MALE MATE PREFERENCE IN THE EASTERN MOSQUITOFISH
(*GAMBUSIA HOLBROOKI)*

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ABSTRACT

As a live-bearing fish, *Gambusia holbrooki* offers unique opportunities to study sexual selection. While males of this species invest little time or energy per offspring, sexual selection theory should predict that if a male is presented with two females of differing size, a preference should be shown for the larger of the two females. Evolution should favor the choice of a larger female because of increased brood size, better health, and ability to survive the environment. Only two studies to date have looked at male mate preferences in *Gambusia holbrooki*, and the results were mixed. In this study, to measure male preference a dichotomous choice test was used with female fish of varying sizes placed in clear containers at opposite ends of an aquarium. The elapsed time a free swimming male spent in "side time" (on same side of aquarium as either female) and "proximity time" (within one male length) was recorded. Experiment 1 looked at male preference for females differing in size by at least 5mm and found that males (n=12) spent significantly more time in close proximity to larger (compared to smaller) females in the first 10 minutes of a 20-minute preference test. The males, however, did not show a significant preference in side time. Experiment 2 sought to increase the size discrepancy between the two females tested and included very large females, as they might provide a supernormal stimulus for the male (n=9). However, the results of Experiment 2 showed no such preference in side time or proximity time. Experiment 3 increased the number of subjects to 27 to provide a more representative sample of female fish a male might encounter in the wild and added a social interaction portion to the testing. Female size differential groupings were broken in to three categories: Small (2-6mm), Medium (8-11mm), and Large (13-17mm). The results of this experiment showed a significant preference for the larger females that was dependent upon the size differential grouping, with proximity time in the medium grouping being most pronounced.

In the social interaction portion of the testing, a significant preference was found for larger versus smaller females, but there was not a significant preference found between the size groupings. As predicted by the sexual selection theory, the results of the aforementioned experiments seem to indicate that a male mate preference for larger females does exist, but only within certain parameters.

ACKNOWLEDGEMENTS

DEDICATION

I would like to dedicate this thesis to my mother and father whose unyielding support made this possible.

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INTRODUCTION

Natural selection and sexual selection are the two major forces behind the evolution of organisms. Natural selection holds that if individuals with certain traits reproduce more than others in a population, then these traits will occur with greater frequency in subsequent generations. As a specific type of natural selection, sexual selection occurs as phenotypic qualities lead to certain individuals having greater access to mates and thus more offspring (Darwin, 1874). Sexual selection influences secondary sexual characteristics of the phenotype such as morphological differences between males and females, or differences between male types.

Darwin (1874) developed the theory of sexual selection because he realized that many characteristics of males and females did not enhance survival. For example, male features such as horns or antlers in ungulates (e. g., deer and gazelles), significantly larger body size in many mammals, or the bright plumage common in birds might actually be detrimental to an individual because they may make it more susceptible to predation or they are costly in terms of energetics. However, such characteristics would be favored if the enhancement of mating success outweighed (or at least equaled) their detrimental effects.

Darwin's (1874) theory of sexual selection is further divided into two types: intersexual selection and intrasexual selection. Intersexual selection influences the evolution of secondary sexual characteristics which determine the relative attractiveness of members of one sex to the other sex within a species. Intersexual selection may give rise to several, often exaggerated, different secondary characteristics, such as size, color, and vocalizations. In many species, the way a female chooses which male to mate with is correlated with these characteristics. The female may choose a larger male over a smaller one, a more vivid male over a duller one, or a

male with a deeper or louder call over a quieter one. Intrasexual selection involves characteristics which affect the outcome of competition among members of one sex for access to members of the other sex. For example, intrasexual selection would operate on physical and behavioral features which helped to determine the outcome of aggressive encounters among males over territories, if possession of or the quality of a territory increased subsequent mating success. If one male is physically able to prevent another male from mating with a prospective female, then he would have a selective advantage despite potential female preferences for other types of males. An excellent example of such behavior is provided when one male bowerbird destroys the bower of another male (Diamond, 1982). The male left with the destroyed bower may lose opportunities to copulate with females, giving a mating advantage to the destroyer at least in the short term.

According to Trivers (1972), sexual selection exists because females are the limiting sex; they invest more in offspring than males do, and many females are unavailable for fertilization because they are caring for young or developing young. This means that females, being the limiting sex, are in high demand which leads to competition among males for access to the females. It has been shown that females invest more in their offspring than males. For example, a female may be pregnant for several months with nutritional requirements for herself as well as the fetus. Also, once the offspring is born, the female of many species is often the primary caregiver and thus expends more time and energy on the offspring.

Trivers (1972) also stated that sexual selection exists because males are not the limiting sex and therefore have developed ornaments for attracting females or taking on other males in contests. For example, Kodric-Brown (1989) found that female guppies (*Poecilia reticulata*) are more receptive to courtship displays from more brightly colored males. In another experiment

extra tail feathers were glued to male African long-tailed widow birds (*Euplectes progne)* and the male birds with the extra plumage had significantly more females nesting in their territories than those with shorter tails (Andersson, 1994). Two other excellent examples of ornaments that attract females were provided by Darwin with the elaborate plumage and displays by male birds such as the quetzal and sage grouse (Darwin, 1874). The male quetzal has ornate tail feathers that can reach two feet in length while the sage grouse has air sacs in his neck that push his neck and chest feathers up when they are inflated. Such ornamentation would impose survival costs, but would likely advance success in the competition for mates.

One theory as to why females choose certain males over others is based on the positive correlation of certain characteristics with increased survival. If the female mates with a certain male, the characteristic associated with increased survival should be passed along to the offspring. For example, Petrie, Krupa, and Burke (1999) found a significant female preference for male peacocks (*Pavo cristatus*) with bigger and brighter tails. The offspring of the larger and more ornate peacocks grew faster and survived longer when released into the wild. Fisher (1958) believed that female preferences initially evolved because the preferred trait is favored by natural selection and hence the offspring are more likely to carry that beneficial trait. The trait must, however, exhibit "truth in advertising" (Zahavi, 1975) or females will stop benefiting from mating with the male exhibiting this trait. If the preferred characteristic does not correlate with actual survival of young, the prevalence of the female preference for the trait will diminish and the frequency of the trait will decline.

If females continue to prefer a certain trait over time the characteristic may become exaggerated over generations. Fisher (1958) referred to this as "runaway selection", and it can be demonstrated with such traits as increased ornamentation size and brighter species coloration.

However, according to natural selection, it is possible that the trait may reduce the male's chance of survival if it requires additional energy for upkeep or increases chance of predation. Such scenarios would reduce the prevalence of the trait over time.

Male Mate Choice

While most studies of sexual selection have focused on male-male competition and female mate choice, male mate choice does sometimes play a role. In species with prolonged courtship and copulation periods or those in which the male makes substantial reproductive investment of some sort, males are expected to be very choosy. When multiple females are available for mating, it would be advantageous for males to select higher quality mates as time spent with one female reduces the chance to be with other females. Successive ejaculates in many species such as goats (*Capra hircus*; Fielden & Berken, 1964), sheep (*Ovis aries*; Synnott, Fulkerson, & Lindsay, 1981), and smooth newts (*Triturus vulgaris*; Halliday, 1976) show reduced numbers of sperm which may decrease the likelihood of insemination as males mate with multiple females. Cohen (1971) found that high sperm count was essential for successful insemination in mammals in general. Derrick and Johnson (1974) found this to be especially true in humans. In addition to the high cost of sperm, human males often devote more to their offspring than the initial investment of gametes (Clutton-Brock, 1989), suggesting that under certain circumstances, male and female investment might actually be closely aligned. This seems to suggest that males and females would both exhibit a high degree of choosiness.

