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Effect of Fluoxetine Hydrochloride on Routine Metabolism of Lambari *(Deuterodon iguape,* Eigenmann, 1907) and Phantom Shrimp (*Palaemon pandaliformis,* Stimpson, 1871)

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HIGHLIGHTS

- Fluoxetine increases the metabolic rate and excretion of ammonia in both species.
- O:N ratio in fish showed higher values in the highest concentrations of fluoxetine.
- The LC50 - 96 hour values of *Palaemon pandaliformis* represented greater toxicity.
- Both species are a good biological model for fluoxetine exposure studies.

Abstract: Fluoxetine is an emerging pollutant that acts as a selective serotonin reuptake inhibitor (SSRI) and being a hydrolytic molecule that is photolytically stable and accumulaties in biological tissues, its disposal in the aquatic environment can interfere with the physiology of fish and shrimp. Thus, the objective of this study was to analyze the effects of fluoxetine on routine metabolism (metabolic rate, specific ammonia excretion and O:N ratio) of *Deuterodon iguape* and *Palaemon pandaliformis*. For this, five groups of each species, were exposed to different concentrations of fluoxetine for 24 hours (*D. iguape*) and 2 hours (*P. pandaliformis*). The results demonstrated that in *D. iguape* exposure to fluoxetine significantly increased both the metabolic rate by 75%, 85%, 55% and 50% for concentrations of 0.05; 0.1; 0.5 and 1.0 mgL^{-1}, respectively, and the specific ammonia excretion by 40%, 48% and 20% for concentrations of 0.05; 0.1 and 0.5 mgL^{-1}, respectively, when compared with their control. The O:N ratio was statistically greater in concentrations of 0.5 and 1.0 mgL^{-1}. Concerning *P. pandaliformis*, exposure to fluoxetine increased metabolic rate at concentrations 30.0 and

60.0 μgL^{-1}, and also increased specific ammonia excretion at concentrations 10.0, 30.0 and 60.0 μgL^{-1}, when compared with the control group. It was concluded that exposure to fluoxetine increases the routine metabolism of both species and that at the concentration 1.0 mgL^{-1}, *Deuterodon iguape* required different energy substrates.

Keywords: metabolic rate; ammonia excretion; O:N ratio; antidepressant; drug.

INTRODUCTION

Fluoxetine is an emerging pollutant that is a selective serotonin reuptake inhibitor (SSRI) and its molecular formula is $C_{17}H_{18}F_3NO$ (molecular weight: 345.79 g mol^{-1}). This chemical, after consumption, is metabolized in the liver to norfluoxetine ($C_{16}H_{16}F_3NO$; molecular weight: 295.305 g mol^{-1}), which also acts as an SSRI with a fluoxetine-like potency [1,2].

The SSRIs target central nervous system neurons by inhibiting the serotonin transporter (SERT) which is responsible for the reuptake of serotonin (5-hydroxytryptamine, 5-HT) by the presynaptic neuron. Consequently, it increases the amount of this neurotransmitter available at synapse, enhancing its effect [3-5]. The 5-HT plays important roles such as wakefulness, aggression, appetite [5] and modulation of motor production [6] and is considered one of the most important neurotransmitters of the animal kingdom [7].Thus, SSRIs are drugs prescribed for the treatment of diseases such as depression, anxiety, panic disorder and obsessive-compulsive disorder [1,8]. Among these drugs, fluoxetine is one of the most prescribed in the world [9].

It is noteworthy that, according to the Organization for Economic Cooperation and Development (OECD), the consumption of antidepressants has increased over 60% in the last decade [10]. Therefore, it is estimated that depressive disorders affect 350 million people worldwide, of which 4.86% are Brazilians [11], and this number will tend to increase in the coming years, since the World Health Organization predicts that depression will be the second largest public health disability in 2020 [12].

Fluoxetine is excreted from the human body primarily through the urinary system, where approximately 10% is eliminated as the parent compound (fluoxetine) and the remainder is eliminated as norfluoxetine [1,13]. Both compounds enter water treatment facilities through both human waste and the disposal of this drug, unused, in the sink or toilet [14]. Kwon and Armbrust [15] warn that this compound is hydrolytic and photolytically stable in water causing a relatively long half-life and is not eliminated after the effluent treatment. As a result, environmental concentrations of this drug are often detected, ranging from 0.012 to 1 μg L^{-1} [16,17], which raises concerns about its potential effects on biota [18].

In addition, it is suggested that entrapment of fluoxetine/ norfluoxetine in cell lysosomes may cause retention in biological tissues [19]. These characteristics increase the probability of the bioaccumulation of these substances in organism target tissues [20,21] like the brain [22,23], muscle and liver [22,24].

Thus, improperly discarding this drug leads to concerns about the health of organisms and, consequently, with the ecosystem. Aquatic vertebrates and invertebrates become susceptible to the action of fluoxetine and norfluoxetine because some of their physiological processes are also regulated by 5-HT [25]. Studies show that fluoxetine exerts anxiolytic effects on fish and may interfere with neuroendocrine stress axis activity [26,27] affecting various levels of neuropeptides such as neuropeptide Y, oxytocin and arginine vasopressin [28]. Henry and Black [29] alert that substances that act on the neuroendocrine system can negatively affect organisms, even at low environmental concentrations. Interference in neuroendocrine stress axis activity influences behavior (aggression, appetite) [5], endocrine and reproductive parameters [30,31], and compromises survival [32] since the stress response helps individuals cope with adverse conditions [33,34].

