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# BIOCHEMICAL MARKER OF ENVIRONMENTAL CHANGES IN ANTARCTIC ANIMALS: SCREENING OF ENERGY METABOLISM ENZYMES OF *Nacella concinna* FOR MONITORING OF INTERTIDAL ENVIRONMENT OF ADMIRALTY BAY, KING GEORGE ISLAND.

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Cecilia Nahomi Kawagoe Suda[1,*], Mariana Feijó-Oliveira[2], Edson Rodrigues Junior[2], Gannabathula Sree Vani[1], Lucélia Donatti[2], Helena Passeri Lavrado[3] & Edson Rodrigues[1]

[1]Instituto Básico de Biociências, Universidade de Taubaté, Av. Tiradentes 500, Centro, CEP 12030-180, Taubaté, SP, Brazil
[2]Departamento de Biologia Celular, Universidade Federal do Paraná, Centro Politécnico s/nº, Jd das Américas, CEP 81990-970, Curitiba, PR, Brazil
[3]Departamento de Biologia Marinha, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, Ilha do Fundão, CEP 21941-902, Rio de Janeiro, RJ, Brazil
*e-mail: cnksuda@hotmail.com

**Abstract:** *The Antarctic limpet Nacella concinna inhabits the intertidal shores of Admiralty Bay. The activities of energy metabolism enzymes of this species were determined in order to evaluate their potential as biochemical markers of intertidal environment changes. The limpets were collected on February 2011 at six intertidal sites at Admiralty Bay: Ullman Point, Botany Point, Punta Plaza, Hennequin Point, Brazilian Refuge 2 and in front of the oil tank of Brazilian Antarctic Station Comandante Ferraz. The enzyme activities were undertaken on the gills and foot tissues of these limpets. There were no significant differences in the activities of malate dehydrogenase, lactate dehydrogenase and hexokinase from foot tissue and citrate synthase, phosphofrutokinase and glucose 6-phosphate dehydrogenase from gills in samples from the six sites. Thus, they are candidates for biochemical markers because these enzyme activities are not affected by small genetic variations between populations, as well as small natural differences in the environment.*

**Keywords:** Antarctica, Intertidal Zone, Biochemical Marker, *Nacella concinna*

## Introduction

Impact of human activities on the environment can be determined by physical and chemical measurements of the contaminants. However, the analysis of biochemical markers, such as enzyme activity, can be a sensitive indicator of penetration of environmental contaminants into tissues and cells of organisms. Biochemical markers can detect sub-lethal effect of contaminants and can contribute for early decision about environmental management (Slatinská *et al.*, 2008). The limpet, *Nacella concinna* occurs in Antarctic and sub-Antarctic rocky intertidal and sub-tidal zones south of 54°S, colonizing areas along the Antarctic Peninsula and adjacent islands, including King George Island (Hoffman *et al.*, 2011). The activities of enzymes of energy metabolism were investigated in *N. concinna* in order to evaluate their potential as biochemical markers of intertidal environment changes caused by natural or anthropic factors.

## Materials and Methods

*N. concinna* were collected in the intertidal zone of Admiralty Bay, King George Island on February 2011. The collection sites were Ullman Point (UP: 62°04'55.5"S 58°21'17.6"W), Botany Point (BO: 62°06'19.4"S 58°21'28.2"W), Punta Plaza (PP: 62°05'28.9"S 58°24'21.3"W), Hennequin Point (HP: 62°07'33.8"S 58°23'36.6"W),

Brazilian refuge, Refúgio 2 (R2: 62°04'16.0"S 58°25'17.6"W) and near the Oil Tank of Brazilian Antarctic Station Comandante Ferraz (OT: 62°4'59.1"S 58°23'23.6"W). The feet and gills were separated and the tissues were homogenized with 50 mM Tris-HCl buffer (pH 7.4). The ratio tissue mass (g) to buffer volumes (mL) were 1:10 and 1:5 for gills and feet, respectively. The homogenized material was sonicated, centrifuged (14000*g*) for 10 minutes and the supernatant was utilized for activity assay of hexokinase (Baldwin *et al.*, 2007), glucose 6-phosphate dehydrogenase (Ciardiello *et al.*, 1995), phosphofrutokinase (Baldwin, *et al.*, 2007), lactate dehydrogenase (Thuesen *et al.*, 2005), malate dehydrogenase (Childress & Somero, 1979) and citrate synthase (Saborowski & Buchholz, 2002).

