EFFECTS OF SIZE AND EXPOSURE TO PREDATION THREAT ON
MATING BEHAVIOR AND PREDATION RESPONSE IN MALE
WESTERN MOSQUITOFISH (GAMBUSIA AFFINIS)

By
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EFFECTS OF SIZE AND EXPOSURE TO PREDATION THREAT ON MATING BEHAVIOR AND PREDATION RESPONSE IN MALE WESTERN MOSQUITOFISH (GAMBUSIA AFFINIS)

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Trade offs between mating and predator avoidance advantages were studied in the western mosquitofish (Gambusia affinis: Poeciliidae). When tested with green sunfish (Lepomis cyanellus) predators, larger male G. affinis were found to have a higher number of escapes compared to smaller males. Smaller males were captured more often than larger males in groups of six and in both complex and simple habitat types. Large males, on average, were found to attempt more matings and spend a larger proportion of time near the female than smaller males. When trials with predators were compared to trials without predators, large males decreased mating attempts and time spent near the female although, small males increased time spent near females. Size of the predator did not affect these behaviors. Fry were reared under varying levels of predator exposure (visual, olfactory, both, or neither) and predation response was measured for each group at maturity. Results were not significant and were confounded by G. affinis size.
ACKNOWLEDGMENTS

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CHAPTER I:
INTRODUCTION

The western mosquitofish (*Gambusia affinis*) is a member of the family Poeciliidae. Characteristics common to this family include internal fertilization, live birth, and sexual size dimorphism. Males are usually smaller than females, and in many species such as the Trinidadian guppy (*Poecilia reticulata*), sailfin molly (*Poecilia latipinna*), and pygmy swordtail (*Xiphophorus nigrensis*) males have elaborate coloring, ornamentation, and courtship displays. In mosquitofish, sailfin molly, and guppy populations, males have different mating strategies associated with size (Hughes, 1985a; Luyten and Lilly, 1986; Ptacek, 1997). Although courtship displays are not as common in mosquitofish (Vaccari *et al.*, 2001) as in guppies, they have been observed in the lab and the field (Peden, 1970; 1975; Hughes, 1985a). In the western mosquitofish larger males are more likely to court a female while smaller males are more likely to pursue a female and attempt forced copulations (Hughes, 1985a; Bisazza and Marin, 1991; Bisazza and Pilastro, 1997). Larger males seem to have an advantage in mating due to advantages in inter-male competition and female preference for larger males (Hughes, 1985a).

In guppies, bright coloration and high courtship rate were positively correlated with predation risks (Farr, 1976; Endler, 1987; Godin, 1995; and many others). Males that live in areas with clear, fast flowing water and low predation risk for adult fish are
often elaborately colored and perform more courtship displays, while males that live in areas with turbid water and high predation risk for adults tend to be drab and rely on forced copulations. Differences in mating behaviors for males have been shown have a genetic basis in *P. reticulata* and *X. nigrensis* (Farr, 1976; 1983; Zimmerer and Kallman, 1989). However, environment may have a greater influence on mating behavior of *P. reticulata* than genetics (Rodd and Sokolowski, 1995). Females in different populations of *P. reticulata* have also been shown to have genetic preferences for the type of males most common in their area (Luyten and Liley, 1985; Breden and Stoner, 1987; Endler and Houde, 1995). It has been suggested that predation has affected female preference in this system (Luyten and Liley, 1985; Breden and Stoner, 1987; Jean-Guy and Briggs, 1996). Predation may also alter mating behaviors in mosquitofish populations (personal observation).

Size differences at maturity have been shown to be genetic between and within some populations of *G. affinis* (Hughes, 1985a). However, recently it has been shown that in male *G. affinis* size is not necessarily entirely genetic but seems to have more to do with the environment. This study showed habitat structure, and predation risk (Fiorillo, 2003) to be the most important variables contributing to adult size. Male western mosquitofish have some degree of plasticity in their life history traits. They experience a drastic decrease in growth rate once they reach sexual maturity, and adult size may vary greatly in a population (Meffe and Snelson, 1989). Males that mature at a younger age are smaller and may trade size for the potential to mate earlier in the season. By the end of the breeding season when larger males become sexually mature, smaller
males are at a disadvantage in intrasexual competition for females. Hughes (1985b) found a delay in maturity for males born later in the breeding season in field populations of *G. affinis*. He attributed this delay to the need for these males to compete against the smaller, already mature, males for access to females. Other advantages, such as an increased ability to sneak copulations (Pilastro et al., 1997), may exist for small males allowing them to continue to achieve matings. The effects of size on mating strategies in mosquitofish have been well studied (Bisazza et al., 1989; Bisazza and Marin, 1991; Hughes, 1985a&b; and others). However, mating success is not the only characteristic affected by size as the ability to escape predation may also be positively correlated with size (Fiorillo, 2003).

Western mosquitofish females are usually between 3 and 4 cm standard length (SL). Males are considerably smaller and rarely reach 3 cm (Meffe and Snelson, 1989). Fish of this small size are prey items throughout their lives to many species of piscivorous fish (Winkelman and Aho, 1993) including the green sunfish (*Lepomis cyanellus*) used in this study. According to theory, age-specific mortality selects for individuals that mature early and mate early when faced with reduced adult survivorship while individuals that experience higher predation threats as juveniles than adults mature later and at a larger size (Law, 1979). The later pattern has also been observed in some poeciliid fishes (Sohn, 1977; Reznick and Endler, 1982). A positive correlation between adult size and predation was observed in bluegill sunfish (*Lepomis macrochirus*) but was caused by increased growth rates (Belk and Hales, 1993). However, the opposite effect was observed in the western mosquitofish. Males exposed to visual and chemical cues of
adult predation delayed maturity and consequently grew larger than control males that were not exposed to predators (Fiorillo, 2003). This suggests that there might be some benefit to increased size in mosquitofish that outweighs the need to mature early and mate before being eaten.

The proximate explanation for the large size of predator-experienced fish may be hormone related. Fishes that are constantly exposed to stress, such as the threat of a predator, increase their production of corticosteroid hormones. An increased level of corticosteroid hormones has been shown to delay sexual maturity in carp (Consten et al., 2001). Fish exposed to high levels of non-lethal predation may suffer from chronic exposure to corticosteroid hormones, and thus mature later, resulting in larger size at maturity. An adaptive advantage for larger sized males might be increased ability to avoid predation. I hypothesized that when threatened by a predator larger male mosquitofish would survive longer and escape a greater number of attacks than smaller males. This study examines survival ability during a predation attempt for large and small males.

In addition to size, predation threat may also influence courtship behavior in mosquitofish. In order to survive and reproduce, many animals must balance the need to be attractive against the need to be inconspicuous to a predator. There are many examples of such trade-offs in fish species. Iowa darters (Etheostoma exile) and male sticklebacks (Gasterosteus aculeatus) were found to decrease courtship activity in the presence of a predator (Candolin, 1997; Chivers et al., 1995). Similarly, dollar sunfish
*Lepomis marginatus* decreased nest-guarding behavior when faced with the threat of predation (Winkelman, 1996).

