

2018

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THE EFFECT OF FEMALE PRIMING ON MALE BETTA SPLENDENS

By

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BA, Millersville University, Lancaster, PA, 2014

Professional Paper

presented in partial fulfillment of the requirements
for the degree of

Master of Arts
In Experimental Psychology

The University of Montana
Missoula, MT

May 2018

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Psychology

The effect of female priming on male *Betta splendens* exposed to Fluoxetine Abstract

Chairperson: Allen Szalda-Petree

Male betta fish are naturally aggressive to attract a mate and defend a territory. This makes them a good model for studying aggression and choice behavior. This study looked at male *Betta splendens* aggressive nature when impacted by priming with a female and exposure to the SSRI drug fluoxetine. Males in the study received a prime before each choice trial, and the latency for each choice was measured and analyzed. We found no significant differences for preference for a mirror versus a non-mirror trail, or latency for choice for the mirror. However, a significant effect was found for fighting data, with primed males displaying more aggressive behavior than the males who were not primed. Another significant effect was also found, showing males exposed to fluoxetine had higher latencies for non-mirror trials than those not exposed to fluoxetine. This provides support for the hypothesis that the drug could impact motor movements and decrease arousal.

Betta fish are an aggressive species of fish commonly used in research to study aggression (Goldstein, 1975). These fish are native to Thailand, where they were once bred for their fighting abilities, and subsequently used in sporting contests, making them a popular figure in their native country (Goldstein, 1975; Abate, 2005). Due to the nature of the contests, the more aggressive fish were desirable since they could defeat their opponent, causing the breeders to selectively breed more aggressive fish, and create a highly aggressive captive population. Also, it has been noted that the betta fish exhibit a modal action pattern in regard to stimuli such as another live fish or a mirror; and that this is not only an effective strategy but reinforcing as well for the fish. Since the fish do not have self-recognition capabilities, they will behave aggressively when exposed to a mirror or another live fish (Balzarini et al., 2014). However, differences in aggressive behavior are found based on testing the fish against a mirror or live fish, suggesting they treat both stimuli differently (Verbeek et al., 2007). Studies have made note of these differences, and even compared wild type betta fish to domesticated strains, finding a similar effect based on context (Verbeek et al., 2007). Therefore, the chance to behave aggressively has been used by many researchers to reinforce the betta fish subjects, and test their aggressive responding for aggression studies.

Due to the aggressive nature of these fish, males are usually housed separately and only encounter females when breeders wish to breed them. The male fish will also behave aggressively toward a female, so breeders must be careful to monitor breeding activity and remove the female directly after she has laid her eggs. However, such careful control does not exist in the wild population still living in Thailand. Therefore, researchers have attempted to create communities of betta fish to study their social behavior in the laboratory and learn more about the aggressive nature of these creatures.

A study conducted by Goldstein (1975) attempted to establish a stable community of male and female betta fish in a large L shaped tank, capable of holding 1100 liters of water. To do this, the fish had to be introduced slowly to establish stability within the population, and dominance hierarchies formed through subsequent fights. Furthermore, if the fish still showed signs of extreme stress they were removed two days later. First, they had to get a stable population of two males, which took several tries, resulting in two deaths and three removals. However, after the stable population of two was established, the researcher was able to reach a population of seven males and eight females without further deaths or removals. Overall, the highest the population of the tank reached was 10 male fish and 10 female fish, but this only lasted a short time, and soon the male population returned to seven. In addition to observing the community, aggressive encounters were measured. It was noted that no fight exceeded 10 minutes and that it was typically newcomers that started the fights in the tank. Typically, these newly introduced male fish would swim around fighting with each male already present in the tank eventually fitting into the hierarchy. The fights also typically consisted of behavior considered to be low-risk, such as lateral displays, rather than high-risk behaviors like biting. In addition to the community experiment, the researcher also conducted another experiment where 20 males were tested in smaller tanks. They paired the males, creating 10 separate fights, and recorded the amount of time the fish fought, and the behaviors they exhibited. They noted that the fish in the smaller tank fought longer, averaging about an hour, and engaged in more high-risk biting behaviors than the males in the larger tank.

Goldstein (1975) concluded that betta fish can coexist together, so long as adequate space is provided for the fish, creating a situation similar to the natural environment for these fish. Also, the researcher disagreed that the betta's territorial response is what was driving the

aggressive behavior he observed, as only one fish was ever recorded to have established a territory, and it was after the social hierarchy had developed. Therefore, the author believes that the dominance hierarchy played an important role in the establishment of territories, and the aggressive responding noted among the fish, since the newcomers were typically the ones starting the aggressive engagements, and the dominant male appeared to have no trouble holding his position among the community. Overall, this study provides an interesting investigation of the social behavior of betta fish, and the social components that affect their aggressive behavior.

As Goldstein (1975) demonstrated, betta fish are capable of living in communities and coexisting according to the dominance hierarchy formed through initial fights. While some fish live primarily in shoals, others like bettas tend to form social preferences and are capable of existing alone or with other fish (Snekser et al., 2006). This has led researchers to investigate social preferences of betta fish to find out more about their social behavior and ability to coexist with others of the same species.

Snekser et al. (2006) investigated the social preferences of male and female betta fish by giving them a choice between shoaling options. To test this, the researchers obtained their participants from a local supplier and housed the females together in a large tank, while the males were placed together in another large tank, but separated into their own individual containers. After an acclimation period, the fish were tested using an apparatus with several chambers that allowed the fish to see a stimulus fish or nothing in some cases. The fish were able to swim a short distance to encounter the two chambers, and the fish were considered to have a preference based on the amount of time they spent near each chamber. Several conditions were established to test the preference for both male and female fish: one female fish vs empty chamber, three female fish vs empty chamber, one male fish vs empty chamber, one female fish

vs three female fish, and one male fish vs three female fish. The data were analyzed for each condition, and several findings emerged. First, for the condition of one female fish vs empty chamber, the female betta fish had a significant preference for the lone female stimulus fish when compared to the empty chamber, while the male betta fish was not found to have a preference. Next, the researchers found that both the male and female betta fish preferred the three female stimulus fish over the empty chamber. In the next test of male betta fish vs empty chamber, the researchers found opposite results for the male and female test fish, with the females showing a preference for the empty chamber, while the males preferred to spend more time near the male stimulus fish. Then, for the test of one female betta fish vs three female betta fish, both male and female test fish were found to prefer the group of females over the lone stimulus female betta fish. Finally, the last test condition of one male fish vs three female fish showed no preference for the test male betta fish, yet the female betta fish were found to prefer the group of females over the lone male stimulus fish.

In addition to investigating the preference in regard to time spent near each chamber, Snekser et al. (2006) also investigated the display behavior of the male and female test fish for each of the five conditions. For the first two conditions, one female betta fish vs empty chamber and three female betta fish vs empty chamber, both the males and females were found to display more at the females than the empty chamber. Then, for the condition for one male betta fish vs empty chamber, the male test betta fish was found to display more at the stimulus male than the empty chamber, and it was noted that the test female betta fish rarely emitted any display behavior toward the male stimulus fish. In the next condition of one female betta fish vs three female betta fish, it was found that the female test fish showed no display preference, while the male test fish preferred to display at the group of stimulus female fish over the lone stimulus

female fish. Finally, in the last condition of one male stimulus betta fish vs three female stimulus betta fish, the males were shown not to have a preference for displaying, while females preferred to display at the group of females over the lone male fish.

Based on the results of both time spent near the chambers and display behavior, Sneksner et al. (2006) concluded that both male and female betta fish have a preference for groups of female fish, over one lone female or nothing. However, the researchers noted that female betta fish tend to be wary of male fish, which would explain why the female test fish consistently preferred either other females or nothing over the male betta stimulus fish. In addition, the male test betta fish had difficulties choosing in the last condition between the lone male and three female betta fish, going back and forth between displaying at the potential mates and rival. Finally, the researchers noted that males appeared to display less aggressively in the presence of the females in the last condition. Therefore, based on the results of this study, the researchers concluded that while betta fish are not considered to be a social species of fish, they do exhibit social behavior, and have social preferences.