In invertebrates, studies show that serotonin plays an important role in physiology and behavior, such as aggression [35], spawning induction [36], swimming [37] and feeding [2]. However, effects of exposure to fluoxetine on aquatic organisms are not yet fully understood [5,38]. Fluoxetine was found between 0.14 and 1.02 μg kg^{-1}, in tissues of fish species: *Ameiurus nebulosus*, *Dorosoma cepedianum* and *Morone americana* [39].

D. iguape, known as lambari, belongs to the order Characiforme. It is commonly found in small rivers and streams of tropical and subtropical regions and is native to the Atlantic Forest watershed [40]. This species is widely used in the fishing market both for human consumption and for use as live bait in recreational fisheries [41,42]; mainly of tucunaré (*Cichla* spp.) and south american silver croaker (*Plagioscion squamosissimus*) [41,43], which helps to reduce juvenile shrimp fishing [44]. *Palaemon pandaliformis* (known as phantom shrimp) is a small crustacean decapod. Having a great osmoregulatory capacity [45], this species

is found in freshwater and estuarine environments from the southern coast of Brazil to Guatemala [46]. Ferreira and coauthors [47] highlights the ecological importance of these organisms in the cycling of organic nutrients, in addition to being part of the diet of fish and birds.

Routine metabolism is a technique that assesses the daily energy expenditure of fish (non-fed) in their routine activity that includes spontaneous swimming movements [48]. Thus, many current studies on aquatic animals use this assessment to measure the toxicity of a chemical [49], such as graphene oxide with trace elements [50], multiwalled carbon nanotubes and carbofuran [51], titanium dioxide nanoparticles [52], nickel [53], perfluorooctane sulfonate [54].

In this context, it was hypothesized that exposure of *D. iguape* and *P. pandaliformis* to fluoxetine hydrochloride would interfere with the neuroendocrine stress axis, causing changes in its metabolism, revealed by the increase in metabolic rate and specific ammonia excretion. In addition, it is believed that to maintain homeostasis, fish and shrimp will use different energy sources revealed by the O:N ratio. The objective of this study was to analyze the sublethal effects of different concentrations of fluoxetine hydrochloride on routine metabolism (metabolic rate, specific ammonia excretion and O:N ratio) of *Deuterodon iguape* and *Palaemon pandaliformis*.

MATERIAL AND METHODS

This study was carried out according to the ethical principles for animal experimentation adopted by the Brazilian School of Animal Experimentation (COBEA) and received authorization (no. 06/2016) from the Ethics Committee in Animal Experimentation of the Fisheries Institute, São Paulo, Brazil.

Deuterodon iguape and *Palaemon pandaliformis* were purchased from a fish farm located on the coast of São Paulo and were kept at the Fisheries Institute (Cananéia Laboratory) Secretary of Agriculture of the São Paulo State, on the southeastern coast of Brazil. Each species remained in different 500L tanks for one week for their acclimatization with constant aeration and daily water change. The freshwater used for maintenance was filtered through three 2µm filters, two 1µm filters and one 0.5µm filter arranged sequentially.

The temperature of the tanks was constantly monitored with a mercury thermometer (accuracy of 0.5 °C), and was maintained at 22.75 °C ± 0.27 °C (mean ± standard deviation). The animals were fed commercial feed once a day during this period. Feeding was suspended 24 hours before the test. No animals were used more than once.

Fluoxetine hydrochloride (N-methyl-3-phenyl-3- [4- (trifluoromethyl) phenoxy] propane-1-amine - $C_{17}H_{18}F_3NO$) was used as a reagent. The choice of this drug was based on its predominance (approximately 68.8%) among antidepressant prescriptions [55]. It is also noteworthy that Brazil has little research focused on the toxicity of medicines in the aquatic environment. Using an analytical balance with a resolution of 0.0001g, the reagent was diluted in a stock solution of 100 mg/ 100 mL of distilled water immediately prior to use to prevent substance degradation . For each species, four different concentrations were tested, as well as a control group (water without the drug). Fluoxetine hydrochloride was introduced at a determined nominal concentration into the aquariums using a 0.1ml precision pipette (accuracy ± 0.8%; precision 0.4%).

A total of 25 lambaris, with a mass of 9.87 g ± 1.66 g (mean ± standard deviation), were randomly divided into 5 glass aquariums, with a capacity of 6 liters (5 lambaris in each aquarium), containing different concentrations of fluoxetine hydrochloride (0.0, 0.01, 0.1, 0.5 and 1 mg L^{-1}). In addition, 25 shrimps were also divided into 5 glass aquariums, with the same capacity (5 shrimps in each aquarium), with different concentrations of fluoxetine hydrochloride (0.0; 5.0; 10.0; 30.0 and 60.0 µg L^{-1}). The animals were kept in this condition for a period of 24 hours (*D. iguape*) and 2 hours (*P. pandaliformis*), at a controlled temperature (22.75 ± 0.27 °C) and without feeding.