In the wild, mosquitofish encounter many predators (Winkelman and Aho, 1993). When this occurs they must reduce their courtship activity in order to be less conspicuous. If the predation threat is intense there may be little or no opportunity for courtship. Mosquitofish reared in the lab that have experience with an individual green sunfish predator were found to reduce mating behaviors in the presence of a green sunfish (personal observation). Males raised without predator experience decreased mating attempts in the presence of a predator, but to a lesser extent than experienced fish. Naïve fish were also smaller in size. It is unclear whether the lack of response to predators was due to small size or lack of predator recognition. It is possible that smaller fish respond to predators differently than larger fish. I hypothesized that smaller and larger male mosquitofish might alter their mating behaviors differently in the presence of large and small predators. This study quantifies the mating behaviors of different sized wild male mosquitofish in the presence of different sized green sunfish predators to test for differential responses to the threat of a predator.

Many cyprinid fish are thought to acquire species-specific predator recognition through experience. Chivers and Smith (1994) observed that fathead minnows (*Pimephales promelas*) displayed no fright response to visual predator cues until after cues were paired with the alarm substance (*shreckstoff*). Mosquitofish have been shown to release shreckstoff when attacked by a predator (Garcia *et al.* 1992) indicating that this species may use olfactory cues to identify danger.
Although it has received considerable attention, alarm substance is not the only means fish have to communicate predation threat. Mathis et al. (1996) found that fathead minnows could learn to recognize a predator by visually observing the response of experienced conspecifics. Because mosquitofish have visual components to their courtship behaviors I hypothesized that they could learn predator recognition through a combination of visual and olfactory cues of conspecifics predation. This study looks for differences in the ability of fish with varying forms of predator experience (i.e. visual chemical or both) to avoid a predation attempt.


CHAPTER II:
GENERAL METHODS

All *Gambusia affinis* used in this study were field caught in May 2003 from aquaculture ponds on the campus of Mississippi State University. Approximately 600 adults were captured using a seine net and 800 juveniles were captured using a dip net. Thirty *Lepomis cyanellus* were captured via a casting net in June 2003 from a pond on the North Farm of the Mississippi State University campus. The pond contained mosquitofish and it can be assumed that they were a source of food for the sunfish.

All fish were transported to the lab in coolers and held for two hours to allow the water temperature to cool to 30° C. At this time mosquitofish were transported to one of 21 fiberglass 140 L aquaria, filled with 128 L of dechlorinated water. To prevent experimental fish from being preyed upon sunfish were held in 20 L plastic tanks placed within the fiberglass tanks. All tanks were heated to 30 C and kept on a 12:12 L:D photoperiod. Mosquitofish were fed daily *ad libitum* Tetramin® flake food and twice weekly frozen brine shrimp. Sunfish were fed daily either live mosquitofish or frozen brine shrimp depending on the treatment. Fish remained in the lab for 158 days. At the end of the experiment all fish were euthanized-using MS-222.

Standard length (SL) was measured for all fish immediately before experiments were run. Standard length was calculated by measuring from the tip of the snout to end of
the caudal peduncle. Prior to each experiment fish were placed into a white metal pan containing 5 cm of water with a ruler affixed to the bottom with aquarium caulking. The pan was placed onto a stand with a digital camera on a stand 60 cm over it. Photos were taken of all fish or groups of fish and SCION imaging software was used to analyze the photos. All fish measurements were taken in centimeters.

All mating experiments took place in a 20 L glass aquarium filled with 18 L of dechlorinated water. The aquarium was divided into two compartments by a clear perforated Plexiglas divider. Mosquitofish were placed in the front compartment and sunfish were placed in the rear. This allowed mosquitofish to experience the visual and chemical cues of the predator while not actually being at risk of predation. After each trial the tank was emptied and rinsed with alcohol to remove chemical cues from previous trials. Because many poeciliids have been shown to alter their mating behavior throughout the day (Endler, 1987) trials took place within the first two hours of turning the lights on. This set up mimics the time of day when fish mate, but the light intensity was constant.

Female *G. affinis* used in mating studies were approximately 3 cm SL. Individuals were field caught and held in a 140 L aquarium without sunfish for a period of 90 days so that all gravid females would give birth at least two weeks prior to the start of any experiment. This was done to ensure that all females were relatively equal in their receptivity and attractiveness to males (see Hughes, 1986; Bisazza *et al.*, 1989).

All predation experiments took place in a 120 L glass aquarium filled with 100 L of dechlorinated water. The tanks contained a heater and were kept at 30 C so that fish
would not be shocked by extreme temperature changes when switched to these tanks from their holding tanks. Although light intensity in the lab is constant, trials took place between 12:00 and 15:00 to mimic the time of day when wild fish engage in the least amount of courtship behavior. (Endler, 1987). For all trials fish were allowed to acclimate for fifteen minutes before being filmed.

Much of the data from previous studies of poeciliid mating and antipredator behaviors were obtained from direct observations of live behaviors in the lab or in the field (Houde and Hankes, 1997; Goodey and Liley, 1986). To ensure that fish would not be disturbed by the human observer all trials in the current study were video recorded and viewed using a video camera and 120 min. 9mm tapes. This method also allows for behaviors to be analyzed in more detail by pausing or replaying the tape.
LITERATURE CITED


CHAPTER III:
EXPERIMENT 1: EFFECTS OF SIZE AND HABITAT COMPLEXITY
ON THE ABILITY TO AVOID PREDATION

Introduction

Mosquitofish have indeterminate growth, but males slow growth considerably once they reach sexual maturity (Meffe and Snelson, 1989). Age and size at sexual maturity is plastic and can vary greatly even in a small population. In general, alternate mating behaviors are correlated with size (Hughes, 1985), although some exceptions may occur. Size-related mating strategies have been extensively studied in poeciliids (Hughes, 1985; Pilastro et al., 1997; Bisazza and Pilastro, 1997; and many others). Although size most likely affects more than just mating success, the effects of size on other aspects of poeciliid biology have been less well studied.

Size may influence other factors affecting mosquitofish fitness such as the ability to escape predation (Farr, 1975). Predation in turn may influence the size of male mosquitofish by speeding up or delaying sexual maturity (Sohn, 1977; Fiorillo, 2003). Age specific mortality models predict that when adults in the population are more heavily preyed upon than juveniles, individuals should become sexually mature as early as possible in order to increase their chance of mating before being eaten (Abrams and
Rowe, 1996). Additionally, it has been suggested that rapid swimming is more energetically costly for larger fish than smaller fish (Hughes, 1985). However, Fiorillo (2003) found that male mosquitofish mature later, and consequently with a larger body size, in a system with adult-specific predation than in a predator free environment.

