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STUDY ON THE EFFECTS OF ULTRAVIOLET RADIATION (UV) AND ORGANIC CONTAMINANTS ON ANTARCTIC MARINE ANIMALS FROM SHALLOW WATERS

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Abstract: *The effects of UV and organic pollutants on coastal species of Antarctic marine organisms are being studied using selected methods for animal behaviour, citogenotoxicity and immunohistochemistry. Results obtained up to the present indicate that the methods are suitable and the species chosen is appropriate to indicate alterations related to local contamination. These kind of procedures are promising for future biomonitoring programs.*

Keywords: Antarctic, Environmental Monitoring, UV, Hydrocarbons, Biomarkers, Genotoxicity, Immunohistochemistry.

Introduction

Antarctica is largely considered to be a pristine environment. However, local contaminations by a wide range of pollutants including hydrocarbons (Cripps & Priddle, 1991), persistent organic pollutants (Fuoco *et al.*, 1996; Weber & Goerke, 2003) and those of sewage effluents (Hughes, 2004; Hughes & Thompson 2004) have been recorded in shallow marine habitats in the vicinities of populated areas such as scientific stations.

The research REDE-2, already finished, and now the INCT-APA (the Brazilian National Institute of Science and Technology – Antarctic Environmental Research) are integrated cooperative programs that are developing comprehensive studies on the state of the environment in Admiralty Bay, under the influence of the Brazilian Station "Comandante Ferraz" (EACF, Portuguese acronym). Results of good quality in a diversity of aspects will serve as a baseline for future monitoring activities.

The present research aims to develop different methods to investigate the effects of pollutants and water contaminated by sewage and petroleum hydrocarbons on fish and amphipods of shallow waters around the EACF, as well as the interactions of these factors with ultraviolet radiation (UV). The genotoxic effects of these parameters are being assessed by the micronucleus (Mn), erythrocyte nuclear abnormalities (ENAs) and the comet assays, which have been successful to investigate damage to fish and amphipods DNA. Animal behavior and imunohistochemistry analysis are also being carried out in order to identify alterations due to pollutants and UV.

The MN assay is a quite simple method to detect genotoxic effects of chemical compounds. They are cytoplasmic chromatin masses similar to a small nucleus, originated from fragments or whole chromosomes that were left behind during cell division. The frequency of micronucleated cells has been employed as a suitable index of chromosome damage for more than 20 years (Ayllon & Garcia-Vazquez, 2000). Nowadays, different studies have also described the presence of other ENAs on cells of fish exposed to genotoxic compounds that can complement the MN assay (Pacheco & Santos, 2002).

The single cell gel electrophoresis or comet assay is also a highly suitable method for evaluating the effects of contamination of aquatic environments on the DNA of organisms (Frenzilli *et al.*, 2009). In this assay, after the

DNA is unwound in a highly alkaline solution, subsequent electrophoresis causes the relaxed and broken fragments of negatively charged DNA to move towards the anode, away from the nucleoids. After staining, the size and intensity of the comet head and tail represent the degree of DNA damage in individual cells.

Recently, immunohistochemistry techniques were included in our work to identify protein activation related to temperature increase, as well as to the metabolic effects of pollutants and UV. Heat shock protein (HSP-70), tumor protein (p-53) and vascular endothelial growth factor (VEGF) are the main focus of these studies.

Materials and Methods

Individuals of the amphipod species *Gondogeneia antarctica* and fish *Trematomus newnesi* were collected in shallow water using a hand sieve or a small otter trawl net. Animals were kept for 5 days in aquaria inside a cold chamber at 0º C and 35 psu to diminish stress due to handling.

Thereafter, the amphipods were transferred to a circular 4L aquarium and exposed to natural light or to different UV intensities, combined with the presence or absence of anthracene used as a standard PAH. The animal behavior was assessed during 5 hours per day through recordings for 3 days, with resting periods in the dark of 19 hours. The aquarium was covered by a metal frame to support blue UV filter sections and/or transparent UV filter sections, and also sections free of filters, so that the animals could swim freely under those areas. Choice of a certain area can indicate avoidance or not to radiation enhanced or not by pollutants. At the end of exposition, the hemolymph was sampled to evaluate genotoxicity through the comet assay.

Amphipods from control places far away from the EACF influence and places in front of the fuel tanks and sewage outlet had the hemolymph sampled for comet assay. Samples of whole animals from the same locations were taken and prepared for immunohistochemistry assays.

Experiments were also made with fishes that were placed inside cages and submerged for 12 days at 1m depth in shallow water in front of the fuel tanks, near sewage outlet and at control places far away from EACF. After exposure, fish blood samples were taken and used for comet assay and MN and ENA assays.

