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Female Mate Choice Copying in Pseudo Wild vs. Truly Wild Poecilia latipinna

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Female Mate Choice Copying in Pseudo Wild vs. Truly Wild *Poecilia latipinna*

by

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Abstract

Mate Choice Copying (MCC) is a non-independent sexually selective behavior that is present in *Poecilia latipinna* (sailfin mollies) and *Poecilia reticulata* (guppies). Experiments were conducted that show that truly wild sailfin mollies express MCC at a higher percent, while pseudo wild exhibit MCC at a lower percentage. Wild sailfin mollies from Mustang Island in Texas and pseudo wild sailfin mollies (12 generations in captivity) with similar morphology were used as the test subjects for the experiment. The testing apparatus was similar to the apparatus Dukatkin used (1992). The more robust MCC behavior exhibited by the truly wild sailfin mollie females switch to the initially rejected male (IRM) from the initially preferred male (IPM) 22% versus the pseudo wild at 14%. This supports that the Mate Choice Copying behavior is diminished in the captivity from the domestication process.

Acknowledgements

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List of Abbreviations

Term	Abbreviation	Definition
Focal female	Ff	Female who's preference that was being timed
Model female	Mf	Female that was placed near Initially Rejected Male (IRM) that was being observed by the Focal female (Ff)
Initially Preferred Male	IPM	Male that received >60% of the Focal female's (Ff's) time of Trial 1 & Trial 2 (T1 & T2) in his zone for the Preference Test (PT)
Initially Rejected Male	IRM	Male that received <40% f the Focal female's (Ff's) time of Trial 1 & Trial 2 (T1 & T2) in his zone for the Preference Test (PT)
Preference Test	PT	A series of 2 ten minute trials that were run to determine which male that the Focal female (Ff) preferred
Mate Choice Copying	MCC	A second series of 2 ten minute trials that were run to determine which male that the Focal female (Ff) preferred that includes the Model female (Mf) in close proximity to the Initially Preferred Male (IPM) during an observation period of 30 minutes
Trial 1	T1	First 10 minute trial for either the Preference Test or Mate Choice Copying Test
Trial 2	T2	Second 10 minute trial for either the Preference Test or Mate Choice Copying Test

Chapter I: Introduction

Females use one of two main strategies to determine mate preference based on independent and non-independent choice (Pruett-Jones 1992). Independent mate choice is when a female's choice is based on the male's secondary sex characteristics and other qualitative traits (Houde 1994, Wiley & Posten 1996, Godin, Herdman & Dugatkin 2005). Traits would include male coloration, size and intensity of courtship display (Reynolds & Gross 1992, Endler & Houde 1995).

Female guppies have evolved a preference to specific traits in male guppies that is reflected in their selection. Preference for males with higher intensity spot coloration and larger size is a significant factor in mate choice (Kodric-Brown 1985, Endler & Houde 1995). The intensity and size of the orange area is a reflection of the male's foraging ability, parasite load and quality. Female's preference of larger-bodied males increases the male's reproductive fitness (Reynolds & Gross 1992). Females can also distinguish specific morphological traits such as tail size and utilization in courtship displays in mate choice preference (Bischoff, 1984). However, these secondary male characteristics are costly to the male in energy and predation (Burke 1982).

A non-independent form of mate choice is when a female bases her decision on watching the selection of another female (Dugatkin 1993, Westneat, Walters, Hatch & Hein 2000). This non-independent selection is referred to as Mate Choice Copying (MCC) and was first identified in wild caught *P.reticulata* (Dugatkin 1992). The conditional probability is based on the female's knowledge of the male's rejection decreasing the probability of mating or the male's mating increasing the probability of her choice (Pruett-Jones 1992). The definition was later revised to include the female's observation of the males mating history as crucial information in determining choice (Dugatkin 1996).

This non-independent form of sexual selection has been seen in many animals, including humans (Waynforth 2007, Place et al. 2010). Mate Choice Copying has been identified in live bearing *Poecilia* fish species, specifically *P. reticulata* (guppies) and *P. latipinna* (sailfin mollies), (Dugatkin 1992, Witte 2006, Elfelt 2014). Guppies and sailfin mollies both Mate Choice Copy when the males are qualitatively closely matched. When the males differ too much in the qualitative traits the females generally rely on independent sexual selection (Dugatkin 1996, Witte & Ryan 1998).

Selection of a mate is costly to a female, both in time and energy. It is suggested that MCC occurs in order to save more time for food foraging, predator avoidance, or when male quality is subtle and difficult to differentiate (Stohr 1998). Guppies have been a subject for various sexually selected male traits and life history studies for over 80 years. Specifically, Dugatkin's initial studies examined MCC in wild guppies from Trinidad.

Female Mate Choice Copying can potentially be affected by the quality of the male and the age of the females. For example the age of the focal female affects Mate Choice Copying. Focal female defined as the female observing and making the dependent choice in males. The model female defined as the female being observed by the focal female. In experiments with a young focal female (Ff) and a more mature model female (Mf) the younger female will make a independent mate choice (Dugatkin 1993, Kodric-Brown 2001). Reversal of the age relationship of the focal female (Ff) and model female (Mf) resulted in an dependent mate choice by the focal female (Ff). The experiment suggests that mate choice copying is a mating strategy employed more by young, inexperienced females in the wild.

Quality of the male also plays a role in the females' Mate Choice Copying behavior. There is a different threshold in regards to orange spot size and intensity for Mate Choice

Copying to occur. The focal female (Ff) would not copy the model female (Mf) if there is more than 40% difference in the size and intensity of the orange color spot. The focal female (Ff) would copy the model female (Mf) if the size and intensity of the orange color spot was within a threshold of 12% - 24% (Dugatkin 1996, Witte 2006). This suggests that the difference in male's quality was dramatic enough that, it could be recognized by the focal female (Ff) and she will make an independent choice. Conversely, when the difference is so subtle and difficult for the focal female (Ff) to distinguish she will make a independent mate choice.

Lafleur and Brooks have repeated Dugatkin's original experiment and were not able to support the MCC behavior (Brooks 1996, Lafleur 1997). This was likely due to differences in the experimental procedure and the guppies' physiological cues. Female members of the Poeciliidae family use chemical cues to indicate receptivity that would influence the strength of the male's courtship display (Brown & Godin 1999). Dugatkin's experimental tank allowed for the dispersion of the chemical cues throughout the tank to the subjects. In Lafleur's tank all compartments had glass segregating the sexes and the water flow. An important difference between Dugatkin and Lafleur's experiments was the use of wild and domestic guppies. Dugatkin used wild caught guppies and Lafleur used highly domesticated "feeder guppies".

The only study known that has been done on the genetic component to MCC behavior was by Dugatkin. The behavior trait could potentially be attributed to two categories: genetic transmission or cultural transmission. Cultural transmission by social learning has been the focus of many behavioral ecologists and biologists' studies. An example would be observation and mimicry or foraging behavior as socially learned (Brown & Laland 2003). In Dugatkin's 1992 study the MCC behavior was observed as an "override" of a female's genetic predisposition preference and therefore make a dependent choice.

The objective of Dugatkin's (2006) study was to identify if the MCC behavior was genetic or cultural. He used 32 pregnant female guppies from Trinidad and Tobago, in a tank isolated from other guppies. One week after parturition the mothers were tested for the MCC behavior. The 83 female offspring were raised in individual tanks until 11 weeks and also tested for the MCC behavior. There was a positive correlation of time between the mother group and daughter group spent with the rejected male near the model female. However, the proportion in time mothers and daughters spent in the rejected male zone, preferred male zone and the neutral zone was not significant. Therefore, concluded that there was no support of Mate Choice Copying in mother and daughter guppies having a genetic or heritable component (Dugatkin & Druen 2007).

