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Predation Stress Induces Mate Choice Copying Behavior in Domestic Guppies (Poecilia reticulata)

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in Domestic Guppies (*Poecilia reticulata*)

by

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Abstract

Mate choice copying has been surmised to be an adaptive alternative mating strategy utilized by females of many species. However, studies have shown that domesticated variants of wild species no longer possess this evolutionary trait. To determine if this trait could be induced, domestic guppies (*Poecilia reticulata*) were exposed to predatory chemical cues for either 3- or 4-day periods. Female domestic guppies underwent preference testing (PT) followed by mate choice copying (MCC) trials pre-stress to establish baseline data and confirm an absence of MCC behavior. After pre-tests, females were placed in a stress tank where water from an aquarium housing a predator (*Crenicichla saxatilis*) was circulated throughout the tank. Females then underwent post-stress preference and mate choice copying tests. None of the females exhibited copying behavior in the pre-stress trials while a 25% incidence of mate choice copying post-stress was observed. The results of this study therefore indicate that predation stress via predatory chemical cues induces MCC behavior and strengthens the theory that this behavior is adaptive in wild populations.

Acknowledgments

To my husband, I could not have done this without your love and support. Thank you for always pushing me to pursue my passions and be my best self.

To my children, you are too young now to understand, but I hope with time you can be proud of what I have accomplished. My hope is that it will serve as a reminder in your lives to always chase your dreams and that you should always strive to continue learning.

To my advisor and committee chair, Dr. Anthony Marcattilio, I cannot fully express how grateful I am for the opportunity you gave me to pursue my graduate degree. I appreciate all the guidance and knowledge you shared along my journey.

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Chapter 1: Introduction

Mate Choice and Sexual Selection

While reproductive tactics vary greatly across the taxa, in many sexually dimorphic species, female mate choice is frequently at the forefront. When the female's selection is based solely on observed qualities of the male it is referred to as an "independent mate choice" (Pruett-Jones, 1992). *Poecilia reticulata*, or guppies, are sexually dimorphic live-bearers where males do not contribute resources to females with exception of their sperm. As such, female mate preference in guppies relies on being able to discern which male's genes will increase their reproductive fitness in their current environment (Real, 1990; Reynolds & Gross, 1992) and will therefore evaluate males based on their physical attributes such as their body length, brightness of the ventral spot, or showy tail (Houde, 1997). Furthermore, the variations in the male's phenotypic expression and parasite load directly contributes to their mating success (Endler, 1983; Houde, 1997; Houde & Hankes, 1997; Kennedy *et al*, 1987), and it has been demonstrated that these energetically costly displays are a sign of health and parasite resistance (Kodric-Brown & Brown, 1984; Zuk *et al*, 1990). By assessing a male's physical qualities along with the vigor of their courtship displays, females can discern which male will best enhance their offspring's fitness (Kodric-Brown, 1985).

Mate Choice Copying

Finding ways to maximize the likelihood of making quality mate choices without increasing costs incurred, such as reduced foraging time or increased risk of predation, is essential for survival in the wild. As such, guppies, among other species, have developed the strategy of utilizing social information in the form of mate choice copying behavior. This nonindependent mating strategy can cause a reversal of choice to a previously rejected male after observing a conspecific choosing that male. Wild *P. reticulata* from Trinidad were first documented as exhibiting this behavior in 1992 by Dugatkin, and additional studies confirmed its prevalence (Dugatkin, 1992; Dugatkin & Godin, 1992). Further research on this behavior, dubbed "Jealousy in Females," has found that it also exists in many other species, including sailfin mollies (Witte & Massmann, 2003; Hill & Ryan, 2006), Japanese quail (White & Galef, 2000), zebra finches (Kniel *et al*, 2015), Norway rats (Galef *et al*, 2008) and even humans (Bowers *et al*, 2012).

While the exact reason that mate choice copying behavior exists is not known, it has been surmised that it is an adaptive reproductive mechanism. Research has shown that juvenile and inexperienced guppies copy the choice of older females while the reverse, old copying young, does not occur (Dugatkin & Godin, 1993). It is supposed that this is a way for young females, who may be poor at discriminating good males from bad males, to learn what are considered desirable traits and will thereby be better able to discern the higher quality male in future mating encounters (Stöhr, 1998). Another reason that mate choice copying is postulated as being adaptive is that it reduces the cost of finding a mate since time used searching for and evaluating potential mates is time that could be spent foraging. It has also been demonstrated that mating and courtship behaviors cause an increase in predation risk (Magnhagen, 1991). Mate choice copying therefore would provide females a way to expedite the mate selection process without sacrificing on quality which, in turn, offers a reproductive fitness advantage.

Domestication and Inbreeding

The process of domestication causes extensive artificial selection on animals to achieve desired phenotypes that results in a multitude of changes. When attempting to achieve direct selection for certain attributes, frequent inadvertent selection also occurs as a byproduct of inbreeding. Consequently, genetic and cultural losses are to be expected during domestication (Huntingford, 2004). Even though domestication is frequently selecting for desirable size or coloration, it can also include behaviors (Jensen, 2006). The aquarium industry is specifically responsible for the domestication of numerous fish species for ornamental purposes, including the guppy (Balon, 2004).

The domestication of guppies has yielded incredibly colorful fish popular among aquarium enthusiasts; however, behavioral changes have also occurred including an absence of antipredator tactics along with a noticeable lack of mate choice copying behavior. Attempts at recreating Dugatkin's experiment in a laboratory environment did not find any mate choice copying behaviors evident (Lafleur *et al*, 1997). In Lafleur's experiment, fish were sourced from local pet stores and as such were domestic variants, whereas Dugatkin used wild populations in his original 1992 study. Besides Lafleur, other researchers have also observed this lack of mate choice copying behavior in domesticated strains of both guppies and sailfin mollies (Brooks, 1996; Brooks, 1999; Croghan, 2012; Elfelt, 2014).

Impact of Stress

In addition to artificial selection altering behaviors, there exists a lack of natural selection pressures experienced by domestics in the aquarium compared to their counterparts living in the wild. Normal stresses on wild animals, such as foraging, shelter or predation, are a non-issue to domesticated animals. Considering the effect stress imposes on animal behavior has been observed across numerous species, it stands to reason that the lack of pressures experienced by domestics would result in added behavioral changes over generations.

In fish, stress often has been studied regarding the effect predation has as a driving force in evolution and how it affects the plasticity of many characteristics. Since guppies rely heavily on chemical cues, the presence of predator kairomones and conspecific alarm pheromones has been shown to have numerous effects on traits and behaviors (Brown & Godin, 1999; Torres-Dowdall *et al*, 2012). When exposed to predation cues, guppies have been shown to alter their color development (Ruell *et al*, 2013), head morphology (Torres-Dowdall *et al*, 2012), size at maturity (Gosline & Rodd, 2008; Torres-Dowdall *et al*, 2012), growth rate (Handelsman *et al*, 2013), how quickly they mature (Dzikowski *et* al, 2004), and both brood size and brood interval (Dzikowski *et al*, 2004). Female mate preference will also switch to favoring less brightly colored males when faced with high levels of predation since brightly colored offspring would have decreased fitness in such environments (Stoner & Breden, 1988). This high level of plasticity, paired with their adaptability, has enabled guppies to be an effectively invasive species across the planet (Deacon *et al*, 2011).