Since betta fish exhibit some social behavior and have been found to prefer groups of females over a single female or nothing, one might wonder how this social behavior is perceived by other betta fish within the vicinity. As mentioned before when examining the study conducted by Sneksner et al. (2006), the males seemed to tone down the aggressive encounters if a female was present, suggesting that the mere presence of the female in the vicinity has an effect on the aggressive responses of the male. This is of interest to researchers since males must prove able to hold and defend a territory to attract a mate, build a bubble nest to hold the eggs until they hatch, and raise the young until they are able to swim away to live on their own (Dzieweczynski et al., 2005). This is particularly important because males hold the sole responsibility for caring for the

offspring until they are able to swim away from the territory and live on their own (Abate, 2005). However, the males will decrease the amount of aggression shown in the presence of a female, which is known as the audience or bystander effect (Dzieweczynski et al., 2005). The betta fish males and females not engaged in the aggressive encounter have been known to observe the aggressive encounter to gain information from the fight, such as potential rivals or mates. Interestingly, the males engaged in the aggressive encounter alter their behavior based on the sex of the audience member, displaying more aggressive behavior in the presence of another male, and less when a female is present. Therefore, researchers have investigated how different audiences have affected the aggressive responding shown in encounters between males, and how the presence and size of a bubble nest can influence the aggression displayed by the fish.

One such study investigating both the effect of audiences and bubble nests was conducted by Dzieweczynski et al. (2005). The researchers obtained a group of fish from a local supplier, and after the acclimation period housed the males in their own tanks with a piece of bubble wrap to facilitate nest building. Furthermore, the males were all measured, and then paired based on color and size. Since the bubble nests were dependent upon the individual fish, the pairs were kept and the bubble nests were recorded for each fish. This created three conditions of no bubble nest, one bubble nest, or two bubble nests. In addition to these conditions, there were also audience conditions of no audience, male audience, or female audience. The subjects were tested and recorded based on these conditions only once, by first exposing the males to the audience tank for five minutes, and removing the partition allowing them to view each other and fight for 20 minutes. After the tests were conducted and the data were analyzed, both the audience and bubble nest conditions yielded significant effects on their own, but an interaction effect was found between the audience and bubble nest conditions. Basically, the presence of the bubble

nests affected the response directed toward the audience member. First, the presence of a bubble nest for both males resulted in less aggressive responding than the other conditions. Also, in the condition where only one male possessed a bubble nest, the males in that condition were found to be more aggressive in the presence of a male or female audience, than no audience member. For the males in the condition of no bubble nests, these males aggressed more when a male audience was present, but not the female audience or no audience. Finally, in each nesting condition, the males were found to spend more time near the audience if the audience member was male, or if there was no audience member. However, this behavior usually occurred after the opponent male stopped responding, leading the other male to direct his attention elsewhere.

Dzieweczynski et al. (2005) determined that context is important for the betta fish aggressive responses. They concluded that the presence of the bubble nest will affect subsequent responses in an aggressive encounter with an audience present and that the aggression is context dependent. The researchers propose that this results from the territorial drive found in this species. Therefore, they accounted for the lower levels of aggression observed in the males who both had bubble nests by arguing that since each male had established a territory, they would be more driven to mate. However, for the males who were in the other conditions of no bubble nests or one bubble nest, the researchers argued these males had not established a territory, thus making them more aggressive in order to attempt to establish the territory. The researchers also noted that the highest level of aggression observed in the condition of two bubble nests was in the presence of the female audience, prompting the researchers to argue that the males wished to chase off the rival to gain access to a mating opportunity. So, the researchers believe their results support the notion that the effect of the audience and presence of a bubble nest create a context that fuels the behavior of the fish in regard to territorial and mating choices.

While it is true that the context is important when studying the aggressive responding of betta fish, other factors should be considered when studying aggression in this animal model. One of the other factors is familiarity, and investigating if betta fish possess an ability to remember other fish from past encounters. Research suggests that betta fish do possess this ability and that it affects their aggressive interactions (Dzieweczynski et al., 2012). Past research has studied the ability of betta fish to recognize other fish they have encountered before, of both genders, and the impact this has on the aggressive reaction the fish displays upon recognizing this familiar fish. As demonstrated by Goldstein (1975), the betta fish can form a dominance hierarchy that dictates social behavior, such as which fish has an opportunity to mate, with most of the fights initiated by the newly introduced fish interjecting itself into the hierarchy. While the dominance hierarchy idea has support, another principle that goes along with Goldstein's (1975) work is that of the dear enemy principle. The dear enemy principle dictates that an animal will fight less aggressively with another animal it is familiar with, which is what a study conducted by Dzieweczynski et al. (2012) was interested in exploring.

In the study conducted by Dzieweczynski et al. (2012), the researchers were interested in studying betta fish aggressive encounters in relation to the dear enemy effect, and how the presence of an audience further affected these aggressive encounters. In order to test this, the researchers obtained a group of fish from Live Aquaria and matched them based on size and color. The fish were acclimated for a period of two weeks after arrival in order to ensure that any gained familiarity would be lost. Then the fish were tested by placing the tanks side by side with an opaque wall preventing the fish from viewing each other, with an audience tank placed below those tanks. Several conditions were created for testing the fish based on time, audience, and familiarity. First, two-time intervals were implemented, with some of the fish being tested four

hours after the initial test, and the others receiving a 24-hour interval between trials. Then for the audience conditions three conditions were used: male audience, female audience, and no audience. Finally, two conditions were set up for familiarity: familiar and unfamiliar. When analyzing the data, the researchers discovered no differences or interactions based on the time conditions. However, the gill flaring behavior observed for the betta fish did show a significant effect based on audience and familiarity, with an interaction effect also present for the two variables. For the unfamiliar males, they spent the most time gill flaring if the audience member was male, but did gill flare more with a female audience member present than no audience at all. Also, it was found that the unfamiliar males exhibited more gill flaring behavior when the female audience was present than the males who were familiar with each other from the past encounter. Finally, the researchers noted the males displayed the most aggression when exposed to a male audience, especially in the unfamiliar condition, while exposure to a female audience instigated more courting behaviors, particularly from the males in the familiar group. The researchers, therefore, suggested that an interaction may be present between these variables.

Therefore, Dzieweczynski et al. (2012) concluded from their results that both context and familiarity are important variables in betta fish aggressive encounters. They noted that consistent with past research males have been shown to decrease aggressive display behavior in the presence of a female betta fish, but that this was only consistent if the opponents were familiar with each other. The researchers suggest this could be due to the benefits the unfamiliar males would stand to gain if they were able to intimidate their opponent to retreat, thus giving them access to the potential mate. This would also serve as support for the variable of context having a significant impact on the behavior exhibited, and the researchers further noted that no gill flaring difference was found between the males when no audience was present for the fight. Therefore,

the males changed their display behavior based on the presence of an audience, which served as the context variable in the study. Overall, the researchers believe the study generates support for the dear enemy notion since significant changes in fighting behavior were observed between the familiarity conditions, with less aggressive responding shown in the familiar condition. Finally, another study conducted by Dzieweczynski and Perazio (2012) yielded results suggesting that familiarity effects extend to audience members as well as opponents. They found different behavior depending on the familiarity with the audience member, which was further affected by sex and the passage of time between exposures.

In addition to a notable change in behavior due to the presence of an audience, betta fish can also be influenced by priming. In order to use priming, the subjects are exposed before the testing condition to the manipulation and then tested with the experimental measures after the priming procedure has been completed. Priming in aggression experiments with betta fish involves the presentation of another betta fish, male or female, for a short period of time before the upcoming aggressive encounter. When a fish is primed before the aggressive encounter the latency to respond is shortened, and there is an increase in aggressive displays (Matos et al, 2003). However, investigations into the effects of priming have found a short time interval for this effect (Bronstein, 1989).

Bronstein (1989), investigated the priming effect of male betta fish, specifically looking for the time interval or the priming effect. The researcher obtained a sample of fish from a local supplier and screened them to ensure that the fish met the aggressive criteria for the study. Then for testing, the fish were placed in the test area for 10 minutes to acclimate, before being exposed to another male for priming, or no male fish for the control group, for 15 minutes. After the 15-minute exposure time, the males waited either zero, one, or five minutes before being exposed to

a male fish for testing. The fish were considered displaying aggressive behavior if they were between a stimulus wall and approach line, and the researcher recorded the gill flaring, lateral displays, and biting behavior in this area. The notion of random assignment was supported for the three experimental groups upon comparing their behavior with the priming experience. Also, the fish in the experimental conditions of exposure to a male fish after zero or one minute of priming were found to display more aggressive behaviors than the other groups. The males in the experimental group that waited five minutes in between priming and the second exposure were found not to be significantly different than the control condition. Therefore, since pheromone removal was controlled for, the researcher concluded that the priming was responsible for the observed behavior differences among the fish. Also, the researcher believes this adds further support to the data that has shown that betta fish have a short memory span, which culminates in a rapid decrease in fighting motivation after the opponent is removed.