The enzyme activities in a specific tissue were compared between different sampling sites by One-way Anova followed by Tukey test or Kruskal-Wallis followed by Dunn's post test ($p < 0.05$). GraphPad Prism 5.00 for Windows (GraphPad Software) was used for the statistical analysis.

## Results

In eukaryotes, at least, cytosolic and mitochondrial isoforms of malate dehydrogenase (MDH) are present. In this work, activities of all isoforms were determined as total activity. The lowest and highest activities of MDH in the gills were observed in the samples collected at UP and PP, respectively. The activity of MDH from feet was not significantly different between collection sites (Figure 1a).

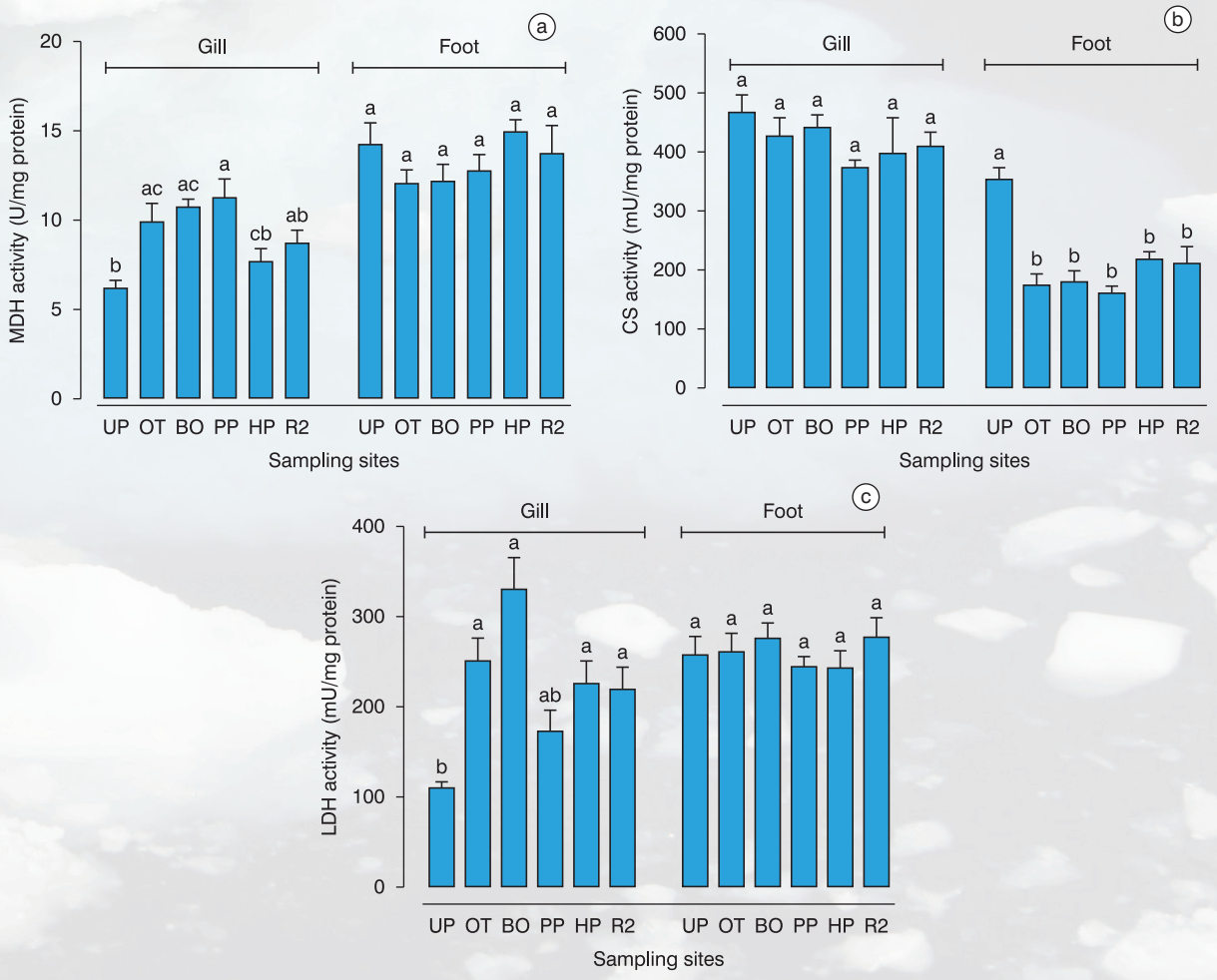


**Figure 1.** Activity (average ± standard error of the mean) of malate dehydrogenase (a), citrate syntase (b) and lactate dehydrogenase (c) from gills and foot muscle of *N. concinna* collected at different sites in Admiralty Bay (UP: Ullman Point, OT: Oil Tank, BO: Botany Point, PP: Punta Plaza, HP: Hennequin Point, R2: Refúgio 2). Different letters above bars indicate significant difference between the sampling sites.

Citrate synthase (CS) is the first enzyme in the citric acid cycle and is an indicator of aerobic metabolism. The CS activity did not vary in samples from different sites except for foot CS from UP (Figure 1b). In general, the activity of gill CS was higher than foot CS, suggesting that aerobic metabolism prevails in gills. Lactate dehydrogenase (LDH) is an enzyme of anaerobic metabolism of pyruvate. The activity in the gills was lowest in *N. concinna* collected at UP. However, for the feet, the level of activity was not significantly different between collection sites (Figure 1c).

Other glycolytic pathway enzymes such as hexokinase (HK) and phosphofrutokinase (PFK) were investigated, glucose 6-phosfate dehydrogenase (G6PDH), an enzyme of pentose phosphate pathway that provides NADPH for antioxidative defense system and intermediary compounds for glycolysis was also investigated (results not shown). The results of HK were similar to LDH in the foot tissue. No difference between collection sites was found in relation to PFK and G6PDH extracted from gills.

