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Waterborne psychoactive drugs impair the initial development of Zebrafish

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ABSTRACT

The contamination of rivers and other natural water bodies, including underground waters, is a current reality. Human occupation and some economic activities generate a wide range of contaminated effluents that reach these water resources, including psychotropic drug residues. Here we show that fluoxetine, diazepam and risperidone affected the initial development of zebrafish. All drugs increased mortality rate and heart frequency and decreased larvae length. In addition, risperidone and fluoxetine decreased egg hatching. The overall results points to a strong potential of these drugs to cause a negative impact on zebrafish initial development and, since the larvae viability was reduced, promote adverse effects at the population level. We hypothesized that eggs and larvae absorbed the drugs that exert its effects in the central nervous system. These effects on early development may have significant environmental implications.

1. Introduction

The contamination of rivers and other natural water bodies, including underground waters, is a current reality. Human occupation and economic activities generate a wide range of contaminated effluents that reach these water resources (Deblonde et al., 2011; Oggier et al., 2010). One of the most dangerous types of contamination is the presence of psychotropic drug residues (Calisto and Esteves, 2009). In fact, this drug class has been detected in natural water bodies in different countries (Borova et al., 2014; Boyd et al., 2003; Kolpin et al., 2002; Van Der Ver et al., 2004) and it is related with increased prescription in last years (Calisto and Esteves, 2009).

Among the psychoactive drugs, we studied three substances: the antidepressant fluoxetine, the anxiolytic diazepam and the atypical antipsychotic risperidone. Fluoxetine belongs to the selective serotonin reuptake inhibitor (SSRI) class and exerts its effects by modulating the synaptic levels of the neurotransmitter serotonin (Brooks et al., 2003). Diazepam is a benzodiazepine drug with effects on the central nervous system (CNS) mediated by the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and its $GABA_A$ receptor (Brunton et al., 2012). Risperidone is an atypical antipsychotic that acts as an inverse agonist at $5\text{-}HT_{2A}$ receptors, decreasing the production of intracellular inositol triphosphate. It also binds to dopamine D2 receptors, decreasing the action of dopamine in the synaptic cleft, thus treating the positive symptoms in psychotic patients (Brunton et al., 2012).

Several deleterious effects of fluoxetine, diazepam and risperidone have been reported in both larvae and adult fish (Oggier et al., 2010; Kohlert et al., 2012; Abreu et al., 2014; Idalencio et al., in

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Table 1
Drugs and concentrations tested.

Drug	Concentrations (μg/L)						[a]Reference
Diazepam	0.0088	0.088	0.88[a]	8.8	88		Ternes et al., 2001
Fluoxetine	0.0099	0.099	0.99[a]	9.9	99		Van Der Ver et al., 2004
Risperidone	0.00033[a]	0.0033	0.033	0.33	3.3	33	Calisto and Esteves, 2009 Borova et al., 2014

[a] reference of environmental concentration.

press; Akande et al., 2010; Richendrfer et al., 2012; Park et al., 2012; Airhart et al., 2007). Studies using eggs and larvae exposure are very important to verify if psychoactive drugs affect embryos and larvae in concentrations that apparently do not exert effects in adult animals. Impacts on hatchability and larvae viability can cause a severe impact at the population level, since the number of individuals generated in each generation decreases. Thus, we tested the hypothesis that different concentrations of fluoxetine, diazepam and risperidone negatively affect the initial development of zebrafish.

2. Materials and methods

2.1. Ethical note

This study was approved by the Ethics Commission for Animal Use (CEUA) at Universidade de Passo Fundo, UPF, Passo Fundo, RS, Brazil (Protocol #7/2013) and met the guidelines of Conselho Nacional de Controle de Experimentacão Animal (CONCEA).

