and communication in juvenile male cowbirds (*Molothrus ater*). *Behavioral Ecology* 13(4), 487-496.

Wich, S.A. & Sterck, A.H.M. (2003). Possible audience effect in Thomas langurs (primates; *Presbytis thomas*), An experimental study on male loud calls in response to a tiger model. *American Journal of Primatology*, 60(4), 155-159.

Witte, K. & Ryan, M.J. (1998). Male body length influences mate-choice copying in the sailfin molly *Poecilia latipinna*. *Behavioral Ecology*, 9(5), 534-539.

Witte, K. & Ryan, M.J. (2002). Mate choice copying in the sailfin molly, *Poecilia latipinna*, in the wild. *Animal Behaviour*, 63, 943-949.

Zajitschek, S.R.K., Evans, J.P., & Brooks, R. (2006). Independent effects of familiarity and mating preferences for ornamental traits on mating decisions in guppies. *Behavioral Ecology*, 17, 911-916.