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Effects of fluoxetine exposure on *Danio rerio*: A biochemical and behavioral perspective

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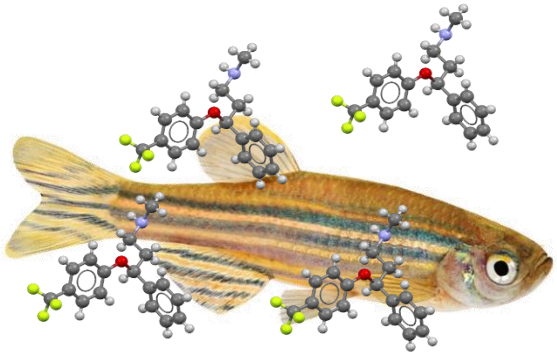
Effects of fluoxetine exposure on *Danio rerio*: A biochemical and behavioral perspective



Chronic exposure (21 days)

Behavioral effects (locomotor activity and thigmotaxis, novel tank and social behavior)

Biochemical effects

(ChE, LDH, GST and GPx)

Low concentrations can induce effects that may have consequences for the fish population

Abstract:

Psychiatric drugs have been increasingly prescribed and, due to a reduced efficiency of waste water treatment plants, their environmental levels have been increasing in aquatic ecosystems. Fluoxetine, selective serotonin reuptake inhibitor (SSRI), is among the pharmaceuticals most commonly detected in the aquatic environment. In this work, the effects of fluoxetine on zebrafish (*Danio rerio*) juveniles were evaluated assessing biochemical endpoints (e.g., effects on biotransformation – glutathione S transferase (GST), neurotransmission – acetylcholinesterase activity (AChE), energy metabolism - lactate dehydrogenase (LDH) and antioxidant defenses - glutathione peroxidase (GPx)) and behavior (swimming behavior, social behavior and thigmotaxis) after 21 days exposure to 0 (control) 0.1, 1 and 10 µg/L. Overall, fluoxetine induced no significant effects on neurotransmission and energy metabolism. The antioxidant enzyme glutathione peroxidase (GPx) and the phase II biotransformation enzyme GST presented decreased activities after exposure to 10 µg/L. In terms of behavior, exploratory and social behavior was not affected. However, the response to light and dark stimuli was affected, with fish exposed to the highest concentration swimming longer distances in the dark period. Overall, the data show that juvenile fish chronically exposed to fluoxetine may exhibit behavioral changes, affecting their ability to respond to environmental stressors and the interaction with other fish.

Keywords: Behavior; biomarkers; chronic effects; pharmaceuticals; selective serotonin reuptake inhibitors.

Introduction

Fluoxetine

- Psychiatric drug;
- Prescribed for the treatment of human depression, obsessive-compulsive disorder, anxiety, compulsive behavior, eating and personality disorders;
- **Mode of action:** selective serotonin reuptake inhibitor (SSRI);

1. Connection to presynaptic receptors
2. Blockage of serotonin (5-HT) transport
3. Accumulation of 5-HT in the synaptic cleft
4. Attenuation of anxiety and depressive symptoms (anxiolytic effect)

