HISTOLOGY AND HISTOCHEMICAL CHARACTERISATION OF THE STOMACH STRUCTURE OF THE ANTARCTIC FISH *Notothenia rossii* (RICHARDSON, 1844) UNDER CONDITIONS OF THERMAL STRESS

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Abstract: *Ecological impacts of climate change on marine environments have been significant in the Antarctic Peninsula. Ecological responses may affect fish fauna from the species level to community level. Our goal was to analyze histological and histochemical aspects of three stomach portions (cardiac, fundic and pyloric) of Notothenia rossii, an antarctic fish, under thermal stress using light microscopy. Fish were collected in Admiralty Bay and taken to the Brazilian Antarctic station "Comandante Ferraz". Fish (n=5/bioassay) were acclimated in seawater tanks (0ºC/35‰) and exposed to temperatures of 0°C (control), 4°C and 8°C during 1, 4, 15 and 30 days. Samples were processed and stained with haematoxylin/eosin, Periodic Schiff acid, Alcian-Blue pH 2.5 and 1.5. Heating caused an increase in the muscle layer of fundic and pyloric regions in samples kept for 1 day at 8°C and 4 days at 4°C, compared with the control group. Expression of neutral glycosaminoglycans was not affected by increasing temperature. Thermal stress caused a reduction in expression of carboxylated and sulfated-glycosaminoglycans in samples kept at 8°C during 1, 4 and 30 days. However, the stomach does not show histological and histochemical standard alterations under different thermal stress conditions and, therefore, we cannot be conclusive about the lack of full functionality.*

Keywords: Nototheniidae, Stomach, Histochemistry, Thermal Stress.

Introduction

Temperature directly or indirectly affects living organisms, determining metabolic rate and the development of digestive chemical reactions and metabolic processes (Kapoor *et al.*, 1975; Ba-Omar & Victor, 2000; Silva & Araújo-Lima, 2003; Kawaguchi, 2007; Handeland, 2008) and increases susceptibility to histopathology in some chronic cases. Nevertheless, there are few studies focused on the morpho-histology (Freiberger, 1996) and functional aspects of the digestive system of Antarctic fishes subjected to thermal stress (Hernandez-Blazquez *et al.*, 2006). Therefore, this study aims to carry out the histological and histochemical characterisation of the stomach structure of *Notothenia rossii* under different conditions of thermal stress.

Materials and Methods

Samples of *Notothenia rossii* were captured with hook and line in Admiralty Bay (Lat.: 61°S and 63°30'S/ Long.: 53°55'W and 62°50'W), in King George Island, South Shetlands Archipelago, Antarctica. The experiment was

approved by the Ethics Committee of the Federal University of Paraná (UFPR) under the number 496.

After collection, fish were maintained for 5 days in 1000-L tanks in Comandante Ferraz Brazilian Antarctic Station under controlled temperature (0 ± 0.5˚C), salinity (35 ± 1.0) and photoperiod (12 hours light/12 hours dark) with constant aeration. *Notothenia rossii* individuals were transferred to 1000-L controled tanks with temperature of 0 ± 0.5°C, 4 ± 0.5°C and 8 ± 0.5°C for 1, 4, 15 and 30 days. A total of 5 animals were assigned for each treatment and they were directly placed into the experimental temperatures. Fish were fed (epaxial fish muscle-based diet) every two days, alternating with an exchange of 50% of the water in the tanks. At the end of each experiment, fish were anesthetized with 1% benzocaine (pv^{-1}), sacrificed by medullary sectioning and immediately dissected. Fragments of three stomach regions (cardiac, fundic and pyloric) were fixed for 24 hours in Bouin for histological and histochemical analysis.

Sections (5 µm thick) were stained with haematoxylin and eosin (HE), PAS – Periodic Acid Schiff (neutral glyconjugates), Alcian Blue pH 2.5 (carboxyl and sulphate-groups of acidic carbohydrates) and Alcian-Blue pH 1.0 (sulphated carbohydrates). Slides were photographed using a Olympus BX40 binocular optical microscope with built-in camera and images were analyzed in Image Pro PluS 6.0 software. In order to determine the thickness of gastric layers of each animal, three images were captured, from which three measures were obtained. Moreover, two images were captured from each animal to determine the secretion of mucous substances through RGB colour systems.

The effect of exposure time and temperature (independent variables) was statistically tested tested using analysis of variance (two-way ANOVA). Tukey's post-hoc test was used for pairwise comparisons of means. Levene's test was used *a priori* to test the homogeneity of variances and data were *log-transformed* in significant cases. The significance level used in the statistical analyses was 5%.

