The Effects of Sex, Predation Risk and Sexual Selection on

Depth Choice in the Mosquito fish, Gambusia affinis.

by

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<u>Abstract</u>

Habitat use by fishes often varies spatially and temporally, and may have fitness consequences depending on the habitat that is chosen. Depth selection is an extremely important habitat choice in the life histories of fishes, and may be influenced by many variables. Some understudied variables that may affect depth choice include predation risk and sex. Furthermore, little is known about the effects of sexual interactions on depth choice. In this experiment, the role of predation risk, sex and sexual interactions on depth choice (quantified by distance from surface and distance from shore) in the mosquito fish, Gambusia affinis was investigated. An experimental apparatus with a depth gradient was used, such that a fish of 10-50 mm Standard Length could choose a distance from surface ranging from 0-42 cm and a distance from shore ranging from 0-120 cm. A MANOVA was performed to determine if predation risk, sex, and sexual interactions had any effect on depth choice. If a significant multivariate interaction of treatment and factors was found, we performed univariate tests for each variable. A second *a priori* test for sexual selection was performed by comparing regressions to determine if a relationship existed between the size of the companion individual and the depth or activity of the focal individual. If any of the regressions revealed a significant relationship between companion size and focal individual depth, ANCOVA analyses were used to test if the sex of the companion individual changed the relationship between companion size and focal individual depth. Finally, regressions were performed to determine whether the focal individuals' size affects its' depth distribution. The MANOVA analysis revealed both sex and predation risk affect the depth distribution of mosquitofish. Although the MANOVA analysis revealed only a trend of companion sex

affecting focal individual depth, the second regression analysis revealed that increasing male size resulting in decreased female distance from surface. The subsequent ANCOVA analysis showed that female reaction to companion size was significantly different in the presence of another female than in the presence of a male. Therefore, it appears as though female *G. affinis* in the current study incur increased predation risk to avoid sexual harassment by males. Although no evidence of a risk behavior trait was found in male *G. affinis*, (i.e. moving deeper in the presence of females versus in presence of other males) females appear to be more receptive to larger males by moving up in the water column, reducing the predation risk they take upon themselves. Since females participate actively in the probability of success of a given male, sexual selection is occurring as active sexual selection rather than passive sexual selection. This experiment illuminates important life history traits of the mosquito fish, a poeciliid species that has been widely introduced for the biological control of mosquitoes and is closely related to at least one endangered species, *Gambusia nobilis*.

Introduction

Movements by fishes among habitats are frequently non-random, with fishes often exhibiting temporal and spatial variation in habitat use. For instance, Xie et al. (2000) found that a community of small fishes was demonstrating seasonal use of habitats. Also, brook char, *Salvelinus fontinalis*, were shown to move inshore at dawn and move back to deeper water at dusk (Bourke et al., 1996). The importance of habitat selection in the life history of fishes is apparent in the consequences it has on their fitness, such as parasite load (Lysne et al., 1998), energetic costs (Twiss et al., 2000) and foraging success (Keast and Fox, 1992).

One way fishes may shift habitats is by adjusting their depth selection. In fact, depth selection may be extremely important in the life history of fishes. For instance, physiological functions such as digestion are facilitated by temperature (Kihara and Sakata, 2001; Temming et al., 2002; Andersen, 2001), which in turn can be related to water depth. Depth selection may also be important in foraging (Freeman and Grossman, 1993), reproduction (Aadland, 1993) and in influencing the composition of fish assemblages (Hyndes et al., 1999; Gido and Matthews, 2000; Lawson et al., 1999), but several other variables may also contribute to depth preference.

Predation Risk

Predation risk is a variable that affects habitat preference, and therefore, may also affect depth selection in fishes. Harvey and Stewart (1991) found that as pool depth increased in streams, the predation risk of tethered stoneroller minnows, *Campostoma anomalum*, creek chubs, *Semotilus atromaculatus*, and striped shiners, *Notropis chrysocephalus* decreased dramatically. This study supports a suggestion by Lyons

(1987) that the depth distribution of an assemblage of fishes may be related to predation risk.