Influencing Behavior

Given the high level of inducible variation from the exposure to predator cues, it raises the question of whether predation stress would affect mating strategies. Studies on mate choice copying behavior in wild guppies showed that the effect of predation stress through visual cues did not increase the incidence of copying (Briggs *et al*, 1996). However, these wild guppies originated from a population that experiences frequent high levels of predation pressures and utilized mate choice copying as a mating strategy prior to the introduction of the pike cichlid in the experiment. The lack of effect on copying might also relate to a diminished interest in mating as female guppies from high-predation populations will exhibit decreased sexual activity and reduced mating preference towards a previously desirable male when faced with predation risk (Godin & Briggs, 1996). Meanwhile, between domestication and lack of natural selection pressures, including predation, domesticated guppies have forgone employing this reproductive tactic. Even still, the high degree of plasticity exhibited by guppies in response to predation stress raises the question of whether exposure to predatory chemical cues could elicit mate choice copying behaviors in domestic guppies.

Chapter 2: Materials and Methods

Test Subjects

This experiment explicitly tested domestic *P. reticulata* response to stress. As such, all fish were obtained from a local pet store: Art, Fish & Soul in St. Cloud, Minnesota. At the onset of the experiment, 40 adult female and 40 adult male guppies were acquired (Figure 2.1). After some natural deaths occurred, an additional 15 adult female guppies were obtained. Instances of reusing females (as models) and males did occur over the course of the experiment, but a minimum of 7 days separated trials using the same individual. Most of the females were gravid when first acquired so the resulting offspring were collected and moved to a 60-gallon holding tank where they reproduced freely. Any additional fish needed for the experiment were from the holding tank. The predator used in this experiment was one pike cichlid (*Crenicichla saxatilis*) which was also acquired from Art, Fish & Soul.

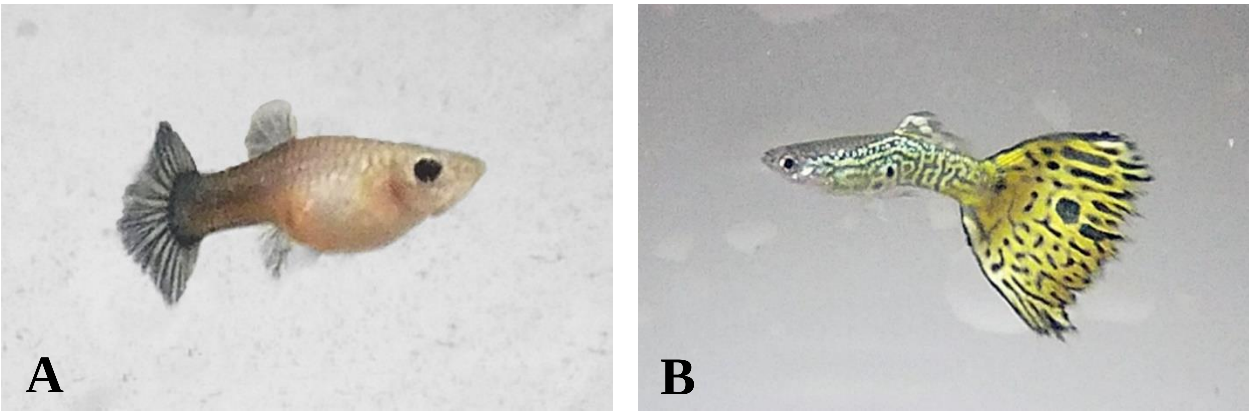

Figure 2.1. Domestic Guppies (*Poecilia reticulata*). A – Female guppies are larger, have more muted coloration, fan shaped anal fins and smaller, less showy dorsal and caudal fins. B – Male guppies are brightly colored, have a gonopodium and have bright, showy dorsal and caudal fins.

Housing

Guppies. Subjects were separated by sex and placed into four 20-gallon tanks upon arrival with 20 fish in each. Shelf paper and cardboard dividers were used to isolate tanks from one another. The aquariums contained natural color gravel, artificial plants, filters, heater, lights and hood. To provide additional cover, short sections of PVC pipe were added to the tanks. All lighting, aquarium and room, was maintained via timers for the duration of the study with a 16:8 light-dark cycle. The tanks were filled with filtered well water and water temperature was maintained between 23 – 26°C with submersible heaters. Water changes occurred weekly replacing 25% of the water. Carbon filters were rinsed weekly during water changes and replaced monthly. API® Aquarium Salt and Seachem Prime® were added to the tanks during initial set up and with each water change. Females were isolated until no longer gravid before trials began.

Pike Cichlid. The pike cichlid (*C. saxatilis*) was housed alone in a 20-gallon aquarium divided with opaque plexiglass and contained natural color gravel, artificial plants, filters, heater, lights and hood (Figure 2.2). The light-dark cycle was kept constant with the guppies via timers, and the water temperature was maintained between 23 – 26°C. The tank was filled with filtered well water and underwent 25% water changes weekly. Carbon filters were rinsed during water changes and replaced monthly. API® Aquarium Salt and Seachem Prime® were added at tank start up and with each water change.

Figure 2.2. Pike cichlid (*Crenicichla saxatilis*). The pike was housed alone in the divided aquarium.

Feeding

Guppies were fed using automatic feeders twice daily with a combination of Tetra® Tropical Crisps and Tetra® Flakes. The pike's diet consisted of live fish added to the tank by the experimenter.

Test Apparatus

Preference and mate choice copying tests were conducted in a 10-gallon tank (51.43 cm x 26.67 cm x 31.91 cm). The tank was divided with 2 translucent plexiglass pieces, yielding three zones: male holding areas on each end that were 10 cm wide and the center zone reserved for the focal female which was a total of 30 cm wide. Lines were marked on the outside of the tank further dividing the center zone into two preference zones that were 7.5 cm each and an indifference or neutral zone that was 15 cm wide (Figure 2.3). A removable upturned clear breeder box was used to contain the focal female during observation periods before being allowed to swim freely and choose a male.

Figure 2.3. Test Apparatus Front View. The red lines indicate the edge of preference zones with the center of the tank reserved as the indifference or neutral zone. The males are contained by clear plexiglass dividers on opposite ends of the tank.

Procedure

Prior to starting the experiments, males were matched for total length and tail length, each within 2 mm, by measuring with a wet plastic metric ruler while contained in a dip net after removal from the tank. Males were also matched by a visual assessment for shape and showiness of their tail. All preference and mate choice copying tests were filmed and repeated with males switching sides to control for any potential bias the females may have towards one side of the tank. The trials were recorded using a Canon Vixia HF R800 which was set up viewing the long side of the aquarium mounted on a tripod. While recording, the experimenter sat quietly away from the aquarium monitoring the experiment with a stopwatch and documenting information.

Preference Test

All fish were first allowed a minimum 30-minute period of acclimation to the tank which enabled the female to observe the two males while she was contained in an upturned clear breeder box prior to commencing preference trials (Figure 2.4). At the end of the acclimation period, the female was released from the breeder box, and a 10-minute choosing period began once the female engaged with the males. The interval spent in each male's preference zone was timed on stopwatches. After 10-minutes elapsed, the female was returned to the upturned breeder box while the male's sides were switched to control for any side bias. The female then had a minimum 10-minute observation period of the males on their new sides before being allowed another 10-minute choosing period, again starting after the female engaged. The time spent with each male from the second choosing period was added with those from the first to determine preference. The process of utilizing time as a measure of preference in guppies has been shown to have a positive correlation with copulation probability (Bischoff *et al*, 1985; Kodric-Brown, 1993). When a preference of >55% was indicated, the chosen male was then labelled the Initially Preferred Male (IPM) and the second male was referred to as the Initially Rejected Male (IRM).

