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Multibiomarker response in ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842) exposed to Reconquista river water

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ABSTRACT

The aim of this paper is to assess the water quality to chemical pollution at Roggero Dam, the headwater of the Reconquista river, and to perform a Cadmium (Cd) contamination pulse simulation through a wide battery of biomarkers which included: genotoxicity and enzymatic biomarker parameters on a neotropical teleost fish namely *Cnesterodon decemmaculatus*. Water samples were taken in order to determine the river's physicochemical profile. An integrative approach was applied using a biomarker index.

The bioassay involved the use of laboratory culture adult animals, acclimatized in moderately hard water (MHW) and fed *ad libitum*. A semi-static 96 h bioassay was conducted and the experimental groups were as follows: [1] river water (Rg); [2] river water + 2 mg/L Cd (RgCd); [3] MHW + 2 mg/L Cadmium (Cd), positive metal control; [4] MHW + 5 mg/L Cyclophosphamide (positive genotoxicity control -CP); [5] MWH, negative control (NC). At the end of the exposure time fishes were sectioned and the following biomarkers were determined: 1) condition factor rate (CF); 2) for the anterior section (A) (head): glutathione (GSH) and protein (Pr) content; 3) for the body midsection (M) (viscera): Pr, GSH, Glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD). Blood samples were also taken from the fish specimens to estimate the frequency of micronuclei (MN) as well as other nuclear abnormalities (NA).

The physicochemical profile of the river water sample indicated high Copper concentrations. CAT and SOD activity and total Pr content did not show any significant changes. GST activity decreased in fish exposed to Rg, while GSH content decreased significantly for all treatments compared to controls in MHW. These results would seem to point to a reduction in cell defense capability as a result of the depletion antioxidants such as GSH. The NA frequency increased significantly in all treated groups while MN frequency was increased only in Cd and CP groups.

Using some the biomarkers measured, a biomarker index was estimated which revealed that fish exposed to Rg were 90% affected or highly affected, while those exposed to RgCd were 80% and Cd 68% affected or highly affected. The obtained results indicate the usefulness of the use of a battery of variables by means of the biomarker index to analyze water quality.

1. Introduction

Bioassays respond to the bioavailable fraction of the toxicants, thus providing environmentally relevant information that complements the physicochemical data with the biological information in order to determine the consequences of exposing organisms to a polluted environment. A biomarker may be defined as any biological response by a test organism after exposure to different stress conditions. Thus, biochemical, physiological, histological, morphological and behavioral measurements used as biomarkers become sensitive tools that can be used to assess the adverse effects of several pollutants on natural populations or communities (Walker et al., 2006; van der Oost et al., 2003).

Recent studies have highlighted the importance of using suitable biomarkers at different biological response levels to provide

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an integrated relative measure of the general health status of natural environments where contaminants are usually present as complex mixtures (Maggioni et al., 2012). In this context, there is no single biomarker that can give a complete diagnosis of environmental degradation.

Cells exhibit enzymatic and non-enzymatic protective mechanisms to counteract factors causing alterations in their critical parameters, beyond a steady-state balance. These are molecules involved in detoxifying xenobiotics or their metabolites into more soluble forms that can be more easily excreted from the body (van der Oost et al., 2003). Many environmental contaminants provoke toxic effects by promoting oxidative stress (Lushchak, 2011). Reactive oxygen species (ROS) are well studied in connection with environmental chemical substances that increase production and lead to effects causing damage to the organism at cellular level. Among them, other pollutants, e.g. heavy metals, herbicides and aromatic compounds produce an imbalance due to excess ROS or oxidant in cell capacity to promote an effective antioxidant response. While organisms have antioxidant defenses against ROS of their own production, environmental pollutants alter this equilibrium leading individuals into an oxidative stress condition. Key biological molecules, notably DNA, proteins, and lipids, can all be adversely affected by ROS (Di Giulio and Meyer, 2008).

Antioxidant enzymes facilitate the removal of resulting increase ROS. For example, superoxide anion (O_2•) can be metabolized by superoxide dismutase (SOD) to H_2O_2. This hydrogen peroxide molecule can then be reduced to H_2O and O_2 by catalase (CAT). Also there are a number of molecules that function as scavengers of free radicals. One of the most abundant and most important molecular antioxidant in cellular cytoplasm is reduced glutathione (GSH). GSH is a low molecular weight thiol that can react directly with ROS species, thereby detoxifying them. In addition, GST (glutathione-S-transferase) catalyzes GSH conjugation with electrophiles and facilitates xenobiotics excretion (Lushchak, 2011).

The micronucleus (MN) assay in peripheral blood erythrocytes is one of the best established in vivo and in vitro cytogenetic bioassay in the Genotoxicology field; this assay provides a convenient and reliable index of both chromosome breakage and/or whole chromosome loss (Fenech, 2000).

