IMPACT ASSESSMENT OF SEWAGE OF BRAZILIAN ANTARCTIC STATION ON THE PLASMA CONSTITUENTS OF ANTARCTIC FISH *Notothenia rossii*

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Abstract: The burning of fossil fuels, sewage dumping and leakage of combustible oil has been polluting Antarctica around the scientific stations and ship anchoring locations. The present work evaluated the effect of sewage effluent of Antarctic Station Commandant Ferraz (EACF) on the plasmatic levels of glucose, triglycerides, cholesterol, total proteins, albumin and globulins of Antarctic fish *Notothenia rossii*. The bioassays were conducted with effluent from the sewage processing station of EACF diluted in seawater. In the presence of sewage, glucose, triglycerides, total proteins and globulins reduced in the plasma of *N. rossii*, where as albumin and cholesterol increased. In this case, the reduction of energy metabolites (glucose and triglycerides) plasmatic levels can be related with the rise in energy demand of *N. rossii* induced by the sewage.

Keywords: Antarctica, Fish Metabolism, Biochemical Marker, *Notothenia rossii*

Introduction

Antarctica was rapidly explored and occupied as soon as it was discovered in the XIX century. In 1959, the Antarctic treaty was officially signed and set forth the rights and duties of all those who occupy this continent. The formal commitment for the preservation and monitoring of Antarctica as a natural reserve restricting the continent to peace and science was explicitly stated in the Environmental protection protocol (Madrid Protocol) and signed by the parties to the Antarctic Treaty of 1991 (Roura & Hemmings, 2011).

The scientific occupation introduced pollutants that were not originally present in Antarctica. The burning of fossil fuels, effluents from the scientific stations/ships and the leakage of combustible oil has contaminated the coastal regions with human sewage, heavy metals and persistent organic pollutants (Tin *et al.*, 2009; Aronson *et al.*, 2011). The global climatic changes and localized pollution around

the scientific stations has raised concerns about its impact on the Antarctic ecosystems (Aronson *et al.*, 2011).

The present study aims to evaluate the chronic effect of sewage effluents of the EACF on the plasmatic constituent's levels of the Antarctic fish *Notothenia rossii*.

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. The fishes were transferred to aquariums at EACF and after 48 hours, anesthetized, measured, weighed, marked and sorted for bioassay. The effluent from the sewage processing station (SPS) of EACF was fed slowly into the tanks for final concentrations of 0.05% (v/v) and 0.5% (v/v), with 10 fish for each experiment. The experimental

controls (EC) were 10 fish kept at thermo-saline conditions of 0-35, without effluents from SPS. The fishes were kept in the experimental condition for 25 days. Every two days the fish were fed with Antarctic fish muscle, corresponding to 1% of the body mass. The blood of the nature control group (NC) fish was collected as soon as the *N. rossii* were removed from the water to minimize the possible effect of stress arising from the capture.

The blood of the fish was obtained by caudal vein puncture with a heparinized syringe. The sample was centrifuged for 5 min at 2000 rpm, the plasma was transferred to cryogenic tubes and frozen in liquid nitrogen. The plasma levels

of glucose, triglycerides, cholesterol, total proteins and albumin were determined by spectrophotometric methods adapted for micro volumes using reagent kits from LabTest Diagnostica S/A. The statistical comparison of the control and experimental groups was done using one way ANOVA followed by a Tukey multiple comparison post tests. The differences were considered significant for $p < 0.05$ (Motulsky, 1995).

Results

The effect of sewage on the levels of glucose, triglycerides and cholesterol are shown in figure 1. The total proteins

Figure 1. Levels of glucose (a), triglycerides (b) and cholesterol (c), proteins (d), albumin (e) and globulins (f) in the plasma of *N. rossii*. Different letters above the bars indicate significant difference ($p < 0.05$) between nature control (NC), experimental control (EC) and sewage groups.

reduction (Figure 1d) observed in the sewage groups was due to reduction in the globulin levels (Figure 1f) and were followed by a rise in the albumin fraction (Figure 1e).

Discussion

The glucose levels in both the sewage groups were less than the experimental control. The difference of the glucose levels in the nature and experimental control indicates that confinement stress results in hyperglycemia (Figure 1a). The difference in glycaemia between the two experimental groups with SPS effluents was not significant. The increase in glucose in vertebrates has been associated with the energy demands of “fight-or-flight” reaction (Pottinger *et al.*, 2000). The reduction in plasma glucose levels of *N. rossii* in experimental conditions with sewage could be due to increased energy demands. The same profile of glucose was observed for triglycerides (Figure 1b). The presence of ample and diffuse adipose tissue, as well as elevated oxidative potential of liver muscle and heart in the Antarctic fish, supports the view of metabolism based on lipids (Crockett & Sidell, 1990; Sidell *et al.*, 1995). The lipid plasmatic transport in the fish is similar to mammals, the very low density lipoprotein (VLDL) being the main transporter of TG, whereas low density lipoprotein (LDL) and high density lipoprotein (HDL) are rich in cholesterol. In fish, the low levels or absence of albumin is compensated by fatty acid transport through HDL (Metcalf *et al.*, 1999; Nanton *et al.*, 2006). The relatively high levels of cholesterol in the plasma of experimental control and sewage, compared to the nature control (Figure 1c), could be related with the rise in the energy demands and the plasmatic transport of fatty acids through HDLs. So, the tendency of cholesterol

levels to rise as a function of sewage concentration could be related with to the possible increase in energy demand. The albumin levels in the vertebrates has been associated with fatty acid, divalent metallic cations transport and blood colloidsmotic pressure (Metcalf *et al.*, 1998). Therefore, the elevation in the albumin levels and reduction in the globulins of *N. rossii* plasma were impacted by experimental confinement and the sewage.

Conclusion

The confinement was capable of inducing rise of glucose, triglycerides (not significant) and cholesterol in plasma of *N. rossii*. In the presence of sewage, the glucose and triglycerides levels suffered reduction, signaling possible rise in energy demand. The elevation of cholesterol levels in the presence of sewage could be related to the lipid relocation with energetic aims. The alterations observed in the presence of sewage were not proportional to the concentration of this effluent. Plasmatic cholesterol and globulins emerge as potential biomarkers of the sewage pollution.

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- A background image of an Antarctic landscape featuring large, white ice floes floating in dark blue water. The ice floes are of various sizes and shapes, some with jagged edges. The water is a deep, dark blue, and the sky is a pale, hazy blue. The overall scene is serene and cold.
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