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Use of integrated biomarker indexes for assessing the impact of receiving waters on a native neotropical teleost fish

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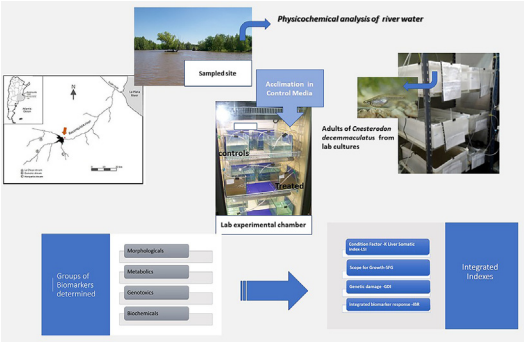
HIGHLIGHTS

- *C. decemmaculatus* from lab cultures were used for river water ecotoxicity evaluation.
- Morphological, metabolic, genotoxic and biochemical biomarkers were applied.
- An integrated biomarkers approach into different indexes is proposed.
- SFG, IGD and IBR are useful indexes for early detecting alterations by exposure.
- In experimental setting, negative effects of river water were found on the test specie.

GRAPHICAL ABSTRACT



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ABSTRACT

In the field of aquatic ecotoxicology, indexes obtained from a battery of biomarkers have proved to be a useful tool for assessing quantifiable and integrated health responses of organisms exposed to pollutants. The objective of this work was to evaluate the effects of exposure to the Reconquista River water (RR) on adults of *Cnesterodon decemmaculatus* using different integrated indexes. We conducted a 12-d laboratory assay involving the exposure of fish to RR, a negative control (moderately hard water - MHW medium), and a positive control (for genotoxicity with MHW + Cyclophosphamide, CP). There were measured metabolic (food intake and specific assimilation, specific metabolic rate, oxygen extraction efficiency, ammonia excretion, and ammonia quotient), genotoxic (comet assay, micronucleus test, and nuclear abnormalities), morphological variables (total length, body and liver weight) and biochemical variables (Electron Transport System - ETS, Acetylcholinesterase activity - AChE, Catalase - CAT, Glutathione-S-transferase - GST, Glutathione content - GSH and tissue proteins). These variables were grouped into different indexes: morphological (Condition Factor - K and Liver Somatic Index-LSI), metabolic (Scope for Growth-SFG), genetic damage (GDI) and integrated biomarker response - IBR (AChE brain, CAT, GST and GSH liver, GSH gills, ETS muscle) indexes. Results indicated that RR water induced metabolic, biochemical and genetic damages. The SFG, GDI and IBR were suitable to assess the effects of exposure to an environmental sample in an integrated approach, reducing uncertainty due to inherent biomarker variability. These indexes have emerged as promising tools for environmental monitoring studies.

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1. Introduction

Biomarkers are used to assess the exposure to and effects of contaminants at the individual, cellular, and subcellular levels, and the integration of specific biomarkers into different types of indexes has become a very important tool for diagnostic purposes (Amiard-Triquet and Berthet, 2015; Colin et al., 2016).

In this study, different types of biomarkers (metabolic, biochemical, enzymatic and non-enzymatic, genotoxic and morphological) were used to assess the integrated response of fish to receiving waters of the Reconquista River under controlled exposure conditions for 12 days. Biomarkers, which were selected based on their capability to respond to contaminant exposure by either specific or general responses, were further grouped into indexes usually used for fish health evaluation. These were Fulton's Condition Factor (K) and Liver Somatic Index (LSI) (morphological); Scope for Growth (SFG) (metabolic); genetic damage (GDI); and the integrated biomarker response index (IBR).

Teleostei's elicit physiological responses to environmental factors such as water quality, food availability, dissolved oxygen, temperature and their combination (Liew, 2012). In this regard, Scope for Growth (SFG), which is defined as the energy available for growth and reproduction after all the physiological demands of respiration and excretion of an individual have been met (Alcaraz and Espina, 1997), provides a rapid and quantitative assessment of fish energy status, being able to establish an alert about changes in growth rate because of exposure to a polluted media.

From a cytogenetic point of view, the alkaline comet assay (CA) (Azqueta and Collins, 2013) and the micronucleus test (MN), which detects chromosomal aberrations and nuclear abnormalities (Fenech, 2000; Mudry and Carballo, 2006) are well-known genotoxicity biomarkers. The results of CA were integrated into the Genetic Damage Index (GDI).

The biochemical biomarkers included indicators of metabolic stress (electron transport system-ETS), biotransformation mechanisms (glutathione S-transferase - GST), antioxidant defense mechanisms (glutathione content-GSH and catalase-CAT), and neurotoxic effects (acetylcholinesterase, AChE). These biomarkers were used to develop an integrated biomarker response index (IBR), which constitutes a practical and robust tool to evaluate the susceptibility to contaminants (Broeg and Lehtonen, 2006; Damiens et al., 2007; Delfino Vieira et al., 2017; Leinio and Lehtonen, 2005).

