EFFECT OF TEMPERATURE, SALINITY AND FLUORIDE ON THE PLASMATIC CONSTITUENTS CONCENTRATION OF ANTARCTIC FISH *Notothenia rossii* (Richardson, 1844)

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Abstract: *The Antarctic marine environment has unique characteristics such as isolation, low and even temperatures, as well as high levels of fluoride in the trophic web. The objective of the present study is to verify the effect of temperature, salinity and dietary fluoride in the diet on the levels of plasma constituents of the fish* ***Notothenia rossii****. The sample collection and the bioassay were conducted at Antarctic scientific station Comandante Ferraz. The fish were acclimated in an aquarium at temperatures of 0 and 4 °C; salinity of 35 and 20 psu, using feed with and without fluoride. The combination of these variables resulted in 8 experimental groups. The calcium serum levels were reduced in hyposaline stress and temperature elevation reduced the plasmatic levels of magnesium and chloride. The trophic fluoride isolatedly was not capable of changing the non-protein electrolytes levels.*

Keywords: Antarctica, biomarker, blood, *Nothothenia rossii*

Introduction

The compiled ichthyofauna data of the south Antarctic Polar Frontier shows the existence of 322 species distributed in 50 families dominated by benthonic fish (70%) of the Notothenioidei family (Eastman, 2005). Even though the marine fish are represented by approximately 16,764 species, the Antarctica ichthyofauna is only 1.9% of this species (Eschmeyer *et al.*, 2010). The low Antarctic fish diversity has been correlated with, the tectonic events which physically isolated this region, low temperatures and the probable alterations occurred in the trophic structure during the Miocene (Eastman, 2005).

The Antarctic demerso-pelagic fish *Notothenia rossii* is one of the four dominant species of the South Shetland Islands (Casaux *et al.*, 1990) and can be easily captured using a fish hook. Its diet is very diversified and includes fish, krill, gastropods, polychaetes among other organisms (Barrera-Oro, 2002). The vertical migration during summer permits its feeding through pelagic organisms (Casaux *et al.*, 1990), especially krill, this explains the high levels of bone tissue fluoride, even though the fluoride tolerance is in study (Camargo, 2003). The thermal metabolic plasticity limit of Antarctic fish has raised questions about the high temperature impact in the adaptation of this ichtyofauna (Mark *et al.*, 2005).

Considered as the most pristine region of the planet, the increase in antrophic activity (scientific and tourism) in Antarctica is a factor of questioning for the scientists. Admiralty Bay in the King George Island, South Shetland Islands Archipelago, has a narrow cove similar to fjords (Barnes *et al.*, 2006) with inshore surface water salinity values fluctuating between 16.4 to 34.2 psu (Romão *et al*,. 2001). Admiralty Bay is an Antarctic Specially Managed Area (ASMA) and shelters five countries scientific stations (Brazil, Ecuador, Poland, Peru and USA).The monitoring of Admiralty Bay ASMA is the main scope of the National Institute of Antarctic Science, Technology and Environmental Research (INCT-APA) of the Brazilian Antarctic Program. Using a long time series of the physical, chemical and biological processes, INCT-APA is interested in understanding the natural and anthropic impacts on the region. The studies about biomarker responses to environmental pollution integrate the activities of INCT-APA. The biomarkers research for ASMA of Admiralty bay has the objective of identifying sufficiently sensible biological responses to differentiate the natural impact for those caused by pollutants. The aim of the present study was to investigate how warming, salinity reduction and the wide fluoride distribution in the Antarctic trophic web will be inducing biochemical responses in the blood of *N. rossii* Antarctic fish.

Material and Methods

Animals

Specimens of *N. rossii* were collected using fish hooks, in Punta Plaza (62º 05' 35.8" S and 58º 24' 11.8" W) and Glacier Ecology (62º 10' 11.9" S and 58º 27' 17.2" W), Admiralty Bay, King George island, Antarctica, at depths of 10-20 m, during the summer of 2009-2010. A total of 8 experiments were carried out in the Commandante Ferraz Antarctic Station. The control conditions were 0 °C, 35 psu and a fluoride free diet. The remaining experiments were done with a combination of conditions: temperature (0 °C and 4 °C), salinity (35 and 20 psu) and diet a with/without fluoride (15 mg/kg fish). Each bioassay was performed on eight fish specimens, all of similar size (475 g ± 350 g) (media ± SD) and kept in experimental conditions for 11 days. At the end of the experiment, the caudal vein was punctured and the blood collected with heparin, centrifuged at 2000 rpm for 5 minutes and the plasma frozen in liquid nitrogen. In the same manner blood samples were collected from five specimens of *N. rossii*, directly from Punta Plaza (PP) and Glacier Ecology (ECO), which are respectively, farther away and close to Arctowski Penguin Rookeries. In this case, the caudal vein puncture was undertaken aboard boat as soon as the fish was taken from the water to register the closest physiological condition.

