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BIOMONITORING OF GENOTOXICITY OF SHALLOW WATERS AROUND THE BRAZILIAN ANTARCTIC STATION “COMANDANTE FERRAZ” (EACF), ADMIRALTY BAY, KING GEORGE ISLAND, ANTARCTICA, USING AMPHIPOD CRUSTACEANS

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Abstract: The comet assay was applied to the biomonitoring of genotoxicity in shallow waters around the Brazilian Antarctic Station “Comandante Ferraz” (EACF). Mean values of DNA damage to animals captured from shallow waters nearby the Fuel Tanks (FT) and Sewage Treatment Outflow (STO) were significantly higher than the values of the controls captured from shallow waters of Punta Plaza (PPL) and Yellow Point (YP), naturally undisturbed places far from the EACF.

Keywords: Polar Environment, Marine Pollution, *Gondogeneia antarctica*, DNA Damage.

Introduction

The Antarctic region is one of the most preserved environments in the world, with around 79 near shore research stations. Environmental impacts resulting from human activities in Antarctica such as fishing, tourism and research are almost inevitable. Sewage outflow from those research stations as well as the use of fossil fuel for power supply are responsible for the contamination of coastal shallow waters (Martins *et al.*, 2012). The Brazilian Antarctic Station “Comandante Ferraz” (EACF) is such an example, located at Keller Peninsula on Admiralty Bay, King George Island, South Shetland, whose the adjacent marine environment is inhabited from shallow waters to 500 m deep by different organisms. Antarctic marine ectotherms are animals usually with a short reproductive season, low larval dispersal, low fecundity as well as subjected to strong seasonal factors such as light intensity and food availability (King & Riddle, 2001). The evolutionary history of adaptation to the low temperature in a stable environment makes these animals sensitive to any disturbances on

their natural lives, thus being also suitable bioindicators for environmental quality measurements. *Gondogeneia antarctica*, the species chosen for this study, is one of the amphipod crustacean abundant in intertidal region of the Antarctica coastal waters, with sedentary habits, feeding on the macroalgae and debris from the surf zone (Opalinski & Jazdzewski, 1978; Jazdzewski, 1993; Opalinski & Sicinski, 1995).

Environmental monitoring of Antarctic regions occupied by signatory countries is an objective of the Antarctic Treatise (Santos *et al.*, 2006; Phan *et al.*, 2007; Gomes *et al.*, 2009, 2012). The Brazilian Antarctic Program has studied methods for environmental monitoring that assesses and mitigates impacts caused by the human presence to the environment and to the organisms that inhabit it (Martins *et al.*, 2012). This study is aimed at the biomonitoring of marine shallow water of the Admiralty Bay around the EACF, by investigating the genotoxicity on *G. antarctica* through the comet assay.

Materials and methods

Groups of *Gondogeneia antarctica* amphipods were captured by hand net from shallow waters of the Admiralty Bay, King George Island, in four different locations (Figure 1). Punta Plaza (PPL) and Yellow Point (YP) are more distant from the EACF and established as control places to be compared with the environmental influence of the Fuel Tanks (FT) and Sewage Treatment Outflow (STO) of the Station.

Environmental samplings were carried out weekly during February 2012 and designated as biomonitorings I, II, III and IV. Four species of *G. antarctica* of each place were selected for the DNA damage assessment by employing the alkaline comet assay described by Singh *et al.* (1988) with slight modifications. Briefly, a small drop of hemolymph was individually sampled and added to a mixture of 10 μL of cooled phosphate buffer solution (PBS - pH 7.4) and 90 μL of low melting point agarose (LMP - 37°C) in PBS, spread on the surface of a glass slide previously covered with the normal melting point agarose (NMP - 60°C). The slides were then transferred to the electrophoretic chamber and an electrophoresis was performed at the pH 13 for 20 minutes. After electrophoresis, the slides were

silver stained (García *et al.*, 2004) and cells were analyzed by visually scoring the comets as belonging to one of five classes, according to tail length and intensity. Each comet class is given a value between 0 (undamaged) and 4 (maximum damage) (Figure 2). One hundred comets were blind scored for each animal and the Index of Damage (ID) was calculated (García *et al.*, 2004), ranging from 0 to 400 arbitrary units. Means ($\pm\text{SD}$) of the hemocyte DNA damages were calculated. Homogeneity of variances was checked through the Levene's test and significant differences between groups were determined through the analysis of variance ANOVA, followed by the Newman Keuls test ($\alpha = 0.05$).

