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Original Article

Effect of testosterone and fluoxetine on aggressive behaviors of fighting fish, *Betta splendens*

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Abstract: Effects of oral administration of testosterone and fluoxetine exposure on aggressive behavior of the fighting fish, *Betta splendens*, were investigated. Testosterone diluted in ethanol and sprayed on pre-weighted pellet to achieve concentrations of 0, 1, 2 and 4 mg/kg of hormone in food. Two main behaviors were recorded: the time in front of mirror and duration of the gill flaring using a mirror 8 and 15 days after the start of the experiment. Then, half of the specimens in each treatment subjected to waterborne fluoxetine at a concentration of 100 µg/L for 24 hours and the behavior was recorded. After 8 days of feeding, the time in front of mirror and duration of gill flaring were not significantly different between the treatments. Duration of the gill flaring increased significantly after 15 days; however there was no significant difference for the behavior in front of the mirror. Over time the aggressive behaviors were reduced significantly after fluoxetine exposure. This study indicated that fluoxetine in the aquatic environment alters the aggressive behaviors of the fighting fish.

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Introduction

The role of sex steroids especially androgens in fish is very clear. In males, this type of hormones are involved in gonadal development, defense of spawning site, mating behavior performance and in some cases parental care (Dey et al., 2010). The hypothalamus-Pituitary-gonad (HPG) axis controls the circulating levels of androgens. Many factors such as environmental conditions (season, temperature, photoperiod, etc) and social status (being dominant or subordinate) can be effective on synthesis and secretion of hormones. Androgens are also important in aggression and spawning behaviors (Dey et al., 2010). Change in androgen levels, alters behavioral consistency of aggression and courtship. Dzieweczynski et al. (2006) reported that male 11-ketotestosterone level increases as a result of being encountered in Siamese fighting fish, *Betta splendens*. Sex reversal is one the main uses of androgens in aquaculture. However, there is little information on aggressive behavior after artificial increase of androgens in fishes.

Natural resource pollution has always been a subject of great interest. The drugs used by humans, may enters to aquatic systems through waste water treatment plant at levels of ng/L to µg/L (Fent et al., 2006). Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) drug with 11% of it excreted from body as parent compound (de Vane, 2000) and in natural system is biologically active because of its resistance to hydrolysis and photolysis (Kwon and Armbrust, 2006). Exposure to fluoxetine disrupt reproductive axis of goldfish, *Carassius auratus* (Mennigen et al., 2010). Fluoxetine at a concentration of 54 µg/L reduced significantly serum testosterone levels after 7 days of exposure. Also, there are some studies on reduction effects of fluoxetine on aggression behaviors in different

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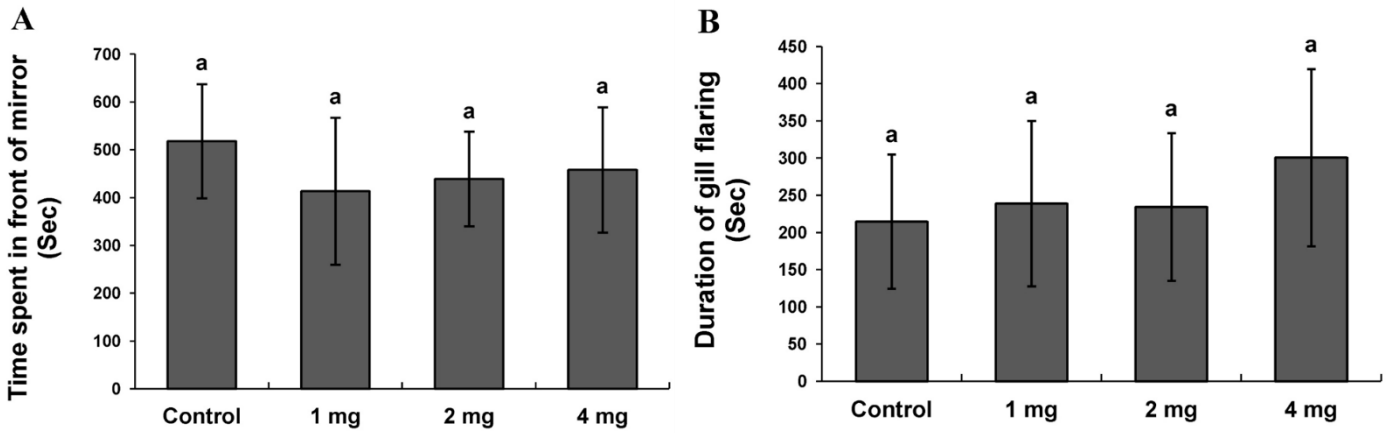

Figure 1. The aggressive behavior of *B. splendens* (A) mean (± SD) of time spent in front of the mirror and (B) duration of gill flaring administered by oral testosterone after 8 days of feeding experiment.

animal taxa such as mammals (Fuller, 1996), birds (Sperry et al., 2003), reptiles (Deckel, 1996) and fish (Lynn et al., 2007).

