Science of the Total Environment 592 (2017) 693–703

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Reproductive effects of oestrogenic endocrine disrupting chemicals in *Astyanax rivularis* inhabiting headwaters of the Velhas River, Brazil

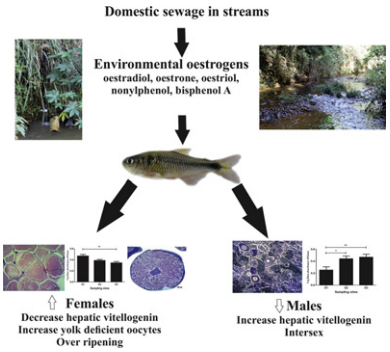
André Alberto Weber [a], Davidson Peruci Moreira [a], Rafael Magno Costa Melo [a], Augusto Bicalho Cruz Vieira [a], Paula Suzanna Prado [a], Mirra Angelina Neres da Silva [b], Nilo Bazzoli [c], Elizete Rizzo [a,*]

[a] Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, C.P. 486, 30161-970, Minas Gerais, Brazil
[b] Departamento of Química, Universidade Federal de Minas Gerais, Belo Horizonte, C.P. 486, 30161-970, Minas Gerais, Brazil
[c] Programa de Pós-graduação em Zoologia de Vertebrados, Pontifícia Universidade Católica de Minas Gerais, PUC Minas, Belo Horizonte 30535-610, Minas Gerais, Brazil

HIGHLIGHTS

- Oestrogenic EDCs were assessed by HPLC/MS in Velhas River headwaters, Brazil.
- Over-ripening and yolk deficient oocytes can be novel biomarkers of oestrogenic EDCs.
- Intersex gonads showed perinucleolar follicles into the seminiferous tubules.
- ELISA assays showed higher hepatic Vtg levels in males from impacted sites.
- Physiological follicular atresia occurred in sites contaminated by oestrogens.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 20 January 2017
Accepted 22 February 2017
Available online 22 March 2017

Editor: D. Barcelo

Keywords:
Endocrine disruption
Vitellogenin
Yolk deficient oocyte
Intersex
Gametogenesis
Spawning

ABSTRACT

The Velhas River is the most polluted river in the state of Minas Gerais, south-eastern Brazil. Due to its historical and environmental relevance, the aim of this study was to evaluate the effects of oestrogenic endocrine disruptors on the reproduction of the lambari *Astyanax rivularis*, a small-sized species found in headwaters of the São Francisco River basin. Quarterly field samplings were carried out during a reproductive cycle in three streams of the upper Velhas River: S1 (reference site) and S2 and S3 (sites contaminated by untreated sewage). The main oestrogenic compounds were evaluated in water using HPLC/MS. Molecular, histological and reproductive biomarkers were assessed in liver and gonad. The results showed higher average concentrations of oestradiol (>200 ng/l) in S2 and S3, oestrone (>250 ng/l) in S2 as well as oestriol (>200 ng/l), bisphenol A (>190 ng/l), and nonylphenol (>600 ng/l) in S3 compared to S1 (<70 ng/l for all compounds). In S2 and S3, there was an increase in the proportion of females, higher ELISA levels of vitellogenin (Vtg) and proteins of the zona radiata (Zrp) in liver males. Insulin-like growth factor (IGF-I) levels were lower in S2 males, which also had a smaller body size, a smaller seminiferous tubule diameter, a higher proportion of spermatogonia, and lower proportion of spermatozoa in relation to S1. Histopathological analyses detected an increase in yolk deficient oocytes and over-ripening in the contaminated sites, and these alterations were associated to a reduction of hepatic Vtg levels and a delay in spawning, respectively. Intersex specimens with perinucleolar follicles in a multifocal distribution in the testis were detected in S2 and S3. These results indicate that chronic exposure to oestrogenic compounds induced endocrine disruption that may affect wild populations of *A. rivularis* in the Velhas River.

