



Journal of
**Fisheries and
Aquatic Science**

ISSN 1816-4927



Academic
Journals Inc.

www.academicjournals.com

Reproduction and Behavioral Responses of Convict Cichlid, *Amatitlania nigrofasciata* to Fluoxetine

¹Tahmineh Latifi, ²Mohammad Navid Forsatkar and ²Mohammad Ali Nematollahi

¹Department of Fisheries, Faculty of Natural Resources, University of Zabol, Zabol, Iran

²Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran

Corresponding Author: Mohammad Ali Nematollahi, Department of Fisheries, Faculty of Natural Resources, University of Tehran, Chamran St., P.O. Box 31585-4314, Karaj, Iran Tel/Fax: +98 26 32245909

ABSTRACT

Widespread prescription of fluoxetine has led to enter into the aquatic environments which act as an endocrine disruptor for fish. This study examined the effect of fluoxetine on the reproductive consequence of convict cichlid (*Amatitlania nigrofasciata*), during three successive cycles of spawning. Following pair-bond formation, pairs were divided into four treatments and exposed to concentrations of 0, 0.54, 5.40 or 54.0 $\mu\text{g L}^{-1}$ fluoxetine in pre-acclimated spawning tanks. Reproduction was assessed by examination of Egg Number (EN), Incubation Period (IP), Hatching Ratio (HR) and Inter-Spawning Interval (ISI). At the last spawning cycle, behavior and physiochemical properties of fish were determined. Hepatosomatic index (HSI) of males/females was insignificant at the end of the experiment; however, condition factor of males showed a significant difference between 0 and 54.0 $\mu\text{g L}^{-1}$ fluoxetine treatments. At 5.40 and 54.0 $\mu\text{g L}^{-1}$ of fluoxetine, testosterone were significantly decreased in males. Both main effects of the fluoxetine concentrations and the spawning cycles were significant in the EN, HR and ISI. IP was significantly affected only by fluoxetine. The behaviors, spawning duration and fanning latency were affected in 54.0 $\mu\text{g L}^{-1}$ fluoxetine but time spent near the substrate and lipping display were not affected. Results indicated that fluoxetine disrupts reproduction features of convict cichlid. However, significant impacts were mainly observed at higher concentrations than those reported in natural environments (0.54 $\mu\text{g L}^{-1}$), so that the impact of fluoxetine on reproductive fitness of cichlid populations might be relatively low.

Key words: Convict cichlid, fluoxetine, reproduction, parental care, behavior

INTRODUCTION

The impact of man-made chemical compounds, specifically pharmaceuticals, on aquatic environments has been the focus of several studies. There are interest on this topic for several reasons: (1) Many of these drugs acts as Endocrine Disrupting Chemicals (EDCs) which interfere with the endogenous endocrine function of exposed organisms (Clotfelter *et al.*, 2004), (2) Behaviors such as reproduction, aggression, predator avoidance, feeding, swimming and schooling behaviors can be altered by the exposure to these compounds (Lynn *et al.*, 2007; Gaworecki and Klaine, 2008; Barry, 2013; Forsatkar *et al.*, 2014b) and (3) Reproduction in aquatic animals can be directly impaired by exposure to EDCs (Weinberger and Klaper, 2013), therefore may be important in regulating population dynamics.

Fluoxetine is a highly prescribed Selective Serotonin Reuptake Inhibitor (SSRI) that is used for treating depression disorders (Schreiber and Pick, 2006). Between 2010 and 2011, fluoxetine was

prescribed 4.2 million times in the UK, an increase of 100,000 on the previous year (Naish, 2013). Detection of fluoxetine in the aquatic environment has been reported at levels between 12-540 ng L⁻¹ (Metcalf *et al.*, 2010). Moreover, fluoxetine can also exert adverse effects on reproduction in both vertebrates and invertebrates. Disruption in the ejaculatory responses in human taking fluoxetine capsules. Fluoxetine decreased sexual motivation in male rats (Bataineh and Daradka, 2007). In fish, fluoxetine inhibited sperm release of male goldfish, *Carassius auratus* (Mennigen *et al.*, 2010). Fluoxetine significantly induced foot detachment from the substrate in five species of marine snails (Fong and Molnar, 2013). Although, few studies showed the main effect of fluoxetine on aquatic organisms, their exposure period was short (Lister *et al.*, 2009; Mennigen *et al.*, 2010; Weinberger and Klaper, 2013; Forsatkar *et al.*, 2014a, b). In the present study, three successive cycles of spawning was investigated by which we can assess the impact of long-term fluoxetine exposure on model fish reproduction.

