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Biochemical biomarkers and metals in *Perna perna* mussels from mariculture zones of Santa Catarina, Brazil

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ABSTRACT

The activity of cholinesterase (ChE), glutathione-*S* transferase (GST), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH) and catalase (CAT) was evaluated in the gill and digestive glands of the *Perna perna* mussel transplanted to three non-contaminated mariculture zones under the influence of distinct physical–chemical characteristics. Differences among sites for ChE, GST and CAT activities in gill, as well as ChE, GST and G6PDH activity in digestive gland of mussels, were found and possibly related to differences in physicochemical characteristics of the sites and/or biological status of the mussels. Mussels that were transplanted to another, more urbanized site (Ponta do Lessa) with similar physicochemical characteristics to one of the farming sites (Sambaqui), was also chosen to evaluate biomarker responses to pollution. Activities of ChE, GST and GR in the digestive glands and CAT in the gills were higher in the polluted site. GR was the only biomarker to be unaltered in different farming sites, but induced in the pollution site. The trace metal concentrations in the mussels were low and unlikely to cause the changes observed in the biomarker levels. The present study strongly suggests that monitoring programs should compare sites with similar physicochemical characteristics when using a complementary biomarker approach. In addition, the baselines for the biomarkers and metal used in the present study can serve as a reference for the monitoring of these mariculture zones in future monitoring programs employing *P. perna*.

1. Introduction

Coastal zones of the Santa Catarina State in Southern Brazil have shown a significant increase in population density in the last decade, with a concomitant increase in sewage production affecting the environment quality (MMARH, 1996). An important economic activity in this region is mollusk farming, mainly of the brown mussel, *Perna perna*, and the pacific oyster, *Crassostrea gigas* (Poli and Littlepage, 1998). Santa Catarina produces more cultured mollusks than any other region in Brazil; therefore, the monitoring of the marine environmental quality is of primordial importance for establishing the most adequate areas for bivalve farming.

Mussels are filter-feeding bio-accumulators that have been used as sentinel organisms in numerous monitoring programs (NOAA, 1995). The analyses of biomarkers in these bivalves have been also incorporated into biomonitoring studies to evaluate the effects of pollutants (Viarengo et al., 2000) in areas contaminated with heavy metals (Najimi et al., 1997; Regoli and Principato, 1995), domestic sewage (Radenac et al., 1998), pesticide residues (Narbonne et al., 1991; Mora et al., 1999) and organic compounds (Akcha et al., 2000).

Cholinesterase (ChE) inhibition in sentinel organisms has been widely used as a marker of exposure to organophosphate and carbamate pesticides in biomonitoring programs (Mora et al., 1999; Walker et al., 1996; Monserrat and Bianchini, 1998) and, altered ChE activity have been also attributed to other classes of contaminants (Payne et al., 1996). Antioxidant enzymes, which help to protect cells against oxyradical damage resulting from exposure to certain pollutants, were also involved (Sies, 1991; Rodríguez-Ariza et al., 1992, 1993; Pedrajas et al., 1993;

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López-Barea, 1995; Bainy et al., 1996). Some transition metals and numerous aromatic compounds can generate reactive oxygen species (ROS) through redox cycling mechanisms (Garcia-Alfonso et al., 1995, 1996). Poorly coupled metabolism of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) can also result in ROS (Bainy et al., 1996). Glutathione-*S* transferase (GST) conjugates different electrophilic compounds with tripeptide glutathione during phase II of biotransformation reactions, enhancing the polarity of these compounds in order to enable their excretion, and have frequently been used as an indicator of increased phase II reactions in contaminant-exposed animals (Dauterman, 1994; Martínez-Lara et al., 1996; Lenartova et al., 1996; García-Alfonso et al., 1998). The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), along with ancillary enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and glutathione reductase (GR) and conjugating enzymes, help to protect organisms from oxidative stress induced by exposure to contaminants in the environment (Akcha et al., 2000; Walker et al., 1996; Sies, 1991; Dauterman, 1994).

Despite the enormous array of data available on the levels of contaminants in mollusks around the world, there is little information about contaminants and/or biochemical responses in bivalves on the Brazilian coast. We previously found changes in GST activity in the digestive gland of *P. perna* transplanted to a site contaminated by domestic sewage and associated with a heavy rainfall period (Bainy et al., 2000). After one year of exposure, the levels of DNA damage increased as well (Almeida et al., 2003).

In this study, we evaluated the enzymatic biomarkers ChE, GST, GR, G6PDH and CAT in two important detoxification organs (the gill and the digestive gland) of *P. perna mussels* transplanted to three farming sites with different physicochemical characteristics. Mussels were also transplanted to a fourth site located in the same section of the bay as one of these mariculture sites, with similar physicochemical characteristics, but contaminated by urban sewage discharge. The first goal of this study was to evaluate which biomarker/organ combination is not erratic in mariculture sites, but instead altered by pollution. The second goal of this study was to show the levels of the metals As, Pb, Cr, Cd, Sn, V, Cu, Hg, Se, Hg, Ni, Zn and Ag in water and mussels kept in these four sites (Curtius et al., 2003). The results generated here can serve as reference for future monitoring studies in this mariculture/urban zone located along the central shore of Santa Catarina, as well as for biomarker studies in other regions of the world.