Results

Notothenia rossii has four histological layers forming the stomach wall: the mucous, the submucous, the muscular and the serous. No significant variation was observed among the groups (0°C, 4°C and 8°C) according to histo-morphometric analyses of mucous and submucous layer thickness of the cardiac, fundic and pyloric regions of the animals subjected to thermal stress. The muscular layer presents an evident thickening in the fundic and pyloric regions of controlled and experimental animals kept for one day at 8°C and four days at 4°C respectively (Figures 1A and 1B).

The data obtained through image analysis did not show significant difference in the expression of neutral glycosaminoglycan in the three portions of the stomach. Tests with Alcian Blue pH 1.0 were negative, indicating that sulphated acid glycosaminoglycan is absent.

Striking differences were observed in the expression of acid glycosaminoglycan in the epithelium of the cardiac

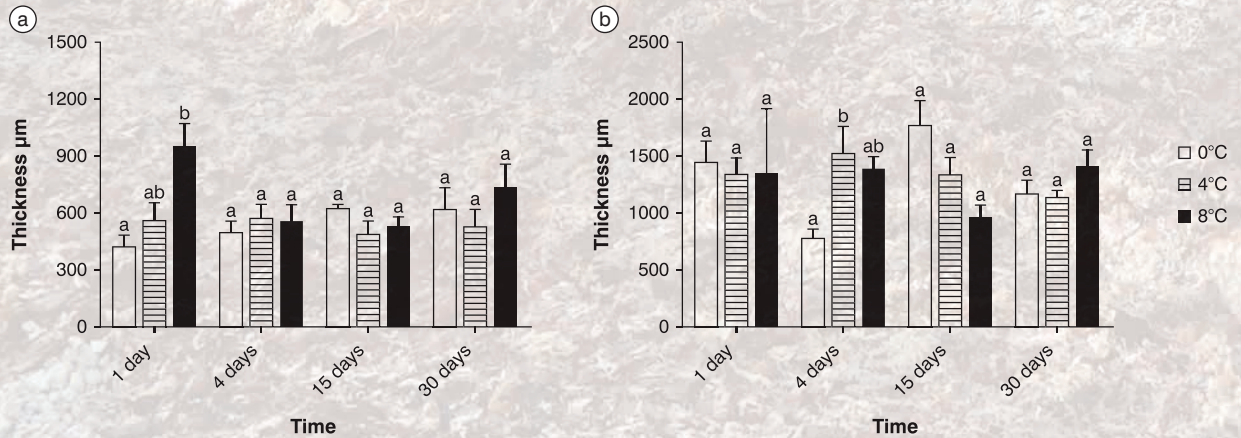

Figure 1. Mean thickness ± SE of the muscle layer. **A.** Fundic and **B.** Pyloric region of the stomach of *Notothenia rossii* exposed to temperatures of 0°C, 4°C and 8°C for 1, 4, 15 and 30 days. Different letters indicate statistical differences in the thickness of the muscle layer due to increase of temperature. (Tukey test, $p < 0.05$).