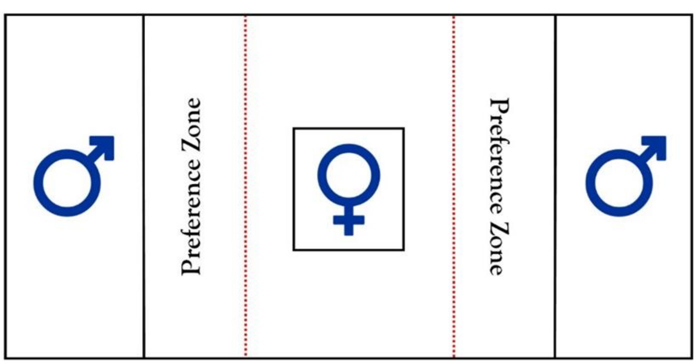

Figure 2.4. Test Apparatus Aerial View Diagram. Focal Female is shown as being contained in the upturned breeder box while the males are located on each end of the tank for the observation and acclimation periods.

Mate Choice Copying Test

After completion of the preference test, upturned breeder boxes were added to each male compartment. A Model Female was added to the side of the Initially Rejected Male. The Model Female was matched for total length, within 2 mm, of the Focal Female to control for age dependent effects as it has been shown that wild guppies will not copy younger females while they more readily copying older females mate choices (Dugatkin & Godin, 1993). A second female, obscured from view of the Focal Female, was added to the preferred male's side to counterbalance for courtship displays. All fish were allowed a minimum 30-minute acclimation and observation period that began after the addition of the Hidden and Model Females. Allowing at least 30-minutes for the viewing period permitted for maximal mate choice copying behavior since it has been shown to plateau beyond 30-minutes (Dugatkin, 1998). The Focal Female was contained for the duration of the viewing period in the upturned breeder box in the center zone of the tank (Figure 2.5).

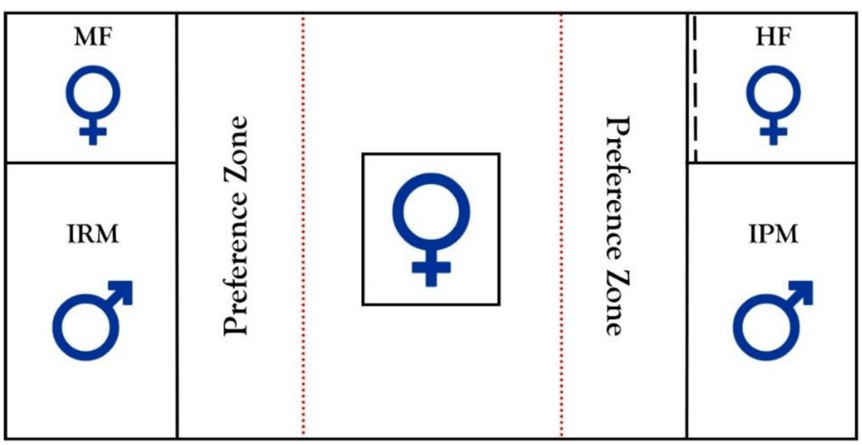

Figure 2.5. Testing Apparatus for Mate Choice Copying Observation Period Diagram. The Model Female (MF) is shown next to the Initially Rejected Male (IRM) within view of the Focal Female (FF) (center of tank). The Hidden Female (HF) is on the side of the Initially Preferred Male (IPM) to control for courtship displays but is obstructed from view (indicated by dashed lines) of the Focal Female.

At the cessation of the observation period, the Model and Hidden Females were removed. The Focal Female was then released from the breeder box, and a 10-minute choosing period began after the female engaged. When time elapsed, the Focal Female was returned to the upturned breeder box and the male's positions were switched, again to control for potential side bias. The Model and Hidden Females were once more added to the tank, with the Model Female again on the side of the Initially Rejected Male. After the females were added, a second observation period began lasting a minimum of 10-minutes. The Model and Hidden Females were then removed. The Focal Female was released from the breeder box and allowed to choose for an additional 10-minutes. Timing started after the female would begin to engage with the males. Utilizing the same procedure as in the Preference Trials, the times spent in each preference zone for the two Mate Choice Copying trials were added.

Stress Exposure

After establishing a lack of mate choice copying behavior in domestic guppies, males were returned to their housing while the females were placed into a stress tank with a recirculating pump that cycled water from the side of a pike cichlid to the guppy side of the tank. The water flowed back to the pike side through holes drilled near the top of the opaque plexiglass divider (Figures 2.6). There were two stress levels assessed: 3- or 4-days. After the stress exposure, the guppies were again tested for preference between 2 new males, matched for size, followed by mate choice copying tests adhering to the previously listed protocols.

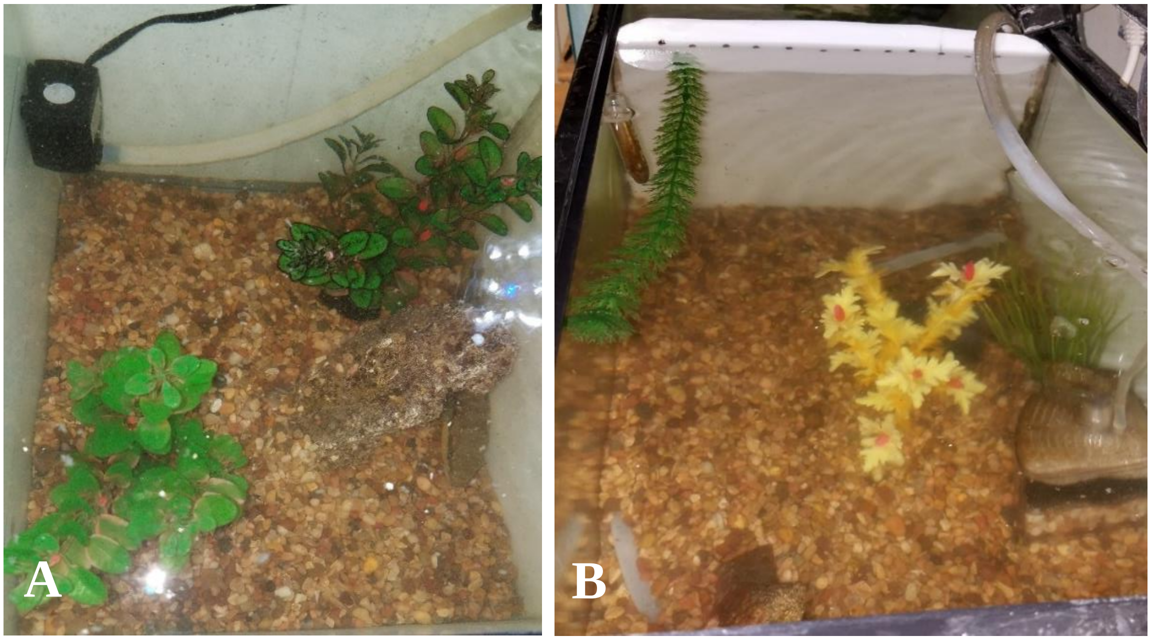

Figure 2.6. Experimental Stress Tank. An 18-gallon aquarium was divided with opaque plexiglass. A – *C. saxatilis* side with natural rock, artificial plants, submersible pump and tubing. B – *P. reticulata* side with opaque plexiglass divider, natural rock, artificial plants and tubing outlet.

