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Differential biochemical responses to metal/metalloid accumulation in organs of an edible fish (*Centropomus parallelus*) from Neotropical estuaries

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ABSTRACT

Metal/metalloid accumulation in fish organs elicits biochemical responses indicating the overall fish and environmental health status. This study evaluated the bioaccumulation of metals and metalloid in relation to a suite of biochemical biomarkers (superoxide dismutase, catalase, glutathione-S-transferase, Na+/K+-ATPase, H+-ATPase, acetylcholinesterase activities and the levels of glutathione, metallothionein, lipid peroxidation and oxidized protein) in different organs of fish, *Centropomus parallelus*, in Vitória Bay and Santa Cruz estuaries (State of Espírito Santo, Brazil) with distinct contamination levels. Metal and metalloid concentrations differ in each organ and were significantly higher in winter than in summer. Chemometric evaluation performed between metal/metalloid accumulation and the biomarkers revealed a complex scenario in which the biomarker responses depend on both metal accumulation and organ/tissue sensitivity. The metal levels in gills indicate fish contamination mainly via water and the low sensitivity of this organ to most metals. Biomarker responses suggested that the metal elimination pathway is through the gills and kidney. The hepatopancreas and kidneys were the most important detoxification organs while muscle was the less reactive tissue. In general, the finding suggested that, *C. parallelus* is partly able to tolerate such metal contamination. However, it is emphasized that the biomarker responses imply an energetic cost and may affect the growth rate and reproduction. Given the ecological and economic importance of *C. parallelus*, the level of toxic metals/metalloids in juvenile fish is an important early-warning for the maintenance, conservation and commercial use of this species.

1. Introduction

Estuaries are characterized by high physical and chemical variability as they constitute an interface between the continent and the sea (Elliott and Whitfield, 2011) and many are influenced by anthropogenic activities, including main contamination sources (Borja et al., 2012; Wolanski and Elliott, 2015). Metal/metalloid inputs in estuarine areas, and their transfer through the trophic web, may disrupt biological processes resulting in toxicity, which may affect the structure of population and community, even in those organisms well-adapted to tolerate such stressors (Elliott and Quintino, 2007). Essential metals play important roles in biological systems, but they become toxic at high levels and non-essential metals can be toxic, disturbing biological processes, even at trace amounts (Mazon et al., 2002; Hartl, 2013; Rosabal et al., 2015).

Metal/metalloid bioaccumulation may differ among organs/tissues depending on the mode of exposure (dietary and/or water), uptake, regulation and excretion mechanisms as well as their roles in these processes (Jarić et al., 2011). Metals may interfere in cellular enzymatic pathways by generating reactive oxygen species (ROS), which promote oxidative stress and degenerative processes in the cells (Oliveira et al., 2010; Carvalho et al., 2012; Sakuragui et al., 2013; Barbee et al., 2014; Brandão et al., 2015; Cappello et al., 2016a, 2016b). ROS can be detoxified by enzymatic and non-enzymatic cell defence systems, including the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) and

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levels of glutathione (GSH) and metallothioneins (MT), among others enzymes and compounds (Storey, 1996). The activation and/or inhibition of these systems reflect the exposure to metals and/or their toxicity (Oliveira et al., 2010; Souza et al., 2013). Studies on wild fish have long emphasized the metal accumulation in the muscles, the edible part of fish consumed by humans (Bosch et al., 2016; Cerveny et al., 2014). However, it is important to analyse other organs/tissues to evaluate metal distribution, clarifying the links between the contamination and adverse responses in fishes (Elliott et al., 1988).

Among fish organs, the gills have important functions such as gas exchange, ion transport and nitrogen excretion, being the first organs exposed to waterborne chemicals; contaminant uptake (including metals) is facilitated by the large surface area and thin water-blood diffusion distance in gills (Fernandes and Mazon, 2003). As well as representing the main route of entry of contaminants in fishes, the gills can also be directly affected by metal and metalloids, triggering antioxidant defence systems and disturbing its metabolic pathways (De Domenico et al., 2013; Cappello et al., 2016b). Furthermore, the high osmoregulatory activity in estuarine fish in the gills and, to a lesser extent, the kidney, increases the tissue susceptibility to water contaminants (Elliott et al., 1988; Monserrat et al., 2007). The liver, the main detoxification organ in all vertebrates, effectively takes up metals/metalloids from the bloodstream, and its dysfunction is an early indicator of the presence of toxicants in the environment (Fonseca et al., 2011; Paul et al., 2014).

In Brazil, the coast of Espírito Santo state (ES) has been impacted by metal and metalloids, such as As, Pb, Cr, Cu, Fe, Al, Zn and Mn, arising from metallurgical industries and harbours for iron export (Santos et al., 2017). The estuaries of Santa Cruz and Vitória Bay have different levels and types of metal contamination. Contamination with K, Ag and Mn has been reported in Santa Cruz, while high levels of Fe, Al and Pb were found in Vitória Bay (Arrivabene et al., 2015; Souza et al., 2013, 2014a, 2014b). Despite the lower levels of As, Pb and Cu reported in Santa Cruz, the bioavailability of such elements was higher in Santa Cruz than in Vitória Bay (Souza et al., 2014b). Numerous biological changes in the biota from these areas were associated with metal contamination (Souza et al., 2014a, 2014b, 2015; Arrivabene et al., 2014, 2015).