The reasons for delay of maturity in environments with heavy adult predation are unknown. Large size as a result of predation is sometimes explained as an attempt to escape gape size (Belk, 1998). However, because male G. affinis rarely exceed 3.0 cm in length, a size small enough for most adult green sunfish to engulf them, escaping gape size does not appear to be a possibility. Fiorillo (2003) suggested that there might be some advantage to increased size such as increased speed or ability to escape predation, but this was not quantified. In this experiment I attempt to quantify the advantages, if any, of larger size when faced with a predation threat.

If larger males do have an advantage in predator avoidance, that advantage coupled with the aforementioned mating advantages (Hughes, 1985; Bisazza and Marin, 1991; Bisazza and Pilastro, 1997; and others) begs the question: why do some males in the same litter of fish mature at smaller body sizes? Considering the many advantages for larger males it would seem that small males have no advantages in terms of reproduction or predator avoidance. However, natural selection is relatively efficient and thus no advantage seems unlikely. It is possible that different-sized mosquitofish have unequal survival across multiple habitat types. Larger males may be able to swim faster than smaller males and thus escape predators better in open water environments. Conversely smaller males may have an advantage in complex habitats that contain many
plants or debris because they have better maneuverability (Pilastro et al., 1997) and their smaller size may allow them to utilize more hiding spaces. Mosquitofish are most often found near shore where the habitat is more complex and it is thought that this behavior may be linked to predator avoidance (Goodyear, 1973). This experiment looks at the survival ability of large and small males in open and complex habitat types. I hypothesized that larger males would survive longer than smaller males in open habitats while small males would survive longer than larger males in complex habitats.

Methods

Approximately 480 adult male mosquitofish were randomly selected from the 600 field collected individuals to participate in predation experiments. Males ranged in size from 1.60 to 2.90 cm. Because size in this species is not separated into discrete classes I used the median size to separate large from small males. Individuals were divided into two groups small (1.60-2.30cm) and large (2.30-2.90cm) and placed into two separate aquaria and held for the duration of the experiment, which ranged from one to four weeks. Fish were categorized as “large” and “small” to ensure equal representation of sizes. Actual SL was used in analysis because size and behavior are a continuum in this species (Hughes, 1985).

Predation experiments took place in two 120 L glass aquaria filled with 100 L of dechlorinated water. Tank one contained only water to simulate an open water environment. While tank two contained two plastic plants affixed to the bottom, two plants affixed to the sides at the surface of the water to simulate algae or floating leaved
aquatic macrophytes, and one plastic log on the bottom. This tank represented the complex habitat found closer to shore.

Ten sunfish measuring between 6 and 7 cm SL were chosen as predators for this experiment. Each day ten male mosquitofish were randomly chosen, size (SL) was measured, and fish were individually tested in one of the two tanks with one of the ten predators. Initially mosquitofish were placed into a perforated plastic container and allowed to float in the tank with the predator for 15 minutes. By this time, in all cases, the sunfish showed interest in the container. This set up allowed the mosquitofish to experience the visual and chemical cues of the predator prior to actually being at risk of predation. Once the fifteen minutes were over the observer released the mosquitofish into the tank and left the room.

Fish were video recorded for ten minutes. After the ten minutes the observer returned and the trial ended. Numbers of escapes, time to capture, and whether or not the mosquitofish was captured were later scored from the videos. If the mosquitofish was not eaten during a trial it remained in the tank until predated. This ensured that all predators were fed every day that experiments took place and to ensure that mosquitofish learning would not be a factor. To increase predator motivation each individual sunfish participated every other day and was rotated between tanks one and two. Fish were chosen from large and small tanks such that there would be an equal number from each tank in each treatment with each predator. Sixty trials were run in both habitat types resulting in 120 trials total.
To simulate a more natural setting, trials were also run using groups of six (three small and three large) mosquitofish per trial. Because of difficulties in distinguishing between similarly sized fish on videotapes, time to capture and number of escapes for each fish could not be recorded. Instead at the end of each trial all fish were categorized as eaten or not eaten. This was done by comparing before and after pictures of the fish using SCION imaging software. Fish that were not captured during tests were placed into a common aquarium and not used in any other experiments. This methodology avoided any effects of learning or habituation to the tank by *G. affinis*. Sixty trials were run (30 in each habitat type).

In trials where mosquitofish were tested individually, ANCOVA was used to determine if habitat structure influenced escape behavior or capture vulnerability. Mosquitofish SL was the covariate. Logistic regression was used to test for effects of size and habitat structure on successful predation, a discrete, binary variable. In the trials where mosquitofish were tested in groups of six Wilcoxon signed rank test was used to compare the mean size of fish eaten vs. not eaten for each group.

**Results**

For males tested singly there was no significant interaction term, but number of escapes was significant for the covariate SL and for habitat complexity. Neither size nor habitat complexity were significant for time to capture (Table 3.1). Larger fish escaped a higher number of attacks than smaller fish and fish escaped more attacks in open than complex habitat (Fig 3.1). Capture probability was also significantly related to SL and
habitat type. Smaller fish were captured more often than larger fish (df = 106, 1, Slope = 0.221, P = 0.023) and all fish were captured more often in open habitat than complex habitat (df = 106, 1, Slope = -0.261, P = 0.007).

In approximately one half of group trials no mosquitofish were captured. These trials were not included in the analysis. The resulting sample sizes were N = 17 for the open habitat and N = 15 for the complex habitat. Smaller fish were captured more often than larger fish (P = 0.002) in both habitat types.

Discussion

Results indicated that larger males escaped a greater number of attacks in both habitat types, whether individually or in a group. This supports the hypothesis of Fiorillo (2003) that large males have an advantage in predator avoidance, at least when the predator is a green sunfish. Thus, it is not necessarily advantageous to speed up maturity in this system because although mosquitofish cannot possibly escape gape size of adult sunfish there is a predator avoidance advantage associated with increased size. Results were consistent for fish tested in groups.

The exact nature of the size advantage is not known. It is possible that smaller fish are easier for sunfish to consume and are thus preferred. This idea is supported by optimal foraging theory, which suggests that predators will preferentially choose prey with a shorter handling time. It could also be possible that larger mosquitofish are faster swimmers and thus can outrun a sunfish. Goodey and Liley (1986) suggested that the fish behavior at the moment of strike is the most important factor determining whether or
not a predation attempt will be successful. Studies of biomechanics would be necessary to determine the physiological causes of these advantages.

While it is known that large males have an advantage over smaller males in mating it also appear that they have an advantage in predator avoidance. I attempted to explain the persistence of small males in the population despite these advantages for larger males by hypothesizing that small males might be better at avoiding predators in complex habitats. I predicted that their small size might allow them to hide in a greater number of places than large males resulting in increased survival time for small males. I found no evidence to support this hypothesis. It may be that the replicas did not properly mimic nature. In a pond a fish as small as a mosquitofish would have seemingly unlimited area to out run a predator, while in the lab there are four walls enclosing the prey species in the same are as the predator ensuring an eventual successful predation.

