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Integrated use of antioxidant enzymes and oxidative damage in two fish species to assess pollution in man-made hydroelectric reservoirs

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ABSTRACT

This study investigated the relationship between contaminant body burden and the oxidative stress status of the gills and livers of two wild fish species in the Furnas Hydroelectric Power Station (HPS) reservoir (Minas Gerais, Brazil). Gills and livers presented similar pathways of metals and organochlorine bioaccumulation. During June, organochlorines were associated with lipid peroxidation (LPO), indicating oxidative stress due to the inhibition of the antioxidant enzymes superoxide dismutase and glutathione peroxidase. In the most polluted areas, metal concentrations in the liver were associated with metallothionein. During December, contaminants in the gills and liver were associated with catalase activity and LPO. Aldrin/dieldrin was the contaminant most associated with oxidative damage in the livers of both species. This integrated approach shed light on the relationship between adverse biological effects and bioaccumulation of contaminants inputted by intensive agricultural practices and proved to be a suitable tool for assessing the environmental quality of man-made reservoirs.

1. Introduction

Man-made hydroelectric reservoirs provide a diverse habitat for aquatic organisms and are ecologically important aquatic systems due to their vertical organization of lentic ecosystems and horizontal organization of lotic ecosystems. These environments are susceptible to human activity, which may be aggravated by the intermittent and relatively low water flow of these reservoirs. In tropical regions where the economy is based on agriculture, intensive agricultural and livestock activities on the lands surrounding the reservoirs generate a high amount of residue that causes water contamination and affects the aquatic biota (Dores et al., 2008; Britto et al., 2012).

The area surrounding the reservoir of the Furnas Hydroelectric Power Station (HPS) is characterized by intense agricultural activity and cattle farming, although isolated spots of native vegetation are still found along the border. The reservoir, which is in proximity to thirty-four small- to medium-sized cities, exhibits marginal soil erosion in some regions. In general, the reservoir shows oligotrophic characteristics, with some regions showing relatively high levels of degradation and mesotrophic and eutrophic characteristics (Heleno, 2004). Furthermore, the water and sediment of the reservoir indicate organochlorine and metal contamination (Sadauskas-Henrique, 2008; Sadauskas-Henrique et al., 2011).

In general, most of the environmental monitoring programs that assess water quality of hydroelectric reservoirs consist of physical and chemical limnological analyses of water and sediment. The evaluation of endemic fauna is rare, and when included, such evaluations are usually restricted to the identification of fish size and species diversity. However, biochemical and cellular responses of the aquatic biota to the presence of contaminants in the water have been important tools for detecting the impact of contaminants in the ecosystem (Fernandes et al., 2013; Porte et al., 2001, 2002). Biochemical reactions to contaminants are usually the earliest biological changes to occur and the etiology of physiological and morphological changes in the animal (Ciccotelli et al., 1998), thus making the reactions important biomarkers to use in biomonitoring programs. Among biochemical biomarkers, the antioxidant defense system plays an important role in maintaining cellular homeostasis and has been used as an indicator of contaminant exposure in a variety of animal species, including fish. Xenobiotic agents that directly affect either the cells or the detoxification process in the

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cells may generate reactive oxygen species (ROS). The cellular antioxidant system acts to neutralize the endogenous and exogenous induction of ROS (Ruas et al., 2008; Simonato et al., 2011); however, the antioxidant system may be either stimulated or inhibited by xenobiotics depending on the intensity and duration of exposure and the susceptibility of the exposed animal (Cossu et al., 2000; Pereira et al., 2007).

The antioxidant defense (AD) system includes many enzymes and compounds that scavenge ROS. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the first line of enzymes of the second phase of the detoxification process. Metallothionein (MT) is a non-enzymatic antioxidant that is involved in the homeostasis of essential metals and shows a high scavenging activity against different free-radical species and heavy metals, including copper and cadmium (Viarengo et al., 1999; Marcon and Wilhelm Fillho, 1999). Oxidative stress occurs when the equilibrium between ROS production and the antioxidant defenses is lost (Ahmad et al., 2000); such stress induces changes to DNA, lipid peroxidation (LPO) and membrane destabilization, which lead to different pathologic processes (Pereira et al., 2011) and fish diseases (Livingstone, 2001).

The present study investigated the oxidative stress status of *Astyanax fasciatus* and *Pimelodus maculatus*, which are two ecologically distinct species that inhabit the reservoir of the Furnas HPS; the relationship between the species and the contaminant body burden was also studied. For this purpose, a set of enzymatic and non-enzymatic antioxidants and the LPO levels were selected as biochemical biomarkers. The gills, which are in direct contact with the environmental water, and liver, which is the major detoxification organ, were analyzed for these biomarkers; additionally, organochlorines and metal bioaccumulation were assessed via chemical analysis. Multivariate analysis was employed to determine the most significant relationships between biomarker responses and analytical chemistry, highlighting spatial and temporal patterns of exposure and effects.

2. Materials and methods

2.1. Study area

Located in Minas Gerais, Brazil (Fig. 1), the Furnas HPS is the result of the damming of the Grande River. It has 1440 km^2 overflow area, including the Sapucaí River, consists of 21 million m^3 of water, and has a perimeter of 3500 km (Furnas Centrais Elétricas, 2006). Water and fish specimens were collected from five sites: site 1, the reference site – FU10, was located at the confluence of the Grande and Sapucaí Rivers [Turvo (S20° 40′ 835″ W46° 13′ 232″)]; site 2, Guapé – FU20 (S20° 44′ 331″ W45° 55′ 800″), and site 3, Porto Fernandes – FU50 (S20° 48′ 826″ W45° 40′ 567″), were both located in the Rio Grande axis; and site 4, Barranco Alto – FU30 (S21° 10′ 510″ W45° 57′ 061″), and site 5, Fama – FU40 (S21° 24′ 074″ W45° 49′ 621″), were both located in the Rio Sapucaí axis (Fig. 1).

