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INTERACTIVE EFFECTS OF WARM ACCLIMATION, LOW SALINITY, AND TROPHIC FLUORIDE ON THE LIVER METABOLISM OF THE ANTARCTIC FISH *Notothenia rossii*

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Abstract: *All Antarctic vertebrates are directly or indirectly exposed to high levels of fluoride present in the exoskeleton of krill. Absence of fluorosis in these organisms indicates high tolerance to this halogen. The present study investigated the effect of fluoride on the hepatic metabolism of N. rossii Antarctic fish. Levels of the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRED) and arginase (ARG) were determined. Specimens were subjected to eight different thermo-saline-trophic conditions, involving the interaction between two temperatures (0ºC or 4ºC), two salinities (20 or 35) and two trophic conditions (with or without fluoride). Although fluoride did not modulate the levels of SOD, CAT and GRED, low salinity reduced hepatic levels of GRED, revealing that this saline condition may be inducing oxidative stress. Fluoride raised arginase levels except in the case of the thermo-saline condition of 4-20. In relation to antioxidant defense, the liver of N. rossii fish was found to be more resistant to toxic effects of fluoride than the liver of non-Antarctic fishes.*

Keywords: Antarctic Fish, Fish Metabolism, *Notothenia rossii*, Oxidative Stress

Introduction

King George Island is located at the northern tip of Antarctic Peninsula and is one of the most sensitive areas to climatic change. Admiralty Bay, a fjord-like bay in King George Island, receives melt waters from various glaciers and the inshore surface water has salinity between 33.4 and 34.6 (Siciński *et al.*, 2011). Global climate change and rapid increase of surface temperatures in the Antarctic Peninsula, particularly in the western sector, has raised questions regarding the impact of warm waters and changes in salinity on the continental shelf fauna (Barnes *et al.*, 2006).

All Antarctic vertebrates directly or indirectly feed on krill and are consequently exposed to elevated levels of fluoride present in the carapace of these crustaceans. Generally, in Antarctic conditions, krill-eating animals have the ability to live with high levels of fluoride in the diet, without any symptoms of fluorosis, even though this halogen can potentially inhibit many metabolic enzymes (Adelung *et al.*, 1985).

The aim of this study was to investigate the thermo-saline-trophic interactions involved in elevated temperatures and low salinity in the presence of fluoride in the diet, and to show how this halogen could modulate the metabolism of *N. rossii*, particularly, the enzymes of argininolytic metabolism (arginase) and oxidative stress (glutathione reductase, superoxide dismutase and catalase).

Materials and Methods

Specimens of *N. rossii* were captured at Punta Plaza, Keller Peninsula, Admiralty Bay, King George Island,

Antarctica, from January to March 2011, at depths of 10 to 20 m using fishing line. Fish were transferred to aquaria at EACF and after 48 hours, anesthetized, measured, weighted, marked and sorted for bioassay. Fish were kept for three days in 1000L tanks (1fish/200L) at thermo saline conditions of 0°C and 35 psu. Tanks were continuously aerated and exposed to photoperiod of 12h. Fish were submitted to 8 different conditions from the combination of two different temperatures (0 or 4°C), salinities (35 or 20), and food (with and without fluoride). The transition from thermo-saline condition of 0–35 (control) to the other experimental conditions took place slowly and gradually over a period of 6 hours. The salinity of 20 was obtained by diluting the seawater with freshwater.

Fluoride was added to the food (skeletal muscle of *N. coriiceps*) by injecting small volumes (80–120µL) of sodium fluoride into it in order to obtain an end dose of 15 mg NaF/kg of body mass. This dose was chosen based on metabolic changes induced by the fluoride in the diet of rats and fishes (Yoshitomi *et al.*, 2006). After the fluoride injection, the food was offered individually to the fish for a maximum period of 30seconds. Fish that refused to feed on one or more occasions were retained in the tanks but were excluded from the analysis. The fish were kept at the experimental condition for 11 days, and subsequently, their liver tissues were frozen in liquid nitrogen for posterior analysis.

Homogenates were prepared in the proportion of 1g of liver to 5 mL of buffer Tris-HCL 50mM, pH 7.4, sonicated for 15 seconds, centrifuged at 12,000 x g for 10 min, and the supernatants used to determine the activities of the enzymes: glutathione reductase (GRED); superoxide dismutase (SOD); catalase (CAT) and arginase (ARG). Enzymatic activities were normalized with the concentrations of proteins determined using the bicinchoninic acid (BCA, kit QuantiPro - Sigma) method. All analytical procedures were carried out as described by Rodrigues-Jr *et al.* (2013).

Results are presented as mean ± SEM (standard error of the mean). Groups were statistical compared using one way ANOVA, followed by multiple pairwise comparisons with Tukey's post-hoc test. Differences were considered significant for $p < 0.05$.

Results

Liver enzyme levels of antioxidant defense and argininolytic metabolism are shown in Figure 1. SOD levels were negatively modulated by trophic fluoride only in the thermo-saline condition 0-20. CAT levels were not affected in any experimental thermo-saline-trophic condition. Fluoride caused a significant raise of arginase levels only in the thermo-saline conditions of 4-35 and 0-20. GRED levels were significantly lower in thermo-saline condition of 0-20 without fluoride (non-trophic fluoride; NTF) in relation to the control (NFT 035). GRED levels increased significantly in the same condition (0-20) with fluoride.