## Discussion

During the austral spring and summer, melt waters from neighboring glaciers and snowfields flow into the intertidal zone at BO and R2. Thus, these are potentially stressful environments for *N. concinna*, which is a stenohaline osmoconformer. Exposition for 96 hours to salinity of 20.9 is lethal for 50% of the animals, and 50% die if exposed to freshwater for 2h and 18 min (Davenport, 2001). PP is far from scientific stations and far from anthropic influence but subject to wave action. HP is a place also exposed to wave action due to currents between Admiralty Bay and Bransfield Strait. OT is the site with anthropic influence whereas UP is a sheltered place far from the scientific station and the anthropogenic influence. A study with isotopic analysis of biotic and abiotic elements from UP indicated that C and N from sewage could have been incorporated into organic matter in front of the Brazilian station but not in UP, suggesting that UP was not impacted by sewage (Corbisier *et al.*, 2010). Perhaps UP is the less stressful environment compared to others sites. Curiously, in UP samples, the high activity of CS and low activity of LDH in gills suggested this tissue has an elevated capacity of aerobic metabolism. However, in foot tissue, activities of both LDH and CS were high suggesting that foot muscles of UP samples may have high potential for aerobic and anaerobic metabolisms. The opposite was found in samples from other sites, where gills probably have elevated potential for both aerobic and anaerobic metabolism, whereas foot muscle apparently has more capacity for anaerobic than aerobic metabolism.

## Conclusion

For monitoring purposes of Admiralty Bay, MDH, LDH and HK from foot tissue and CS, PFK and G6PDH from gills are candidates for biochemical markers since their levels were not significantly different at the several sampling sites. The ideal biochemical marker molecules for detecting environmental change should be sensitive to contaminants but should not be affected by small genetic variation between populations living in the area and/or by small natural variations in the environment. The activity of some energy metabolism enzymes of *N. concinna* from UP were different from those from other sampling sites at Admiralty Bay.

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