Fish size may determine the relative predation risk at various depths. For instance, Power (1984) suggested that larger loricariid catfishes avoided shallow water due to predation risk from fishing birds, while smaller loricariid catfishes were restricted to shallow water by larger piscivorous fishes. The correlation of increasing depth with increasing size is not rare. According to Heincke's law (after Heincke, 1913; cf. Cushing, 1981), the mean size of fishes in the marine environment increases with depth. For instance, while investigating the distribution of 25 species of teleosts in four habitat types of a subtropical bay, Blaber and Blaber (1980) found that within individual species, juveniles preferred shallower water than adults. Moreover, the size-depth pattern is not just relevant in the marine environment. Hendry et al. (2001) found that both male and female sockeye salmon, Oncorhynchus nerka, oriented themselves according to depth and size while breeding, such that the largest salmon occupied the deepest water. Maximum pool depth was also shown to predict fish size in Tennessee streams (Harvey and Stewart, 1991). Clearly, depth choice and predation risk are often related to body size, but more investigation into this subject is needed.

Sex

In addition to predation risk, sex may also contribute to habitat associations in fishes. Abrahams (1989) tested the conformity of guppies, *Poecilia reticulata*, to an Ideal Free Distribution (IFD) using two feeders which supplied different amounts of food each day. An IFD occurs when organisms are distributed throughout a habitat depending upon the amount of resources available at a particular site (Fretwell and Lucas, 1970). For

example, if there are two sites, and 70% of the food is available at Site 1 and 30% is available at Site 2, 70% of the organisms should be found at Site 1 and 30% should be found at Site 2. Abrahams (1989) found that although both male and female guppies roughly conformed to an IFD during the first day, females increased their conformity to an IFD in subsequent days as opposed to male guppies which continued to shuttle between feeders and differ from the expectations of an IFD. Abrahams (1989) suggested that the temporal difference in conformity to IFD may have been due to females utilizing the previous days' information on how the resources were distributed in foraging decisions, while the males may acquire mate-locating benefits from sampling several environments (and not conforming). Therefore, male guppies may have temporally differential habitat associations compared to females due to potential social interactions.

Although there have been studies showing differential habitat preferences between the sexes for reptiles (Tiebout and Anderson, 2001), invertebrates (Merilaita and Jormalainen, 1997), birds (Przybylo and Merila, 2000) and mammals (Chamberlain et al., 2002), there have been relatively few studies comparing habitat use between sexes in fishes. Recently, using a stomach content analysis and parasite species analysis, the three-spine stickleback, *Gasterosteus aculeatus*, was shown to have differential habitat use by sex; males utilized benthic habitats while females utilized pelagic habitats (Reimchen and Nosil, 2001). Also, Eckert and Stewart (2001) suggested that whale sharks, *Rhincodon typus*, may segregate by sex, although the evidence was inconclusive. In another study, transmitter-tagged male dogfish, *Squalus acanthius*, had spatially and temporally different behavior patterns than female dogfish, also implying sexual habitat segregation (Sims et al., 2001). The authors suggested that if sexual segregation is

occurring, it may be due to females needing to conserve energy by limiting multiple matings. Reasons for sex specific differences vary, but one hypothesis, i.e. the intersexual niche differentiation hypothesis, suggests sexual size dimorphism allows for each sex to utilize a different ecological niche (Selander, 1966). For sexually dimorphic species, a factor in the intersexual niche differentiation hypothesis may be differential predation risk. Dill (1986) suggested that the habitat preference of a sex class of fish may be dependent on the predation risk of the particular sex class, not the species as a whole. Furthermore, as shown above, predation risk may be the determining influence resulting in the size gradient of fishes following the depth gradient (Harvey and Stewart, 1991; Lyons, 1987; Powers, 1984). Therefore, a sexually specific depth preference may be shown in size dimorphic fishes due to predation risk. Overall, investigations into reasons for possible differential habitat use due to sex in fishes have been relatively rare. *Sexual Interactions*

Although investigations into the role of sex in habitat selection have been rare, to my knowledge, studies investigating the effect of sexual interactions on habitat selection of fishes are non-existent. Little is known about how the presence or absence of the opposite sex may affect habitat choice in fishes. However, differential habitat preferences due to differential predation risks may offer a mechanism for sexual selection. Many studies have shown that females prefer males that have apparently "risky" traits. This is known as the handicap principle (Zahavi, 1975). The handicap principle states that, to minimize the likelihood of false signaling, animals have developed traits that put an extra fitness burden on the signaling individual. The extra fitness burden advertises that the individual is of high genetic quality, and able to incur an

additional fitness burden yet still survive to reproductive age. For instance, both predators and female guppies have been shown to prefer male guppies with the most ornamentation (Endler, 1980; Godin and Briggs, 1996). Ornamented males are selected against by predators, but selected for by females, so only those ornamental males with high genetic fitness would be able to survive predation long enough to reproduce. These "risky" traits are not restricted to being expressed morphologically. For instance, some small fishes exhibit predator-approaching behavior, where the individual will approach a predator, possibly to obtain information about the predator's status or deter predator attack (Godin and Dugatkin, 1996; Milinski, 1987). Female guppies have been shown to prefer males that are able to take a greater risk in predator approaching behavior by moving closest to the predator (Godin and Dugatkin, 1996). This is an example of a behavioral expression of a "risky" trait facilitating sexual selection.