Another study conducted by Matos et al. (2003), investigated the effect of priming on aggressive encounters using the audience effect, with two separate experiments. In the first experiment, four conditions were created: no audience, audience visible for only 5 minutes preexposure, audience visible for only 10-minutes opponent faceoff, and audience present the whole time. For the second experiment, three conditions were created: both males exposed to the audience, both males exposed to an empty tank, and one male exposed to the audience while the other is exposed to an empty tank. Both experiments were tested using the same method, which involved first exposing the test males to the audience for five minutes, and then removing the partition between them allowing them to fight for 10 minutes. For experiment one, a significant difference was found for latency to first bite in the conditions preexposure and exposure throughout to the audience, when compared to the conditions no audience or only exposure

during the fight to the audience. Then for experiment two, the most aggression was found when both fish received preexposure to the audience, or in the fish who received the preexposure in the condition where only one fish was preexposed.

Matos et al. (2003), concluded that the preexposure, or priming, with the male audience was what led to the increased aggression, and decreased latency to first bite, that was observed in the study. In addition, it was found that even though the audience was not present during the fight for the preexposure only group in experiment one, this group still showed a latency to engage in the first bite, demonstrating the effect of the mere presence of an audience on the betta fish aggressive behavior. This could also be tied to the aggressive displays directed at the audience member in the preexposure group. Furthermore, as past research has demonstrated, betta fish tend to start fights out with lower level aggressive behaviors, such as lateral displays, and move toward more aggressive behaviors, such as biting, throughout the fight. However, the males who received preexposure displayed at both their opponent and at the audience member, suggesting that they would be moving toward more aggressive behaviors during the test fight. Also, it was noted that the fish who received the preexposure to the audience lost their encounters more often than those who were not preexposed, suggesting that the primed fish were unable to maintain their aggressive displays throughout both the priming and subsequent fight afterward. Finally, the researchers observed that the less aggressive fish matched the level of aggression displayed by the more aggressive fish for the audience, presumably to avoid being seen as the less aggressive fish, and to avoid injury. Past research has shown that the less aggressive fish, or the loser of an encounter, is more likely to be approached by a male audience member when the fight is over, thus adding another benefit to matching the aggression shown during the fight. Overall, the results of the study led the researchers to conclude that priming

does have an effect on increasing the aggressive behavior exhibited by the betta fish and that the presence of a male audience member increases the amount of biting behavior, which is considered a higher risk aggressive behavior, between the opponent males.

As the research up to this point has shown betta fish behavior can be manipulated using natural methods, such as context, audience effect, and priming. However, research conducted in recent years has focused on other methods of manipulating betta fish behavior, such as the use of antidepressants and anti-anxiety medications, classified as Selective Serotonin Reuptake Inhibitors (SSRI) (Dzieweczynski and Hebert, 2012). This research has become increasingly important over the years, as it explores the potential effects such SSRI's have when introduced into waterways, through wastewater treatment plants, on the aquatic life living there (Dzieweczynski and Hebert, 2012). These SSRI's are designed to disrupt the endocrine system and bring balance to people suffering from depression and anxiety, but to animals not suffering from these disorders the effects could be severe. Recent work has been devoted to discovering the potential effects wildlife experience from these SSRI's, and how it affects behaviors such as aggression, mating, and general activity. Studies have found that SSRI's such as fluoxetine start to affect the aquatic subjects very quickly, sometimes within a matter of hours. These SSRI's have been found to affect reproductive growth, decrease testosterone levels, affect prey capture and predator evasion, interfere with normal swimming patterns, and affect aggression. Mennigen et al. (2008) and Mennigen et al. (2011) found effects of fluoxetine on goldfish reproduction. In addition, work with stickleback's exposed to the SSRI Prozac have shown that the exposed fish build lower quality nests, and are slightly less aggressive when it comes to defending them (Sebire et al., 2015). Furthermore, past studies with betta fish have shown that SSRI's lower aggression levels in this species, thus negatively impacting their ability to defend a territory

(Forsatkar et al., 2014). Overall, the effects of SSRI's can disrupt many behaviors necessary to the survival and continuation of a variety of species. Since aquatic species in the wild are being exposed to excess serotonin in their natural environment, researchers such as Dzieweczynski and Hebert (2012), and Eisenreich and Szalda-Petree (2015) have looked at how an excess of serotonin affect the natural behavior of an aquatic model, the betta fish. While Dzieweczynski and Hebert (2012) focused on specific aggressive behaviors toward a dummy model, Eisenreich and Szalda-Petree (2015) focused more on instances of aggression and latency to engage in the aggressive behavior.

The effect of the SSRI fluoxetine on betta fish behavior was explored in a study conducted by Dzieweczynski and Hebert (2012). The researchers obtained male betta fish from Live Aquaria and used bubble wrap to facilitate nest building amongst the fish, purposely not testing the fish until they had created a bubble nest. Each day before the trials were conducted the males were monitored to ensure they were tending to their bubble nest, and a 10-minute priming exercise was conducted. For the priming exercise the opaque walls were removed, allowing the fish to be exposed to the neighboring fish. Then for testing the fish received three baseline tests to establish a pattern of responding without the SSRI's influence. These baseline tests were conducted using both a dummy male and female mold, painted to look like a real fish. After the three baseline tests were completed, the researchers exposed the betta fish to the fluoxetine agent by introducing 20 μL to the tank of each male, culminating in an overall concentration of 0.54 $\mu\text{g L}^{-1}$. The fish were tested three times, with both the baseline and testing conditions occurring every other day. The researchers speculate that since 30 percent of the water was changed daily, the concentration of fluoxetine varied between 8–23 ng L^{-1} throughout the experiment. In addition to the experimental group exposed to the SSRI, a control group was

also used that also underwent the same procedure, but without any SSRI exposure. The behavior for both groups was recorded and analyzed. For the control group, no significant differences were found between the first and last three trials. The researchers found no significant effects for fluoxetine on the behaviors the males directed at the female dummy, or the amount of time that the males spend near their bubble nest. However, the male-directed behaviors of gill flaring and fin spreading were significantly impacted by the exposure to fluoxetine. This was also shown in the repeatability ANOVA tests used to analyze the data, which yielded a decrease in male-directed behaviors, and an increase in female-directed behaviors after SSRI exposure.

Dzieweczynski and Hebert (2012) concluded that the SSRI fluoxetine had an effect on the consistency of the behavior observed in the betta fish tested in the study. They noted that the male betta fish that were exposed to the SSRI had lower levels of aggression afterward, while the control fish remained the same, suggesting the effects were SSRI related. However, it was noted that the males exposed to the SSRI increased the amount of behaviors directed at the female dummy, suggesting that the SSRI impacts more than just the aggression system in the fish. This could be further impacted by an individual's sensitivity to the SSRI, as fish living in polluted areas have provided interesting research findings in terms of individual and population effects of chemical agents in the environment. In addition, it was noted that the group exposed to the SSRI showed a decrease in fin spreading and gill flaring, but not biting and tail beating behaviors. The researchers gave several possible explanations for this, including individual fighting style and another possible system, aside from serotonin, for controlling these behaviors. Furthermore, the researchers tested the prevalence of behaviors during the baseline condition and looked at individual differences in fighting style. Since the males who were exposed to fluoxetine did not demonstrate any negative correlations when the behavior was analyzed, the researchers

concluded that the SSRI affected each fish differently, and changed the consistency of the aggressive response the males exhibited. However, while the researchers anticipated that the male-directed behaviors might change after exposure to the SSRI, they did not anticipate that the males would change their female directed behaviors. They gave several tentative explanations, such as behavior consistency affecting attractiveness to a potential mate, but believe more research should be conducted to investigate this effect. Overall, the researchers believe that this study provides evidence that the SSRI fluoxetine has an impact on the amount of aggression demonstrated in the male betta fish when exposed for a short time period, showing a drop in the consistency of the aggressive behavior. This could affect mating when coupled with behavioral consistency, as territory establishment and defense is important for betta fish reproduction.

