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DNA and Lipid Damage in the Brown Mussel *Perna perna* from a Contaminated Site

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Much work has focused on DNA and lipid damage resulting from interactions among reactive oxygen and nitrogen species (ROS/RNS) and associated oxidants produced during biotransformation of certain xenobiotics (Livingstone 2001). The presence of DNA strand breaks is generally determined by gel separation after denaturation or unwinding under alkaline conditions, or by the Comet assay, which measures the electrophoretic migration of relaxed or fragmented DNA away from the nuclei of cells immobilized in agarose (Steinert 1999). More recently, the evaluation of modified DNA bases such as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) has proved to be a good indicator of oxidative stress caused by xenobiotic exposure in marine organisms (Mallins and Haimanot 1990; Canova et al. 1998; Rodríguez-Ariza et al. 1999; Livingstone 2001). Moreover, membrane lipid oxidation induced by ROS and RNS, leads to the formation of fatty acid hydroperoxides and other reactive products, including a wide range of aldehydic compounds, which can be measured in invertebrates as indicators of pollutant injury (Pellerin-Massicote 1994). In this work, we compared the levels of 8-oxodGuo and lipid peroxidation product levels in mussels *Perna perna* transplanted from a reference to a contaminated site on the Santa Catarina Island.

The reference site, located at the mussel farming area of the Federal University of Santa Catarina, on Sambaqui beach, was selected based on a previous report about the quality of seawater for mollusk farming in this region (Cerutti 1996). Mussels from this site were placed on nylon nets and transferred to the contaminated site (at North Bay, Figure 1). Some studies have indicated that North Bay is critically affected by pollution since this area is the main target for the urban wastewater discharges of Florianópolis city (Cerutti 1996, Benato 1999). Higher levels of nickel, copper, cadmium, and manganese were observed in the seawater collected at this site when compared to the reference site, respectively at 3, 6.53, 2.5 and 0.33-fold (Pozebon 1998). In addition, a previous study suggested that the urban wastewater discharges associated with the elevated rainfall index in this area caused changes in biochemical defense systems in transplanted mussels (Bainy et al. 2000).

MATERIALS AND METHODS

Mussels Perna perna of similar length (3 to 4 cm) were collected and transplanted from a clean (reference) to a contaminated site at Santa Catarina Island (Florianópolis, SC, Brazil, figure 1). After 12 months of exposure, mussels were collected in both reference

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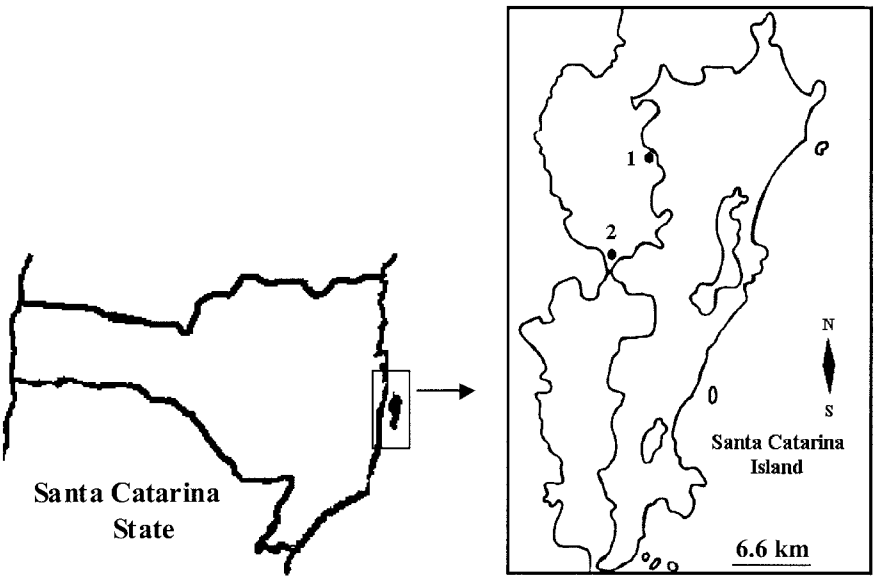

Figure 1. Map of the Santa Catarina State and the Florianópolis city (Santa Catarina Island), indicating the reference site (1, Sambaqui beach) and the contaminated site (2, North Bay).

and contaminated sites and their digestive glands, gills and mantle tissues were dissected and immediately immersed in liquid nitrogen (-196°C). Mussels from both sites were collected on the same day, respectively at 9:00 and 10:30 A.M. The water temperature was 18 and 19.5 °C and the pH was 8.0 and 7.8 in the contaminated and reference sites, respectively. According to data provided by the *Fundação do Meio Ambiente* (FATMA), at the day of collection, the levels of faecal coliforms were 11000 and 230 coliforms/100mL of water (median of 5 different collected aliquots) in contaminated and reference sites, respectively. Levels higher than 1000 coliforms/100mL of seawater indicate that the area is unsuitable for bathing.