Data Collection and Analyses

The amount of time the Focal Female spent in each male's preference zone was recorded in seconds (s) with the totals added between the two preference trials and the two mate choice copying trials. A preference threshold was established as time spent with a particular male as being >55% of the total time spent between both males (Equation 1). Any females not meeting this threshold were excluded from the results. Instances of side bias or lack of choice also resulted in exclusion. When preference was indicated, mate choice copying trials were run and Equation 1 was again utilized to determine the percent of time the Focal Female spent with each male. Male 1 and Male 2 were used as the designations for each male prior to preference being established. T1 and T2 indicates time in seconds for trial 1 and trial 2, respectively, for either the Preference Test (PT) or Mate Choice Copying Test (MCC).

Equation 1: Percentage Equation

$$Percent\ of\ Total\ Time = \frac{Male\ 1\ T1\ (s) + Male\ 1\ T2\ (s)}{Male\ 1\ T1\ (s) + Male\ 2\ T1\ (s) + Male\ 1\ T2\ (s) + Male\ 2\ T2\ (s)}$$

The percentage of time spent with the Initially Rejected Male (IRM) from Preference Trials (PT) was subtracted from the percentage of time that the IRM received during the Mate Choice Copying (MCC) Trials (Equation 2). A positive integer indicated an increase in the percent of time that the Initially Rejected Male received from the Focal Female. These values were compared pre- and post-stress.

Equation 2: Percent Change between Mate Choice Copying and Preference Trials

$$\Delta = IRM\ MCC\ \% - IRM\ PT\ \%$$

Pre-Stress preference and mate choice copying trials had 16 fish meeting the criteria of a preference threshold of >55% and no side bias shown. There were 8 females meeting the criteria in the post-stress 3-day exposure and 12 females after a 4-day stress exposure. For paired t-tests on the percent change, only those females with complete pre-stress and post-stress data were utilized. In the Pre-Stress to 3-Day group, there were 6 females, and the Pre-Stress to 4-Day had 8 females. A pooled analysis paired t-test was run on the 14 Pre-Stress data points to the 14 Post-Stress data points for percent change. All confidence levels were set to an alpha of 0.05.

Chapter 3: Results

Behavioral Changes

Females exposed to predatory stress exhibited behavioral changes that included increased antipredator tactics, such as dashing and freezing behaviors along with shoaling tendency. Females did not swim freely throughout the tank and would instead stay fixed near the edges and corners of the aquarium, utilizing cover when possible. Their movements were seemingly agitated compared to their non-stressed counterparts who swam freely in their aquarium. Where non-stressed fish would exhibit begging behaviors for food, stressed females would instead take cover when the experimenter approached their aquarium. After stress exposure, females were also more distressed during preference and mate choice copying trials. There were multiple instances where the females required 10+ minutes after being released from the upturned breeder box before they would engage with males. Some occurrences resulted in incomplete trials due to the female hiding and refusing to move for the duration of the trial.

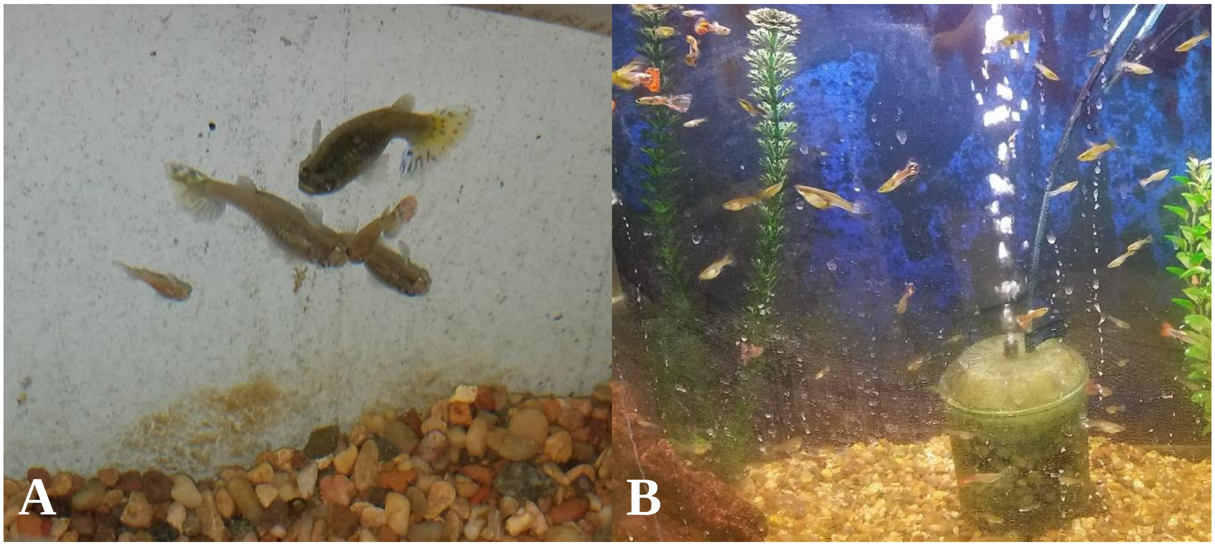

Figure 3.1. Behavioral Changes. A – Females exposed to predation stress exhibited frequent shoaling and freezing behaviors. B – Non-stressed fish swam freely throughout their aquarium.

Percent Change

The percent change (determined using Equation 2) was compared for the Pre-Stress, 3-Day and 4-Day stress exposure data. A general trend can be seen showing that as stress exposure increased, so did the percent of time spent with the Initially Rejected Male (Figure 3.1) suggesting there is a positive correlation of stress on potential incidence of mate choice copying behavior.

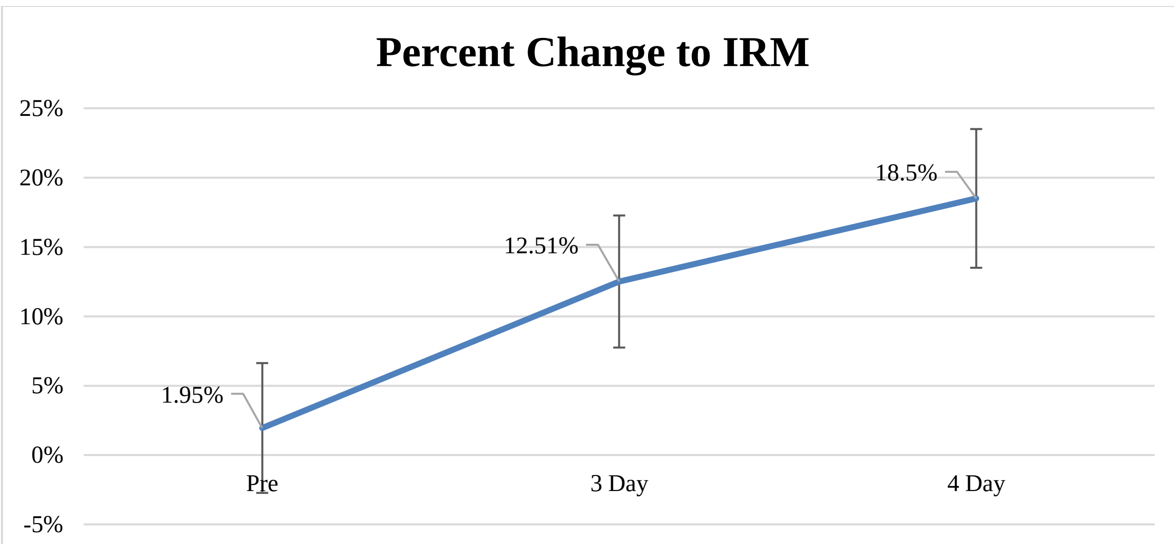

Figure 3.2. Percent Change to IRM. Data from the pre-stress exposure was pooled (n = 16) and compared to the 3-Day (n = 8) and 4-Day (n = 12) stress exposures showing that percent of time spent with the Initially Rejected Male increased as the length of stress exposure.