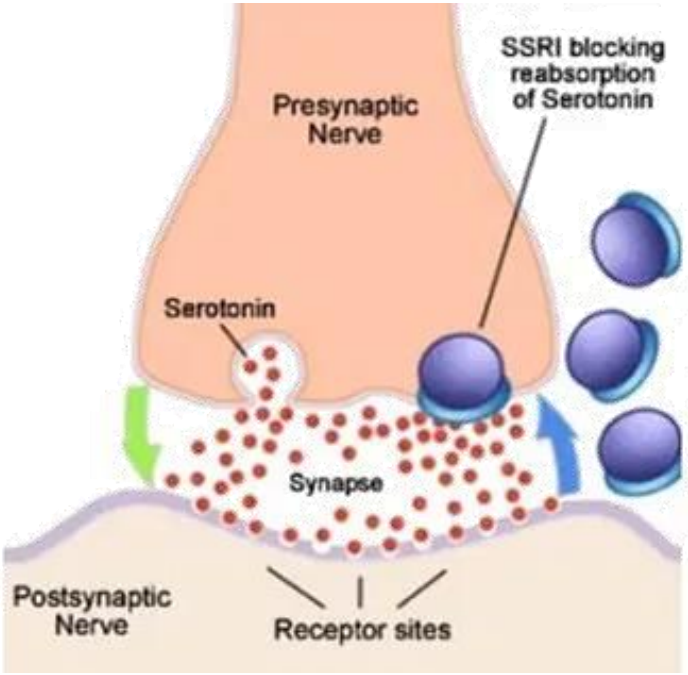


Fig. 1: SSRI mode of action
(Source: https://medicoapps.org/m-selective-serotonin-reuptake-inhibitors-ssri/)

Introduction

- **Levels in the aquatic environment:** 0.00015 to 32.2 µg/L (WWTP, surface, groundwater and drinking water) (Mezzelani et al., 2018; Silva et al., 2012);

- There are previous studies that have shown that fluoxetine can be toxic to fish after acute and chronic exposures;

- However, in these studies, higher concentrations are used, resulting in changes at different biological levels such as:
  - gene transcription;
  - neuronal markers;
  - enzymatic activities;
  - endocrine processes (e.g. cortisol levels);
  - histological alterations.

- In addition, there are studies that also indicate the effect of this drug on behavior (e.g., locomotor activity, stress response, eating, aggression and social behavior).

Introduction

Main objective: Evaluate the effects of a chronic exposure to low concentrations of fluoxetine at the biochemical and behavioral levels in juvenile zebrafish.

Duration: **21 days**

Concentrations: **0.1**, **1** and **10 µg/L** of fluoxetine

Behavior analysis (locomotor activity and thigmotaxis, novel tank, aggressiveness (mirror test) and social behavior) assessed **after 12- and 21-days exposure**

Biochemical endpoints (ChE, LDH, GST and GPx) were assessed after **21- days exposure,** using the whole body of the fish

