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Bioaccumulation and biochemical response in South American native species exposed to zinc: Boosted regression trees as novel tool for biomarkers selection

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ABSTRACT

The aim of this study was to evaluate the response of a wide battery of biomarkers in two native species, the freshwater shrimp *Palaemonetes argentinus* and the macrophyte *Potamogeton pusillus*, experimentally exposed to zinc in order to establish the potential use of selected species as bioindicators of aquatic pollution. For this purpose, we propose the use of integrated biomarker index (IBR) with a previous selection of biomarkers using boosted regression trees (BRTs) as a new tool in ecotoxicology. Organisms were collected from a reference site, acclimated and exposed at relevant environmental zinc levels (control, 5, 50 and 500 μg Zn L^{-1}) for 96 h. Biomarkers were measured in cephalothorax and abdomen of shrimp as well as in leaf, stem and root of plants.

Significant zinc accumulation was observed in cephalothorax of *P. argentinus* from 50 μg Zn L^{-1} and from 5 μg Zn L^{-1} in stem and root of *P. pusillus*, when compared with control condition. Those effect biomarkers with significant differences among treatments were pre-selected to run out the BRTs model for each species. In *P. argentinus*, microsomal acetylcholinesterase activity, metallothioneins and superoxide dismutase activity measured in cephalothorax, as well as glutathione reductase activity in abdomen, showed the higher capacity to explain or predict the zinc exposure concentration. On the contrary, in *P. pusillus*, only chlorophyll *a* measured in leaf and H_2O_2 measured in root were the more representative of exposure concentrations, at least, within the biomarkers tested in the present study. Thereafter, IBR was calculated with the selected biomarkers in *P. argentinus* and showed in a sole value the organism stress, which also correlates with zinc exposure and accumulation.

Natives species tested displayed a sensitive response to metal exposure, which represents an important characteristic for biomonitoring programs. Our findings suggest that the BRTs and IBR are useful and robust run tools to select the better biomarkers in toxicological studies and indicate the organism stress.

1. Introduction

Physical and especially chemical methodologies quantify pollutants in detail, but lack the ability to judge their toxicity impact on biota. Therefore, biomarkers, more precisely biochemical, physiological and histological responses of organisms, were developed to assess the impact of environmental pollutants in terms of exposure and/or damage to organisms (Lam and Gray, 2003; Van der Oost et al., 2003).

Many authors have pointed out the importance of the use of a wide battery of biomarkers when assessing the biological effects in impacted environments, since a single biomarker may not reflect the health status of a sentinel species (Cazenave et al., 2009 and

Abbreviations: Accu, zinc accumulation; α-Toco, alpha-tocopherol; BRTs, boosted regression trees; cAChE and mAChE, cytosolic and microsomal acetylcholinesterase; CAT, catalase; cBChE, cytosolic butyrilcholinesterase; cGST and mGST, cytosolic and microsomal glutathione-S-transferase; ChE, cholinesterase; Chl, chlorophyll; CP, carbonyl content in proteins; CV, cross validation; dw, dry weight; GPx, glutathione peroxidase; GR, glutathione reductase; H_2O_2, hydrogen peroxides; IBR, Integrated Biomarkers Response; ICP-MS, inductively coupled plasma-mass spectrometry; MTs, metallothioneins; P, insoluble fraction; Pheo, pheophytins; POD, guaiacol peroxidase; ROS, reactive oxygen species; S, soluble fraction; SOD, superoxide dismutase; TBARs, thiobarbituric acid-reactive substances; ww, wet weight; [Z1], 5 μg Zn L^{-1}; [Z2], 50 μg Zn L^{-1}; [Z3], 500 μg Zn L^{-1}.

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other authors therein referenced). However, the interpretation of data provided by such a multibiomarker approach is difficult without an integrated overview that globally assesses the potential influence of contaminants and environmental conditions in an organism. In this context, stress indexes, as the Integrated Biomarkers Response (IBR), constitute practical and robust tools to assess the susceptibility to pollutants using multiple biomarker responses (Beliaeff and Burgeot, 2002; Serafim et al., 2012). Nevertheless, the calculation of IBR needs a previous selection of biological responses to be considered. Beliaeff and Burgeot (2002) suggested that only careful selection of an appropriate combination of biomarkers can provide information about global adverse environmental effects. Previous studies about the use of IBR do not explain how to select biomarkers to be included in this mathematical tool index. Usually, those biomarkers are selected according to capability to be measured. Although, which are the best biomarkers? How should be carried out an independent selection of them? In this way, the use of boosted regression trees (BRTs) could be a valid tool to select those biomarkers to be used in IBR, with higher capacity to predict contaminant exposure. Boosted methods such as BRTs differ substantially from regression-based methods such as Generalized Linear Models and General Additive Models that have been used widely over the last decade (Yuan, 2004; Iwasaki and Brinkman, 2015). While these latter methods seek to identify a single "best" model describing relationships between the response and the predictor variables, boosting progressively builds a sequence of models of increasing complexity, each one fitting the training data slightly better than its predecessor (Leathwick et al., 2008).