Although results of this experiment were not significant there may be advantages for smaller fish in other forms of predator avoidance. Small fish may be better able to avoid predation from other species of predator such as snakes, birds, or turtles. Trexler (1994) found that herons preferred larger male sailfin mollies. Future studies could investigate the survivability of different sized mosquitofish when faced with a diverse suite of predators.
LITERATURE CITED


Table 3.1. ANCOVA for time to capture and number of escapes in complex and simple habitats for males tested alone

<table>
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<th>Source</th>
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<td>G. affinis SL</td>
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<td>Habitat Type</td>
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Figure 3.1. Number of escapes in open (black circles) and complex (white triangles) habitat as a function of mosquitofish SL (cm) for trials where mosquitofish were tested individually. Fish survived a greater number of attacks in open vs. complex habitat, and smaller males were captured significantly more often than larger males (Table 3.1).
CHAPTER IV:

EXPERIMENT 2: EFFECTS OF PERCEIVED PREDATION THREAT ON MATING BEHAVIOR IN MALE MOSQUITOFISH

Introduction

In many animals survival depends on the ability to balance the need to be attractive to the opposite sex against the need to be inconspicuous to a predator. There are many examples of such trade-offs in fish species. Iowa darters (*Etheostoma exile*) and male sticklebacks (*Gasterosteus aculeatus*) were found to decrease courtship activity in the presence of a predator (Candolin, 1997; Chivers *et al.*, 1995). Similarly, dollar sunfish (*Lepomis marginatus*) decreased nest-guarding behavior when faced with the threat of predation (Winkelman, 1996). In guppies the prevalence of bright coloration and intensity of courtship displays have been shown to be negatively correlated with predation risks (Farr, 1976; Endler, 1987; Godin, 1995). Predation may also alter mating behaviors in mosquitofish in the lab (personal observation).

Many aquatic prey species have been shown to perceive the level of predation risk and adjust their investment in defense behaviors accordingly (Tollrian, 1993; Van
Buskirk and Arioli, 2002; Turner et al., 2003). These adjustments usually involved changes in feeding rates, but the trend may be present in courtship behaviors as well. In a preliminary study Nootz and Basolo (2003) found that large Montezuma swordtail (*Xiphophorus montezumae*) males spent more time near females than small males when there was no predator in the environment. When the same fish were tested while viewing a predation attempt on a television monitor, small males increased their time spent near the female while large males decreased time spent near the female. This was presumably because small males were either less sensitive to predation risk or because large males were decreasing their female guarding behavior. Small immature sunfish are unable to catch a large male mosquitofish and often will not pursue them, while very large sunfish often will preferentially pursue a larger mosquitofish (personal observation). Thus, it would seem advantageous for mosquitofish to recognize the potential threat that different size sunfish pose to them and adjust their behaviors accordingly.

Because many Poecilliids have multiple mating tactics which vary in successful copulation rates and conspicuousness it is important that individuals use the appropriate tactic at the most opportune time. Some species such as the Trinidadian guppy and sailfin molly, rely heavily on very elaborate ornamentation and courtship displays to achieve copulations. *G. affinis* also has a courtship display (Peden 1970) which involves the male orienting himself sideways towards the female and arching his body in an ‘S’ shape called the sigmoid display. If the female is receptive she will arch her body and swim away from the male with exaggerated tailbeats then stop and lift her tail while flexing it to one side (Hughes, 1985; Bisazza and Marin, 1995). The male will then swim
towards her and insert his gonopodium into her gonopore. This behavior has been observed in the lab and the field, but it is not the primary means of insemination (Hughes, 1985). Female *G. affinis* are rarely receptive so males rely primarily on gonopodial thrusting (Peden, 1975) also called sneak (Endler, 1983), rape (Farr, 1980) or forced copulations (Hughes, 1985). This type of copulation consists of the male orienting himself behind the female then swinging the gonopodium towards her and attempting to insert his gonopodium into her gonopore without her cooperation (Bisazza *et al*., 1989).

Western mosquitofish, like all Poeciliids have internal fertilization, live birth and sexual size dimorphism (Meffe and Snelson, 1989). The female is the larger sex sometimes doubling the SL of the male and weighing up to five times more (Bisazza and Pilastro, 1997). Recently, in attempts to explain reversed sexual size dimorphism in poeciliids, it has been suggested that small size in males might confer an advantage in insemination probability (Bisazza and Marin, 1995). In the closely related eastern mosquitofish (*Gambusia holbrooki*) small males have been found to have such an advantage (Bisazza, 1993; Bisazza and Marin, 1995). Females in this species are rarely, if ever, cooperative during copulation (Bisazza *et al*., 1989). The advantage observed was an increased success in gonopodial thrusting for small males presumably due to their stealth and maneuverability. Results were obtained from laboratory experiments where males were tested with females individually, but in a natural setting there would likely be at least two males around any receptive female. Males will guard females from other males and larger males can greatly restrict access of smaller males (Hughes, 1985; Yan, 1987). Sometimes smaller males are only able to achieve < 10% of the matings (Bisazza
and Pilastro, 1997). Therefore, while it may be advantageous in terms of mating opportunities to be smaller than the female, it is not necessarily advantageous to be smaller than the other males.

Reproductive advantages for smaller males may be positively correlated with predation. In *P. reticulata* the proportion of courtship displays to gonopodial thrusting is associated with the existence of different types of predators (Far, 1975; Endler, 1987; Godin, 1995). Males living in areas with high predation tend to be drab and rely on forced copulations while males living in low predation areas are often elaborately colored and perform more courtship displays. Additionally, females of this species were found to either become sexually unreceptive or switch preferences from brightly colored to drab males after being exposed to a predator (Gong and Gibson, 1996). This suggests that the advantages associated with different mating tactics may be environmentally determined.

My study looks at the trade offs between reproductive behavior and predator avoidance. I hypothesized that in the presence of a threatening predator both large and small mosquitofish males would perform less courtship behaviors and more sneak copulations. I also looked for different responses by different sized males to different sized predators. I hypothesized that individual male *G. affinis* might be able to assess the likelihood that a specific predator will capture them and adjust courtship behaviors accordingly. I predicted that larger males would decrease mating behaviors in the presence of a larger predator, but not a smaller one and conversely, smaller mosquitofish would reduce mating behaviors in the presence of smaller predators, but not larger ones. Finally, my study looks for reproductive advantages for males who mature early and are
thus smaller than other males. I predicted that smaller males might be able to gain a
greater number of copulations than larger males in the presence of large predators while
larger males would monopolize copulations in the presence of smaller or no predators.