Convict cichlid (*Amatitlania nigrofasciata*), is a freshwater monogamous biparental cichlid from Central America. In the aquarium, pair fish lay their eggs in a small cave or similar hiding place before meticulously cleaning their chosen site (Townshend and Wootton, 1985). Once laid, one or both parents will take care for the eggs, regularly fanning them to keep well oxygenated water passing over them and chasing away any fish that approaches (Wisenden *et al.*, 1995). Convict cichlid are one of the most popular cichlids kept in captivity and easily spawn in the laboratory, making this species an important model to study fish reproduction (Bockelman and Itzkowitz, 2008). However, to our knowledge, there are only few studies evaluating the effect of EDCs on the reproduction of this species. Piron (1978) introduced the convict cichlid as a good candidate for laboratory toxicity testing; particularly in studies focused on assessing the effects on breeding performance. Ozoh and Jacobson (1979) reported that copper exposure decreased hatching success and increased the number of abnormal larvae. The convict cichlid was chosen by Newsome (1980) as a suitable species to assess toxicity of trisodium carboxymethyloxysuccinate because of its small size, lack of exacting water quality requirements and easy breeding in the laboratory. Recently, Da Cuna *et al.* (2013) reported that *Cichlasoma dimerus*, a very close species to convict cichlid, exposed to endosulfan evidenced a disruptive effect of this organochlorine pesticide on reproductive axis. On the other hand, convict cichlid males and females need to invest into the reproduction process since this species elaborate reproductive behaviors, such as territory defense and brood care (Mackereth and Keenleyside, 1993). Therefore, any potential external factor that may affect physiological parameters can interfere with the reproductive process by changing systemic hormone levels and responsiveness.

Following the identified need by Brooks (2014) to produce further results of fluoxetine effects on different aquatic animals, the aim of this study was to investigate the effect of fluoxetine at environmentally relevant dose (0.54 µg L⁻¹) to one hundred times higher concentrations (54.0 µg L⁻¹) on testosterone levels, spawning behaviors and reproduction outputs of convict cichlid at three successive spawning.

MATERIALS AND METHODS

Fish: Thirty five matures convict cichlid approximately 6-7 months old were purchased from a commercial supplier, Isfahan, Iran. The fish were kept in mixed-sex in two 200 L glass holding tanks for twenty days before the experiment. Fish were fed twice daily with frozen bloodworm (Mahiran, Iran) and commercial pellet (BioMar, France), *ad libitum*. Temperature was 28±1°C and lightening as 12 L/12 D with 50% changing water every 3 days during the pre-adaption period.

Pair bonding and fluoxetine exposure: Convict cichlids ($n = 24$) were allowed to form pairs which were then transferred to spawning tanks (45 L; 30×30×50 cm). From the fish stock, twelve pairs were formed in 12 different spawning tanks. The standard length of males and females were 6.27 ± 0.43 cm and 5.08 ± 1.13 cm (Mean±SD), respectively. The tanks contained a clay pot as spawning substrate and had an airstone for aeration. Water temperature was maintained at $28 \pm 1^\circ\text{C}$, pH and dissolved oxygen were 7.6 and 8 ± 0.5 mg L⁻¹, respectively. We allowed pairs to spawn once before initiating the experiment. After 1 day from this spawning, fish pairs were divided into 4 groups and each group was exposed to 0, 0.54, 5.40 or 54.0 µg L⁻¹ fluoxetine (Pharmaceutical Company of Dr. Abidi; Tehran, Iran). Three replicates were considered for each exposure concentration. Doses were chosen according to environmental concentrations (0.54 µg L⁻¹) and higher doses (5.40 and 54.0 µg L⁻¹) were the ones used in other studies (Mennigen *et al.*, 2010). Whole spawning tank's water was renewed every 3 days and treated again with the adequate amount of fluoxetine. It warranted us to keep constant fluoxetine concentration in the tanks throughout the exposure experiment. A stock solution of 1 mg fluoxetine/mL distilled water was created at the dates of water renewal. Three successive spawning for each pair in treatments were used to evaluate reproductive performance differences between the treatments. The average completion time of spawns for each treatment are listed in the results section.

After each spawning, the substrate with eggs was removed from the tank, photographed and transported into a 101 tank containing fluoxetine with the same initial concentration. The container received constant low aeration and was treated with methylene blue (1 ppm) to inhibit *Saprolegnia* infection. The number of eggs was calculated based on manual computation of eggs in the picture using Paint software. After hatching, embryos were siphoned out in the white plate; photographed and then hatching rate was calculated as before.

Reproductive traits, including total number of produced eggs (EN), Incubation Period (IP), Hatching Rate (HR) and interval (days) between each spawning event (ISI) were used to evaluate the reproductive performance differences between treatments.

Behavioral assessment: At the third spawning activity, we measured spawning duration of pairs once the first performance of male in the spawning substrate until leaving the nest (clay pot) to protect his territory. One hour post-spawning, pair behaviors were recorded during 15 min using the Nikon video-recorder. The time that females spent near to the spawning substrate, lipping and duration of fanning of eggs were scored according to Townshend and Wootton (1985).