Potential confounding factors would be that the mother cohort ages used was unknown and likely varied. All focal females and model females in the daughter generation were 11-week-old virgins with no clear "mature" female to model. The female subject times were grouped three ways: time near initially rejected male, time near initially preferred male and time in the neutral zone. Times near the initially rejected male between both subject groups were positively correlated. The mother/daughter groups' times spent with the initially preferred male had no correlation. It was commented that the time in the neutral zone and time with the initially preferred male differed greatly between the two subject groups. The acclimation time of the virgin daughter group spent in the neutral zone is much greater than the mother group. This could potentially skew the data in the initially preferred male group.

The correlation of both the subject groups' time spent with the initially rejected male is suspect to support that the MCC behavior does have a genetic component. A future study should take the above confounding variables in account when comparing different generations of

females for Mate Choice Copying in domestic and wild *Poecilia*. MCC behavioral studies combined with genetic studies will give feedback and strengthen studies on behavior (Vakirtzis 2011).

The genetic component to mate choice copying behavior has also been thought to be polygenic within a population. Females can be divided into 'choosers' and 'copiers' and some females could exhibit both categories dependent on the frequency in the population and environment (Vakirtzis 2011). Considering that this trait is heritable and females can potentially switch from 'chooser' independent mate choice or 'copier' dependent mate choice suggests environment and epigenetics plays a role. Epigenetics is a heritable phenotypic trait that is the result of a change in a chromosome and not the DNA sequence (Berger, Kouzarides, Shiekhattar & Shilatifard 2009). Studies support that an altered epigenome effects mate choice. In rats that have in utero exposure to endocrine disrupting chemicals altering the epigenome will not be chosen by females up to three generations after the exposure event (Crews, Gore, Hsu, Dangleben, Spinetta, Schallert, Anway & Skinner 2007). Chemical exposure events and environmental exposure to high stress such as captivity can potentially have an affect on the epigenome and thus mate choice (Freil & Fraga 2012, Danchin & Wagner 2009, Parsons 1990)

The green sailfin mollies are *Poecilia* fishes have been taken from the wild and domesticated in captivity for desired morphological traits. The domestication process has drastically changed the phenotype and behavior of the fish. Unpublished research shows that domestic *P. reticulata* no longer exhibit the MCC behavior (Croghan 2012). Successive research shows that MCC behavior occurs in wild type *P. latipinna* but very weakly in domesticated *P. latipinna* (Elfelt 2014). Sailfin mollies taken from the wild and kept in captivity for 12

generations will be considered pseudo wild. Sailfin mollies taken from the wild in the first generation will be considered truly wild.

The purpose of this MCC experiment is to replicate the intraspecific MCC behavior in wild caught and pseudo wild sailfin mollies. Once established, the experiment will also examine the effect of domestication on MCC through a series of cross breeding procedures. Pseudo wild will be bred with truly wild sailfin mollies. As a control a pseudo wild group and a truly wild group of sailfin mollies will also separately bred for comparison do determine that captivity is not influencing the results.

Chapter II: Materials and Methods

Test Subjects

A total of 40 wild sailfin mollies, 20 male and 20 female, were obtained from Goliad Farms as young adults. The wild caught sailfin mollies were acquired from Mustang Island in Texas in June of 2015, refer to Figure 1 & 2. Wild males were more colorful with dark spots on both sides of their bodies and a modified anal fin, gonopodium. The amount of coloring in the wild males varied only slightly between individuals. All wild caught females were similar in coloring and only varied in size.

Figure 1. Young Male Wild Caught Sailfin Molly (Photographed by Charles Reitka)

Figure 2. Young Female Wild Caught Sailfin Molly (Photographed by Charles Reitka)

The green sailfin mollies are hybrids that have been selectively bred in captivity for 12 years, refer to Figure 3. They are hybrids of *Poecilia latipinna, P.velifera* and crossed with other wild *P.latipinna* to improve the strain. The term "pseudo wild" is used because the fish were from a population of wild caught sailfin mollies and commercial for 12 years. Green sailfin mollies were chosen due to their similar size and coloration to that of the wild caught sailfin mollies. Females had a larger, heavier body than the males. The green sailfin mollies were also obtained from Goliad Farms.

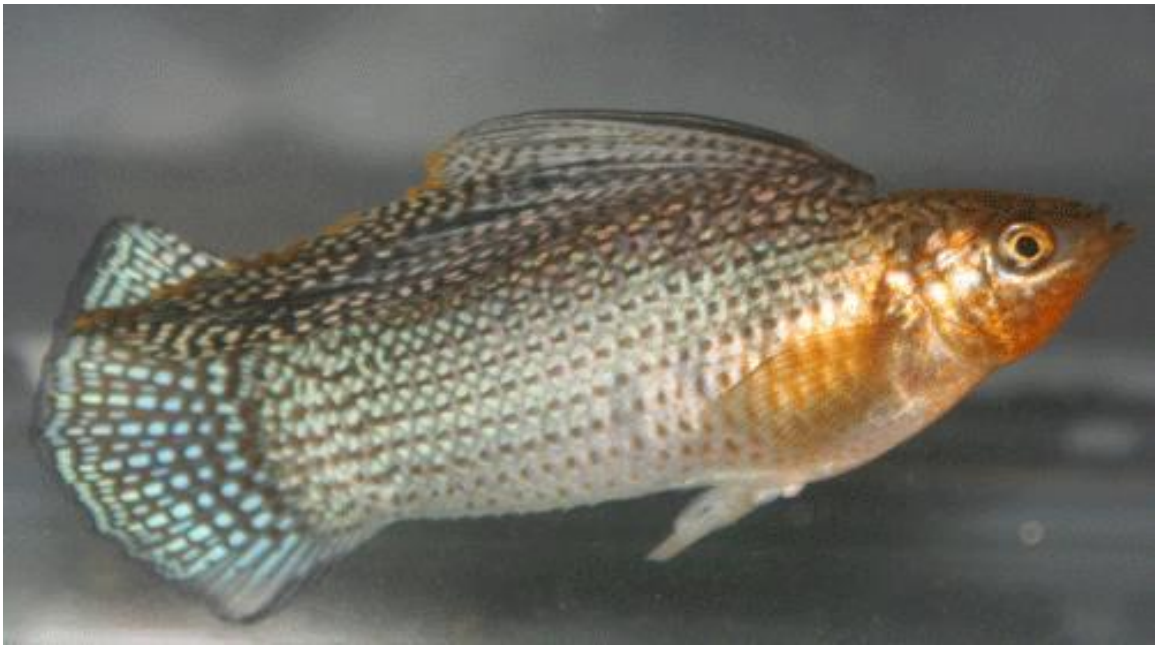

Figure 3. Young Male Domestic Green Sailfin Molly (Photographed by Charles Reitka)

Figure 4. Young Female Domestic Green Sailfin Molly (Photographed by Charles Reitka)

Housing

Once the fish arrived in the lab sexes were segregated and quarantined for a 28-day period to control for gravid females. Females remained quarantined until they had gone 28 days without a gravid spot. It is important that females are not pregnant for the experiment so they

will be receptive to males (Ptack & Travis 1998). Since females can hold sperm for 3-4 cycles (Moyle 1976; Burgess 1980). A total of five 28-day quarantine cycles were conducted. Young were segregated after 3 weeks. During the experiment the fish will be segregated no more than 3 days time from one another.

After the (maximum) 3-day segregation period, fish were deposited into their permanent aquaria, which consisted of seven 10-gallon (37.85L) tanks, one 20-gallon (75.18L) tank, two 40-gallon (150.36L) tanks and two 60-gallon (227.1L) tanks to accommodate the 80 fish. Filters were not needed but were provided for each tank and filter cleaning occurred every two weeks. Aeration for the tanks was also provided via a manifold and central aerator. The filtered water was switched out at 25% per tank, weekly and Aqua Safe aquarium salt was used. The pH was checked with a pH meter and the salinity (10 ppm) with a hydrometer. Water temperature was maintained at 24°C with heaters when the room temperature was not adequate.