After exposure, the fish and shrimps were acclimated, separately, in cylindrical glass respirometers for 1 hour with continuous water circulation to alleviate the stress caused by handling. Then, the water supply was suspended, and the respirometer was closed so that the organisms could consume the oxygen in the known water volume for a period of one hour. The respirometers were protected by a barrier to isolate the animals from possible movement in the laboratory.

A water sample was then taken from each respirometer. The difference between oxygen and ammonia concentrations determined at the beginning and end of confinement was used to calculate the metabolic rate (mLO_2 $g^{-1}h^{-1}$) and specific ammonia excretion (mg L^{-1}) during the period, considering the volume of the respirometer, the wet weight of the animal and the confinement time. To minimize the effects of low oxygen concentration and metabolite accumulation on metabolism, the duration of the experiment was regulated so that the final oxygen concentration was greater than 70% of its initial concentration. Dissolved oxygen was

determined by the Winkler method and ammonia excretion by the Nessler method (Standard Methods for the Examination of Water and Wastewater).

The atomic oxygen consumption rate (OCR) and ammonia excretion rate (AER) (O:N ratio) was calculated using the following equation O:N ratio = (17 x OCR) / (16 x AER) [56].

The data were evaluated according to means and standard deviations obtained by one-way ANOVA followed by Tukey's post-test after verification of normal distributions (Kolmogorov-Smirnov test) and homoscedasticity (Levene test). Differences were considered significant when $p < 0.05$.

RESULTS

In *Deuterodon iguape,* the metabolic rate increased significantly in all groups exposed to different fluoxetine concentrations when compared to the control group (ANOVA; $p < 0.05$) (Figure 1). The increase of the groups exposed to fluoxetine was by 75%, 85%, 55% and 50% for concentrations of 0.05; 0.1; 0.5 and 1.0 mg L^{-1}, respectively. The data indicate a tendency to decrease the metabolic rate with higher concentrations (0.5 and 1.0 mg L^{-1}) when compared to lower concentrations (0.05 and 0.1 mg L^{-1}).

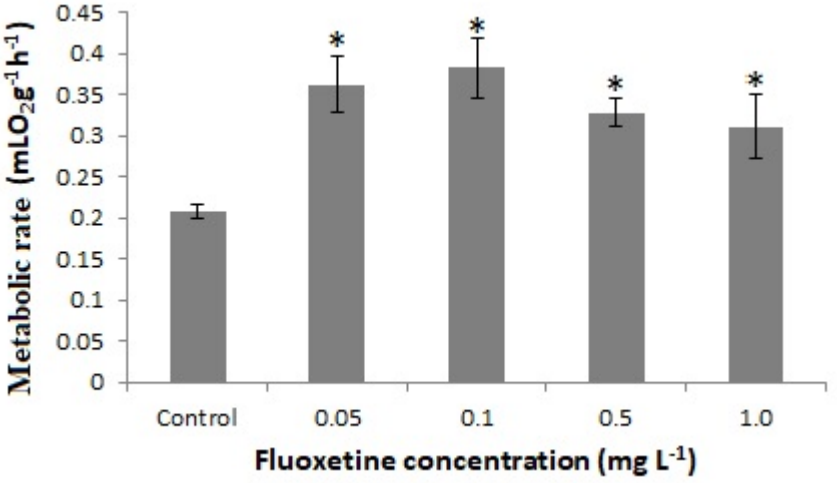

Figure 1. Mean of metabolic rate ($mLO_2g^{-1}h^{-1}$) relative to different fluoxetine concentrations (mgL^{-1}) of *Deuterodon iguape*. The bars are the respective standard deviations. *Indicates statistical difference compared to the control group.

A statistically significant increase of the specific ammonia excretion in the exposed groups were observed at concentrations of 0.05, 0.1 and 0.5 mg L^{-1} fluoxetine when compared to the control group (ANOVA; $p < 0.05$), in *Deuterodon iguape*. However, the group exposed to 1.0 mg L^{-1} fluoxetine showed no statistical difference (Figure 2). The increases were by 40%, 48% and 20% for fluoxetine concentrations of 0.05; 0.1 and 0.5 mg L^{-1}, respectively, alerting to the same trend of metabolic rate; where, the increase is greater at lower concentrations (0.05 and 0.1 mg L^{-1}) than at higher concentrations (0.5 and 1.0 mg L^{-1}).