Size may be one of the variables that choosy males look for when selecting a mate. It is possible that larger size may be preferred by males in certain species for the same reasons females prefer larger size in their male mates. Large female size is thought to be correlated with overall health, foraging ability, ability to avoid predation, and number of eggs (e.g., in

Gambusia; Bisazza, Marconato, & Marin, 1989). Possibly because they only have a limited

supply of sperm and mating-plug secretions, males of some other species are highly selective

with respect to choice of mates (Shine, Phillips, Waye, Lemaster, & Mason, 2003). Red-sided

garter snakes (*Thamnophis sirtalis parietalis*) provide an example of this male mate preference

(Shine & Mason, 2001). The male red-sided garter snake tends to prefer large rather than small

females and as male size increases, so does his preference for a larger female. Male Pacific blue-

eye fish (*Pseudomugil signifier)* also show a significant preference for larger females (Wong &

Jennions, 2003). In simultaneous choice tests, the male fish spent a greater proportion of their

time courting large, as compared to small, females. Such evidence suggests that both female and

male mate choice may be occurring within sexual selection in some species.

Studying Sexual Selection in Poeciliidae

   Many sexual selection studies have been performed on the family of fish called Poeciliidae

for numerous reasons. One of the reasons for studying this family of fish is the frequent

exhibition of sexual behavior by males in both laboratory and natural settings (Martin, 1975).

Another reason for studying Poeciliidae is the females' increased investment time per offspring

compared with most other fish species. Differing from other fish species, which lay eggs and

invest little energy per offspring, Poeciliidae give birth to live offspring and are therefore called

livebearers. Female mate choice, therefore, becomes important because of this increased

investment. Poeciliidae have also found to be able to survive harsh conditions and easily kept in

lab settings (Hughes, 1985).

   Female mate choice has been studied in many different poecillid fish for the reasons

mentioned above. Bishoff, Gould, & Rubenstein (1985) found that female guppies prefer males

with larger tails as opposed to those males with smaller tails. The results held true even when

the tails of the larger were surgically shortened; that is, the females chose the males with the larger tail regardless of the size of the rest of the fish. Basolo (1990) looked at the preferences of female swordtails*, Xiphophorus helleri*, and found that females prefer males with long tails as opposed to those with short tails.

In a study on *Jenynsia multidentata*, another live-bearing species, Bisazza, Manfredi, and Pilastro (2000) examined male mating success, male-male competition, female mate choice, and male mate choice in order to gain a better understanding of both intersexual and intrasexual selection. The mating success of males was determined by observing one male with one female and scoring the number of attempted inseminations by the male in a free swimming condition. A negative correlation was found between male size and mating success, due to the ability of the smaller males to sneak up on the females and force insemination. In order to test the competition between males, two males differing in body size (the smaller male measuring 80% in body size as compared with the larger male) in an aquarium with one or two females. Copulatory attempts and aggression between males were recorded in ten-minute intervals. It was found that the larger male succeeded in dominating access to the female and was more aggressive.

In order to determine a female mate choice for *J. multidentata* in their study, Bisazza et al. (2000) used a dichotomous choice test to allow for the female to choose between two males differing in size while controlling for other variables which may have influenced the results. The two males in this study were housed in separate compartments on each side of the tank and the position of the female was recorded, in relation to the males, every 30 seconds for 30 minutes. The dichotomous choice test design allows for the female to view and compare the two males and her preference can be determined through observations of her behavior. A slight preference was found for the larger males. Similarly, male mate choice for female size was studied by

placing two females into a tank with a single male and recording the number of mating attempts. One male was introduced into the aquarium and the number of his mating attempts with the two females was counted. Males longer than 23 mm made more mating attempts towards the larger of two females, whereas males 23 mm or shorter selected the smaller one. Such findings seem to support the possibility of male mate choice occurring within Poeciliidae.

Bisazza et al. (2000) used a dichotomous choice design in the aforementioned study in order to collect data based on the amount of time females will spend in proximity to males (female choice) and the time males spend near females (male choice). This design allows for greater control over the experiment, but is criticized for not recording direct observations of sexual behavior (Houde, 1997). By placing the males, for example, in separate compartments on each side of the tank, the design controls for the effects of male-male competition (intrasexual selection) in the experiment while leaving female mate choice (intersexual selection) free to occur. A few studies have compared data from dichotomous choice tests to more direct measures of mate choice and found consistent results, thus providing evidence for the external validity of such methodology. For example, Kodric-Brown (1992) examined male mating success in guppies (*P. reticulata)* by performing a dichotomous choice test to determine a female preference between two males and then releasing the three fish and scoring various behavioral patterns. The patterns included courtship displays and copulation attempts. The results of the experiment showed the same female preference for certain males in the dichotomous choice test as well as in the recording of behavioral patterns in free-swimming/social conditions.

McPeek (1992) also used a dichotomous choice test in his study to determine male mate preference in eastern mosquitofish between two females differing only in size. After the choice test was performed, the three fish were released to allow for direct interaction. Behavioral

patterns such as a chase (defined as a rapid movement toward a fish that caused the other fish to swim away) and the number of times the male attempted to copulate with each female were recorded. A copulation attempt was recorded when a male extended his gonopodium (a modified anal fin used for insemination) toward the female while chasing her. As with the other study mentioned above, no difference was found in mate preference between the dichotomous choice test and in the social interaction tests. As these studies show, the dichotomous choice design appears to be a valid test for determining mate choice by either males or females.

Studying Sexual Selection in Mosquitofish

The poecillid fish being examined in the present study are the same as mentioned above in the McPeek (1992) study, *Gambusia holbrooki*, or eastern mosquitofish. Eastern mosquitofish are found primarily in the southeastern United States, but have been introduced to many different parts of the world as a means of mosquito control (Courtenay, 1984*). G. holbrooki* are usually grayish in color and may have small, black spots on their upper body and fins (Martin, 1975). Eastern mosquitofish range from Maryland to southern Florida on the east coast and can be found as far west as the Mississippi River (Gilbert & Williams, 2002). *G. holbrooki* exhibit reverse size dimorphism with females measuring an average standard length of 35 millimeters and males an average standard length of 25 millimeters. Males stop growing once sexual maturity is reached, while females continue to grow indefinitely (Peden, 1973).

Males do not exhibit secondary sexual characteristics in order to attract females, but instead rely on forced insemination in order to deliver sperm. The males use a modified anal fin called a gonopodium to inseminate the female at her urogenital opening (Karplus & Algon, 1996). The male can be identified by this gonopodium and his smaller size. Females can be identified by an egg spot on the lower abdomen that darkens and swells during pregnancy.

Several studies in the early 1990s looked at intrasexual selection with *G. holbrooki*. Bisazza and Marin (1991) placed several males with a single female and found that the largest male was usually able to monopolize access to the female. However, body size was negatively correlated with the outcome of sexual behavior. As the length of the male increased, the frequency of success (defined as contact between genitalia) declined. Bisazza (1993) looked at male competition and female mate choice in *G. holbrooki* and found similar results. In the first experiment in this study, three males and three females were chosen at random and placed in a large tank to simulate their natural environment. Similarly, the results of this test showed a negative correlation between number of matings and male standard length. In another experiment in the study, Bisazza put two to five males in a tank with a single female and found that a hierarchy was quickly formed and the largest male spent most of his time behind the female to prevent copulatory attempts by the smaller males. The dominant male in this experiment was recorded as attempting over 90% of all copulations. Bisazza's study showed that smaller males were more efficient than larger males at inseminating a female, but only in an area of low male competition.

Intersexual selection in the form of female mate choice has been studied in *G. holbrooki*, including female preference for males based on size, ornamentation, or courtship display. Bisazza & Marin (1991) found no female preference for large or small males in groups of adult females that (a) had been housed with males, (b) had been male deprived for 30 days, (c) had been male deprived for 4 months, or (d) were virgin. Across female groups, females showed no preference for spending time with either large or small males during dichotomous choice tests when males differed in size by at least 5 millimeters. The dichotomous choice test consisted of a tank divided into three departments by panes of glass with the female in the center area while

one male was placed in each of the two outer areas. The males were kept separate in order to control for the effects of intrasexual competition.

Contrary to Bisazza and Marin's (1991) findings, McPeek (1992) noted that large females and large males tended to associate in the field, and that females appeared to prefer larger males in laboratory tests based on the amount of time that females spent near the larger males. Bisazza & Pilastro (1997) found that females did prefer larger males when presented with two males simultaneously. It was also found that brood size increases with female length, but that larger males do not transfer more sperm than smaller males. This increase in brood size could be associated with a possible male mate preference for larger females in intersexual selection.