The present study aimed to first increase the plasma levels of androgens by diets containing different amounts of testosterone and then the fish were exposed to waterborne fluoxetine. Finally, the effect of both experiments on aggression behaviors of *Betta* fish was investigated. Results of this study can be used for better understanding of relationship between physiology and behavior in fish and determine the adverse effect of pollutant in non-target species.

Material and methods

Fish: Forty mature male *B. splendens* with a mean weight of 1.64 ± 0.46 g were obtained from a local distributor. The specimens were transported to laboratory and kept individually in a 1 lit opaque container. Water temperature was 26 °C and photoperiod set as 12D:12L. The specimens were fed one or two times daily for two weeks using 0.9 mm commercial pellet (BioMar, Norway).

Food and stock solution preparation: We used four concentrations: 0, 1, 2 and 4 mg/kg of hormone in food. These levels selected because it was found that 3 or 4 mg/kg of 17α-methyltestosterone (via inert food) resulted in 100% masculinization of fighting fish fry (Kipouros et al., 2011). For this, different amount of testosterone (Andriol TestocapsTM) dissolved in ethanol and sprayed on pre-weighted pellet. Fish were fed daily 3% of body mass for 15 days and then half of them exposed for 24 h in waterborne fluoxetine at a concentration of 100 µg/L.

Experimental protocol and behaviors: Male specimens were divided into four groups. On days 8, 15 and 16 of the experiment, fish were transported to 25 lit experiment tanks. After 15 min acclimatization, a 16 × 24.5 cm mirror was placed in one side of the tank and male behavior was recorded in 10 min. Two main behaviors, i.e. duration of gill flaring and time spent in front of the mirror was measured. The gill flaring is known to be associated with fight outcome (Clotfelter et al., 2007) being fish reflected in opercular activity. Also, response to the mirror show the importance of fighting for the male specimens.

Statistics: The data were statistically analyzed using one-way ANOVA in SPSS v. 19.0. Duncan's multiple range test was used to identify significant differences between treatments ($\alpha<0.05$).

Results

Androgen administration: After 15 days, fishes were tested two times on day 8 and 15 of the experiment. On the 8th day of the experiment, the time spent in front of the mirror ($F= 1.220$, $p= 0.317$; Fig. 1A) and duration of gill flaring ($F= 1.205$, $p= 0.322$; Fig. 1B) had no significant difference between the treatments. There was no significant differences in time spent in front of the mirror on day 15 ($F= 0.508$, $p= 0.679$;

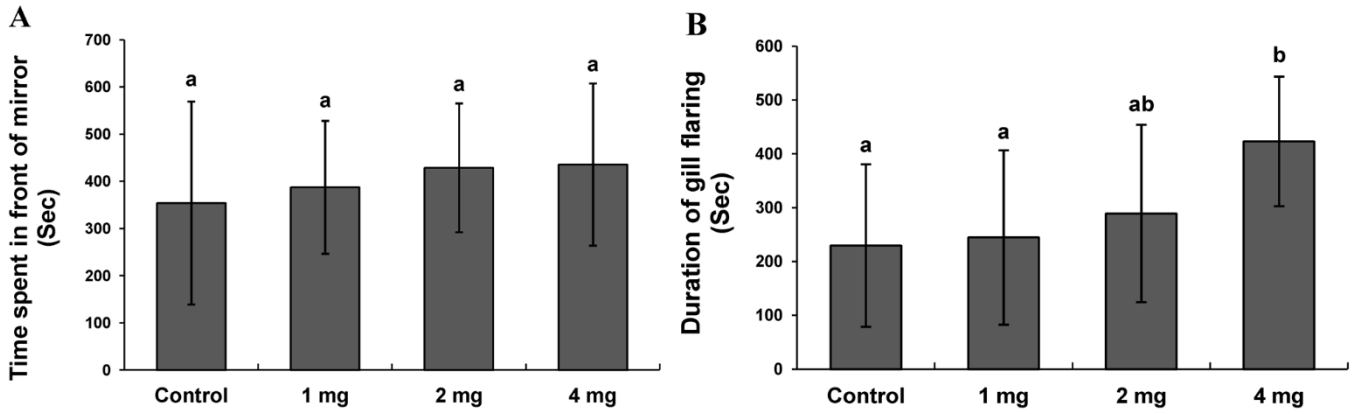

Figure 2. The aggressive behavior of *B. splendens* (A) mean (± SD) of time spent in front of the mirror and (B) duration of the gill flaring administered by oral testosterone after 15 days feeding experiment.

