Prozac Alters Reproductive Performance and Filial Cannibalism in Male Fighting Fish, *Betta Splendens*

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ABSTRACT

Background: Fluoxetine (Prozac^{TM}) is one of the most popular antidepressant that can be released to aquatic systems via sewage-treatment effluents. It is suspected to provoke substantial effects in the aquatic environment.

Methods: In spawning tanks, specimens were exposed to concentrations of 0 and 0.54 $\mu g l^{-1}$ fluoxetine from male introduction until the larvae had hatched. Prior to spawning, nest area and time spent for nest building were measured. Also, spawning duration, number of copulations per spawning and eggs per copulation, total produced eggs and hatching rate were recorded.

Results: The number of copulations, eggs per copulation and total produced eggs did not differ between the two treatments. Fluoxetine treatment significantly decreased the nest size, time spent for nest building and spawning duration. Also hatching rate was significantly lower during fluoxetine treatment than in the control condition. Notably, five fluoxetine treated males cannibalized their eggs and larvae.

Conclusion: We showed that environmental exposure of fighting fish to fluoxetine potentially alters specific aspects of nest building and sexual behavior and, as a consequence, reproductive output.

Keywords: Animal Sexual Behavior, Fighting Fish, Filial Cannibalism, Fluoxetine, Reproduction.

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INTRODUCTION

Adaptive physical and physiological conditions are among the major factors influencing successful reproduction [1]. Therefore, any potential external factor that may affect physiological parameters can interfere with the reproductive process by changing systemic hormone levels and responsiveness. Fluoxetine (Prozac^{TM}) is one of the most popular antidepressant drugs from the class of pharmaceuticals known as Selective Serotonin Reuptake Inhibitors [2]. In comparison to other treatment options for depression, fluoxetine is widely prescribed for patients with depressive disorders. Some of the consumed drug is released via urine in the form of the original compound or its biologically active metabolites [3]. Some studies have examined the destructive effects of fluoxetine on fish reproduction [2, 4]. These studies suggested that fluoxetine can potentially disrupt reproduction in fish. However it must be noted that although endocrine disrupting chemicals like fluoxetine may have an impact on whole body systems [5], small behavior changes can be important and even alterations in some components of behavioral sequences may be informative about the disruptive effects.

Fighting fish (*Betta splendens*) is a model species for behavioral and physiological research due to their specific display of aggressive behavior during reproduction [6]. The effects of different chemical compounds on fighting fish behavior have also been studied [7- 10]. In these studies fluoxetine had suppressive effects on aggression and courtship behavior in male fighting fish; however it is unknown whether fluoxetine interferes with male sexual motivation or with male brood care behavior. So far, behaviors such as the number and the duration of spawning bouts have not been studied in fighting fish; to investigate the effects of fluoxetine on these behaviors, we measured mating and nesting behaviors of male fighting fish that were either exposed or not exposed to fluoxetine.

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MATERIALS AND METHODS

Fish

Forty mature male *B. splendens* were purchased from a local distributor. The fish were transported to the laboratory and kept individually in 1 L opaque containers two weeks before the experiments. The mean weight of fish was 1.64 ± 0.46 g. The water temperature was 26 ± 1 ℃ and the photoperiod was set at 12 h light, 12 h dark. In addition, thirty adult females were purchased from another distributor. They were kept as groups of 15 fish in two 20L tanks under the same conditions as the male containers. The fish were fed two times daily with 0.9 mm commercial pellet and frozen blood worms.

Experimental Treatments

From the fish stock, 14 males, which had bubblenests, were selected for future spawnings. They were exposed to concentrations of 0 μgl^{-1} and 0.54 μgl^{-1} fluoxetine in spawning test tanks. For testing behavioral changes within-individuals, each male was exposed to two treatments, we first tested the 14 fish in 0 μgl^{-1} fluoxetine (control treatment) for 6 days long and then the same fish were exposed to 0.54 μgl^{-1} fluoxetine for another six day. Time between the two tests was one week. On day one we added 12.15 µg fluoxetine (1.22 mg fluoxetine was added to 10 ml distilled water, then 100 µl of it added to tanks) to the 36 lit tanks with 22.5 lit water (water depth was 20 cm) for a final concentration of 0.54 ugl^{-1}. This concentration previously showed significant effect on fighting fish behaviors [9].