Hormone assay, condition factor and hepatosomatic index: One day after the last spawning, fish were anesthetized with clove powder, after measurement of body weight and total length, the blood of different sex of each pair in the treatments was drawn from the caudal peduncle with heparin-coated syringe (less than 4 min). The blood samples were centrifuged at 3000×g for 15 min at 4°C and the plasma collected and stored at -80°C until assay. Plasma concentration of Testosterone (T) was measured using enzyme-linked immunosorbent assay kits (Uscnlife, Wuhan, China), following the manufacturer's instructions. Condition Factor (CF) was calculated as:

$$CF = \frac{\text{Body weight (g)}}{\text{Total length}^3 \text{ (cm)}} \times 100$$

The specimens were finally sacrificed by decapitation and then livers were removed and weighed to calculate:

$$\text{HSI} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

Statistics: A repeated measures analysis of variance (ANOVA) and the Greenhouse-Geisser test (GG) were used in this study. The four fluoxetine concentrations were the between-subject factors. The second factor was the spawning activities which were repeatedly recorded 3 times and was applied within-subjects. Reproductive behavior and hormone levels were analyzed using one-way ANOVA followed by Duncan's multiple comparison test. Data is presented as mean value±standard error. The software used for the statistical analyses was the SPSS v. 19.0 and all statistical tests were considered significant at $p < 0.05$.

RESULTS

Table 1 shows the morphological and endocrinological traits of fish at the end of the experimental period. No significant differences between the exposed treatments were found in the final weight (males: $F = 2.214$, $p = 0.164$ and females: $F = 1.56$, $p = 0.273$), total length (males: $F = 2.534$, $p = 0.13$ and females: $F = 2.712$, $p = 0.115$) and HSI (males: $F = 0.907$, $p = 0.479$ and females: $F = 1.579$, $p = 0.269$). CF was significantly different between males of 0 and $54.0 \mu\text{g L}^{-1}$ treatments ($F = 2.686$, $p = 0.117$) with a significant increase in the $54.0 \mu\text{g L}^{-1}$ group.

There was no difference between testosterone levels of females ($F = 2.082$, $p = 0.181$). However, testosterone concentration between males was found to be different significantly ($F = 18.266$, $p = 0.001$) with lower values in the 5.40 and $54.0 \mu\text{g L}^{-1}$ treatments with respect to 0 and $0.54 \mu\text{g L}^{-1}$ groups.

Significant effects of both fluoxetine concentration ($F = 93.66$, $p < 0.0001$) and spawning activities (GG epsilon = 0.939; $F = 22.31$, $p < 0.0001$) were reported on the EN (Fig. 1). The interaction was also significant ($F = 7.638$, $p < 0.001$). The mean ENs produced in the third spawning activities were 246 ± 6 , 231 ± 6 , 197 ± 10 and 140 ± 18 for 0, 0.54, 5.40 and $54.0 \mu\text{g L}^{-1}$ fluoxetine treatments, respectively (Fig. 1). The total number of eggs produced in the third spawning activity in the 0, 0.54, 5.40 and $54.0 \mu\text{g L}^{-1}$ fluoxetine treatments were 2217, 2080, 1769 and 1262, respectively. The effects of fluoxetine concentrations on the IP were statistically significant ($F = 11.48$, $p < 0.0001$). However, the spawning activities (GG epsilon = 0.928; $F = 1.346$, $p > 0.05$) and the interaction were similar in all treatments ($F = 2.069$, $p > 0.05$). Time needed to hatch embryos were 48.1 ± 0.9 , 48.2 ± 0.8 , 50.1 ± 0.9 and 59.3 ± 1.7 h for 0, 0.54, 5.4 and $54.0 \mu\text{g L}^{-1}$ fluoxetine treatments, respectively (Fig. 1). The results also showed significant effects of fluoxetine treatments.

Table 1: Effects of fluoxetine on somatic indices of *A. nigrofasciata*

Fluoxetine ($\mu\text{g L}^{-1}$)	Body weight (g)		Total length (cm)		HSI (%)		CF (%)		Testosterone (ng mL ⁻¹)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0.00	6.71±0.44	4.95±0.26	7.62±0.52	6.06±0.42	1.94±0.02	1.89±0.03	1.56±0.23 ^a	2.30±0.37	9.43±1.1 ^b	4.02±0.18
0.54	6.60±0.32	4.95±0.39	7.61±0.37	5.97±0.29	1.89±0.06	1.92±0.03	1.51±0.17 ^{ab}	2.34±0.16	9.42±0.48 ^b	3.96±0.24
5.40	6.02±0.41	4.39±0.27	7.10±0.24	5.15±0.44	2.00±0.09	1.96±0.04	1.68±0.06 ^{ab}	3.41±0.71	5.33±0.5 ^a	3.68±0.21
54.0	5.55±0.22	4.27±0.19	6.39±0.22	4.90±0.18	2.02±0.04	2.01±0.04	2.13±0.15 ^b	3.64±0.23	3.26±0.69 ^a	3.29±0.27

Data is expressed as Mean±SE (n = 3 pairs per condition). Different letters indicate statistically different values, $p < 0.05$, HSI: Hepatosomatic index, CF: Condition factor