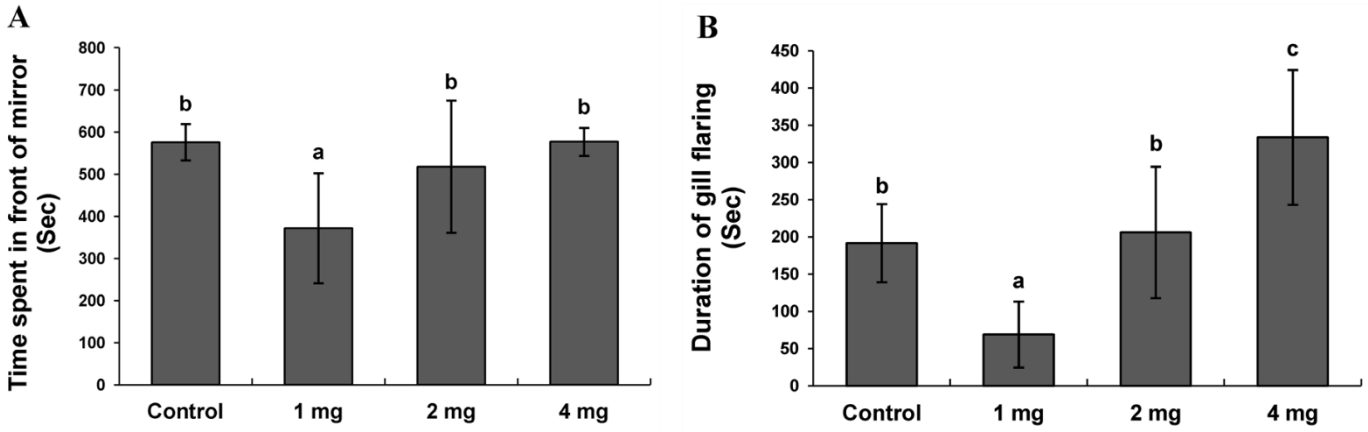

Figure 3. The aggressive behavior of *B. splendens* (A) mean (± SD) of time spent in front of the mirror and (B) duration of the gill flaring following 24 h fluoxetine exposure at 100 µg/L.

Fig. 2A) but there was a significant difference for duration of the gill flaring (F= 3.414, p= 0.028; Fig. 2B).

Fluoxetine exposure: Following 24 h exposure, five fish from each treatment was examined for aggressive behaviors. There was significant difference in the time spent in front of the mirror among treatments (F= 4.181, p=0.023; Fig. 3A). Also, fluoxetine significantly affected the duration of gill flaring behavior (F= 11.326, p=0.000, Fig. 3B).

Discussion

Increasing entrance of pharmaceuticals into the aquatic environment has attracted the attention of workers on their adverse effect. These compounds disrupt endocrine system as well as behaviors. Result of this study showed that orally administration of testosterone increases aggression behavior of fighting fish after 15 days feeding trial. This result is in agreement with the same previous studies (e.g. Cardwell and Liley, 1991; Pankhurst and Barnett, 1993), which have correlated the aggressive behavior with circulating androgens. In an African cichlid fish, *Astatotilapia burtoni*, the dominant male had more circulating levels of testosterone in comparison to sub-ordinate fish (Parikh et al., 2006). Also, it is showed that at the time of social contrast, androgens concentrations increase (Dzieweczynski et al., 2006). These researchers reported that circulating levels of 11-ketotestosterone increased after observing each other in fighting fish. Therefore, a high initial concentration of testosterone in plasma and increased after the introduction of the mirror, makes a significant difference in the incidence of aggressive behaviors after 15 days feeding trial.

On 8th day of the experiment showed that there was not a significant differences between treatments in any of the measured behaviors. This showed that

short term intake of androgen could not influence on the fish behaviors. This is noted that until the end of the experiment, there was no significant difference between treatments for the time spent in front of the mirror. This showed the importance of the conspecifics that expressed aggressive behavior in male fighting fish (personal observation); so, all the males were similar in exhibiting of this behavior.

After fluoxetine exposure, the aggressive behavior has been reduced in compared to the previous measurements. This is consistent with other studies. Perreault et al. (2003) reported that territorial aggression in a coral reef fish (*Thalassoma bifasciatum*) reduced after long-term and acute fluoxetine administration. Short-term exposure of male fighting fish to fluoxetine concentration at 3 μg/ml reduced the expression of specific aggressive behaviors (Lynn et al., 2007). At the present study, there is a significant difference between treatments after exposure to fluoxetine and this was very obvious at 1 mg/kg of testosterone in food for both behaviors. Fluoxetine in both injected (Clotfelter et al., 2007) and exposure (Gaworecki and Klaine, 2008) forms caused reduction in serotonin and its metabolite, 5-hydroxyindoleacetic acid levels within the brain. Therefore, serotonergic pathway reduction and its interaction with increase testosterone levels, maybe the main reason of significant reduction of aggressive behaviors of fighting fish in 1 mg/kg treatment. Further physiological studies are needed to clarify the relationship between testosterone and fluoxetine interactions.

In conclusion, the present study indicates that exogenous testosterone resulted in increase of aggression in fighting fish. However, waterborne fluoxetine cause reduction in these behaviors. Therefore, due to the increasing prescribing different drugs, their consequences in the environment and finally established methods should be investigated.

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