Methods

Thirty sexually mature *G. affinis* (10 females and 20 males) were selected based
on size to participate in the mating experiment. Males were measured (SL) and defined
as small (1.60-2.20cm) or large (2.30-2.90cm). Females were all approximately 3 cm
long. Fish were divided into 10 groups containing one small and one large male and a
female. For each trial a randomly selected group was placed into the testing aquarium
and allowed to acclimate until fish were observed to swim freely. At this time a *Lepomis
cyanellus* predator was added to the back compartment of the tank. At the beginning of
each trial the observer left the room and mating behavior was video recorded for ten
minutes.

Behaviors analyzed from videos included number of courtship displays, number
mating attempts, number of guarding behaviors, and percent time spent near the female
by each male (For a more detailed explanation of behaviors analyzed see Table 4.1.).
Percent time spent near the female was calculated by dividing time spent near the female
by each individual male by the total time both males spent near the female. This was
done to account for differences in activity levels of different females. Percent times were
arcsine transformed for analysis.
Each group of mosquitofish was tested with each of five predators ranging in size from 4.5-10cm (SL). Additionally each group received one trial with no predator as a control. Each group was tested once per day and trials were run in random order. Each group participated in six trials resulting in 60 total trials.

Analysis of covariance (ANCOVA) was used to test the relationship between size (SL) of the sunfish and mosquitofish size (SL) for each behavioral variable. A significant interaction would indicate that different sized mosquitofish are responding to the size of the sunfish in different ways. Multivariate analysis of variance (MANOVA) was used to test for an overall effect of predation on a suite of behavioral characteristics. Individual F-tests were then examined to determine which variables showed the strongest response to predation. A sequential Bonferroni adjustment of alpha (Rice, 1989) was used to determine the significance of univariate F-tests that were carried out for multiple behaviors within the same experimental design (Table 4.3).

Results

The MANOVA was significant based on Wilk’s Lambda (df = 13.3, 4, Wilk’s lambda = 0.624, P >0.001). Both large and small males decreased courtship displays and guarding behaviors from control to experimental trials. These behaviors were seldom observed in the presence of a predator (Fig. 4.1 and 4.2). Large and small males had different responses to number of mating attempts and time spent near the female (Fig 4.3 and 4.4). Larger males decreased the number of mating attempts and percent of time
spent near the female from control to experimental trials while small males increased these behaviors from control to experimental trials.

Slopes were homogeneous for the relationship between sunfish size and the effect of mosquitofish size on the four behavioral variables (df = 116, 1, F = 0.490 P = 0.479 for courtship displays, df = 116, 1, F = 0.979, P = 0.324 for mating attempts, df = 116, 1, F = 0.316, P = 0.575 for guarding behavior, and df = 116, 1, F = 0.008, P = 0.927 for percent of time spent near the female). After removing the interaction term, mating attempts and percent time spent near the female were significantly related to mosquitofish size. Guarding behaviors and courtship displays were not significantly related to mosquitofish size (Table 4.2). Larger males performed more mating attempts and spent a larger percentage of time near females than smaller males regardless of the size of the predator (Fig. 4.5 and 4.6). Predator size had no effect on the behavioral variables (Table 4.2).

Discussion

While results of this experiment do not support the hypothesis that small and large mosquitofish assess their risk of being eaten by the predator and respond by decreasing mating behaviors in accordance with threat level, there was evidence for differential responses to predation threat for large and small males. When a predator was present both sizes decreased courtship displays, and large males decreased mating attempts and percent of time near the female. Small males however, increased percent of time spent near the female when a predator was present. Small males also increased mating
attempts, but this was not significant. Similar results were observed by Reynolds (1993). He observed that under low light intensity, which he considered low predation risk, larger male *P. reticulata* had a mating advantage over smaller males. But when the light intensity was switched to high (high predation risk), larger males decreased their courtship behaviors and thus, reduced their advantage over small males.

The reasons that small fish are increasing time spent near the female when a predator is present are unclear. It could be that small fish are at a lower risk of predation than large fish, but this is not likely considering that small fish were captured more often than larger fish in experiment one. Another explanation might be that during normal mating events larger males are restricting access to the female, but when a predator is present, large males decrease these activities. This hypothesis is supported by the decrease in guarding behaviors exhibited by large males between control and experimental treatments (Fig. 4.3).

It has been shown that courtship displays and guarding can increase predation risk for animals performing these behaviors (Sih, 1994) and possibly for the females that the behaviors are directed towards (Magurran and Nowak, 1991). Thus Sih (1994) predicted that animals that are at risk of predation should either stop these “risky” behaviors or switch to a safer tactic such as sneak copulation. Results of my study are consistent with this hypothesis. Both large and small males decreased courtship and guarding behaviors to almost zero when predators were present (Fig. 4.3 and 4.4).

It is interesting to note that some of the predators used in this study were too small to engulf the large male mosquitofish. These predators would most certainly have gone
after the smaller male if given the opportunity, but in the presence of these predators large males still decreased mating behaviors while the smaller male did not. It is unclear why large males would be more sensitive to predation threat than small males. Perhaps because larger males have the mating advantages when predators are not present and when females are receptive and do not need to take high risks to secure a mating opportunity. Conversely smaller males must seize every opportunity to mate that is presented to them. It would be interesting to see if the extra opportunity to mate that small males have in the presence of a predator translates into increased insemination success for small males in high predation environments.
LITERATURE CITED


Table 4.1. Descriptions of behaviors analyzed from videos during mating trials

<table>
<thead>
<tr>
<th>Behaviors Analyzed</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Courtship displays</td>
<td>Male orients himself sideways in front of the female and lowers his gonopodium (Peden, 1970).</td>
</tr>
<tr>
<td>Mating attempts</td>
<td>Male orients himself behind the female swings the gonopodium forward and attempts to insert it into her gonopore (Bisazza et al., 1989).</td>
</tr>
<tr>
<td>Guarding behaviors</td>
<td>Preventing the other male from approaching the female by chasing and or biting him (Bisazza and Marin, 1995).</td>
</tr>
<tr>
<td>Percent time near female</td>
<td>Total time spent within one body length of the female oriented towards. Percent time was calculated from the total time for both males combined.</td>
</tr>
</tbody>
</table>
Table 4.2. ANCOVA for mating attempts, courtship displays, percent time spent near the female, and guarding behaviors for large and small male mosquitofish with sunfish SL as the covariate. Mating attempts and percent time near the female are significant for male mosquitofish SL. Slopes were homogeneous for all variables, so the interactions between the main effect and covariate were excluded from the model.