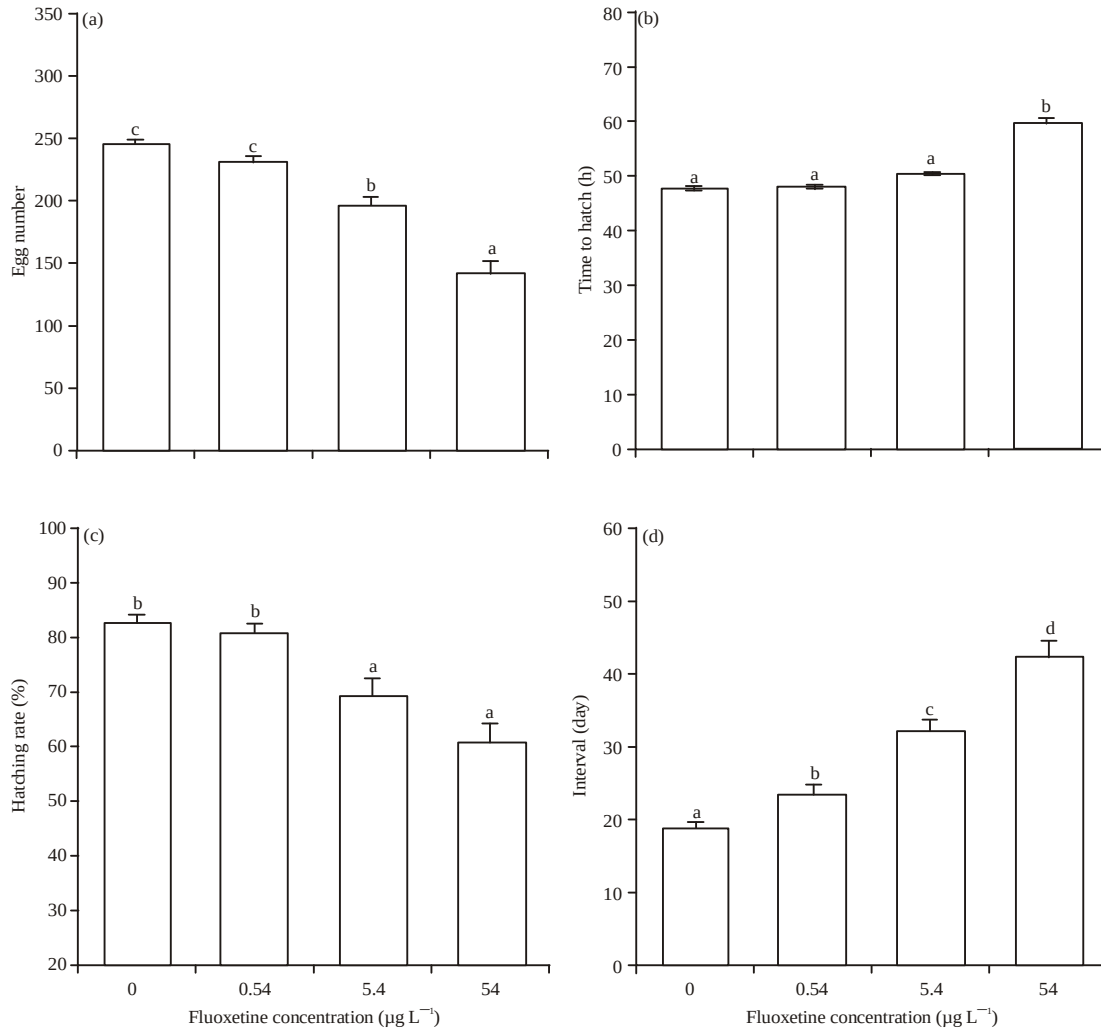


Fig. 1(a-d): Reproductive parameters (a) Egg number, (b) Time to hatch (h), (c) Hatching rate (%) and (d) Interval (day) of *A. nigrofasciata* exposed to different concentrations of fluoxetine (n = 3). Data are of expressed as the Mean±SE (n = 3 samples). Different letters indicate statistically different values, $p < 0.05$

($F = 13.5$, $p < 0.0001$) and the spawning activities (GG epsilon = 0.836; $F = 4.962$, $p < 0.05$) on the HR. The interaction was also significant ($F = 3.095$, $p < 0.05$). The mean HR in the 0 µg L⁻¹ fluoxetine treatment for the third spawning activities ($82.66 \pm 2.21\%$) was higher than that in the 0.54 µg L⁻¹ ($80.7 \pm 2.2\%$), 5.4 µg L⁻¹ ($69.3 \pm 5.7\%$) and 54.0 µg L⁻¹ ($60.9 \pm 6.5\%$; Fig. 2).

The effects of both the fluoxetine doses ($F = 59.17$, $p < 0.0001$) and the spawning activities (GG epsilon = 0.997; $F = 107.05$, $p < 0.0001$) were significant on the ISI. The mean ISI in the 54.0 µg L⁻¹ fluoxetine (42.2 ± 3.4 days) was longer than that in the 5.4 µg L⁻¹ (32.0 ± 2.4 days), 0.54 µg L⁻¹ (23.6 ± 2.0 days) and 0 µg L⁻¹ (18.9 ± 1.2 days) fluoxetine treatments (Fig. 1). The three spawning cycles took the pairs exposed to 54.0, 5.4 and 0.54 µg L⁻¹ fluoxetine were 127 ± 3 , 9.00 ± 3.05 and 70.7 ± 4.1 days, respectively. The 0 µg L⁻¹ fluoxetine treatment took only 56.7 ± 5.5 days to achieve 3 spawning activities.