Plasma assay

The plasmatic electrolytes chloride, magnesium, calcium and inorganic phosphate were assay spectrophotometrically by methods of the mercuric thiocyanate, xylidyl blue, O-cresolphthalein complexone, phosphomolybdate, respectively (Burtis & Ashwood, 1994). The spectrophotometer reading was carried out in 384 wells micro plates using the micro plate reader Fluostar of BMG.

Statistical analysis

The differences between the control (0 °C and 35 psu) and the experimental group as well as the nature controls were tested at 5% significance levels using t-test with Welch's correction.

Results and Discussion

The concentration of the plasmatic constituents of the specimens of *N. rossii* from bioassays, as well as for the control of the nature from Punta Plaza (PP) and the Glacier Ecology (ECO) are summarized in Figure 1.

Independently, the fluoride was not able to change the plasmatic levels of calcium, inorganic phosphate, magnesium and chloride. Directly or indirectly all the Antarctic vertebrates consume krill, so consequently are exposed to high levels of fluoride present in this euphausiid exoskeleton. The apparent lack of symptoms for fluorosis, rouse questions about the protective adaptive mechanisms

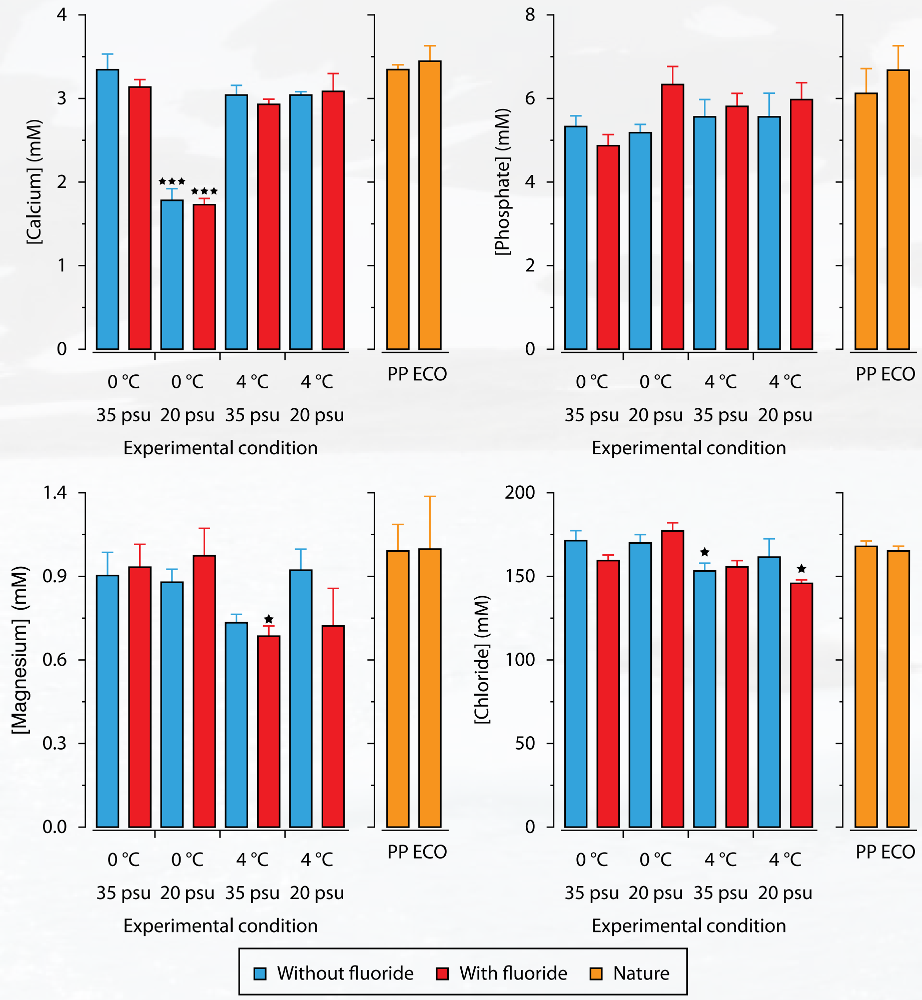

Figure 1. Plasma levels of chloride, inorganic phosphate, magnesium, and calcium in the Antarctic fish *Notothenia rossii* subjected to thermal and salinity stress. Fluoride was supplied by trophic pathway. Results expressed as mean ± standard error of the mean.

against the toxic effect of fluoride (Yin *et al.*, 2010). Differences were also not observed between the means values of controls, experimental and nature (PP and ECO) groups. The choice of ECO and PP was taken into account considering the probable effect of fluoride present in high concentrations in the soils and sediments near the Penguin Rookeries (Xie & Sun, 2003), on the plasmatic levels of non-protein electrolytes.