Results and Discussion

1. Locomotor activity

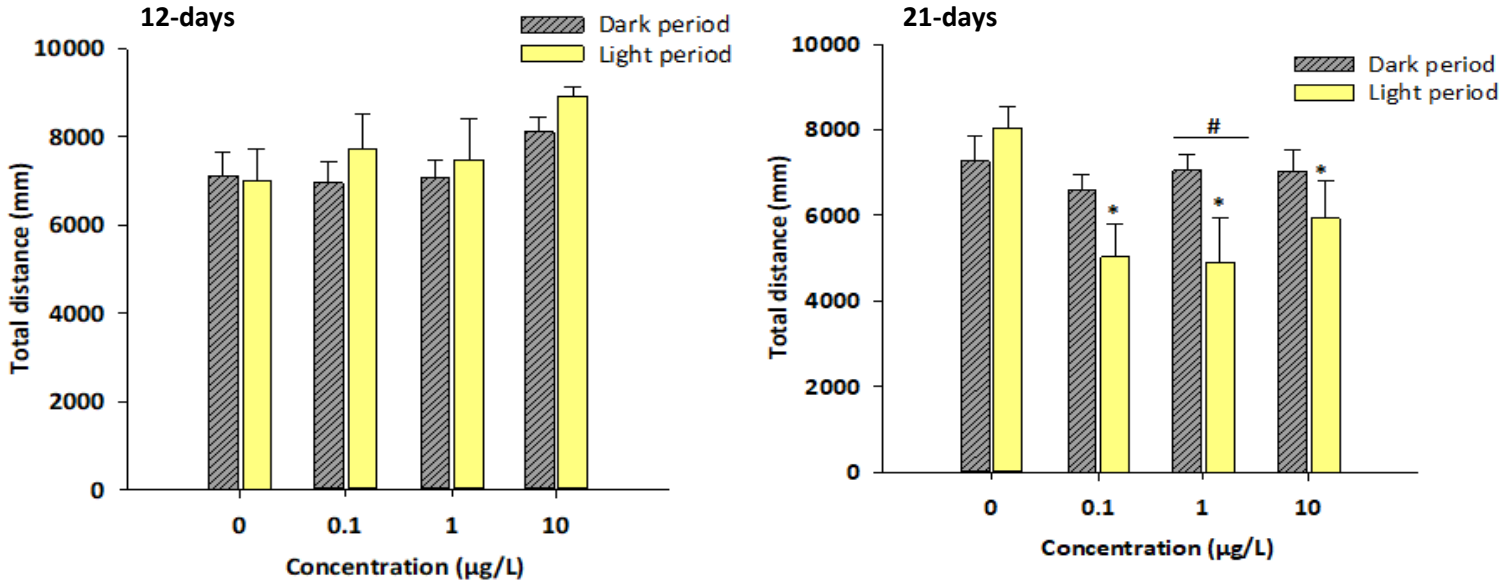


Fig. 2: Locomotor activity of juvenile zebrafish after 12 and 21 days (mean value ± standard error (SE)).

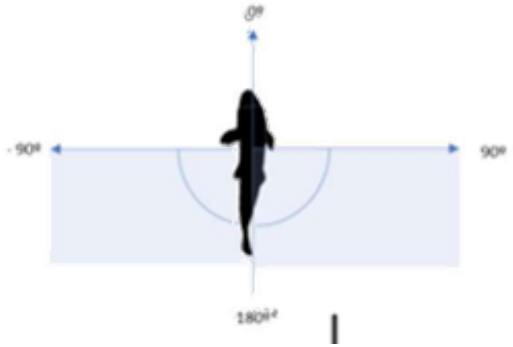
- **After 12-days exposure:** fish exposed to 10 µg/L of fluoxetine tended to swim longer distances in the dark and light periods, although without statistical significance;
- **After 21-days exposure:** fish exposed to fluoxetine showed significantly depressed swimming activity in light periods compared to control.

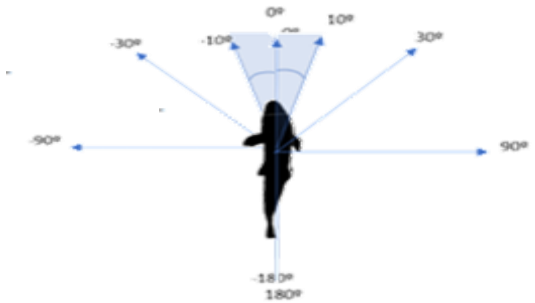
Zebrafish larvae exposed to several concentrations of the same drug (0.0003, 0.03, 0.43 and 4.48 µg/L) for 96h, reduced stress-related swimming activity in the dark period (Zindler et al., 2020).

Results and discussion

1. Locomotor activity (Path angles)

Class 1 angles (-90 to -180° and 90 to 180°)	Class 4 angles (-10° to 10°)
High amplitude angles are indicators of **erratic swimming behavior** (stress behavior)	**Low amplitude angles** are indicative of **straight movements/relaxation**
The decrease in their frequency manifests the anxiolytic effect of fluoxetine on the stress behavior of exposed juveniles.	**The increase during the dark period reveals a decrease in stress behavior in exposed juveniles.**