Each tank consisted of, live and artificial plants and rock formations in order to provide cover. The aquarium lights were set on a timer for 12:12 light/dark cycle to induce sexual activity, (Shubel, 1995; Goliad, 2014). Shelf paper was applied in between tanks to isolate groups from one another.

Feeding

An automatic commercial feeder fed the large group fish tanks at 6:00 a.m. and 6:00 p.m. When the fish are in temporary glass jar housing the experimenter will feed individual fish at the same time as the commercial feeder. The fish received a combination of high protein pellets and live brine shrimp fry. Live plants, *Najas guadalupensis* (guppy grass) was available in large tanks to supplement the fish's diet.

Apparatus

The experimental apparatus has five chambers, two static partitions and three dynamic partitions within the 38-Liter tank, refer to Figure 5. The two end chambers house two males, while the next two inner chambers are dedicated as the preference zones and utilized when the focal female (Ff) is choosing a specific male. The males were selected to be within 2 mm of body length and similar color intensity. The final chamber was centered in the middle of the tank (the neutral zone) and houses a Plexiglas square, where the focal female was confined during experimental observations.

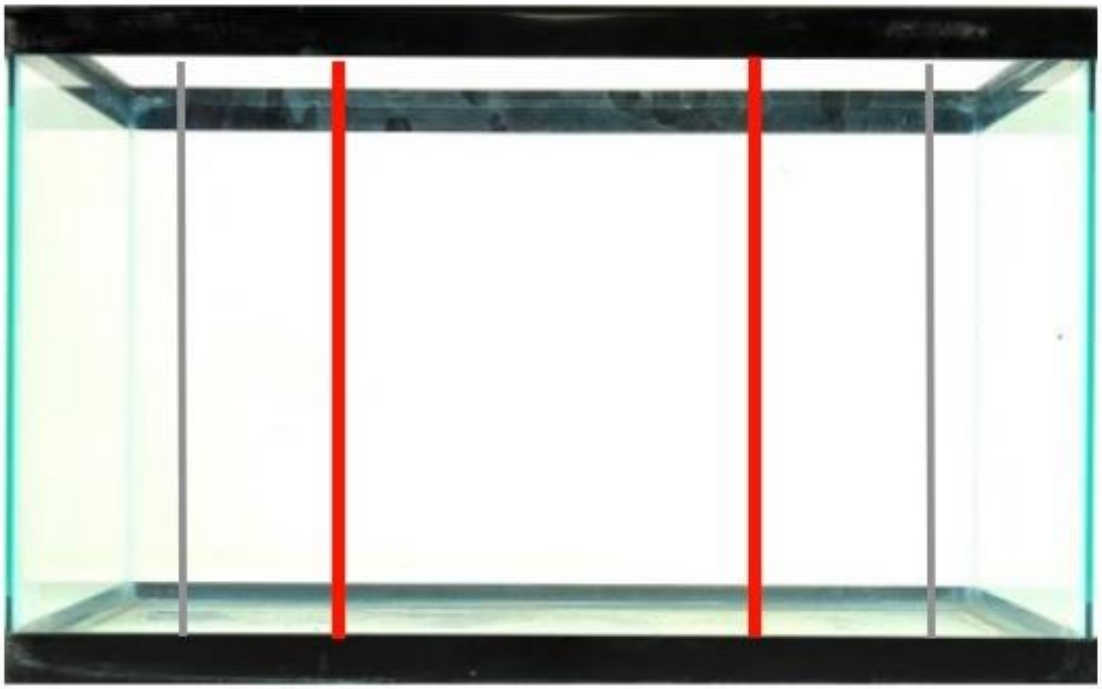

Figure 5. Front View of Apparatus Testing Tank. Red lines are marks on the outside of the tank that designate the preference zones. The grey lines represent the clear Plexiglas dividers for the males in the tank.

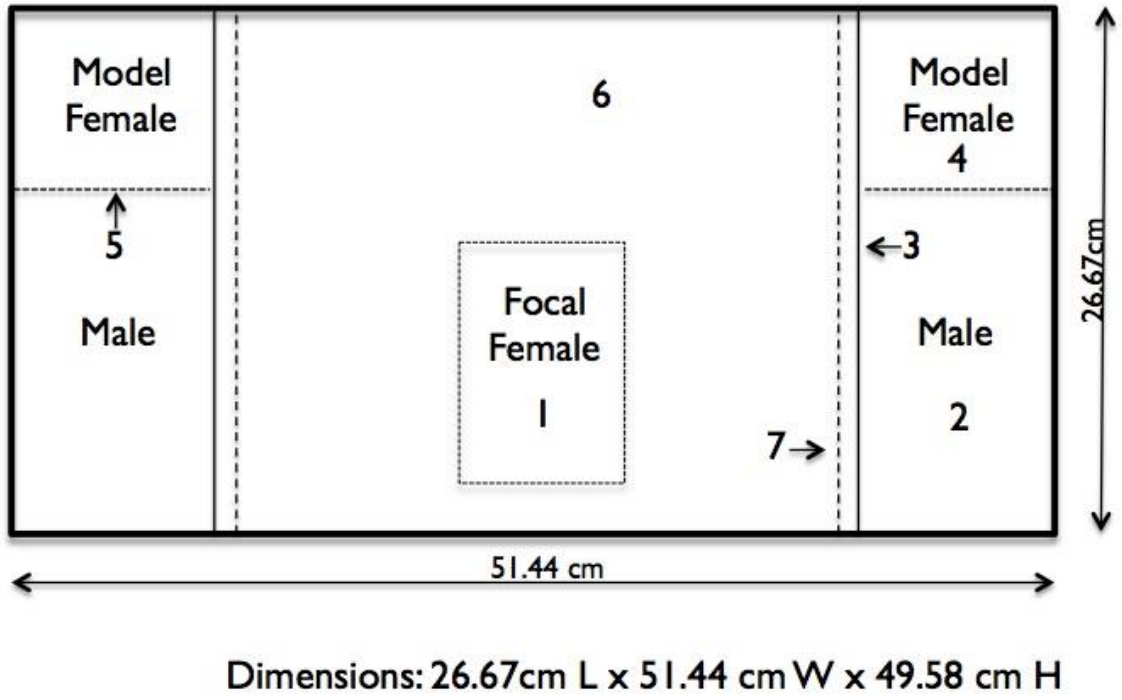

Figure 6. Aerial View of Apparatus Testing Tank, Illustrating Partitions. The clear plastic feeder box zone (1) is where the focal female (Ff) is restrained during the experimental observations. Zone (2) is where the males are partitioned by Plexiglas (3) from the females on either side of the apparatus. Zones (4) are where the model females (Mf) are restrained on either side of the apparatus by Plexiglas dividers (5). Zone (6) is the neutral zone. The Ff view of the males is regulated by black, opaque, removable Plexiglas (7).

Individual length measurements were also taken before each trial to the nearest millimeter. The fish were briefly removed from the tank and kept in the net to reduce stress, and measured with a ruler. Opaque Plexiglas partitions were used during the trial when fish were moved to reduce stress. All trials were conducted from 8 a.m. to 5 p.m. and video recorded.

Control Trials

Control experiments were conducted before preference and mate choice copying tests to determine if side preference occurs and differences in fish activity. In the control tests the same timing as the preference and mate choice copying test with pseudo wild and truly wild mollies. Focal female (Ff) control tests consisted of timed trials with only the Ff in the tank. The model

female (Mf) control test consisted of timed trials with the Mf and two males. The male control test consisted of the timed trials with the Ff and Mf.

All control, preference and mate choice copying trials were modeled after Elfelt's 2014 trials. The same apparatus was used, with the exception of replacing the Plexiglas dividers due to their age and loss of transparency. The same procedure, rest times and trial times were used and detailed as follows.

