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Bisphenol risk in fish exposed to a contamination gradient: Triggering of spatial avoidance

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ABSTRACT

Bisphenol A (BPA) is an emerging contaminant widely used in various industrial products. Sublethal toxicity of BPA on aquatic organisms is expected to occur at a concentration of around 500 μg L^{-1}, which is much higher than environmentally realistic concentrations found in water bodies (up to 0.41 μg L^{-1}). However, there is no information concerning how a BPA contamination gradient could affect the spatial displacement of organisms. We hypothesized that fish might be able to detect an environmentally realistic BPA contamination gradient and avoid potential toxic effects due to continuous exposure. Therefore, the objectives of this work were: (i) to determine if BPA could trigger an avoidance response in the freshwater fish *Poecilia reticulata*; (ii) to assess whether BPA-driven avoidance occurs at environmentally relevant concentrations; and (iii) to estimate the population immediate decline (PID) at the local scale, considering avoidance and mortality as endpoints. Avoidance experiments were performed in a seven-compartment non-forced exposure system, in which a BPA contamination gradient was simulated. The results indicated that BPA triggered avoidance in *P. reticulata*. In a traditional forced acute toxicity test, lethal effects in 50% of the population occurred at a BPA concentration of 1660 μg L^{-1}, while in the non-forced system with a BPA concentration gradient, avoidance of 50% of the population occurred at a concentration four orders of magnitude lower (0.20 μg L^{-1}). At environmentally relevant BPA concentrations, PID was mainly determined by the avoidance response. Avoidance in *P. reticulata* populations is expected to occur at BPA concentrations below those that cause sublethal effects on fish and are considered safe by international agencies (≤1 μg L^{-1}). The approach used in the present study represents a valuable tool for use in environmental risk assessment strategies, providing a novel and ecologically relevant response that is complementary to traditional ecotoxicological tests.

1. Introduction

The compound Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane) is widely found in aquatic environments, due to its use in the manufacture of many products including food and beverage packaging, flame retardants, adhesives, building materials, electronic components, and paper coatings (Fromme et al., 2002; Liu et al., 2017). In 1993, around 640,000 metric tons of BPA were produced worldwide, of which 0.017% was released as pollutants in the air, water bodies, and effluents (Staples et al., 1998). Delfosse et al. (2012) showed that the production of BPA exceeded 3 million tons per year. BPA has been found in drinking water after treatment, but at low concentrations (0.003 μg L^{-1}; Fan et al., 2013). However, in surface waters, BPA concentrations have typically been found in the range from 0.0005 to 0.41 μg L^{-1}, while concentrations in effluents have been reported to vary from 0.018 to 0.702 μg L^{-1} (Fromme et al., 2002). Two more recent studies have shown average BPA concentrations of 0.026 μg L^{-1} in Taihu Lake in China (Liu et al., 2017) and 0.045 μg L^{-1} in the Paraíba do Sul River in Brazil (Silva et al., unpublished data).

The presence of BPA in water bodies is of concern, because the compound can cause lethal and sublethal toxic effects in aquatic organisms (Staples et al., 1998; Mathieu-Denoncourt et al., 2016). The mean lethal concentrations (LC_{50}) of BPA for aquatic invertebrates vary from 960 to 2700 μg L^{-1}, while the values for fish range from 6800 to

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17,900 μg L^{-1}. Chronic effects in aquatic organisms have been reported to occur at concentrations from 500 to 780 μg L^{-1} (Mathieu-Denoncourt et al., 2016). Chronic effects of BPA in fish include negative feedback on the gonadotropin-releasing hormone (GnRH) system in females (Qin et al., 2013), perturbation of molecular pathways (Chen et al., 2015), prevention of innate regenerative cell responses (such as in hair cells), and detrimental effects in sensory systems (Hayashi et al., 2015). The vast majority of research focusing on the adverse effects of BPA has involved the use of concentrations many times higher than those found in natural water bodies. Although the "worst scenario" approach (using unrealistic concentrations of BPA) can help in predicting the potential long-term risk of BPA in the absence of any control strategy, the inability of ecotoxicological responses to detect toxicity at environmentally realistic concentrations prevents understanding of the actual risk of BPA.

A second problem (besides concentrations) that contributes to the lack of realism in ecotoxicological studies arises when exposure to contaminants is simulated assuming a homogeneous distribution of the contamination. In aquatic systems, contaminants often disperse, forming a dilution gradient, so the induced stress tends to be negatively correlated with the distance from the source of the contamination (Araújo et al., 2016a; López-doval et al., 2017). In the case of mobile organisms, their displacement to locations distant from the contamination source prevents continuous exposure to contaminants. Therefore, the use of toxicity tests involving the long-term exposure of organisms under confined conditions, with no possibility of escape, could be considered unrepresentative of real exposure scenarios. In order to achieve better simulation of real contamination scenarios, where a contamination gradient is expected, a non-forced exposure system should preferably be employed (Moreira-Santos et al., 2008; jo et al., 2014a, 2016a; ; Silva et al., 2017). This new approach using a non-forced exposure system has been used in ecotoxicology as a complementary tool to analyze the ways that contaminants can affect the spatial distributions of organisms (Lopes et al., 2004). This changes the focus of environmental risk assessment from toxicity caused by continuous exposure at a specific level to contamination-driven habitat selection. In an experimental non-forced system with multiple compartments, organisms are free to move within a contamination gradient, hence simulating a heterogeneous aquatic environment. Evidence of contamination-driven avoidance has been found for different organisms exposed to various stress-inducing agents (see the review by Araújo et al., 2016a), and studies have shown that before sublethal or lethal effects occur, organisms move towards more favorable habitats (Rosa et al., 2012; Silva et al., 2017; Araújo et al., 2018).

