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Analyses of paralytic shellfish toxins and biomarkers in a southern Brazilian reservoir

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ABSTRACT

The Alagados Reservoir (Brazil) is an important source for the supply of water, recreation and fishery. Since 2002, the occurrence of cyanobacterial blooms (paralytic shellfish toxins – PST producers) have been noted. This study was aimed at the monitoring of PST occurrence in the Reservoir's water and fish. Biomarkers such as ethoxyresorufin-O-deethylase (EROD), glutathione S-transferase (GST), catalase (CAT), and acetylcholinesterase (AchE) activities, lipoperoxidation (LPO), histopathology, and comet assay were analyzed in fish. Water and fish were sampled in spring, summer and autumn. The PST concentrations in water were 5.15, 43.84, and 50.78 ng equiv Saxitoxin/L in the spring, summer and autumn, respectively. The PST muscle concentration was below the limit for shellfish. Gonyautoxins (GTX) were found in water samples and fish muscle, and GTX 5 was the major analogous found in muscle. In the summer samples, the LPO, genetic damage, and the GST and AchE activities increased while in the autumn an increase in EROD activity and genetic damage were observed. In all samplings, histopathological alterations in the fish gills and liver were found. The results showed a seasonal variation in the fishes health, which could be related also to farming activities and to the contaminants bioavailability during the year.

1. Introduction

The alteration of landscapes and the pollution of water resources by both natural and human-made processes have serious ambient, economic, and public health consequences. Thus, the use of water, either for regular supply or for generation of energy, must be managed in a responsible manner.

The enrichment of nutrients in aquatic ecosystems, especially those containing phosphorus and nitrogen, is an important factor that leads to the eutrophication of these systems and to an accelerated growth of algae (Briand et al., 2003). Cyanobacterial proliferations, also known as blooms, cause negative impacts on the ecosystem, on the health of animals living in these systems, and on human populations that use these water bodies for water supply and/or recreational purposes (Chorus and Bartram, 1999; Wiegand and Pflumacher, 2005). Cyanobacteria that produce poisonous toxins (cyanotoxins) have been reported from around the world over the past few decades (Carmichael,

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1994; Codd et al., 2005). Cyanotoxins are classified on the basis of their chemical (cyclic peptides, alkaloids, or lipopolysaccharides) or toxicological (hepatotoxins, neurotoxins, or dermatotoxins) properties (Patocka, 2001).

In 1999, Lagos et al. published the first report describing the production of saxitoxin analogs by the freshwater cyanobacterium *Cylindrospermopsis raciborskii* in South America. Because the occurrence of blooms due to the proliferation of this species was observed in different reservoirs in Brazil (Chellappa and Medeiros Costa, 2003; Tucci and Sant'Anna, 2003; Yunes et al., 2003; Sperling et al., 2008), saxitoxin analysis of water assumed great importance.

The Alagados Reservoir supplies water to three cities in the State of Paraná in southern Brazil. Electrical energy generation, recreation, and fishing are the other important activities associated with the reservoir. Farming activities and disordered occupancy near the reservoir are the main causes of water eutrophication. Consequently, frequent cyanobacterial blooms, with concomitant production of saxitoxins, have occurred since 2002 (Yunes et al., 2003). Analyses conducted over the past several years have shown cyanobacterial levels as high as 8×10^5 cells/ml (Instituto Ambiental do Parana, 2007).

Saxitoxins comprise a group of more than 20 molecules with a tetrahydropurine structure, which are also known as paralytic shellfish toxins (PST) due to their occurrence and association with seafood. They are produced by some species of dinoflagellates and cyanobacteria and can be classified into four groups based on their chemical structure as follows: saxitoxins (STX, decarbamoyl *saxitoxin*-dcSTX, neosaxitoxin-neoSTX, decarbamoyl neosaxitoxin-dcneoSTX, and nonsulfated STX); gonyautoxins (GTX 1 to 6, dcGTX 1 to 4, and single-sulfated GTX); C-toxins (C 1 to 4, doubly sulfated C-toxins); and other variants identified in *Lyngbya wollei* (LWTX 1 to 6).