Bisazza, Marconato, & Marin (1989) looked for such a male mate preference in *G. holbrooki* when presented with two differentially sized females simultaneously. The females differed in size by an average of 11 millimeters and were matched by size of gravid spot and degree of abdomen distension. The first experiment utilized a dichotomous choice test in which each male was placed in a 20 x 20 x 20 cm aquarium with the front facing a 20 x 10 x 20 cm glass tank which housed the females. The tank with the females was separated into two compartments so the females could not interact. The test lasted ten minutes and the time the male spent within one body length of the front glass next to each female was recorded. The results of this experiment showed that 13 out of 14 males spent more time closer to the larger female and overall, 70.9% of male time was spent with the larger female. In the second experiment 40 pairs of females that differed in length from 1 to 25 millimeters were tested. The same procedure was used with new males and a male mate preference for larger females was found in 36 out of the 40 pairs available. The results of this experiment showed that the males not only have a significant preference for larger females, but the time spent with the female increased as the size difference

increased.

    McPeek (1992) also tested for male mate choice in *G. holbrooki*, but found different results. An aquarium was divided into three compartments by plexiglass dividers, and the females were housed in the two outer compartments and the male was housed in the middle compartment. Preference by males was measured by recording the time the male spent in each half of the compartment. The middle compartment was divided into two sections by a string taped on the outside of the tank. Each trial was 10 minutes and fish were chosen randomly from stocks of fish which had never been exposed to each other. In the experiment a single male was given a choice between two females in one of two treatments. The first was similar sized females in which there was less than 1.0 millimeter of difference between the females. The second was different sized females in which the two fish differed by 5.0 to 8.0 millimeters. Twelve males total were tested in each treatment. The males in this study, however, did not show any preference for the larger of two females presented.

Purpose

    The two studies to date looking at male mate choice of the eastern mosquitofish found different results. Bisazza et al. (1989) found a significant male preference for larger females, whereas McPeek (1992) found no such preference. Thus, one purpose of the current study was to replicate the Bisazza et al. (1989) and McPeek (1992) studies while controlling for factors which may have influenced the results. To better control for factors which may have influenced the results from the two studies, in this study I: 1) only used males which have not been tested in previous experiments (in each of the studies mentioned above males were retested); 2) had a representative sample of female sizes, including very large females which may have been represented in the Bisazza et al. and McPeek studies, but were not reported; 3) allowed males to

come into olfactory contact with female fish during the dichotomous choice test which was not done in the Bisazza et al. study; 4) added a social interactions component in order to determine if there are any problems with the dichotomous choice test design of this experiment and to add a more naturalistic component to increase validity; 5) obtained further data from a new population as suggested by McPeek (1992) that would either support or refute his notion of differences being due to the origination of the fish. In the present study, I hypothesized that the male mosquitofish would prefer the larger of the two females presented simultaneously while controlling for the possible effects of intrasexual competition between the females. For the free swimming portion of Experiment 3, I hypothesized that males would attempt more gonopodial thrusts, spend more time within a fish length, and nip the larger of two females when presented with them simultaneously. The free swimming portion of this experiment was added as difficulties in finding a clear answer may stem from problems in the design of the dichotomous choice test. However, the free swimming portion also introduced the possibility of intrasexual competition influencing the results.

Experiment 1

Method

Subjects

Subjects for this study were 24 female and 12 male *Gambusia holbrooki* collected from freshwater ponds located on the campus of the University of North Carolina Wilmington. All fish were given an acclimation period of at least 7 days in the community tanks in the UNCW psychology department animal housing area. Within each testing group, the three fish were taken from separate community tanks (never housed together before testing). Gestation of the paired female fishes was matched as closely as possible looking for the presence and size of anal

spots as well as the degree of distension in the abdominal area. The length of each fish was measured from the tip of the mouth to the end of the caudal fin. Methods of subject collection and treatment were approved by the university's Institutional Animal Care and Use Committee.

Apparatus

Testing was conducted in a 10-gallon (38 liter) tank which contained gravel, a heater, thermometer, and aeration filters. The community tanks also included artificial plants. The testing and community tanks had black plastic lining on three sides to minimize the effects of external stimuli. The community tanks for the male and female fish were cleaned once a week in order to maintain a pH range of 6.6-7.0 and temperature within the tanks was held between 70-78 deg F.   Fish were fed Tetramin Tropical which is a commercial flake food.

The testing tank (see Figure 1) was divided into two halves front to back, separated by a black partition. Testing took part in the front part of the tank. During testing each female was kept within a clear, plastic container (8 cm x 8 cm x 11 cm) which was then clipped to the upper inside lip of the tank. One container was clipped on the left and the other container on the right. Each container was perforated in order to allow for water flow between the tank and the containers. Dark lines are drawn on the outside front of the tank one inch outside the edges of the containers as to measure proximity time. To aid with visibility, a hood with a 15-watt fluorescent bulb covers the testing tank. A computer with an event recorder was used to measure the dependent variables.

Procedure

Each testing group consisted of two females and one male with each fish taken from a separate community tank. Females were matched on the size and darkness of anal spots and amount of abdominal distension, but varied in size to assess male preference as shown. Small
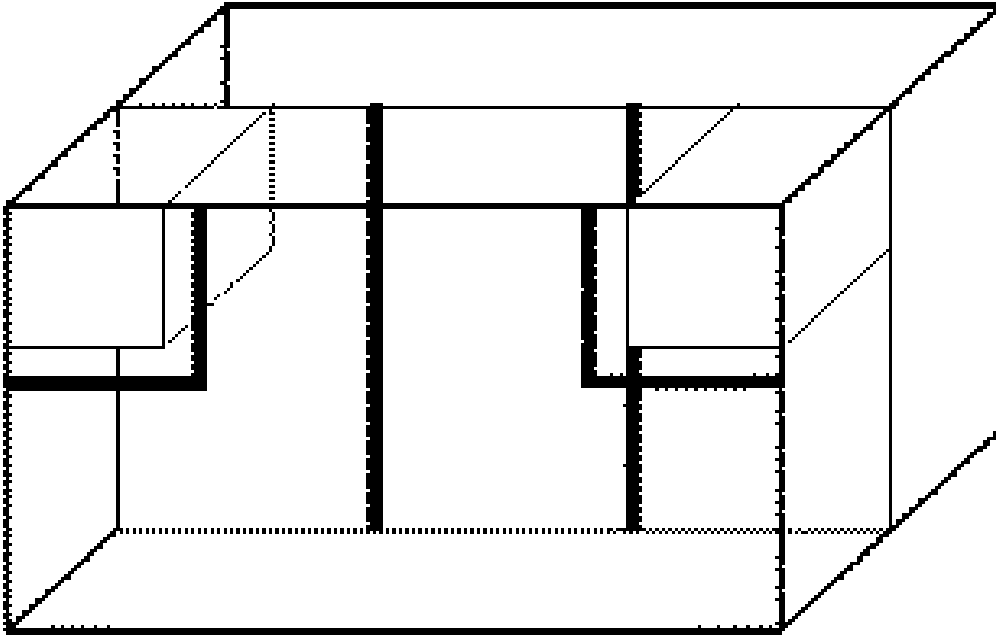
Figure 1. The testing tank. Dark parallel lines in middle are the side lines; dark lines close to the compartments are proximity lines.

females measured at least 5 mm less in length than the larger of the females tested.

Before testing began, pH and temperature were recorded for the three community tanks as well as the testing tank to ensure that conditions were similar. Overhead lights within the testing room were turned out and the 15-watt fluorescent bulb in the hood was turned on. The laptop computer was turned on and the event recorder program was started.