Research has shown that fluoxetine does have an impact on aggression in betta fish, yet the way the fish are affected needs more exploration (Dzieweczynski, and Hebert, 2012). The betta fish share many of the same brain pathways as mammals, and serotonin is known to affect the fish similar to mammals both philologically and behaviorally (Eisenreich and Szalda-Petree, 2015). SSRI's that manipulate serotonin can affect the fish in a variety of ways, with the two most common hypotheses supporting either motor sedation or a lowered level of arousal to cues present in the environment. Many studies have looked into these hypotheses, and support for each has been generated in a variety of animal models (Aristieta, 2014).

Eisenreich and Szalda-Petree (2015) examined motor changes in fish exposed to fluoxetine. Results showed increased motor sedation as dosage increased. Each fish was placed in a dosing chamber with 200 mL of water each day, resulting in a 10 μ mol concentration of the fluoxetine. The procedure used for testing was that of an ABA design. For the experiment a t-shaped maze had the top portion blocked off to form an alleyway. The fish were tested using

discrete trials, where either a checkerboard or blue pattern were used to signal the presence of a mirror or white wall at the end of the alley, creating a goal box between the patterns and mirror or white wall. These conditions were counterbalanced across subjects and served as a signal for excitatory mirror trials and inhibitory white wall trials. The fish started the trials in the start box, in front of which a guillotine door was placed, and lifted to allow the fish access to the alleyway for testing. The latency and aggressive reactions of the fish were recorded, with a latency of two and a half minutes set as the maximum. The analysis revealed less time taken to aggress and more aggressive responding being present in the baseline conditions, while the experimental condition yielded longer latencies for both inhibitory and excitatory trials.

Eisenreich and Szalda-Petree (2015) concluded that while it appeared some motor effects could be present; they are only tentatively arguing for that hypothesis. Also, the researchers acknowledge that the fluoxetine may have lowered arousal levels for the excitatory mirror trials, noting that the fish decreased aggressive responding and patrolling behavior while under the influence of the fluoxetine. In addition, the researchers found that the fish became accustomed to the daily switching of first running with excitatory or inhibitory trials, based on patrolling behavior exhibited by the fish in the inhibitory trials. Therefore, the researcher's findings continue to support both of the hypotheses of motor sedation and lowered arousal, putting forth a possible explanation of the fluoxetine interfering with dopamine brain pathways. The dopamine pathways have a connection to both motor movement, and reward learning, which fits with the data obtained in this study. So, based on the results of this study, the researchers conclude there may be a connection between serotonin and the dopamine brain pathways, with regard to motor and reward learning.

Much of the research reviewed has focused on male *Betta splendens* and aggressive behavior. However, the presence of female *Betta splendens* is important when considering the male aggressive response (Dzieweczynski et al., 2005). Males exhibit this aggressive reaction to other males as a way to establish dominance and gain a territory to attract a mate (Goldstein, 1975). By only focusing on the male fish without the influence of the female the results may be lacking important contextual cues tied to aggressive behavior (Dzieweczynski et al., 2005). If males are exhibiting aggressive behavior with the end goal of attracting a mate, and subsequently producing and raising young, then it would be important to include female conspecifics as part of the context when testing the aggressive behavior of the fish (Dzieweczynski et al., 2005).

The present experiment examined the effects of female presence and fluoxetine on aggressive responding to a mirror in male *Betta splendens* using a Go-No go task. It is hypothesized that:

- subjects in the female primed group will have lower latencies to enter the goal box with the mirror compared to the non-primed group.
- subjects in the fluoxetine group will have higher latencies to enter the goal box with the mirror compared to the non-fluoxetine group.
- subjects in the fluoxetine group will exhibit less aggressive behavior toward the mirror compared to the non-fluoxetine group.

Methods

Participants

Sixty male and nine female *Betta splendens* were obtained from Live Aquaria of approximately five cm long, red and blue coloration. All fish were maintained on a 12 hr

dark/light cycle, housed individually, and the water temperature of the tanks was maintained at 76-82 °F.

Material/Apparatus

Each fish was housed individually in their own tank of approximately 67 cm x 40 cm x 18 cm (L x W x H), containing approximately 20 liters of water treated with water conditioner to remove chlorine. Each tank also contained brown gravel, a T-maze, a heater, a thermometer, and a bubbler hooked up to an airstone. The alleyway maze in which the fish lived, measured approximately 53 cm x 20 cm x 10 cm (L x W x H). The fish were maintained on a diet of Tetra min betta pellets, receiving about eight pellets a day.

A stock solution of 0.5 mmol was created using the SSRI fluoxetine, obtained from TCI America. The fish were exposed to the stock solution by mixing 4 ml of the stock solution with 196 ml of the tank water from individual fish in a separate dosing chamber, resulting in a 10 µmol concentration of fluoxetine.

Procedure

Male subjects were screened for aggression and matched on latency to aggress toward a mirror. Matched sets of four males were randomly assigned to the four groups: Control – No Fluoxetine group (C-NF), Control – Fluoxetine group (C-F), Experimental – No Fluoxetine group (E-NF), and the Experimental – Fluoxetine group (E-F). E-NF and E-F subjects were exposed to a female prior to each trial, while groups C-NF and C-F subjects were exposed to an empty chamber before each trial.

The daily testing consisted of 10 trials, with five trials involving the mirror condition, and the other five involving the timeout condition. Fish in the fluoxetine groups (C-F and E-F) were

exposed to fluoxetine three hours before the beginning of each daily testing session. The fluoxetine exposure consisted of placing the fish in a dosing container with 196 ml of their own tank water and 4 ml of the fluoxetine solution for thirty minutes, resulting in a 10 μmol concentration of fluoxetine. For fish in the Experimental groups (E-F and E-NF) a female conspecific was placed into a clear holding chamber directly adjacent to the start box (see Figure 1). Before each trial, fish in the Experimental groups were exposed to the female conspecific for 30 seconds. Fish in the Control groups (C-F and C-NF) were exposed to an empty holding chamber for 30 seconds. Once the 30 seconds elapsed the male's view of the holding chamber was blocked with a white wall and the trial began. A unique discriminative stimulus pattern was associated with each reward condition (mirror or timeout). The mirror or timeout conditions were counterbalanced across days such that the mirror condition is presented first every other day.

The format for all trials consisted of placing the fish into the start box and then raising the dividing door between the start box and the holding box for 30 seconds (see Figure 1). The courting behavior exhibited by the male was recorded as either yes courting occurred, or no courting did not occur. After 30 seconds the dividing door between the start box and the holding box was lowered and the door between the start box and alleyway was raised. The amount of time it takes for the male to swim into the goal box of the alleyway was recorded. The fighting behavior of the male was also recorded as either yes aggressive responding was present, or no aggressive responding was not present.

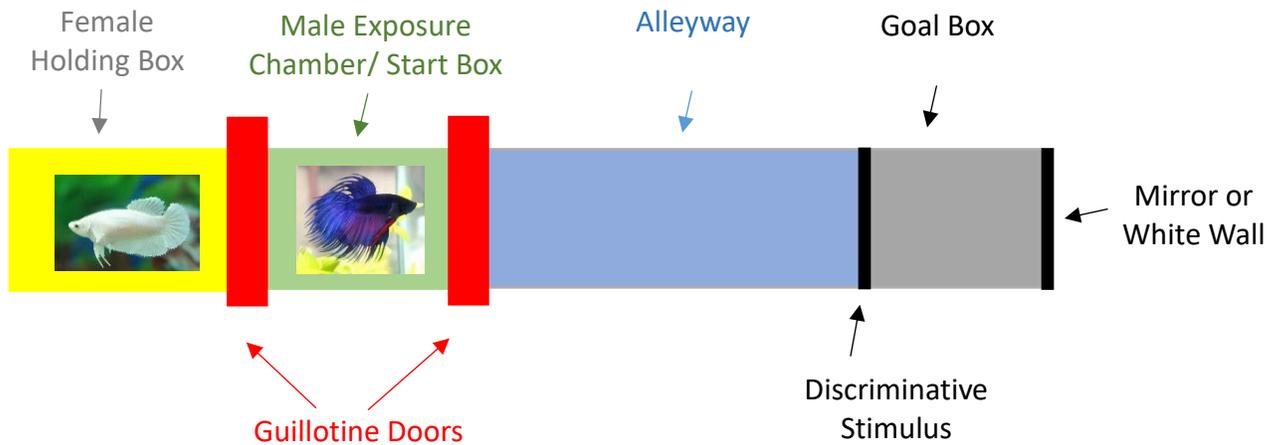


Figure 1. Testing apparatus

Analysis

These data for analysis included latency to enter the goal box for both mirror and non-mirror trials, preference ratio for the mirror/non-mirror condition, and presence of fighting behavior. Group means were calculated for each dependent variable for each test day and then the group means were averaged across the last five testing days for analysis. To calculate the average for the fight behavior, the fish were scored each trial as 0 for fighting behavior not present and 1 for fighting behavior present. The preference ratio was calculated by dividing the non-mirror latency by the sum of the non-mirror and mirror latencies for that day ($S^- / (S^- + S^+)$). Thus creating a ratio bound between 0 and 1 with a higher ratio indicating a higher preference for the mirror.

