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NATURAL AND ANTHROPIC IMPACT ASSESSMENT ON BIOCHEMICAL AND HISTOPATHOLOGICAL BIOMARKERS OF FISHES AND INVERTEBRATES AT COASTAL REGION OF ADMIRALTY BAY – KING GEORGE ISLAND

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Edson Rodrigues[1], Lucélia Donatti[2], Gannabathula Sree Vani[1], Helena Passeri Lavrado[3], Flávia Sant'Anna Rios[2], Cecília N.K. Suda[1], Cláudio Adriano Piechnik[2], Cíntia Machado[2], Edson Rodrigues Júnior[2], Mariana Feijó de Oliveira[1], Flávia Baduy Vaz da Silva[2], Luciana Badeluk Cettina[2]

[1]Laboratório Bioquímica e Ecofisiologia de Organismo Antárticos, Universidade de Taubaté, Taubaté, SP, Brazil
[2]Laboratório de Biologia Adaptativa, Departamento de Biologia Celular, Universidade Federal do Paraná, Curitiba, PR, Brazil
[3]Laboratório de Benthos, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
e-mail: edsonrod@unitau.br, rodedson@gmail.com

Marine environments near the scientific stations and local anchoring of ships are considered potential pollution sites in Antarctica. Human waste, the burning of fuel oil and its possible leakage from ships during transport to the stations are major sources of pollution. The metabolic responses and histopathology of fish and benthic invertebrates of Admiralty Bay, King George Island, are a part of environmental monitoring research proposed in Module 3 INCT-APA for the region. The Antarctic fish *Notothenia rossii* and *Notothenia coriiceps* and invertebrates *Nacella concinna* were selected as target organisms for studies of biomarker responses during the XXVIII Brazilian expedition to Antarctica. The aim of these studies is to establish a baseline for biochemical and histopathological biomarkers and to understand, through bioassays, the effect of pollutants on biological responses of Antarctic organisms. Bioassays with pollutants (fuel oil, sewage and heavy metals) aim to distinguish the biological responses caused by seasonal variations in natural environmental conditions from those imposed by the presence of pollutants in Admiralty Bay ASMA.

In aquatic environments, the absorption of toxic substances, mainly by phytoplankton and zooplankton, results in the high concentration of these substances in organisms belonging to higher trophic levels in the food chain.

These substances then eventually reach a large number of organisms, including fish and invertebrates. Through the use of different biomarkers it is possible to detect the presence of pollutants in water and sediment, in the organisms that inhabit these environments.

Fish and invertebrates are good bioindicators of environmental quality in aquatic ecosystems, since they occupy different positions in the food chain and have varying spatial distribution. These features allow for a direct and indirect evaluation of the health of several components of the aquatic environment.

Using histopathological, cellular, biochemical and molecular analysis, the health of fishes and invertebrates, collected directly from nature or through the bioassays, can be evaluated. The histology can be used as a tool for identifying biomarkers of fish fauna and invertebrates.

The lesions detected in cells, tissues or organs exposed to pollutants represent an integration of the cumulative effects of these substances at the biochemical and physiological levels (Meyer *et al.*, 2002).

Biomarkers are not normally associated with severe adverse effects caused by toxic stress agents. The early biomarker response is much more important as a marker of future adverse biological effects than as an indicator of serious environmental problems. The effects caused by toxic stress agents are often compensated or repaired at the molecular level, without compromising the higher level of biological performance of the organism (Lam and Gray, 2003).

Considered the best preserved region of the planet, pollution in Antarctica is restricted to areas surrounding the scientific stations and places frequented by research ships and tourists (Kennicutt II, 1995). The wreck of Paradise Bay in 1989 and the leakage of 600,000 liters of fuel oil and the subsequent contamination of the organisms in the subtidal and intertidal zone with hydrocarbons, highlights the risk of pollution from the sinking ships (Kennicutt II *et al.*, 1992).

The stress effects of pollutants on the metabolism of fish and invertebrates have been used as biomarkers of anthropogenic activity in monitoring projects (Regoli *et al.*, 2002; van der Oost *et al.*, 2003).

The Antarctic Specially Managed Area (ASMA) of Admiralty Bay is formed by glaciers and narrow bays, similar to the fjords, and occupies a total area of 362 km^2 (Arigony-Neto *et al.*, 2004). During the summer, several species of birds and marine mammals migrate to these places for reproduction. This area also houses three scientific stations, two with ongoing activities throughout the year, including the Brazilian station Comandante Ferraz.

The field activities of the biomarker team were initiated during the summer of XXVIII Brazilian Antarctic Expedition (November 2009 - March 2010).