The percent change was also compared for paired data utilizing only females with complete pre- and post-stress preference and mate choice trials. Three groupings were compared: Pre-Stress to 3-Day, Pre-Stress to 4-Day and the pooled data for Pre-Stress to Stress (Figure 3.2). All females exhibited an increase in time spent with the Initially Rejected Male after Mate Choice Copying Trials; however, it was a 4-Day stress exposure that resulted in the greatest increase at 15.93%.

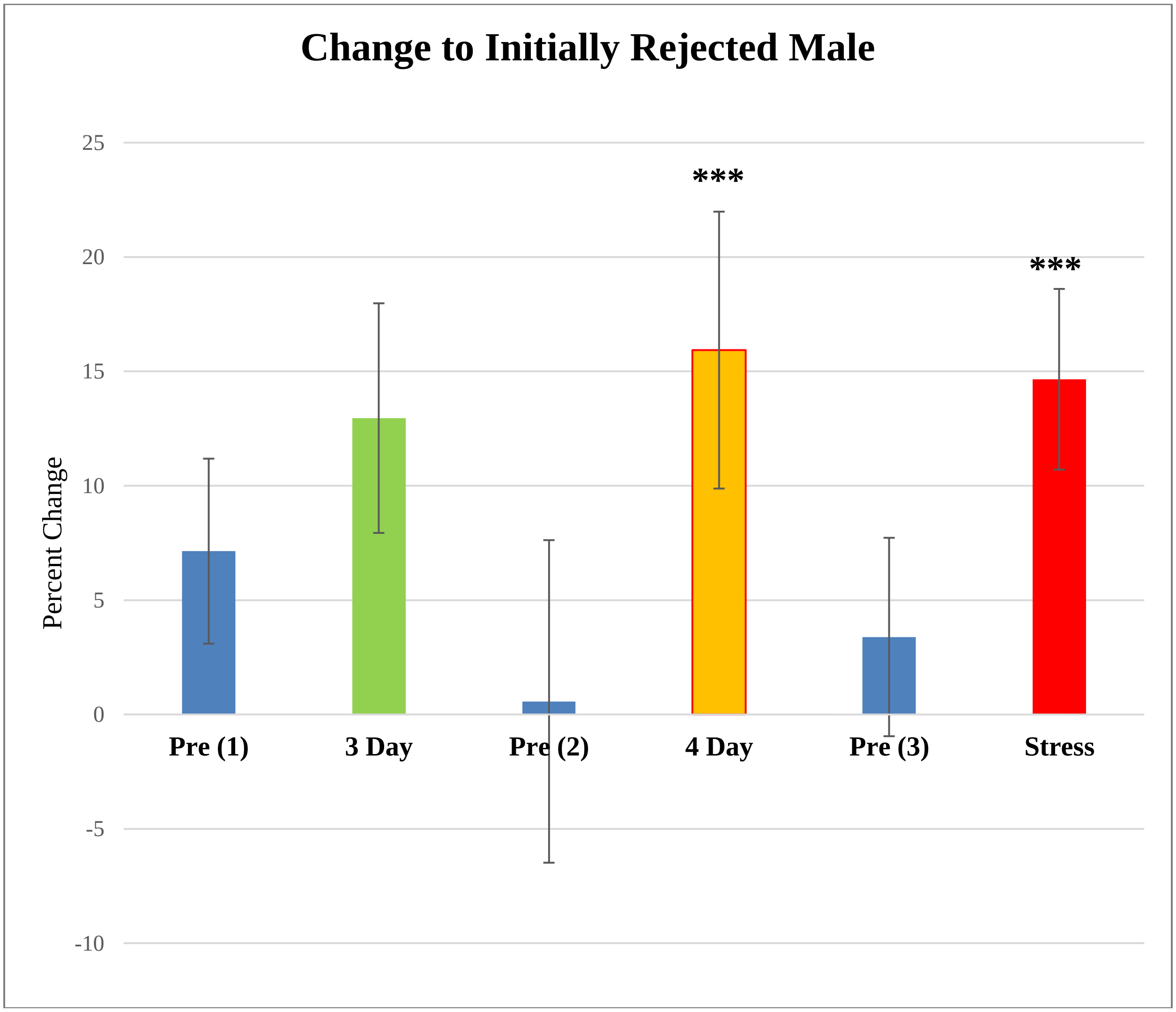

Figure 3.3. Percent Change to the Initially Rejected Male Before and After Stress Exposure. Pre (1) to 3-Day had an n = 6 and Pre (2) to 4-Day had an n = 8. Pre (3) and Stress were the pooled groups with an n = 14.

Paired t-tests for the Pre-Stress to 3-Day (df = 5) did not show significance for the percent change (t = –1.4359, p = 0.105). However, the percent change was significant for both the Pre-Stress to 4-Day (df = 7) paired data (t = –2.7538, p = 0.014) and the pooled Pre-Stress to Stress (df = 13, t = –3.0128, p = 0.005) (Table 1).

Mean Time Spent

Data was also evaluated for the time (s) spent with the males in preference versus mate choice copying trials at each treatment level (Figure 3.3). In the Pre-Stress trials (n = 16), there was a negligible change in the mean time spent with both the IPM and the IRM between Preference and Mate Choice Copying Trials (IPM = – 4.34 (s), IRM = + 6.93 (s)). After stress exposure, the IPM had considerable decreases to time from the Preference to the Mate Choice Copying trials (3-Day = – 93.80 (s), n = 8, and 4-Day = – 49.43 (s), n = 12), and the IRM had significant increases to the time spent (3-Day = + 47.95 (s), 4-Day = + 108.64 (s)).