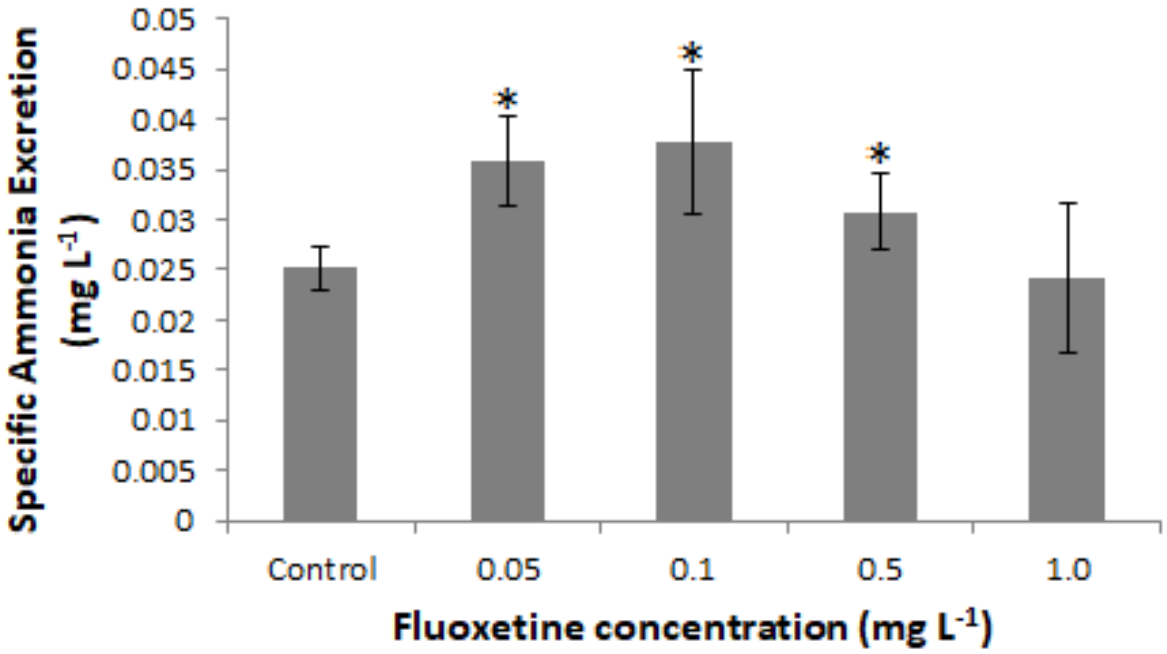

Figure 2. Mean of specific ammonia excretion (mgL^{-1}) relative to different fluoxetine concentrations (mgL^{-1}) of *Deuterodon iguape*. The bars are the respective standard deviations. *Indicates statistical difference compared to the control group.

By analyzing the atomic oxygen consumption ratio (OCR) to ammonia excretion ratio (AER) (O:N ratio) in *Deuterodon iguape*, it was shown that no statistical differences were found in the exposed groups with the lowest concentrations of fluoxetine (0.05 and 0.1 mg L^{-1}) when compared to the control group. On the other hand, the groups exposed to the highest fluoxetine concentrations (0.5 and 1.0 mg L^{-1}) had a statistical difference when compared to the control (ANOVA; $p < 0.05$) (Figure 3).

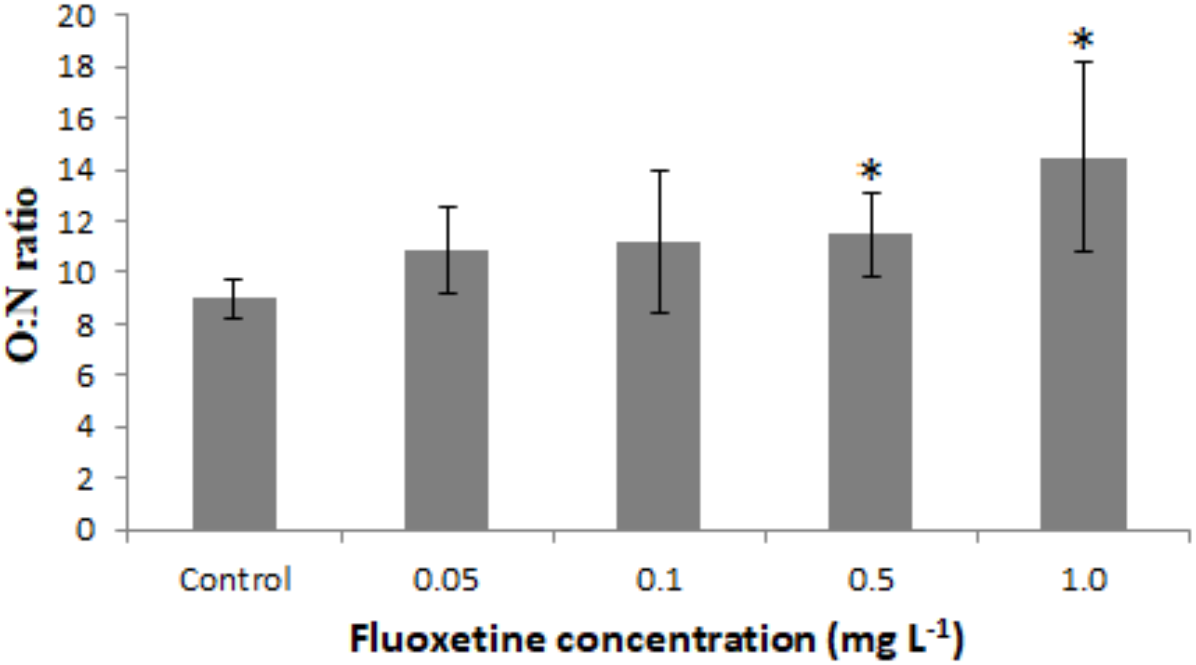

Figure 3. Mean of atomic ratio of oxygen consumed to ammonia nitrogen excreted (O:N) relative to different fluoxetine concentrations (mgL^{-1}) of *Deuterodon iguape*. The bars are the respective standard deviations. * Indicates statistical difference compared to the control group.