Other sexual preferences have been found in poeciliids. Many female poeciliids have been shown to prefer larger males. For instance, Hughes (1985) found when female *G. affinis* were deprived of contact with males for at least 30 days, females preferred larger males. Interestingly, when females were not deprived, and therefore not receptive, smaller males had a copulatory success rate equal to larger males (Hughes, 1985). Using a computer model, Bisazza and Marin (1995) showed that under natural conditions in a congener, smaller *G. holbrooki* males would have a reproductive advantage, probably due to their greater agility and ability to approach a female undetected. Therefore, it might be expected that females of this genus would use some strategy to exert more control over their mate choice. In fact, females may allow larger males to stay closer than small males to insure insemination by larger males and limit the overall number of matings (McPeek,

1992; Bisazza and Marin, 1995). Since this suggests an active form of sexual selection, the distinction between active and passive sexual selection will be defined. Active sexual selection will be defined as any activity by Sex "A", the degree of which will vary with regard to the presence or degree of a particular trait found in individuals of Sex "B". Passive sexual selection will be defined as an activity by Sex "A" stimulated by the presence of Sex "B", but does not vary with regard to any particular trait of Sex "B". For fishes, and specifically poeciliids, passive sexual selection could occur if females occupy deeper water due to their larger size, and the higher predation risk for the smaller males makes the possibility of females mating with males who are larger or "riskier" more probable. Alternatively, females may actively select larger males by moving into shallower water in the presence of larger males, if they are present there.

Study Organism

A sexually dimorphic species that has been widely introduced as a biological control of mosquitoes is *Gambusia affinis*, the mosquitofish (family Poeciliidae). As in other poeciliid species, fertilization is internal, with sperm transfer accomplished by the gonopodium (a modified anal fin) (Rosen and Bailey, 1963). Although subtle female mate choice has been suggested in this species (Hughes, 1985), the majority of copulations are forced with the last male to mate with the female siring most of the brood (Constantz, 1984). Males provide no parental care and females retain the fertilized eggs until the offspring are self sufficient (Constantz, 1989). Males in this genus do not grow much, if at all, after maturity while females continue to grow with larger females producing larger clutches (Reznick, 1981; Yan, 1986). *G. affinis* males (15-25 mm) are

considerably smaller than females (25-40 mm), and therefore may be subjected to a higher predation risk from piscivores in deeper waters.

The purpose of this study is to test the hypotheses that predation risk and sex affects the depth, distance from shore and activity of *Gambusia affinis*, the mosquito fish. Furthermore, I will test the hypothesis that a sexually specific depth choice may offer a mechanism for either active or passive sexual selection.

Materials and Methods

Fish Collection and Maintenance

Two species of fishes were used in this experiment: the mosquito fish, *Gambusia affinis*, as the study organism, and the spotted bass, *Micropterus punctulatus*, as the predator in predation risk treatments. *G. affinis* and *M. punctulatus* were collected with seine and dip nets from the Pecos River, near the Highway 60 bridge, De Baca County, New Mexico (N 34° 28.405', W 104° 15.618'). Fishes were transported in an aerated fish hauler to the Behavioral Ecology Laboratory at Eastern New Mexico University and kept in 76 l aquaria at constant temperature ($26^{\circ} \pm 1^{\circ}$ C) and photoperiod (14 hours light: 10 hours dark) to control for seasonal influence. Water was treated with Super Strength ® tap water conditioner to remove chlorine, chloramines and heavy metals. *M. punctulatus* were kept individually and *G. affinis* were kept in similar sized groups of no greater than 60 fish. Sex ratios of *G. affinis* were approximately 1 male:10 females in "female" holding tanks, and vice versa in "male" holding tanks to minimize the number of fish required for the experiment. One fish of the opposite sex was left in the tank to prevent the fish used in the experiment from "shutting down" sexually, as found in *Centropygi*

potteri (Lutnesky, 1992). Furthermore, the biased sex ratio would maximize any sexual selection response by females which have been shown to exhibit preferences for some traits in males when deprived of contact with males for more than 30 days (Hughes, 1985). Females and males occupying opposite-sex holding tanks were not used in the experiment. Five times weekly, *G. affinis* were fed Tetraflakes *ad libidum* and *M. punctulatus* were fed feeder fish (including *G. affinis* within 24 hours of predation risk trials). Water exchanges (10% partials) were performed weekly.