Results

A 2 (Female groups) x 2 (Drug groups) between subjects ANOVA conducted on the latency to enter the goal box for the mirror condition revealed a non-significant main effect for drug group ($F(1,56)=3.115$, $p=0.083$, $\omega^2=0.032$), a non-significant main effect for female group

($F(1,56)= 2.737, p=0.104, \omega^2= 0.027$), and a non-significant Drug X Female interaction ($F(1,56)=2.662, p=0.108, \omega^2= 0.025$). See Table 1 and Figure 2 for descriptive statistics.

Table 1. Group descriptive statistics for the mirror raw latency data averaged across the last 5 days of testing.

		No Female	Female
No Drug	Mean	103.36	65.20
	SD	42.42	50.65
	N	15	15
Drug	Mean	104.91	104.65
	SD	40.51	45.67
	N	15	15

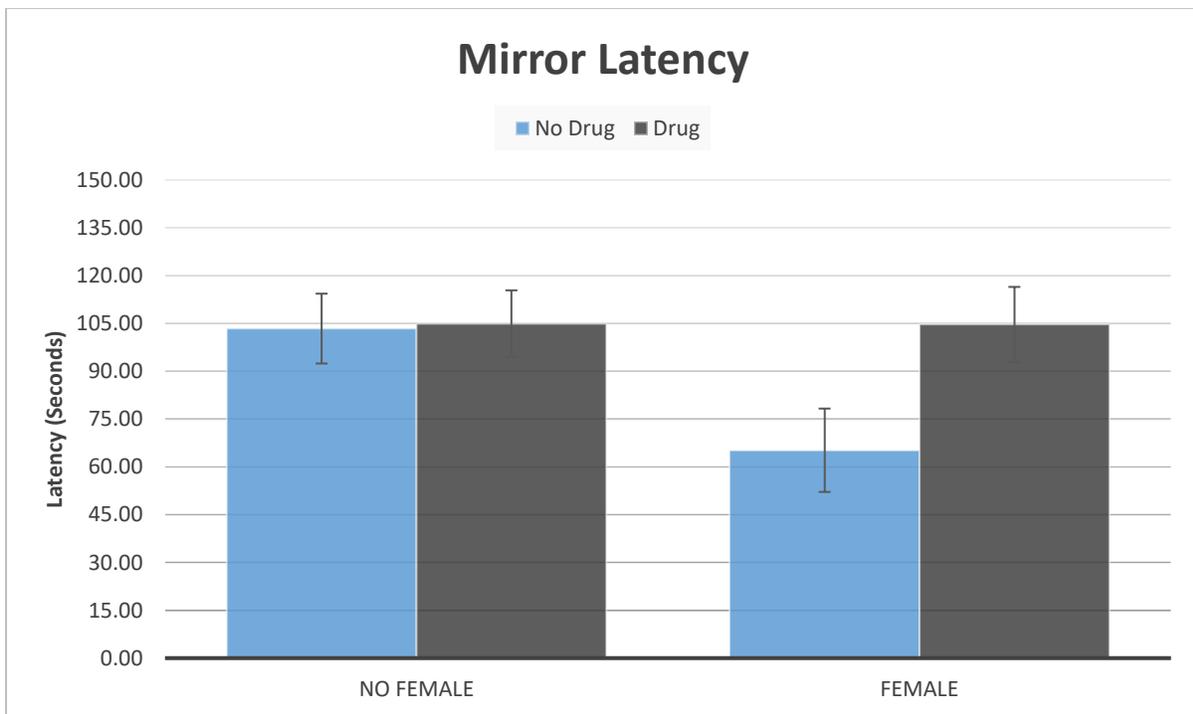


Figure 2. Group descriptive statistics for the mirror raw latency data averaged across the last 5 days of testing.

A 2 (Female groups) x 2 (Drug groups) between subjects ANOVA conducted on the latency to enter the goal box for the non-mirror condition revealed a significant main effect for

drug group ($F(1,56)= 19.938, p< 0.001, \omega^2= 0.239$), a non-significant main effect for female group ($F(1,56)= 1.039, p=0.312, \omega^2< 0.000$), and a non-significant Drug X Female interaction ($F(1,56)=1.186, p=0.281, \omega^2= 0.002$). See Table 2 and Figure 3 for descriptive statistics.

Table 2. Group descriptive statistics for the non-mirror raw latency data averaged across the last 5 days of testing.

		No Female	Female
No Drug	Mean	80.76	62.21
	SD	34.26	33.52
	N	15	15
Drug	Mean	110.46	111.07
	SD	31.08	37.14
	N	15	15

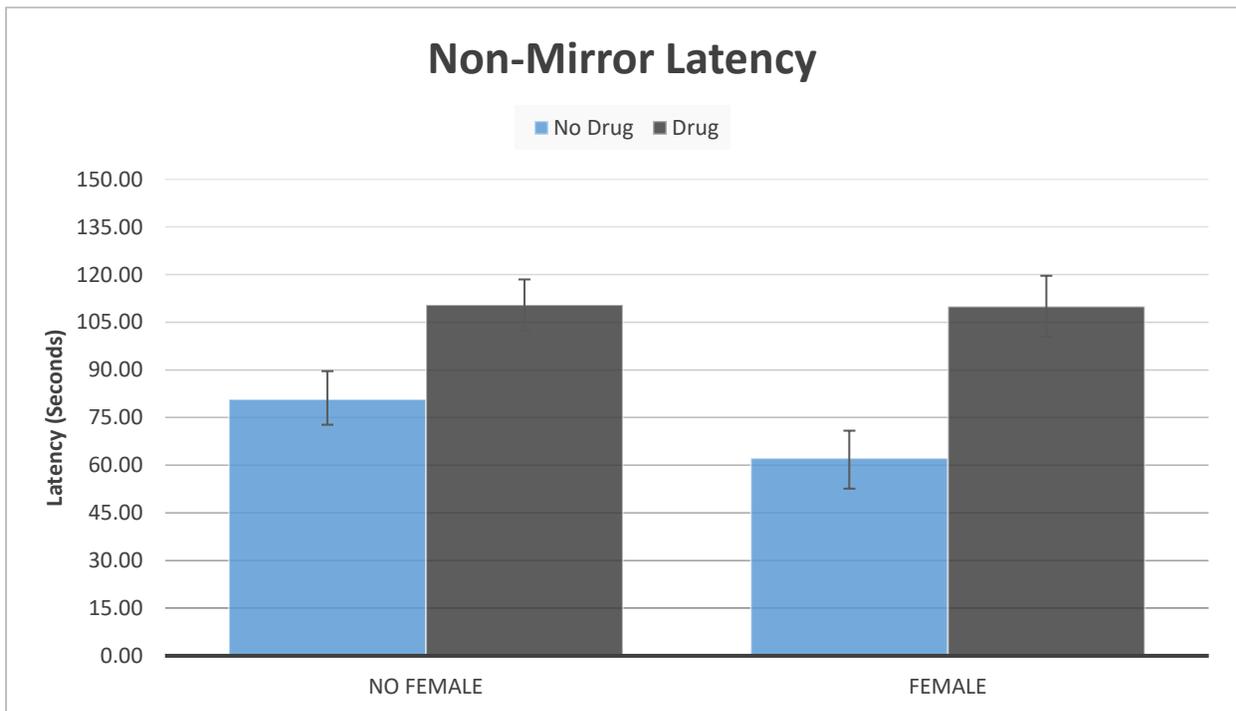


Figure 3. Group descriptive statistics for the non-mirror raw latency data averaged across the last 5 days of testing.