<table>
<thead>
<tr>
<th></th>
<th>G. affinis SL</th>
<th>Sunfish SL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mating attempts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>17.707</td>
<td>1.636</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.001</td>
<td>0.203</td>
</tr>
<tr>
<td><strong>Percent time near female</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>4.538</td>
<td>0.000</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.001</td>
<td>0.997</td>
</tr>
<tr>
<td><strong>Courtship displays</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>0.996</td>
<td>0.481</td>
</tr>
<tr>
<td>P</td>
<td>0.320</td>
<td>0.489</td>
</tr>
<tr>
<td><strong>Guarding Behaviors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>1.839</td>
<td>0.930</td>
</tr>
<tr>
<td>P</td>
<td>0.178</td>
<td>0.337</td>
</tr>
</tbody>
</table>
Table 4.3. Individual f-tests for the effect of predation on four mating behaviors. Alpha levels have been adjusted using the sequential Bonferroni adjustment correction. Asterisks indicate significant p-values after sequential Bonferroni adjustments (significance level = 0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent time near female</td>
<td>1/136</td>
<td>75.419</td>
<td>0.049</td>
</tr>
<tr>
<td>Mating attempts</td>
<td>1/136</td>
<td>30.139</td>
<td>0.010*</td>
</tr>
<tr>
<td>Courtship displays</td>
<td>1/136</td>
<td>9.256</td>
<td>0.004*</td>
</tr>
<tr>
<td>Guarding behaviors</td>
<td>1/136</td>
<td>7.801</td>
<td>0.039*</td>
</tr>
</tbody>
</table>
Figure 4.1. Mean number of guarding behaviors for large (white triangles) and small (black circles) males in control vs. experimental trials.

Figure 4.2. Mean number of courtship displays for large (white triangles) and small (black circles) males in control vs. experimental trials.
Figure 4.3. Mean percent of time spent near the female for large (white triangles) and small (black circles) males in control vs. experimental trials.

Figure 4.4. Mean number of mating attempts for large (white triangles) and small (black circles) males in control vs. experimental trials.
Figure 4.5. Mean number of mating attempts for small and large males in the presence of predators.

Figure 4.6. Mean percent time spent near the female for large and small males in the presence of predators.
CHAPTER V: EXPERIMENT 3:
EFFECTS OF PREDATOR EXPERIENCE ON THE ABILITY TO AVOID PREDATION

Introduction

Some animals are thought to acquire predator recognition through experience while others have an innate fright response to any unfamiliar organism. Recently many animals that were thought to have innate anti-predator responses have been shown to learn predator avoidance mechanisms through experience, including some invertebrates (Rochette et al., 1998). The existence of learned predator recognition has been well documented in fishes (Magurran, 1989; 1990; Chivers and Smith, 1993; 1994; and others) and has primarily focused on the minnow family, Cyprinidae.

Because of their small size and the occurrence of cannibalism in mosquitofish, individuals are subjected to predation threats throughout their lives. Schooling behavior in mosquitofish has been shown to be innate (Breder and Halpern, 1946). Intuitively one would assume that as a common prey item, G. affinis should have an innate form of predator recognition as well. However, research suggests that mosquitofish may not have an innate ability to recognize piscivorous predators (Peterson et al. unpubl.) or to most
efficiently avoid those predators (Goodyear, 1973). This may be true for other poeciliids as well. Populations of *P. reticulata* living in areas with high predation have better developed schooling and anti-predator responses than populations where there are few predators that specialize on small fish (Luyten and Liley, 1985). This suggests that there may be some amount of learning involved in predator avoidance behaviors, although these anti-predator behaviors might also be innate.

Learned predator recognition has not been as extensively studied in the poeciliids, as in cyprinids, but a notable exception is *P. reticulata*. It was initially thought that guppies have an innate anti-predator response (Breeden *et al.*, 1987). However, *P. reticulata* chased by conspecifics as juveniles were found to require significantly more attacks prior to capture than naïve *P. reticulata* when exposed to a piscivorous fish. These results suggest that although predator recognition may be innate some learning of predator avoidance behaviors may exist (Goodey and Liley, 1986). Peterson *et al.* (unpubl.) examined the mating behaviors of male mosquitofish that had been raised with and without exposure to green sunfish predator cues. In the presence of a predator, naïve male mosquitofish attempted to mate significantly more than predator-experienced males. Additionally naïve males did not show any predator avoidance behaviors. These results suggest that mosquitofish do learn to recognize potential predators.

The mechanisms by which cyprinids learn have been well studied. Chivers and Smith (1994) observed that fathead minnows (*Pimephales promelas*) have no reaction to visual predator cues until after cues are paired with the alarm substance. Alarm substance (*shreckstoff*) is a form of chemical communication. Fish release this chemical
when a predator attacks them. This type of communication is thought to be the primary means of transferring information in many aquatic species. However, it is not clear if chemical communication is the primary mechanism for predator recognition in *G. affinis*.

Although it has received considerable attention, alarm substance is not the only means fish have to communicate predation threat. Mathis et al. (1996) found that fathead minnows could learn to recognize a predator by observing the response of experienced conspecifics. It is possible that *G. affinis* could learn predator recognition in a similar manner.

My study looks at the survivability of fish that have experienced various cues of predation during development. I attempt to tease apart the visual and chemical cues of predation to see which cues are important for learning in the mosquitofish. I hypothesized vision and olfactory cues might both be important in predator recognition and that fish that were familiar with predators might survive longer than fish that were not. Therefore I predicted that fish reared in tanks where they could experience visual and chemical cues of predation would survive longer than fish reared in tanks where they were only exposed to visual or chemical cues singly and these would survive longer than individuals exposed to no cues at all.

*Methods*

Eight hundred fry between the ages of one and fourteen days were collected from an aquaculture pond on the campus of Mississippi State University. Fry were used rather than adults because adults have unknown histories, and some fish that learn about
predators do so as fry (Magurran, 1990). After being transported to the lab individuals were randomly selected and assigned to one of the following five treatments (four replicates per treatment): 1) No predators; 2) Visual and chemical predation cues (\textit{L. cyanellus} separated by clear perforated plastic and fed live \textit{G. affinis}); 3) Visual predation cues only (\textit{L. cyanellus} separated by clear solid plastic and fed live \textit{G. affinis}); 4) Chemical predation cues only (\textit{L. cyanellus} separated by dark perforated plastic and fed live \textit{G. affinis}); 5) Predator, but no predation (\textit{L. cyanellus} separated by clear perforated plastic fed frozen brine shrimp). Each replicate contained forty mosquitofish. Fish were reared in these aquaria for three months to allow them to reach sexual maturity. Maturity was determined by visual examination of the gonopodium following the methods of Fiorillo (2003).