Among genotoxicity biomarkers, in vivo micronucleus assay is widely employed to evaluate various chemicals and polluted aquatic environments such as rivers (Arkhipchuk and Garanko, 2005). During the micronuclei assay, some authors have observed the occurrence of other nuclear abnormalities (NA) seems to be a useful indicator for estimating DNA damage too, and suggest these should be taken into consideration during conventional micronucleus analysis. Such NA is connected to cell division failures and cell death processes, as well as to genotoxicity and/or mutagenicity and may complement the MN scoring in routine assays for genotoxicity screening (da Silva Souza and Fontanetti, 2006; Lajmanovich et al., 2014). The NAs type like lobed, bi-nucleate, kidney-shaped nuclei, etc. estimations, represent an alternative to MN testing and overcome any possible lack of sensitivity in connection with the low MN frequency found in wild fish populations (Guilherme et al., 2008).

In their natural environment organisms exhibit an integrated response to multiple pollutant contamination from their environment, in an effort to detoxify and recover their homeostatic balance. This disturbance involves different biological processes at differing organizational levels. Thus, the employ of several biomarkers can provide a better approximation for characterize the toxicity of a xenobiotic under study.

The Reconquista river is the second most contaminated watercourses in Argentina, having been the disposal point over many decades for numerous contaminating effluents of urban, agricultural, cattle-farming and industrial origin. It should be noted that 90% of industrial waste produced in the area is dumped directly into watercourses (Salibián, 2006). Several studies have been previously conducted on the Reconquista river using physicochemical water quality assessment and chronic and acute toxicity bioassays on amphibians and fish under field and laboratory conditions (i.e. Ferrari et al., 2005; de la Torre et al., 2007; Ossana et al., 2013). The water quality index (WQI) for the high basin of this river indicates slight pollution values (Rigacci et al., 2013), yet highly stressful conditions have been found in animals exposed to these waters (Ossana et al., 2013; Ossana, 2011; de la Torre et al., 2005).

Cnesterodon decemmaculatus (Poeciliidae, Cyprinodontiforme) is a widely distributed species in Neotropical America, densely populating a large variety of South American water-bodies including the middle stretch of the Reconquista river (Ferrari, 2015). It is a small viviparous, micro-omnivorous, benthic-pelagic, non-migratory fish found in both pristine and severely degraded habitats (Hued and Bistoni, 2005). According to Gopalakrishnan et al. (2008) to be of practical use in ecotoxicological assessment bioassays, a candidate species, should be not only sensitive to potential contaminants, but also relatively easy to collect from the field (i.e. abundant) as well as amenable to routine maintenance, culture and rearing in the laboratory (Somma et al., 2011). Furthermore, several studies have found this species a suitable test organism in acute and chronic toxicity bioassays (de la Torre et al., 2002, Menéndez-Helman et al., 2012; Vera-Candioti et al., 2010, 2013; Mastrángelo and Ferrari, 2013). *C. decemmaculatus* is also one of the neo-tropical fish species recommended for use in standardized monitoring bioassays (IRAM, 2008).

Cadmium (Cd) is a non-ferrous metal for which no physiological function has been demonstrated. In aquatic environments, concentrations of this metal have been increased due to anthropic activities. It is one of the most toxic metals for aquatic organisms and, though a non-essential element, follows the metabolic pathway of essential elements such as zinc, copper and calcium (Mebane, 2006). Numerous effects have been observed among aquatic vertebrates exposed to Cd, including: changes in swimming activity and abnormal swimming patterns (Eissa et al., 2010; Sloman et al., 2003), alterations in gill morphology (Ferrari et al., 2009), alterations in energy balance (Ferrari et al., 2011), increase in antioxidant enzyme activity (Almeida et al., 2002; Dabas et al., 2012), increase in micronuclei frequency (Ossana et al., 2009), etc. This metal has been proposed and used as a reference toxic element for *C. decemmaculatus* in acute lethality bioassays on juvenile animals (de la Torre et al., 1997; Mastrángelo and Ferrari, 2013) and is currently being assessed for use as a positive control for early effect endpoints.

This study considers the use of an integrative biomarker approach to analyzing surface water effects in the headwater of the Reconquista river basin, and to perform a Cadmium (Cd) contamination pulse simulation on adult *Cnesterodon decemmaculatus*. The aim of the study was to assess water quality through a wide battery of biomarkers including genotoxic, enzymatic and non-enzymatic biomarkers on a neotropical teleost fish.

2. Material and methods

2.1. Water sampling

Water samples were taken from the body of water formed at the Roggero dam (D: 34°40′16.47″ S; 58°52′46.19″ W) (Fig. 1), located in the boundary area between the upper and middle basins of the Reconquista river. Although built to reduce overflow due to flooding, the reservoir could be considered a depuration system

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