Gills and foot muscle of the limpet *N. concinna* were collected as part of biomarker studies of the costal intertidal zones (Figure 1).

Further, samples of biological fluids (blood and bile) and tissues (liver, epaxial muscle, brain, kidney and gills) were

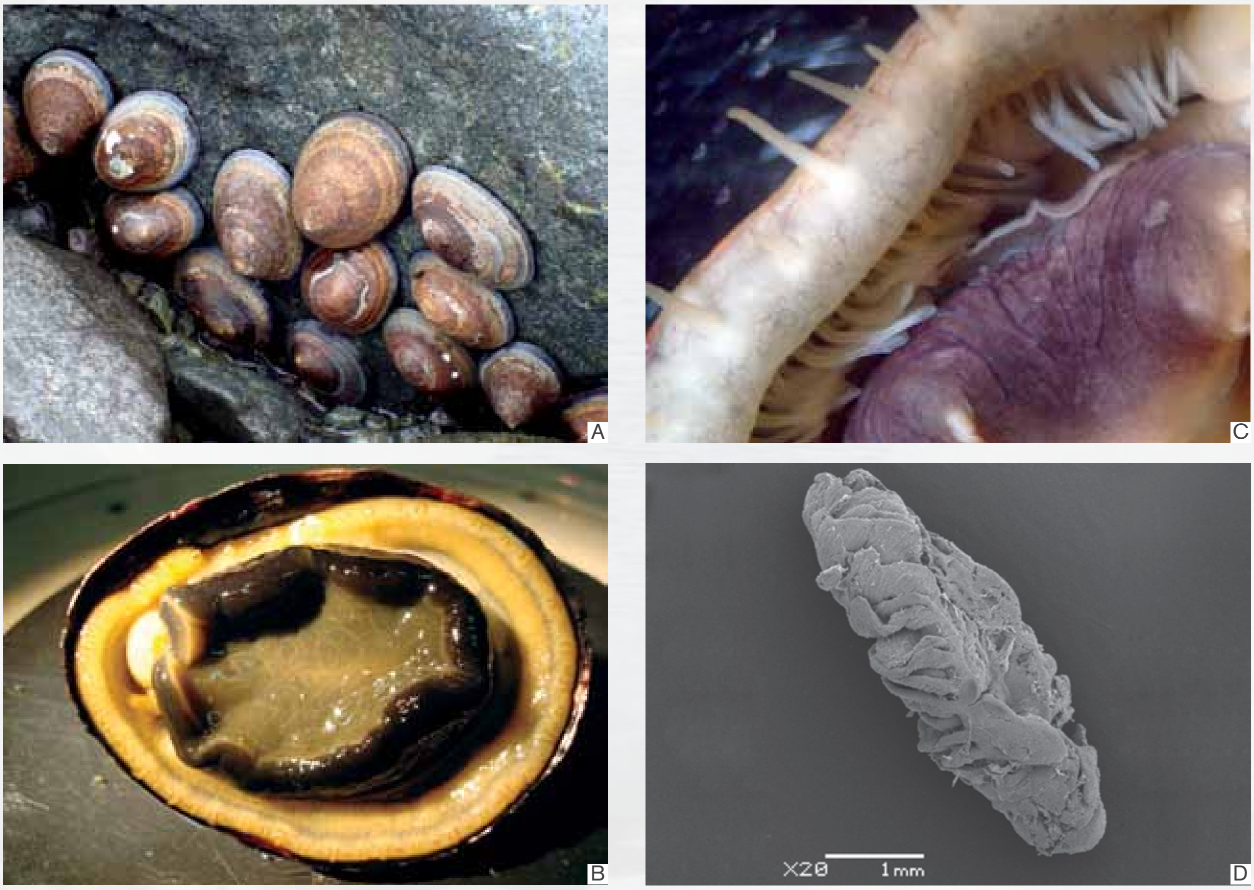

Figure 1. *Nacella concinna* occurs in patches in the intertidal zone (A). The foot muscle (B) and gills (C) were obtained by dissection. The scanning electron microscopy (D) showed the respiratory lamellae.

Figure 2. Obtaining *N. coriiceps* tissues for morphological and biochemical analysis.

collected from the Antarctic fish *N. coriiceps* and *N. rossii* (Figure 2) as part of biomarker studies of the subtidal.

In both cases, tissues and fractionated biological fluids (plasma and bile) were collected, and frozen immediately in liquid nitrogen. For histopathological analysis, tissues were fixed in Alfac or in Karnovisky.

All biological material was transported to our laboratories at the University of Taubaté and the Federal University of Paraná.

The sampling sites (Figure 3) were selected based on their proximity to pollution sources, penguin colonies and large areas without human and/or ornithogenic influence.