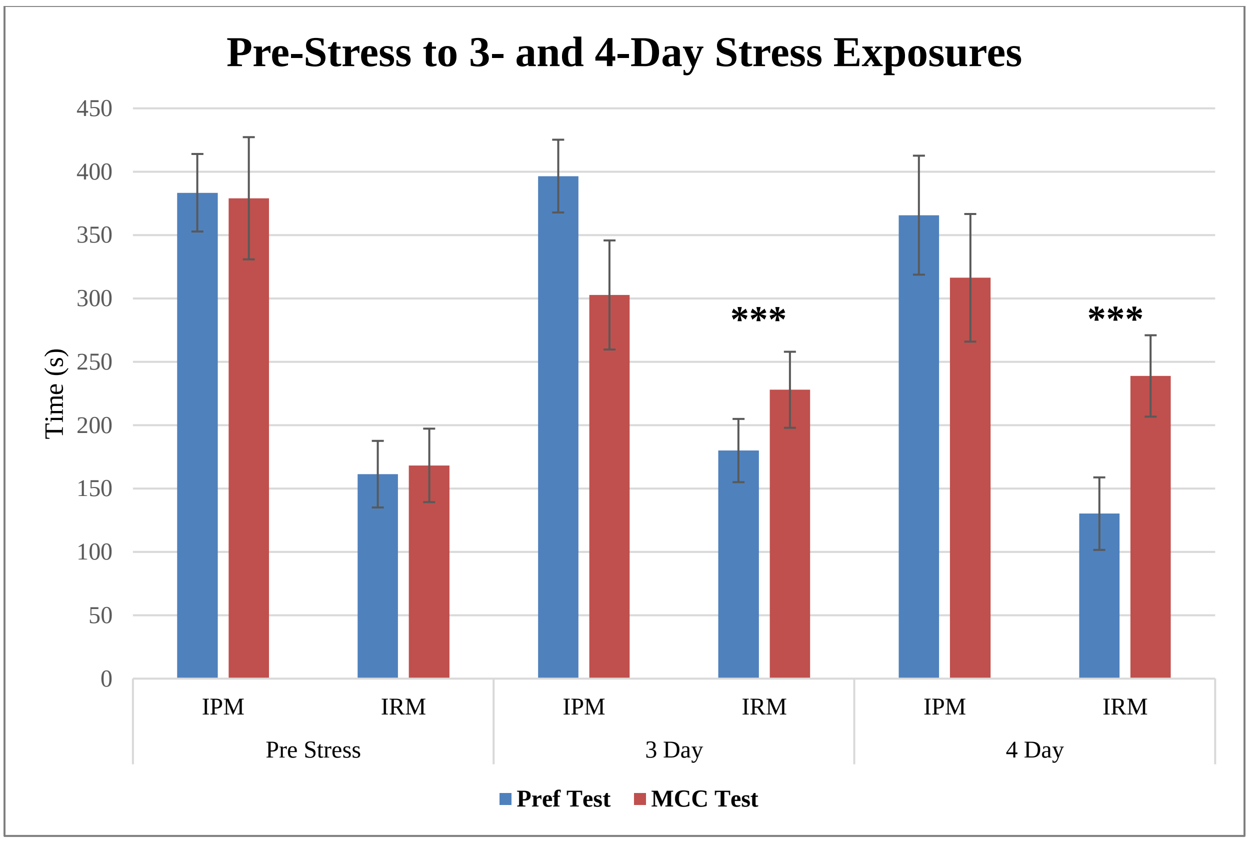

Figure 3.4. Pre-Stress to 3- and 4-Day Stress Exposure. Mean time (s) that the female spent with the Initially Preferred Male (IPM) to the Initially Rejected Male (IRM) in Preference and Mate Choice Copying (MCC) tests.

Paired t-tests were used to compare all times between Preference and Mate Choice Copying Trials (Table 3). Significance was noted for the IRM 3-Day Preference to MCC (df = 7, t = –2.7562, p = 0.014) and the IRM 4-Day Preference to MCC (df = 11, t = –2.8543, p = 0.008). There was not significance for the decrease in time from the IPM Preference to MCC for either the 3-Day (df = 7, t = 1.5970, p = 0.077) nor the 4-Day (df = 11, t = 1.1712, p = 0.133).

Data was also pooled to compare Pre-Stress (n = 16) versus the Stress (n = 20) time spent with the Initially Preferred Male and the Initially Rejected Male in Preference and Mate Choice Copying trials (Figure 3.4). As was expected, the negligible change in the time spent during PT to MCC for either the IPM or the IRM was not significant. However, there was dramatic changes in the time spent in the post-stress PT to MCC for both the Initially Preferred and Initially Rejected Males (IPM = - 67.18 (s), IRM = + 84.38 (s)). Paired t-tests were used to analyze the time (s) and significance was indicated in both the IPM from Preference to MCC trials (df = 19, t = 1.9793, p = 0.031) and the IRM from Preference to MCC trials (df = 19, t = –3.4605, p = 0.001) (Table 3).

Lastly, employing the previously established threshold for preference as >55% of the time spent, there were no observed instances of copying behavior in the pre-stress trials (n=16). However, both the 3-day (n=8) and 4-day (n=12) exposures had 25% of the females tested exhibit copying behavior after observing the model female interacting with the Initially Rejected Male. Thus, predation stress via chemical cues did induce mate choice copying behavior in domestic guppies.

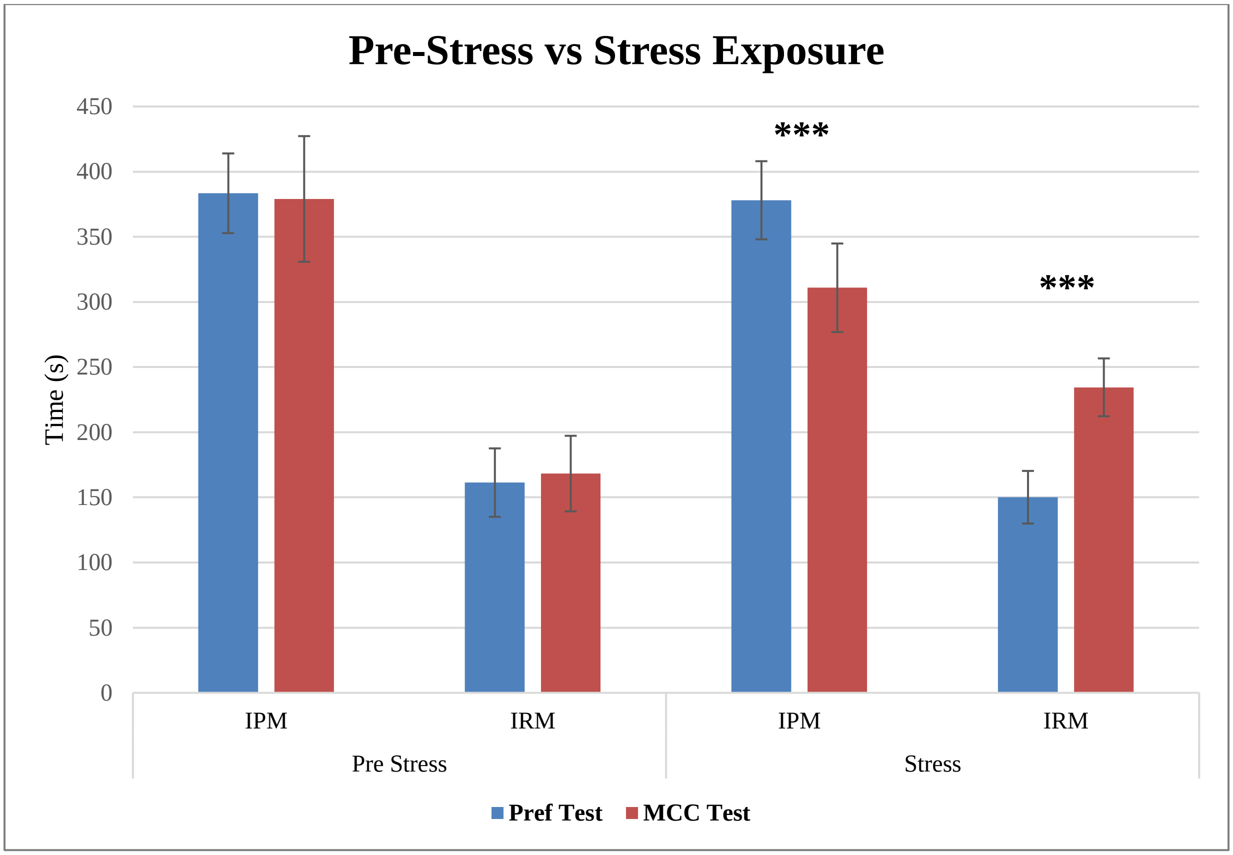

Figure 3.5. Pooled Pre-Stress to Stress Exposure. Mean time (s) that the female spent with the Initially Preferred Male (IPM) and the Initially Rejected Male (IRM) in Preference and Mate Choice Copying (MCC) tests. Paired t-tests indicated a significant change from Preference to MCC for both the IPM and the IRM post-stress.

