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The impact of organochlorines and metals on wild fish living in a tropical hydroelectric reservoir: bioaccumulation and histopathological biomarkers

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HIGHLIGHTS

- Multiple contaminants accumulation in wild fish causes histological changes.
- Metals and organochlorines have similar bioaccumulation pattern in gills and liver.
- Contaminant bioaccumulation was higher in the liver than in the gills.
- The histopathological indices in the liver were greater than those of gills.
- Gills and liver lesions are useful for the discrimination of contaminated field sites.

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ABSTRACT

This study evaluates the contaminants in water and their bioaccumulation in the gills and liver of two ecologically distinct fish species, *Astyanax fasciatus* and *Pimelodus maculatus*, living in the reservoir of the Furnas hydroelectric power station located in Minas Gerais in the southeastern Brazil. The histological alterations in these organs are also examined. Water and fish were collected in June and December from five sites (site 1: FU10, site 2: FU20, site 3: FU30, site 4: FU40 and site 5: FU50) in the reservoir, and agrochemicals and metals selected based on their use in the field crops surrounding the reservoir were analyzed in the water and in the fish gills and livers. The concentrations of the organochlorines aldrin/dieldrin, endosulfan and heptachlor/heptachlor epoxide as well as the metals copper, chromium, iron and zinc in the gills and livers of both fish species were higher in June than in December; the liver accumulated higher concentrations of contaminants than the gills. The organochlorine metolachlor was detected only in the liver. The histological pattern of changes was similar in both species with regard to contaminant accumulation in the gills and liver. Fish from FU10, the least contaminated site, exhibited normal gill structure and moderate to heavy liver damage. Fish collected at FU20 to FU50, which were contaminated with organochlorines and metals, showed slight to moderate gill damage in June and irreparable liver damage in the livers in June and December. The histological changes in the gills and liver were suitable to distinguishing contaminated field sites and are therefore useful biomarkers for environmental contamination representing a biological end-point of exposure.

1. Introduction

Aquatic ecosystems and wildlife are continuously threatened by anthropogenic activities. In the reservoirs erected to generate hydroelectricity, the aquatic ecosystems are phenotypically intermediate between those of lakes and rivers with specific limnological characteristics due to the intermittent water flow (Straskraba and Tundisi, 1999). Although the generation of electricity does not change the water quality, the inadequate use of the soil surrounding the reservoir may contribute to aquatic environmental degradation due to the domestic, industrial and agricultural effluents that are produced as a consequence of an increasing population and increased economic development.

The Furnas hydroelectric power station (HPS) reservoir is one of largest in southeastern Brazil and was formed by the damming of two rivers: the Grande River and the Sapucai River. The reservoir has a 1440 km^2 overflow area containing 21 million m^3 of water with a perimeter of 3500 km, and it is bordered by 34 small to medium-sized

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cities, most of which engage in intense agricultural and cattle farming activities. The limnological, physical and chemical variables of the reservoir water are within the range of the limits recommended by the Brazilian Environment National Council for water biota preservation (CONAMA 357, 2005) and exhibit oligotrophic characteristics (Heleno, 2004). However, the dendritic morphology of the reservoir favors horizontal variation in the water quality, and some regions are highly degraded and exhibit mesotrophic- and eutrophic characteristics (Sá Junior, 1994; Heleno, 2004; Negreiros et al., 2010). The presence of trace metals (aluminum, chromium, copper, iron and zinc) and organochlorines (aldrin/dieldrin, endosulfan, heptachlor/heptachlor epoxide and metolachlor) in the water and sediment of different regions of the reservoir highlights the dissimilarity between the Grande and the Sapucai rivers (Sadauskas-Henrique, 2008; Sadauskas-Henrique et al., 2011).

Chronic exposure to multiple contaminants in water, even at low levels, affects the resident biota, including the fish. Contaminant accumulation in the tissues induces changes in the biochemistry and physiology of the cells (Boon et al., 2002; Fernandes et al., 2007). Previous studies of fish collected from the Furnas HPS reservoir reported changes in blood variables and in the induction of oxidative stress in the blood, gills and liver of fish (Sadauskas-Henrique et al., 2011; Sakuragui et al., 2013) as well as inhibition of Na^+/K^+-ATPase and alterations in the surface architecture of pavement and chloride cells in the gills (Fernandes et al., 2013). Such biochemical and physiological alterations may lead to histological changes in these organs.

Histological biomarkers are an intermediate level of biological organization (between the molecular and individual levels), and they are useful tools for integrating the cumulative effects of biochemical and physiological alterations (Myers and Fournie, 2002). Chemical tissue analyses and histological biomarkers permit the identification and evaluation of the impact of contaminants present in aquatic ecosystems in addition to the degree of environmental pollution; thus, they provide important information for the generation of public policies for monitoring and conservation of biota (Van der Oost et al., 2003). In fish, the gills and liver are the main target organs used to evaluate water quality. The gills are the main sites for gas exchange as well as acid–base and ionic regulation and are the first organs to contact the contaminants. The large surface area of the gills that is in contact with water and the very thin diffusion distance between water and blood favor the uptake of contaminant molecules dissolved in water. The contaminant uptake from water into a fish by the gills depends on the movement of the water with dissolved contaminants through the gill lamella and the diffusion of the contaminants across the water and the gill epithelium and from the gill into the blood (McKim and Erickson, 1991). The physicochemical and biological mechanisms that influence the diffusion of contaminants across the biological membranes of gills include the concentration of the contaminant in the water, its molecular weight and volume, the charge on the molecule and its lipid solubility (Spacie and Hamelink, 1982) in addition to the flow of water and blood to and from the gills, which maintains the diffusion gradient. The liver has numerous functions: it is the main organ involved in the metabolism of proteins, lipids and carbohydrates; it plays an important role in the storage and distribution of compound reserves; and it has a key role in xenobiotic biotransformation, detoxification and excretion.