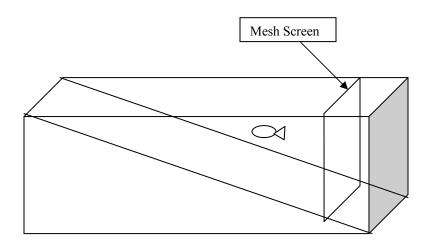


Figure 1. Sloped-bottom apparatus. Zone grid not pictured.

Experimental Apparatus

The experimental apparatus (Figure 1) was made from 0.6 cm Plexiglas. Sand substrate was adhered to the sloped bottom of the apparatus with a thin coat of aquarium sealant. A black mesh screen was placed across the deep end of the apparatus forming a compartment for the predator in the predation risk treatments. The compartment constituted 18 cm of the 143 cm length of the apparatus. The mesh allowed the *M. punctulatus* to be presented visually and olfactorily to the *G. affinis*. Permanent black marker was used to draw 10 cm x 10 cm zones on the outside of each sidewall of the apparatus providing the observer a visual scale to measure the distance from surface and distance from shore of a focal individual. For each trial the water level inside the apparatus ranged

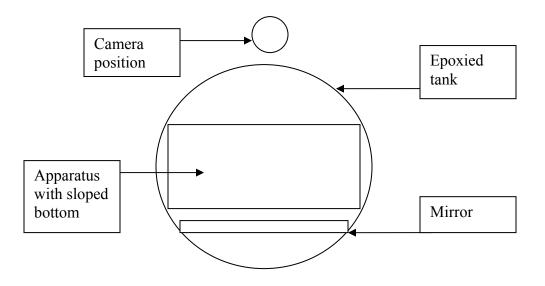


Figure 2. Experimental Apparatus inside epoxied, circular tank.

from 0-42 cm in distance from surface and 0-125 cm in distance from shore. Although it confined the fish, the apparatus was not water tight, and therefore, it was placed in a large epoxied, circular tank (0.60 m deep x 1.58 m dia., Fig. 1b). All observations were made remotely, and recorded, using a digital video camera on a tripod. A large mirror was placed in the circular tank at an angle (\sim 45°) across from the camera such that it allowed

observation and measurement of the depth of the focal individual. The sole light source during the trials was a 500-Watt halogen work light placed directly over the apparatus at a height of approximately two meters.

Sex of focal individual	Male			Female				
Paired With	Male		Female		Male		Female	
Predation Risk	Yes	No	Yes	No	Yes	No	Yes	No
Number of Trials	30	30	30	30	30	30	30	30

 Table 1: Experimental Design. The number and type of trials intended.

Experimental Protocol

From January 3, 2003 to April 4, 2004, a single experiment was performed with G. affinis, using a fully crossed factorial design, consisting of eight treatments with 30 trials per treatment (Table 1). Treatments consisted of all possible combinations of sex of a focal individual (male or female), predation risk (presence or absence) and sex of the companion fish (male or female). All trials were performed indoors, in random temporal order. The tank and apparatus were drained and rinsed after each trial to remove olfactory cues, since G. affinis has been shown to respond to pheromones (Lutnesky and Adkins, 2003; Park & Propper, 2002). For each trial, two G. affinis were taken from randomly chosen holding tanks and placed in the experimental apparatus. After an acclimation period of at least 30 minutes, each trial was recorded by a digital video camera for 13 minutes. The first three minutes of the recording of each trial was not used in the behavioral observations to minimize the measurement of behavior caused by the disturbance of manually activating the camera. G. affinis return to previous behavior patterns, e.g. copulatory attempts and normal swimming patterns, within a few minutes when briefly disturbed (Smith and Belk, 2001; personal observation). In treatments

where predation risk was present, a randomly chosen *M. punctulatus* was placed behind the mesh screen after the acclimation period. In same-sex treatments a focal individual was chosen randomly. After completion of each trial, the length and wet weight of each fish was determined using a scale and calipers. The activity of the focal individual, defined as the number of times the focal individual would move to another zone, and the time spent in each zone to the nearest tenth of a second were recorded. Furthermore, both distance from surface and distance from shore were recorded and used to analyze depth preference. The Index of Spatial Position (ISP), a measure used for the average distance from surface or distance from shore, was calculated by the equation:

$$ISP = \frac{\sum_{i=1}^{n} i x_i}{S}$$

where x_i = time in ith zone, S = seconds in observation period and n = the number of zones (Oyodamari, 1999).

