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# The SSRI fluoxetine exhibits mild effects on the reproductive axis in the cichlid fish *Cichlasoma dimerus* (Teleostei, Cichliformes)

Luciana S. Dorelle [a], Rodrigo H. Da Cuña [a], Graciela Rey Vázquez [a], Christian Höcht [b], Akio Shimizu [c], Griselda Genovese [a], Fabiana L. Lo Nostro [a, *]

[a] *Laboratorio de Ecotoxicología Acuática, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires & Instituto de Biodiversidad y Biología Experimental y Aplicada IBBEA, CONICET-UBA, C1428EGA, Buenos Aires, Argentina*
[b] *Departamento de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos (UBA), C1113AAD, Buenos Aires, Argentina*
[c] *National Research Institute of Fisheries Science, Fisheries Research Agency, Kanazawa, Yokohama, 236-8648, Japan*

## HIGHLIGHTS

- The purpose of this study was to evaluate the endocrine disrupting potential of fluoxetine (FLX) over the reproductive axis in a cichlid fish.
- Fluoxetine injection caused an increase on LH levels in females of *C. dimerus.*
- Testis abnormalities were observed in FLX-exposed fish males.
- FLX is acting as a mild endocrine disrupting compound in adults of *C. dimerus.*

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## ABSTRACT

Among the wide variety of pharmaceuticals released into the environment, Fluoxetine (FLX), a selective serotonin reuptake inhibitor, is one of the most prescribed for the treatment of major depression. It inhibits serotonin (5-HT) reuptake at the presinaptic membrane, increasing serotonergic activity. In vertebrates, including fish, the serotonergic system is closely related to the Hypothalamic Pituitary Gonadal (HPG) axis which regulates reproduction. As FLX can act as an endocrine disrupting compound (EDC) by affecting several reproductive parameters in fish, the aim of this study was to provide an integral assessment of the potential effect of FLX on the reproductive axis of the Neotropical freshwater fish *Cichlasoma dimerus*. Adult fish were intraperitoneally injected with 2 $\mu g\ g^{-1}$ FLX or saline every third day for 15 days. No significant differences were found on serotonergic turnover (5-HIAA/5-HT ratio). Pituitary βLH content in FLX injected females was significantly higher than control females; no significant differences were seen for βFSH content. Sex steroids remained unaltered, both in males and females fish, after FLX treatment. No plasma vitellogenin was induced in treated males. Some alterations were seen in testes of FLX injected males, such as the presence of foam cells and an acidophilic PAS positive, Alcian-Blue negative secretion in the lobular lumen. Although there is no clear consensus about the effect of this drug on reproductive physiology, these results indicate that FLX is acting as a mild EDC in adults of *C. dimerus*.



## 1. Introduction

Thousands of pharmaceutically active compounds are commercially produced nowadays, among which a wide variety of substances designed to improve animal and human health can be found (Rehman et al., 2015; Wen et al., 2014). In addition, widespread usage of these substances (both prescribed and over the counter) has generated a significant discharge of pharmaceuticals and their metabolites into sewerages systems, leading to their detection in water -even in drinking water- in the last few years due to the improved sensitivity of analytical methods (World Health Organization, 2011). In this context, many of these substances are today considered to be contaminants of emerging concern (CECs), according to Sauvé and Desrosiers (2014), since these chemicals or

* Corresponding author.
*E-mail address:* fabi@bg.fcen.uba.ar (F.L. Lo Nostro).

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materials -whether naturally occurring, manufactured or man-made- have now been discovered in the environment, and their toxicity and/or persistence can potentially alter the metabolism of organisms. Once a pharmaceutical is consumed and excreted, it remains in sewage as it cannot be completely removed from effluents at wastewater treatment plants (WWTPs) (Blair et al., 2013). In consequence, these pollutants can reach aquatic environments, where they can affect non-target organisms, such as fish, amphibians and invertebrates (Segura et al., 2009).

One of these pharmaceuticals, Fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), is the active ingredient of commercial antidepressants such as Prozac®, which are commonly prescribed not only for treatment of major depression but also for other psychological disorders such as obsessive compulsive disorder, bulimia nervosa and panic disorder (Burt et al., 2007). The mode of action of SSRIs in the human central nervous system (CNS) was characterized based on their effects on several biological mammalian models (Bymaster et al., 2002). In brain serotoninergic synapses, the neurotransmitter serotonin (5-hydroxytriptamine, 5-HT) is released into the synaptic cleft, where it interacts with a variety of membrane receptors on the postsynaptic membrane, provoking the corresponding response. The stimulus is ended by 5-HT re-uptake on the presynaptic membrane by the serotonin transporter (SERT). All SSRIs act by blocking SERT, hence increasing brain serotonergic activity (Koch et al., 2002; Wong et al., 1995). Since SERT is blocked by FLX, less 5-HT is taken up by the presynaptic neuron and metabolized by monoamine oxidase (MAO), resulting in an altered ratio between the levels of 5-hydroxyindoleacetic acid (5-HIAA), the main 5-HT metabolite, and 5HT in the brain (5-HIAA/5-HT ratio), a parameter which is known as brain 5-HT turnover (McDonald et al., 2011).