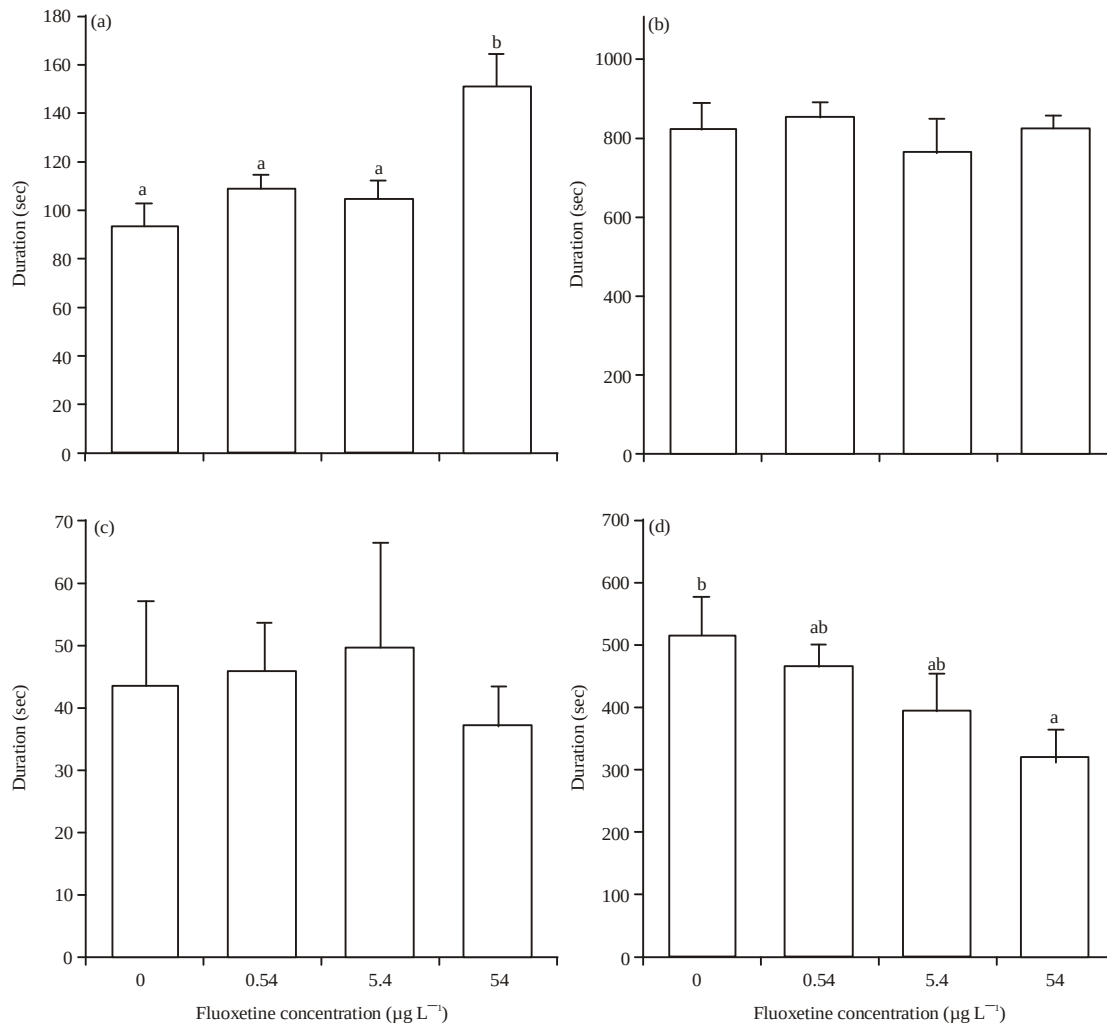


Fig. 2(a-d): Mean±SE duration of measured behaviors, (a) Spawning duration, (b) Time spent near the substrate, (c) Lipping and (d) Fanning of *A. nigrofasciata* exposed to the different fluoxetine concentration at the third spawning. Different letters indicate statistically different values, $p < 0.05$

During the third spawning activity, reproductive behaviors were assessed in pairs in all treatments. Spawning duration was significantly different between 0 and 54.0 µg L⁻¹ treatments ($F = 4.17$, $p = 0.047$; Fig. 2a). However, the time spent near the spawning substrate by females ($F = 0.26$, $p = 0.84$; Fig. 2b) and duration of lipping ($F = 0.2$, $p = 0.89$; Fig. 2c) were similar between treatments. Females under 0 µg L⁻¹ of fluoxetine (control) presented higher fanning activity (513 ± 63 sec) in comparison to other treatments (464 ± 35 , 395 ± 57 and 323 ± 40 for 0.54, 5.40 and 54.0 µg L⁻¹ fluoxetine treatments, respectively; Fig. 2d).