In a previous study, we reported that the fat snook fish (*Centropomus parallelus*) inhabiting these areas showed biological anomalies, such as changes in the activity of antioxidant enzymes (SOD and CAT) and the biotransformation enzyme (GST) in the hepatopancreas and gills, in addition to erythrocyte anomalies, moderate damage in liver and metal bioaccumulation in muscles (Souza et al., 2013). These findings led to the following questions: 1) Does metal distribution and accumulation differ among fish organs? 2) How does each organ respond to metal accumulation? 3) Are there seasonal differences in the bioaccumulation and physiological responses in fish?

In this context, metals and metalloid accumulation in the gills, hepatopancreas, kidneys and muscle have been determined together with biochemical enzymatic and non-enzymatic biomarkers in juvenile fat snook, *Centropomus parallelus* Poey 1860 (Centropomidae) from Vitória Bay and Santa Cruz estuaries, ES, Brazil. This was allowed all aspect to be integrated to identify metal and metalloid accumulation and organ biochemical and physiological responses. *C. parallelus* is a protandric top-predator fish, which does not undergo migratory cycles during its juvenile stage (Volpe, 1959; Taylor et al., 2000). As an estuarine resident, *C. parallelus* has been proposed as a possible bioindicator in Brazilian coastal regions (Rocha et al., 2007).

2. Materials and methods

2.1. Fish sampling

Samples from gills, kidney, hepatopancreas and muscles used in this study were from the same juvenile male *C. parallelus* of the previous study (Souza et al., 2013). *C. parallelus* (n = 40, ten fishes for each site and season; body mass = 150 ± 30 g; total length = 15 ± 5 cm) were collected in two seasons (winter 2009 and summer 2010) in Vitória Bay (20° 19′S and 40° 20′W) and Santa Cruz estuary (19° 58′S and 40° 07′W), ES, Brazil (Fig. 1) in September of 2009 and March of 2010. Fishes were killed by medullary section and field-dissected. The gills, hepatopancreas, kidneys (posterior region) and muscle were removed using plastic instruments to avoid contaminating the samples, stored in plastic tubes (Eppendorf or Falcon tubes depending on the size), immersed in liquid nitrogen immediately after collection to stop the metabolic activity and so transported to the laboratory, and stored at − 80 °C until analysis.

Vitória Bay is an estuarine complex formed by five rivers showing environmental degradation caused by harbour and industrial activities, including air pollution by smoke metallic particles; the Santa Cruz estuary is formed from two rivers with a large mangrove area (natural reserve). Water samples were taken approx. 1 m below the surface in pre-cleaned recipients. Physical and chemical data corresponding to water samples were reported in Souza et al. (2013).

2.2. Multi-elemental analyses

Ultra-pure water (resistivity > 18.2 MΩ cm^{-1}; ≤ 5 µg L^{-1} TOC) was obtained from a purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Multi-element standard solution Merck VI CertiPUR® was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid (63.7%) sub-boiling grade was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distiller, Córdoba, Argentina). The purity of nitric acid was verified by Mass Spectrometry Inductively Coupled Plasma (ICP-MS), Agilent 7500cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE). Filters (0.45 µm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). All glassware and plastic bottles and containers were cleaned overnight in dextran 10%, scrubbed, washed with tap water, then left overnight in nitric acid 10%, washed first with deionized water and finally with ultrapure water. ICP probes and pipes were of PTFE, previously washed as described above, changing the concentration of nitric acid to 35% and then in 20% sulphuric acid, as the method EPA 200.8 for metal analysis (EPA, 1994).

For multi-elemental analysis tissue samples (n = 5 animals per site and season) were dried at 37 °C until constant weight and stored at room temperature. Samples in triplicate were ground and homogenised with a mortar and digested (0.1 g from each organ) according to Chappaz et al. (2012), using 4 mL nitric acid (ultra-pure, sub boiling grade) and 1 mL hydrogen peroxide (30%, Merck), in pre-cleaned PTFE tubes (Savillex) at constant temperature (90 °C) during 24 h. Controls were prepared using the same protocol without sample (only reagents) (Monferrán et al., 2016). Digested samples were stored at 4 °C until analysis for B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg and Pb.

The concentrations of elements were determined in triplicate from each sample and the repeatability of ICP-MS measurements was generally ≥ 97%. Analytical quality assurance (QA) and quality control (QC) were done using a certified reference material (CRM): typical diet NIST1548a) and bovine muscle (NIST 8414) and the recoveries of each material were 94 ± 17% and 91 ± 13%, respectively. Three spiked samples were prepared for gills, kidney, hepatopancreas and muscle samples by adding standard solutions (10 mg/L and 10 µg/L) containing all the elements analysed to 0.1 g of dried tissue. Spiked samples are important to verify whether the digested matrix can influence in the metal analysis (Monferrán et al., 2016). The average recovery of these assays was 87 ± 16%.

2.3. Biochemical analyses

Individual samples of gills, hepatopancreas, kidney and muscle from each animal were homogenised (n = 5 animals per site and season). The total protein in each sample was determined according to Bradford