In this context, the present study was designed to integrate the histological changes in gills and liver with the chemical analysis of these organs in two native fish species, the lambari, *Astyanax fasciatus*, and the mandi, *Pimelodus maculatus*, which inhabit the reservoir of the Furnas HPS. The histological changes permit evaluation of the effect of multiple contaminants on the structure of these organs and the possible harmful effects on fish health. Both fish species are omnivorous and have different feeding habits: *A. fasciatus* is a benthic–pelagic species, and *P. maculatus* is a benthic and bottom-feeding species (Esteves and Galetti, 1995; Ramos et al., 2011). These species are widely distributed throughout the reservoir and do not exhibit long distance migratory behavior (Costa et al., 2013), making them useful for comparing different sites in the same aquatic system.

2. Materials and methods

2.1. Study area and water and fish sampling

Water and specimens of *A. fasciatus* (n = 20/site, Body mass [M_B] = 37.8 ± 2.6 g, total Length [Lt] = 14.3 ± 0.3 cm) and *P. maculatus* (n =15/site, M_B = 182.3 ± 32.9 g, Lt = 25.1 ± 1.4 cm) were collected from five sampling sites in the reservoir of the Furnas HPS, Minas Gerais, Brazil (Fig. 1) in June (winter, dry season) and December (summer, wet season) of 2006. Site 1: FU10–Turvo (the reference site) is located at the confluence of the Grande and Sapucaí Rivers (S20° 40′ 835″ W46° 13′ 232″); site 2: FU20–Guapé (S20° 44′ 331″ W 45° 55′ 800″) and site 3: FU50–Porto Fernandes (S20° 48′ 826″ W45° 40′ 567″) are located in the Grande River axis; while site 4: FU30–Barranco Alto (S21° 10′ 510″ W45° 57′ 061″) and site 5: FU40–Fama (S21° 24′ 074″ W45° 49′ 621″) are located in the Sapucaí River axis (Fig. 1). Collected fish were anesthetized with benzocaine (0.1 g L^{-1}) and killed by medullar sectioning. The gills and liver were removed and systematically sampled. Tissue samples for chemical analyses were stored at −80 °C, and samples for histological analyses (5 samples/organ/fish/site) were immediately fixed with 2.5% glutaraldehyde buffered at pH 7.3 in 0.1 M phosphate buffer solution or Bouin's solution. Water samples (3 L) were collected (0.20 m below the surface water) into pre-cleaned amber glass bottles (0.2–1.0 L) and preserved according to the analyses to be done: physicochemical variables, metals and agrochemicals. All water samples were stored at ~ 4 °C immediately after collection until laboratory analysis. The agrochemicals evaluated in the water and in the gills and liver of fish were those generally applied to the field crops cultivated around the reservoir, the organochlorines: the 2,4-dichlorophenoxyacetic acid, alachlor, atrazine, hexachlorobenzene, lindane (gamma-BHC), metolachlor, methoxychlor, permethrin, propanil, simazine, aldrin, dieldrin, chlordane, endosulfan, endrin, heptachlor and heptachlor epoxide, pentachlorophenol and dichlorodiphenyltrichloroethane (DDT isomers), and the non-organochlorines: molinate, pendimethalin, trifluralin and bentazon (non-organochlorine contaminants).

2.2. Chemicals

All reagents for metal analysis were of analytical grade. n-Hexane, dichloromethane, diethyl ether (pesticide grade), sulfuric acid 95%–97%, acetone and anhydrous sodium sulfate for analysis were from Sigma Aldrich (Milwaukee, WI, USA). The purity of the solvents was determined by gas chromatography coupled to electro capture detection (GC–ECD). The standard 2,4-dichlorophenoxyacetic acid, alachlor, atrazine, hexachlorobenzene, lindane (gamma-BHC), metolachlor, methoxychlor, permethrin, propanil, simazine, aldrin, dieldrin, chlordane, endosulfan, endrin, heptachlor and heptachlor epoxide, pentachlorophenol and dichlorodiphenyltrichloroethane (DDT isomers), molinate, pendimethalin, trifluralin and bentazon, and element standards were from Sigma Aldrich (Milwaukee, WI, USA). Florisil powder (Merck; 0.150–0.250 mm in diameter) was used for residue analysis quality assurance and was baked at 150 °C for 4 h to ensure dryness.

2.3. Water chemical analyses

Dissolved oxygen (DO), conductivity, temperature and pH were measured in the field using a multi-parameter water analyzer (YSI, 600XL). Alkalinity was determined as described by Golterman et al. (1978), and hardness was determined following APHA (1992) methodologies. Concentrations of nitrogen in the forms of ammonium, nitrite and nitrate were determined using a colorimetric method (Mackereth et al., 1978). The concentrations of agrochemicals and metals were determined by the laboratory BioEng Ltda (São Carlos, SP, Brazil).