DISCUSSION

The present study investigated the effects of fluoxetine exposure on the production status of convict cichlid. Egg numbers laid by females significantly decreased at higher doses of fluoxetine

across all spawning cycles. The negative role of fluoxetine on egg production has been shown in other fish species such as zebrafish, *Danio rerio* (Lister *et al.*, 2009). The endocrine mechanism of fluoxetine disruption on females was also investigated elsewhere (Mennigen *et al.*, 2008). The two higher doses (5.40 and 54.0 $\mu\text{g L}^{-1}$ fluoxetine) showed the greater impact on egg production. These results are in agreement with Lister *et al.* (2009) that reported a reduction in egg production and cumulative egg production in zebrafish during 7 days exposure to 32 $\mu\text{g L}^{-1}$ fluoxetine. However, fluoxetine did not have a significant effect on reproductive outputs of Japanese medaka, *Oryzias latipes* (Foran *et al.*, 2004). It must be noted that the highest concentration of fluoxetine in their study was 5 $\mu\text{g L}^{-1}$ and in the present study, we used about 11 fold higher fluoxetine concentration.

During the three spawning cycles, fluoxetine at higher concentrations had significant negative effects on the time required for hatching and hatching rate at all spawning activities. Hatching time depends on water quality and broodstock condition (Brooks *et al.*, 1997). In our study, after eggs were laid, they were immediately transferred to another container with standard water quality. Therefore, the increase in the time needed to hatch embryos can be attributed to the direct effect of fluoxetine on eggs. Lower hatching rate after 54.0 $\mu\text{g L}^{-1}$ fluoxetine exposure on the third spawning is partially caused by the accumulation of fluoxetine in parent fish. Although we did not measure this accumulation, fluoxetine accumulation has been reported from fish exposed in the environment (Brooks *et al.*, 2005). Other authors also found the fluoxetine accumulation in the body only after a few days of exposure (Clotfelter *et al.*, 2007).

Fernandes *et al.* (2011) reported that SSRIs (fluvoxamine and fluoxetine) are the strongest inhibitors of the key enzymes involved in the androgen synthesis. Based on our results, exposing of convict cichlid to waterborne fluoxetine results in a 76.5% decrease in circulating levels of testosterone (from 9.73-3.26 ng mL⁻¹) of males at a fluoxetine concentration of 54.0 $\mu\text{g L}^{-1}$. A similar concentration of fluoxetine significantly reduced circulating testosterone levels and milt release of mature male goldfish after 14 days of exposure (Mennigen *et al.*, 2010). Sex steroids play a crucial role in sexual maturation (Chang *et al.*, 2013). Further, it is reported that the levels of sex steroids could affect the quality of gametes during gametogenesis (Kime *et al.*, 1999). Consequently, the decreased levels of testosterone may be one of the main reasons for the reduced hatching rate observed in the present study.

However, Condition Factor (CF) of males exposed to 54.0 $\mu\text{g L}^{-1}$ fluoxetine was significantly reduced in comparison to the control treatment. The CF is a common indicator of physiological fitness in fish health (Anderson *et al.*, 2003) and shows the amount of food intake and protein budgets (Smolders *et al.*, 2003). Beside, HSI is considered a good indicator of exposure to environmental contaminants (Du *et al.*, 2009). Although no significant effect of fluoxetine on HSI has been found in males or females, a significant decrease in males CF at high fluoxetine exposure was evidenced showing the improper performance of specimens in food metabolism. Fluoxetine indirectly affects food intake in fish (Mennigen *et al.*, 2009). Fluoxetine exposure at concentrations of 10 and 100 $\mu\text{g L}^{-1}$ increased the time required for the fathead minnows (Weinberger and Klaper, 2013) to eat daphnids. The role of quantity and quality of food in reproduction of several species has been proved (Izquierdo *et al.*, 2001). Accordingly, it is likely that the effect of fluoxetine on body condition reduced the reproductive outcomes of convict cichlid pairs. The amount of food intake was not our goal in the present study; therefore further experiment is needed to assess this factor.

Some reproduction behaviors of convict cichlid were affected after fluoxetine exposure. These results are surprising because the total number of produced eggs by exposed pairs was lower than

by control specimens. The increase in the time needed for spawning along with reduction in the number of laid eggs indicates a general disruption of fluoxetine on fish. Parental care response is extremely related to androgen levels (Smith, 1969). Lower testosterone levels of males in higher dosages of fluoxetine may reflect the lower propensity of these males to exhibit paternal care. Subsequently, reduced protective behaviors in female can be due to (1) The interaction with the male sexual reluctance or (2) The direct effect of fluoxetine. The sexual reluctance of fish species in the presence of fluoxetine to exhibit spawning behaviors was also shown in previous studies (Dziewieczynski and Hebert, 2012; Forsatkar *et al.*, 2014a, b). Fluoxetine concentrations as low as $1 \mu\text{g L}^{-1}$ were found to significantly impact mating behavior, specifically nest building and defending in male fathead minnow (Weinberger and Klaper, 2013). The reduced ability of females exposed to $54.0 \mu\text{g L}^{-1}$ fluoxetine to protect her brood may have been related to side effects of fluoxetine such as decreased total locomotion (Kohlert *et al.*, 2012), plasma androgen levels (Mennigen *et al.*, 2010) and/or male sexual motivation which is strongly related to circulating androgen concentrations (Safarinejad, 2008).

Interval length between spawning cycles was also affected by fluoxetine concentration. The greater amount of time needed for spawning in fluoxetine treated fish suggests fewer opportunities for each pair to spawn in the presence of fluoxetine. As mentioned earlier, parental body condition is extremely affected by environmental pollution (Scott and Sloman, 2004). As a consequence, spawning periodicity was also impacted by fluoxetine in our study.