2. Materials and methods

2.1. Experimental design and sampling

The brown mussels, *P. perna* (30–40 mm), were collected at the Sambaqui Beach mariculture site (SAM), located in the North Bay of Florianópolis, SC, Brazil (27°28′30″S; 48°33′40″W; Fig. 1), close to the Mollusk Marine Laboratory (LMM, CCA, Federal University of Santa Catarina, UFSC). One group of mussels was kept at this farming site in a long line system, while other groups were transplanted to two other economically important farming zones of Santa Catarina State in the South Bay of Florianópolis (Ribeirão da Ilha Beach—RIB), approximately 15 miles away from SAM, and outside the Florianopolis Bays (Pinheira Beach—PIN), approximately 30 miles away from SAM.

A fourth group was transplanted to Ponta do Lessa (PL), a contaminated site which constantly receives untreated domestic sewage discharge from the city of Florianópolis (SC, Brazil). This area is frequently monitored by the *Fundação do Meio Ambiente* (FATMA: The Environmental Foundation), and the contamination is confirmed by much higher fecal coliform levels in the seawater compared to mariculture sites in Santa Catarina (Almeida et al., 2003). This contaminated site possesses physicochemical characteristics similar to SAM, since is located in the same region in the North Bay of Florianópolis, approximately 6 miles away from SAM. Based on this information, PL was considered a contaminated site, and SAM was considered a reference site for biomarker comparison using mussels.

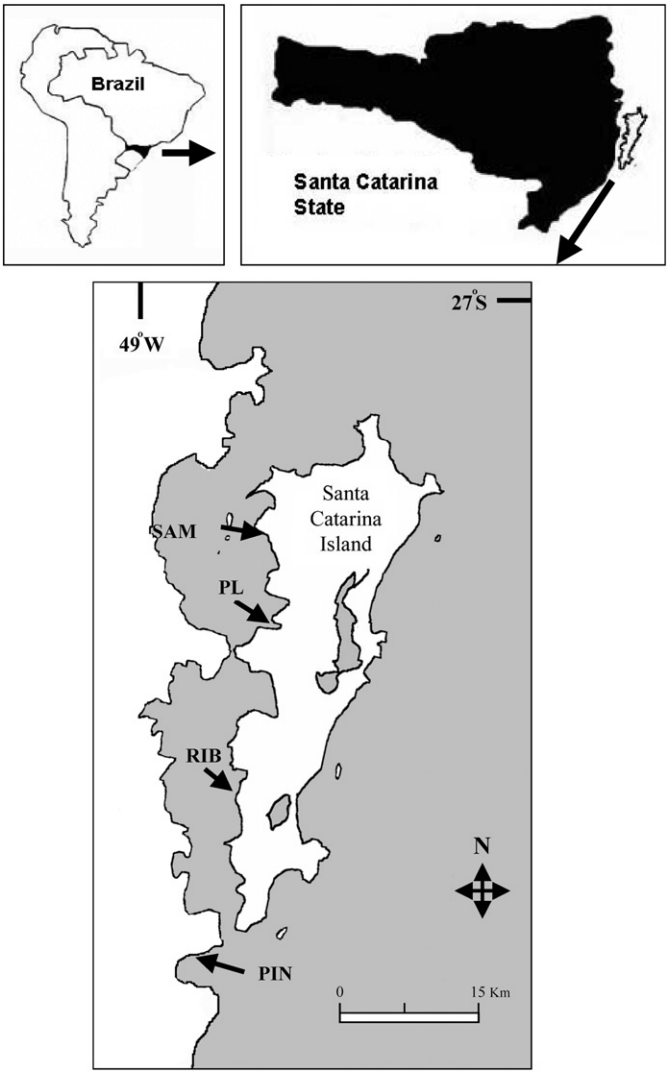

Fig. 1. Map of Santa Catarina Island showing the farming sites Pinheira Beach (PIN), Ribeirão da Ilha Beach (RIB) and Sambaqui Beach (SAM), and the contaminated site, Ponta do Lessa (PL).

After six months in the four sites (November/1999 to April/2000), twenty mussels from each site were collected (size 70–120 mm) in order to measure the biochemical parameters, and thirteen mussels were collected in order to analyze the levels of trace elements. Temperature, pH, turbidity and chlorophyll-*a* levels in the water were determined at the three farming sites (but not in PL) using standard methods (Strickland and Parsons, 1972; Lorenzen, 1967; Littlepage, 1998).

2.2. Biological parameters of mussels

After collection, shell length, soft tissue weight and gonad maturation stages (GMS) were recorded for each mussel. Mussels of both sexes were sampled in similar proportion for each site (50% male and 50% female). GMS was identified macroscopically following Lunetta (1969), and were found to be at IIIA for the full ripe or spawning gonads, IIIB for completely regressed gonads and IIIC for partially ripe gonads at the early gametogenic stage. The gill and digestive glands were excised from the mussels, immediately frozen in liquid nitrogen, and stored at −85 °C for subsequent biochemical analysis.

2.3. Homogenization and centrifugation of the samples

Mussel organs were homogenized (1:4 w/v) in a 20 mM Tris–HCl buffer containing 1 mM EDTA, 0.5 M sucrose, 1 mM DTT, 0.1 M PMSF, 0.15 M KCl pH 7.6, and using a Tissue Tearor (Biospec Prod. Inc.). The homogenate was divided in two parts: 1 ml was centrifuged at 9000*g* for 30 min at 4 °C before measuring the ChE activity in the supernatant (Najimi et al., 1997). The remaining homogenate was