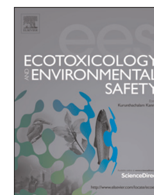
Ecotoxicology and Environmental Safety 111 (2015) 23–31

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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



Environmental effects on the gills and blood of *Oreochromis niloticus* exposed to rivers of Bahia, Brazil

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ARTICLE INFO

Article history:
Received 20 April 2014
Received in revised form
14 September 2014
Accepted 23 September 2014
Available online 31 October 2014

Keywords:
Gill histopathology
Hematology
Contamination
Environmental biomarkers
Freshwater fish
Field study

ABSTRACT

Through the integration of chemical, biochemical and morphological analyses, this study investigated the effects of multiple pollutants on environmental biomarkers, such as gill histopathological changes and hematological and biochemical parameters, in *Oreochromis niloticus* exposed to four sites in the Jacuipe and Subaé rivers over seven days. Sediment analyses identified Sapelba as the most contaminated site, followed by Oliveira de Campinhos, Santo Amaro and Jacuípe. Water analyses revealed aluminum, iron and manganese at all sites. Aluminum and other metal were also detected in the gills of fishes. Fish exposed to the Sapelba site exhibited significant necrosis formation, as well as higher hematological parameters and trend to increase of cortisol levels. However, filament epithelium proliferation was higher at the Oliveira de Campinhos and Santo Amaro sites, at which the lowest levels of the hematological variables were observed. Multivariate analysis grouped some gill histopathological changes together, such as epithelial detachment with edema and lamellar epithelial proliferation with the lamellar fusion of adjacent filaments, revealing relationships among them. Positive associations were identified between sediment contamination and necrosis and cortisol, while water contamination was related with filament epithelium proliferation, aneurism, lamellar fusion and several hematological parameters. Furthermore, relationships between blood parameters and gill histopathological changes demonstrated a joint physiological response that may have resulted from environmental variables such as dissolved oxygen. The results exhibited the direct influence of xenobiotics on these biomarkers but also highlighted the need to consider the complexity of environmental factors to optimize the adoption of these environmental predictive tools.

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1. Introduction

One of the most common routes of exposure to xenobiotics in aquatic organisms is the direct intake of particles through gill surfaces (Geffard et al., 2003; Grosell et al., 1998; Handy, 1993). Due to their large surface area exposed to the external environment, gills become the first targets of waterborne pollutants, causing the appearance of histopathological changes (Mallatt, 1985). These lesions can be considered either as direct damage to gill tissue inflicted by xenobiotics or as a primary defense mechanism against the entry of these substances within the organism, and they are recognized as biomarkers of environmental pollution (Monteiro et al., 2008; Garcia-Santos et al., 2007; Alazemi et al., 1996). According to Mallatt (1985), structural alterations of gills such as edema in the filament epithelium and lamellar epithelial detachment can be caused by exposure to heavy metals. Other histopathological changes findings have been described in fish exposed to several xenobiotics, including epithelial necrosis, vasodilation, hypertrophy and hyperplasia of the lamellar and filamentar epithelia (Arellano et al., 1999, Monteiro et al., 2008).

Because the gill is multifunctional, histopathological changes in this organ may lead to the impairment of several functions, including gas exchange, ion regulation and excretion of metabolites. Specifically in relation to fish respiratory physiology, changes in gill tissue are characterized by an increased diffusion distance for oxygen (water–blood) and a smaller respiratory surface, which can lead to functional hypoxia (Richards et al., 2009). Hypoxia associated with the presence of xenobiotics in the aquatic environment triggers several physiological responses, generally designated as stress responses. In fish, neuroendocrine responses, designated as primary stress responses, include the release of hormones such as cortisol. Secondary stress responses then occur,

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http://dx.doi.org/10.1016/j.ecoenv.2014.09.022
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consisting of various biochemical and physiological adjustments such as the mobilization of plasma glucose to meet increased energy demands (Wendelaar Bonga, 1997; Iwama et al., 2004) and changes in hematological parameters (Davison et al., 1993). These changes are also recognized as environmental biomarkers and were investigated in the present study.

These biomarker responses have been linked with various pollutants under controlled laboratory conditions, including copper (Monteiro et al., 2008; Nussey et al., 1995), lead (Olojo et al., 2005; Patnaik et al., 2011; Mager and Grosell, 2011), cadmium (Patnaik et al., 2011; Srivastava and Iishra, 1979), mercury (Ribeiro et al., 2006), nickel (Athikesavan et al., 2006), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Weber et al., 2011; Soengas et al., 1996; El-Shahawi et al., 2010). The knowledge of these responses in aquatic natural systems are scarce but take into account the dynamics of the river, its ecological processes and the interactions between pollutants in the environment. These studies are, therefore, more ecology relevant, as their results better describe the conditions of the natural environment (Yasser and Naser, 2011; Camargo and Martinez, 2007; Thiyagarajah et al., 1996; Nero et al., 2006; Larsson et al., 1984; Bainy et al., 1996).