A 2 (Female groups) x 2 (Drug groups) between subjects ANOVA conducted on the fish preference for the mirror vs non-mirror conditions revealed a non-significant main effect for drug group ($F(1,56)= 2.810$, $p= 0.099$, $\omega^2= 0.028$), a non-significant main effect for female group ($F(1,56)= 2.946$, $p=0.092$, $\omega^2= 0.030$), and a non-significant Drug X Female interaction ($F(1,56)= 1.163$, $p=0.285$, $\omega^2= 0.003$). See Table 3 and Figure 4 for descriptive statistics.

Table 3. Group descriptive statistics for the preference data averaged across the last 5 days of testing.

		No Female	Female
No Drug	Mean	0.45	0.52
	SD	0.09	0.14
	N	15	15
Drug	Mean	0.52	0.54
	SD	0.09	0.09
	N	15	15

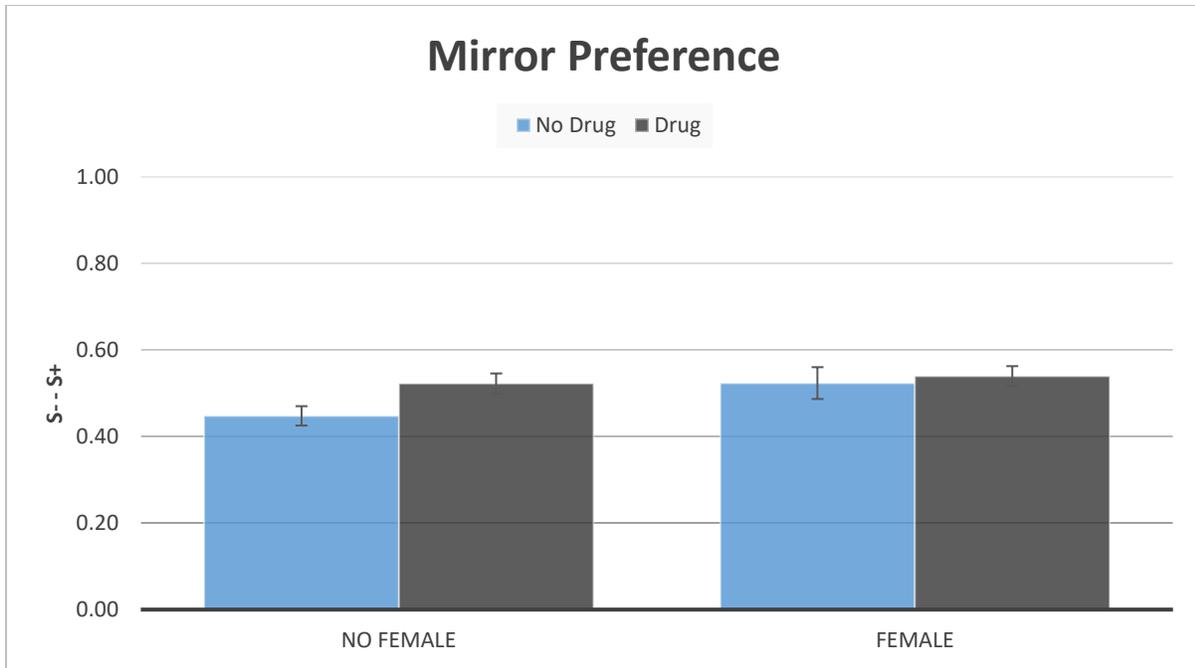


Figure 4. Group descriptive statistics for the preference data averaged across the last 5 days of testing.

A 2 (Female groups) x 2 (Drug groups) between subjects ANOVA conducted on the fight behavior revealed a significant main effect for drug group ($F(1,56)= 7.793, p= 0.007, \omega^2= 0.091$), a significant main effect for female group ($F(1,56)= 6.397, p=0.014, \omega^2=0.072$), and a non-significant Drug X Female interaction ($F(1,56)= 3.303, p=0.075, \omega^2= 0.031$). See Table 4 and Figure 5 for descriptive statistics.

Table 4. Group descriptive statistics for the fight data averaged across the last 5 days of testing.

		No Female	Female
No Drug	Mean	0.18	0.49
	SD	0.30	0.33
	N	15	15
Drug	Mean	0.11	0.16
	SD	0.24	0.22
	N	15	15

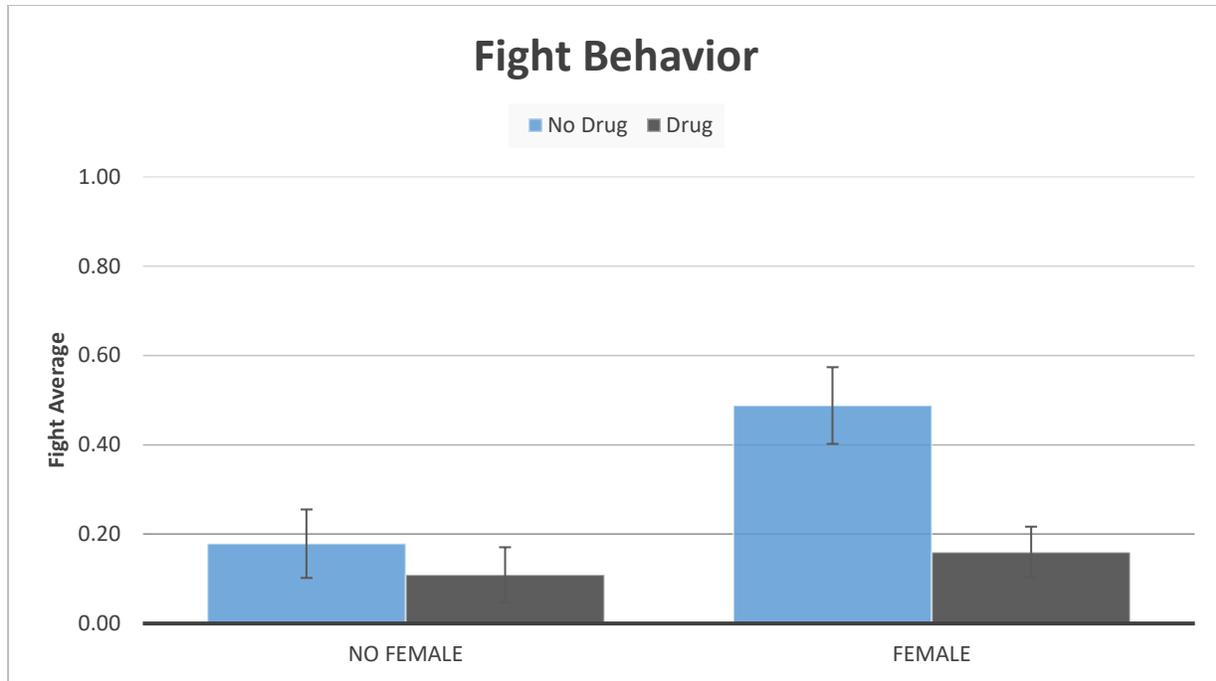


Figure 5. Group descriptive statistics for the fight data averaged across the last 5 days of testing.

Discussion

Of the three hypotheses proposed only one was supported. Specifically, subjects in the fluoxetine groups exhibited less aggressive behavior toward the mirror compared to the non-fluoxetine groups (hypothesis 3). Hypothesis 1 and 2 were not supported. Specifically, subjects in the female primed group did not have lower latencies to enter the goal box with the mirror compared to the non-primed group and subjects in the fluoxetine groups did not have higher latencies to enter the goal box with the mirror compared to the non-fluoxetine group.

The failure to support Hypothesis two is inconsistent with previous research (Balzarini et al., 2014; Verbeek et al., 2007; Eisenreich, Greene, and Szalda-Petree, 2017). Eisenreich, Greene, and Szalda-Petree (2017) showed a significantly shorter latency to enter the goal box for the mirror for the non-fluoxetine group ($\bar{x} < 15$ s) when compared to the fluoxetine group ($\bar{x} < 120$ s). In the present study, the failure to show a shorter latency to enter the goal box for the mirror

condition in the no fluoxetine group (\bar{x} =103.36 s) compared to the fluoxetine group (\bar{x} =104.91 s) suggests the mirror condition was not excitatory/reinforcing. However, while not a significant difference, the female primed groups did show a shorter latency to enter the mirror condition goal box for the no fluoxetine group (\bar{x} =65.20 s) compared to the fluoxetine group (\bar{x} =104.45 s) which suggests the mirror condition was excitatory/reinforcing.