Serotonin-producing neurons in the CNS have been extensively studied and characterized in every group of vertebrates, including bony fish. In teleosts, three different populations of serotonergic neurons have been identified: pretectal, hypothalamic and raphe nuclei (Lillesaar, 2011). These neurons not only differ on their location in the brain but also on their physiological role (Ekström and Van Veen, 1984; Lillesaar et al., 2009). The pretectal area is known to be involved in the integration of visual inputs and motor behaviour (Wullimann, 1998), whereas hypothalamic serotonergic neurons are suggested to sense cerebrospinal fluid (CSF) and be involved in the release of substances into the circulation due to their proximity to the ventricular system (Lillesaar, 2011), some authors even including this population in the group of CSF-contacting cells, which were described in several species (Vigh and Vigh-Teichmann, 1998). The raphe contains a population of serotonergic neurons whose projections reach all areas in the brain, including the olfactory bulb, telencephalon, hypothalamus and spinal cord (Lillesaar et al., 2009). In humans, 5-HT is related to mood, aggression, sexual drive and appetite (Tops et al., 2009), and there is evidence that it also exerts control over hypothalamic regulation of pituitary secretion of several hormones, such as adrenocorticotrophin, prolactin, growth hormone, and the gonadotrophins (GtHs): Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) (Frazer and Henzler, 1999).

As in other vertebrates, reproduction in fish is highly regulated by the Hypothalamic Pituitary Gonadal (HPG) axis (Hachfi et al., 2012). Regulation of reproductive anatomy, physiology and behaviour in fish are closely related to the serotonergic system (Khan and Thomas, 1993; Winberg et al., 1997), mainly through the releasing of Gonadotrophin Releasing Hormones (GnRH) from the hypothalamus (Senthilkumaran et al., 2001; Yu et al., 1991). In zebrafish, *Danio rerio*, 5-HT receptors are expressed in the hypothalamus (Norton et al., 2008), suggesting a possible co-expression with GnRH, as was described in mammals (Bhattarai et al., 2013). Khan and Thomas (1993) demonstrated in Atlantic croakers, *Micropogonias undulates*, injected with 5-HT an increase in LH levels through potentiation of GnRH effects. Moreover, perfusion of goldfish, *Carassius auratus*, pituitary fragments with 5-HT, resulted in a dose-dependent increase of LH and decrease of GH release (Somoza and Peter, 1991), and this action seemed to be mediated by 5-HT2 subtype receptors, as it was blocked by a specific antagonist (Prasad et al., 2015).

Previous studies indicate that FLX can alter reproductive parameters in fish. Exposure to FLX generated an increase on plasmatic levels of 17β-estradiol (E2) in males of *C. auratus* (Mennigen et al., 2010) and females of Japanese medaka, *Oryzias latipes* (Foran et al., 2004). However, other authors observed a reduction of E2 levels, and a decrease on gene expression of aromatase and gonadotrophin receptors in the ovaries of *D. rerio*, which inhibited egg production (Lister et al., 2009). With regard to the effect of FLX on vitellogenin (VTG) synthesis, while one study reported the abnormal presence of plasmatic VTG in males and alterations in secondary sex characteristics of fathead minnow, *Pimephales promelas* (Schultz et al., 2011), another study reported that plasmatic VTG was not induced by the presence of waterborne FLX in *O. latipes* males (Foran et al., 2004). Additionally, a wide variety of behaviour alterations were observed due to FLX, which include either a decrease (Barry, 2013; Dzieweczynski and Hebert, 2012; Kania et al., 2012) or an increase in territorial aggression (McDonald et al., 2011), an anxiolytic effect (Ansai et al., 2016), a lower predator avoidance (Painter et al., 2009; Weinberger II and Klaper, 2014), and a decrease in swimming patterns, such as swimming speed and distance to the nearest neighbour (Barry, 2013).

In this context, cichlid fish, a diverse family of teleosts are commonly used as an experimental model since they have a distinctive pattern of social hierarchies and territorial aggression, which regulates several physiological processes: fertility, gonadal maturation and even neurogenesis and stress levels (Maruska and Fernald, 2013; Ramallo et al., 2014). Particularly, *Cichlasoma dimerus* (Heckel, 1840) (Cichliformes; Cichlidae) (Nelson et al., 2016), a Neotropical freshwater fish endemic to the Paraná basin, is easy to keep in captivity under controlled environmental conditions. Having *C. dimerus* been successfully used as a model in aquatic ecotoxicology testing (Da Cuña et al., 2013, 2016; Genovese et al., 2012, 2014; Meijide et al., 2016; Piazza et al., 2015; Rey Vázquez et al., 2016), it is included on local environmental regulations for developing acute toxicity tests in freshwater fish (IRAM, 2008).

Based on the aforementioned evidence, we hypothesized that FLX has the ability to alter serotoninergic brain activity, which in turn impacts on the reproductive physiology of the cichlid fish *C. dimerus*.

## 2. Materials and methods

### 2.1. Animals

Sexually mature male and female *Cichlasoma dimerus*, were captured at Esteros del Riachuelo, Corrientes, Argentina (27°35′S, 58°45′O). Fish were transferred to the laboratory facilities, where they were held in 200 L aquaria for at least 4 weeks under controlled conditions (aeration, light and temperature). Animals were fed daily with commercial food (Tetra food® sticks).

All experiments were conducted in accordance to international standards on the care and use of fish in research and testing according to the guide for the care and use of laboratory animals (National Research Council, 2011), as well as being in compliance with the local Ethical Committee (CICUAL, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires).