*Environmental Toxicology and Chemistry*—Volume 38, Number 9—pp. 1902–1910, 2019
Received: 15 January 2019 | Revised: 20 February 2019 | Accepted: 8 May 2019


Environmental Toxicology

# The Psychoactive Drug Escitalopram Affects Foraging Behavior in Zebrafish (*Danio rerio*)

Sebastian Vedel Nielsen, Marie Frausing, Per Guldhammer Henriksen, Kristian Beedholm, and Erik Baatrup*

Zoophysiology Research, Department of Bioscience, Aarhus University, Aarhus, Denmark

**Abstract:** Selective serotonin reuptake inhibitors (SSRIs) are psychoactive pharmaceuticals that have been detected intact in natural waters globally. Laboratory experiments have reported that several SSRIs inhibit fish foraging behavior, but data for the SSRI escitalopram are lacking. The objectives of the present study were to determine whether escitalopram affects feeding behavior in zebrafish and whether possible sex differences exist. We exposed female and male zebrafish (*Danio rerio)* to 0.00, 0.10, and 1.50 μg/L of escitalopram in flow-through tanks for a 3-wk exposure period. We used a video tracking system with high temporal and spatial resolution to collect data on zebrafish swimming patterns in test tanks containing a food source. The results show a more pronounced effect of escitalopram in males compared with females. At the assumed most environmentally relevant concentration (0.10 μg/L), male average feeding time/visit and maximum feeding duration were significantly reduced by 27 and 42%, respectively. In addition, male total feeding duration was also significantly reduced (by 73%) at the highest concentration (1.50 μg/L). In females, only the maximum feeding duration was significantly reduced (by 41%) in the 0.10 μg/L treatment group. Hence, we reject our initial hypothesis that female feeding behavior is more vulnerable to escitalopram. There was no effect of escitalopram on length or weight among the experimental groups. The present study demonstrates that escitalopram, like other SSRIs, can inhibit fish foraging behavior and therefore potentially disturb natural food chains. Finally, our study suggests that SSRIs can both be sex and behavior specific. *Environ Toxicol Chem* 2019;38:1902–1910. © 2019 SETAC

**Keywords:** Ecotoxicology; Behavioral toxicology; Escitalopram; Selective serotonin reuptake inhibitor; Zebrafish

## INTRODUCTION

The selective serotonin reuptake inhibitors (SSRIs) have gained increasing popularity as psychopharmaceuticals for the treatment of depression and other mental diseases. Despite pharmacological and pharmacokinetic differences (Baumann 1996), they all share a common mechanistic functioning trait, which is a prolonged residence of extracellular serotonin (5-HT) in the synaptic cleft through inhibition of the 5-HT active transporter protein (SERT). Among the SSRIs, escitalopram, the S-enantiomer of the 1:1 racemate citalopram, has the highest selectivity for 5-HT in the central nervous system (Plenge and Mellerup 1997; Owens et al. 2001; Sánchez et al. 2003a). Human SSRI use is reflected in environmental registrations, and the potential ecological consequences for aquatic wildlife have recently been partially revealed. In Scandinavia, citalopram has been quantified in sewage treatment plant effluents at levels ranging from 9.2 to 720 ng/L (Vasskog et al. 2006, 2008; Wahlberg 2008; Fick et al. 2011; Krog et al. 2015), and similar levels have been detected globally (Silva et al. 2012). Furthermore, samples from surface freshwaters in Denmark and Sweden contained citalopram concentrations ranging from 3 to 92 ng/L and 6.6 to 210 ng/L, respectively (Fick et al. 2011; Kragelund et al. 2015). To the best of our knowledge, there are no data on the presence of escitalopram in the environment. The SSRIs were developed for humans but may equally influence aquatic organisms at different trophic levels and ultimately disturb natural food webs and thus aquatic communities. It has been demonstrated that SSRIs bioconcentrate in different fish tissues, particularly the brain, and in other aquatic organisms in the aquatic food chain (Brooks et al. 2005; Nakamura et al. 2008; Schultz et al. 2010; Grabicova et al., 2014, 2015; Boström et al. 2017). The SERTs are comparable across different vertebrate taxa, and the 5-HT system is remarkably conserved in animal evolution (Wang et al. 2006; Lillesaar 2011). Among a wide array of physiological states, functions, and behaviors

This article includes online-only Supplemental Data.
* Address correspondence to baatrup@bios.au.dk
Published online 6 August 2019 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.4474

(Kreke and Dietrich 2008; Lillesaar 2011; Gaspar and Lillesaar 2012; Maximino et al. 2013; Prasad et al. 2015; Winberg and Thörnqvist 2016), serotonergic activity has been implicated in the control of food intake in fish (Matsuda 2009; Kellner et al. 2015). Previous fish studies have demonstrated that the SSRI fluoxetine can regulate neuropeptides as well as metabolic parameters and related enzymes controlling energy balance (Mennigen et al. 2009, 2010). Most of the relevant SSRI studies have used fluoxetine, but sertraline and citalopram have also been reported to inhibit fish foraging behavior (Stanley et al. 2007; Valenti et al. 2009; Mennigen et al. 2009, 2010; Hedgespeth et al. 2014; Weinberger and Klaper 2014); data on possible similar effects of escitalopram are lacking. Food intake determines the ability to maintain internal homeostasis and the allocation of energy to growth and reproduction. Thus, appropriate feeding behavior is vital for individual survival, development, and Darwinian fitness.

We recently reported the first results of the effects of escitalopram on fish behavior, indicating a negative ecological impact of this SSRI on wild fish species (Nielsen et al. 2018). That study demonstrated disturbed spontaneous swimming behavior in zebrafish (*Danio rerio*) and a possible negative effect on growth in both sexes after a 3-wk exposure. After exposure to 1.50 µg/L of escitalopram, females in particular displayed a higher activity level in the test tank center at the expense of thigmotaxis compared with unexposed individuals (Nielsen et al. 2018).

The present study aimed to evaluate the effects of escitalopram on fish foraging behavior and growth. Zebrafish of both sexes were exposed to nominal escitalopram concentrations of 0.00, 0.15, and 1.50 µg/L for 3 wk in flow-through water tanks. In contrast to other SSRI studies, we used a video tracking system with high temporal and spatial resolution to quantify the feeding behavior profile of the test fish. The impact of escitalopram on growth was evaluated by measuring the weight and length of the zebrafish before and after exposure. In our previous study (Nielsen et al. 2018), we found that general swimming behavior in females was much more vulnerable to escitalopram exposure than it was in males. We therefore also postulated a more pronounced response in female feeding behavior compared with males.

## MATERIALS AND METHODS

### Setup and experimental design

Four hundred adult 4-mo-old wild-type zebrafish (*D. rerio)* of both sexes were obtained from Credo Fish and kept in a 130 × 90 × 30 cm (1.1 fish/L) stainless-steel stock tank at the Institute of Bioscience, Aarhus University, Aarhus, Denmark. The fish were acclimatized for at least 9 d before experimentation. The photoperiod was 12:12-h light:dark, with a gradual increase in light intensity in the morning to simulate sunrise. A header tank supplied water to the stock tank and exposure tanks, which contained aerated, demineralized water mixed with local tap water (16:1) and added saline (NaCl) to give a conductivity of 223 ± 11 µS $cm^{-1}$. The stock tank water was constantly circulated through a biofilter. Approximately 30% of the stock water was exchanged twice weekly (on Mondays and Fridays). Heating elements were used to maintain the water temperature in the stock tank at 27 ± 0.5 °C. The zebrafish were fed daily with TetraMin® (Tetra Werke) flake fodder, adjusted so that all food was eaten within 5 min (the 5-min rule; Lawrence 2007), but not eaten within 1 min. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The experiments were conducted in accordance with the guidelines of The Danish Animal Experiments Inspectorate (permission 2012-15-2934-00246).

Six glass exposure tanks (47 × 29 × 19 cm), each containing approximately 26 L of water, were used for the 3 treatment groups (2 tanks/treatment). A peristaltic pump (Ole Dich Instrument Makers) ensured a constant flow of water from the common header tank to the 6 exposure tanks at a flow rate of approximately 47 L/d. The water of this continuous flow-through system left the tanks through an outlet with a mesh to retain the fish. Heating elements kept the water temperature constant at 27 °C.

### Escitalopram exposure

The 3 zebrafish treatment groups were exposed to nominal concentrations of 0.00, 0.15, and 1.50 µg /L, respectively, of escitalopram oxalate ((*S*)-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, oxalate; CAS 128196-01-0; purity 99.8% w/w), kindly donated by Lundbeck Pharma (Copenhagen, Denmark). A stock solution was prepared 1 d before the 3-wk exposure period by dissolving 107 mg escitalopram oxalate in 1000 mL Milli-Q water, stored in the dark at 5 °C. Two working solutions (2 L) were made from this stock solution with escitalopram concentrations at 55.3 and 564.5 µg/L. Both working solutions were prepared fresh every week and kept in the dark at 4 °C. A programmable peristaltic pump (Ismatec IPC-N; IDEX Health and Science) was used to dose the 2 working solutions and a control solution constantly with Milli-Q water at 126 mL $d^{-1}$ to the inlet water using syringe needles.

Each of the 3 treatment groups consisted of 2 exposure tanks, for a total of 6 tanks. Individual fish were preliminarily sexed by their typical secondary sexual characteristics. A typical male zebrafish has nonvisible papillae, a slim body shape, a reddish coloration, and a large anal fin with distinct markings. A typical female zebrafish has a large visible urogenital papilla, a round body shape, a bluish coloration, and indistinct anal fin coloration (Eaton and Farley 1974; Brion et al. 2004; Nash et al. 2004). Then female and male fish were sequentially distributed to the 6 exposure tanks so that each tank contained 15 presumed females and 15 presumed males (1.1 fish/L, for a total of 180 fish). Later, after behavioral measurements, we determined the true gender by identification of the gonads. Behavioral measurements of each treatment group were completed in 2 d, and thus we delayed the start of exposure for each treatment by 2 d, to ensure the same exposure period for the 3 treatment groups.

Just before the exposure period, the length and weight of the zebrafish were measured. One by one, the zebrafish were

 

anesthetized in a benzocaine solution of 0.1 g/L, as recommended (Matthews and Varga 2012; Sneddon 2012; Olt et al. 2016) and then weighed. To measure length, the left side of each zebrafish was photographed with an Olympus SZ 40 dissection microscope equipped with a Moticam 1000 1.3 M Pixel USB 2.0 camera. Fish lengths were determined using Motic Images Plus 2.0 ML software (Motic China Group). During the exposure period, zebrafish were fed daily at 2 PM with TetraMin flake fodder corresponding to 5% of total fish weight. Fifteen minutes after feeding, feces and remaining fodder were removed from the exposure tanks. The oxygen content in the exposure tanks was 98.0 ± 0.3% of air saturation, which is within the parameters of Organisation for Economic Co-operation and Development test guideline 210 (1992); the pH was 7.02 ± 0.01. As defined by the Tetratest Kit (Tetra Werke), levels of ammonium (<0.25 mg/L), nitrate (<25 mg/L), and nitrite (<0.3 mg/L) were consistently acceptable. Water samples from each exposure tank were collected weekly (on Wednesdays) for quantification of the actual escitalopram concentrations. The flow-through system ensured that the aquaria concentrations of escitalopram were constant during the exposure period. The procedure for analysis is described in Nielsen et al. (2018). Briefly, water samples were immediately frozen and stored at −18 °C before analysis by high-performance liquid chromatography–mass spectrometry (HPLC–MS) without any further sample preparation. Twenty-five µL were injected into the HPLC column. The HPLC–MS system used consisted of a Hewlett-Packard 1100 series chromatograph equipped with a QuadPump, a degasser, a column oven (ColComp), an Autosampler (Automatic Liquid Sampler), and a Mass Selective Detector (MSD). Data were collected using the HP ChemStation software, Ver 6.03 (Hewlett-Packard). The analytical column was a reversed phase Kinetex C18 (Phenomenex) column (4.6 mm i.d. × 50 mm, 2.6-µm particles). A linear gradient system was applied. Eluent A consisted of 0.1% v/v formic acid in Milli-Q water, and eluent B consisted of 0.1% v/v formic acid in methanol. The gradient was applied from 30% B to 90% v/v B from 0 to 4 min, maintained at 90% B for 1 min, and then returned to 30% B v/v over 0.5 min. The flow rate was 0.5 mL/min. The MSD was equipped with an electrospray interface and was used in positive mode for single ion monitoring detection of the mass:m/z = 325.1.

## Swimming and feeding behavior measurements

The day before the behavior measurements, the zebrafish were fed 25% of the normal food amount to stimulate appetite and feeding behavior. Feeding behavior of 2 zebrafish was measured simultaneously in 2 test tanks (28 × 21 × 13 cm) each containing approximately 3.8 L of water at 27 °C. A 2-cm-diameter fodder ball, composed of TetraMin flake fodder embedded in gelatin, was placed on a stick in the middle of each tank. Initially, each treatment group consisted of approximately 30 males and 30 females. Half an hour before the 30-min measuring period, one presumed male and one presumed female were transferred to the 2 test tanks containing clean fresh water.

The general swimming and feeding behaviors of male and female zebrafish were quantified. A detailed description of the general swimming behavior recording is given in Nielsen et al. (2018). In brief, the test tanks were placed on a glass plate illuminated from below, producing clear silhouettes of the 2 fish when viewed from above by the camera. The camera signals were analyzed using the MOTIO vision system (Department of Biological Sciences, University of Aarhus, Aarhus, Denmark), establishing the positions of the 2 fish at a frame rate of 15 Hz. The interior of each of the 2 test tanks was framed by a software window area of interest (AOI), giving a spatial pixel resolution of 0.39 mm of the visual field. Our quality criterion was that the fish should be visible for at least 70% of the recording time to qualify for further analyses. A second 100 × 100-mm software window region of interest (ROI) framed the centrally placed food source (Figure 1A). Eight behavioral parameters were selected for the analyses of general swimming behavior in the whole tank area (area of interest), including: 1) total swimming distance (m); 2) maximum swimming velocity maintained for 1 s (mm $s^{-1}$); 3) average swimming velocity (mm $s^{-1}$); 4) turning rates swum (degrees $s^{-1}$); 5) turning bias between right (negative degrees $s^{-1}$) and left turns (positive degrees $s^{-1}$); 6) time spent in tank center (region of interest [s]); 7) swimming distance in region of interest (m); and 8) number of visits to the region of interest. Feeding behavior was quantified by: 1) number of visits to the fodder ball; 2) average duration of feeding visits (s); 3) maximum feeding duration (s); and 4) total duration of feeding episodes (s). A feeding event was recognized and quantified when the 2 silhouettes of zebrafish and fodder ball merged in the region of interest (Figure 1), defined as an active feeding behavior.

## Determination of length, weight, and gender

After the behavioral measurements, the 2 zebrafish were euthanized in 0 °C water, wiped off, weighed, and

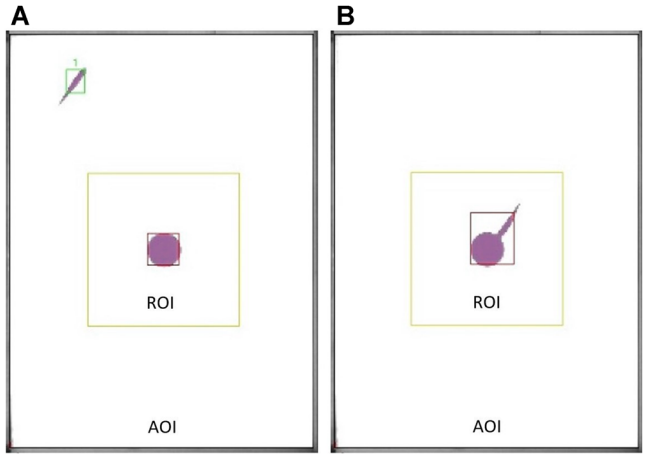


**FIGURE 1:** The zebrafish and fodder ball (**A**) were identified as a dark silhouettes against the light background. When the fish optically merged with the fodder ball (**B**) within the region of interest (ROI), the combined area exceeded the area range set for the fish. This was marked in the data file, from which the number and duration of feeding events were calculated. The area of interest (AOI) represents the whole observation area.

 

photographed as described above in the *Swimming and feeding behavior* section. "Before treatment" and "after treatment" pictures of the fish were used to identify each zebrafish. The ornamentation of the fish body, especially the stripes on the tail fin, was used to positively identify each individual, and thus possible effects of the exposures on length and weight could be established. The gender of the 2 fish was finally established by examination of the gonads.

### Statistical analysis

Initially, data were tested for normality (Kolmogorov–Smirnov and Shapiro–Wilk tests) and homogeneity of variance (Levene's test). When data did not comply with normality, simple log-transformations were applied resulting in normality. Comparisons of general swimming behaviors were accomplished using a multivariate general linear model with Tukey's honestly significant difference test as the post hoc test and fish weight as the covariate. Given that there were no interactions between sex and treatment for the general swimming parameters, the 2 genders were pooled and analyzed together. For the feeding behavior endpoints, there were significant interactions between sex and treatment. Accordingly, female and male zebrafish were treated separately using analysis of variance and Tukey's honestly significant difference post hoc test. All statistical tests were performed in SPSS 25.0 for Windows (IBM), and data are presented as mean values ± standard error of the mean with a significance level of 0.05. There were no statistical differences between the 2 tanks from each treatment for any of the measured endpoints. Data from the 2 tanks were therefore pooled. In total, 69 males and 71 females were monitored in the test tanks. In the statistical analysis of zebrafish swimming behavior, 20, 23, and 22 females and 22, 23, and 22 males in the 0.00, 0.10, and 1.50 μg/L treatment groups, respectively, met the 70% visibility criterion and consequently were used for further analysis (Table 1). The numbers of female and male zebrafish used in the analyses of the 6 feeding parameters were 18, 20, and 19 and 16, 18, and 17 for the 3 treatment groups, respectively (Tables 2 and 3). The exception was number of visits to the fodder ball, for which the distribution was 21, 22, and 22 females and 19, 22, and 20 males for the control groups and the 2 escitalopram concentrations, respectively.

## RESULTS

### Actual water concentrations of escitalopram

The actual escitalopram concentrations for the nominal concentrations of 0.00, 0.15, and 1.50 μg/L treatment groups were 0.00 ± 0.00, 0.10 ± 0.003, and 1.50 ± 0.104 μg/L, respectively. In the following text below and in the figures, the actual concentrations of 0.00, 0.10 and 1.50 μg/L are given.

### Mortality

During exposure, a total of 15 females and 8 males died (corresponding to 13% of all exposed zebrafish). Specifically, the lethality distribution was 2 males and 5 females in the