Because the No Female/No Fluoxetine group failed to replicate previous research interpretation of the present results is difficult. However, even if the No Female/No Fluoxetine group had performed as expected the latencies for the fluoxetine groups were higher than expected. Balzarini et al. (2014) used a mirror and found that their fish elicited aggressive responding when exposed to the mirror. The fish typically exhibit a fixed action pattern of aggression upon seeing the mirror, and this aggressive behavior is considered reinforcing for the fish (Balzarini et al., 2014). Despite the positive result found by Balzarini et al., not all researchers advocate for the use of mirrors as the reinforcer for the fish. Previous work by Verbeek et al., (2007) has shown that betta fish respond differently to mirror stimuli and live conspecifics potentially due to the phase locked nature of the mirror stimulus. Therefore, it is possible that the mirror is not the correct choice for reinforcement in this context. Further, there was no significant difference when comparing mirror vs non-mirror preference for any of the groups, thus the mirror as the manipulation did not provide motivation to significantly decrease latency when paired against a non-mirror condition.

The failure to support Hypothesis one is inconsistent with previous research (Dzieweczynski et al., 2005; Sneksler et al., 2006; Eisenreich, Greene, and Szalda-Petree, 2017). While consistent with the increased aggression shown during male/male interactions in Dzieweczynski et al. (2005) and Sneksler et al. (2006), the latency data is higher than that found

in Eisenreich, Greene, and Szalda-Petree (2017). The latency for the no fluoxetine group ($x=103.36$) compared to the female group ($x=65.20$) is higher, but not enough to be significant. However, both the fluoxetine groups have similar means ($x=104.91$ and $x=104.65$), suggesting the prime and mirror were not reinforcing for these groups.

Though it is difficult to interpret the results due to the higher latencies found for the No Fluoxetine/No Female group when compared to Eisenreich, Greene, and Szalda-Petree (2017), other factors could have contributed to the inconsistent results. It is possible that the duration of the female exposure was too extensive and produced an attenuation in mirror excitation/reinforcement. The aggressive nature of the male *Betta splendens* is tied to the mating and territorial responses of the species (Dzieweczynski et al., 2005). Males in the species are responsible for obtaining a territory and raising the young, while the female's only contribution is the eggs during mating (Dzieweczynski et al., 2005). In the present study, males were primed with a female based on the premise that the presence of a female would create a more natural context for the aggressive behavior. The females were selected to provide added motivation to the male to encourage aggressive responding in the presence of a rival and obtain a territory to attract a mate. Females were presented at the beginning of each trial, so it is possible that the males were overexposed to the female, taking their focus off the need to chase away a potential rival and splitting it between fighting and attracting the mate, like the results of Snekser et al. (2006). Snekser et al. (2006) found that males given the option of fighting a male and courting with a female at the same time split their time between courting and fighting the rival, instead of focusing on one or the other. Therefore, it is possible that since the males in this study were exposed to the female before each trial instead of once at the beginning before trials started that

the males' motivation was split between the mate and potential rival instead of just chasing away the rival.

Eisenreich, Greene, and Szalda-Petree (2017) found a significant effect of latency when exposing the fish to fluoxetine, which was not replicated in this study. One potential explanation for this is that the males were overexposed to the female, as Eisenreich, Greene, and Szalda-Petree (2017) did not use female fish as a manipulation. In addition to overexposure to the female fish, the males were exposed to only a mirror, not other live fish., while Eisenreich, Greene, and Szalda-Petree (2017) used both mirrors and live fish. The use of only mirrors helped control for size and color differences, but provided the same condition for the fish each time which could lead to habituation.

Habituation of the fish to their mirror reflection could cause a steady decrease in aggressive responding over time. Based on the results of Dzieweczynski et al. (2012) this would maximize a male's chances of choosing to aggress against an unfamiliar opponent, thus saving energy by responding less to familiar fish. Dzieweczynski et al. (2012) studied the dear enemy effect, and concluded that males are less aggressive to familiar fish than unfamiliar fish. Since the males were shown a mirror presentation each day they would become familiar with their mirror image and could become less aggressive, as suggested by the dear enemy effect. This would also shift the fish's behavior between the competing responses of courting and fighting more toward courting. Finally, Matos et al. (2003) found that males who were preexposed to an audience lost more aggressive encounters than those who were not preexposed. The males therefore lost motivation over time, leading to the higher loss rate in the aggressive encounters. This could suggest that by preexposing the males beforehand to the females, the males had less motivation to dedicate to the aggressive encounter itself, causing the motivation to be split

between the two potential actions and not leaving enough to properly engage in an aggressive encounter.

Both Dzieweczynski and Hebert (2012) and Eisenreich and Szalda-Petree (2015) have found significant decreases in aggressive displays in betta fish when fish are exposed to the SSRI fluoxetine. In these studies, the fluoxetine exposed males' aggressive behavior toward a mirror and live conspecifics was severely dampened when compared to other male fish not exposed to the fluoxetine. This study did not show a significant difference in the latency to aggress toward the mirror when comparing fish exposed to fluoxetine with the fish not exposed to fluoxetine. Also, the fish exposed to fluoxetine showed no significant difference in preference for the mirror or non-mirror conditions.

This could be due to the motor impact the SSRI fluoxetine has on the fish (Eisenreich and Szalda-Petree, 2015). Eisenreich and Szalda-Petree, 2015 found significantly less motor movement when comparing the fish on fluoxetine to the fish not exposed to fluoxetine. They utilized a grid system and counted the amount of grid crossings for each fish in a two-minute period. When looking at the data collected in this study there was no significant difference between the means for the two fluoxetine groups, however, there was also a time limit on each trial. Each fish was given 150 seconds to complete each trial, and the means for both groups average over 100 seconds. Overall there is only about a 5 second difference between the mirror and non-mirror trials for both groups exposed to the drug. However, since both groups already have high means that does not leave much time for improvement. Also, there is an effect for the motor sedation in the data for the non-mirror latency. There was a significant effect for the latency of the non-mirror with the fish not exposed to fluoxetine having a faster latency than the

fish exposed to fluoxetine. Therefore, a drug effect was present for the non-mirror latency showing a significantly higher latency for the drugged fish.

Dzieweczynski and Hebert (2012) and Eisenreich and Szalda-Petree (2015) also argue for a decreased arousal hypothesis, with both studies finding evidence to support this claim. In this study the males' arousal levels were tested in several ways: latency for both mirror and non-mirror trails, preference for mirror and non-mirror, and fighting behavior exhibited when exposed to the mirror. The males in the fluoxetine condition were predicted to score lower on each of these measures, and while not significant for the latency measures, the data did reveal significantly lower fighting behavior for the fish in the fluoxetine groups. This would show a decrease in arousal levels when comparing the fluoxetine fish to the fish in the non-fluoxetine groups.

Therefore, Hypothesis 3: subjects in the fluoxetine group will exhibit less aggressive behavior toward the mirror compared to the non-fluoxetine group, was supported by the data. The data collected was binary data, with 0 coding no fight behavior present, and 1 coding fight behavior present. If the fish showed any signs of aggression while exposed to the mirror the fish scored a 1, while the opposite is true for a score of 0. Fish in the fluoxetine groups showed significantly less fight behavior when exposed to the mirror as predicted. A drug effect was clearly present in the fight behavioral data, and shows support for both the motor and arousal hypotheses (Dzieweczynski and Hebert, 2012; Eisenreich and Szalda-Petree, 2015). The lack of aggressive behavior exhibited toward the mirror could be due to decreased levels of arousal and desire to fight, along with an overall decrease in motor activity akin to what was observed for the latency to enter the goal box in the drug groups. Both could be responsible for the profound

decrease in aggression seen in the groups exposed to fluoxetine, with this study presenting an argument for both hypotheses.

The data yielded additional findings not hypothesized before collection, one being that males in the no drug condition were significantly faster when swimming down to the goal box for the non-mirror than the drug condition males. Betta fish are naturally aggressive, and the presence of another male is enough to cause the males to exhibit a modal action pattern (Goldstein, 1975; Abate, 2005). This modal action pattern is also often used to reinforce the fish, as it provides a natural form of reinforcement that is easy for researchers to attain and present (Balzarini et al., 2014). While fish in this study showed no significant preference for the mirror or non-mirror condition, when comparing the non-mirror condition between groups there is a significant difference with the males in the no drug condition showing lower latencies than the fish in the drug condition. Therefore, the mirror was not as reinforcing as predicted based on the lack of significant preference data, and the lower latencies found for the fish in the no drug groups. The fight data still clearly shows a difference for the drug with the fish in the no drug groups having significantly more fighting behavior present, but the latency to be aggressive does not hold a significant difference like it does for the non-mirror condition.