Preference Test

The preference test (PT) was conducted to determine which male the Ff preferred. The males were measured then recorded and were within 3 mm of each other to be used as pairs for the PT. The PT measures the female preference of one male over another. Preference is defined as greater than 60% of the time with a given male. Preference was determined by adding the times in each males' zone for the 2 trials and then males were assigned initially preferred male (IPM) or initially rejected male (IRM). In order for the female to be considered in a zone her gill mark had to cross the red zone demarcation before timing of the male's zone could begin.

Before individuals were placed in the apparatus black Plexiglas screens were in place and were continuously used to minimize distress. In the central Plexiglas square a measured female was introduced for 30 minutes to acclimate to the apparatus and also to observe the two males after the black Plexiglas screens were removed. After 30 minutes trial 1 began.

Trial 1. After the acclimation time Ff was released in the neutral zone, with access to the preference zones for 10 minutes. With 2 stopwatches an observer measured the amount of time the female spent in the two preference zones and the neutral area. The trial was also time stamped and video recorded. When the 10-minute trial was over the black Plexiglas screens were

reintroduced, the Ff was returned to her Plexiglas square. Males were also netted and sides were placed in the opposite side. Times were recorded on a testing sheet and converted into seconds.

Trial 2. After 5 minutes of rest the black Plexiglas screens were removed and a 10-minute observation time began. At the end of the 10-minute observation time the Ff was released in the neutral zone. With 2 stopwatches an observer measured the amount of time the female spent in the two preference zones and the neutral area. The trial was also time stamped and video recorded. Times were recorded on a testing sheet and converted into seconds. Times for T1 & T2 were added together in seconds to determine initially preferred male (IPM) or initially rejected male (IRM).

Mate Choice Copying Test

The mate choice copying test was conducted to determine if the Ff would switch her preference to the IRM that was observed in close proximity to a model female. The model female (Mf) was within 3 mm in size from the Ff. It was considered 'copying' if the focal female chooses the previously rejected male >60% and personal preference (no mate choice copying) if she stays with the previously preferred male.

The main difference between the PT and MCC trials is the introduction of a Mf. When all the black Plexiglas screens are installed both male compartments are divided with a clear Plexiglas screen. The MF is placed near the IRM and for the 30-minute acclimation time and be observed by the Ff. All the black Plexiglas screens were put into place and the Mf was removed. The black Plexiglas screens were removed and then the Ff was released for a first trial.

Trial 1. The Ff was released in the neutral zone, with access to the preference zones for 10 minutes. With 2 stopwatches an observer measured the amount of time the female spent in the two preference zones and the neutral area. The trial was also time stamped and video recorded.

When the 10-minute trial was over the black Plexiglas screens were reintroduced, the Ff was returned to her Plexiglas square. Males were also netted and sides were placed in the opposite side. Times were recorded on a testing sheet and converted into seconds.

Trial 2. With black Plexiglas screens in place the Mf was again placed next to the IRM. The black Plexiglas screens were removed and a 10-minute observation time began. At the end of the 10-minute observation time the black Plexiglas was returned and the Mf removed. The screens were removed and the Ff was released in the neutral zone. With 2 stopwatches an observer measured the amount of time the female spent in the two preference zones and the neutral area. The trial was also time stamped and video recorded. Times were recorded on a testing sheet and converted into seconds. Times for T1 & T2 were added together for the MCC tests and statistical analysis followed.

Statistical Analysis

A paired sample t-test was run for preference and for the mate choice copying test in Excel. T-tests were also run to compare times for both trials for both the preference and mate choice copying experiments. Percent change was determined for each individual to determine the degree of mate choice copying. A one-way ANOVA was run to determine if there was a significant difference between the groups in SSPS. A post hoc multivariate Tukey test was run to determine where the significant difference was on the pseudo wild, truly wild and Elfelt's domestic times in percent of time in seconds in SSPS.

Breeding

Females were separated into their own birthing jar 25 days after copulation. Females were removed from young fry no more than 24 hours after birth and placed into a community tank. Once sex was determined the young fry were separated into males and females. The

progeny (F1 generation) was selected based on the afore mentioned MCC procedures to be bred for an F2 generation. The F1 generation had the same selection process and numbers for breeding pairs. Figure 7 below illustrates the domestic and wild crosses for both species.

Figure 7. Breeding Crosses of (2) truly wild$_{male}$/(10)truly wild$_{female}$, (2)pseudo wild$_{male}$/(10)pseudo wild$_{female}$, 2)pseudo wild$_{male}$ /(10)truly wild$_{female}$ (2)truly wild$_{male}$/(10)pseudo wild$_{female}$ for P. latipinna.

Males were chosen based on mating preference score. Females were chosen and segregated based on their MCC ability scale rating. The breeding pairs were to result in the P1 generation of a male: female 1:5 ratio with a total of 40 pregnant females. Females were kept in the tank with a grate placed in a wedge shape at the bottom of the tank. Young fry were able to swim through the grate and adults were not. After 3 weeks fry were collected and placed in their own tank. Once sex was determined the young fry were separated into males and females.

The progeny (F1 generation) was to be selected based on the MCC experiments to be bred for an F2 generation. The F1 generation had the same selection process and numbers for breeding pairs. The F1 generation was to consist of full sibling mating to produce the F2 generation.

Chapter III: Results

During the control experiments truly wild fish needed more time to acclimate to the testing tank. Once the dark dividers were removed from the apparatus with only a focal female and 2 males the fish of both sexes would freeze. The amount of truly wild fish or sex in the tank, did not matter, the response was similar. The males engaged faster than the females. The maximum time for males to engage was less than 3 minutes once they noticed a female. Females took on average 10:23 minutes, on average to engage with males once the dividers were removed. Females would stay close to an edge and freeze at the bottom of the tank. Based on this information timing for the truly wild preference and mate choice-copying test did not begin until females engaged with the males. The pseudo wild males and females did not have issues freezing and quickly engaged in courtship once the dividers were removed. Once engaged, neither the pseudo nor truly wild mollies showed a side preference during the trials.

Preference was determined by adding the time in seconds (s) the Ff spent in each males' preference zones for both preference trials, T1 & T2. Whomever the female spent more time with was labeled the initially preferred male (IPM) and the other the initially rejected male (IRM) for the mate choice copying tests. Paired sample t-tests were run on the Ff's time with males to determine time in seconds that the Ff spent in T1 & T2 with each male. The two trials were conducted to determine if side biased was present in the Ff. There were no trials that had Ffs with side biased.

Paired t-tests were used to determine significance in the preference tests (PT) of the change in Ff choice in time with the IPM (T1 & T2) & IRM (T1 & T2) for both trials. Paired t-tests were used to compare IPM (T1 + T2) & IRM (T1 + T2) to determine if the Ff time spent

with the IPM & IRM was significant. Paired t-tests were also used to determine if the time spent in the neutral zone was significant for preference and mate choice copying test trials.

In the mate choice copying test preference (MCC) was determined by adding the time in seconds (s) the Ff spent in each males' preference zones for both mate choice-copying trials, T1 & T2. To determine if mate choice copying was present a t-test compared the Ff's time with the IPM in the preference test versus the mate choice copying test. Then a t-test compared the Ff's time with the IRM in the preference test versus the mate choice copying test. A significant increase in the amount of time the Ff spent with the IRM from the preference test versus the mate choice copying test indicates the presence of mate choice copying. An increase in the amount of time the Ff spent with the IPM from the preference test versus the mate choice copying test indicates mate choice copying is not present.

Pseudo wild Ffs in the preference test spent significantly more time (225 seconds) ($p = 0.006$) with one male (IPM) over another (IRM) (Table 2). The pseudo wild Ffs in the mate choice copying test had a significant decrease in the amount of time spent with the IPM (138 seconds) ($p = 0.01$) (Table 2 & Figure 8). The Ff increased the amount of time spent with the IRM by 145 which seconds was also significant ($p = 0.002$) (Table 2). The time the pseudo wild Ff spent in the neutral zone in the preference test compared to the mate choice copying test was not significant ($p = 0.81$)(Table 2). The paired t-test comparing the preference test IPM combined times with the IRM combined times were significant ($p = 0.001$) (Table 2). This supports that in pseudo wild females mate choice copying is present.