The Reconquista River is a peri-urban watercourse in Buenos Aires Province (Argentina). It is the second most contaminated water body in Argentina because it receives complex pollutant mixtures from poorly treated or untreated domestic, agricultural and industrial sewages over many decades (Ferrari, 2015). The river extends along 82 km and its basin covers 1574 km^2; it encompasses 20 districts inhabited by >4.5 million people (INDEC, 2010), representing the third most populated area in the country. Many studies have been conducted in the Reconquista river to determine physicochemical parameters of water quality and to assess toxicity by means of chronic and acute bioassays on amphibians, fish and amphipods under field and laboratory conditions (de la Torre et al., 1997; Ferrari et al., 2005; Giusto et al., 2014; Ossana et al., 2013).

The Neotropical poecilid *Cnesterodon decemmaculatus* has been widely used in ecotoxicological evaluations throughout its geographic range (de la Torre et al., 2005; Menéndez-Helman et al., 2012; Vera-Candioti et al., 2014). In particular, it has been used at both juvenile and adult stages in ecotoxicity studies showing the harmful effects of exposure to surface waters of the Reconquista River, both experimentally and in the field (de la Torre et al., 2007; Ossana et al., 2016; among others).

The work reported in this paper is part of a series of studies made in our laboratory, which were designed to contribute to the knowledge of *C. decemmaculatus* used as test organism exposed to receiving waters. In the present study we evaluated the effect of exposure to water samples from the Reconquista River on *C. decemmaculatus* adults using a battery of biomarkers integrated into different indexes.

2. Materials and methods

2.1. Exposure media and water sampling

The water sample (one liter for pesticides, and four liters for physicochemical analysis) was collected in the headwaters of the Reconquista River, at the Roggero dam (S 34° 41′ 03.5″and W 58° 51′ 15.5″), which is naturally inhabited by *C. decemmaculatus*. The sample was filtered and stored at 4–8 °C and remained there a maximum of nine days, when the second water renovation was done. The exposure began 24 h after water sample. Prior to the bioassay and before any water renovation, it was aerated, brought to experimental temperature (22–24 °C), and used as the exposure medium (RR). The control medium was reconstituted moderately hard water (MHW; pH 7.4–7.8; hardness: 80–100 mg $CaCO_3$ L^{-1}; alkalinity: 60–70 mg $CaCO_3$ L^{-1}) (USEPA, 1993).

2.2. Physicochemical analysis of the samples

Conductivity, pH, dissolved oxygen (DO) and temperature of the water used in the experiment were measured in situ using a portable device (HqD Field case Hach). A sample was transported to the laboratory under adequate refrigeration to measure the 5-day Biochemical Oxygen Demand (BOD_5), hardness, chloride concentration (Cl^-), alkalinity, turbidity, conductivity, pH, ammonium (NH_4^+), Chemical Oxygen Demand (COD) and metals concentrations (As, Cu, Cr, Cd and Pb). All measurements were carried out in triplicate and was determined based on the standard methods (APHA-AWWA WEF, 2005). Metals were measured by atomic absorption spectrophotometer (Perkin Elmer; AAnalyst 200 model,) equipped with hollow cathode lamps. Certified standards were used (1000 mg/L, for each element, from Merck). Results are expressed as the mean value of three readings. Water river samples were screened for the following pesticides and metabolites: atrazine, glyphosate, AMPA, chlorpyrifos, λ cyhalothrin, cypermethrin, α endosulfan, β endosulfan, α lindane, β lindane, ϒ lindane, aldrin, Dieldrin, endrin, p-p′ DDT, o-p′DDT, p-p′DDE, o-p′ DDE, p-p′DDD, heptachlor, heptachlor epoxide.

Glyphosate and AMPA analysis were performed using high-performance liquid chromatography and mass spectrometry (HPLC-MS) after derivatization with 9 fluorenylmethoxylcarbonyl chloride (FMOC-CL). An Agilent 1100 liquid chromatograph model was used for detection and quantification with ESI ionization source operating in negative mode, coupled to an Agilent model VL single quadrupole mass spectrometer (Agilent Technologies Inc., Miami, FL, USA). Chromatographic separation was performed in a C18 X-SELECT™ column using methanol and nanopure water gradient, with NH_4Ac as ionization additive. Glyphosate and AMPA were quantified by means of an external calibration curve. The limit of detection (LD) was 0.5 μg L^{-1} and the limit of quantification (LQ) was 0.8 μg L^{-1}, both for AMPA and glyphosate.

Analysis of the other pesticides was performed using gas chromatography (Agilent 6890N, equipped with a spitless injector at 250 °C and a HP-5 column of 30 m; coupled to an electron capture and phosphorous/nitrogen selective detectors) after liquid-liquid extraction with dichloromethane (APHA, 2005) and clean-up with Fluorisil (USEPASW-846, M 3620C). The LD was 0.1 and 0.001 μg L^{-1} and the LQ was 0.2 and 0.003 μg L^{-1} for atrazine and the others, respectively.

We calculated the following water-quality indexes (WQIs): WQI-b is an indicator of industrial pollution (Lacoste and Collasius, 1995) based on DO, COD and the major heavy metals (in this case As and Cu), while WQI-a (Berón, 1984) is an indicator of domestic pollution,