Chapter 4: Discussion

While the exact reason for mate choice copying behavior in wild populations is still unknown, three different hypotheses have been proposed which include: allowing for more time spent foraging, assisting poor discriminators of male quality in making good mate decisions, and reducing the increased risk of predation from courtship and mating behavior. The present study assessed the predation hypothesis and found that mate choice copying behavior was induced in domestic guppies (*Poecilia reticulata*) via exposure to predatory chemical cues. These results strengthen the theory that this behavior exists in wild populations because it is adaptive to their natural environment (Magnhagen, 1991).

The ability to regulate behavior to match the level of predation aligns well with the threat-sensitive predator avoidance hypothesis (Brown *et al*, 2009). Behavior can be more readily and efficiently altered in ecological time to the present perceived risk of mortality from predation when compared to other antipredator tactics such as phenotypic plasticity (Álvarez & Nicieza, 2003; Lima & Dill, 1990). However, despite domestication resulting in morphological and behavioral changes to the species, this study demonstrated that domestic *P. reticulata* still possess the ability to identify and respond to allopatric predator kairomones as well as conspecific alarm pheromones with both increased antipredator behaviors and the burgeoning return of a socially influenced mating strategy.

While domestication eliminated the need, and consequently, the expression of mate choice copying behavior, exposure to predatory chemical cues resulted in its resurgence. The predation exposure in this study simulated a potential cost of mating and resulted in a 25% incidence of mate choice copying behavior post-stress. On the other hand, wild guppies have been shown to copy between 60-80% of the time after viewing a model female (Briggs *et al,* 1996; Dugatkin, 1992; Dugatkin & Godin, 1992); therefore, these results therefore necessitate further investigation on the effect of increased predation exposure to determine if the frequency of mate choice copying could match that of wild populations. It also merits determining how long mate choice copying behavior would continue to be expressed in domestics at specified post-stress intervals (e.g. one day, one week, etc.), as well as any possible transgenerational and/or genetic implications of the stress exposure. When considering the interpretations of these results, there is the potential that the increase to the Initially Rejected Male observed in this study could have been linked to an antipredator tactic related to attempted shoaling behavior to the side of the Model Female. This possibility could be controlled in future studies by including a smaller, juvenile female near the Initially Preferred Male as research has shown that older females will not copy younger (Dugatkin & Godin, 1993).

Although the data presented supports predation as a factor influencing mate choice copying behavior, it does not negate potential impact from other natural selection pressures such as loss of foraging time or being a poor discriminator of mate quality. The game theory analysis of non-independent choice predicts that when there is a cost to mating, a mixed population of copiers and choosers will be present (Pruett-Jones, 1992). As the present study demonstrated, these natural pressures and costs shape wild versus domestic guppies differently in relation to their effect on mate choice copying. Therefore, further investigation should consider if additional forms of stress would evoke copying behavior in domestic guppies.

References

Álvarez, D. and Nicieza, A. (2003). Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *Journal of Fish Biology, 63*, 1565-1577.

Balon, E. (2004). About the oldest domesticates among fishes. *Journal of Fish Biology*, *65* (Supp. A), 1-27.

Bischoff, R., Gould J. and Rubenstein D. (1985). Tail size and female choice in the guppy (*Poecilia reticulata*). *Behavioral Ecology & Sociobiology, 46*, 169–175.

Bowers, R., Place, S., Todd, P., Penke, L. and Asendorpf, J. (2012). Generalization in mate-choice copying in humans. *Behavioral Ecology, 23*(1), 112-124.

Briggs, S., Godin, J. and Dugatkin, L. (1996). Mate-choice copying under predation risk in the Trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology, 7*(2), 151-157.

Brooks, R. (1996). Copying and the repeatability of mate choice. *Behavioral Ecology and Sociobiology, 39*(5), 323-329.

Brooks, R. (1999). Mate choice copying in guppies: females avoid the place where they saw courtship. *Behaviour*, *136*(4), 411-421

Brown, G. and Godin, J. (1999). Chemical alarm signals in wild Trinidadian guppies (*Poecilia reticulata*). *Canadian Journal of Zoology, 77*, 562-570.

Brown, G., Macnaughton, C., Elvidge, C., Ramnarine, I., and Godin, J. (2009). Provenance and Threat-Sensitive Predator Avoidance Patterns in Wild-Caught Trinidadian Guppies. *Behavioral Ecology and Sociobiology*, *63*(5), 699-706.

Croghan, K. (2012). Sexual selection in domestic guppies (*Poecilia reticulata*). (*Unpublished Master's thesis*). St. Cloud State Univ., St. Cloud, MN.

Deacon, A., Ramnarine, I. and Magurran, A. (2011). How Reproductive Ecology Contributes to the Spread of a Globally Invasive Fish. *PloS ONE, 6*(9). doi:10.1371/journal.pone.0024416

Dugatkin, L. (1992). Sexual selection and imitation: Females copy the mate choice of others. *The American Naturalist*, *139(*6), 1384-1389.

Dugatkin, L. (1998). Genes, copying, and female mate choice: shifting thresholds. *Behavioral Ecology, 9*(4), 323-327.

Dugatkin, L. and Godin, J. (1992). Reversal of female mate choice by copying in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London B, 249*, 179-184.

Dugatkin, L. and Godin, J. (1993). Female mate copying in the guppy (*Poecilia reticulata*): age-dependent effects. *Behavioral Ecology, 4*(4), 289-292.

Dzikowski, R., Hulata, G., Harpaz, S. and Karplus, I. (2004). Inducible reproductive plasticity of the guppy *Poecilia reticulata* in response to predation cues. *Journal of Experimental Zoology, 301A*, 776-782.

Elfelt, R. (2014). Female mate choice copying in domestic vs wild *Poecilia latipinna*. (*Unpublished Master's thesis*). St. Cloud State Univ., St. Cloud, MN.

Endler, J. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes, 9*(2), 173-190.

Galef, B., Lim, T. and Gilbert, G. (2008). Evidence of mate choice copying in Norway rats, *Rattus norvegicus*. *Animal Behaviour, 75,* 1117–1123.

Gosline, A. and Rodd, F. (2008). Predator-induced plasticity in guppy (*Poecilia reticulata*). Life history traits. *Aquatic Ecology, 42,* 693-699.

Godin, J. and Briggs, S. (1996). Female mate choice under predation risk in the guppy. *Animal Behaviour, 51*(1), 117-130.

Handelsman, C., Broder, E., Dalton, C., Ruell, E., Myrick, C., Reznick, D. and Ghalambor, C. (2013). Predator-induced phenotypic plasticity in metabolism and rate of growth: Rapid adaptation to a novel environment. *Integrative and Comparative Biology, 53*(6), 975-988.

Hill, S. and Ryan, M. (2006). The role of model female quality in the mate choice copying behaviour of sailfin mollies. *Biology Letters, 2,* 203-205.

Houde, A. (1997). *Sex, color and mate choice in guppies*. Princeton, New Jersey: Princeton University Press.