DNA was isolated using the chaotropic NaI method (Wang et al. 1994; Helbock et al. 1998) with some modifications. Briefly, 500 mg of tissues were homogenized in 10 mL of buffer A (320 mM sucrose, 5 mM $MgCl_2$, 10 mM Tris HCl, 0.1 mM desferoxamine and 1% Triton X 100, pH 7.5). After centrifugation at 1500 g for 10 min, the pellets were suspended in 5 mL of 10 mM Tris-HCl buffer, pH 8.0, containing 5 mM EDTA, 0.15 mM desferoxamine and 10% SDS. The RNAse A (150 µL, 1mg/mL) and RNAse T1 (40 µL, 1000 U/mL) in 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA and 2.5 mM desferoxamine were added and the reaction mixture was incubated at 37 °C. After 1 hour, 150 µL proteinase K (20 mg/mL) was added followed by additional incubation at 37°C for 1 h. After centrifugation at 5000 g for 15 min, the liquid phase was collected and 3 mL of 7.6 M NaI was added, followed by the addition of 3 mL of isopropanol. The content in the tube was well mixed by inversion until a whitish precipitate appeared. The precipitate was collected by centrifugation at 5000 g for 15 min and washed with 3 mL of isopropanol 40% (w/v), followed by 3mL of ethanol 70% (w/v). After additional centrifugation at 5000 g for 15 min, the DNA pellet was

solubilized in 300 μL of desferoxamine (0.1 mM). The DNA concentration was measured spectrophotometrically at 260 nm and its purity was assessed by ensuring that $A_{260}/A_{280}>1.75$. DNA (100 μg) was diluted in 200 μL of deionized water, followed by the addition of 4 μL of 1 M sodium acetate buffer (pH 5.5) plus 5 units of nuclease P_1 and incubated at 37°C for 30 min. A total of 20 μl of 1 M Tris-HCl buffer (pH 7.4) and 2 units of calf intestinal alkaline phosphatase were then added, followed by 1 h incubation at 37°C according to Fiala et al. (1999). Chloroform was then added (1:1 vol), the samples were centrifuged and the aqueous layer collected for the analysis.

The level of 8-oxodGuo was measured by High Performance Liquid Chromatography coupled to electrochemical detection (HPLC/ECD) using a Shimadzu model LC-10AD/VP pump with a Phenomenex Spherex 5 C18 column (250 X 4.6 mm i.d., 5 μm particle size), a Shimadzu SPD-10AV/VP UV detector at 254 nm and an electrochemical coulometric detector (ESA *coulochem II 5021* Massachussetts, USA) with potentials set at 120 and 280 mV in electrodes 1 and 2, respectively (Laws and Adams 1996; Rodriguez-Ariza et al. 1999; Beckman et al. 2000). Shimadzu Class-LC10 1.6 software was used to calculate the peak areas. The mobile phase consisted of potassium phosphate buffer (0.05 M, pH 5.5) containing 10% methanol pumped at a flow rate of 1 mL/min. The level of dGuo in the hydrolysate was simultaneously quantified using an UV detector set at 254 nm.

Lipid peroxidation products were evaluated by the thiobarbituric acid reactive substances (TBARS) method, as described by Ghatak and Ho (1996). Tissues were homogenized in 0.25M sucrose solution (tissue to sucrose solution ratio = 1:50). 500 μL of whole tissue homogenate were mixed with 100 μL of 10% SDS, 1.5 mL of 200 mM sodium acetate (pH 3.75), and 1.5 mL of 1% aqueous solution of thiobarbituric acid. The reaction mixture was brought up to 4 mL with MilliQ water, and heated in a boiling water bath for 60 min at 100°C. The colored derivative was extracted with 1 mL *n*-butanol and quantified at 532 nm, in terms of a malonaldehyde standard.

Statistical analyses were performed with the aid of the Micrococal Origin 6.0 software (Northampton, MA, USA). Results are presented as mean $\pm$ standard deviation. Significant differences between different groups were studied using *t*-test and one-way analysis of variance, and only $p < 0.05$ was accepted as significant.