Assessing the toxicity of fluoxetine in shrimps, it was observed that the 96-hour LC50 values of *Palaemon pandaliformis* displayed a greater toxicity (Table 1).

Table 1. Percentage mortality (%) of shrimp exposed to increasing concentrations of Fluoxetine (µgL^{-1}) for 96h and the average lethal dose (CL50 with 95% confidence limit) calculated by Spearman-Karber analysis.

Time of exposure (h)	0	5	10	30	60	100	CL50 Fluoxetine (µg L^{-1})
24	0.00	0.00	0.00	6.66	100	100	39.61 (35.61-44.86)
48	0.00	0.00	0.00	20	100	100	35.47 (29.48-42.68)
72	0.00	0.00	6.66	40	100	100	27.93 (21.66-36.02)
96	0.00	0.00	6.66	46.6	100	100	26.31 (20.33-34.06)

Moreover, the metabolic rate increased significantly in the groups exposed to the highest concentrations of fluoxetine (30.0 and 60.0 µg L-1) when compared to the control group (ANOVA; p <0.05) in *Palaemon pandaliformis* (Figure 4).

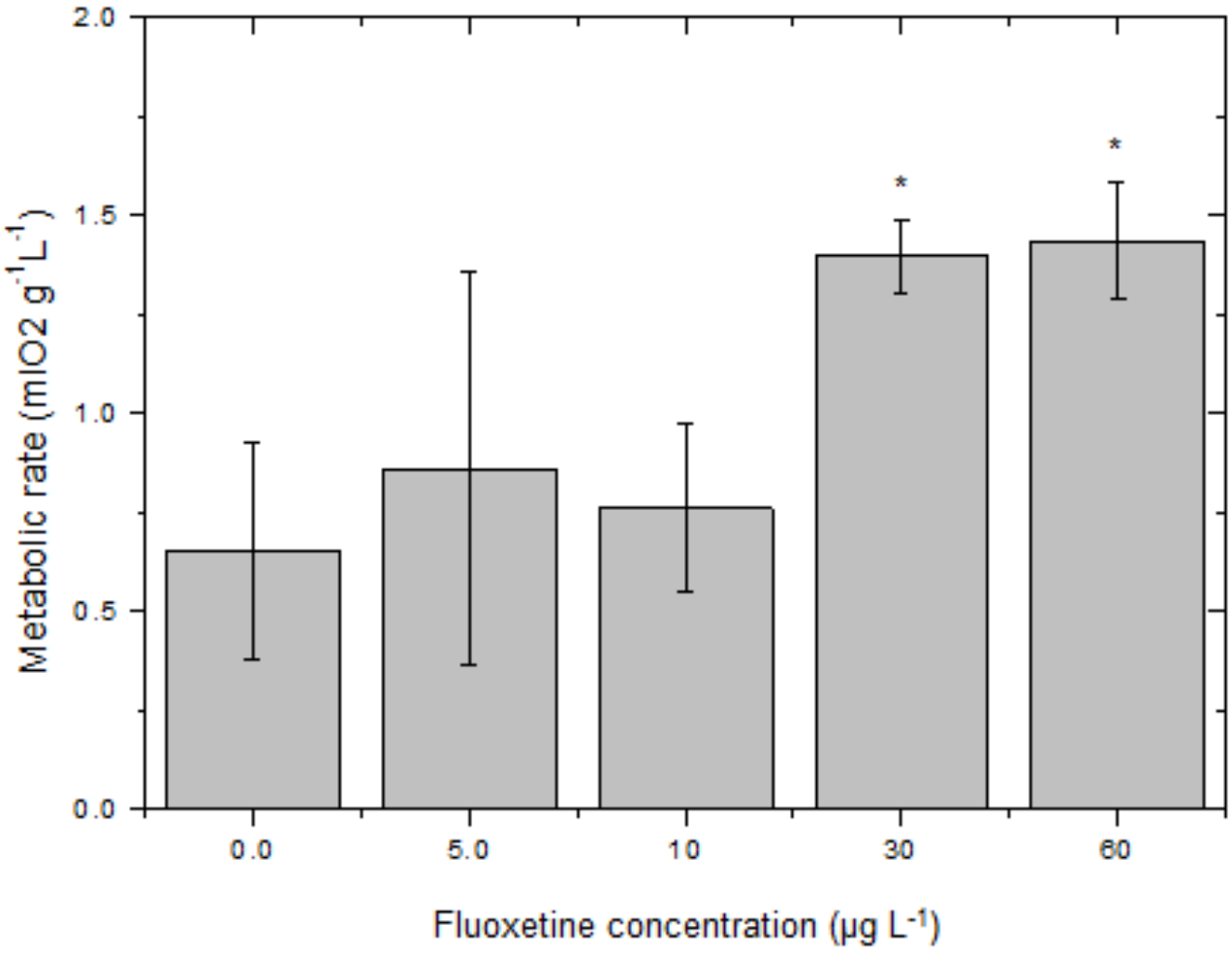

Figure 4. Mean of metabolic rate (mLO_2 g^{-1}h^{-1}) relative to different fluoxetine concentrations (µgL^{-1}) of *Palaemon pandaliformis*. The bars are the respective standard deviations. *Indicates statistical difference compared to the control group.