Results and discussion

1. Locomotor activity (Path angles- class 1)

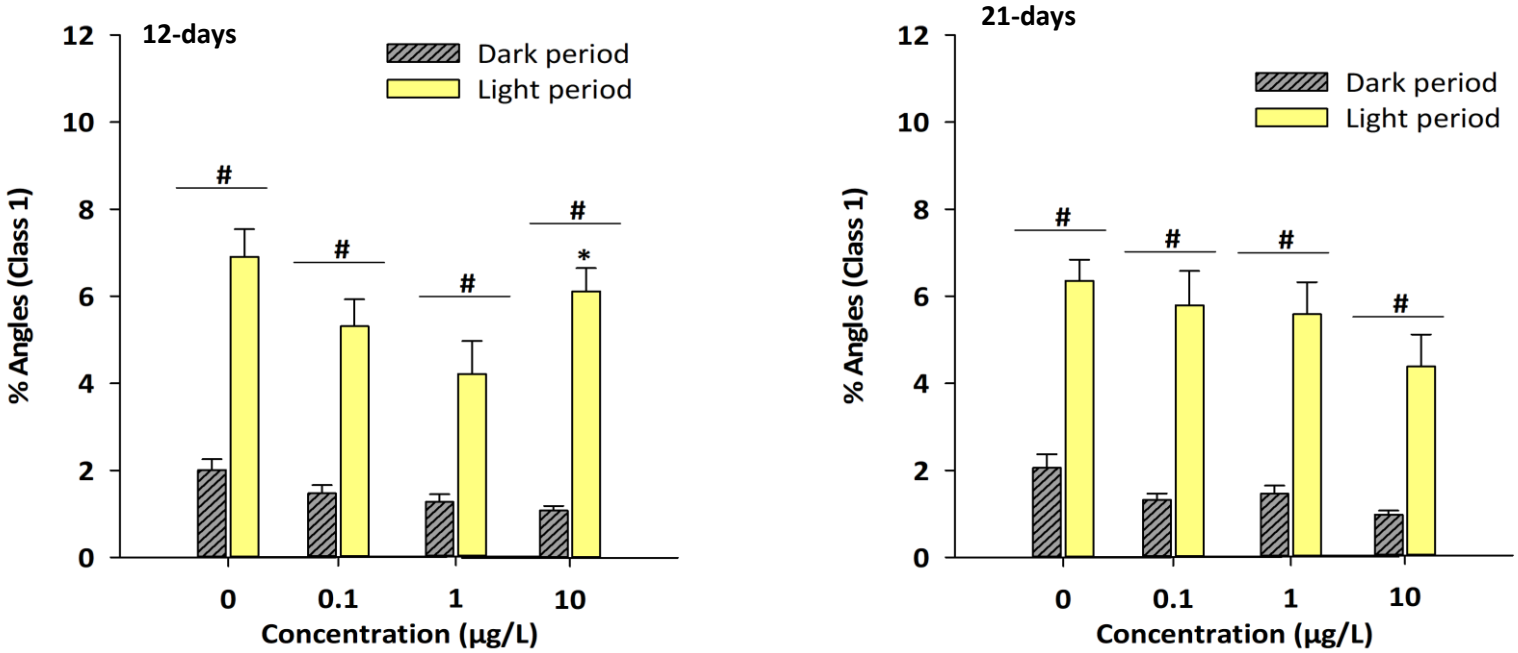


Fig. 3: Percentage of class 1 angles frequency (mean values ± standard error) after 12 and 21 days.

- **After 12-days exposure:** tendency to decrease the frequency of class 1 angles (high amplitude angles) in the dark period for all fluoxetine tested concentrations (without statistical significance). In the light, this trend was also observed for but only for 0.1 and 1 µg/L of fluoxetine. However, for the highest tested concentration (10 µg/L of fluoxetine) there was a significant increase in frequency, compared to the other fluoxetine concentrations;
- **After 21-days exposure:** there was a trend (without statistical significance) of decreasing frequency in the light period for all fluoxetine concentrations.