Statistical Analysis

All data were found to be normal or transformed to fit a normal curve. Data were analyzed using Systat 7.0©. First, a multivariate analysis of variance (MANOVA) was used to analyze the effects of the factors sex, predation risk and sexual interactions on the choice of distance from surface, distance from shore and activity in *G. affinis*. Any significant result from the MANOVA was then tested by ANOVA. Using a three factor ANOVA, the influence of the three factors were simultaneously tested on depth preference and activity level, with each factor having two levels: sex (male and female), companion sex (female and male) and predator (presence and absence). If a significant multivariate interaction of treatment and factors was found, we performed univariate tests for each variable. The MANOVA and subsequent ANOVA tests allowed us to test for sexual selection through the factor of sexual interactions.

A second *a priori* analysis of sexual selection was performed to approach the problem from a different direction. Regressions were performed to determine if a relationship existed between the size of the companion individual and the depth or

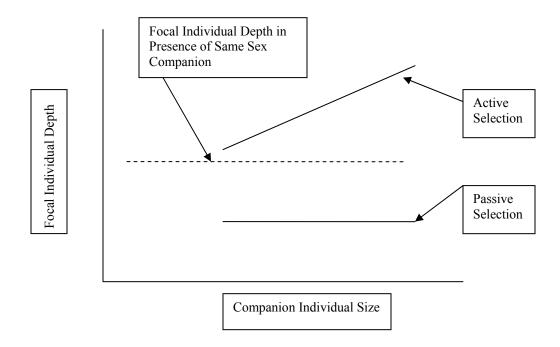


Figure 3. The hypothetical effect of companion size on focal individual depth if sexual selection is occurring. If no size based sexual selection is occurring, there will be no significant difference between regression lines when the focal individual is in the presence of same vs. opposite sex individuals in the ANCOVA analyses.

activity of the focal individual. If any of the regressions revealed a significant relationship between companion size and focal individual depth, ANCOVA analyses were used to test if the sex of the companion individual changed the relationship between companion size and focal individual depth. To distinguish between active and passive sexual selection, any regressions of companion size vs. focal depth were graphed with a baseline focal depth determined from focal individual depth preference in the presence of a companion of the same gender. If the two regression lines had different slopes active sexual selection was occurring while passive sexual selection was occurring if the regression lines had statistically identical slopes yet different y-intercepts (Figure 3). If the regression lines were statistically identical, no sexual selection would be occurring. Finally, to determine if the focal individuals' size affected their depth, regressions were performed.

Results

The MANOVA revealed significant results for two factors: predator presence, sex and their interaction (Table 2). The subsequent univariate analyses for the significant factors revealed that *G. affinis* were found significantly closer to shore (Figure 4, P<0.00001, n=120) and closer to the surface (Figure 5, P<0.00001, n=120) in the presence of a predator. Furthermore, *G. affinis* were significantly more active when no predator was present (Figure 6, P<0.00001, n=120). There were significant differences between the sexes as well, in both depth and activity. Males were significantly more active than females (Figure 6, P=0.00359, n=120), but stayed significantly closer to shore than females (Figure 4, P=0.01191, n=120). A significant interaction between predator and sex, as factors, was found for the distance from shore of the focal individual (P=0.03312, n=120). Although the MANOVA results for sexual interactions was not significant as a factor, a trend was revealed suggesting the possibility of some effect of companion sex on focal individual depth (Table 2, P=0.11507, n=120). However,

Factor	Wilk'	Variables tested	F-statistic	df	p-value	
~	Lambda	with ANOVA				
Sex	0.91626		5.23211	4, 229	0.00047	
		Zone from Shore	6.42572	1, 232	0.01191	
		Zone from Surface	3.29763	1, 232	0.07067	
		Activity	8.65636	1, 232	0.00359	
Predator	0.71424		22.90512	4, 229	< 0.00001	
		Zone from Shore	41.91281	1,232	< 0.00001	
		Zone from Surface	67.98843	1,232	< 0.00001	
		Activity	24.23220	1, 232	< 0.00001	
Companion Sex	0.96823		1.87831	4, 229	0.11507	
Companion Sex	0.90025		1.07051	1, 229	0.11207	
Predator * Sex	0.94709		3.19863	4, 229	0.01397	
		Zone from Shore	4.59398	1,232	0.03312	
		Zone from Surface	0.00000	1, 232	0.99949	
		Activity	1.63413	1, 232	0.20241	
Predator *	0.99251		0.43175	4, 229	0.78562	
Companion Sex				,		
Sex * Companion	0.98697		0.75560	4, 229	0.55520	
Sex Predator * Sex *	0.98816		0.68609	4, 229	0.60225	
Companion Sex	0.20010		0.00007	1, 22)	0.00220	

Table 2: Results of MANOVA for all factors and their interactions. If the MANOVA was significant (P<0.05), ANOVA's were performed for each variable.

although the regression analyses of sexual selection revealed no effect of female size on male depth, a significant effect of male size on female distance from surface was found (Table 3). An ANCOVA comparing female distance from surface as an effect of companion female size versus companion male size revealed a significant difference between the slopes (Figures 7, 8; P=0.001, t=18.61109). While focal females did not significantly change their distance from surface preference in the presence of females,

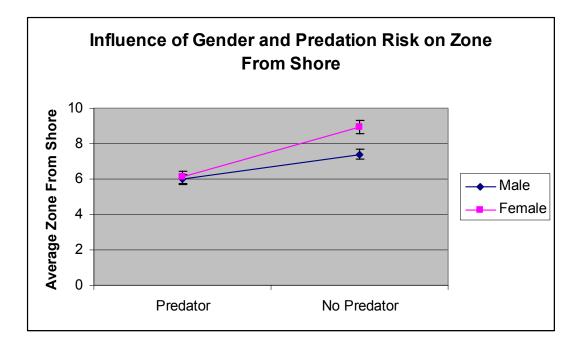


Figure 4: Influence of Sex and Predation Risk on Zone from Shore in *G. affinis* in the presence of a companion individual of the same sex. Average number of zones from shore for the sex and predation risk noted.

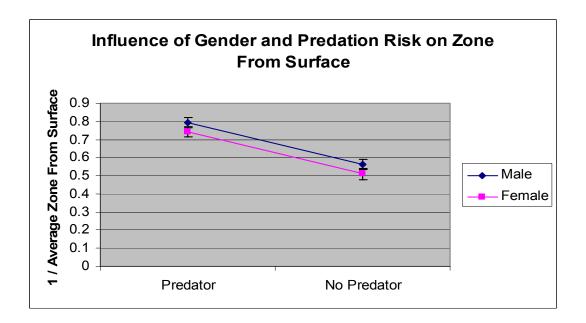


Figure 5: Effect of Sex and Predation Risk on the Zone from the Surface in *G. affinis* in the presence of a companion individual of the same sex. Average number of zones from surface for the sex and predation risk noted.

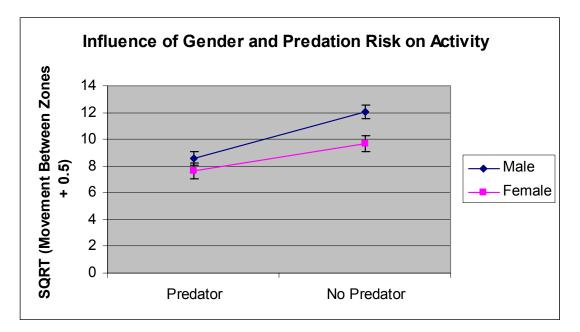


Figure 6: Influence of Sex and Predation Risk on Activity Level in *G. affinis*. Average movement between zones for the sex and predation risk noted.

	Com	panion Weigh	t	Companion Length			
Focal Individual	P-value	R-squared	Df	P-value	R-squared	df	
Zone from Shore							
Male	0.694	0.006	29	0.304	0.038	29	
Female	0.251	0.048	28	0.071	0.118	28	
Zone from Surface							
Male	0.590	0.010	29	0.992	3.31E-06	29	
Female	0.038	0.150	28	0.025	0.173	28	
Activity							
Male	0.101	0.004	29	0.992	3.26E-06	29	
Female	0.293	0.043	28	0.108	0.093	28	

Table 3. The effect of companion size on the distance from shore, the distance from surface and the activity of the focal individual. Data are from regression analyses.

focal females moved significantly higher in the water column as male size increased (Figures 7, 8; P=0.03770, R^2 =0.15032). The size of the focal individual did no determine distance from shore or distance from surface preferred by the focal individual.