**TABLE 1:** Swimming behavior in female and male (pooled) zebrafish[a]

| Behavioral component | Treatment (μg/L) | Measure ± SEM | Test | Comparison (μg/L) | Tukey's post hoc *p* value |
|---|---|---|---|---|---|
| Swimming distance (m) | 0.00 ($n$ = 20) | 123.0 ± 4.2 | GLM | 0.00–0.10 | 0.001* |
| | 0.10 ($n$ = 23) | 145.8 ± 5.0 | | 0.00–1.50 | 0.325 |
| | 1.50 ($n$ = 22) | 131.8 ± 3.5 | | 0.10–1.50 | 0.055 |
| Maximum velocity (mm $s^{-1}$) | 0.00 ($n$ = 20) | 277.9 ± 14.5 | GLM | 0.00–0.10 | 0.401 |
| | 0.10 ($n$ = 23) | 299.4 ± 9.5 | | 0.00–1.50 | 0.977 |
| | 1.50 ($n$ = 22) | 281.3 ± 11.5 | | 0.10–1.50 | 0.522 |
| Average velocity (mm $s^{-1}$) | 0.00 ($n$ = 20) | 72.7 ± 2.9 | GLM | 0.00–0.10 | 0.009* |
| | 0.10 ($n$ = 23) | 83.8 ± 2.7 | | 0.00–1.50 | 0.506 |
| | 1.50 ($n$ = 22) | 76.9 ± 2.4 | | 0.10–1.50 | 0.146 |
| Turning rate (degrees $s^{-1}$) | 0.00 ($n$ = 20) | 262.6 ± 7.1 | GLM | 0.00–0.10 | 0.065 |
| | 0.10 ($n$ = 23) | 239.9 ± 6.8 | | 0.00–1.50 | 0.323 |
| | 1.50 ($n$ = 22) | 248.0 ± 9.0 | | 0.10–1.50 | 0.701 |
| Turning bias (degrees $s^{-1}$) | 0.00 ($n$ = 20) | –1.05 ± 1.14 | GLM | 0.00–0.10 | 0.302 |
| | 0.10 ($n$ = 23) | 1.61 ± 1.28 | | 0.00–1.50 | 0.352 |
| | 1.50 ($n$ = 22) | 1.46 ± 1.38 | | 0.10–1.50 | 0.996 |
| Time in ROI (s) | 0.00 ($n$ = 20) | 296.3 ± 30.5 | GLM | 0.00–0.10 | 0.142 |
| | 0.10 ($n$ = 23) | 227.1 ± 21.4 | | 0.00–1.50 | 0.261 |
| | 1.50 ($n$ = 22) | 238.4 ± 24.4 | | 0.10–1.50 | 0.948 |
| Path in ROI (m) | 0.00 ($n$ = 20) | 20.2 ± 1.9 | GLM | 0.00–0.10 | 0.991 |
| | 0.10 ($n$ = 23) | 19.9 ± 1.5 | | 0.00–1.50 | 0.466 |
| | 1.50 ($n$ = 22) | 17.4 ± 1.5 | | 0.10–1.50 | 0.535 |
| No. of visits to ROI | 0.00 ($n$ = 20) | 209.7 ± 11.9 | GLM | 0.00–0.10 | 0.008* |
| | 0.10 ($n$ = 23) | 270.0 ± 16.9 | | 0.00–1.50 | 0.979 |
| | 1.50 ($n$ = 22) | 205.8 ± 12.6 | | 0.10–1.50 | 0.003* |

[a]Swimming behavior measurements after 21 d of exposure to 0.00, 0.10, and 1.50 μg/L escitalopram.
*$p < 0.05$.
SEM = standard error of the mean; ROI = region of interest; GLM = general linear model.

**TABLE 2:** Feeding behavior measurements in female zebrafish[a]

| Behavioral component | Treatment (µg/L) | Measure ± SEM | Test | *F* value | Comparison (µg/L) | Tukey's post hoc *p* value |
|---|---|---|---|---|---|---|
| No. of feeding visits | 0.00 ($n = 19$) | 96.8 ± 26.5 | ANOVA | 0.891 | 0.00–0.10 | 0.902 |
| | 0.10 ($n = 22$) | 65.1 ± 13.2 | (log trans.) | | 0.00–1.50 | 0.999 |
| | 1.50 ($n = 20$) | 112.1 ± 29.0 | | | 0.10–1.50 | 0.919 |
| Average duration of feeding visits (s) | 0.00 ($n = 16$) | 0.360 ± 0.031 | ANOVA | 0.180 | 0.00–0.10 | 0.383 |
| | 0.10 ($n = 18$) | 0.298 ± 0.023 | (log trans.) | | 0.00–1.50 | 0.169 |
| | 1.50 ($n = 17$) | 0.288 ± 0.033 | | | 0.10–1.50 | 0.855 |
| Maximum duration of feeding visits (s) | 0.00 ($n = 16$) | 1.62 ± 0.26 | ANOVA | 0.032 | 0.00–0.10 | 0.027* |
| | 0.10 ($n = 18$) | 0.96 ± 0.10 | | | 0.00–1.50 | 0.174 |
| | 1.50 ($n = 17$) | 1.17 ± 0.15 | | | 0.10–1.50 | 0.682 |
| Total feeding duration (s) | 0.00 ($n = 16$) | 45.4 ± 15.2 | ANOVA | 0.753 | 0.00–0.10 | 0.742 |
| | 0.10 ($n = 18$) | 24.7 ± 5.59 | (log trans.) | | 0.00–1.50 | 0.860 |
| | 1.50 ($n = 17$) | 39.1 ± 9.64 | | | 0.10–1.50 | 0.976 |

[a]Feeding behavior after 21 d of exposure to 0.00, 0.10, and 1.50 µg/L escitalopram.
*$p < 0.05$.
SEM = standard error of the mean; ANOVA = analysis of variance.

control groups, 4 females exposed to 0.10 µg/L escitalopram, and 6 males and 6 females exposed to 1.50 µg/L escitalopram.

## Length and weight

The number of test fish used in the analyses of length and weight included 23 females and 23 males in the control groups, 24 females and 24 males exposed to 0.10 µg/L, and 22 individuals of each sex exposed to 1.50 µg/L escitalopram. Escitalopram had no measurable effects on length or weight in female or male zebrafish (Figure 2).

## Swimming and feeding behavior

The general swimming behavior demonstrated no significant effects of escitalopram on the following parameters: mean maximum swimming velocity maintained for 1 s (mm $s^{-1}$), turning rate/s swum (degrees $s^{-1}$), turning bias between right (negative degrees $s^{-1}$) and left turns (positive degrees $s^{-1}$), time spent in the central area of the tank (region of interest), and distance swum in the region of interest (data not shown). In contrast, fish exposed to 0.10 µg/L escitalopram traveled a significantly ($p = 0.001$) longer distance (145.8 ± 5.0 m) in the test tank compared with the 123.0 ± 4.2 m of the unexposed individuals (Table 1). This 0.10 µg/L group also swam with a significantly ($p = 0.009$) higher average velocity (83.8 ± 2.7 mm $s^{-1}$) than the control group (72.7 ± 2.9 mm $s^{-1}$). In contrast, between controls and the fish exposed to 1.50 µg/L escitalopram, there was no statistical difference in swimming distance (131.7 ± 3.5 m) and average swimming velocity (76.9 ± 2.4 mm $s^{-1}$; Table 1).