The specific excretion of ammonia in the groups exposed to the highest concentrations of fluoxetine (10.0, 30.0 and 60.0 µg L^{-1}) was statistically higher when compared to the control group (ANOVA; p <0.05), in *Palaemon pandaliformis* (Figure 5).

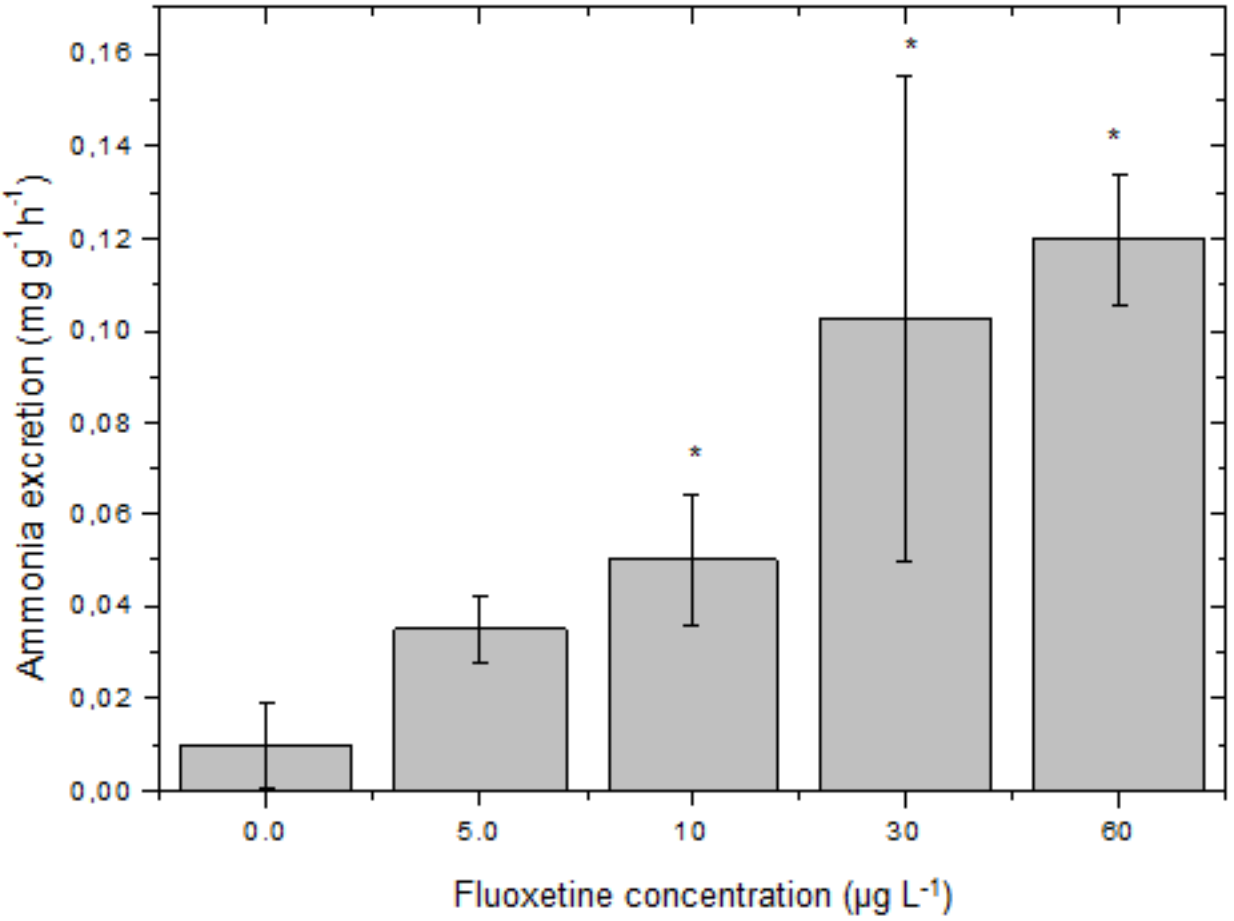

Figure 5. Mean of specific ammonia excretion (mgL^{-1}) relative to different fluoxetine concentrations (µgL^{-1}) of *Palaemon pandaliformis*. The bars are the respective standard deviations. *Indicates statistical difference compared to the control group.