The male fish was given a five minute acclimation period in the testing tank. After the five minute habituation, females were put in at exactly the same time and a 10-min test began to record the location of the male in relation to each female. Side time was recorded as duration in seconds and began when the male's nose passed the line on the black partition which separates the tank into thirds. Proximity time was recorded when the nose of the male fish breached the black lines drawn on the outside of the tank. After the 10-min time period, the females were switched to the opposite ends of the tank at the same time. A second 10-min test took place and the results were recorded. After scores were recorded for the second 10-min period, the females were released to measure how much time a male spent with each female or both. After each trial, all three fish were measured in millimeters and put in a used tank not to be used again. Percent agreement was measured with at least two researchers recording side time and proximity time for this experiment with interrater reliability above 90%. Interrater reliability was determined by having two researchers in the testing room recording for side time or proximity time simultaneously using stopwatches.

Results and Discussion

As Table 1 shows, the larger females averaged 35.3 mm in length while the smaller females averaged 29 mm. As seen in Figure 2, males (n=12) spent significantly more time in close proximity to larger compared to smaller females in the first 10 minutes of a 20-minute preference

Table 1

Size in millimeters of female fish tested in Experiment 1

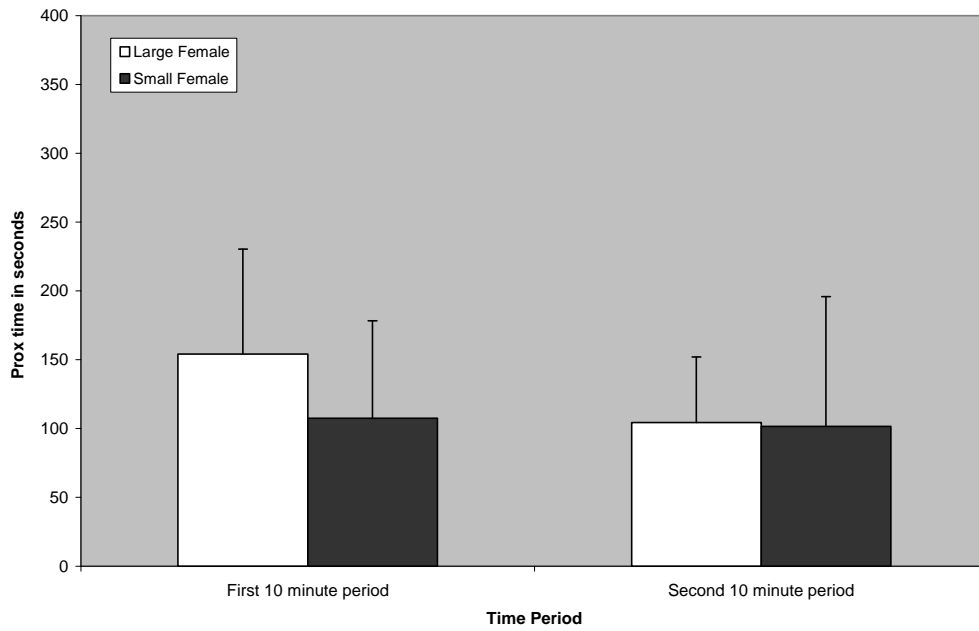| Test	Large Female	Small Female	Size Differential
1	26	21	5
2	32	27	5
3	26	22	4
4	26	21	5
5	35	27	8
6	36	29	7
7	38	31	7
8	39	32	7
9	50	43	7
10	43	36	7
11	33	27	6
12	40	32	8
Average	35.3	29	6.3

Figure 2. Means and Standard Deviations for the proximity time in seconds that males spent

near large females and small females during Dichotomous Choice Test.
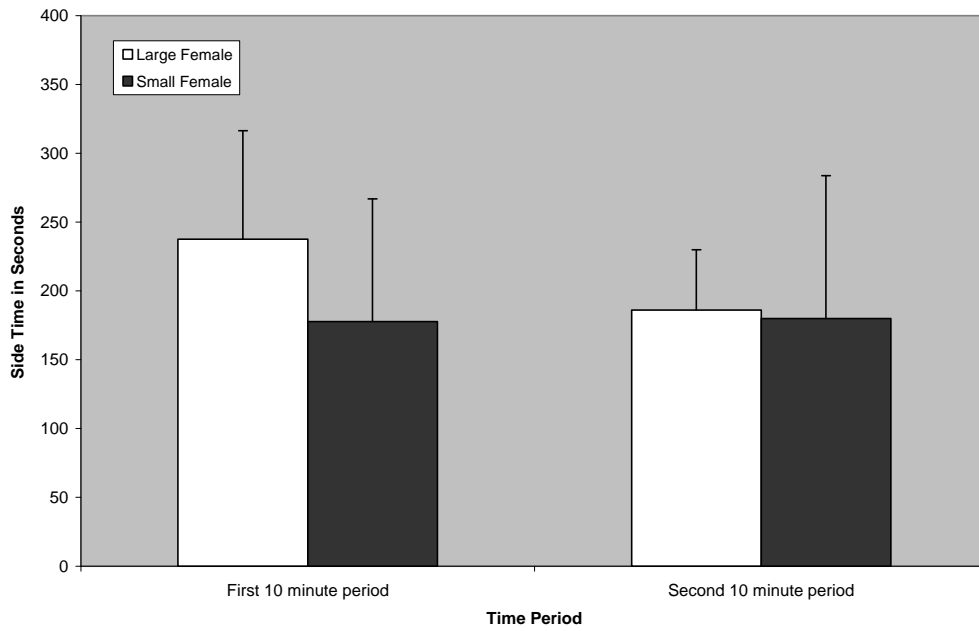
Figure 3. Means and Standard Deviations for the side time in seconds that males spent near large

females and small females during Dichotomous Choice Test.

dependent t-test ($t(11)=1.98$, $p=.035$), but not in the second 10 minutes ($t(11)=.1$, $p=.46$). Male preference for the larger females (see Figure 3) approached significance as measured by side time ($t(11)=1.53$, $p=.078$) in this first 10 minute period, but not for the second 10 minute period ($t(11)=.17$, $p=.43$) of the 20-minute test.

Though the results of this experiment did not show the significant preference in all situations for larger females as the Bisazza et al. (1989) study found, male preference for larger females in proximity time was found to be significant during the first 10-min period. Thus, unlike McPeek's (1992) study, the current findings seem to suggest that some male preference for size is influencing male mate choice. The results of this experiment do not provide the clear-cut answers to the questions put forth by the different findings presented by Bisazza et al. (1989) and McPeek (1992) in their respective experiments. Rather, the findings seem to suggest the need for further exploration of this question. Perhaps even larger females will need to be used in future experiments as enhanced size may represent a trigger of some sort. Actual female size was not reported in the studies mentioned above and it seems possible that not all size fish were represented.

Females may choose males with exaggerated features simply because such signals indicate the presence of fitness benefits that enhance the reproductive success of choosy females. (Andersson, 1994). As noted in the introduction above, larger size and more elaborate ornamentation are two of the most prevalent features that females prefer (Fisher, 1958). If the male signal and the female preference both have a genetic basis, choosy females will, on average, pair with males with exaggerated secondary sexual characteristics. The mate preference and the signal will become genetically coupled as a result of this process.

Exaggerated secondary sexual characteristics may be a result of a predisposition towards

supernormal stimuli. The idea of supernormal stimuli was coined by Tinbergen (1948) and

refers to a stimulus that produces a more vigorous response than the normal stimulus eliciting

that particular response. Egg retrieval by oystercatchers (*Haematropus ostralegus*) offers an

example of supernatural stimuli as females of this species prefer to retrieve eggs even from

outside the nest if they are several times larger than normal (Tinbergen, 1948). Another example

is provided by young herring gulls (*Larus argentatus argentatus*) that obtain food by pecking at

the parent's bill until it regurgitates. The young gull directs its pecking at a red spot at the tip of

the lower mandible. Tinbergen and Perdeck (1950) showed that both the spot and the bill shape

are signals for the response. Models that were much longer and thinner than the natural bill as

well as targets that exaggerated the natural red spot were much more effective at eliciting the

chick's response. In sticklebacks (*Gasterosteus aculeatus*), both male and female mate choice is

influenced by supernormal stimuli. Rowland (1989) showed that males prefer dummies whose

size range greatly exceeded that of females in a natural population.