Feeding behavior was more affected by escitalopram in males than in females. In males, a significant reduction in 3 of 4 of the selected foraging parameters was evident for at least one of the 2 escitalopram concentrations. At the lowest escitalopram concentration (0.10 µg/L), male average feeding time/visit and maximum feeding duration were significantly reduced (Figure 3E and F and Table 3). Also in males, a 3-wk escitalopram exposure to 1.50 µg/L resulted in a significant reduction in 3 of 4 feeding parameters, the only exception being males' number of feeding visits (Figure 3D–F and Table 3). However, there was a strong trend toward reduced feeding visits in males with increasing escitalopram concentrations (Figure 3D). Males' number of feeding

**TABLE 3:** Feeding behavior measurements in male zebrafish[a]

| Behavioral component | Treatment (µg/L) | Measure ± SEM | Test | *F* value | Comparison (µg/L) | Tukey's post hoc *p* value |
|---|---|---|---|---|---|---|
| No. of feeding visits | 0.00 ($n = 21$) | 85.3 ± 19.9 | ANOVA | 0.160 | 0.00–0.10 | 0.253 |
| | 0.10 ($n = 22$) | 46.4 ± 10.2 | (log trans.) | | 0.00–1.50 | 0.187 |
| | 1.50 ($n = 22$) | 39.9 ± 8.04 | | | 0.10–1.50 | 0.978 |
| Average duration of feeding visits (s) | 0.00 ($n = 18$) | 0.434 ± 0.038 | ANOVA | 0.009 | 0.00–0.10 | 0.039* |
| | 0.10 ($n = 20$) | 0.318 ± 0.019 | (log trans,) | | 0.00–1.50 | 0.010* |
| | 1.50 ($n = 19$) | 0.308 ± 0.030 | | | 0.10–1.50 | 0.835 |
| Maximum duration of feeding visits (s) | 0.00 ($n = 18$) | 1.84 ± 0.25 | ANOVA | 0.002 | 0.00–0.10 | 0.014* |
| | 0.10 ($n = 20$) | 1.07 ± 0.11 | (log trans.) | | 0.00–1.50 | 0.004* |
| | 1.50 ($n = 19$) | 0.96 ± 0.09 | | | 0.10–1.50 | 0.859 |
| Total feeding duration (s) | 0.00 ($n = 18$) | 50.7 ± 14.2 | ANOVA | 0.039 | 0.00–0.10 | 0.096 |
| | 0.10 ($n = 20$) | 18.3 ± 4.6 | (log trans.) | | 0.00–1.50 | 0.049* |
| | 1.50 ($n = 19$) | 13.5 ± 2.4 | | | 0.10–1.50 | 0.939 |

[a]Feeding behavior after 21 d of exposure to 0.00, 0.10, and 1.50 µg/L escitalopram.
*$p < 0.05$.
SEM = standard error of the mean; ANOVA = analysis of variance.

 

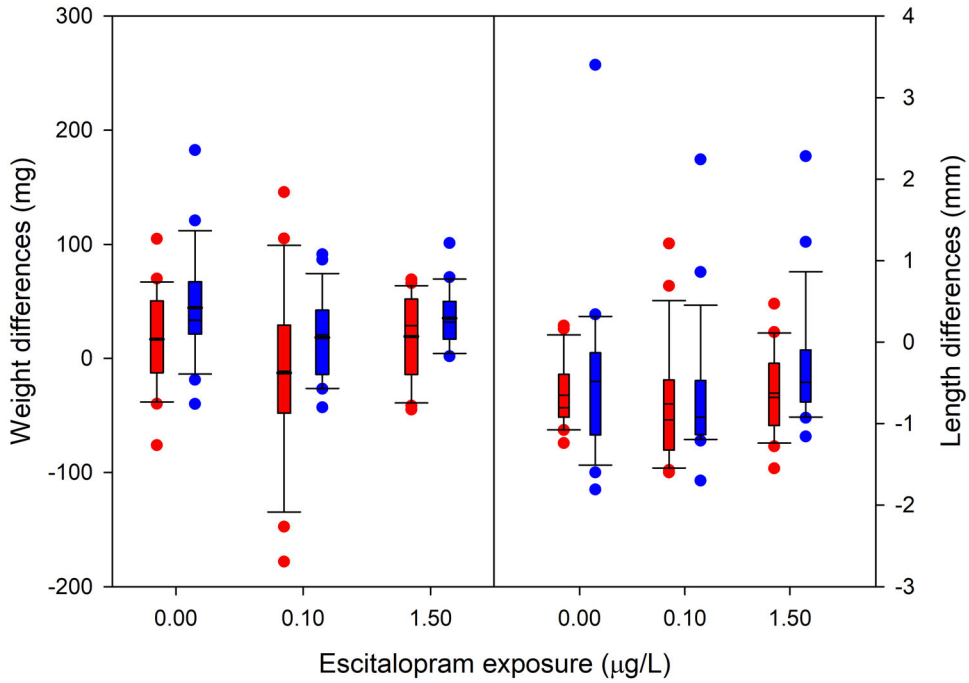


**FIGURE 2:** Box plot with median value, variation, and outliers, showing the differences in weight and length of female (red) and male (blue) zebrafish before and after 21 d of exposure to 2 concentrations of escitalopram and one control. There were no significant treatment effects.

visits decreased from 85 in the control group to 46 and 40 in the 0.10 and 1.50 µg/L treatment groups, respectively (Table 3). In females, only the maximum feeding duration at the lowest concentration (0.10 µg/L) was significantly reduced compared with unexposed individuals (Table 2).

## DISCUSSION

### *Mortality*

Mortality was relatively high in the present study compared with our previous experiment with escitalopram and zebrafish, in which no mortality was observed during the 3-wk exposure using similar concentrations and experimental design (Nielsen et al. 2018). The underlying cause of these lethal occurrences is unknown, but one obvious difference between the 2 studies is the anesthetization with benzocaine prior to the exposure period in the present study. However, it is worth noting that the swimming behavior of the surviving fish was comparable to that found in our previous study (Nielsen et al. 2018).

### *Length and weight*

Escitalopram had no measurable effects on length and weight among the treatment groups. This may well be due to the relatively short exposure period. Another study with the SSRI fluoxetine using goldfish (*Carassius auratus*) found weight loss in both sexes after 28 d of exposure to 54 µg/L (Mennigen et al. 2010). However, Kellner et al. (2015) found no effect on growth rate in the three-spined stickleback (*Gasterosteus aculeatus*) after 21 d of citalopram exposure (nominal concentrations of 0.15 and 1.50 µg/L in ambient water). No differences in weight were observed in rat experiments with citalopram and escitalopram after 5 wk of daily injections of the 2 drugs (Montgomery et al. 2001; Sánchez et al. 2003b). Future SSRI studies are needed in this regard.

### *Swimming behavior*

Swimming behavior is a widely used endpoint of chemical impacts on fish (e.g., Kellner et al. 2015; Storgaard et al. 2017; Nielsen et al. 2018). Kalueff et al. (2013) provide a comprehensive catalog of zebrafish behaviors. The presence of the fodder ball in the test tank center apparently had an overall stimulating effect on the swimming pattern including total swimming distance, average velocity, time spent in the region of interest, distance swum in the region of interest, and number of visits to the region of interest compared with the control groups in our previous study with no fodder ball in the test tanks (Nielsen et al. 2018). This finding indicates that the energy source and diffusive chemical cues in the water stimulated the general swimming and foraging behaviors of zebrafish.