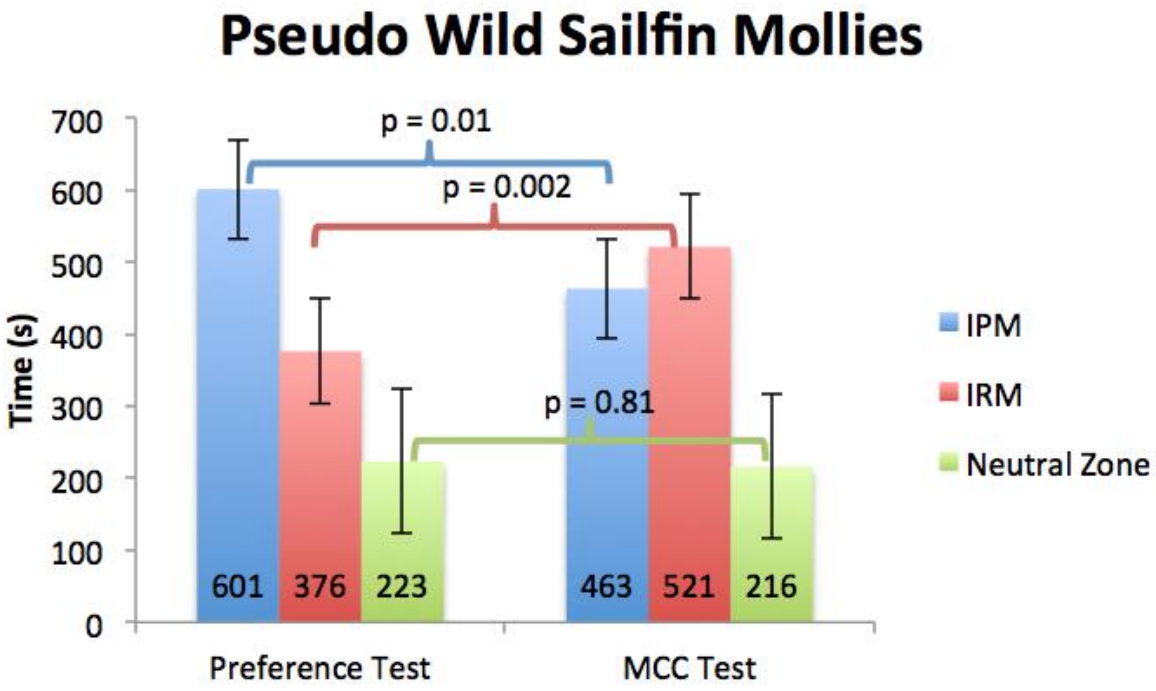

Figure 8. Pseudo Wild Focal Female Results of the Preference Test and Mate Choice Copying Test. Mean time (s) that the pseudo wild focal female spent with the initially preferred male (IPM) and the initially rejected male (IRM) in the preference test and the mate choice copying test (MCC). Time Ff spent in neutral zone is also averaged and included. Ff change in preference for the (IRM) in the (MCC) test is present. Error bars represent standard deviations.

Truly wild Ffs in the preference test spent significantly more time (266 seconds) ($p = 0.001$) with one male (IPM) over another (IRM) (Table 3 & Figure 9). The truly wild Ffs in the mate choice copying test decreased the amount of time by 153 seconds they spent with the IPM, which was significant ($p = 0.01$) (Figure 9). The Ff increased the amount of time they spent with the IRM by 208 seconds and was also determined to be significant ($p = 0.002$) (Table 3 & Figure 9). The time the truly wild Ff spent in the neutral zone in the preference test compared to the mate choice copying test was not significant ($p = 0.34$) (Table 3). The paired t-test comparing the

preference test IPM combined times with the IRM combined times were significant (p = 0.001 (Table 3). This supports that in truly wild females mate choice copying is also present.

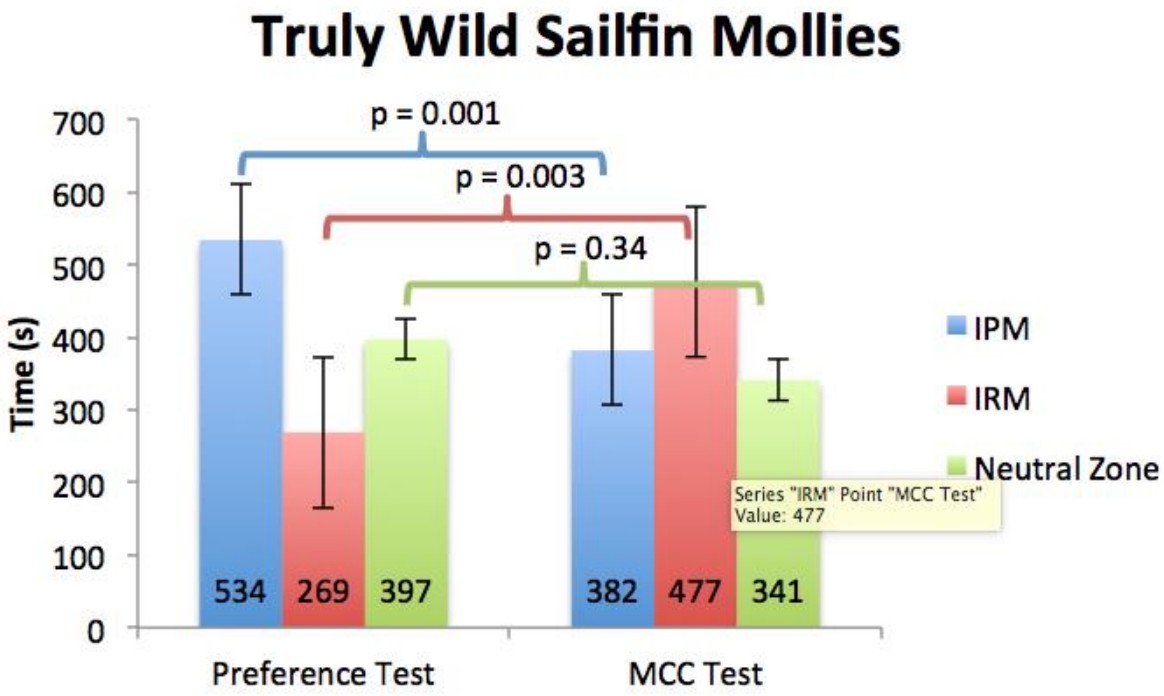

Figure 9. Truly Wild Focal Female Results of the Preference Test and Mate Choice Copying Test. Mean time (s) that the pseudo wild focal female spent with the initially preferred male (IPM) and the initially rejected male (IRM) in the preference test and the mate choice copying test (MCC). Time Ff spent in neutral zone is also averaged and included. Ff change in preference for the (IRM) in the (MCC) test is present. Error bars represent standard deviations.

Paired T-tests on times in the neutral zones within the pseudo wild and truly wild groups did not have significant values. The comparison of the pseudo wild and truly wild groups' preference test neutral zone times were significantly different p = 0.02 (Table 4). Pseudo wild sailfin mollies spent 174 more seconds in the neutral zone during the preference tests. The comparison of the pseudo wild and truly wild groups' mate choice copying test neutral zone

times were significantly different $p = 0.03$ (Table 4). Pseudo wild sailfin mollies spent 125 more seconds in the neutral zone during the mate choice copying tests. Showing in both tests that truly wild sailfin mollies spend more time in the neutral zone than the pseudo wild by 229 seconds with a significance value of $p = 0.01$ (Table 4).

In order to determine the individual's degree of mate choice copying a percent range was determined. First, the time the Ff spent with the IRM in the preference trials was converted into a percent by dividing the time of both trial 1 and trial 2 by the total time the female spent in both male preference zones (Equation 1).