Here, the environmental scenario was the Subaé river that suffer along its route by release of domestic sewage effluent by the cellulose and paper industries, as Sapelba and Penha, and proximity to the Brazilian Company of Lead (COBRAC), a disabled metallurgical which is considered an important source of metal contamination in the region of Santo Amaro, Bahia (Hatje et al., 2006).

Thus, this study was performed in the river Subaé with the aim of evaluating the effects of environmental conditions, concentrations of metals and persistent organic pollutants on biomarkers such as gill histopathological changes, hematological parameters, cortisol and glucose, thereby allowing a practical assessment of these biomarkers as predictive tools of environmental pollution.

The fish *Oreochromis niloticus* was employed as an experimental model because it is are easy to acquire in aquaculture systems and be of use in ecotoxicological studies in Brazil (Franco et al., 2010; Fuzzinato et al., 2013).

2. Materials and methods

2.1. Experimental model

Forty male juveniles (8.45 ± 1.28 cm, 9.15 ± 3.37 g) of *Oreochromis niloticus* (Linnaeus, 1758) were acclimated for one month in six tanks with a density of 3 g/l of water. Each week the water was renewed in approximately 70% of the total volume of each tank.

2.2. Experimental design

Ten fish were placed individually into cylindrical baskets of 30 cm in length and 10 cm in diameter, made from plastic screens with a 0.5 cm mesh to allow the free passage of water. The cages were spaced one meter apart and placed approximately 20 cm below the water surface, and they were then fixed on the borders of the rivers. The animal use was approved by the local ethics committee on the animal use (CEUA, IBIO, UFBA, 05/2012).

Four experimental groups were exposed to the Jacuípe or Subaé rivers, located in the northeastern state of Bahia, Brazil. In the Subaé river, three sites were chosen for exposure. The first site, designated as Sapelba (S), is located at the intersection of the BR-101 road with the Subaé river, close to the Sapelba paper industry in Feira de Santana (12°21′57,8″S; 38°52′3,9″O). The second site is located in the Oliveira de Campinhos district (OC), in Santo Amaro,

Fig. 1. Exposure site locations: (J) Site Jacuípe, Jacuípe river; (SA) Site Santo Amaro, Subaé river; (OC) Site Oliveira de Campinhos, Subaé river; (S) Site Sapelba, Subaé river. All sites were located in the north Recôncavo watershed, Bahia, Brazil.

at the junction of the BA-084 road with the Subaé River (12°25′56″ S; 38°48′2″W). The third site is located near Santo Amaro city (SA), downstream of the Penha packaging industry and near the effluent of the COBRAC metallurgical complex (12°32′21,9″S; 38°43′37,6″O). The single site at the Jacuípe river (J) is located in the Nazaré do Jacuípe district in São Sebastião do Passé (12°29′ 48,5″S; 38°36′42,1″W) (Fig. 1).

Each group was exposed to the water for seven days in the field, and the animals were then collected and taken to the Laboratory of Animal Physiology (LAFISA), Federal University of Bahia (UFBA), Salvador, in water from their respective collection sites. Tissue processing for the biological analyses was then conducted (item 2.5).

2.3. Water parameters and contamination

For water quality analysis, three samples of 1 L each from each experimental site were packed in plastic containers. The water samples were processed and analyzed for ammonia, ammonium ion, nitrate, phosphate, oxygen chemical and biochemical demand (OCD and OBD) and the presence of metals. The dissolved oxygen (DO), temperature, salinity, pH and total dissolved solids (TDS) of the water at each exposure site were determined on the first and last day of animal exposure using a Hanna® multiparameter measurer.

For ammonia determination (USEPA 350.2 method, U.S. Environmental Protection Agency, 1979), 100 mL of water from each experimental site was distilled in an alkaline medium under enclosed reflux. After this procedure, 25 mL of the sample was analyzed by nesslerization at 400 nm. The reading of ammonium ion concentration was taken in 25 mL of the distilled sample using specific ion electrodes for the determination of NH_4^+. To stabilize the result, 1 mL of ionic strength adjuster solution was added to each sample.

To determine the concentration of nitrate (SM 4500-NO_3E method; APHA, 2011), 25 mL of the filtered sample was passed through a column of cadmium with 75 mL of ammonium chloride and EDTA, and the reading was conducted using a spectrophotometer at a wavelength of 540 nm.

For phosphate analysis (4500-P method; APHA, 2011), a 50 mL aliquot of each sample was digested in a plate with an acid medium.

Oxygen chemical demand was determined by spectrophotometry at a wavelength of 600 nm after acid digestion of the sample under closed reflux for 2 h at 150 °C (SM 5220 method; APHA,

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