All these toxins block neuronal transmission by binding to site 1 of the voltage-gated Na^+ channels in nerve cells, causing neurotoxic effects (Patocka, 2001; Briand et al., 2003; Wiegand and Pflumacher, 2005). Moreover, there are evidences of STX being a gating modifier of hERG K, and that can block L-type Ca^{+2} currents in myocytes (Wang et al., 2003; Su et al., 2004).

The aim of this study was to analyze the presence of PSTs in both water and muscle tissue of a fish species from the Alagados Reservoir and to assess the toxic impact of this water on a selected group of biomarkers during different seasons of the year. In this study, the activities of ethoxyresorufin-O-deethylase (EROD) and glutathione S-transferase (GST) were estimated to identify the changes in hepatic biotransformation; hepatic lipoperoxidation (LPO) and activity of catalase (CAT) were evaluated to investigate the disturbances in cellular oxidative stress. Brain and muscle tissues were used to study the acetylcholinesterase (AchE) activity. Histopathology studies of the gill and liver were used as physiological and morphological biomarkers due to the constant contact of the gill with water and the importance of the liver as the major center of xenobiotic metabolism.

Finally, the genotoxic effect was applied as an indicator of general damage and its assessment was carried out using the comet assay.

2. Material and methods

2.1. Fish

Geophagus brasiliensis (Perciformes, Cichlidae) is a native fish widely distributed in Brazil. It is the most common species in the Alagados Reservoir and was hence used in this study to analyze the effect of pollutants present in the Alagados waters.

Specimens of *G. brasiliensis* and samples of water were collected from the Alagados Reservoir in November 2007 (corresponding to spring in the southern hemisphere), February 2008 (summer), and May 2008 (autumn). Fish were anesthetized with 2% benzocaine and then killed by medullar section. The total length of each fish was measured, and blood was collected for the comet assay. The third-left gill arch and a liver fragment were sampled for histopathological studies. Samples of the brain, liver, and axial muscle were frozen at −70 °C for biochemical analyses. The muscle was also collected and stored in the dark at −20 °C for PST analysis.

2.2. Water analysis

The water was harvested always at two different points: at the deepest point of the Reservoir (near the dike, P1) and at the edge (P2), a shallow point at a distance of 720 m from P1. The water was harvested always at 11 a.m. at a depth of 50 cm under the water surface (euphotic zone). One sample was preserved with a Lugol solution for studying the cyanobacterial count (Utermöhl, 1958), and another was stored at 4 °C for 24 h until used for analysis of PSTs by high-performance liquid chromatography (HPLC). The corresponding results were expressed as the mean values of the two points (P1 and P2).

The pH, temperature, and levels of dissolved oxygen in the water were measured during the samplings.

2.3. Determination of PSTs in water and fish-muscle tissue

Water (500 ml) was first lyophilized, then resuspended in acetic acid (0.5 M), and stored at −20 °C for subsequent analysis. The water samples were filtered with cellulose acetate filters (0.45 μm) before HPLC analysis.

Pools of approximately 10 g of fish muscle were used to determine the PST content in each group. The pools were homogenized in HCl (0.1 N) and then centrifuged at 10,000 × *g* at 19 °C for 10 min. The supernatants were filtered with cellulose acetate filters (0.45 μm) before HPLC analysis.

Analyses of the two classes of PST (GTX and STX) in water and fish-muscle tissue were carried out by HPLC with postcolumn oxidation using a fluorescence detector, as described by Oshima (1995). PST standards, from the *National Research Council*, Canada, were also run before and after sample analyses. The detection limits were determined as follows: STX, 0.89 ng/L; NeoSTX, 2.33 ng/L; GTX 1, 1.03 ng/L; GTX 2, 0.72 ng/L; GTX 3, 0.21 ng/L; GTX 4, 0.24 ng/L, and GTX 5, 0.01 ng/L.

The concentrations of each toxin analog, as determined by chromatographic analyses, were converted to equivalents of STX (STX eq.) for comparing the toxicity of each variant with that of STX (Hall et al., 1990).