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Review

Environmental pollution affects molecular and biochemical responses during gonadal maturation of *Astyanax fasciatus* (Teleostei: Characiformes: Characidae)

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ABSTRACT

Endocrine disrupting compounds (EDCs) have the potential to alter fish reproduction at various levels of organization. The aim of this study was to assess the impact of a natural environment with heavily anthropogenic influence on the physiological processes involved in reproduction in the freshwater fish lambari (*Astyanax fasciatus*) using different biomarkers. Adult males and females were collected in different seasons from two distinct sites in the same watershed: Ponte Nova Reservoir (PN) considered a pristine or small anthropogenic influence reference point; and Billings Reservoir (Bil), subjected to a large anthropogenic impact. Biological indices, such as hepatosomatic index and gonadosomatic index (GSI), gonadal histomorphology, fecundity, and biomarkers such as plasma levels of estradiol (E2) as well as hepatic gene expression of its alfa nuclear receptor (ERα), were analyzed. Hepatic vitellogenin (VTG) gene expression was evaluated in both sexes, as an indicator of xenoestrogen exposure. Females collected at PN presented a typical annual variation reflected in GSI, whereas for those sampled at Bil the index did not change through the seasons. The higher concentration of E2 in males collected at Bil during spring/2013, together with the detection of VTG gene expression, suggest the presence of EDCs in the water. These EDCs may have also influenced fecundity of females from Bil, which was higher during winter and spring/2013. Gene expression of ERα and ovarian morphology did not differ between fish from both sites. Water conditions from Bil reservoir impacted by anthropic activity clearly interfered mainly with biomarkers of biological effect such as plasma E2 levels and absolute and relative fecundity, but also altered biomarkers of exposure as VTG gene expression. These facts support the notion that waterborne EDCs are capable of causing estrogenic activity in *A. fasciatus*.

1. Introduction

The introduction of contaminants into the environment due to anthropogenic activities may cause adverse effects on human health as well as wildlife populations (Vázquez et al., 2009). Among the contaminants affecting physiological processes of the aquatic biota are endocrine disrupting compounds (EDCs), which are defined as compounds which can interfere with the synthesis, secretion, transport, binding, action or elimination of naturally secreted hormones. A large number of EDCs present in natural environments originate from improper elimination from sewage in wastewater treatment plants (WWTPs) (Tan et al., 2007; Hassell et al., 2016). EDCs can act on different pathways involving many physiological processes, such as reproduction, mimicking estrogen hormones (Vázquez et al., 2009),

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affecting sexual differentiation (Baroiller and Guiguen, 2001), gonadal development (Vested et al., 2014), and inducing vitellogenin synthesis in males (Moncaut et al., 2003; Vetillard and Bailhache, 2006). Several types of compounds are able to act as EDCs and affect reproductive processes in fish, including non-steroidal anti-inflammatory drugs (NSAIDs) (Fernandes et al., 2011; Ji et al., 2013) and metals (Driessnack et al., 2016), displaying estrogen-like activity and interference with estradiol receptors (Orn et al., 2006; Xu et al., 2008; Flick et al., 2014) and vitellogenin (VTG) synthesis (Scholz et al., 2004; Muncke and Eggen, 2006; Salierno and Kane, 2009). These studies have shown that different pollutants can deleteriously alter several physiological processes involved in reproduction at different organizational levels, potentially leading to population losses.

The presence of different EDCs in the aquatic environment has been well characterized in several studies around the world (Kinney et al., 2006; Kellar et al., 2014; Dias et al., 2015; Gorga et al., 2015; Minh et al., 2016). A wide range of water contaminants with EDC potential, such as NSAIDs (ibuprofen and diclofenac), that may bioaccumulate in organisms have been reported in Brazilian reservoirs, as is the case for the Billings reservoir (Bil) located in the metropolitan region of São Paulo, Brazil (Almeida and Weber, 2005). Bil reservoir is characterized by an intense anthropogenic activity, such as domestic sewage disposal and industrial effluents, deforestation and extensive land use (Mariani and Pompêo, 2008; Moschini-Carlos et al., 2010). Metals such as lead, chromium, mercury and zinc have also been detected in fish tissues since the 1980s at several points in this reservoir (Rocha et al., 1985), like the Taquacetuba and Bororé branches, where metal water concentrations are above those recommended by resolution of the Special Secretariat for the Environment. In fact, Oliveira (2012) detected high chromium concentrations in the liver of 60% of the sampled individuals of acará, *Geophagus brasiliensis*, 67% of trahira, *Hoplias malabaricus*, 98% of lambari, *Astyanax* sp. and 100% of tilapia, *Tilapia rendalli*, which are in disagreement with the limit established by ANVISA Decree 55.871/65 (National Health Surveillance Agency). In addition, in the Taquecetuba branch, the concentration of metals (lead, copper, mercury, nickel and zinc) in the water has also been reported above those allowed by CONAMA Resolution 357 (Gomes et al., 2015).

