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Original Article

Fluoxetine alters reproductive performance of female fighting fish, *Betta splendens*

Mohammad Navid Forsatkar*¹, Tahmine Latifi², Soheil Eagderi¹

¹Department of Fisheries, Faculty of Natural Resources, the University of Tehran, Karaj, Iran.

²Department of Fisheries, Faculty of Natural Resources, University of Zabol, Zabol, Iran.

Abstract: This study was aimed to investigate the effects of waterborne fluoxetine on the reproduction performance of female fighting fish (*Betta splendens*). For this purpose, mature, ready for spawning females were exposed to concentrations of 0, 0.54 and 54.0 µg/l fluoxetine for 7 days. Then they were introduced into the spawning tank containing pre-acclimated male and reproductive consequences including number of copulations per spawning, number of eggs per copulation, duration of spawning, fecundity and hatching rate were assessed. Fluoxetine concentration of 54.0 µg/l, was significantly affected on the number of produced eggs per copulation, fecundity and hatching rate. In addition, the mean number of copulations per spawning was not different between treatments but significantly different for the spawning duration between control and 54.0 µg/l treatments. The results suggest that fluoxetine can impacts on reproductive performance of female fighting fish at concentrations greater than those found in the aquatic environments.

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Introduction

Selective Serotonin Reuptake Inhibitors (SSRIs) are common treatment for alleviating depressive disorders (Stroud, 2012). SSRIs block the serotonin transporter from taking-up serotonin released by pre-synaptic serotonergic neurons from synaptic junctions within the brain. This action results increasing serotonin concentrations within the synapse (Kwon and Armbrust, 2006). Fluoxetine is one of the most prescribed SSRI antidepressant that metabolize in liver (Mennigen et al., 2010). Following human excretion, fluoxetine enter into the aquatic environments through sewage (Kwon and Armbrust, 2006). Potentially, it has an ability to accumulate in body which brain and liver are the main sites for its accumulation in non-target organism including fish (Brooks et al., 2005). Fluoxetine has been identified in the list of chemicals of concern, suggested to have the potential to impact wildlife populations (Weinberger II and Klaper,

2014).

Several works have indicated the negative effect of fluoxetine on fish reproduction. Mennigen et al. (2008) administered fluoxetine to female goldfish (*Carassius auratus*) and found that plasma estradiol levels and expression of estrogen receptors are reduced significantly after its injections. Fluoxetine significantly reduced cumulative egg production in Zebrafish (*Danio rerio*; Lister et al., 2009). Also, fluoxetine concentrations as low as 1 µg/l, that is found in many freshwater environments, was found effective on mating behaviour, specifically nest building and defending in male fathead minnow (*Pimephales promelas*; Weinberger II and Klaper, 2014). However, fluoxetine did not have a significant effect on reproductive properties of Japanese Medaka, *Oryzias latipes* (Foran et al., 2004). In addition, fluoxetine impairs the reproductive axis of male fish (Mennigen et al., 2010), that were previously reported in human and

* Corresponding author: Mohammad Navid Forsatkar
E-mail address: nforsatkar@ut.ac.ir

other mammals (e.g. Bataineh and Daradka, 2007; Safarinejad, 2008).

Fighting fish (*Betta splendens*), is a suitable model to study the impacts of pollutant on reproductive traits (e.g. Clotfelter and Rodriguez, 2006). Female fighting fish spawns under bubble nest constructed by male that cares brood in the bubble and exhibits some levels of aggression in contrast to any intruder (Jaroensutasinee and Jaroensutasinee, 2003). Fluoxetine in both administration forms of injection (Clotfelter et al., 2007) and exposure (Dzieweczynski and Hebert, 2012), reduces the aggressive behaviour of male fighting fish; however, there is no information about the effect of fluoxetine on female reproductive traits. Therefore in this study, female fighting fish exposed to sub-lethal waterborne fluoxetine and then its reproductive consequences including fecundity, hatching rate, the number of copulations with its mate, the number of released eggs per copulation and whole spawning duration were investigated.