Equation 1: IRM % time for Preference Trial

$$\text{IRM \% time for Preference Trial} = \frac{(\text{IRM T1 time (s)} + \text{IRM T2 time (s)})}{(\text{Total time in (s) IPM T1} + \text{IRM T1} + \text{IPM T2} + \text{IRM T2})}$$

Then, to determine the time the Ff spent with the IRM in the mate choice copying trials was converted into a percent by dividing the time of both trial 1 and trial 2 by the total time the female spent in both male preference zones during the mate choice copying tests (Equation 2).

Equation 2: IRM % time for Mate Choice Copying Trial

$$\text{IRM \% time for Mate Choice Copying Trial} = \frac{(\text{IRM T1 time (s)} + \text{IRM T2 time (s)})}{(\text{Total time in (s) IPM T1} + \text{IRM T1} + \text{IPM T2} + \text{IRM T2})}$$

Finally, the IRM % for the mate choice copying trial was subtracted by the initial IRM % for the preference trial to determine the percent change in time (Equation 3).

Equation 3: IRM % Change

IRM % change = IRM % time for Mate Choice Copying Trial - IRM % time for Preference Trial

The results were then applied to Table 1 to determine degree of mate choice copying and assigned a category that was similar to Croghan's 2012 domestic guppy study. A maximum value of 100% to 75% was determined to be a 'Very Strong' degree of mate choice copying and a 'Strong' degree was determined to be 74% to 50%. A 'Moderate' degree was 49% to 25% and a 'Mild' degree 24% to 11%. Values below 10% were considered to have no mate choice copying. Either there was not significant change in preference from the preference test to the mate choice copying tests or the Ff did not change her preference from her originally IPM. A lack of change was represented by a negative change in percent. The degree category 'None' includes both weak mate choice copying and sailfin mollies that kept their original preference.

Table 1. Degree of Mate Choice Copying Categories (Croghan 2012)

Category	Degree Percent Range
Very Strong	100% to 75%
Strong	74% to 50%
Moderate	49% to 25%
Mild	24% to 11%
None	10% and below

The fifteen Ff pseudo wild times in the IRM zones were converted in a percent change from the preference trial to the mate choice copying trial. The pseudo wild sailfin mollie individuals for this study did not have any that were a degree of mate choice copying 'Very Strong' or 'Strong' categories. However, four individuals were 'Moderate' and five individuals were considered in the 'Mild' range. There were six individuals that kept their original preference for the IPM and showed no mate choice copying (Figure 10). Out of the six individuals three individuals were 10% to 0% and three had a negative change in percent indicating that they spent even more time in the MCC trials with their original preference.

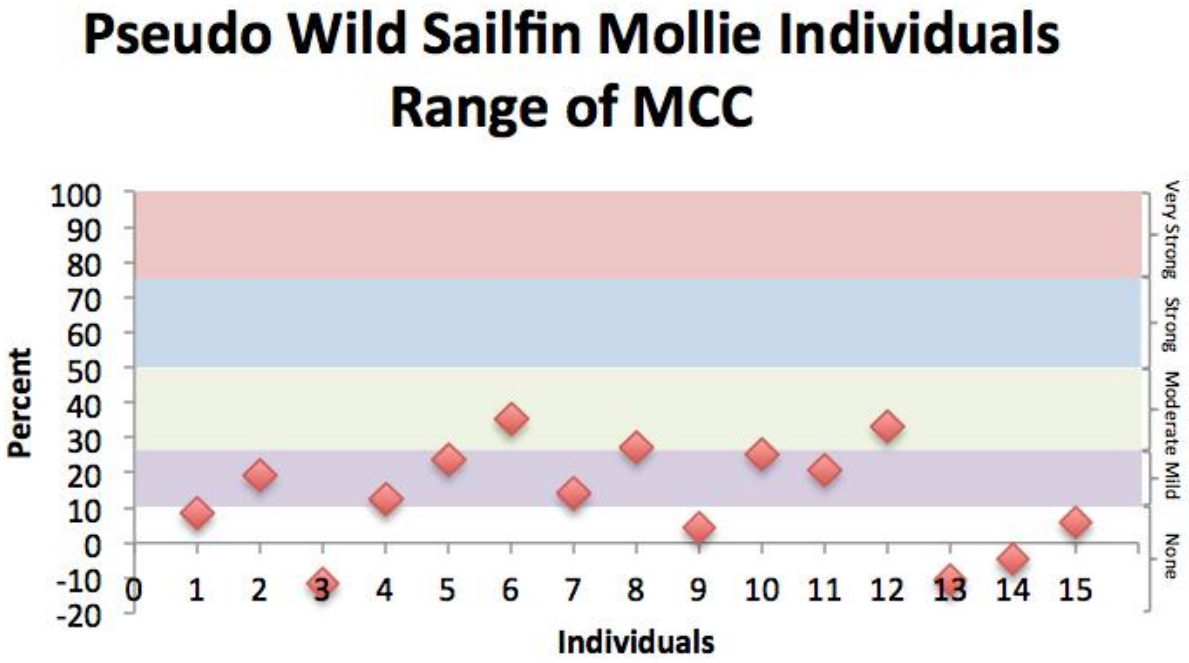

Figure 10. Pseudo Wild Sailfin Mollie Individual Percent of Mate Choice Copying with the Degree Range. Individual times for Ff preference and mate choice copying testes were run in Excel to Equations 1, 2, & 3 then Table 3 was applied to determine degree of mate choice copying. There are 0 = Very Strong, 0 = Strong, 4 = Moderate, 5 = Mild and 6 = None, individuals in the different degrees of mate choice copying.

The fifteen Ff truly wild times in the IRM zones were converted in a percent change from the preference trial to the mate choice copying trial. The truly wild sailfin mollie individuals for this study did not have any that were a degree of mate choice copying 'Very Strong' category. However, a single individual was in the 'Strong' category, five individuals were 'Moderate' and six individuals were considered in the 'Mild' range. There were three individuals that kept their original preference for the IPM and showed no mate choice copying (Figure 11). Out of the three individuals, two individuals were 10% to 0% and one had a negative change in percent indicating that she spent even more time in the MCC trials with their original preference. The single

individual in the ‘Strong’ category can be considered and outlier at 67%. The majority of the individuals in the next category of ‘Moderate’ are within a consistent range in relation to one another.