Water and sediment monitoring studies led by the State Government of São Paulo demonstrated the presence of several contaminants and 21 out of 27 points analyzed were considered eutrophic (CETESB, 1996). In a later study, the Taquacetuba branch was considered super-eutrophic (Gomes et al., 2016), evidencing that Bil contains a large diversity of pollutants. Only a few fish species such as tilapia (*Oreochromis niloticus*), lambari (*Astyanax* sp.) and catfish (*Rhamdia* sp.) can be found in this water body as representative species (Castro et al., 2009). However, when the ecosystem is degraded, as is the case of Bil, it is difficult to understand whether the effects observed in organisms that inhabit in this area, are due to the action of contaminants or due to other stressors, like hypoxia or changes in the food chain (Hook et al., 2014). Then, in order to evaluate the effect of environmental pollution on those species, biomarkers of biological effects and exposure have to be used (Broerg et al., 2005; Hook et al., 2014). According to Hook et al. (2014), biomarkers of biological effects are indicators of physiological or biochemical changes as a consequence of exposure, such as condition indices or hormonal circulating levels; while biomarkers of exposure are defined as single or multiple pollutants with similar modes of action than can show an early response to contaminants and are typically specific to a class of contaminants, e.g., the induction of the vitellogenin in males induced by estrogenic compounds.

Astyanax fasciatus, commonly known as red-tailed lambari, is one of the species frequently found in Bil. reservoir. It is a small omnivorous fish considered an important prey for carnivorous species (Vilella et al., 2002; Gurgel, 2004). This species presents different reproductive strategies, which may vary according to the environmental conditions, presenting both asynchronous or synchronous oocyte development (Silva et al., 2010). Its distribution is very wide, reaching all of Central and South America, being found in both clean and polluted areas. All these features contributed to choose *A. fasciatus* as a sentinel species for environmental and toxicological test studies (Schulz and Martins-Júnior, 2001; Alberto et al., 2005; Carrasco-Letelier et al., 2006; Prado et al., 2011). Therefore, the aim of this study was to assess the impact of a heavily anthropogenic impacted environment on the physiological processes involved in reproduction in the freshwater fish lambari (*Astyanax fasciatus*) using different biomarkers at different organizational levels.

2. Material and methods

2.1. Study area

The study area comprised two important reservoirs of the Tietê River Basin, located in the Metropolitan Region of São Paulo (MRSP) in the State of São Paulo, Southeastern Brazil (see map at Gomes et al., 2015). Ponte Nova reservoir (PN), located at the Upper Reaches of the Tietê River Basin, is part of an environmental protected area occupied by rainforests, preserved ciliary forests and flood plains along the water course. Based on these characteristics, the PN reservoir was considered in this study as a reference site with minimal anthropogenic impact. Samples were collected in the Rio Claro branch (PN, 23°34′36.5″S, 45°54′23.9″W), considered in a previous study as mesotrophic according to its trophic state index (TSI) (Table 1).

Bilings reservoir (Bil), located inside the MRSP, is a heavily impacted area due to its used for generation of electric power and as a public water supply. This reservoir is considered the second largest water reservoir of the MRSP, with a drainage area of 1560 km^2 and a water surface of 106.6 km^2 (Marceniuk and Hilsdorf, 2010). The Bil reservoir has seven branches with spatially differentiated biotic and pollutant characteristics, such as domestic sewage and industrial effluents, deforestation and uncontrolled soil occupation (Gomes et al., 2015). Samples were collected in the Taquacetuba branch (Bil

Table 1
Hydrogeomorphical, physical, chemical, and biological characterization of the Ponte Nova (PN) and Billings (Bil) reservoirs.

Variables	PN	Bil
Surface area (km^2)	25.7	106.6
Water retention time (days)	720	80
Elevation (m)	840	745
Temperature (°C)	16–23	17–25.5
pH	6.5	7.5–9.5
Chlorophyll-a (μg L^{-1})	0.7–4.1	33.3–867.0
Nitrate (μg L^{-1})	9.5–200	288.9–1045.9
Nitrite (μg L^{-1})	2.0–9.0	8.1–25.7
Total nitrogen (μg L^{-1})	500.00–900.0	430.0–473.6
Total phosphorus (μg L^{-1})	8.0–20.0	54.6–402.2
TSI	Mesotrophic	Super–hypereutrophic
Ibuprofen (ng L^{-1})	NF	10.0–78.2
Diclofenac (ng L^{-1})	NF	8.1–394.5
Total cadmium (mg/kg)	< 0.005	0.005
Total lead (mg/kg)	< 0.01	0.01–0.06
Total copper (mg/kg)	< 0.005	0.005–0.1
Total mercury (mg/kg)	< 0.0002	0.0002–0.27
Total nickel (mg/kg)	< 0.01	0.01–0.11
References	Gomes et al. (2015); Carvalho (2003)	Almeida and Weber (2005); Carvalho (2003); Gomes et al., (2015, 2016)

TSI Trophic State Index; NF not found.