Comet assays were performed as described by Singh *et al.* (1988), with modifications, and DNA damage was established by calculating the Index of Damage (ID) of 100 scored comets per animal (García *et al.*, 2004). MN and ENA were quantified by assessing 2000 erythrocytes per individual (Phan *et al.*, 2007). The ENAs were classified as kidney-shaped (K), lobed (L) and segmented (S) (Ayllon & Garcia-Vazques, 2000).

Preliminary immunohistochemical studies are being carried out in order to identify and quantify the expression of HSP-70, p-53 and VEGF proteins, using appropriated methodologies.

Means (SD±) calculated from ID, MN and ENAs data were tested for normality and homogeneity of variances, and submitted to Kruskal-Wallis ANOVA, followed by Mann-Whitney U test and Newman Keuls multiple comparison non-parametric tests. Differences of $p < 0.05$ were considered as significant.

Results

Phase I experiment results showed that the UV and the anthracene affects the survival and behavior of Antarctic amphipods. In normal conditions, amphipods always prefer blue filter shadowy places. Animals subjected to UV and anthracene showed an altered behavior on the first day, probably due to stress. The surviving stronger animals reestablished normal behavior by the second day. On the third day the animals lost their mobility. The avoidance behavior to UV may exist, although it is still to be confirmed by the coming results of experiments without blue filters.

Data of comet, MN and ENAs assays of amphipods *G. antarctica* obtained at the laboratory and from the biomonitoring natural areas are being analysed. Preliminary results of the comet assay showed significant high ID on the DNA of *G. antarctica* sampled from places close to the fuel tanks and sewage outlet, in relation to control places (Figure 1). The ID was higher in animals from the sewage outlet in comparison to those from fuel tanks areas.

DNA damage was significantly higher in fish exposed to the waters in front of the fuel tanks and near the sewage outlet when compared to those of fish exposed to control areas. Differences in DNA damage between fish maintained at the sewage outlet and those in the water in front of the fuel tanks were also significant in both experiments performed (Figure 2).

Slides with tissue of amphipods for immunohistochemical studies are being prepared. At the moment, preliminary

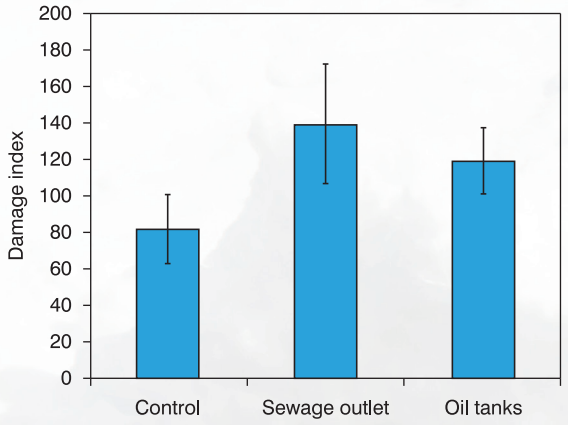

Figure 1. DNA damage index of amphipods *G. antarctica* captured from shallow water around the EACF.

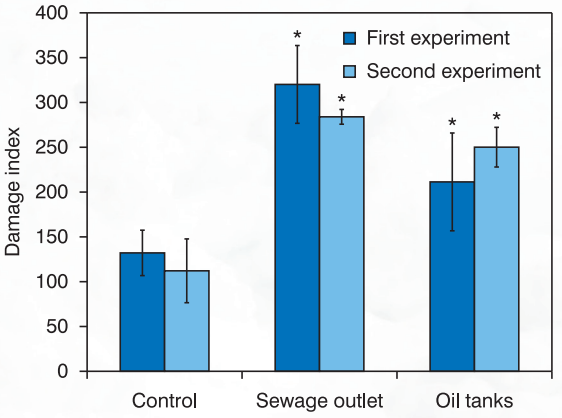

Figure 2. DNA damage index of *T. newnesi* exposed to water at different sites. Data are showed as means ± standard deviations (n=5). * Significantly different to control.