To determine the effects of predator experience (i.e. predator recognition) on escape ability fish were randomly chosen (20 from each treatment) and exposed to a single individual \textit{L. cyanellus}. Methods were identical to those used in open habitat tests for males tested singly in experiment one (see Chapter 3). Time to capture and number of escapes was quantified from video recordings. Size (SL) was measured prior to testing. Trials were run every day for ten days in random order and ten similar sized sunfish (6-7 cm SL) were used in all trials. Because size of male \textit{G. affinis} was a significant factor in survival ability in experiment one I used an ANCOVA to test for differences in rearing regime on number of escapes and time to capture. Mosquitofish size was the covariate. One extreme outlier was discounted resulting in 99 total trials (Fig. 5.5).
Results

There was a significant interaction between mosquitofish SL and rearing regime for number of escapes (df = 87, 4, F = 2.646, P = 0.039). Thus, the homogeneity of slope assumption was violated and ANCOVA could not be used in this analysis. I then regressed number of escapes by SL for each rearing regime independently. The slopes for number of escapes as a function of SL were different for each rearing treatment, but tended to be positive for olfactory, olfactory/visual with predation, and visual indicating that larger fish survived a higher number of attacks than smaller fish. Slopes were negative for no predator and olfactory/visual without predation indicating that smaller fish survived a higher number of attacks (Fig. 5.1-5.5). However, slopes were not significantly different than zero, except for the olfactory/visual (no predation) treatment (Fig. 5.4). Excluding the outlier did not change the slope.

To test for differences in rearing regimes among treatments where size was not a significant factor I ran an ANOVA for number of escapes excluding the olfactory/visual treatments. Results were marginally significant for the rearing regime (df = 73, 3, F = 2.554, P = 0.062). I ran a Tukey’s post hoc comparison to determine which treatments were statistically different from one another. Fish reared in olfactory treatments survived a nearly significantly greater number of attacks than fish reared in visual only treatments (0.052). No other treatments were statistically different from one another (Fig. 5.6).

There was not a significant interaction between mosquitofish SL and rearing regime for time to capture (df = 87, 4, F = 2.071, P = 0.091). There were also no
significant main effects for time to capture (df = 91, 4, F = 1.152, P = 0.104 for rearing regime, and df = 91, 1 F = 2.691, P = 0.337 for SL).

Discussion

Based on the results obtained in experiment one, I expected the slope of the regression of number of attacks on to mosquitofish SL to be positive in all rearing regimes. This result was obtained for fish reared with visual cues only, olfactory cues only, and both visual and olfactory cues combined. However, fish reared without a predator and fish reared with visual and olfactory cues of a predator fed frozen brine shrimp showed negative slopes indicating that in these rearing regimes smaller fish are escaping a greater number of attacks compared to larger fish.

It is difficult to explain why size dependent responses differed between olfactory/visual no predation treatments and all other treatments. Although there were no significant differences between size and rearing regime for the fish used in this experiment (P = 0.108), there were no males in either the control or the visual/olfactory (no predation) treatment that were larger than 2.21 cm. In all other rearing regimes some males were larger than 2.30 cm. In experiment one the average size of fish captured in single trials was 2.35 cm and in group trials it was 2.25 cm. The number for group trials is likely lower because sunfish had a choice among fish and preyed on smaller ones. However, while sunfish preferred smaller fish in group trials they rarely ate the smallest fish presented to them. Sunfish usually ate the fish closest to 2.3 cm. This may suggest that there is an optimum prey size for sunfish. If this is true it is possible that fish reared
in control and olfactory visual (no predation) experiments were simply below the preferred size. The smaller fish in this study were extremely small when compared to wild populations and perhaps they had a higher time to capture and number of escapes because the predators were not as motivated to catch them. Thus, the significant interaction may simply be an artifact of the overall small size of fish reared without cues of predation although exclusion of the fish above 2.21 cm would not have significantly changed the slopes for any treatment with a positive slope. Further experiments should be performed to test for an optimum prey size in *L. cyanellus*.

Although no treatments were statistically different from the control Fish reared in olfactory treatments were significantly different than fish reared in visual treatments (Fig. 5.6). This result is likely due to low sample size or high variance among predators. As predicted there was a trend for fish that received olfactory cues to survive a higher number of attacks compared to fish that did not. The trend for fish reared in olfactory, olfactory/visual (predation) tanks to have higher mean number of escapes and time to capture indicates that olfactory cues may be important in predator recognition and development of successful avoidance behaviors. However, visual cues do not appear to be important in predator recognition as fish reared without ever seeing a sunfish predator survived a greater number of attacks than fish reared in any other treatment.

It would intuitively seem that time to capture and number of escapes would be closely related; however in this experiment number of escapes was significant while time to capture was not. One factor affecting the significance of results in this experiment may be high variance between treatments. This high variance may be the due to a small
sample size, or it could be due to individual variation between predators in their foraging efficiency and/or motivation. In future experiments it may be helpful to use fewer predators and increase the number of trials.
LITERATURE CITED


Figure 5.1. Number of Escapes as a function of size (SL) for *G. affinis* reared in control treatments (Slope = -0.271, P= 0.249).
Figure 5.2. Number of escapes as a function of size (SL) for *G. affinis* reared in olfactory treatments (Slope = 0.014, P = 0.953).
Figure 5.3. Number of escapes as a function of size (SL) for *G. affinis* reared in visual treatments (Slope = 0.380, *P* = 0.109).
Figure 5.4. Number of escapes as a function of size (SL) for G. affinis reared in visual/olfactory treatments where predators were fed frozen brine shrimp (Slope = -0.659, P = 0.002). Arrow indicates data point that was removed from analysis as an outlier (note increased scale).
Figure 5.5. Number of escapes as a function of size (SL) for *G. affinis* reared in visual/olfactory treatments where predators were fed live *G. affinis* (Slope = 0.016, P = 0.665).
Figure 5.6. Number of escapes for *G. affinis* by rearing regime. Fish reared with only olfactory cues of predation survived a greater number of attacks than fish reared with only visual cues of predation. Significantly different treatments are indicated by letters A or B.
CHAPTER VI:
CONCLUSIONS

Many recent studies have indicated that predator-prey interactions can drive the evolution of life history traits in a variety of aquatic organisms (Relyea and Werner, 1999; Peckarsky et al., 2002; Fiorillo, 2003; and others). One such life history trait is body size at maturity. Size in male poeciliids is a complex trait affecting and affected by many factors. Predation can affect the size at maturity (Fiorillo, 2003), and mating behavior of male poeciliids (Hughes, 1985a), but mating behavior itself is affected by predation (Endler, 1995; Nicoletto and Kodric-Brown, 1999). The relationships between size, mating behavior, and predation threat are so interwoven that it is very difficult to separate them and understand how each one operates independently and together to shape the behavior of the western mosquitofish. In P. reticulata there has been an attempt to consider multiple traits and how they work together to shape the morphology of populations (Endler, 1987; 1995; Rodd and Sokolowski, 1995; Nicoletto and Kodric-Brown, 1999; and others). Previous experiments involving Gambusia have tended to consider factors such as predation threat or size independently (Hughes, 1985a&b; Bisazza and Pilastro, 1997; Pilastro et al., 1997) but for an exception see Fiorillo (2003). In this study I have attempted to examine the trade offs between mating advantages and
predator avoidance advantages in *G. affinis* to understand why there are two mating strategies associated with size in this species.