RESULTS AND DISCUSSION

Table 1 shows the level of 8-oxodGuo in digestive gland, gill and mantle of mussels from contaminated and reference sites. With regard to basal levels, 8-oxodGuo was higher in mantle tissue and digestive gland than in gills, probably due to composition and functional differences between the three tissues (i.e., antioxidant and lipid content, metabolic rate and DNA repair activity). Moreover, it has been shown that the digestive gland is the main target tissue for biotransformation in mussels (Livingstone and Pipe 1992), evidenced by higher activities of Phase I enzymes than other tissues. Interestingly, the mussel *P. perna* has about 10-fold lower levels of 8-oxodGuo than *Mytilus galloprovincialis*, as reported by Canova et al. (1998) and Akcha et al. (2000). On the other hand, mussel *P. perna* showed similar levels of 8-oxodGuo than levels observed in the digestive gland of the mangrove mussel *Mytella guyanensis* (Torres et al. 2002).

Table 1. Levels of 8-oxodGuo in digestive glands, gills and mantle tissues of mussels collected in the reference and polluted sites. Data are expressed as residues of 8-oxodGuo/10^6 dGuo.

Tissue	Reference	Contaminated
Digestive glands	12.8 ± 5.4 (8)	* 24.2 ± 4.8 (10)
Gills	5.8 ± 2.2 (10)	* 9.4 ± 3.0 (9)
Mantle tissue	13.6 ± 3.9 (8)	18.1 ± 6.6 (8)

* Statistical differences ($p<0.05$). Numbers in parenthesis indicates the number of samples analyzed.

After 12 months of exposure, mussels from the reference site showed 12.8 ± 5.4 residues of 8-oxodGuo/10^6 dGuo in digestive gland and 5.8 ± 2.2 residues of 8-oxodGuo/10^6 dGuo in gills. In contrast, mussels from the contaminated site showed 24.2 ± 4.8 residues of 8-oxodGuo/10^6 dGuo in digestive glands and 9.4 ± 3.0 residues of 8-oxodGuo/10^6 dGuo in gills, representing levels of 8-oxodGuo 1.9-fold and 1.6-fold higher than control site, respectively. No differences were observed in the levels of 8-oxodGuo of mantle tissue between the groups. Based on these results we suggest that there is an association between contaminant exposure and increased oxidative DNA damage. Accordingly, Canova *et al.* (1998) observed higher levels of 8-oxodGuo in gills and digestive glands of mussels exposed to benzo[*a*]pyrene. Torres et al. (2002) observed higher levels of 8-oxodGuo in digestive glands of mussels *M. guyanensis* collected at a polluted mangrove. Mallins and Haimanot (1990) detected a significant increase in the levels of 8-oxodGuo in fishes collected at a contaminated area. In addition, Rodríguez-Ariza et al. (1999) observed elevated levels of 8-oxodGuo in the fish *Sparus aurata* exposed to dieldrin, paraquat and copper or when sampled at contaminated areas. Contrariwise, Akcha et al. (2000) observed no differences in the levels 8-oxodGuo in gills of *M. galloprovincialis*.

Table 2 shows the levels of lipid peroxidation in digestive glands, gills and mantle of mussels from the reference and contaminated sites. No statistical differences were observed in the levels of lipid peroxidation between digestive gland and gills of the mussels, respectively from contaminated and reference sites. However, mussels from the contaminated site showed higher levels of lipid peroxidation in mantle tissue (15.5 ± 5.9 nmol of TBARs/mg tissue) than in mussels from the reference site (4.5 ± 1.9 nmol of TBARs/mg tissue). It has been demonstrated that digestive gland and mantle tissues possess higher levels of polyunsaturated fatty acids (PUFA) than gills, and that the digestive gland contains higher amounts of lipophilic antioxidants than mantle tissue (Ribera et al. 1991). This hypothesis could explain the fact that only mantle tissues from the mussels kept at the contaminated site showed elevated levels of lipid oxidation products. The high antioxidant levels in the digestive gland could account for the lack of significant differences in the lipid peroxidation levels observed in this tissue.

Data presented here indicate that DNA and lipid damage levels in mussels *Perna perna* are affected after 12 months exposure to urban contamination. As the collections were carried out in the summer, which corresponds to the major tourist activity in Florianópolis city, augmented drainage of the urban wastewater to the contaminated site could account for some of the observed differences. A seasonal study needs to be done

Table 2. Levels of TBARS in digestive glands, gills and mantle tissues of mussels from the polluted and reference sites. Values are expressed in nmoles of TBARS/g of tissue.

Tissue	Reference	Contaminated
Digestive glands	6.5 ± 1.8 (9)	4.6 ± 2.9 (10)
Gills	4.3 ± 2.3 (10)	3.2 ± 0.9 (10)
Mantle tissues	4.5 ± 1.9 (10)	* 15.5 ± 5.9 (10)

* Statistical differences ($p<0.05$). Numbers in parenthesis indicates the number of samples analyzed.

to clarify whether these changes occurs throughout the year.

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