In South America, the use of biomarkers to evaluate aquatic contamination is increasing, but not always in native species (Carriquiriborde and Bainy, 2012). The study of biomarkers in native bioindicators results relevant since the sensibility to a toxic compound is specie dependent (Van der Oost et al., 2003) and only few previous studies indicated negative effects of water contamination on native biota, particularly for invertebrates and plants (Hued and Bistoni, 2005; Brodeur et al., 2011). The decapod *Paleomonetes argentinus* showed sensitivity to pollution in laboratory test (Galanti et al., 2013; Griboff et al., 2014; Bertrand et al., 2015), and consequently some authors have proposed that this species might be used as a bioindicator crustacean to provide information on environmental quality (Montagna and Collins, 2007). This species is known to be widely distributed in South America from estuaries to large river systems in Brazil, Uruguay and Argentina, among others countries (http://www.iucnredlist.org; Spivak, 1997). In a similar way, *Potamogeton pusillus* is proposed as sentinel organism as it is an aquatic macrophyte of ecological importance within the aquatic ecosystem, providing shelter and habitat for young fishes and other aquatic animals. Additionally, *Potamogeton* genus has a cosmopolitan distribution (Novara, 2003, http://www.iucnredlist.org).

Environmental exposure to metal pollutants can provoke several toxic effects on individuals and may have community wide consequences (Wallace et al., 2000). Several investigations around the world showed the occurrence of various levels of heavy metal in aquatic areas with the predominance of Zn, Pb, Cd, Cu and Cr (Cheung et al., 2003; Smolders et al., 2003; Zhou et al., 2008).

Therefore, the aims of this work were: (1) evaluate the response of biomarkers of exposure and effect in two native species, the freshwater shrimp *P. argentinus* and the macrophyte *P. pusillus*, experimentally exposed to zinc; (2) apply an integrated biomarker index (IBR) with a previous selection of biomarkers using boosted regression trees as a new tool in ecotoxicology, in order to determine the potential use of selected species as bioindicators of aquatic pollution.

2. Materials and methods

2.1. Reagents and materials

All reagents were of analytical grade supplied by Sigma–Aldrich, Merck and Sintorgan (Argentina). Ultra-pure water (Arium 611 UV system, Sartorius) was used to prepare standard solutions, dilutions, blanks and artificial freshwater employed for metal exposition. All glassware and aquaria were appropriately washed to avoid metal contamination.

AccuStandard® atomic absorption spectrometry standard solution (1000 mg L^{-1} in 1% nitric acid) was used as stock solution for calibration of metal quantification equipment.

2.2. Specimens

Adult freshwater shrimps, *P. argentinus*, and macrophyte, *P. pusillus*, were collected from a reference site located in Suquía River basin (Córdoba, Argentina; Monferrán et al., 2009; Galanti et al., 2013). Shrimps were acclimated during two weeks in 40 L glass aquaria filled with artificial freshwater and maintained at constant laboratory temperature (25 ± 1 °C), under a light/dark regime of 12 h:12 h and fed daily *ad libitum* with commercial food for fish (Vita Fish) complemented until 54% proteins through the addition of lyophilized shrimp (Griboff et al., 2014). Zinc content of the formulated diet was 0.066 μg Zn mg^{-1} food. Plants were also placed into a 40 L tank but containing 10% Hoagland's solution and sediment (1/4) from the same sampling area. Plants were grown for two weeks under a light/dark photoperiod of 14 h:10 h before starting the exposures (Monferrán et al., 2012).

2.3. Experimental exposure

Two days before the beginning of metal exposition, organisms were relocated into the exposure aquaria. Adults (body length >2.2 cm) of *P. argentinus* (0.195 ± 0.011 g wet weight (ww); 2.815 ± 0.050 cm) were transferred to aquaria filled with artificial freshwater (ultra-pure water containing 0.100 g L^{-1} sea salt, 0.200 g L^{-1} $CaCl_2$ and 0.103 g L^{-1} $NaHCO_3$), maintaining a relation of two individuals per liter. Meanwhile, plants were kept in 1 L beakers (three plants per beaker, 5–8 g ww per liter) containing 10% Hoagland's solution prepared without Zn.

The experimental design involved four experimental conditions: Control (not metal exposed) and organisms exposed to 5 μg Zn L^{-1} (7.65×10^{-2} μM, [Z1]), 50 μg Zn L^{-1} (7.65×10^{-1} μM, [Z2]) and 500 μg Zn L^{-1} (7.65 μM, [Z3]). Zinc exposition was carried out for 96 h at similar temperature and photoperiod than during acclimation. Heavy metal concentrations tested were selected according to relevant environmental levels (1.9–12,000 μg L^{-1} in contaminated environments; Cheung et al., 2003; Smolders et al., 2003) and the concentration of Zn established for the protection of the aquatic biota in freshwaters by the Argentinean Environmental Water Quality Guidelines (9.7–45 μg L^{-1} depending on the water hardness; AEWQG, 2003).

For each treatment, 7–11 organisms were used to measure Zn accumulation, while 4–7 individuals were used to assess the other biomarkers responses. Organisms were taken randomly from nine aquaria or 21 beakers prepared for each exposure condition. A total of 166 shrimps and 128 plants were used for the experimental exposure. All measurements were performed in triplicate.

A stock metal solution was prepared using $ZnSO_4 \cdot 7H_2O$ (99.5%, Merck) and specific aliquots were taken to provide nominal metal concentrations. Exposure concentrations were tested at time 0 and 96 h for each experimental condition. Zinc concentrations in exposure media were measured on an inductively coupled plasma–mass spectrometry (ICP–MS) (Agilent Technology