It is believed that the reversed sexual size dimorphism in the poeciliid family is due to an increased ability for small males to copulate when the female is not receptive (Bisazza and Pilastro, 1997). Large body size in females is also thought to increase fertility (Breeder and Rosen, 1966). However, the reasons for differences in size and mating tactics among males within a population are less well understood. There are advantages and disadvantages associated with size for reproduction, (Hughes, 1985a&b; Bisazza and Marin, 1991; Bisazza and Pilastro 1997; and others) predator avoidance, and probably other as yet unexplored areas. The advantages and disadvantages for each trait may also vary spatially and temporally. For example, larger males have an advantage in mating when females are receptive, but smaller males have an advantage when females are not receptive (Bisazza and Pilastro, 1997; Pilastro *et al.*, 1997).

Pilastro *et al.* (1997) found that smaller male *G. holbrooki* have increased insemination success compared to larger males. However, this may be due to the lack of receptivity in females used in their study as females that are not receptive do not respond to courtship displays and larger males lose their advantage in mating (Hughes, 1985a). When smaller males successfully mate it is largely due to their ability to maneuver and hide from the female’s view, thus increasing their success at performing sneak copulations. In *G. affinis*, small males were likewise shown to have an advantage when females were non-receptive (Hughes, 1985a). However, this advantage is negligible because females who are non-receptive are often either gravid or have sperm from a
previous mating. Smaller male western mosquitofish probably do not have the same advantage in insemination as eastern mosquitofish because females of *G. affinis* species do respond to courtship and males compete for access to the female. However, the current study suggests that when a predator is present, non-gravid females might be less receptive and larger males less inclined to guard them giving small males more opportunities to sneak copulations. Genetic testing would be needed to determine if smaller *G. affinis* actually increased their rate of insemination in the presence of predators.

Switches in mating tactics associated with predation have been noted in other poeciliid species. Magurran and Nowak (1991) found that female guppies spend less time avoiding copulation when a predator is present and that males exploit this trait by increasing their rate of gonopodial thrusting in the presence of a predator. This does not seem to be the case for mosquitofish. Although no fish performed courtship displays in the presence of a predator, larger males did not compensate by increasing gonopodial-thrusting rates. Conversely, large males actually decreased mating attempts in the presence of a predator. Small males increased the number of mating attempts in the presence of a predator, but this was not significant. These results may suggest that larger mosquitofish males do not need to trade vigilance for mating opportunities because they dominate matings when no predator is present. Smaller males on the other hand appear to be at a higher risk of predation than larger males, yet they increase time spent near the female when a predator is present.
I do not have the necessary data to determine the cause of the increase in time spent by smaller males near the female in the presence of a predator. One possible explanation is that small males attempted to stay near the female for protection. However, given that smaller males seem to have a higher risk for predation than larger males and females this explanation does not seem likely. An alternative explanation is that in the presence of the predator the larger male and the female are distracted from the smaller male. Results from Experiment 2 showed that during a predation attempt larger males are not attending the female as vigorously as when no predator is present. The female is likely engaged in predator avoidance behaviors as well. Thus, small males may be taking advantage of their only opportunity to mate even though doing so may increases their risk of being predated.

Factors such as predation can affect organisms to a greater or lesser extent in different habitat types. Although I did not find that smaller fish surpassed larger fish in predator avoidance in complex habitats, I did find that all fish survived longer in tanks with structure. Fish in these treatments also had a lower number of attacks compared to fish in open habitat. These results are consistent with hypotheses considering vegetation as refugia for prey (Jackson et al., 2001). Although not quantified in my experiment, this reduction in attacks may result in less stress on the prey. Woodley and Peterson (2003) found that killifish (Fundulus majoralis) reduced predator avoidance behaviors in predation experiments with vegetation compared to trials with no vegetation. They also found reductions in oxygen consumption rates for fish that had refugia compared to fish without refugia, indicating decreased stress levels. It would be interesting to see if
mosquitofish reared in the presence of predators and with and without refugia would show the same delay of maturity in high predation environments seen by Fiorillo (2003) or if the refugia would reduce predator related stress resulting in earlier dates of maturity.

Since post-release survival rates are a concern for fisheries scientists, the mechanisms through which fish learn to recognize and successfully avoid predators can provide valuable information to this industry. Knowledge of the cues that are associated with predator recognition is essential when building models to “teach” hatchery-reared fish about potential predators. I was unable to answer the question of which cues were important in mosquitofish predator recognition. Chivers and Smith (1994) demonstrated that minnow alarm substance is necessary for learning predator recognition, but chemical cues of a predator were not sufficient. Although results from my study were not conclusive, they do suggest that conspecific alarm substance is not necessary for predator recognition in mosquitofish. Fish that received visual and chemical cues of a predator, but no alarm substance, behaved similarly to fish that received alarm substance. However, further tests with larger sample sizes and fewer predators may yield significant results.

Kelley et al. (2003) examined how social learning affects antipredator behaviors in guppies. They concluded that guppies could improve antipredator behaviors when presented with visual cues of predation in the presence of experienced fish. Results of my study although not conclusive, suggested that visual cues of predation alone might not be sufficient to reduce predator capture success of mosquitofish. This suggests that social learning through observation of experienced individuals might be an important factor.
I compared the time to capture and number of escapes for mosquitofish in the open habitat in experiment one with that of fish reared in the lab under varying levels of predation exposure in experiment three. Methods in both experiment one and three were identical and the same predators were used for both experiments. The exception was that fish in experiment one were wild reared and fish in experiment three were laboratory reared. Statistical tests could not be run without committing pseudo-replication. However, I found that wild fish on average had much higher times to capture and numbers of escapes than laboratory reared fish. This is consistent with research done by Goodey and Liley (1986) in which male guppies who had been chased by conspecifics as juveniles were found to survive a higher number of escapes and have longer times to capture than those that were not. Similar results were found in steelhead trout (*Oncorhynchus mykiss*) (Berejikian, 1995). Mosquitofish juveniles are often chased and sometimes cannibalized by adults in the wild. It is possible that this behavior benefits surviving juveniles in predator avoidance as adults. Future experiments should examine the effects of conspecific chasing on predator avoidance ability in *G. affinis*.

It has also been shown in some fishes that innate predator avoidance mechanisms may be altered over generations without exposure to predators. Berejikian (1995) found that steelhead trout (*Oncorhynchus mykiss*) descended from wild populations survived predation significantly better than hatchery descended fish. Because fish used my experiment were first generation lab fish, no comparisons of this nature can be made. Experiments involving laboratory strains of mosquitofish and wild strains reared under
similar conditions could show if there is any genetic component to predator avoidance behaviors.
LITERATURE CITED

Berejikian, B. A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. Canadian Journal of Fisheries and Aquatic Science 52:2476-2482.