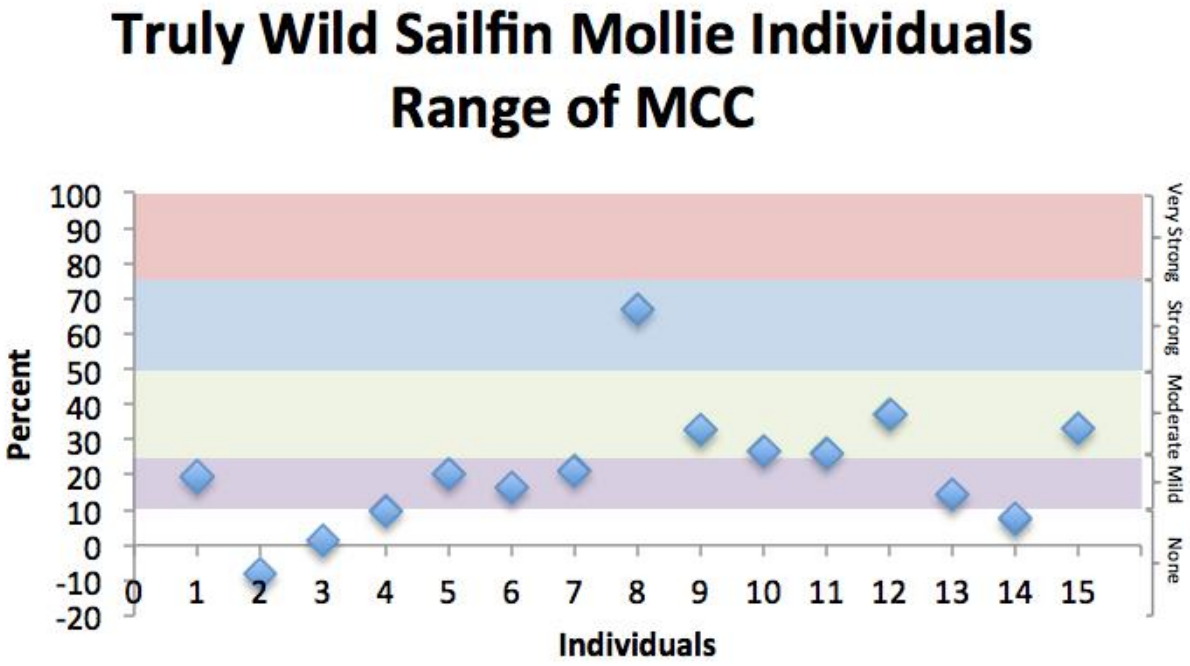

Figure 11. Truly Wild Sailfin Mollie Individual Percent of Mate Choice Copying with the Degree Range. Individual times for preference and mate choice copying testes were run in Excel to Equations 1, 2, & 3 then Table 3 was applied to determine degree of mate choice copying. There are 0 = Very Strong, 1 = Strong, 5 = Moderate, 6 = Mild and 3 = None, individuals in the different degrees of mate choice copying.

The degree of percent of mate choice copying mean was taken for the truly wild and pseudo wild groups. The average change in percent for the 4 sailfin mollie groups was then plotted (Figure 12). Two data points (squares) are from Elfelt’s 2014 data on sailfin mollies mate choice copying change in percent. The average change in percent for the truly wild, pseudo wild and Elfelt’s (2014) ‘wild’ group show mate choice copying in the ‘Mild’ degree category. The

truly wild group had a change in percent average of 22% a dramatic difference from the pseudo wild group 14%. A paired T-test comparing Elfelt's 'wild' and the pseudo wild groups percent change was not significant p = 0.69. Elfelt's (2014) 'domestic' group average change in percent 2% supports that the group did not exhibit the mate choice copying behavior. Further tests were run to determine if there was a significant difference between the groups.

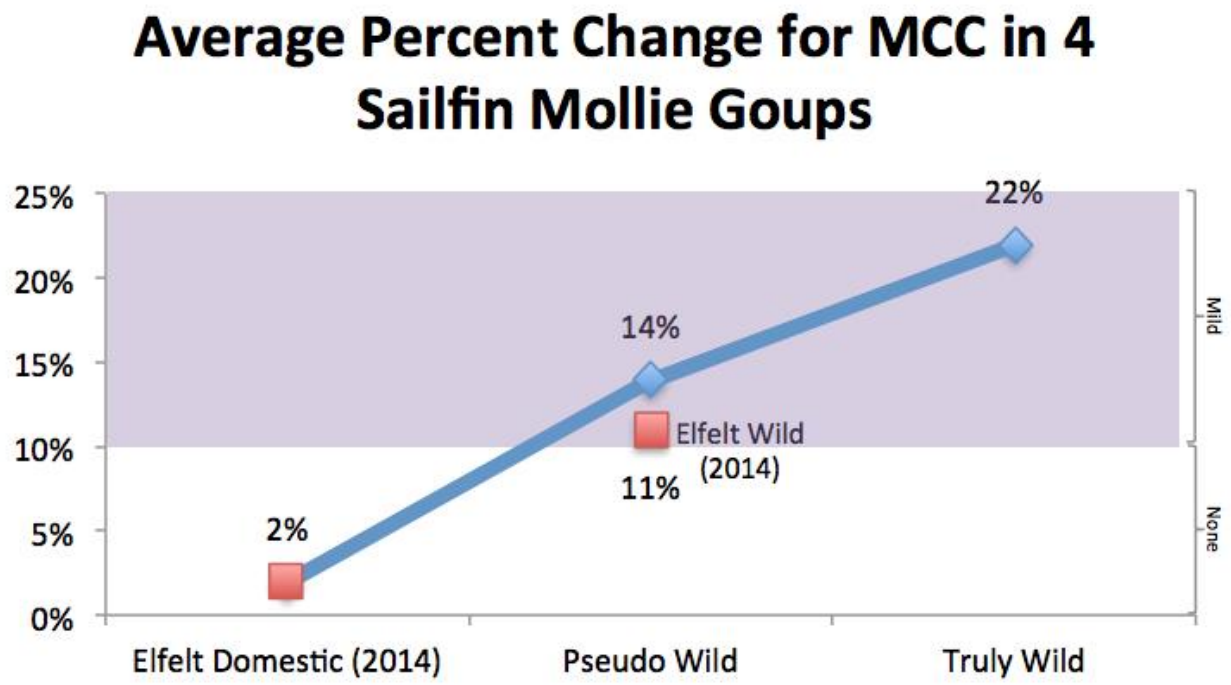

Figure 12. Average Percent Change for MCC in 4 Sailfin Mollie Groups. Average change in percent of time that the pseudo wild and truly wild Ff spent with the initially preferred male and initially rejected male between the preference test and mate choice-copying test shown in Figures 11 & 12. Elfelt's data from 2014 is included as the first data point (square) in the line. Elfelt's 'wild' (2014) data point (square) is included for comparison.

A one-way ANOVA was run and did show significance between the 3 groups at p = 0.022 (Table 5). As a result the Tukey Post Hoc test was run for multiple variants statistical analysis to compare the 3 groups ((1) Elfelt's domestic, (2) pseudo wild and (3) truly wild sailfin

mollies) to determine where the significance occurred. The Tukey analysis showed a significance between Elfelt's domestic and the truly wild sailfin mollies at $p = 0.016$ (Table 6). The analysis supported that there is a significant difference in the mean values for percent change in mate choice copying for the domestic and truly wild groups.

The cross breeding experiments were unsuccessful. The only groups to produce another generation of males and females were the two controls, truly wild females bred with truly wild males and pseudo wild females bred with pseudo wild males. The cross breeding of truly wild males with pseudo wild females resulted in a generation of only females. The cross breeding of pseudo wild males and truly wild females resulted in internal fertilization with no live young produced.

Chapter IV: Discussion

The results support that non-independent form of mate choice copying was present in pseudo wild sailfin mollies (Figure 8, Table 2). Previous experiments by Elfelt 2014, support that MCC behavior occurs in wild type (pseudo wild) *P. latipinna* but very weakly in domesticated *P. latipinna* (Elfelt 2014). Also, unpublished research shows that domestic *P. reticulata* no longer exhibit the MCC behavior (Croghan 2012). The results also support that truly wild sailfin mollies non-independent form of mate choice copying was also present (Figure 9, Table 3). This non-independent form of mate choice is when a female bases her decision on watching the selection of another female (Dugatkin 1993, Westneat et el. 2000) was first documented in wild caught *P.reticulata* (Dugatkin 1992).

The preference test for both the pseudo wild and truly wild supports that sailfin mollies show preference of one male over another. Truly wild sailfin mollies at 22% exhibit stronger mate choice copying behavior than the pseudo wild sailfin mollies 14% (Figure 12). Truly wild sailfin mollie females switched their preference from the IPM to the IRM at higher time duration with the presence of a model female. The higher percent in truly wild sailfin mollies suggests that they mate choice copy at a more robust rate. Results suggest that domestication by living in captivity even for as few as 12 generations can result in a drop in mate choice copying behavior.

Individuals that did not change their preference that received negative percent values were lumped together with individuals that weakly mate choice copied and were in the degree category of 'None' (Figure 10 & 11). In the pseudo wild half of the individuals in the 'None' were in the negative values. In the truly wild only one of the three had negative change in percent values. These females exhibited an independent mate choice behavior and did not follow the social cues to switch their mate choice (Pruett-Jones 1992). Possibly, the females could

discern that they were older than the Mf or that the males had a significant quality difference that influenced their independent mate choice (Houde 1994, Wiley & Posten 1996, Godin, Herdman & Dugatkin 2005). The low ratio of individuals in the truly wild group that received a negative score further supports more robust degree of mate choice copying in truly wild sailfin mollies.