* Corresponding author.
E-mail address: ictio@icb.ufmg.br (E. Rizzo).

http://dx.doi.org/10.1016/j.scitotenv.2017.02.181
0048-9697/

1. Introduction

Untreated domestic sewage discharge into rivers and streams contains substances which have the ability to interact with the endocrine system of animals called endocrine disrupting chemicals (EDCs) (Babin et al., 2007). Among the EDCs, there are the oestrogenic endocrine disruptors, a subclass of compounds including both natural and synthetic oestrogens (Campbell, 2003). Natural oestrogens are mainly related to human metabolism, and population growth in areas adjacent to water bodies causes an increase of these substances in rivers, streams, lakes, and reservoirs. Synthetic substances from various sources such as agricultural, pharmaceutical, and industrial products that mimic the action of natural oestrogens (xenoestrogens) are also found in the aquatic environment (Barber et al., 2012).

EDCs can act in two critical windows of the development: sexual differentiation and gonadal maturation (Bahamonde et al., 2013). Exposure to EDCs in the early stages of development results in irreversible changes in tissue differentiation, which affect sex and in a long-term the reproductive potential of the offspring. In adults, endocrine disruption may be reversible, but chronic exposure to EDCs may compromise the viability of gametes and the sustainability of the species in its habitat (Denslow and Sepúlveda, 2007). Vitellogenin (Vtg) and zona radiata proteins (Zrp) are biomarkers widely used in endocrine disruption studies (Bahamonde et al., 2014; Kidd et al., 2007; Prado et al., 2014, 2011; Randak et al., 2009; Schultz et al., 2013). These proteins are synthesised in the liver of females under the regulation of 17β-oestradiol (E2), and males produce significant quantities of Vtg and Zrp upon exposure to oestrogenic endocrine disruptors (Bahamonde et al., 2014, 2013; Desforges et al., 2010; Prado et al., 2011; Tyler and Jobling, 2008). Zrp expression precedes that of Vtg, thus its expression in males is considered an early signal of the presence of oestrogenic compounds in water (Arukwe and Røe, 2008).

Besides Vtg and Zrp, insulin-like growth factors (IGFs) are targets of oestrogenic endocrine disruptors, such as E2 and 17α-ethinylestradiol (EE2), and other compounds released into the aquatic environment (Prado et al., 2014; Shved et al., 2008). IGFs are produced mainly in the liver under regulation of growth hormone and changes in expression of these proteins may affect gametogenesis and reproduction of fish species (Berishvili et al., 2006; Reinecke, 2010). Growth factors, specifically IGF-I, have various functions, such as growth regulation as well as mitogenesis, differentiation, and apoptosis inhibition in gonads (Reinecke, 2010; Shved et al., 2008).

Reproductive biomarkers such as the gonodosomatic index, fecundity, proportion of intersex fish, and sex ratio are also affected by oestrogenic EDCs (Denslow and Sepúlveda, 2007). Intersex specimens present male and female germ cells in the same gonad in gonochoristic species, and this condition can be induced by water contaminated with oestrogenic and anti-oestrogenic compounds. (Bahamonde et al., 2015; Tyler and Jobling, 2008). In South America, studies on endocrine disruption affecting the wild fish fauna are scarce and intersex gonads have been reported in few species (Chiang et al., 2015; Kinnison et al., 2000; Prado et al., 2014, 2011).

The lambari *Astyanax rivularis* (Lütken, 1875) is a small-sized, gonochoristic species of the Characidae family, inhabiting creeks and streams with strong currents and high altitudes in the upper Velhas River, São Francisco River basin (Lima et al., 2003). This species can reach up to 15 cm in total length and is omnivorous with opportunistic feeding habits, consuming allochthonous and autochthonous items (Vieira et al., 2015). The lambaris have asynchronous oocyte development, spawn in batches in a prolonged breeding season (Veloso-Júnior et al., 2009), and are suitable species to be used as a sentinel model for ecotoxicological studies (Prado et al., 2014, 2011).