   Experiment 2 will test whether male mate choice in *G. Holbrooki* is influenced by

"supernormal stimuli" in the form of very large female size differences. A preference for larger

size females of this species might be due to the positive correlation between female size and

number of offspring produced (Bisazza & Pilastro, 1997). I predict that males will show a

significant preference for very large females versus smaller females as they will represent a

"supernormal stimulus" for size.

<div align="center">Experiment 2</div>

<div align="center">Method</div>

Subjects

   Subjects for Experiment 2 were 18 female and 9 male *G. holbrooki* collected from freshwater

ponds located on the campus of the University of North Carolina Wilmington. All fish tested were from different locations on the UNCW campus and were never housed together in the laboratory prior to testing. Gestation of the paired females was matched as closely as possible looking at anal spots and distension.

Apparatus

The same apparatus used in Experiment 1 was used in Experiment 2.

Procedure

Differences in female body size were manipulated in order to measure male mating preferences in intersexual selection. The procedure for experiment 2 was identical to that used in experiment 1 except that the large females used were from a group of females in the lab that measured between 44-52 mm in standard length.

Results and Discussion

As shown in Table 2, the larger females averaged 47 mm in length while the smaller females averaged 34 mm. Unexpectedly, in Experiment 2, as seen in Figures 4 and 5, males (n=9) did not show a significant preference for the larger females over the smaller females; for the first ten-minute period, neither side time (t(8)=.37, p=.36) nor proximity time (t(8)=.58, p=.29) were found to be significantly different; for the second ten-minute period, neither side time (t(9)=.49, p=.32) nor proximity time (t(9)=.1, p=.47) were found to be significant. As Figure 4 shows, there was only a small difference in side time spent closer to the larger female compared to the smaller female during the first or second ten-minute period. Figure 5 also shows there was not a significant difference in proximity time between different sized females for the first or second ten-minute period.

The results of Experiment 2 seem to raise more questions than provide answers to the

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Table 2

Size in millimeters of female fish tested in Experiment 2

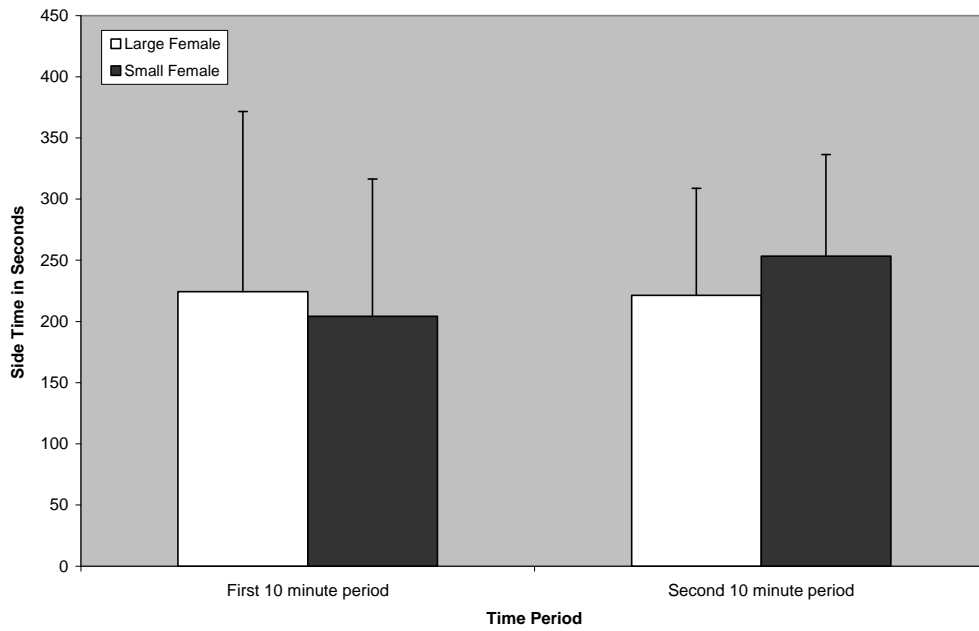| Test	Large Female	Small Female	Size Differential
1	47	33	14
2	44	33	11
3	52	35	17
4	45	38	7
5	49	35	14
6	47	33	14
7	47	28	19
8	46	35	11
9	46	38	8
Average	47	34.2	12.8

Figure 4. Means and Standard Deviations for the side time in seconds that males spent near large

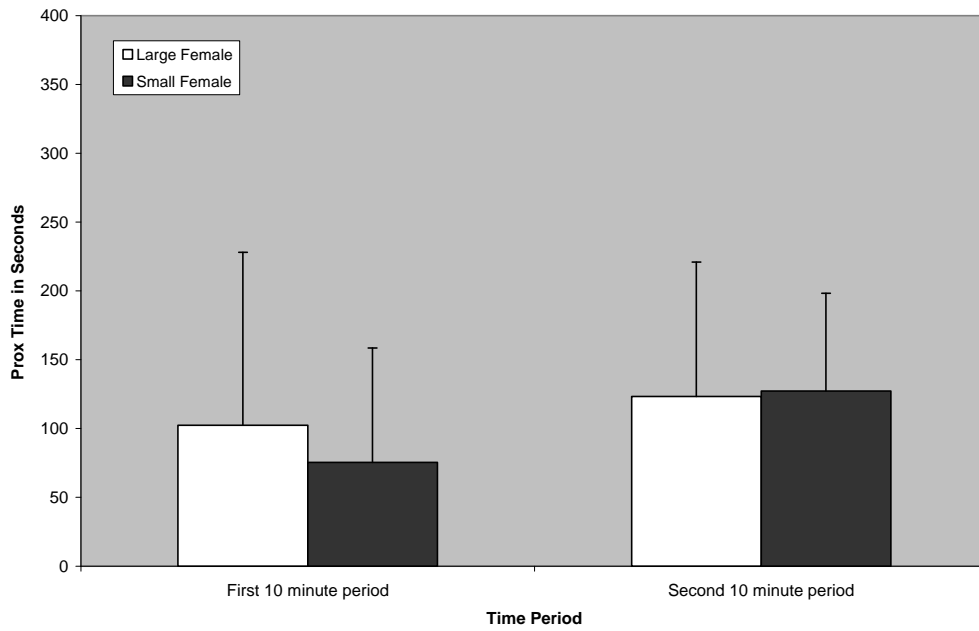females and small females during Dichotomous Choice Test.

Figure 5. Means and Standard Deviations for the proximity time in seconds that males spent

near large females and small females during Dichotomous Choice Test.

question of male mate preference for size in the eastern mosquitofish. In this experiment the males did not show a significant preference for the very large female as predicted. The findings, as with Experiment 1, suggest the need for further exploration of this question. Thus in Experiment 3, I proposed to replicate Experiments 1 and 2 and include a free swimming test after the dichotomous choice test to measure social behaviors and interactions. It seemed possible that difficulties in finding a consistent answer to this question may have stemmed from problems in the dichotomous choice design of this experiment. By measuring behavior in free swimming conditions, I hoped to understand more about male mate choice by observing the actual interactions among the fish, including sexual behavior, in Experiment 3. I also limited the dichotomous choice test to 10 minutes since, based on findings in the previous experiments, any differences should be apparent in the initial 10-min period. I also expanded the number of males tested in each size differential level to make sure that size differences in large and small females were balanced. With the changes mentioned above, I hypothesized that a male mate preference for larger females would be found.

Experiment 3

Method

Subjects

Subjects for Experiment 3 were *G. holbrooki* collected from freshwater ponds located on the campus of the University of North Carolina Wilmington. All fish tested were from different locations on the UNCW campus and were never housed together in the laboratory prior to testing. Gestation of the paired females was matched as closely as possible looking at anal spots and distension. The very large females (44-52mm) from Experiments 1 and 2 were reused for testing due to limited availability while no other fish from Experiment 1 or 2 were reused.

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Apparatus

The same apparatus used in Experiments 1 and 2 was used in Experiment 3, but during the

free swimming condition, the stimulus boxes were removed after the females were released into

the front half of the 10 gallon testing tank.