DISCUSSION

Aquatic environments are being contaminated by drugs and their correlates, and among them is fluoxetine hydrochloride [57], which was used in the present study. This drug is considered an emerging environmental contaminant, as it is increasingly present in the waters of the world [58] and arouses the attention of sanitation agencies because it has lipophilic characteristics and low biodegradability in the environment [59].

Moreover, knowing that the anatomical organization of brain serotonergic systems is remarkably conserved among vertebrates [60], implying that the functions of the 5-HT neurotransmitter may also have been conserved as revealed in recent studies with teleosts [61-63], the fish, *Deuterodon iguape,* was chosen as a biological model, and it is an endemic species of small rivers and coastal streams of the state of São Paulo [64,65]. Apparently, the neurotransmitter 5-HT also plays an important role in aquatic invertebrates, such as crustaceans, mediating many physiological and behavioral processes [66]. Therefore, we used the shrimp, *Palaemon pandaliformis,* as a biological model for the present study, it is a species native to Brazil. Both species were shown to be sensitive to exposure to the different concentrations of fluoxetine hydrochloride revealed by the increase in routine metabolism (metabolic rate and specific ammonia excretion).

Another serotoninergic process in fish is the excretion of ammonia, which is the main product of fish excretion [67-69], through the gills. This excretion is attributed to urea transport protein (tUT), which is regulated by the hormone cortisol and the 5-HT neurotransmitter [70]. On the one hand, cortisol stimulates Na+/ K+-ATPase activity in gills [71], stimulating Na^+, Cl^- and Ca^{2+} absorption [72] and increasing the cell chloride area in the gills resulting in the inhibition of ammonia excretion [73,74]. On the other hand, high concentrations of 5-HT stimulate ammonia excretion [75,76]. The fluoxetine, which blocks the stress response by increasing the action of 5-HT, reduces the influx of Na^+ and K^+ by increasing ammonia excretion

[77]. This was corroborated in the present study, where exposure to fluoxetine for 24 hours increased specific ammonia excretion at three concentrations tested (0.05; 0.1; 0.5 mg L^{-1}) in *Deuterodon iguape*.

Increased metabolic rate and ammonia excretion may indicate the need for additional energy to maintain homeostasis [78]. Thus, the atomic oxygen: nitrogen ratio (O:N) is an indicator of metabolic processes using different energy substrates in various environments [56].

The present study revealed that exposure to concentrations of 0.05; 0.1; 0.5 mg L^{-1} fluoxetine, in addition to the control group, the O:N ratio was maintained in the range of 3-16 indicating, according to Mayzaud and Conover [56], preferential oxidation of protein substrate, preserving the energy balance of the fish [79]. It is noteworthy that the group exposed to 0.5 mg L^{-1} of fluoxetine significantly increased protein substrate oxidation for obtaining energy in relation to the control group. This result warns of an increase in energy requirements which is reflected in the results obtained in the group exposed to 1.0 mg L^{-1} of fluoxetine.

Although the average O:N ratio of the group exposed to 1.0 mg L^{-1} fluoxetine was 14.46 remaining in the oxidative range preferentially of protein substrate, it is observed in the standard deviation (± 3.69) that the O:N ratio of some individuals in the group reached the 16-60 range which reveals a mixed oxidation of protein and lipid substrates [56].

Thus, the data on the O:N ratio demonstrated the need for the use of different metabolic substrates as energy sources in a dose-dependent manner during exposure to fluoxetine. These data reinforce the tendency for a decreased metabolic rate and ammonia excretion at higher fluoxetine concentrations (0.5 and 1.0 mg L^{-1}). However, it is noteworthy that no individual reached the range of more than 60, which would represent oxidation of predominant lipid substrate to meet high energy demands [56].

The results of this study confirm that the fluoxetine was toxic to *P. pandaliformis* and *D. iguape*, which are ecologically and economically important shrimp species in the coastal waters of Brazil. The most sensitive specie to fluoxetine was the shrimp. The effects of fluoxetine on crustaceans is not well documented yet, but there is a study for *C. dubia* and *D. magna*. For example, the 96-hour LC50 of fluoxetine for *C. dubia* was 820 µg L^{-1} and for *D. magna* it was 720 µg L^{-1} [13]. In addition, 96-hour LC50 values of fluoxetine for the fish, *P. promelas*, was 705 µg L^{-1} [13]. In this study, fluoxetine exhibited a greater toxicity on *Palaemon* in which the 96-hour LC50 values were 26.31 µg L^{-1}. Brooks and coauthors [13] and Henry and coauthors [29] worked on another crustacean species and found that the mortality of *C. dubia* increases with increasing SSRIs concentration exposure [29].

It is known that serotonin physiologically regulates biological activities, controlling the release of neurohormones from the X-organ/ sinus gland complex [79,80]. This complex, in crustaceans, produces hormones related to reproduction, nutrient metabolism and growth; and the most well-known of these hormones which possesses a quick response is the Crustacean Hyperglycemic Hormone (CHH) [81].