Discussion

In response to the presence of an aquatic predator, both male and female *G. affinis* move up in the water column and toward shore, suggesting aquatic predation risk increases with distance from shore and distance from the surface in this fish. The movement up and in toward shore may be considered anti-predator behavior for *G. affinis*. This is consistent with findings by Smith and Belk (2001) that mosquitofish moved lower in the water column as the danger posed by a predator decreased. Furthermore, activity was also reduced for *G. affinis* in the presence of a predator, suggesting that reduced activity is also an anti-predator behavior for *G. affinis*. Indeed, reduced activity is a common anti-predator behavior among fishes (Farr, 1972; Forsgren and Magnhagen, 1993; Chivers et al., 2001; Godin and Briggs, 1996). For instance, Godin and Briggs (1996) found that in a predator-wise population of the guppy, *P. reticulata*, females significantly reduced their sexual activity level in response to a predation risk.

A sex specific difference in depth preference of *G. affinis* was also found. Males were found significantly closer to the shore and the surface than females. There are several possible explanations for this result. The inter-sexual niche differentiation hypothesis suggests that sexual size dimorphism leads to utilization of different

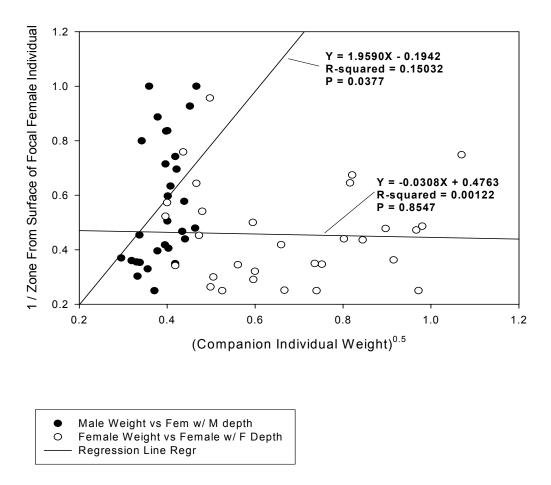


Figure 7. Comparison of the Effect of Companion Size and Gender on Female Depth Preference. In presence of another female, female zone from surface is not affected by companion size. However, in presence of male companion, females decrease their zone from surface with increasing male size.

trophic niches (Selander, 1966). However, Gluckman and Hartney (2000) found there were no significant differences in the diets of males and females of a congener, *G. hubbsi*. Furthermore, small and large *G. holbrooki* have been found to share a extremely similar dietary overlap (Stoffels and Humphries, 2003). A trophic niche separation hypothesis explaining the difference in depth selection of male and female *G. affinis* seems unlikely. Alternatively, predation risk may be the determining factor in the sexually specific depth choice. Since males approach females from below and behind when attempting a forced copulation (Martin, 1975), males may assume a greater predation risk than females at a similar distance from shore when attempting copulations. Interestingly, a female-biased sex ratio has often been found among populations of *G*. *affinis* (Artom, 1924; Hildebrand, 1927; Hughes, 1985). A handicap trait of higher predation risk for males than females, at depth may explain this phenomenon to some extent. In fact, some evidence suggests handicap traits may results in a female-biased sex ratio. Marcias-Garcia et al. (1998), found that snakes disproportionately preyed on male Amarillo fish, *Girardinichthys multiradiatus*, due to their sexually-selected large fins which inhibit predator avoidance maneuvers.

Alternatively, size may play a role in the sexually specific locations of the mosquitofish. Since females are larger than males, their relative predation risk in deeper water may be lower (Power, 1984). However, the lack of a size-depth relationship within each sex infers that size does not explain the difference in depth choice between the genders. One possible explanation is that the scale of the zones used in this study was not sensitive enough to detect a size-depth relationship. But this seems unlikely since the zones were sensitive enough to detect the sex-specific depth difference, as well as a significant relationship between companion male size and female depth.

An alternative to both size determined depth preference and depth choice as a handicap trait in *G. affinis* is that females may be forced to accept a higher predation risk by moving out and down in the water column to avoid sexual harassment by males. Male *G. affinis* will often attempt up to one copulation attempt per minute in laboratory settings (Martin, 1975; Houde, 1997). Sexual harassment by males of many species carries significant costs to females including increased energy expenditure, possible

disease transmission, decreased foraging efficiency and increased predation risk (e.g. Daly, 1978; Martens and Rehfeld 1989; Magnhagen, 1991; Magurran and Seghers 1994a; Watson et al 1998; Pilastro et al., 2003). Female G. affinis may be forced to incur added predation risk to minimize these costs. Consistent with this hypothesis is that in the immediate presence of a predator, female G. affinis move to a distance from the surface and distance from shore not significantly different from males. The reduction or disappearance of emale mate preference in the presence of a predator has been shown in several fishes (e.g. Godin and Briggs, 1996; Forsgren and Magnhagen, 1993). The cost associated with an immediate predation risk are apparently greater than the cost associated with sexual harassment from males. Other ways of minimizing the costs of male harassment may be found in this genus. G. holbrooki females have been shown to follow larger males, which often dominate and reduce copulation attempts by smaller males (Bisazza and Marin, 1991). Furthermore, female G. holbrooki may aggregate to reduce foraging costs of sexual harassment (Pilastro et al., 2003). Such a wide array of strategies to reduce the costs of sexual harassment suggest these costs are high and it may be beneficial to the females to accept, to a point, increased predation risk to avoid these costs.