Escitalopram at the lowest concentration increased the distance traveled and the average swimming velocity of the fish. At the highest escitalopram concentration, there were no differences in swimming behavior compared with the control fish. In our previous study (Nielsen et al. 2018), we found a comparable response pattern, and we also found an increased boldness in the zebrafish exposed to escitalopram. Together, increased swimming activity and boldness might increase vulnerability to predation.

 

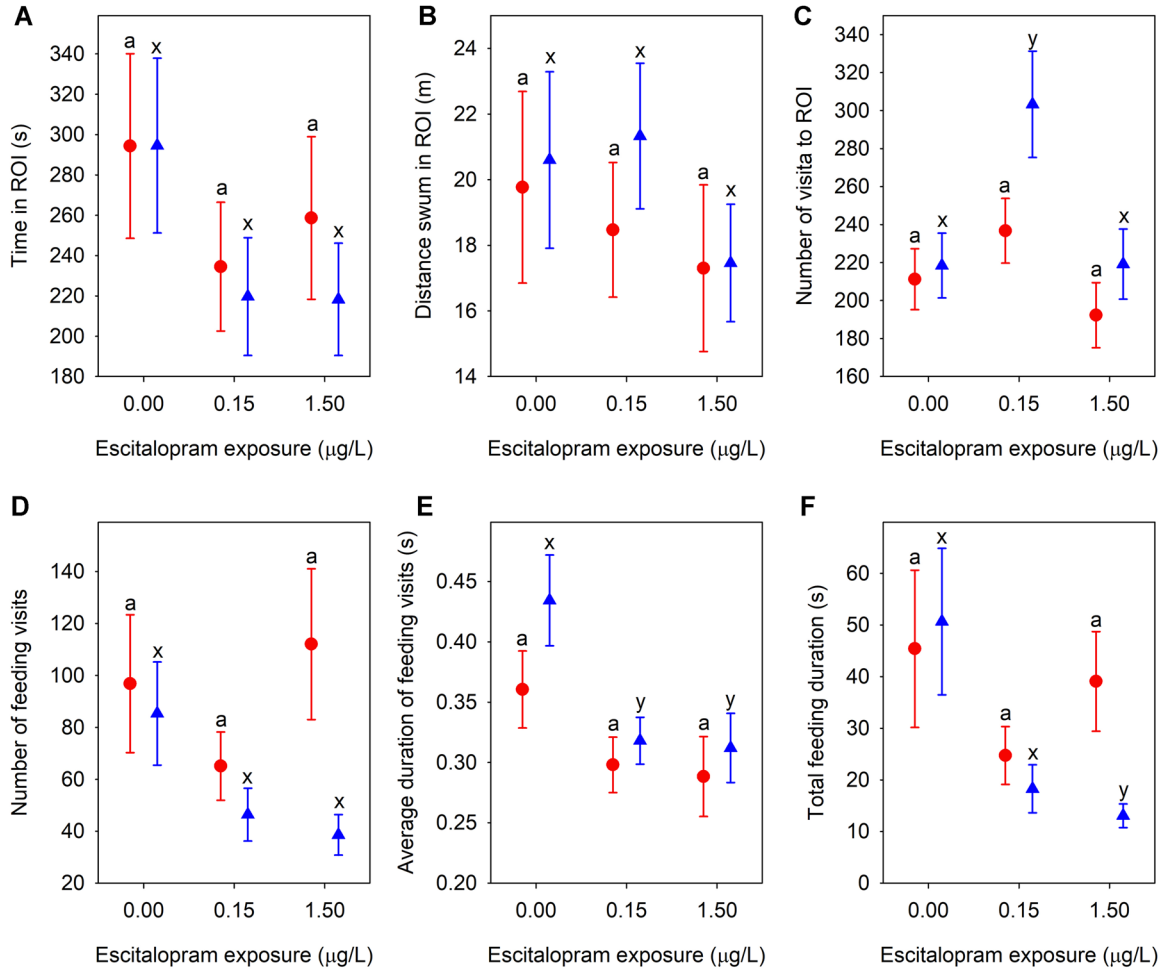


**FIGURE 3:** (A–F) Feeding behavior measurements in female (in red) and male (in blue) zebrafish after 21 d of escitalopram exposure at actual concentrations of 0.00, 0.10, and 1.50 µg/L. Feeding behavior measurements correspond to a quantification of merging silhouettes of test fish and the fodder ball in the test tank center (ROI = region of interest). Values are presented as means ± standard error of the mean, and different letters indicate significant differences (analysis of variance; Tukey post hoc test) with the significance level at 0.05.

## *Feeding behavior*

The analyses of escitalopram effects on zebrafish foraging behavior demonstrated a more pronounced inhibiting effect in males than in females, which negated our initial hypothesis. Suppressed foraging behavior at the lowest concentration (0.10 µg/L) is of concern in terms of possible ecological consequences.

The inhibiting effect of escitalopram on fish foraging behavior supports the findings of other SSRI experiments with different fish species (Stanley et al. 2007; Mennigen et al. 2009, 2010; Valenti et al. 2009; Hedgespeth et al. 2014; Weinberger and Klaper 2014), as well as with higher vertebrates including humans (Luo and Li 1990; Grignaschi et al. 1998; Li et al. 1998; Zendehdel et al. 2013). These results suggest that the inhibiting effect on food intake from increased 5-HT activity is conserved in the vertebrate phylum. Furthermore, SSRIs can inhibit territorial aggressiveness in fish (Lepage et al. 2005) and the ability to capture living prey (Gaworecki and Klaine 2008), which will most likely contribute to a more ineffective foraging behavior. Finally, it was found that SSRI exposure to other aquatic organism such as tadpoles (*Rana pipiens*) reduced weight gain, possibly due to decreased feeding time in exposed individuals (Foster et al. 2010). An inhibiting effect of escitalopram and other SSRIs on foraging behavior might result in a vertical echo in the trophic composition of wildlife aquatic food webs. However, the data we present are insufficient to conclude that escitalopram affects either zebrafish navigation after food or handling time of the food source. On the other hand, the data imply that foraging behavior is more sensitive to SSRIs than growth, which supports other SSRI experiments evaluating fish foraging behavior and growth (Stanley et al. 2007; Valenti et al. 2009; Kellner et al. 2015). Still, the possibility cannot be excluded that prolonged exposure will result in inhibition of growth. A fluoxetine study with male and female goldfish (*C. auratus*) demonstrated a decrease in both food intake and weight gain after 28 d of exposure to 54 µg/L (Mennigen et al. 2010). To our knowledge, experiments demonstrating sex-specific effects of SSRIs on fish feeding behavior are still lacking.