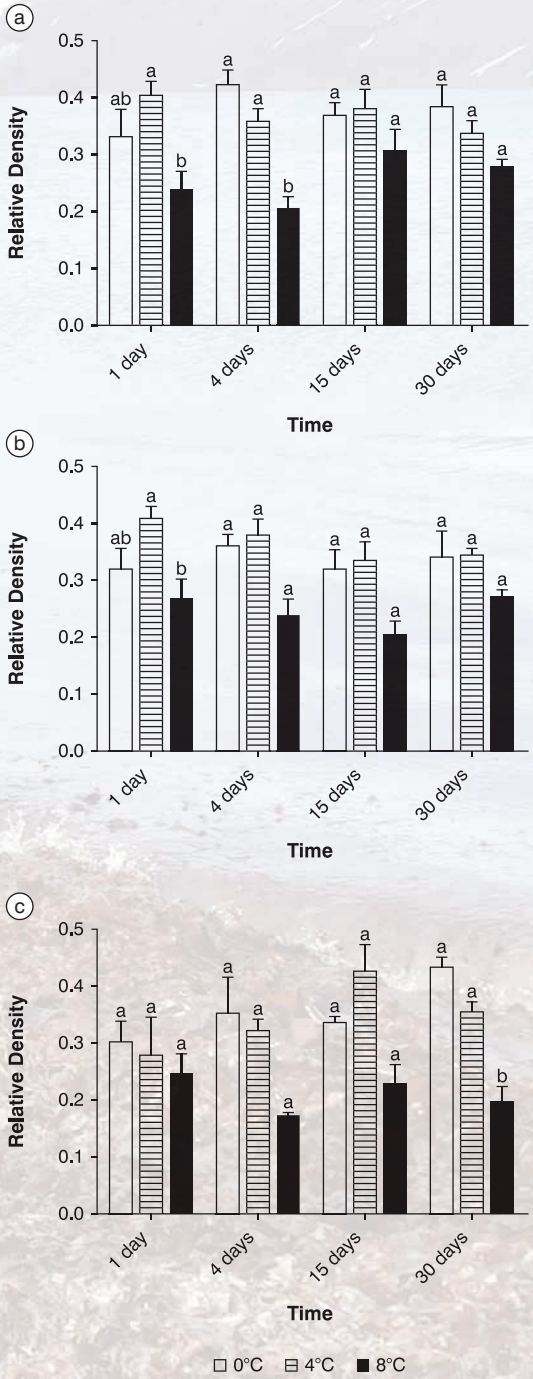

Figure 2. Relative density of acidic glycosaminoglycans (Alcian blue pH 2.5). **A.** Cardiac; **B.** Fundic and **C.** Pyloric region of the stomach of *Notothenia rossii* exposed to temperatures of 0°C, 4°C and 8°C for 1, 4, 15 and 30 days. Notice the significant difference represented by different letters in **A.** groups of 1 and 4 days, **B.** 1 day and **C.** 30 days. (Tukey test, $p < 0.05$).

and fundic regions of animals kept for one day at 4°C and 8°C (Figure 2A and 2B). Animals kept for four days at 8°C showed a meaningful decrease in the expression of acid glycosaminoglycan in the cardiac region when compared to the control and 4°C groups. A slight presence of acid glycosaminoglycan was noticed in the epithelium of the pyloric region of animals kept at 8°C for 30 days (Figure 2C).

Discussion and Conclusions

Temperature is a relevant environmental factor for fishes in the sense that influences nourishing rates, action of digestive enzymes, gastric and intestinal motility, secretion rate of digestive juice and intestine absorption rate (Kapoor *et al.,* 1975; Silva & Araújo-Lima, 2003), and may lead to changes in metabolic responses. Studies on stomach histology of fish under the effects of thermal stress are missing, but studies with mammals indicate that alterations in mucous layers may, in the long term, compromise gastric evacuation, which, in turn, tends to lead to increased acidic concentration inside the stomach and, consequently, gastric ulcers (Meyer, 1999).

In this paper, it was possible to observe that the thickness of the muscular layer of the fundic region increased in the fishes exposed for one day to 8°C heating, as well as in the pyloric region of animals kept for four days at 4°C. The muscular layer of the pyloric region is thicker than that of the cardiac and fundic regions, which suggests that there is a facilitation of ejection of food through peristaltic activity towards the intestine (Rodrigues and Menin, 2008). Bastos-Ramos *et al.* (2000) demonstrated that the increase in temperature leads to a proportional muscle relaxation on the intestine smooth muscle of the *Notothenia neglecta* (*coriiceps*) and of the *Chaenocephalus aceratus*.

According to Domeneghini *et al.* (2002), most teleosts have gastric glands in cardiac and fundic regions. In the current study, gastric glands were detected in the three regions of the stomach, just as it happens in *Trycomycterus brasiliensis* (Oliveira-Ribeiro & Fanta, 2000). Gastric epithelial coating shows positive PAS apical mucous substances that serves particularly forfunctions such as lubrication, digestion, absorption of carbohydrates and fatty acids, control of infectious diseases, colonisation of harmful and opportunistic microorganisms and regulation of the gastric pH (Cao & Wang, 2009; Díaz

et al., 2008). Such roles may be affected in *N. rossii* that underwent a heating-up of 8°C, given that decrease of acidic mucous (carboxylate and sulphate) was observed, although secretion of neutral mucous did not occur with the temperature elevation.

The data obtained indicate that the stomach does not show histological and histochemical standard alterations, hindering the conclusive assertion that its total functionality is not taking place, despite the fact that the animals experienced thermal stress. Other studies have been conducted with Antarctic fish demonstrating the thermal window and metabolic adjustments adopted by these organisms under increased temperature.

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