Results and discussion

1. Locomotor activity (Path angles- class 4)

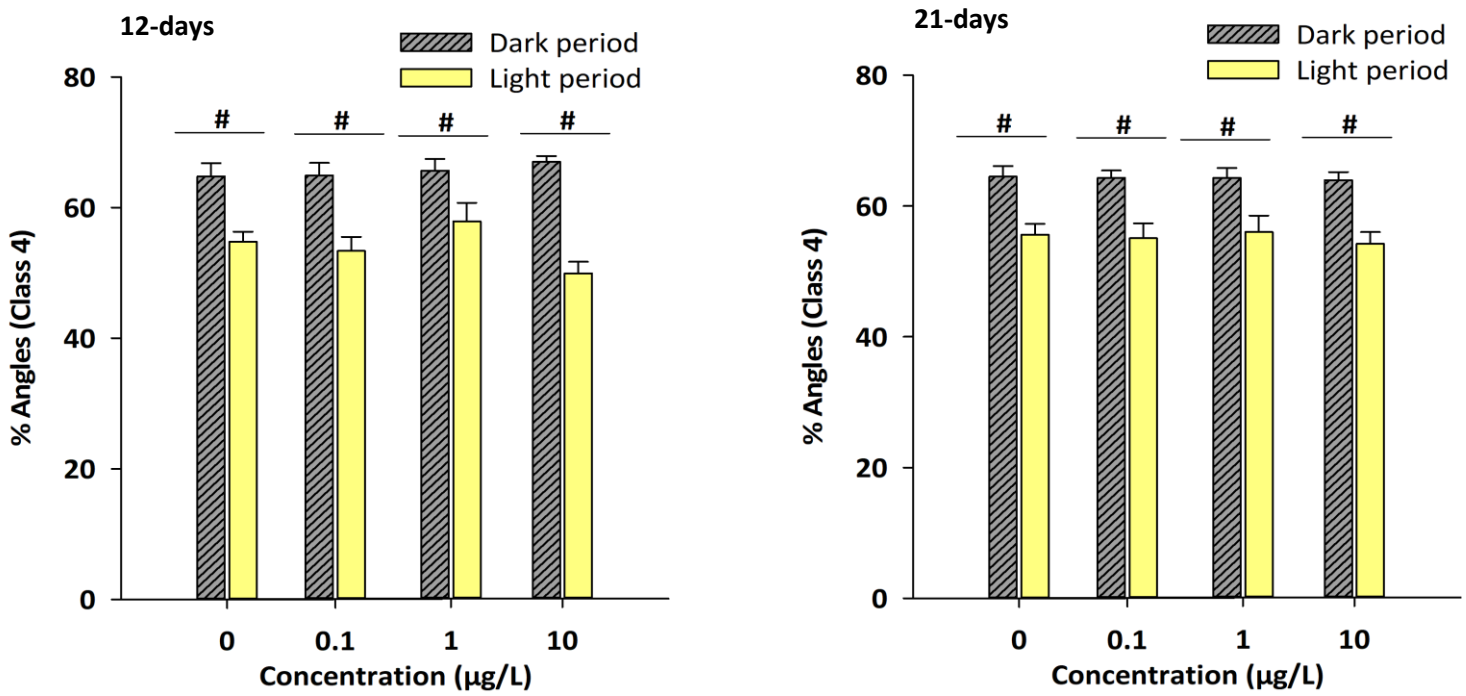


Fig. 4: Percentage of class 4 angles frequency (mean values ± standard error) after 12 and 21 days.

- **After 12- and 21-days exposure:** no effects of fluoxetine exposure were observed in this class of path angles.

Results and discussion

2. Thigmotactic behavior

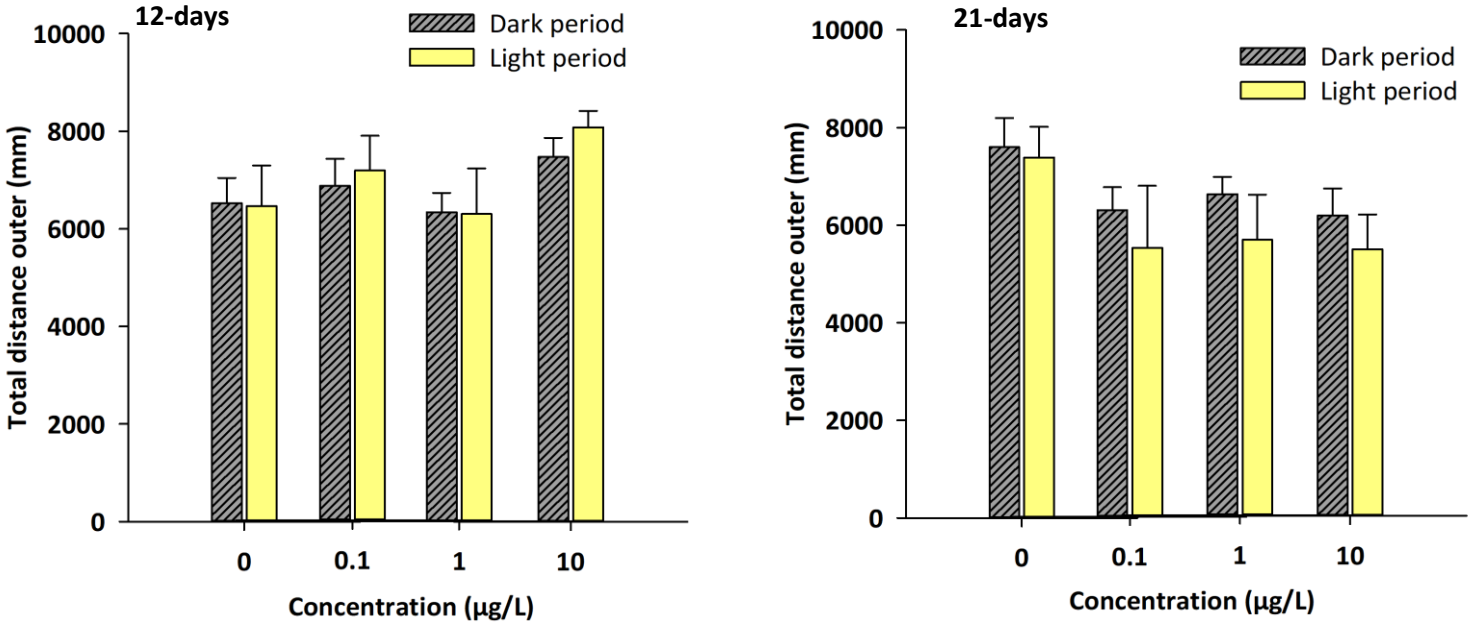


Fig. 5: Thigmotactic behavior of juvenile zebrafish after 12 and 21 days (mean value ± standard error (SE)).

- **After 12- and 21-days exposure:** no effect of fluoxetine exposure was observed on thigmotactic behavior.

Results and discussion

3. Novel tank/ Exploratory swimming test (21 days exposure)

- The time fish spent swimming in the three areas was not affected by fluoxetine exposure.

Drugs with anxiolytic properties have the ability to modify this behavior, leading the fish to explore the new space sooner and spend less time on the bottom (Bencan et al., 2009; Egan et al., 2009; Levin et al., 2007)

In the present study, no clear effects of fluoxetine were observed

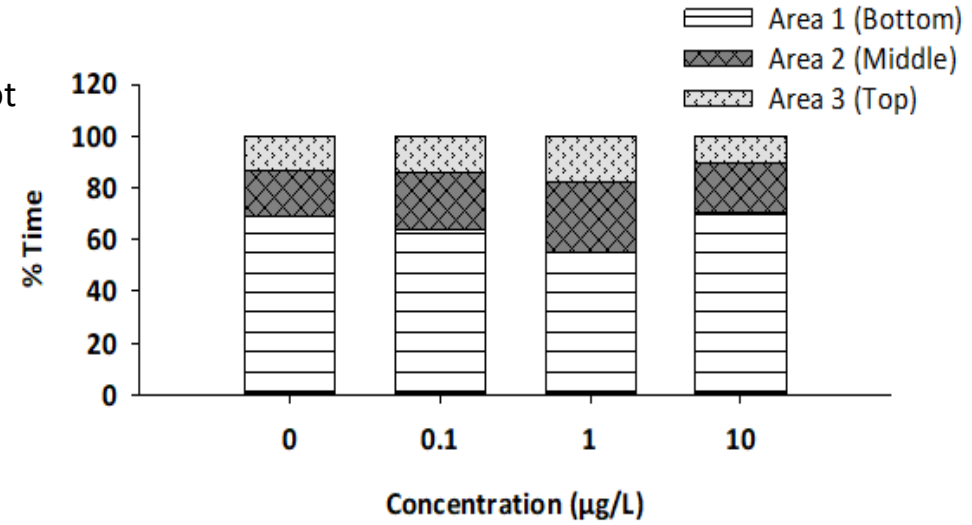


Fig. 6: Novel tank/ Exploratory Swimming Test: swimming behavior of juvenile zebrafish, with three layers (bottom, middle and top) (mean value ± standard error (SE));

This may have occurred due to the **great variability of the data**, making it impossible to observe clear trends and, therefore, the **number of samples (N) should be increased** in future work for a better robustness of the data.

Results and discussion

4. Social test (21 days exposure)

- Fish exposed to fluoxetine seem to spent more time in the area near the shoal (area 1), with no significant effects of fluoxetine detected.

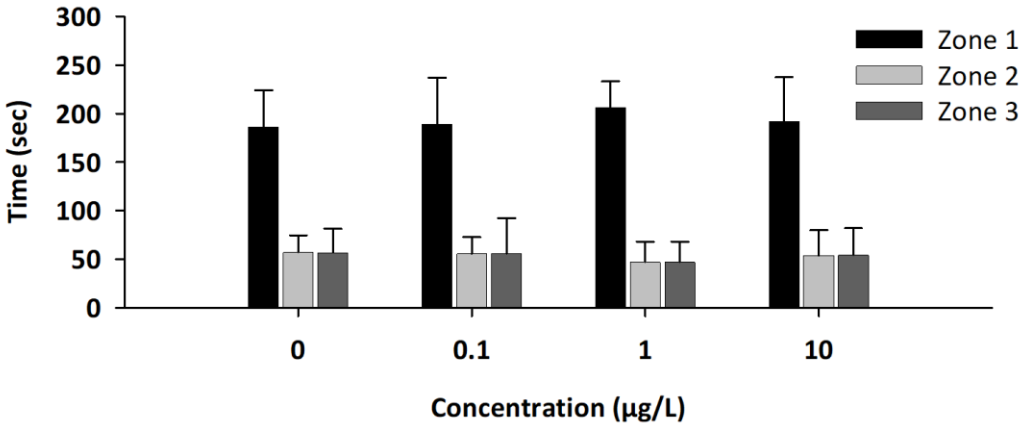


Fig. 7: Social behavior of juvenile zebrafish discriminated between zone 1 - Proximity Zone, zone 2 - Neutral zone near the shoal and zone 3 - Neutral zone away from the shoal (mean value ± SE).

Other results

50 µg/L fluoxetine in a 15-minute exposure decreased social interaction in adult zebrafish, spending less time near the shoal (Giacomini et al., 2016).

Results and discussion

5. Biochemical analysis (21 days exposure)

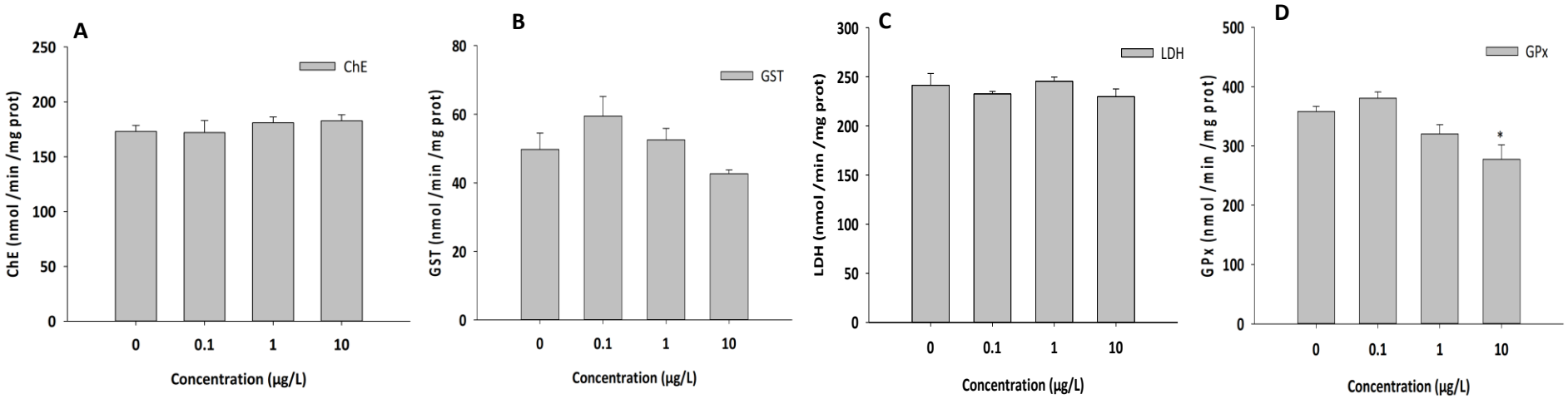


Fig. 8: Effects of chronic exposure to sublethal fluoxetine concentrations on juvenile zebrafish enzymatic activities (mean values ± standard error): A – Cholinesterase (ChE) activity; B – Glutathione-S-transferase (GST) activity; C – Lactate Dehydrogenase (LDH); D - Glutathione peroxidase (GPx) activity.

- Exposure to fluoxetine slightly decreased the activity of the GST, at the highest concentration, although non significantly;
- A similar response was observed in GPx activity for 1 and 10 µg/L of fluoxetine, with significant differences only observed for the highest concentration (10 µg/L of fluoxetine);
- No effect of chronic exposure on ChE and LDH activities.

Results and discussion

5. Biochemical analysis (21 days exposure)

Reduction in the enzymatic activity of GST and GPx at the highest concentration

There are authors who have reported a decrease/ inhibition of GST activity.

- In juveniles *Argyrosomus regius*, an exposure for 15 days at 0.3 and 3 µg/L of fluoxetine resulted in inhibition of GST activity (Duarte et al., 2020);

- A chronic exposure of 42 days, GST activity decreased at the fluoxetine concentration 200 µg/L in the liver and gills of juveniles of *Pseudorasbora parva* (Chen et al., 2018);

- Several studies have reported the effects of fluoxetine on oxidative stress, with an increase or decrease in the activity of the enzymes CAT, SOD, GSH and GPx, depending on the exposure duration, concentrations of fluoxetine and organisms used (Cunha et al., 2016; Ding et al., 2016; Duarte et al., 2019; Pan et al., 2018);

- The effects on GST activity are due to a possible increase in oxidative stress, induced by exposure to fluoxetine, as observed in Chen et al. (2018).

Conclusions

- Exposure to low concentrations of fluoxetine can cause relevant behavioral and biochemical effects in juvenile zebrafish;
- Exposure to fluoxetine caused effects on locomotor activity in response to light stimulation;
- Exposure to the highest concentration (10 µg/L of fluoxetine) caused effects on oxidative stress;
- These are important results, which clearly demonstrate the potential effects of exposure to fluoxetine in the juvenile stages, and it is necessary to consider this scenario in environmental risk assessments.

Acknowledgments

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