Material and methods

Fish: Forty mature female *B. splendens* were obtained from a local pet store. They kept into two 20 L tanks filled with dechlorinated tap water for 14 days. Temperature and photoperiod set as $27 \pm 1^\circ\text{C}$ and 12L: 12D, respectively. Fish were fed twice a day with commercial pellet (Biomar, Germany) and frozen blood worms. In addition, 44 males were purchased from a local pet store for future spawning activities. They also fed twice a day with same food as females.

Experimental protocol: Thirty gravid females from the stock were selected and randomly distributed into three 20 L glass tank (ten fish per tank) exposing with concentrations of 0, 0.54 and 54.0 $\mu\text{g/l}$ fluoxetine for 7 days before spawning. The concentration of 0.54 $\mu\text{g/l}$ is considered as natural occurrence of fluoxetine (Fent et al., 2006); also we applied 54.0 $\mu\text{g/l}$ to examine the acute toxicity of fluoxetine.

For spawning activity, thirty 36 L glass tanks equipped to a float substrate for building bubble nest,

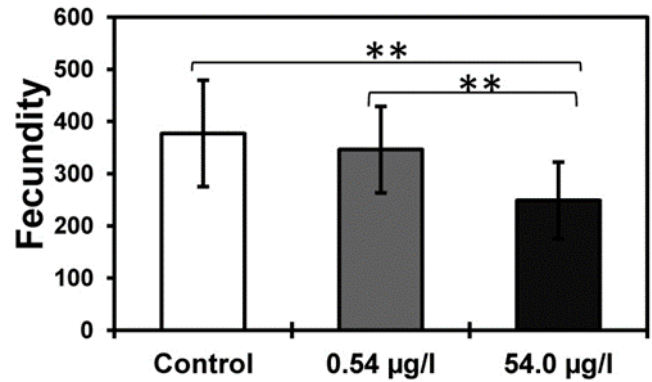


Figure 1. Effect of 7 days fluoxetine exposure on the fecundity of female *Betta splendens*. Data are means \pm SD (n = 10; ***P < 0.01).

a half flower pot for female hiding and a heater to kept temperature at 27°C were used. After nest building by male, each female was introduced into the spawning tank. They were spawned after about one day; however, because of egg mustering behaviour in fighting fish, direct fecundity calculation was impossible. Therefore, we calculated the number of copulations per each spawning activity and the number of laid eggs by female in copulations. Finally, the fecundity was estimated in each spawning as: number of eggs per copulation \times number of copulations. Duration of spawning was evaluated as an indicator of female readiness for spawning activity. Also, hatching rate was investigated to examine the quality of eggs (Schreck et al., 2001).

Statistics: All data were tested for normality and homogeneity of variances using Kolmogorov-Smirnov and the Levene test, respectively. Then, they were examined using a one-way ANOVA following Duncan's multiple range test ($P < 0.05$). All statistical analyses were performed using the statistical software of SPSS, version 19.

Results

There were no significant differences in the body length of females at the beginning of experiment ($F = 0.280$, $P = 0.758$). After 7 days exposure period, the fecundity of 54.0 $\mu\text{g/l}$ treatment was significantly different in compared to another exposed treatment ($F = 5.938$, $P = 0.007$; Fig. 1). However, the fecundity was lower in 0.54 $\mu\text{g/l}$ treatment (346.3 ± 82.89) than

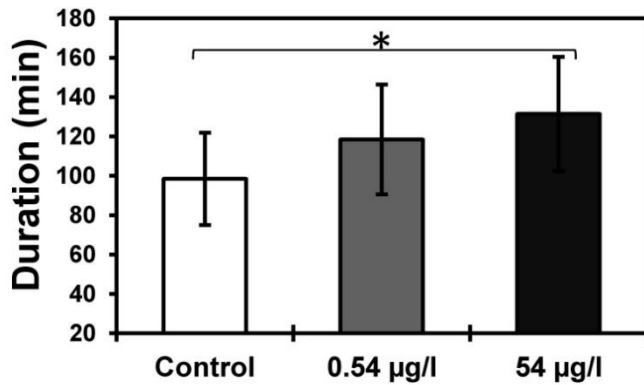


Figure 2. Spawning duration (\pm SD) of female *Betta splendens* after 7 days exposure to fluoxetine (n= 10; * P <0.05).