Contrary to most other SSRI studies assessing fish behavior, the present study demonstrates sex-dependent responses, which highlights the importance of separating the 2 sexes during

 

a study. However, the more pronounced inhibiting effect on the foraging behavior in males compared with females is at variance with our initial expectation based on our previous study, in which females were the more sensitive sex in terms of boldness (Nielsen et al. 2018). This finding indicates that SSRI effects might depend both on gender and on the behavior under evaluation, introducing a greater complexity in predicting SSRI responses in fish. Nevertheless, sex-specific patterns are worth exploring in future studies with escitalopram and other SSRIs.

In conclusion, a 3-wk exposure period with escitalopram predominantly inhibited male zebrafish foraging behavior at concentrations that might be environmentally relevant, given that escitalopram is discharged in similar quantities as other SSRIs. No effects on weight gain or length differences were found in either sex, but this factor definitely needs further study. Our results indicate that presumed relevant escitalopram concentrations have a negative impact on feeding behavior, in accordance with several other SSRI studies. Escitalopram and other SSRIs in polluted waters might have impacts on different trophic levels and thereby possess the potential to disturb natural food chains through inhibited foraging behavior. It remains to be determined whether inhibited foraging behavior translates into reduced food intake. Finally, in fish species, the behavioral consequences after SSRI exposure might be specific to sex, behavior, and particular SSRI, which further complicates the predictions in ecotoxicological SSRI studies assessing behavioral effects. The environmental relevance of SSRI impacts on fish foraging behavior, growth, and other behaviors remains to be explored.

*Supplemental Data*—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4474.

*Acknowledgment*—The present study was supported by the Faculty of Science and Technology, Aarhus University, Aarhus, Denmark. The authors are grateful to Lundbeck Pharma, Copenhagen, Denmark, for donating the pharmaceutical escitalopram oxalate.

*Data Accessibility*—Behavior data for all animals are available in the Supplemental Data.

## REFERENCES

Baumann P. 1996. Pharmacology and pharmacokinetics of citalopram and other SSRIs. *Int Clin Psychopharmacol* 11(Suppl 1):5–11.

Boström ML, Ugge G, Jönsson JÅ, Berglund O. 2017. Bioaccumulation and trophodynamics of the antidepressants sertraline and fluoxetine in laboratory-constructed, 3-level aquatic food chains. *Environ Toxicol Chem* 36:1029–1037.

Brion F, Tyler C, Palazzi X, Laillet B, Porcher J, Garric J, Flammarion P. 2004. Impacts of 17β-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (*Danio rerio*). *Aquat Toxicol* 68:193–217.

Brooks BW, Chambliss CK, Stanley JK, Ramirez A, Banks KE, Johnson RD, Lewis RJ. 2005. Determination of select antidepressants in fish from an effluent-dominated stream. *Environ Toxicol Chem* 24:464–469.

Eaton RC, Farley RD. 1974. Spawning cycle and egg production of zebrafish, *Brachydanio rerio*, in the laboratory. *Copeia* 1974:195–204.

Fick J, Lindberg RH, Kaj L, Brorström-Lundén E. 2011. Results from the Swedish National Screening Programme 2010 (Subreport 3. Pharmaceuticals). Swedish Environmental Research Institute, Stockholm, Sweden.

Foster HR, Burton AG, Basu N, Werner EE. 2010. Chronic exposure to fluoxetine (Prozac) causes developmental delays in *Rana pipiens* larvae. *Environ Toxicol Chem* 29:2845–2850.

Gaspar P, Lillesaar C. 2012. Probing the diversity of serotonin neurons. *Philos Trans R Soc B* 367:2382–2394.

Gaworecki KM, Klaine SJ. 2008. Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. *Aquat Toxicol* 88:207–213.

Grabicova K, Lindberg RH, Östman M, Grabic R, Randak T, Joakim Larsson DG, Fick J. 2014. Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. *Sci Total Environ* 488–489:46–50.

Grabicova K, Grabic R, Blaha M, Kumar V, Cerveny D, Fedorova G, Randak T. 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. *Water Res* 72:145–153.

Grignaschi G, Invernizzi RW, Fanelli E, Fracasso C, Caccia S, Samanin R. 1998. Citalopram-induced hypophagia is enhanced by blockade of 5-HT1A receptors: Role of 5-HT2C receptors. *Br J Pharmacol* 124:1781–1787.

Hedgespeth ML, Nilsson PA, Berglund O. 2014. Ecological implications of altered fish foraging after exposure to an antidepressant pharmaceutical. *Aquat Toxicol* 151:84–87.

Kalueff AV, Gebhardt M, Stewart AM, Cachat JM, Brimmer M, Chawla JS, Craddock C, Kyza EJ, Roth A, Landsman S, Gaikwad S, Robinson K, Baatrup E, Tierney K, Shamchuk A, Norton W, Miller N, Nicolson T, Braubach O, Gilman CP, Pittman J, Rosemberg DB, Gerlai R, Echevarria D, Lamb E, Neuhauss SCF, Weng W, Bally-Cuif L, Schneider H. 2013. Towards a comprehensive catalog of zebrafish behavior 1.0, and beyond. *Zebrafish* 10:70–86.

Kellner M, Porseryd T, Porsch-Hällström I, Hansen SH, Olsén KH. 2015. Environmentally relevant concentrations of citalopram partially inhibit feeding in the three-spine stickleback (*Gasterosteus aculeatus*). *Aquat Toxicol* 158:165–170.

Kragelund C, Litty K, Lindholst S, Langerhuus AT, Møller T, Rasmussen HU, Sundmark K, Sund C, Escolar M, Bester K. 2015. Miljø-og energieffektiv rensning af miljøfremmede stoffer i særligt belastet spildevand. Danish Nature Agency, Ministry of the Environment, Copenhagen, Denmark.

Kreke N, Dietrich DR. 2008. Physiological endpoints for potential SSRI interactions in fish. *Crit Rev Toxicol* 38:215–247.

Krog GF, Borksted B, Holm AG, Pedersen BM, Nielsen U, Jensen LM. 2015. NOVANA-Screening for humane lægemidler i vandmiljøet. Danish Nature Agency, Ministry of the Environment, Copenhagen, Denmark.

Lawrence C. 2007. The husbandry of zebrafish (*Danio rerio*): A review. *Aquaculture* 269:1–20.

Lepage O, Larson ET, Mayer I, Winberg S. 2005. Serotonin, but not melatonin, plays a role in shaping dominant–subordinate relationships and aggression in rainbow trout. *Horm Behav* 48:233–242.

Li DL, Simmons RM, Iyengar S. 1998. 5HT1A receptor antagonists enhance the functional activity of fluoxetine in a mouse model of feeding. *Brain Res* 781:121–128.