The upper Velhas River is located in a transition area between the Atlantic Forest and Cerrado biomes, and both types of vegetation are considered biodiversity hotspots, due to the high number of endemic species and excessive loss of habitat (Myers et al., 2000). Despite its ecological and historical significance as one of the main routes of the gold trade in the 18th century, the Velhas River is the most polluted river in the state of Minas Gerais, due to a population of over 5 million people living in the surroundings of its basin (IBGE, 2014).

Thus, the aim of this study was to investigate the reproductive biology of *Astyanax rivularis* exposed to domestic sewage in streams of the upper Velhas River, using histological, morphometric and molecular approaches.

2. Materials and methods

2.1. Study area

The Velhas River has an average annual water flow of 631 m^3/s and drains an area of 27,867 km^2 in the central area of the state of Minas Gerais, south-eastern Brazil. The headwaters of the Velhas River, at 1520 m above sea level, are located in Andorinhas Park, municipality of Ouro Preto, a UNESCO World Heritage site for its historic buildings and culture.

For this study, three streams of the upper Velhas River were chosen for fish sampling. One stream with low anthropogenic interference (S1, reference) and two receiving municipal domestic sewage (S2 and S3) (Fig. 1). The pollution sources of each sampled stream are described in Table 1.

2.2. Fish collection

Fish collection procedures followed the ethical principles established by the Brazilian College of Animal Experimentation (COBEA), and the study was approved by the Ethics Committee on Animal Use (CEUA, protocol N° 189) of the Federal University of Minas Gerais, Brazil. A total of 1129 females and 265 males of *A. rivularis* were caught during quarterly samplings encompassing a reproductive cycle. At each sampling site, the fish were caught using 100 m (10 gill nets of 10 m) gillnets with a 0.8–1.5 cm stretched mesh size deployed for about 12 h in pools of the streams. Alive fish were euthanized with immersion in eugenol 85 $mg \cdot L^{-1}$. Total length (TL; 0.01 cm), body weight (BW; 0.01 g), gonad weight (GW; 0.001 g), and liver weight (LW; 0.001 g) were measured and the following biological indices were calculated for each fish: gonadosomatic index (GSI = 100 GW/BW), liver somatic index (LSI = 100 LW/BW), and Fulton condition factor (K = 100 BW/TL^3).

During fish samplings, the temperature, pH, dissolved oxygen concentration, conductivity, water flow, and turbidity were measured at each collection site using a multi-parameter Horiba U51 probe, a General Oceanics flowmeter, and a Quimis turbidimeter.

2.3. Main oestrogens in the water

For analysis of the main oestrogenic endocrine disruptors, standards of oestrone (E1), oestradiol (E2), oestriol (E3), bisphenol-A (BPA), and nonylphenol (NP), were purchased from Sigma-Aldrich (St. Louis, MO, USA), all with ≥97% purity. Calibration curve, standard mass and target ions (m/z) of each compound are shown in Table S1 (Supplementary material). During samples collection, 500 ml surface water were obtained in amber glass, three in June (dry season) and three in December (rainy season) from each sampling site and immediately cooled to 4 °C for posterior analyses using high performance liquid chromatography (HPLC). The compounds contained in the samples were extracted by solid phase (SPE C18), dried, and eluted in 1 ml of methanol in a microcentrifuge tube. Aliquots were again dried by evaporation and resuspended in 100 μl of methanol in each tube, and were subsequently shaken in a vortex. Then, 20 μl of the samples were injected into a Shimadzu LCMS-IT-TOF (225-07100-34) liquid chromatograph equipped with a DGU-20A3 degasser, two LC-20AD pumps, a SIL-20A autosampler, a SPD-10A UV–Vis detector, and a CBM-20A communication