Houde, A. and Hankes, M. (1997). Evolutionary mismatch of mating preference and male colour patterns in guppies. *Animal Behaviour, 53*, 343-351

Huntingford, F. (2004). Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology, 65* (Supp. A), 122-142.

Jensen, P. (2006). Domestication – From behaviour to genes and back again. *Applied Animal Behaviour Science, 97*, 3-15.

Kennedy, C., Endler, J., Poynton, S. and McMinn H. (1987). Parasite Load Predicts Mate Choice in Guppies. *Behavioral Ecology and Sociobiology, 21*(5), 291-295.

Kniel, N., Dürler, C., Heckt, I., Heinbach, V., Zimmermann, L. and Witte, K. (2015) Novel mate preference through mate-choice copying in zebra finches: sexes differ. *Behavioral Ecology, 26*(2), 647-655.

Kodric-Brown, A. (1985). Female Preference and Sexual Selection for Male Coloration in the Guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology, 17*(3), 199-205.

Kodric-Brown, A. (1993). Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration, and courtship. *Behavioral Ecology and Sociobiology, 32*, 415–420.

Kodric-Brown, A. and Brown, J. (1984). Truth in Advertising: The Kinds of Traits Favored by Sexual Selection. *The American Naturalist, 124*(3), 309-323.

Lafleur, D., Lozano, G., and Sclafani, M. (1997). Female mate-choice copying in guppies, *Poecilia reticulata*: a re-evaluation. *Animal Behaviour, 54*, 579-586.

Lima, S. and Dill, L. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology, 68*(4), 619-640.

Magnhagen, C. (1991). Predation risk as a cost of reproduction. *Trends in Ecology and Evolution, 6(*6), 183-186.

Pruett-Jones, S. (1992). Independent versus nonindependent mate choice: Do females copy each other? *The American Naturalist, 140*(6), 1000-1009.

Real, L. (1990). Search Theory and Mate Choice. I. Models of Single-Sex Discrimination. *The American Naturalist, 136*(3), 376-405.

Reynolds, J. and Gross, M. (1992). Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proceedings of the Royal Society B, 250*, 57-62.

Ruell, E., Handelsman, C., Hawkins, C., Sofaer, H., Ghalambor, C. and Angeloni, L. (2013). Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. *Proceedings of the Royal Society B, 280*, 2012-2019.

Stöhr, S. (1998). Evolution of mate-choice copying: a dynamic model. *Animal Behaviour, 55,* 893-903.

Stoner, G. and Breden, F. (1988). Phenotypic Differentiation in Female Preference Related to Geographic Variation in Male Predation Risk in the Trinidad Guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology, 22*(4), 285-291.

Swaney, W., Cabrera-Álvarez, M., and Reader, S. (2015). Behavioural responses of feral and domestic guppies (*Poecilia reticulata*) to predators and their cues. *Behavioural Processes, 118*, 42-46.

Torres-Dowdall, J., Handelsman, C., Reznick, D. and Ghalambor, C. (2012). Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*). *Evolution, 66*(11), 3432-3443.

White, D. and Galef, B. (2000). 'Culture' in quail: social influences on mate choice of female *Coturnix japonica*. *Animal Behaviour, 59*, 975-979.

Witte, K. and Massmann, R. (2003). Female sailfin mollies, *Poecilia latipinna*, remember males and copy the choice of others after 1 day. *Animal Behaviour, 65*, 1151–1159.

Zuk, M., Thornhill, R., Ligon, J. and Johnson, K. (1990). Parasites and Mate Choice in Red Jungle Fowl. *American Zoologist, 30*(2), 235-244.

Appendix

Table 1. Results of Excel T-tests for Two Paired Samples comparing Percent Change for Mate Choice Copying Tests (MCC) to Preference Tests (PT) in Pre-Stress versus Stress.

Paired t-Tests for Percent Change	Df	t Stat	P-Value (1-tailed)
Pre-Stress to 3 Day	5	-1.4359	0.105
Pre-Stress to 4 Day	7	-2.7538	**0.014**
Pre-Stress to Stress	13	-3.0128	**0.005**

Table 2. Paired Sample Descriptive Statistics for Pre (1), 3 Day, Pre (2), 4 Day, Pre (3) and Stress. Pre (3) and Stress include pooled data from Pre (1) with Pre (2) and 3 Day with 4 Day.

Group Name	N	Mean	Std Dev	Std Err	Confidence Interval (95%)
Pre (1)	6	7.1409	9.9065	4.0443	-3.2554 – 17.5371
3 Day	6	12.9581	12.2965	5.0200	0.0537 – 25.8624
Pre (2)	8	0.5707	19.9468	7.0523	-16.1052 – 17.2467
4 Day	8	15.9308	17.1230	6.0539	1.6157 – 30.246
Pre (3)	14	3.3865	16.2287	4.3373	-5.9837 – 12.7567
Stress	14	14.6568	14.777	3.9493	6.1248 – 23.1888
Total	28	9.0216	16.275	3.0757	2.7108 – 15.3324

Table 3. Results of Excel T-tests for Two Paired Samples comparing Preferences Tests (PT) time in seconds (s) and Mate Choice Copying Tests (MCC) time in seconds (s).

Paired t-Tests for PT (s) to MCC (s)	P-Value (1-tailed)
IPM 3 Day PT to IPM 3 Day MCC	0.077
IRM 3 Day PT to IRM 3 Day MCC	**0.014**
IPM 4 Day PT to IPM 4 Day MCC	0.133
IRM 4 Day PT to IRM 4 Day MCC	**0.008**
IPM Pre-Stress PT to IPM Pre-Stress MCC	0.462
IRM Pre-Stress PT to IRM Pre-Stress MCC	0.424
IPM Stress PT to IPM Stress MCC	**0.031**
IRM Stress PT to IRM Stress MCC	**0.001**

Table 4. Results of Excel T-tests for Two-Samples Assuming Equal Variances comparing Preferences Tests (PT) time in seconds (s) for Pre-Stress to Stress and Mate Choice Copying Tests (MCC) time in seconds (s) for Pre-Stress to Stress for the Initially Preferred Male (IPM).

Two Sample t-Tests for IPM PT (s) to PT (s) and MCC (s) to MCC (s)	P-Value (1-tailed)
PT – IPM Pre-Stress to IPM 3 Day	0.393
PT – IPM Pre-Stress to IPM 4 Day	0.372
PT – IPM 3 Day to IPM 4 Day	0.314
MCC – IPM Pre-Stress to IPM 3 Day	0.160
MCC – IPM Pre-Stress to IPM 4 Day	0.192
MCC – IPM 3 Day to IPM 4 Day	0.426

Table 5. Results of Excel T-tests for Two-Samples Assuming Equal Variances comparing Preferences Tests (PT) time in seconds (s) for Pre-Stress to Stress and Mate Choice Copying Tests (MCC) time in seconds (s) for Pre-Stress to Stress for the Initially Rejected Male (IRM).

Two Sample t-Tests for IRM PT (s) to PT (s) and MCC (s) to MCC (s)	P-Value (1-tailed)
PT – IRM Pre-Stress to IRM 3 Day	0.328
PT – IRM Pre-Stress to IRM 4 Day	0.217
PT – IRM 3 Day to IRM 4 Day	0.118
MCC – IRM Pre-Stress to IRM 3 Day	0.106
MCC – IRM Pre-Stress to IRM 4 Day	0.059
MCC – IRM 3 Day to IRM 4 Day	0.409