2.2. Fish and water collection

Lambari (*Astyanax fasciatus*, $n = 20$/site, Wt = 37.8 ± 2.6 g, Lt = 14.3 ± 0.3 cm) and mandi (*Pimelodus maculatus*, $n = 15$/site, Wt = 182.3 ± 32.9 g, Lt = 25.1 ± 1.4 cm) specimens, along with water samples, were collected in June and December of 2006. The fish were anaesthetized and then killed by medullar section in accordance with the national guidelines for animal experimentation. Samples of the gills and liver were immediately frozen for chemical and biochemical analyses.

2.3. Water analysis

Dissolved oxygen (DO), conductivity, temperature and pH were measured in the field using a multi-parameter water analyzer (YSI 600XL, YSI Inc, USA). Alkalinity and total phosphorus were determined as described by Golterman et al. (1978). Total hardness was determined following the APHA (1992) methodology, and ammoniacal nitrogen, nitrite and nitrate were determined using the colorimetric method (Mackereth et al., 1978).

2.4. Gill and liver chemical analysis

For metal analyses, the gill and liver samples were weighted, dried at 60 °C until a constant weight was reached, and digested with a solution (1:1) of ultra-pure H_2SO_4 (96%) and H_2O_2 (30%). Metal concentrations were determined with the standards SW84603050/3051 (USEPA, 1986) by using an atomic absorption spectrometer (Gemini AA 12/1475, Varian, USA). The aluminum concentration was determined by using eriochrome cyanine R.

The pesticide residue in the gills and liver was determined using a gas-chromatograph coupled with a mass spectrometer (GS–MS) according to USEPA protocols. A liquid–solid extraction was performed using styrene/dimethylbenzene as solid phase and dichloromethane/acetone as liquid phase; then, the GC–MS analysis was performed using a TurboMass Gold system (Perkin Elmer, USA).

2.5. Gill and liver assay

Gill or liver samples were homogenized in 0.1 M sodium phosphate buffer (pH 7.0) at a ratio of 1:10 w/v for LPO levels and SOD, CAT and GPx activity assays. The homogenates were centrifuged at 12,000 *g* at 4 °C, and the supernatants were used for biochemical assays, which were measured spectrophotometrically (Libra S32, Biochrom, USA) at 25 °C. Total protein content in each sample was determined according to the Bradford method (Bradford, 1976) by using a Dynex MR-HD microplate reader (Dynex Technologies, UK) at 595 nm with bovine serum albumin as a standard.

Following the methodology of Jiang et al. (1992), LPO in the gills and liver was assessed by Fe^{2+} oxidation in the presence of xylenol orange (FOX, ferrous oxidation-xylenol orange assay). The homogenized samples were treated with 10% trichloroacetic acid and centrifuged. The supernatants were applied to a solution containing 900 μL of FOX reagent in 90% (v/v) methanol and incubated at 37 °C for color

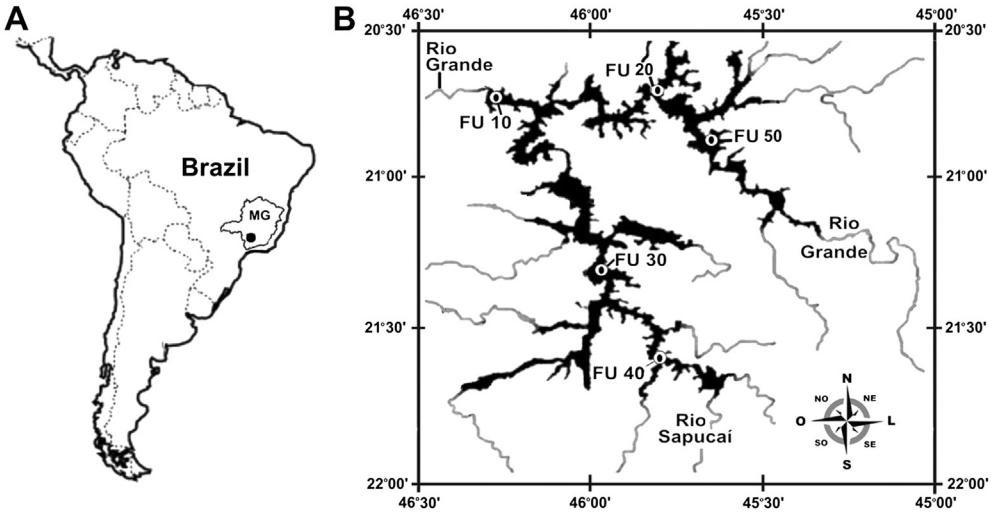

Fig. 1. A. Localization of the Furnas Hydroelectric Power Station reservoir in Minas Gerais (MG) State (●), Brazil; **B**. Map of the Furnas Hydroelectric Power Station reservoir, Minas Gerais, Brazil, showing the sites of water and fish collection (●): FU10 (Turvo), FU20 (Guapé), FU30 (Barranco Alto), FU40 (Fama), and FU50 (Porto Fernandes).