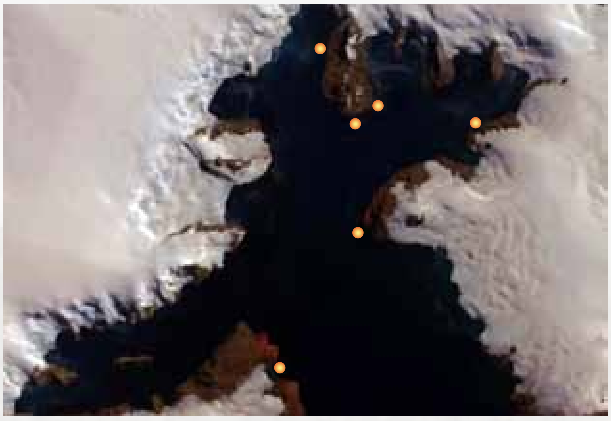

Figure 3. Sampling sites of fishes and limpets at Admiralty Bay – King George Island.

These initial studies were conducted to establish the natural levels of major biochemical biomarkers (enzymes of energy metabolism, antioxidant defenses, xenobiotic metabolism and osmoregulator responses) and histology (normal morphological patterns) in tissues of *N. rossii*, *N. coriiceps* and *N. concinna*, as a baseline for monitoring the Admiralty Bay ASMA.

The activities of hexokinase (HK), glycogen phosphorylase (GPAs), enolase (ENO), phospho-fructokinase (PFK), lactate dehydrogenase (LDH), citrate synthase (CS) and malate dehydrogenase (MDH) are being determined as potential markers of energy metabolism; gill ATPase Na/K as a marker for osmoregulation response; etoxyresorufindietilase (EROD) and glutathione-S-transferase as markers of stage I and II metabolism of xenobiotics, respectively, glucose-6-phosphate dehydrogenase (G-6-PDH), superoxide dismutase (SOD) and catalase (CAT) as markers of antioxidant defense, arginase (ARG) as a marker of the metabolism of nitric oxide, polyamines and phospho-L-arginine. The high-pressure chromatography (HPLC) of bile, to be done at IOUSP, will also establish the hepatic excretory profile for the xenobiotic metabolism.

In tissues, hyperplasia, fusion, aneurysm and gill epithelium detachment are some of the changes that will be analyzed. Further, the fish hepatic tissue will be examined for, melano centres - macrophages, necrosis foci, leukocyte infiltration and vacuolization, among others (Mallatt, 1985; Roberts, 2001). This is because the liver is an important organ in toxicology studies. This organ performs many vital functions in animals, among them the detoxification of the body, with the biotransformation of xenobiotics (Hinton and Laurén, 1990).

Gills, in turn, are a way for the soluble xenobiotics action (Stentiford *et al.*, 2003); they are in direct contact with the environment and any damage to their structure may interfere with respiration and ionic homeostasis (Van Den Heuvel *et al.*, 2000).

Initial Histological studies of the livers of *N. rossii* and *N. coriiceps* revealed that hepatocytes have a polyhedral shape, with single central spherical shaped nucleus with one or more nucleols. The predominant chromatin in the hepatocyte nuclear region is euchromatic and is widely dispersed. The heterochromatin concentration is much less and appears only is some nuclear regions. The hepatic tissue has vessels of many different sizes, distributed in the