The lower activity level in females than in males may be explained by the need to minimize the predation risk in deeper water, i.e. other anti-predator behaviors are needed to make up for being in deeper water. Prey species may often change or eliminate behavior in response to predation risk. For instance, Magurran and Seghers (1990) found that in some populations, male guppies respond to the presence of a predator by

significantly reducing their courtship displays and significantly increasing gonopodial thrusting.

A second hypothesis to explain higher activity levels in males than females is that males are increasing their predation risk as a way of exhibiting a handicap trait. Several handicap traits such as predator-approaching behavior (Godin and Dugatkin, 1996) and ornamentation (Endler, 1980; Godin and Briggs, 1996) have been found in guppies. It is possible that males use activity level as a behavioral display of rigor. Higher activity levels would indeed be one way to reduce false signaling.

Finally, a third hypothesis explaining the higher level of activity for males than females is that males may increase their fitness by moving to different habitats and increasing the probability of encountering new mating opportunities. For instance, Griffiths and Magurran (1998) presented evidence male guppies trade off school fidelity for mating opportunities (see also Abrahams, 1989). Of course, these hypotheses are not mutually exclusive.

Although the MANOVA analysis only suggested a trend between companion sex and depth choice, the second analysis using ANCOVA and regression analyses revealed, in the absence of a predator, the regression slopes of female depth in response to companion male size versus companion female size were significantly different. Furthermore, females moved into shallower water as male size increased, indicating active sexual selection. Although the avoidance of sexual harassment may cause females to move into deeper water, the overall costs may decrease as male size increases due to a 'good genes' benefit. Indeed, McPeek (1992) found larger male *G. affinis* were associated with the larger females and suggested this may be due to female preference for

larger males. Although females may prefer larger males, in *G. holbrooki*, male gonopodial thrusting success is inversely related to size, with smaller males more successful than larger males (Bisazza and Marin, 1994). Mechanisms, such as using depth to select larger males, allowing more control over their mate choice, would presumably be selected for by females.

However, this preference may not simply be due to a 'good genes' hypothesis. Larger males may have a lower sexual harassment cost than small males. Small male *Gambusia* have been shown to attempt to mate significantly more forced copulations than larger males (Hughes, 1985). Furthermore, larger males may reduce the total number of copulation attempts experienced by a female through dominant interactions with smaller or less dominant males (Hughes, 1985; Pilastro et al., 2003). Females of other taxa have been shown to use males as shields. For example, in the water strider, *Aquarius remigis,* females allow smaller males to ride on longer bouts of copulation because they act as lighter, less costly shields to ward off harassment by other males (Sih et al., 2002). However, since only one male was placed in the experimental tank with the female in the our study, this explanation seems unlikely.

Interestingly, although female *G. affinis* are exhibiting sexual selection, no male mate preference was seen, in agreement with Hughes (1985). However, Bisazza et al. (1989) found male *G. holbrooki* preferred larger females, suggesting divergent mate choice between the two species.

Conclusion

Studies of depth selection in fishes have been relatively rare (see Smith and Belk, 2001; Power, 1984). The use of depth in anti-predator behavior has been suggested but

there has only been limited evidence to support this hypothesis. Furthermore, although very few studies have shown a difference in depth choice for males and females, our study may suggest it is widely prevalent, with the species exhibiting this behavior ranging from teleosts such as *G. affinis* to elasmobranchs (Simms et al., 2001). One reason for the sex-specific depths suggested by Simms et al. (2001) is that females need to limit multiple matings, a conclusion supported by female *G. affinis* in the current study incurring increased predation risk to avoid sexual harassment by males. Although no evidence of a risk behavior trait was found in male *G. affinis*, (i.e. moving deeper in the presence of females versus in presence of other males) females appear to be more receptive to larger males by moving up in the water column, reducing the predation risk they take upon themselves. Since females participate actively in the probability of success of a given male, sexual selection is occurring as active sexual selection rather than passive sexual selection.

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