Lillesaar C. 2011. The serotonergic system in fish. *J Chem Neuroanat* 41:294–308.

Luo S, Li TSE. 1990. Food intake and selection pattern of rats treated with dexfenfluramine, fluoxetine and RU 24969. *Brain Res Bull* 24:729–733.

Matsuda K. 2009. Recent advances in the regulation of feeding behavior by neuropeptides in fish. *Ann N Y Acad Sci* 1163:241–250.

Matthews M, Varga ZM. 2012. Anesthesia and euthanasia in zebrafish. *ILAR J* 53:192–204.

Maximino C, Lima MG, Araujo J, Oliveira KRM, Herculano AM, Stewart AM, Kyzar EJ, Cachat J, Kalueff AV. 2013. The serotonergic system of zebrafish: Genomics, neuroanatomy and neuropharmacology. In Hall S, ed, *Serotonin: Biosynthesis, Regulation, and Health Implications*. Nova Science, New York, NY, USA, pp 53–67.

Mennigen JA, Harris EA, Chang JP, Moon TW, Trudeau VL. 2009. Fluoxetine affects weight gain and expression of feeding peptides in the female goldfish brain. *Regul Pept* 155:99–104.

Mennigen JA, Sassine J, Trudeau VL, Moon TW. 2010. Waterborne fluoxetine disrupts feeding and energy metabolism in the goldfish *Carassius auratus*. *Aquat Toxicol* 100:128–137.

 

Montgomery SA, Loft H, Sánchez C, Reines EH, Papp M. 2001. Escitalopram (S-enantiomer of citalopram): Clinical efficacy and onset of action predicted from a rat model. *Pharmacol Toxicol* 88:282–286.

Nakamura Y, Yamamoto H, Sekizawa J, Kondo T, Hirai N, Tatarazako N. 2008. The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish. *Chemosphere* 70:865–873.

Nash JP, Kime DE, Van der Ven LTM, Wester PW, Brion F, Maack G, Stahlschmidt-Allner P, Tyler CR. 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environ Health Perspect* 112:1725–1733.

Nielsen SV, Kellner M, Henriksen PG, Olsén H, Hansen SH, Baatrup E. 2018. The psychoactive drug escitalopram affects swimming behaviour and increases boldness in zebrafish (*Danio rerio*). *Ecotoxicology* 27:485–497.

Olt J, Allen CE, Marcotti W. 2016. In vivo physiological recording from the lateral line of juvenile zebrafish. *J Physiol* 594:5427–5438.

Organisation for Economic Co-operation and Development. 1992. Test No. 210: Fish, early life stage toxicity test. *OECD Guidelines for the Testing of Chemicals*. Paris, France.

Owens MJ, Knight DL, Nemeroff CB. 2001. Second-generation SSRIs: Human monoamine transporter binding profile of escitalopram and R-fluoxetine. *Biol Psychiatry* 50:345–350.

Plenge P, Mellerup ET. 1997. An affinity-modulating site on neuronal monoamine transport proteins. *Pharmacol Toxicol* 80:197–201.

Prasad P, Ogawa S, Parhar IS. 2015. Role of serotonin in fish reproduction. *Front Neurosci* 9:195.

Sánchez C, Bergqvist PBF, Brennum LT, Gupta S, Hogg S, Larsen A, Wiborg O. 2003a. Escitalopram, the S-(+)-enantiomer of citalopram, is a selective serotonin reuptake inhibitor with potent effects in animal models predictive of antidepressant and anxiolytic activities. *Psychopharmacology (Berl)* 167:353–362.

Sánchez C, Gruca P, Papp M. 2003b. R-citalopram counteracts the antidepressant-like effect of escitalopram in a rat chronic mild stress model. *Behav Pharmacol* 14:465–470.

Schultz MM, Furlong ET, Kolpin DW, Werner SL, Schoenfuss HL, Barber LB, Blazer VS, Norris DO, Vajda AM. 2010. Antidepressant pharmaceuticals in two US effluent-impacted streams: Occurrence and fate in water and sediment, and selective uptake in fish neural tissue. *Environ Sci Technol* 44:1918–1925.

Silva LJG, Lino CM, Meisel LM, Pena A. 2012. Selective serotonin re-uptake inhibitors (SSRIs) in the aquatic environment: An ecopharmacovigilance approach. *Sci Total Environ* 437:185–195.

Sneddon LU. 2012. Clinical anesthesia and analgesia in fish. *J Exot Pet Med* 21:32–43.

Stanley JK, Ramirez AJ, Chambliss CK, Brooks BW. 2007. Enantiospecific sublethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. *Chemosphere* 69:9–16.

Storgaard MS, Sanderson H, Henriksen PG, Fauser P, Östin A, Baatrup E. 2017. Suppressed swimming activity in zebrafish (*Danio rerio*) exposed to 1,4,5-oxadithiepane, a sulphur mustard degradation product. *Global Security: Health, Science and Policy* 4:22–28.

Valenti TW, Perez-Hurtado P, Chambliss CK, Brooks BW. 2009. Aquatic toxicity of sertraline to *Pimephales promelas* at environmentally relevant surface water pH. *Environ Toxicol Chem* 28:2685–2694.

Vasskog T, Berger U, Samuelsen P-J, Kallenborn R, Jensen E. 2006. Selective serotonin reuptake inhibitors in sewage influents and effluents from Tromsø, Norway. *J Chromatogr A* 1115:187–195.

Vasskog T, Anderssen T, Pedersen-Bjergaard S, Kallenborn R, Jensen E. 2008. Occurrence of selective serotonin reuptake inhibitors in sewage and receiving waters at Spitsbergen and in Norway. *J Chromatogr A* 1185:194–205.

Wahlberg C. 2008. Naturvårdsverket, Stockholm vatten, and IVL Svenska miljöinstitutet Avloppsreningsverkens förmåga att ta hand om läkemedelsrester och andra farliga ämnen: Redovisning av regeringsuppdrag. 512-386-06 Rm. Swedish Environmental Protection Agency, Stockholm, Sweden.

Wang Y, Takai R, Yoshioka H, Shirabe K. 2006. Characterization and expression of serotonin transporter genes in zebrafish. *Tohoku J Exp Med* 208:267–274.

Weinberger II J, Klaper R. 2014. 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). *Aquat Toxicol* 151:77–83.

Winberg S, Thörnqvist P-O. 2016. Role of brain serotonin in modulating fish behavior. *Curr Zool* 62:317–323.

Zendehdel M, Mokhtarpouriani K, Babapour V, Baghbanzadeh A, Pourrahimi M, Hassanpour S. 2013. The effect of serotonergic system on nociceptin/orphanin FQ induced food intake in chicken. *J Physiol Sci* 63:271–277.

 