Neutral zone times and freezing behaviors are also important factors to consider in mate choice copying. Truly wild sailfin mollies did spend more time on average in the neutral zone than the pseudo wild sailfin mollies (Table 4). After review of video the time in the neutral zone, the females were observed making slight movements and observations of the two males. These movements were confined to the neutral zone. The truly wild movements were much less aggressive than that of the pseudo wild group. A T-test comparing total times for truly wild and pseudo wild, PT and MCC trials were significant ($p = 0.01$).

Also, in the control trials truly wild sailfin mollies spent more time frozen at the bottom of the tank on trials. The freezing behavior was when the black Plexiglas screens were initially removed. The sudden movement instigated the behavior. Pseudo wild did not exhibit the freezing behavior on any of the trials but would engage in courtship displays immediately. Truly wild sailfin mollie time in the neutral zone and freezing behavior can be due to their lack of time in captivity. Pseudo wild sailfin mollies spent more time in tanks in captivity with no predation stress and human interactions. Females in the wild mate choice copy to reduce predation time and increase foraging time (Stohr 1998). The truly wild mollies exhibit more cautious behaviors in the neutral zone and freezing still exhibiting the predator evasion behavior (Briggs, Godin & Dugatkin 1996).

The second purpose of this MCC experiment was to replicate the intraspecific MCC behavior in wild caught and pseudo wild sailfin mollies. Then cross breeding experiments were

to be conducted to examine the effect of domestication on MCC. The control groups for the breeding experiments produced offspring that had the potential to be bred for another generation but did not. Due to this lack of success the breeding experiments were not continued. The focus became difference in domestication in pseudo wild and truly wild sailfin mollies affect on the MCC experiments.

Although, cross breeding experiments did not work for the truly wild females and pseudo wild males (green sailfin mollies) the truly wild male and pseudo wild female cross did produce a generation of all females. The all female cross breeding group was from 3 different clutches of young from the ten pseudo wild females. A repeat of this breeding cross experiment would be interesting to see if an all female cohort would be produced. Then a follow up with subsequent mate choice copying trials should be done to see if they have a similar mean and percent change to the previous truly wild and pseudo wild generation.

Future experiments should include switching the truly wild sailfin mollies from Mustang Island to a parent species of an available domesticated species so breeding would be possible. The truly wild did have more fry in number that were smaller than the domesticated green sailfin mollies. The larger fry were difficult to impossible for the truly wild sailfin mollies to birth causing a lack of a next generation in the male pseudo wild with the female truly wild species. Also, in future experiments should have several generations tested of the truly wild sailfin mollies to see how many generations in captivity it requires diminish the mate choice copying behavior.

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Appendix A

Table 2. Results of Excel T-test for Pseudo Wild Sailfin Mollies. Table of comparing Preferences Tests and Mate Choice Copying Tests for the Pseudo Wild Focal Female P-Values.

Pseudo Wild Sailfin Molly Paired T-tests	P-Value
PT - Paired T-Test of (s) w/ IPM in T1 with (s) w/ IPM in T2	0.75
PT - Paired T-Test of (s) w/ IRM in PT1 with (s) w/ IRM in PT2	0.22
PT - Paired T-Test of (s) spent with IPM in both T1&2 with (s) spent with IRM in both T1&2	0.006
MCC - Paired T-Test of (s) w/ IPM in T1 with (s) w/ IPM in T2	0.60
MCC - Paired T-Test of (s) w/ IRM in T1 with (s) w/ IRM in T2	0.79
MCC - Paired T-Test of (s) w/ IPM in T1+T2 with (s) w/ IRM in T1+T2	0.39
Paired T-Test of (s) spent with IPM in both PT1.T1&T2 with (s) spent with IPM in both MCC PT2.T1&T2	0.01
Paired T-Test of (s) spent with IRM in both PT1.T1&T2 with (s) spent with IRM in both MCC PT2.T1&T2	0.002
Paired T-Test of (s) spent with IPM in both PT+MCC T1+T2 with (s) spent with IRM in both PT+MCC T1+T2	0.001
Paired T-Test of (s) spent in Neutral Zone PT T1&T2 with (s) seconds spent in Neutral Zone MCC T1&T2	0.81

Table 3. Results of Excel T-tests for Truly Wild Sailfin Mollies. Table comparing Preferences Tests and Mate Choice Copying Tests for the Truly Wild Focal Female P-Values.

Truly Wild Sailfin Molly Paired T-tests	P-Value
PT - Paired T-Test of (s) w/ IPM in T1 with (s) w/ IPM in T2	0.78
PT - Paired T-Test of (s) w/ IRM in PT1 with (s) w/ IRM in PT2	0.93
PT - Paired T-Test of (s) spent with IPM in both T1&2 with (s) spent with IRM in both T1&2	0.001
MCC - Paired T-Test of (s) w/ IPM in T1 with (s) w/ IPM in T2	0.49
MCC - Paired T-Test of (s) w/ IRM in T1 with (s) w/ IRM in T2	0.31
MCC - Paired T-Test of (s) w/ IPM in T1+t2 with (s) w/ IRM in T1+T2	0.50
Paired T-Test of (s) spent with IPM in both PT1.T1&T2 with (s) spent with IPM in both MCC PT2.T1&T2	0.001
Paired T-Test of (s) spent with IRM in both PT1.T1&T2 with (s) spent with IRM in both MCC PT2.T1&T2	0.003
Paired T-Test of (s) spent with IPM in both PT+MCC T1+T2 with (s) spent with IRM in both PT+MCC T1+T2	0.002
Paired T-Test of (s) spent in Neutral Zone PT T1&T2 with (s) seconds spent in Neutral Zone MCC T1&T2	0.34

Table 4. Results of Excel T-test for Neutral Zone

Neutral Zone- Pseudo Wild & Truly Wild Sailfin Molly Paired T-tests	P-Value
Pseudo Wild/Truly Wild Neutral Zone - Paired T-Test of (s) w/ PT T1& T2 + MCC T1 & T2	0.01
Pseudo Wild/Truly Wild Neutral Zone - Paired T-Test of (s) w/ IPM in Preference T1 + T2	0.02
Pseudo Wild/Truly Wild Neutral Zone - Paired T-Test of (s) w/ IPM in MCC T1 + T2	0.03

Table 5. One-way ANOVA Results in SSPS for 3 Groups, (1) Elfelt's Domestics, (2) Pseudo Wild, (3) Truly Wild Sailfin Mollies.

ANOVA

V2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2658.210	2	1329.105	4.202	.022
Within Groups	13283.253	42	316.268		
Total	15941.463	44			

Table 6. Tukey Post Hoc Test for Multiple Variants in SSPS. The test compares (2) pseudo wild sailfin molly females with (3) truly wild sailfin molly females, (1) Elfelt's domestic with (3) truly wild sailfin molly females and (1) Elfelt's domestic with (2) pseudo wild sailfin molly females

Multiple Comparisons

Dependent Variable: V2

Tukey HSD

(I) V1	(J) V1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	9.9889238741600	6.4937707451832	.284	-5.787651610326	25.765499358646
	3.0	18.814267756960 0*	6.4937707451832	.016	3.037692272474	34.590843241446
2.0	1.0	-9.9889238741600	6.4937707451832	.284	-25.765499358646	5.787651610326
	3.0	8.8253438828000	6.4937707451832	.371	-6.951231601686	24.601919367286
3.0	1.0	-18.814267756960 00*	6.4937707451832	.016	-34.590843241446	-3.037692272474
	2.0	-8.8253438828000	6.4937707451832	.371	-24.601919367286	6.951231601686