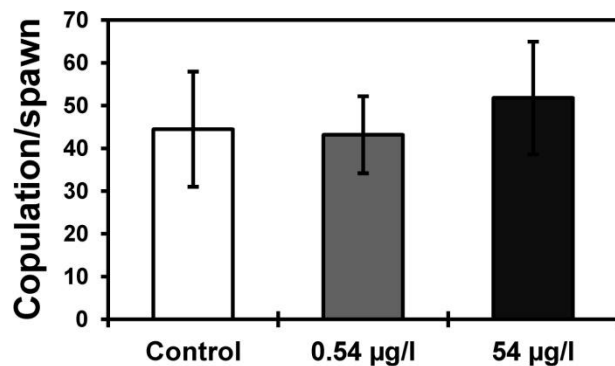


Figure 3. Means \pm SD of the number of copulations per spawning in female *Betta splendens* after 7 days exposure to fluoxetine (n= 10).

that of control one (377.2 ± 101.93), but not significantly different ($P= 0.434$).

The duration of spawning were significantly different between control treatment and females exposed to $54.0 \mu\text{g/l}$ fluoxetine ($F= 3.825$, $P= 0.034$; Fig. 2). This duration was recorded 98.50 ± 23.45 , 118.5 ± 27.89 , and 131.50 ± 28.96 min for control, $0.54 \mu\text{g/l}$ and $54.0 \mu\text{g/l}$ treatments, respectively.

Figures 3 and 4 show the number of copulations per spawning and average number of eggs per copulation, respectively. After 7 days exposure to fluoxetine, there was insignificant differences of the mean number of copulations per spawning between treatments ($F= 1.478$, $P= 0.246$), this index was higher (51.8 ± 13.17) in $54.0 \mu\text{g/l}$ treatment than two others (44.5 ± 13.46 , and 43.2 ± 9.01 for control and $0.54 \mu\text{g/l}$ treatments, respectively).

The mean number of eggs per copulation was significantly lower in $54.0 \mu\text{g/l}$ treatment than that of control and $0.54 \mu\text{g/l}$ treatments ($F= 9.063$, $P=$

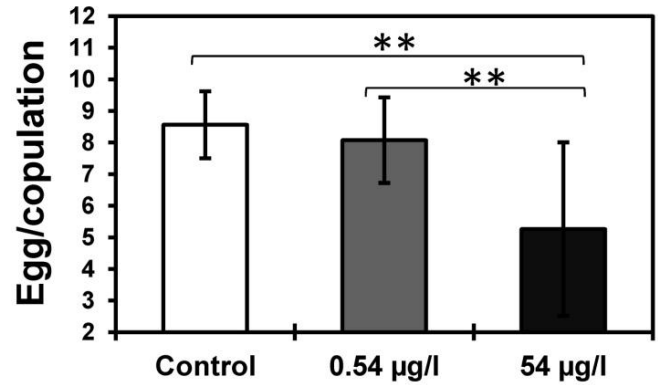


Figure 4. Average total amount of eggs laid in copulations of female *Betta splendens* after 7 days exposure to fluoxetine. Data are means \pm standard deviation (n= 10; ** P <0.01).

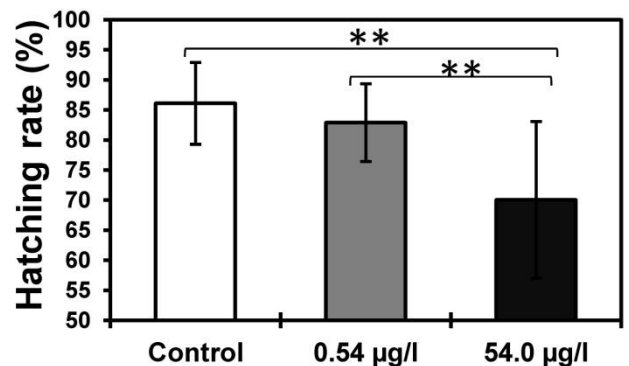


Figure 5. Means \pm SD of the hatching rate in female *Betta splendens* after 7 days exposure to fluoxetine (n = 10; ** P <0.01).