Spawning Tanks and Spawning Activity

Seven tanks were considered for spawning and reproduction data collection. A piece of 8 × 8 cm styrofoam was placed on the water surface close to one end of the tank for building a bubblenest. To induce spawning activity, a female ready for spawning was inserted into the tank one day after male introduction. After spawning, the female was removed from the tank.

Bubblenest Habitat

On the morning of the third day after introduction of male just prior to the spawning time, nest area and time spent for nest building were noted. Nest size was measured by vernier callipers (Jaroensutasinee and Jaroensutasinee, 2001). To measure time spent for nest building we recorded behavior using a Nikon Coolpix P6000 video recorder for two independent bouts of 10 minutes for each male in the two treatments.

Reproductive Output

Because of egg mustering behavior in fighting fish, the calculation of direct fecundity was impossible. Therefore, we noted the number of copulations during each spawning activity and the number of eggs laid by the female in the copulation. Finally, proximate fecundity in each spawning was evaluated as, number of eggs per copulation × number of copulations. We used term “copulation” to explain process where the male wrapped himself around the female, so that his vent is in close proximity to hers. Also, number of released eggs in this process, considered as the number of eggs per copulation. Duration of spawning was recorded for both treatments as an indicator of intensity of male spawning activity. Finally, mean hatching rates were measured by counting the number of larvae that hatched per nest as a proportion (%) of eggs in the nest.

Statistics

Data were tested for normality using Kolmogorov-Smirnov test. All data were normally distributed. Then, behaviors were compared between control condition and fluoxetine treatment with paired-t tests using SPSS, version 19.

RESULTS

Reproductive Output

Total egg production by the female of each pair was not changed due to fluoxetine treatment ($t= -0.193$, $p= 0.850$; Figure 1A). However, hatching rate was significantly lower after fluoxetine exposure as compared to the controls ($t= 5.395$, $p< 0.0001$; Figure 2). Notably five males in the fluoxetine treatment cannibalized their own eggs; so we excluded them from this test. Fluoxetine exposure did not affect the mean number of copulations per spawning activity ($t= -1.464$, $p= 0.167$; Figure 1C) and the average number of eggs per copulation ($t= 1.916$, $p=$

0.078; Figure 1D); however, the average spawning duration was higher in fluoxetine treated than in control pairs (t= -2.350, p= 0.035; Figure 1B).

Nest Building Behavior

Eleven of the 14 nests of fluoxetine exposed males were limited to the artificial nest building substrate. Prior to spawning, the bubblenest from males in the control treatment were significantly bigger than from fluoxetine exposed males (with ranges of 34.06 to 257.48 cm^2 and 32 to 64 cm^2, respectively; t = 2.722, p = 017; Figure 1E). On the morning of the third day of exposure, time spent for nest building was significantly higher during the control treatment than during fluoxetine treatment (t= 5.663, p= 0.000; Figure 1F).

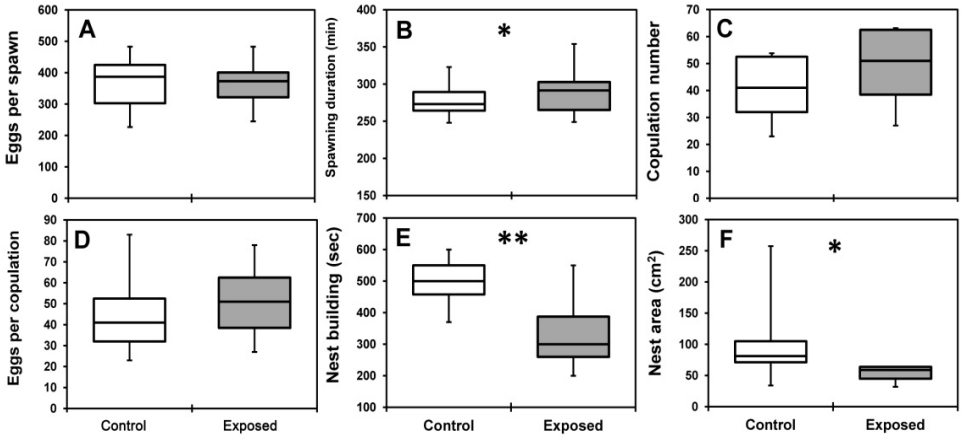

Figure 1. Median ± quartiles, ranges of (A) the number of eggs per spawning activity, (B) duration of spawning, (C) number of copulations in each spawning, (D) number of eggs per copulation, (E) time spent for building the nest, and (F) bubblenest area (cm^2) of fighting fish (*Betta splendens*) experimental pairs exposed to concentrations of 0 ('Control') and 0.54 μgl^{-1} fluoxetine ('Exposed'). * p< 0.05; ** p< 0.01.