2.2. Animals

Male and female adult zebrafish (*Danio rerio*) were placed in breeding tanks specifically designed to prevent egg predation by separating adults from eggs. This was achieved by inserting a grid with a mesh-size of 1 × 1 mm inside each breeding tank. During the early morning, adult animals were removed and embryos collected by siphoning. Fertilized eggs were washed to remove debris and transferred to cell culture plates. Plates were maintained in an incubator at 28 °C and monitored daily until 5 dpf. Unfertilized and dead embryos were identified and removed.

2.3. Drugs and concentrations tested

Diazepam (DZP, Union Chemical, 5 mg/mL) and fluoxetine (FLU, EMS, 20 mg/mL) were used at five different concentrations, one reported in the environment, two higher and two lower concentrations, while for risperidone (RISP, JANSSEN, 1 mg/mL), because the environmental concentration is very low, five higher concentrations were used. The concentrations are shown in Table 1.

2.4. Experimental design

Three experiments were carried out, each one with a specific drug. The embryos ($n = 10$) were placed in 24-well plates (5 ml/well) containing water for maintenance (control group) or water + drug (RISP, FLU or DZP). The plates were capped and kept in a water bath at 28 °C. Embryos and larvae were evaluated during the first 5 days of development, taking into account the mortality outbreak, heart rate and length. Just embryos up to 3 hpf were used (Kimmel et al., 1995). See the schematic view of experimental design in Fig. 1.

2.5. Specific procedures

We classified as "hatched eggs" when the exposure of all or most of the tail was present. The calculation of mortality rate was based on the number of dead embryo/larvae in relation to live ones. Embryos were considered dead when had no transparency, coagulated, with absence of somites formation and with absence of cardiac motion and blood circulation. The absence of reflex response to stimulation or eggs not hatched after 72 h were also considered as parameters for evaluation of mortality. The heart rate was evaluated at 49 hpf in all groups. This phase was

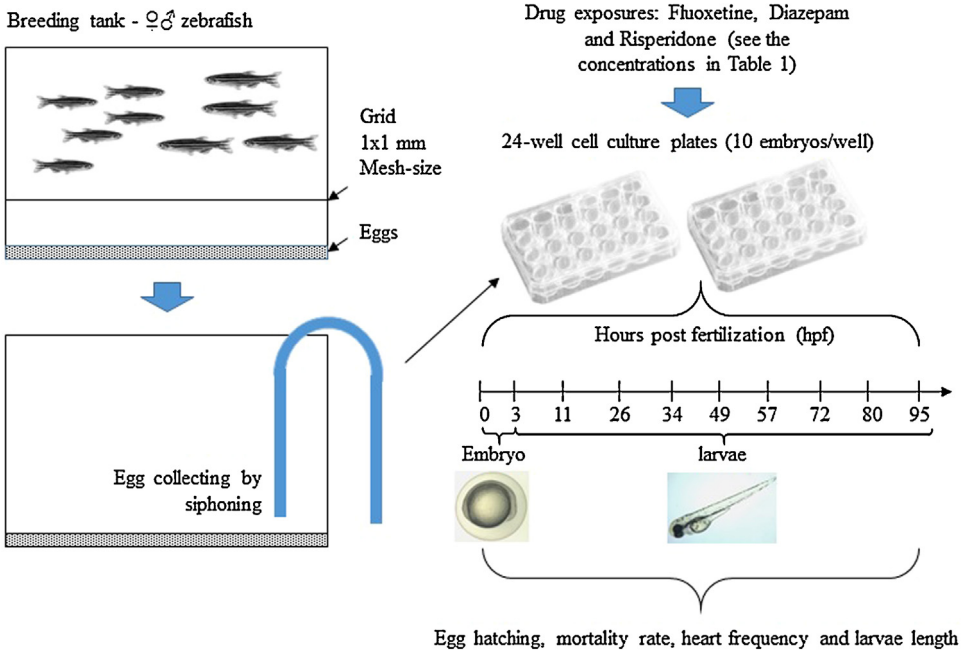

Fig. 1. Schematic representation of the experimental design.