0.001). This ranged from 5.26 ± 2.74 in fish exposed to $54.0 \mu\text{g/l}$ treatment to 8.56 ± 1.05 in control one. The hatching rate was significantly different among treatments ($F= 8.404$, $P= 0.001$; Fig. 5). This was 70.05 ± 13.02 , 82.9 ± 6.45 and 86.11 ± 6.80 for $54.0 \mu\text{g/l}$, $0.54 \mu\text{g/l}$ and control treatments, respectively.

Discussion

Fluoxetine causes some disruption in different aspect of fish life (e.g. Mennigen et al., 2010, 2008; Clotfelter and Rodriguez, 2006). Based on the results, the fecundity of exposed females to $54.0 \mu\text{g/l}$ fluoxetine was significantly lower than that of control and $0.54 \mu\text{g/l}$ treatments. Females are responsible for the production of good quality eggs that are sufficient to guarantee future generations. Any disturbance to this task could alter offspring success and have population consequences. Lister et al. (2009) founded the significant reduction in cumulative egg production of Zebrafish after 7 days exposure to $32 \mu\text{g/l}$ fluoxetine. They showed that

fluoxetine decreases both ovarian estradiol concentration and expression of FSHr and LHR receptors. In the present study, we did not measure the plasma levels of sexual hormones and expression of reproductive genes; however, it is unquestionable that fluoxetine decreases ejaculatory response in human (Safarinejad, 2008), and milt release (Mennigen et al., 2010), and egg production (Lister et al., 2009) in fish. Complex processes are involved to produce mature gametes (Maruska and Fernald, 2011); therefore, fluoxetine may reduce the fecundity of female fighting fish due to change in circulatory concentrations of the sexual hormones and disruption of reproductive axis.

Despite of the lower fecundity of females exposed to 54.0 µg/l fluoxetine, they had more number of copulations in compared to control and 0.54 µg/l treatments, but not significantly different. The average number of released eggs per copulation in 54.0 µg/l treatment was significantly lower than that of others. In addition, the spent time by females for spawning in 54.0 µg/l treatment was significantly higher than that of control one. These results indicate that higher levels of fluoxetine is caused females to produce lower eggs in longer spawning duration. Based on results, the females of 54.0 µg/l treatment copulate with its mate discontinuously and many of their copulations are terminated without any egg production. Occurrence of secondary sexual traits in Mosquito fish, *Gambusia affinis* delayed after exposure to fluoxetine (Henry and Black, 2008). Also, there are reports showing indolence or less basal swimming (Kohlert et al., 2012) and aggressive behaviour (Dzieweczyński and Hebert, 2012) in male fighting fish after fluoxetine treatment. Therefore, many unsuccessful copulations resulting less eggs production even during longer spawning period could be due to demolition of reproductive axis that led to postponing secondary sexual traits and non-proper spawning behavior. These results suggest that acute concentration of fluoxetine causes reduction in libido and impotence of female fighting fish.

The hatching rate was decreased significantly after

exposure to 54.0 µg/l fluoxetine. Schreck et al. (2001) reported that low quality gametes lead to reduction of hatching rate. Also, maternal fluoxetine administration in mice showed side effect on her generation (Santos Gouvêa et al., 2008). However, fluoxetine exposure had insignificant effect on hatching of Japanese medaka (*Oryzias latipes*; Foran et al., 2004). This controversial results may be explained by different concentration of fluoxetine. The maximum fluoxetine level used by Foran et al. (2004) was 5 µg/l which approximately 11 folds lower than our maximum concentration. It is obvious that hatching success of fighting fish depends on environmental conditions such as temperature (Forsatkar and Nematollahi, 2013) and photoperiod (Giannecchini et al., 2012). However, these factors were similar in treatments; therefore decreased hatching rate may be a secondary effect following reduces of female condition.

In conclusion, this study demonstrated the exposure of sexually mature female fighting fish to acute concentration (54.0 µg/l) of fluoxetine is led to decrease of reproductive consequences in compared to 0 and 0.54 µg/l treatments. These findings suggest that fish can be affected by far below lethal doses of fluoxetine. Also, the observed results of this study indicate a dose dependent effect of fluoxetine on reproductive traits of female fighting fish. In addition, these results invite more attention to fate of pharmaceuticals in the aquatic ecosystems.

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