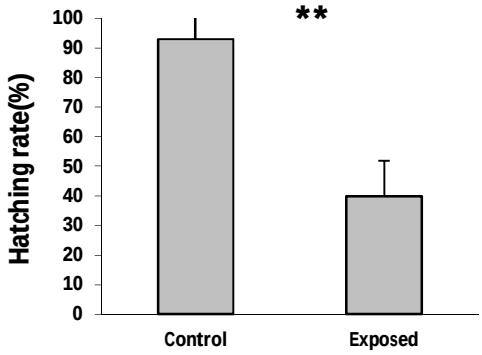

Figure 2. Mean (±SD) of the hatching rate of fighting fish (*Betta splendens*) experimental pairs exposed to concentrations of 0 ('Control') and 0.54 μgl^{-1} fluoxetine ('Exposed'). ** p< 0.01.

DISCUSSION

Waterborne fluoxetine affected male reproductive performance and may even led to filial cannibalism in *B. splendens*. Fluoxetine also disrupt the reproductive axis of female fish [11,12]; however, the focus of this study was on male reproductive behavior and thus, we did not expect that the number of eggs produced by female fighting fish would be affected after only one day exposure to low concentration of 0.54 μgl^{-1} fluoxetine. With respect to effects on female reproductive output, our results are in accordance with previously reported studies. For example, Lister et al. [4] showed that the effect of fluoxetine on zebrafish (*Danio rerio*) was not significant at low concentrations (0.32 and 3.2 μgl^{-1}). Body size is one of the main factors affecting fecundity in female fish [13]; nevertheless, the effect of body size of females may be excluded as in our study females were of similar sizes.

Pairs which were exposed to fluoxetine treatment took longer for spawning than control fish. Based on our observations, males in the fluoxetine group did not have bubblenest of optimal consistence and size to store eggs at the time of spawning. As a consequence, during

copulations some eggs fell down to the bottom of the tank and the male had to invest time for collecting the eggs and return them to the bubblenest. The reduced ability of males exposed to fluoxetine to construct their nest may have been related to the side effects of fluoxetine such as decreased total locomotion [8], plasma androgens levels and/or male sexual motivation [2].

The lower number of eggs produced per copulation during fluoxetine treatment may demonstrate a reduced 'libido' in the fluoxetine exposed males because first, they did not build suitable nests even with a female present; second, courtship displays were changed [9]. In the male goldfish, *Carassius auratus*, fluoxetine exposure significantly decreased milt release and circulating testosterone concentration [2].

Hatching rate was significantly different in the two groups. Fluoxetine with direct action on embryo development may cause abnormalities [14]. When fish embryos show some degree of abnormality or even mortality, their father counters with cannibalism which results in a decrease in offspring reproductive value following reduction in brood size [15]. This may explain the filial cannibalism we observed in fluoxetine treated males but not in control fish. Consumption of fluoxetine exposed abnormal eggs reduced the remaining number of eggs with a capacity for hatching in the fluoxetine group. Also in other fish species, such as *Abudefduf sexfasciatus* [16], experimental brood reduction leads to increased cannibalism. Another main argument for filial cannibalism in fish is insufficient parent energy [15]. It is worth noting that the latest ('younger') eggs of a clutch have higher nutritional value [15]; therefore, this may be an additional explanation for the total filial cannibalism we observed in five fluoxetine exposed males. Typically, male fighting fish do not cannibalise their eggs and larvae [17]. Thus, it can be deduced that fluoxetine has encouraged male to eat his eggs and larvae.

CONCLUSION

It is concluded that fluoxetine both had direct effects on egg mortality and hatching rate, and indirect effects on reproductive output by potentially inducing egg cannibalism. Taken together, fluoxetine in aquatic environments may significantly affect the reproductive performance of male fighting fish. Our results underline the importance of fluoxetine as an endocrine disrupting substance particularly for aquatic organisms.

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