Procedure

Differences in female body size were manipulated in order to measure male mating

preferences in intersexual selection. The procedure for Experiment 3 was identical to that used in

Experiment 1 and 2 except the dichotomous choice test (DCT) was limited to 10 minutes. In

addition to the 10-min dichotomous choice test, a free swimming test was performed to measure

social behaviors between the three fish. To quantitatively measure the amount of time the male

spends with each female or both, instantaneous scans of proximity were conducted for ten

minutes in 10-second intervals (Lehner, 1996). In order to be in proximity to a female, the male

had to be within one body length which was defined as the body length of the male being

observed.

In addition to proximity scans, other social behaviors, nips and gonopodial thrusts, were

recorded. McPeek (1992) defined a nip as actual or attempted oral contact. As stated earlier, a

gonopodial thrust occurs when a male extends his gonopodium more than 90 degrees away from

his body within proximity of another fish (Martin, 1977). After testing, the length of each fish

was measured. Once measurement was completed, the fish were released and not tested again.

A 3 X 2 mixed design was used. The between subjects variable was the size differential

grouping of the females (3 levels: 2-6mm, 8-11 mm, 13-17mm) and the repeated measure was

the identity (size) of the stimulus female (large versus small). In the DCT, the two dependent

variables were side time and proximity time. A 3 X 2 mixed design ANOVA was conducted to

analyze the data collected from the dichotomous choice test.

For the free swimming portion of the experiment, a 3 X 2 mixed design was also used for each portion with the between subjects and the repeated measure variables being the same as above. The dependent variables for the recorded sexual behaviors were the number of gonopodial thrusts and nips recorded in the ten-minute period. The dependent variable for the instantaneous scans portion of the experiment was the number of proximity scans recorded in relation to the large or small female during the same ten-minute period.

Two researchers recorded side time and proximity time for 30 minutes with interrater reliability above 90%. Interrater reliability was determined by having two researchers in the testing room recording for side time or proximity time simultaneously using stopwatches. For the free swimming portion of the experiment at least two researchers recorded scans, nips, and gonopodial thrusts with interrater reliability above 90 percent agreement for 30 minutes. Interrater reliability was determined by having two researchers individually score the social behaviors and the results compared.

<div align="center">Results and Discussion</div>

As shown in Table 3, the small female difference group used females that averaged 38 mm for the large females and 35 mm for small females. The medium difference group averaged 42 mm for the large females and 32 mm for the small. Lastly, the large difference group of females averaged 44 mm for the large females and 29 mm for the small females. Figures 6 and 7 show means and standard deviations for the time spent in proximity time and side time to the large versus small females in each size differential grouping. As seen in Figure 6, males spent more time in proximity to the larger female in all three size difference groupings with the discrepancy being the greatest in the medium size differential grouping. There was a main effect of size

Table 3

Size in millimeters of female fish tested in Experiment 3

Small Difference Group

Test	Size of Larger Female (mm)	Size of Smaller Female (mm)	Size Difference (mm)
1	35	33	2
2	28	26	2
3	39	37	2
4	31	28	3
5	43	40	3
6	42	39	3
7	41	37	4
8	48	43	5
9	39	33	6
Group Avg	38	35	3

Medium Difference Group

Test	Size of Larger Female (mm)	Size of Smaller Female (mm)	Size Difference (mm)
10	46	38	8
11	47	39	8
12	38	29	9
13	33	24	9
14	45	35	10
15	46	36	10
16	49	39	10
17	34	23	11
18	39	28	11
Group Avg	42	32	10

Table 3 *cont'd*

Size in millimeters of female fish tested in Experiment 3

Large Difference Group

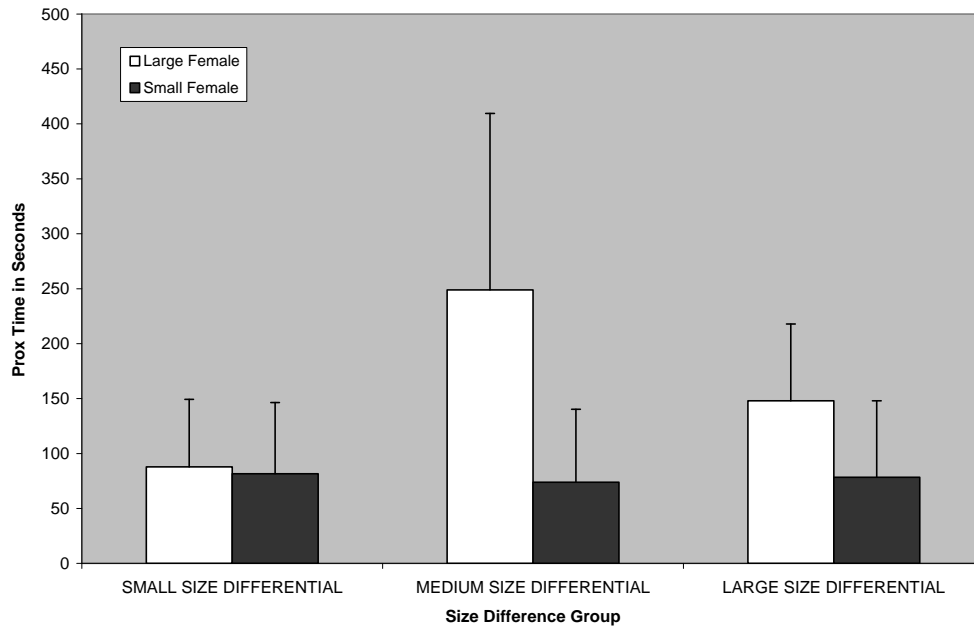| Test	Size of Larger Female (mm)	Size of Smaller Female (mm)	Size Difference (mm)
19	44	31	13
20	36	22	14
21	43	29	14
22	50	35	15
23	50	35	15
24	44	29	15
25	37	21	16
26	43	27	16
27	46	29	17
Group Avg	44	29	15

Figure 6. Means and Standard Deviations for the proximity time in seconds that males spent near large females and small females during Dichotomous Choice Test.
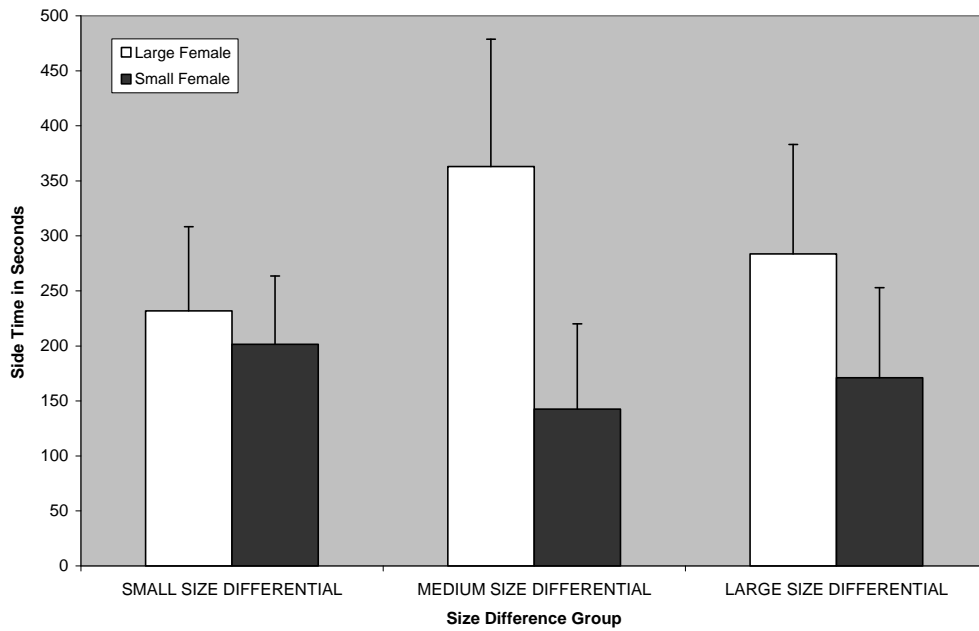
Figure 7. Means and Standard Deviations for the side time in seconds that males spent near large

females and small females during Dichotomous Choice Test.

grouping [F (2, 24) = 10.65, $p < .05$] on time spent in close proximity, as well as a main effect of female size [F (1, 24) = 3.797, $p < .05$], and an interaction between size grouping and female size [F (2, 24) = 3.686, $p < .05$]. The interaction effect indicated that male preference depended on whether the female size disparity was small, medium, or large to analyze the interaction. Dependent t-tests were performed on time spent in proximity for each disparity grouping. For the small grouping there was not a significant preference in proximity time (t(8)=.253, p=.807). For the medium size grouping there was a significant preference for larger females in proximity time (t(8)=2.65, p=.029). For the large size grouping there was not a significant preference in proximity time (t(8)=2.27, p=.053).