The low salinity expressively decrease the levels of plasmatic calcium, whereas chloride and magnesium levels were reduced by thermal stress. Fluoride was capable to induce reduction of chloride plasmatic levels, only under thermal and salinity stress. It is well known that warming tends to reduce the blood osmolality of Antarctic fish, without an increase in cortisol or hematocrit, indicating that the acclimatization to warming is not mediated by stress response (Hudson *et al.*, 2008). As the osmolality is principally maintained by NaCl (Dobbs III & DeVries, 1974), chloride reduction in the *N. rossii* blood should be expected by warming (Figure 2), even though studies with *Notothenia neglecta* showed that low salinity did not significantly change the levels of Na^+ and Cl^- in the blood after 10 days exposure to ≅ 16 psu (Romão *et al.*, 2001).

Calcium and magnesium have a key role in a large range of physiological processes. The renal tissue of Antarctic fish is capable of excreting magnesium and chloride in the

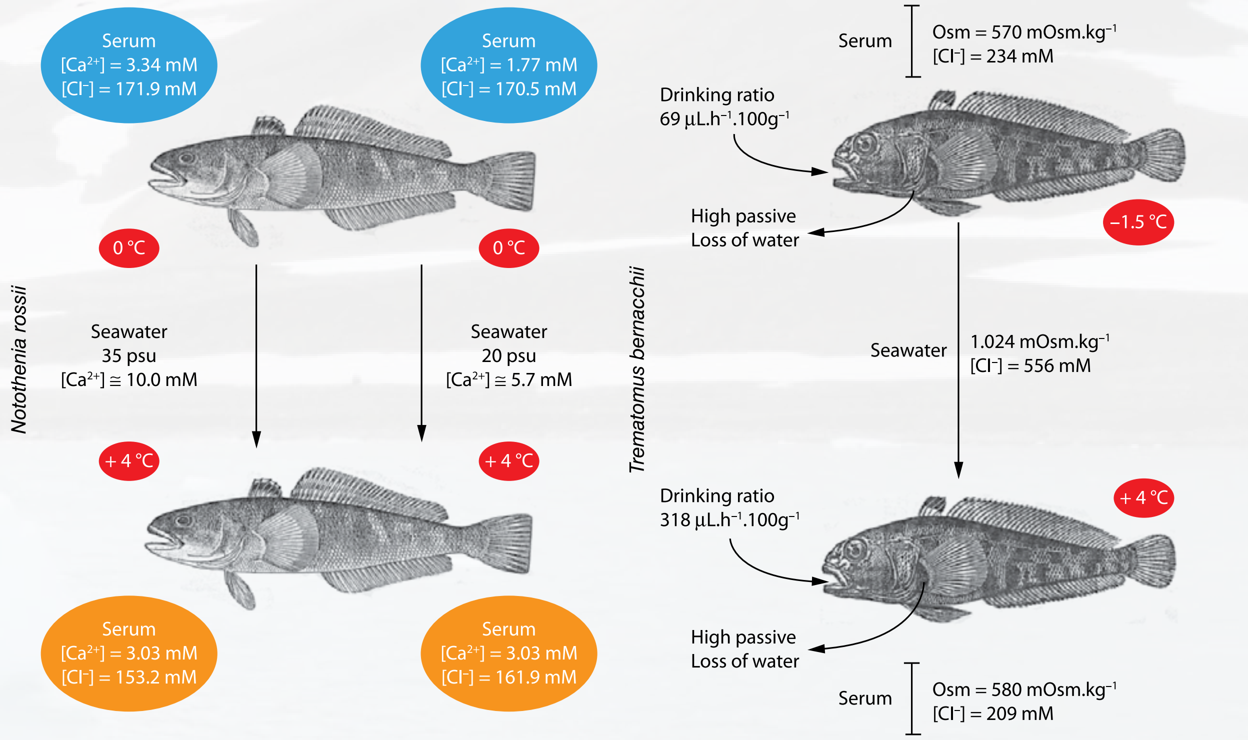

Figure 2. Interrelation between hyposmotic acclimation, drinking ratio, osmolality and serum levels of calcium, chloride and sodium. The metabolic answer of hyposaline and thermal acclimation effect of Antarctic fish *Notothenia rossii* is shown on the left. The warm acclimation effect on osmolality and drinking ratio of *Trematomus bernacchii* is shown on the right (data from Petzel (2005) and fish images from (Fischer & Hureau, 1985)).

urine against a gradient concentration as part of control mechanisms evolved in maintenance of blood osmolality (Dobbs III & DeVries, 1974). In warm acclimation of Antarctic fish, Petzel (2005) observed that the blood osmolality reduction was accompanied by a rise in drinking ratio and reduction of chloride and sodium serum levels (Figure 2).