There is a potential outside factor that has been considered when looking at the latency for both the mirror and non-mirror condition, which is a lighting issue that became prominent toward the end of data collection. The light in the room containing the two groups not exposed to the female fish did experience some issues, notably flickering, which may have an impact on the results. Fish are sensitive to light cycles, and for this experiment were maintained on a 12 hr dark/light cycle. However, the flickering light could have caused an issue with the fish's production of melatonin, related to serotonin which is affected by fluoxetine (Kulczykowska et

al., 2010). Increases or decreases of this neurotransmitter can affect a fish's natural behavior, which is why this drug was selected (Kulczykowska et al., 2010). However, the neurotransmitter can be manipulated naturally with light cycles, making the flickering light a problematic complication, particularly for the control group (Kulczykowska et al., 2010). No definite conclusions can be drawn about the potential effects of the light, and as such it is just considered a potential factor impacting the fish housed in this specific room.

The other unexpected finding was that the males primed with a female displayed significantly more aggressive behavior than males not primed with a female. The males were primed before each trial for 30 seconds, and then once they entered the goal box they were given the mirror or non-mirror for 30 seconds. Therefore, the males spent the same amount of time with the females that they did with their rival opponent. The males that were primed with the female had higher aggressive scores (ranged from 0 to 1 based on scoring scale) than those not primed with the female. The female fish is an important drive to the male's choice to engage in aggressive behavior as the male needs to find and defend a territory to attract a mate (Dzieweczynski et al., 2005). The male performs all necessary acts to raise the babies, including protecting them from potential danger and other males, while the females only contribution is to lay the eggs (Dzieweczynski et al., 2005). Males viewed a female before each trial to provide the motivation for the male to choose to act aggressively and chase away a potential rival. The males in this group did show significantly more accounts of aggressive behavior than the males in the group that did not receive the prime. This shows an effect of the female, and provides support for Dzieweczynski et al. (2005) hypothesis that the female is an important contextual factor in male fish aggression. There were 3 females per group with one loss, leaving 8 females overall, and the

females were rotated each day so the male only saw each individual female every 3 days to balance out individual courting differences that could invoke bias.

Female betta fish are an important component when studying the male betta fish aggressive responding behavior. Male fish have a complex mating and fighting ritual that consists of very similar behaviors: biting, lateral displays, tail beats, and gill flaring (Dzieweczynski et al., 2005). Therefore, the male can both court and fight at the same time, by directing his behaviors between a mate and a rival. The presence of a female can affect the level of aggression the male displays, and so her presence can have great impacts on the nature of an aggressive encounter (Dzieweczynski et al., 2005). As seen with the data collected in this study the females had a profound effect on the choice each male made to be aggressive, with more males choosing to be aggressive in the groups primed by the females.

This study does not have specific courting and fighting data, which would be broken down into the 4 components of courting and fighting: biting, lateral displays, tail beats, and gill flaring. This is due in part to the high volume of subjects in the study and the amount of trials each fish performed daily. Also, a working system for recording the fish during the trials was not developed, with some fish taking an hour each day to complete all 10 trials. Therefore, the data was coded during data collection using the 1 and 0 system instead of using specific behavioral counts or times. In the future, a study utilizing less fish would be beneficial for a study using video recording to compare these 4 separate behaviors. Based on the data presented by Eisenreich and Szalda-Petree (2015) a motor effect was detected, but no data for the difference in the 4 courting and fighting behaviors were collected. Having data comparing these behaviors would be beneficial in looking at exactly what motor movements are being affected by the drug. Future studies may need to design a new testing apparatus that is more video recording friendly,

as the t-mazes smaller but narrow size can make it difficult to zoom in when recording the small fish. Also, a program capable of recording such large data files on the tablet computers used for data collection has not been developed, particularly a program that can record in the background while the actual program used for data collection is running. Therefore, a new recording program or software will have to be developed along with the potential changes in equipment.

Another potential change in future studies regarding the female would be to decrease the amount of priming each male receives, or change the equipment to make the female more involved in the overall design. Currently, the males were primed with the female before each trial, and while each male was exposed to the females on a rotating schedule each male saw each female approximately 6 times. Dzielwczynski et al. (2012) found evidence that males habituate to each other, and show a decrease in aggressive behavior when fighting a familiar male as opposed to an unfamiliar male. It is unknown if the males habituate to the females like they do to opponent males. As was stated previously this dear enemy effect could have caused habituation to the fish's mirror reflection, and the males only saw their reflection for 5 trials as opposed to the 10 daily trials that they were exposed to the female. As such it is possible that the males were overexposed to the female and would benefit from less exposures, such as 1 exposure before the 10 trials began. Alternatively, if the design of the experiment was changed to include the female as more than a prime before trials, such as showing the male subject a female and mirror at the same time, then the amount of female presentation could potentially remain the same. In this way, the female is directly motivating the male to fight the mirror in the moment, instead of before the trial begins. It also means the female can see how the male reacts to the mirror, which was not utilized in this study as the female was serving only to motivate the male through

priming before the trial. This change may motivate males who were not consistent fighters to be more consistent since an audience, the female, is present (Matos et al., 2003).

Finally, the SSRI fluoxetine showed a significant impact on the fish's choice to respond aggressively to the mirror stimulus. The fish exposed to fluoxetine were given a consistent dose daily for a 30-minute period, so the dosing was consistent across fish. While researchers like Eisenreich and Szalda-Petree (2015) have also been known to use the same procedure as this study to expose the fish to the drug, other studies such as Dzieweczynski and Hebert (2012) have placed the drug directly into the fish's home tank and estimated the amount of drug concentration based on water changes and evaporation. However, each method has yielded similar results and behavioral effects. The fluoxetine in these studies, regardless of the administration method, has impacted the aggressive behavior of the male, decreasing fish arousal and impacting the fish's motor abilities. This can be seen in the fight data collected here. The fish had significantly lower fight scores than the fish not exposed to the drug, and while there is no data on the impact on specific behaviors the data does support an overall effect on aggressive choice. Overall the study yielded both a drug main effect and a female main effect on the choice to engage in aggressive behavior, but not a Drug X Female interaction effect. Therefore, this provides extra support for the motor sedation hypothesis due to the additional impact of the female prime, which was designed to increase the arousal of the male fish and encourage the choice to engage in fighting behavior.

However, it is unknown exactly how much of the drug was directly absorbed by the male during the dosing period. There is no way of measuring the exact dosage each fish's body uptakes. The most likely way to check the individual dosage of each fish would involve taking the entire body of the subject and liquifying it, following with a test to check the drug's dosage

concentration. Eisenreich and Szalda-Petree (2015) and Dzieweczynski and Hebert (2012) also do not have data to determine the exact amount of absorption for each individual subject. If the equipment is obtainable it would be beneficial to check the overall amount of drug found in each individual subject. This could then be compared to the subject's data to see if higher amounts of the drug cause more motor sedation and decreases arousal levels when exposed to both a female and a rival. This would provide further insights into the drug's effects, and how it accumulates in the fish's system overtime. The fish may all have a similar absorption rate, but if the fish were found to have a bimodal distribution of drug absorption we could compare fish based on high vs low absorption. This would be helpful in comparing fish latency and preference data using the unsupported hypotheses in this study.

Overall this study yielded interesting results about the choice to aggress in betta fish, and how that choice can be manipulated using female priming and the SSRI fluoxetine. Improvements were suggested for future studies to try and improve testing methods, such as decreasing the amount of exposure to the female fish, and modifying recording equipment to better capture the fish's individual behaviors. Also, the current drugging method ensures that each individual fish receives the same amount of drug for a set period each day, but there is no way of measuring how well each individual fish is absorbing the drug. This measure would greatly improve current knowledge of the drug's effects on behavior by comparing fish with low and high absorption rates, or show us the variability in the species if the absorption rate is similar between subjects. Finally, this study provides evidence for the motor sedation hypothesis for the drug with the use of the female to increase the male's arousal levels even when exposed to the drug.

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