Results

The mean DNA Index of Damage (ID) of *G. antarctica* assessed at the four sampling places is presented in the Figure 3. Differences in the ID were not significant between sampling places for the biomonitorings I, II and III. The DNA damages of *G. antarctica* collected from water in front of fuel tanks and at the sewage outflow (FT and STO) increased significantly as compared to those animals from control sites (PPL and YP), in the biomonitoring IV. Neither

Figure 1. Map of the Keller Peninsula, King George Island, Antarctic, showing the sampling places: YP: Yellow Point; FT – Fuel Tanks; STO – Sewage Treatment Outflow; PPL – Punta Plaza.

differences between PPL and YP, nor between FL and STO were significant. By comparing the biomonitorings, significant differences on the hemocytes DNA ID of *G. antarctica* sampled from PPL and YP were noted (Figure 4). The mean ID of the biomonitorings III and IV decreased relative to those from the biomonitoring I and II at the PPL. For the YP site, the highest ID value was on the biomonitoring I.

Discussion and Conclusion

Biomonitoring studies require systems that quantitatively and qualitatively describe the environment. Organisms

that are in direct contact with pollutants may be suitable bioindicators (Rajaguru *et al.*, 2003). DNA damage is a primary concern for the assessment of pollution-related stress in living organisms (Klobučar *et al.*, 2003). Comet assay have been applied to assess the effects of genotoxicity in different forms of marine organisms (Hartl *et al.*, 2007; Taban *et al.*, 2004), including crustaceans (Rocha *et al.*, 2012).

In the present study, the comet assay was successfully applied to the biomonitoring of genotoxicity in shallow waters around the EACF. There was a general tendency

Figure 2. Classification of DNA damage of hemocytes of *Gondogeneia antarctica*: 0 – not damaged; 1 – weakly damaged; 2 – damaged; 3 – very damaged; 4 – severely damaged.

Figure 3. Mean ($\pm$ SD) DNA Index of Damage of *Gondogeneia antarctica* in the four biomonitorings at the different sampling places. Different letters denote significant differences between places.

Figure 4. Mean ($\pm$ SD) DNA Index of Damage of *Gondogeneia antarctica* at the same sampling places at different biomonitorings. Different letters denote significant differences between the biomonitorings.

of increasing in the DNA damages of *G. antarctica* collected from FT and STO sites. However, results were significant only in the biomonitoring IV, comparative to PPL and YP control sites. Biomonitoring IV was carried out at the end of a period when the EACF research station operated with many people, resulting in an increase of contaminants discharge that caused the effect shown on Figure 4. Differences presented on Figure 4 demonstrate the high data variability that masked the significant results on the biomonitorings I to III. In spite of the proximity of the sample sites, the present results are related to the contamination process from distinct sources. Different hydrocarbon compounds have been found on the sea bed as well as in the water samples nearby the fuel tanks, as a result from the direct input by fuel leaking or from the combustion of fossil fuels (Bícego *et al.*, 2009). Highest values of fecal sterols were found nearby the EACF sewage effluent outflow, although lower than those of the 2005-2006 measurements, when the improvements on the sewage treatment system started to operate (Martins *et al.*, 2012).

Results presented so far, emphasizes the importance of biomonitoring the shallow waters in order to assess the

environmental impacts of the human presence on Antarctic ecosystems. The amphipod *G. antarctica* responded satisfactorily as a bioindicator of aquatic pollution, as well as the employment of the comet assay for the assessment of genotoxicity, through the DNA damage on their hemocytes.

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