The calcium entrance to the blood in marine teleosts is basically through intestine (drinking). Gills and kidneys have a central role in calcaemia control and are capable of actively excreting this metallic cation (Pinto *et al.*, 2010). The hypocalcaemia of *N. rossii* acclimated a 0 °C e 20 psu can be due to low calcium concentration in the seawater at 20 psu. Although in warm and hyposaline acclimation (4 °C e 20 psu), *N. rossii* calcaemia was maintained close to control levels (0 °C e 35 psu). In this case, the thermic stress (4 °C) could be causing reduction of blood osmolality and increase the drinking rate of *N. rossii* compensating the low calcium concentration in the 20 psu seawater through a rise in drinking volume (Figure 2).

Conclusion

The present study revealed that warming, hyposalinity and trophic fluoride interfere with plasmatic non protein electrolytes levels of Antarctic fish *N. rossii*. Considering the variables studied and blood parameters analyzed the plasmatic calcium stand out as an excellent biochemical biomarker of hyposaline stress.

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References

Barnes, D.K.A.; Fuentes, V.; Clarke, A.; Schloss, I.R. & Wallace, M.I. (2006). Spatial and temporal variation in shallow seawater temperatures around Antarctica. Deep-Sea Research Part II: Topical Studies in Oceanography, 53(8-10): 853-65.

Barrera-Oro, E. (2002). The role of fish in the Antarctic marine food web: differences between inshore and offshore waters in the southern Scotia Arc and west Antarctic Peninsula. Antarctic Science, 14(4): 293-309.

Burtis, C.A. & Ashwood, E.R. (1994). Tietz Textbook of Clinical Chemistry. Second. Saunders Company, Philadelphia, Pennsylvania. 2326p.

Camargo, J.A. 2003. Fluoride toxicity to aquatic organisms: a review. Chemosphere, 50(3): 251-64.

Casaux, R.J.; Mazzotta, A.S. & Barrera-Oro, E.R. (1990). Seasonal aspects of the biology and diet of nearshore nototheniid fish at Potter Cove, South Shetland Islands, Antarctica. Polar Biology, 11(1): 63-72.

Dobbs III, G.H. & DeVries, A.L. (1974). Renal function in Antarctic teleost fishes: Serum and urine composition. Marine Biology, 29(1): 59-70.

Eastman, J.T. (2005). The nature of the diversity of Antarctic fishes. Polar Biology, 28(2): 93-107.

Eschmeyer, W.N.; Fricke, R.; Fong, J.D. & Polack, D.A. (2010). Marine fish diversity: history of knowledge and discovery (Pisces). Zootaxa, 2525: 19-50.

Fischer, W. & Hureau, J.C. (1985). FAO Species identification sheets for fishery purposes. Rome: FAO. 470p.

Hudson, H.A.; Brauer, P.R.; Scofield, M.A. & Petzel, D.H. (2008). Effects of warm acclimation on serum osmolality, cortisol and hematocrit levels in the Antarctic fish *Trematomus bernacchii*. Polar Biology, 31(8): 991-7.

Mark, F.; Hirse, T. & Pörtner, H. (2005). Thermal sensitivity of cellular energy budgets in some Antarctic fish hepatocytes. Polar Biology, 28: 805-14.

Petzel, D. (2005). Drinking in Antarctic fishes. Polar Biology, 28(10): 763-8.

Pinto, P.I.S.; Matsumura, H.; Thorne, M.A.S.; Power, D.M.; Terauchi, R.; Reinhardt, R. & Canário, A.V.M. (2010). Gill transcriptome response to changes in environmental calcium in the green spotted puffer fish. BMC Genomics, 11: 476.

Romão, S.; Freire, C.A. & Fanta, E. (2001). Ionic regulation and Na^+,K^+-ATPase activity in gills and kidney of the Antarctic aglomerular cod icefish exposed to dilute sea water. Journal of Fish Biology, 59: 463-8.

Xie, Z. & Sun, L. 2003. Fluoride content in bones of Adelie penguins and environmental media in Antarctica. Environmental Geochemistry and Health, 25(4): 483-90.

Yin, X.; Chen, L.; Sun, L.; Wang, M.; Luo, H.; Ruan, D.; Wang, Y. & Wang, Z. 2010. Why do penguins not develop skeletal fluorosis? Fluoride, 43: 108-18.