Our results demonstrate that exposure to fluoxetine reduced the reproductive outputs of convict cichlid. Egg production and hatching rate decreased after administration of fluoxetine evidencing the inhibitory action of this highly prescribe SSRI on the reproduction of this species. In addition, the higher fluoxetine dose was more susceptible to induce disruption in reproduction outputs of convict cichlid. Future experiments should be address the relationship between fluoxetine and alterations in behavior of parents at different stages of reproduction, i.e., after spawning and hatching of embryos.

REFERENCES

- Anderson, M.J., D. Cacela, D. Beltman, S.J. The and M.S. Okihiro *et al.*, 2003. Biochemical and toxicopathic biomarkers assessed in smallmouth bass recovered from a polychlorinated biphenyl-contaminated river. *Biomarkers*, 8: 371-393.
- Barry, M.J., 2013. Effects of fluoxetine on the swimming and behavioural responses of the Arabian killifish. *Ecotoxicology*, 22: 425-432.
- Bataineh, H.N. and T. Daradka, 2007. Effects of long-term use of fluoxetine on fertility parameters in adult male rats. *Neuroendocrinol. Lett.*, 28: 321-325.
- Bockelman, A.K. and M. Itzkowitz, 2008. Males respond differently than females to mate loss in the biparental convict cichlid fish. *Behaviour*, 145: 313-325.
- Brooks, S., C.R. Tyler and J.P. Sumpter, 1997. Egg quality in fish: What makes a good egg? *Rev. Fish Biol. Fish.*, 7: 387-416.
- Brooks, B.W., C.K. Chambliss, J.K. Stanley, A. Ramirez, K.E. Banks, R.D. Johnson, R.J. Lewis, 2005. Determination of select antidepressants in fish from an effluent-dominated stream. *Environ. Toxicol. Chem.*, 24: 464-469.
- Brooks, B.W., 2014. Fish on prozac (and Zoloft): Ten years later. *Aquatic Toxicol.*, 151: 61-67.
- Chang, J., S. Liu, S. Zhou, M. Wang and G. Zhu, 2013. Effects of butachlor on reproduction and hormone levels in adult zebrafish (*Danio rerio*). *Exp. Toxicol. Pathol.*, 65: 205-209.

- Clotfelter, E.D., A.M. Bell and K.R. Levering, 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals. *Anim. Behav.*, 68: 665-676.
- Clotfelter, E.D., E.P. O'Hare, M.M. McNitt, R.E. Carpenter and C.H. Summers, 2007. Serotonin decreases aggression via 5-HT_{1A} receptors in the fighting fish *Betta splendens*. *Pharmacol. Biochem. Behav.*, 87: 222-231.
- Da Cuna, R.H., M. Pandolfi, G. Genovese, Y. Piazza, M. Ansaldo and F.L. lo Nostro, 2013. Endocrine disruptive potential of endosulfan on the reproductive axis of *Cichlasoma dimerus* (Perciformes, Cichlidae). *Aquatic Toxicol.*, 126: 299-305.
- Du, Y., X. Shi, C. Liu, K. Yu, B. Zhou, 2009. Chronic effects of water-borne PFOS exposure on growth, survival and hepatotoxicity in zebrafish: A partial life-cycle test. *Chemosphere*, 74: 723-729.
- Dziewieczynski, T.L. and O.L. Hebert, 2012. Fluoxetine alters behavioral consistency of aggression and courtship in male Siamese fighting fish, *Betta splendens*. *Physiol. Behav.*, 107: 92-97.
- Fernandes, D., S. Schnell and C. Porte, 2011. Can pharmaceuticals interfere with the synthesis of active androgens in male fish? An *in vitro* study. *Marine Pollut. Bull.*, 62: 2250-2253.
- Fong, P.P. and N. Molnar, 2013. Antidepressants cause foot detachment from substrate in five species of marine snail. *Marine Environ. Res.*, 84: 24-30.
- Foran, C.M., J. Weston, M. Slattery, B.W. Brooks and D.B. Huggett, 2004. Reproductive assessment of Japanese medaka (*Oryzias latipes*) following a four-week fluoxetine (SSRI) exposure. *Arch. Environ. Contam. Toxicol.*, 46: 511-517.
- Forsatkar, M.N., M.A. Nematollahi and B.M. Amiri, 2014a. Prozac alters reproductive performance and filial cannibalism in male fighting fish, *Betta splendens*. *Iran. J. Toxicol.*, 8: 1109-1113.
- Forsatkar, M.N., M.A. Nematollahi, B.M. Amiri and W.B. Huang, 2014b. Fluoxetine inhibits aggressive behaviour during parental care in male fighting fish (*Betta splendens*, Regan). *Ecotoxicology*, 23: 1794-1802.
- Gaworecki, K.M. and S.J. Klaine, 2008. Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. *Aquatic Toxicol.*, 88: 207-213.
- Izquierdo, M.S., H. Fernandez-Palacios and A.G.J. Tacon, 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 197: 25-42.
- Kime, D.E., J.P. Nash and A.P. Scott, 1999. Vitellogenesis as a biomarker of reproductive disruption by xenobiotics. *Aquaculture*, 177: 345-352.
- Kohlert, J.G., B.P. Mangan, C. Kodra, L. Drako, E. Long and H. Simpson, 2012. Decreased aggressive and locomotor behaviors in *Betta splendens* after exposure to fluoxetine. *Psychol. Rep.*, 110: 51-62.
- Lister, A., C. Reagan, J. Van Zwol and G.D. Kraak, 2009. Inhibition of egg production in zebrafish by fluoxetine and municipal effluents: A mechanistic evaluation. *Aquatic Toxicol.*, 95: 320-329.
- Lynn, S.E., J.M. Egar, B.G. Walker, T.S. Sperry and M. Ramenofsky, 2007. Fish on Prozac: A simple, noninvasive physiology laboratory investigating the mechanisms of aggressive behavior in *Betta splendens*. *Adv. Physiol. Educ.*, 31: 358-363.
- Mackereth, R.W. and M.H. Keenleyside, 1993. Breeding territoriality and pair formation in the convict cichlid (*Cichlasoma nigrofasciatum*; Pisces, Cichlidae). *Can. J. Zool.*, 71: 960-967.
- Mennigen, J.A., C.J. Martyniuk, K. Crump, H. Xiong and E. Zhao *et al.*, 2008. Effects of fluoxetine on the reproductive axis of female goldfish (*Carassius auratus*). *Physiol. Genom.*, 35: 273-282.
- Mennigen, J.A., E.A. Harris, J.P. Chang, T.W. Moon and V.L. Trudeau, 2009. Fluoxetine affects weight gain and expression of feeding peptides in the female goldfish brain. *Regul. Pept.*, 155: 99-104.