Figure 7 shows that males spent more time with the larger females in side time for all three size differential groupings. Like proximity time, the discrepancy in side time between the large and small female appear to be most pronounced in the medium size differential grouping. ANOVA confirmed that for side time, there was a main effect of female size [F (2, 24) = 14.76, $p < .05$], but not for female grouping [F (1, 24) = 3.386, $p > .05$] or for the interaction between the two [F (2, 24) = 3.044, $p > .05$].

For the free swimming data analysis, the means and standard deviations for gonopodial thrusts, nips, and instantaneous proximity scans are shown in Figures 8, 9, and 10. As shown in Figure 8, the male aimed more gonopodial thrusts at the females when the size differential was medium. For gonopodial thrusts, there was a main effect of female size [F (1,24) = 15.42, $p < .05$] with the male preferring the larger female, but not of size grouping [F (2,24) = .18, $p > .05$] or of the interaction between the two [F (2,24) = .18, $p > .05$].

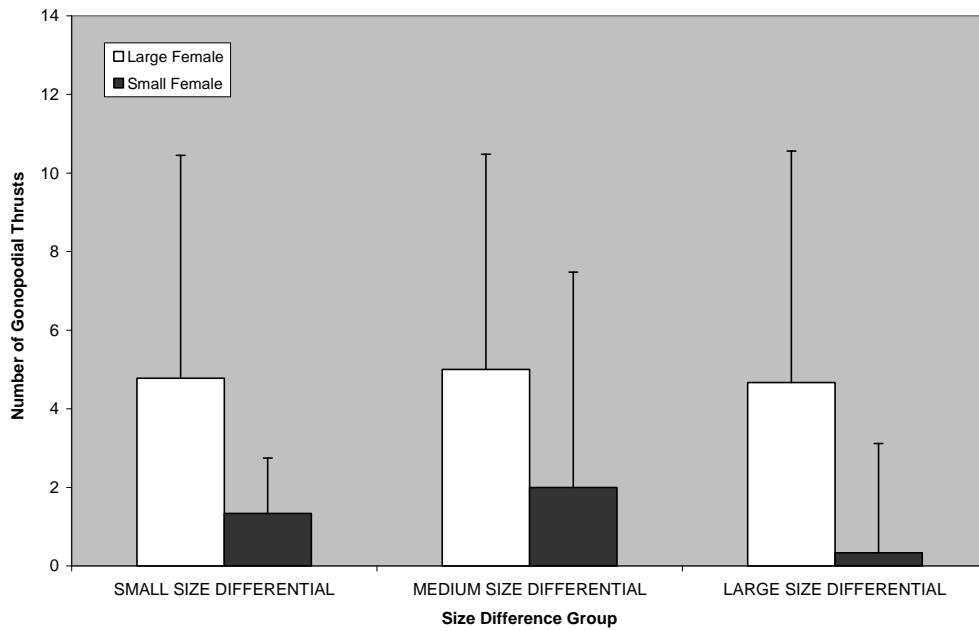Figure 9 shows that the male nipped the larger of the two females in all three size differential

Figure 8. Means and Standard Deviations for the number of gonopodial thrusts that males aimed

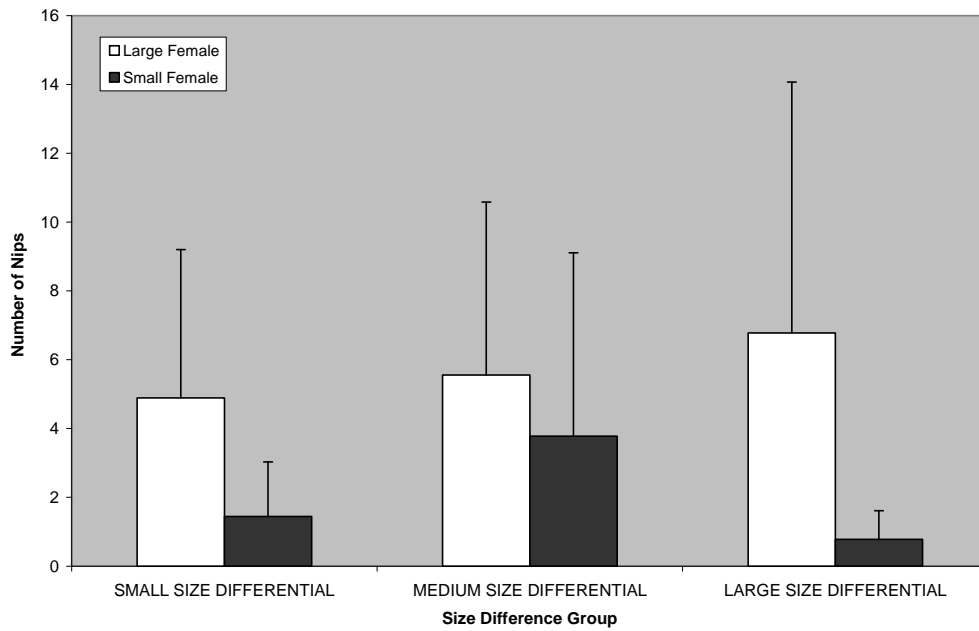towards large females and small females of size difference groupings.

Figure 9. Means and Standard Deviations for the number of nips that males aimed towards large females and small females of size difference groupings.
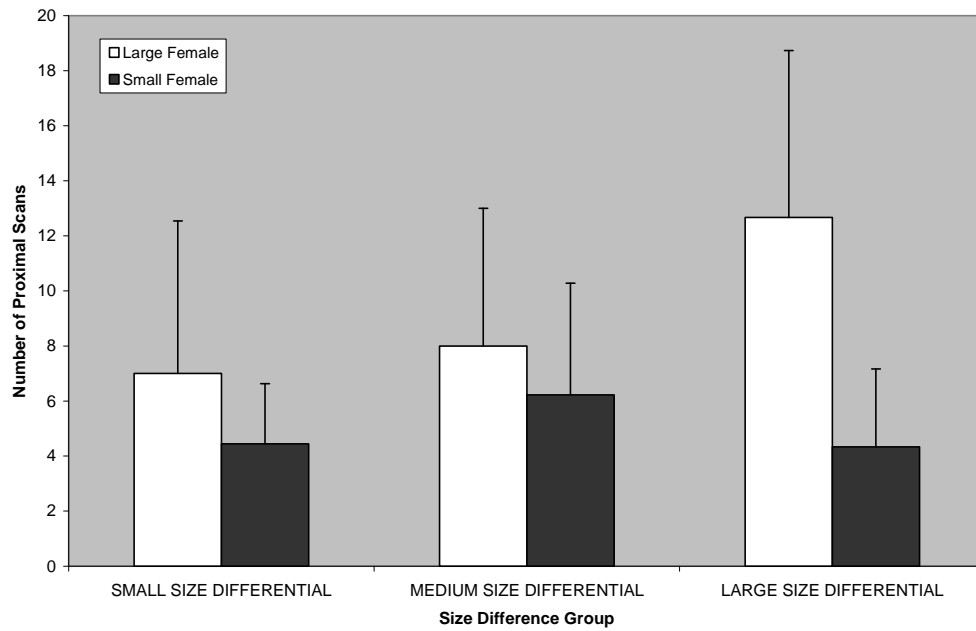
Figure 10. Means and Standard Deviations for the number of proximal scans recorded that males

were in proximity to large females and small females of size difference groupings.

groupings. For nips, there was a main effect of female size [$F(1,24) = 16.03$, $p < .05$] as the males preferred the larger female, but not of size grouping [$F(2,24) = .33$, $p > .05$] or of the interaction between the two [$F(2,24) = 1.73$, $p > .05$].