Discussion

In recent years, several investigations have shown that fluoride can induce oxidative stress, lipid peroxidation and the raise of carbonyl proteins content in many organism tissues (Barbier *et al.*, 2010). However, Antarctic organisms that feed on krill, apparently, do not show signs of fluorosis despite the high levels of fluoride in the exoskeleton of krill (Xie & Sun, 2003). Under thermo-saline condition close to that found in nature (0-35), fluoride could not modulate the levels of SOD, CAT, GRED and Arginase. In rat liver, trophic fluoride was able to reduce antioxidant defense and intensify lipid peroxidation (Inkielewicz-Stepniak & Czarnowski, 2010). Chronic exposure of *Cyprinus carpio* to fluoride reduces hepatic levels of SOD, elevates MDA and results in liver damage (Cao *et al.*, 2013). Although fluoride was could not modulate the levels of SOD and GRED, low salinity was caused a reduction in hepatic levels of GRED, revealing that this saline condition may be inducing oxidative stress.

In the case of non-ureotelic animals, tissue arginase activity has been associated with the maintenance of levels of L-arginine and with synthesis control of nitric oxide (Que *et al.*, 2002). In the presence of trophic fluoride, the liver of *N. rossii* raised the levels of arginase, except under the thermo-saline condition of 4-20. As fluoride directly inhibits arginases (Rodrigues *et al.*, 2009), increased arginase levels can be offsetting this inhibitory effect. Thus, the higher levels of arginase would maintain properly the L-arginine metabolism. However, the interaction thermo-saline condition of 4-20 prevented the induction of higher levels of arginase in the presence of trophic fluoride.

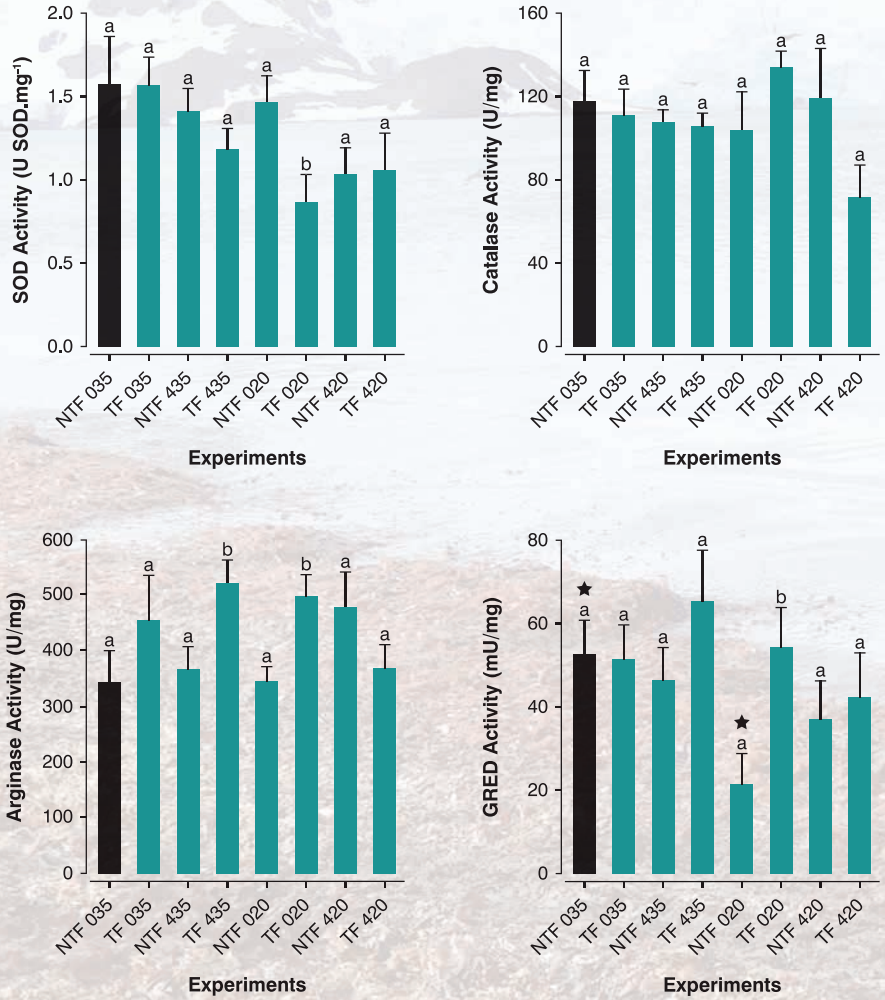

Figure 1. Levels of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRED) and arginase (ARG) in hepatic tissue of *Notothenia rossii*. Different letters indicate significant differences between the trophic fluoride (TF) and non-trophic fluoride (NTF) in the same thermo-saline condition. Asterisk (★) shows significant difference between control NTF 035 and the other thermohaline conditions without fluoride in the diet (NTF).

Conclusion

The present study showed that trophic fluoride did not reduce the levels of liver enzymes SOD, CAT and GRED in *N. rossii*. The maintenance of levels of these enzymes showed that fluoride does not compromise the antioxidant defense of *N. rossii*. However, the modulation of higher levels of arginase in the presence of fluoride may be compensating the direct inhibition of arginase by fluoride.

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THEMATIC AREA 4

ENVIRONMENTAL MANAGEMENT