- Mennigen, J.A., W.E. Lado, J.M. Zamora, P. Duarte-Guterman and V.S. Langlois *et al.*, 2010. Waterborne fluoxetine disrupts the reproductive axis in sexually mature male goldfish, *Carassius auratus*. *Aquatic Toxicol.*, 100: 354-364.
- Metcalfe, C.D., S. Chu, C. Judt, H. Li, K.D. Oakes, M.R. Servos and D.M. Andrews, 2010. Antidepressants and their metabolites in municipal wastewater and downstream exposure in an urban watershed. *Environ. Toxicol. Chem.*, 29: 79-89.
- Naish, J., 2013. The jekyll and hyde happy pill: It's brought relief to millions but is linked to suicide, low libido and birth defects and we still don't know how Prozac works. <http://www.dailymail.co.uk/health/article-2275333/Prozac-Its-brought-relief-millions-linked-suicide-low-libido-birth-defects.html#axzz2KNt7FK00>
- Newsome, C.S., 1980. A multigeneration fish toxicity test as an aid in the hazard evaluation of aquatic pollutants. *Ecotoxicol. Environ. Saf.*, 4: 362-369.
- Ozoh, P.T. and C.O. Jacobson, 1979. Embryotoxicity and hatchability in *Cichlasoma nigrofasciatum* (Guenther) eggs and larvae briefly exposed to low concentrations of zinc and copper ions. *Bull. Environ. Contam. Toxicol.*, 21: 782-786.
- Piron, R.D., 1978. Breeding the convict cichlid (*Cichlasoma nigrofasciatum*) for use in laboratory fish toxicity tests. *J. Fish Biol.*, 13: 119-122.
- Safarinejad, M.R., 2008. Evaluation of endocrine profile and hypothalamic-pituitary-testis axis in selective serotonin reuptake inhibitor-induced male sexual dysfunction. *J. Clin. Psychopharmacol.*, 28: 418-423.
- Schreiber, S. and C.G. Pick, 2006. From selective to highly selective SSRIs: A comparison of the antinociceptive properties of fluoxetine, fluvoxamine, citalopram and escitalopram. *Eur. Neuropsychopharmacol.*, 16: 464-468.
- Scott, G.R. and K.A. Sloman, 2004. The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicol.*, 68: 369-392.
- Smith, R.J.F., 1969. Control of prespawning behaviour of sunfish (*Lepomis gibbosus* and *L. megalotis*). I. Gonadal androgen. *Anim. Behav.*, 17: 279-285.
- Smolders, R., G. de Boeck and R. Blust, 2003. Changes in cellular energy budget as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). *Environ. Toxicol. Chem.*, 22: 890-899.
- Townshend, T.J. and R.J. Wootton, 1985. Adjusting parental investment to changing environmental conditions: The effect of food ration on parental behaviour of the convict cichlid, *Cichlasoma nigrofasciatum*. *Anim. Behav.*, 33: 494-501.
- Weinberger II, J. and R. Klaper, 2013. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish *Pimephales promelas* (fathead minnow). *Aquatic Toxicol.*, 151: 77-83.
- Wisenden, B.D., T.L. Lanfranconi-Izawa and M.H. Keenleyside, 1995. Fin digging and leaf lifting by the convict cichlid, *Cichlasoma nigrofasciatum*: Examples of parental food provisioning. *Anim. Behav.*, 49: 623-631.