As shown in Figure 10, the male was recorded in proximity to the larger female more often in all three size differential groupings with the largest discrepancy between the large and small females. For instantaneous scans, there was a main effect of female size [$F(1,24) = 14.26$, $p < .05$] with the male preferring the larger female, but not of size grouping [$F(2,24) = 1.59$, $p > .05$] or of the interaction between the two [$F(2,24) = 1.91$, $p > .05$].

The results of Experiment 3 seem to support sexual selection theory in the prediction that males should favor the choice of a larger female because of increased brood size, better health, and ability to survive the environment. Overall, the male spent more side time near the larger female in 20 out of 27 tests (74.1%) and more proximity time near the larger female 21 out of 27 tests (77.8%). Binomial tests revealed a significant preference for the larger female in side time, $z = 2.505$, $p < 0.01$, and for the larger females in proximity time, $z = 2.909$, $p < 0.01$. Out of the 16200 seconds that the males were in the tank with the two females for 27 tests, the males spent 7906.4 seconds on the side of the larger female (48.8%), 4635.9 seconds on the side of the smaller female (28.6%), 4362.9 seconds within proximity to the larger female (26.9%), and 2109 seconds within proximity to the smaller female (13%).

Looking at the proximity scans which were conducted after the above test, it was found that out of 837 possible scans that the male was within proximity to the larger female 244 times (29.2%), within proximity to the smaller female 140 times (16.7%), within both the smaller and the larger 94 times (11.2%), and within neither 359 times (42.9%). Looking at the results from the behavioral recordings, the male gave more nips to the larger female 18 out of 27 tests

(66.7%) and out of the 203 nips recorded in the tests 149 were towards the larger female (73.4%). In only 2 out of the 27 tests (7.4%) were more nips directed towards the smaller female. Also from the behavioral recordings, the male had more gonopodial thrusts in 20 out of 27 experiments and out of the 163 gonopodial thrusts recorded, 130 (74.1%) were towards the larger female. In only 1 test out of 27 (3.7%) did the male direct more gonopodial thrusts towards the smaller female.

However, the results of the Experiment 3 dichotomous choice tests seem to show a male preference for larger females of this species exists only within certain parameters. After the female size differential and female size become too large, the male preference starts to decline. The results of the dichotomous choice in Experiment 3 show this clearly as the average time spent with the larger female in the medium size difference group was 220 seconds more for side time and 175 seconds more for proximity time versus the smaller female. In the large size difference group the male averaged 113 seconds closer to the larger female for side time and 70 seconds closer for proximity time versus the smaller female. Though the male still seems to spend more time closer to the larger female in the large size difference group, the preference does decrease compared to the results of the medium size grouping.

<center>General Discussion</center>

It seems possible the very large females used in this study were larger than the females the males would typically encounter in the wild which have an average standard length of 35 millimeters (Peden, 1973). The larger females represented in Experiment 2 averaged 47 millimeters and the larger females represented in the large difference group in Experiment 3 averaged 44 millimeters. Females housed in the laboratory may grow to greater sizes than what would typically be encountered in their natural habitat because they have a regular supply of

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food and are not threatened by predators. Houde (1997) noted that female guppies (*P. reticulata*) can be much larger than males and sometimes behave aggressively toward them. Under conditions when a large female was housed with a single male, the female often chased and nipped at the male. Houde stated that the male appeared "fearful" (p. 99) even of receptive females and avoided rather than courted the females. Any approach by the female resulted in a rapid retreat by the male.

These very large females could possibly be seen as a predation threat and cause some males to keep a greater distance from them. Nesbit and Meffe (1993) found cannibalization does occur within *G. holbrooki* in the wild. In addition, the rate of cannibalizing increased as the size of the consumer increased in this study. The authors found an increase in the rate of cannibalism for fish equal to and greater than 26.5 millimeters. As stated earlier, males grow until sexual maturity is reached and then cease growing while females continue growing throughout their lives (Peden, 1973). The average standard length for males is 25 millimeters which suggest that females may be more likely to cannibalize than males due to their greater size. Cannibalism has also been reported in several other poeciliid fishes in nature including *Gambusia affinis* (Seale, 1917; Krumholz, 1948; Harrington & Harrington, 1982). Several other species of poeciliids are known to cannibalize in captivity, including *P. reticulata* (Shoemaker, 1944) and *Poeciliopsis occidentalis* (Meffe, 1984). These studies seem to suggest once a female attains a certain size, she may become a predation threat to a relatively smaller male. It may benefit a male to keep more distance between himself and the larger female, at least initially. This increased distance could have affected the average time spent with larger females in this study.

In the free-swimming portion of Experiment 3 the results were slightly different than the dichotomous choice test. A significant preference for the larger females in the largest size

difference grouping was found for gonopodial thrusts, nips, and proximity time during scans. In the medium size difference grouping a significant preference was only found for larger females in relation to gonopodial thrusts. However, significant findings in proximity time during scans could have been caused by the larger female staying close to the male with intrasexual competition playing a role. It also seems possible that nips could act as a defense against a perceived predatory threat from a very large female. Gonopodial thrusts seem to represent a more accurate measure for sexual behavior than nips or chases. However, the frequency of gonopodial thrusts could be influenced by the size of the male in many poeciliid species (Houde, 1997). Bisazza (1993) noted smaller males in a mixed population did attempt more gonopodial thrusts in the form of sneak copulations than larger males in the group. This seems to suggest that other factors could influence the number of attempted gonopodial thrusts by a male.

Even with the aforementioned differences in size grouping results, a preference for larger females versus smaller females is present throughout Experiment 3. These results support the study put forth by Bisazza et al. (1989) that males should prefer a larger female versus a smaller one when presented simultaneously. The results of Experiment 3 provide further evidence in support of sexual selection theory which predicts a male mate preference will exist when other variables are controlled. The results of this study also refute those put forth by McPeek (1992) in that no male mate preference exists in this species. McPeek postulated that the difference between his study and others may be due to divergent sex ratios. Due to moderate climatic conditions, McPeek found a sex ratio of 1:1 (male to female) in Florida due to fewer exposures to extreme temperatures which allowed for more males to survive from season to season. He contrasted this to an unpublished study in Indiana in which a sex ratio of 1:5 (male to female) was found where colder temperatures led to harsher conditions which decreased male survival

rate from season to season. These differences in sex ratios could result in males in one population having more females to choose from and therefore may exhibit a preference for larger females. The males from the population with the balanced sex ratio may be less inclined to discriminate between females as there are fewer chances to mate. The sex ratio for the Bisazza study in Italy is not known.

As mentioned earlier, the differences in the studies could be a result of one study providing a more representative sample of female size in native populations. McPeek (1992) only had females which differed in size between 5.0 to 8.0 millimeters and the sizes of the female fish were not published. In contrast, the Bisazza et al. (1989) states that all female sizes were equally represented in order to best simulate the native population. The present study also attempted to equally represent the native population as well as put forth all possible size difference combinations for the males to choose from.

Possible future research might try to incorporate a way to represent weight and/or girth in addition to length in the female subjects. Females have been found to weigh 5 times as much as a male and it is unclear if any of the aforementioned studies fully represented an accurate weight distribution for this species (Gilbert & Williams, 2002). It might also be of value to correlate male size with female size preference as McPeek (1992) noted that larger males and females tended to congregate in the free swimming portion of his experiment. It seems possible that larger males might prefer larger females while smaller males might prefer smaller females. Lastly, a more accurate way to gauge where the female is at in its reproductive cycle may help to definitively answer the question of male mate choice in this species. Attempting to match up abdominal distension and the darkness and size of the gravid spot simply by visual inspection may not present the male to choose between two females who differ only in size.

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