Thus, exposure to fluoxetine maintains the hyperglycemic effect of serotonin resulting in physiological changes that increase metabolic costs in invertebrates. Lange and coauthors [82], analyzing small crustaceans of *Gammarus pulex* exposed to concentrations of 10-100 ng L^{-1} of fluoxetine, observed increased ventilation showing signs of stress. In addition, exposure to fluoxetine also alters the swimming behavior of invertebrates. Castro-Català and coauthors [83] observed an increase in the swimming speed of the small freshwater crustacean, *Gammarus pulex*, exposed to concentrations of 100 ng L^{-1} of fluoxetine.

Studies with mussels, *Lampsilis fasciola*, demonstrated that exposure to fluoxetine (concentration of 22.3 µg L^{-1}) increased the distance covered by these animals; however, the authors warn that the movements were more irregular with changes in position behavior and movement time [84].

Nevertheless, an increase in the metabolic rate of *Palaemon pandaliformis* at the highest concentrations of exposure (30.0 and 60.0 µg L^{-1}) as evidenced in the present study, may be related to the need for meeting the increase in metabolic costs, increasing the rate of glycolysis [85].

Ammonia, the final product of protein catabolism, is responsible for more than half of nitrogenous residues released by decapod crustaceans [86]. Therefore, the results obtained in the present study, where the increase in the specific ammonia excretion was related to the increase in the metabolic rate, can be justified by the prolonged action of serotonin through the influence of fluoxetine, which results in a hyperglycemic effect in which the organism increases the catabolism of one of the most commonly used energy substrates, amino acids, to maintain high glucose levels [87].

In addition, several studies show that different contaminants such as lead [49], nitrite [88], cadmium and zinc [89] can increase the excretion of ammonia in crustaceans reinforcing the thesis that their response is to eliminate the compound from their body more quickly [49,90].

Given the fact that exposure to the highest concentrations of fluoxetine causes an increase in routine metabolism both in vertebrates (*Deuterodon iguape*) and in invertebrates (*Palaemon pandaliformis*), in just

24 hours and 2 hours of exposure, respectively, we warn concerning the ecological risk of this medication in aquatic communities.

Although energy expenditure, as a response to stress, is necessary for the body to balance itself, Ceccarelli and coauthors [91] point out that if the scenario persists, characterizing a situation of chronic exposure, other processes may be compromised such as reproduction, growth and resistance to diseases, which can lead to death [92]. Schultz and coauthors [31], exposing *Pimephales promelas* fish to a concentration of 28 ng L^{-1} for 21 days observed impairment in testicular morphology. Upon exposure to a fluoxetine concentration of 69 µg L^{-1}, *Potamopyrgus antipodarum* showed a decrease in reproduction after 42 days [83]. When exposing *Oryzias latipes* to a concentration of 5 µg L^{-1} of fluoxetine, Foran and coauthors [93] observed an increase in circulating plasma estradiol levels.

In addition to the need for more research on aquatic animals exposed to fluoxetine for acute and chronic periods, Mesquita and coauthors [94] also highlight the importance of studies on toxicological interactions. Nevertheless, the already known changes in the exposure of aquatic organisms to the drug, fluoxetine, concern additional changes in the aquatic communities through both the vertical structure, causing an imbalance in the predator-prey relationship, and also in the horizontal structure, in which the animals compete for the same food resource [95].

Fluoxetine and norfluoxetine hydrochloride act and bioaccumulate in the fish brain as shown in studies with wild fish, in which noted concentrations of these compounds ranged between 1.58 and 8.86 ng g^{-1} [96]. Thus, this drug enhances the effect of the 5-HT neurotransmitter [5], which is responsible for the neuroendocrine modulation of physiological processes in the brain, leading to concerns about its interference with these processes [97]. In addition, vertebrate brains are metabolically one of the most active organs and are extremely sensitive to energy metabolism disorders [98,99], accounting for 2.7-3.4% of total body energy consumption in ectothermal vertebrates [100].

It is known also that besides the brain, the liver is also a key organ involved in the regulation of energy metabolism in fish. As in the brain, Brooks and coauthors [96] detected a liver bioconcentration within the range of 1.34 and 10.27 ng g^{-1} for fluoxetine and norfluoxetine, respectively. Smith and coauthors [101] believe that bioaccumulation is a result of slower metabolism of fluoxetine in the liver in fish compared to mammals.

Thus, the possible action and bioaccumulation of fluoxetine in a *Deuterodon iguape*'s brain and liver results in altered energy metabolism, in which both organs need more energy, not only for a compensatory response caused by stress, but also as a result of decreased oxygen solubility in salt water [74,102,103]. Thus, data from the present study demonstrate an increase in metabolic rate at all fluoxetine concentrations tested (0.05, 0.1, 0.5, 1.0 mg L^{-1}) within 24 hours of exposure.

CONCLUSION

Exposure to fluoxetine hydrochloride increases metabolic rate and specific ammonia excretion of *Deuterodon iguape* and *Palaemon pandaliformis* after 24 and 2 hours of exposure, respectively.

The highest concentration of fluoxetine hydrochloride tested (1.0 mg L^{-1}) required of the *Deuterodon iguape* different energy substrates (protein and lipid) to maintain its homeostasis.

Results show that *Deuterodon iguape* and *Palaemon pandaliformis* are good biological models for fluoxetine exposure studies.

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