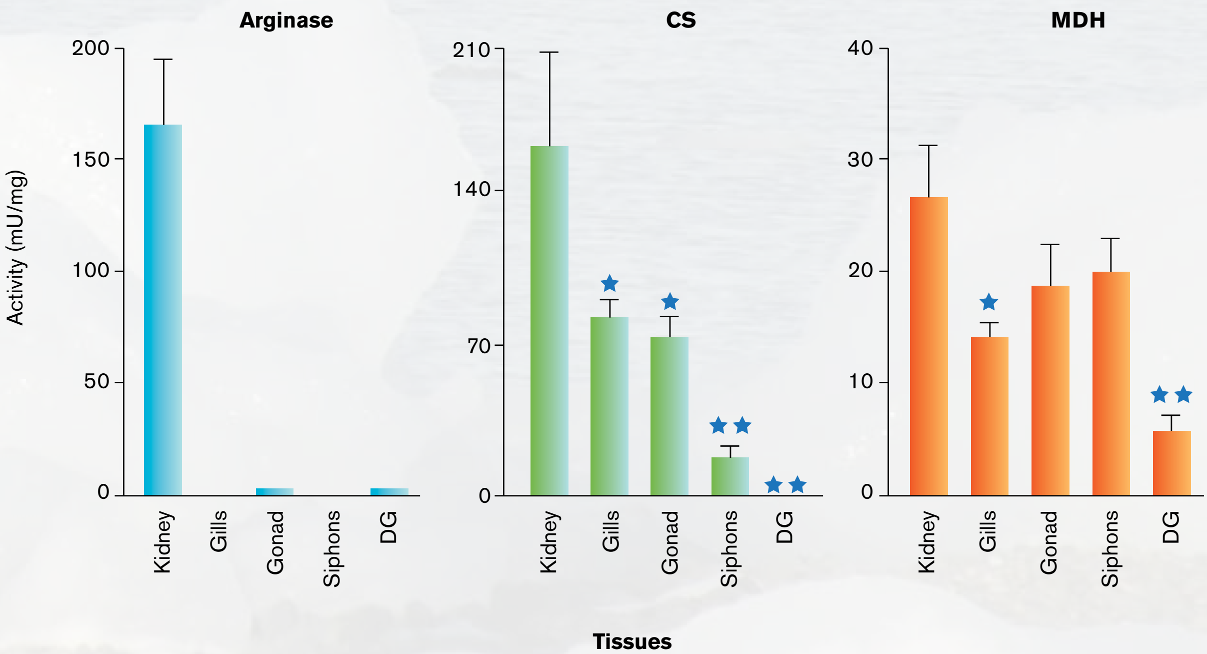

Figure 4. Tissue levels of the enzymes arginase, citrate synthase and malate dehydrogenase for the bivalve *L. elliptica* (Rodrigues *et al.*, 2009).

hepatic parenchyma. The central lobular vein, branches into sinusoid penetrates its wall (Figure 2).

The morphological structure of gills of these fish follows the standard pattern of teleosts, formed by arches filaments and lamelle. Gill epithelium is composed of various types of cells, particularly squamous lining cells, chloride and mucus-producing cells (Figure 2).

The monitoring of the subtidal of Admiralty Bay will also include studies of the bivalve filter feeder *Laternula elliptica*. Studies of specimens collected in Potter Cove, close to Admiralty Bay, revealed that this bivalve meets the necessary prerequisites for monitoring the region, as it has the capacity to bioaccumulate heavy metals and polycyclic aromatic hydrocarbons (PHAs) (Curtosi *et al.*, 2010; Rodrigues *et al.*, 2007).

During the austral winter, this bivalve reduces its filtration rate, retracts its siphon to a position below the sediment surface and is in a state of dormancy using proteins as the main energy source (Rodrigues *et al.*, 2007).

Accordingly, the renal tissue has a high potential for generating ATP and argininolytic capacity compared to the gills, gonads, siphon and digestive gland (Figure 4) (Rodrigues *et al.*, 2009).

The kinetic evidence of two or more forms of arginase in the renal tissue and elevated resistance to inhibition by heavy metals (Figure 5) as compared to the behaviour of the zebra mussel arginase could be related to the living habits of bivalve and the naturally elevated levels of heavy metals in the coastal waters of some regions of the Antarctic Peninsula and adjacent islands.

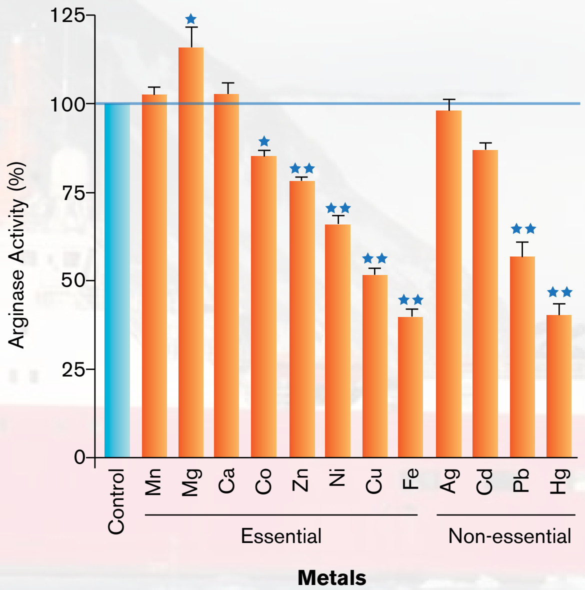

Figure 5. Effect of metal ions on the renal arginase activity of *L. elliptica*. Argininolytic basal activity is represented by the control that probably contains residual amounts of Mn^{2+}, the probable physiological divalent cation. The remaining activities were determined in the presence of metal ions to a final concentration of mmol.L^{-1}. (Rodrigues *et al.*, 2009).

Consequently, the physiological studies with *L. elliptica* has clarified important metabolic aspects related to the living habits of the organism and could also provide important clues for defining additional biomarkers for monitoring the region.

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