The hypothesis tested in the present study is that in heterogeneous environments with environmentally realistic BPA contamination gradients, fish might be able to rapidly move to less contaminated areas. Although such displacement is not considered a toxic effect, since the organisms escape from the contaminated region, contamination-driven habitat selection can have severe environmental consequences at the local scale, with declines in fish populations. Therefore, the objectives of this work were: (i) to determine whether BPA could trigger an avoidance response in the freshwater fish *Poecilia reticulata*, inducing its displacement to less contaminated areas; (ii) to assess whether BPA-driven avoidance occurred at environmentally relevant concentrations; and (iii) to estimate the population immediate decline (PID) at the local scale (proposed by Rosa et al., 2012) caused by exposure to BPA, by integrating both mortality and avoidance responses during a short-term exposure. The sensitivity of the BPA-induced avoidance response was compared with other ecotoxicological responses reported elsewhere, and evaluation of risk was performed using the concentrations considered safe by international agencies.

The fish *P. reticulata* was selected as a test organism, since it is widely used in ecotoxicological tests, is easy to cultivate under laboratory conditions (De Liguoro et al., 2012; Pelli and Connaughton, 2015), and has shown avoidance behavior in the presence of contamination (Silva et al., 2017).

2. Materials and methods

2.1. Test organism

A license for the use of *P. reticulata* was obtained from the Ethics Committee for the Use of Animals at the Institute of Biosciences of the University of São Paulo (Protocol 236/2015 – IB/USP). The fish were obtained from the Agency of Agribusiness and Technology (Pindaminhangaba, Brazil), where they were reared in 500 L tanks under natural conditions. Only fish of 2–3 months of age and 1.2 ± 0.3 cm in size were selected. The organisms were then taken to the Ecotoxicology Laboratory of the University of São Paulo (USP, Lorena-SP, Brazil) and acclimated to the test conditions in 60 L aquaria for at least one week before the tests (OECD, 2000). During this time, the fish were fed with TetraMin flakes, and no mortality was observed. The culture water was obtained from a well and was filtered through a filter containing activated charcoal. The aquarium water was constantly aerated with an air diffuser and was changed by 30% every week. Dissolved oxygen (DO), pH, and conductivity were monitored daily (Hanna HI-9811-5 multi-parameter instrument and AlphaKit) and presented values of 6.4 ± 0.4 mg L^{-1}, 7.0 ± 0.5, and 120 ± 20 μS cm^{-1}, respectively.

2.2. Bisphenol A (BPA)

For the acute toxicity and avoidance tests, stock solutions of BPA (99%, Sigma-Aldrich) were prepared in culture water at concentrations of 40,000 and 2000 μg L^{-1}, respectively. Methanol (4 mL L^{-1}) was used as a solubilization agent in all the tests (controls and treatments). The concentrations used in the tests were prepared using culture water. Quantification of the target compound was performed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). The analyses employed an Agilent 1200 chromatograph equipped with a binary pump, automatic injector, and thermostatically-controlled column compartment. Chromatographic separation was performed at 25 °C using a Zorbax SB-C18 column (2.1 × 30 mm, particle size of 3.5 μm). The mobile phase consisted of ultrapure water (eluent A) and methanol (eluent B), previously filtered through membranes with 0.2 μm pore size. The eluent contained 0.01% ammonium hydroxide as an additive to favor the formation of ions. Detection and quantification employed an Agilent 6410 B triple quadrupole mass spectrometer. The analytical curves were constructed using the peak areas obtained for different concentrations of the compound. The limits of detection and quantification were 6.4 and 21.2 ng L^{-1} (r^2 = 0.991), respectively. The concentrations employed in this work were checked at the end of the tests, as described above.

2.3. Acute lethal toxicity tests (forced exposure)

Forced static acute tests of BPA exposure were performed using a population of 10 fish per treatment (n = 3) in 1 L capacity aquaria. The animals were exposed for 72 h at a temperature of 23 ± 2 °C, with a photoperiod of 12 h: 12 h (light: dark), without feeding. A BPA stock solution (40,000 μg L^{-1}) was prepared on the same day as the tests, and the concentrations used in the experiments were 0 (control), 1000, 5000, 10,000, 20,000, and 40,000 μg L^{-1}. The aquaria were constantly aerated in order to maintain a DO concentration of > 6 mg L^{-1}. Measurements of pH, conductivity, and DO were made at the end of each test.

2.4. Non-forced exposure system

Avoidance tests were conducted in a multi-compartment system with dimensions similar to those described by Araújo et al. (2014b)