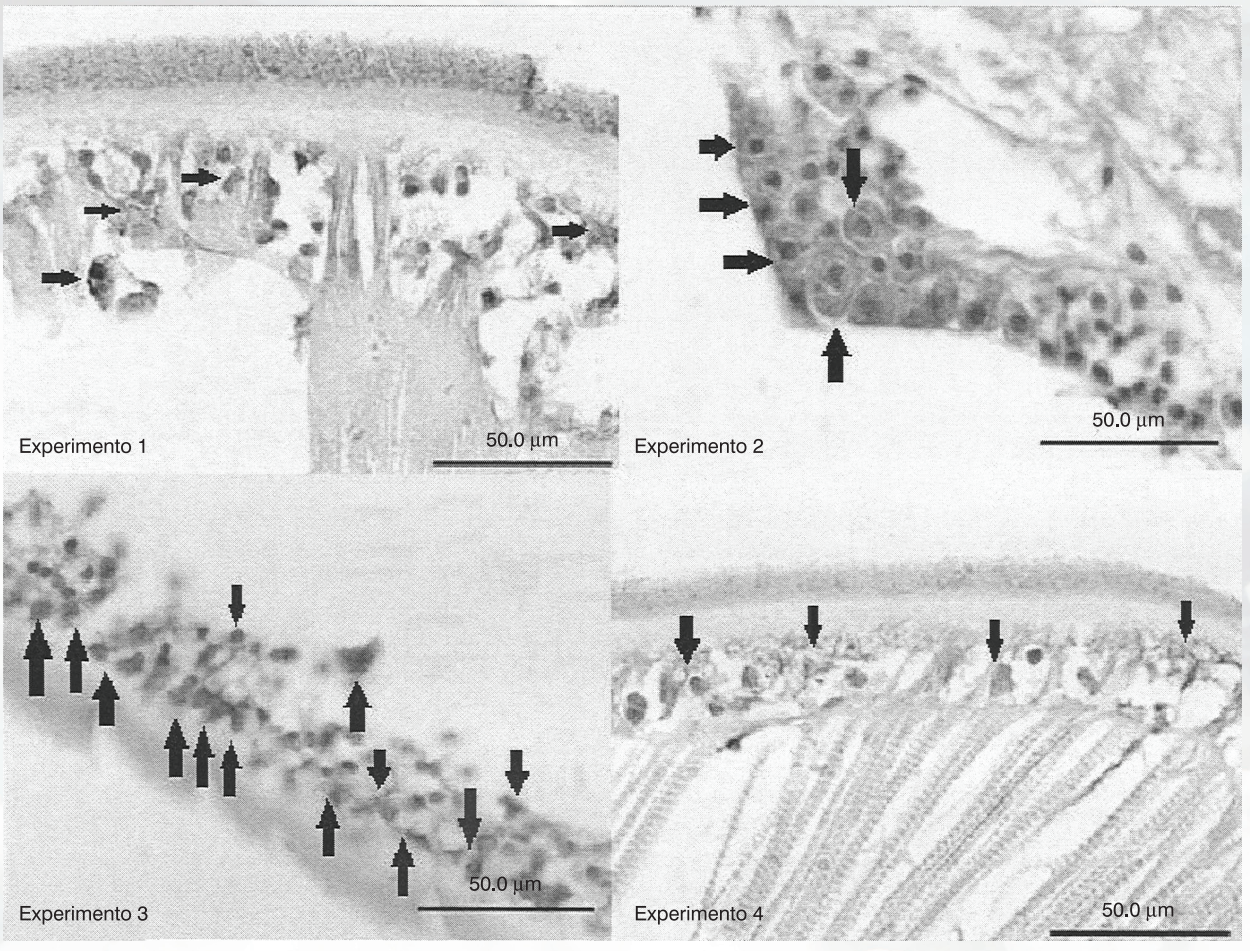

Figure 3. Immunohistochemical assay for the p-53 protein of *G. antarctica*. Arrows indicate the protein expression. Experiment 1 (light control): animals under natural light; Experiment 2: animals exposed to anthracene; Experiment 3: animals exposed to anthracene plus UV; Experiment 4 (dark control): animals kept in dark.

results were obtained for p-53 protein of animals exposed in laboratory to UV and to anthracene (Figure 3).

Discussion and Conclusion

In spite of being scarce, the importance of behavior studies rely on the fact that they can detect early signals of sublethal effects of contaminants and other environmental factors (Little *et al*., 1982), such as UV and HPAs. At this moment, behavior data of (phase I) were analyzed. They were obtained in experiments with amphipods exposed to PAR and UV radiations, in the presence or absence of the anthracene, under frames composed of transparent, blue radiation filters and without radiation filter. Other experiments are being conducted in order to assess the animal behavior under UV and natural light only (phase II), without the synergism with the PAHs, and without the presence of blue filters. In spite of being preliminary, immunohistochemical data on p-53 protein can be visualized in preparations and its expression was higher in animals subjected to the UV combined with anthracene. Data of other experiments and those sampled from the environment are being processed.

Results obtained so far demonstrate that both the animals and methods employed are suitable for the assessment of the environmental quality, being capable of detecting the contamination effects of sewage discharge as well as those of petroleum origin. Nevertheless, many data have been obtained and are to be processed by trained researchers. At the moment, there is a lack of qualified researchers sponsored by the INCT-APA that could help our laboratory in this task.

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