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Biochemical responses in farmed mussel *Perna perna* transplanted to contaminated sites on Santa Catarina Island, SC, Brazil

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Abstract

The effects of contaminants on the biochemical parameters of the intensively farmed mussel *Perna perna*, are unknown. The aim of this study was to compare biochemical responses in mussels held in clean and contaminated sites in Santa Catarina Island, Brazil. Mussels were transplanted from a farming area, Ratones Grande Island (RGI), to two contaminated sites, Itacorubi (ITAC) and Hercílio Luz Bridge (HLB). A reference group was kept at RGI. After 150 and 180 days of exposure, the digestive glands of the mussels were analyzed for catalase, glucose 6-phosphate dehydrogenase (G6PDH) and glutathione *S*-transferase (GST) activities. No changes were observed in the catalase activity, in both periods. Low G6PDH activity was observed in mussels transplanted for 150 days at the ITAC site. Increased GST activity was observed in mussels from ITAC and HLG sites after 180 days. These responses are probably related to the augmented discharges of domestic effluents associated with elevated rainfall index.

Keywords: Antioxidant enzymes; Aquaculture; Biomarker; Effects — biochemical; Enzymes; Florianópolis; Monitoring; Mussel; *Perna perna*; Transplant

1. Introduction

Mussel farming in Santa Catarina state (SC) in southern Brazil has increased significantly in the last 5 years with more than 7500 t of the marine mussel *Perna perna*

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produced in 1997 (Costa, Grumman, Neto & Rockanski, 1998). Effluent discharges in coastal zones may affect the health and marketability of farmed mussels; however, information on the levels of contaminants in the farming areas and related biological responses in the mussel *P. perna* is lacking. In this study, we evaluated the use of biomarkers for determining the effect of contaminant exposure on mussels.

Catalase is an antioxidant which decomposes hydrogen peroxide generated within cells. Glucose 6-phosphate dehydrogenase (G6PDH) is the key regulatory enzyme of the pentose-phosphate shunt and is necessary for the regeneration of reducing power nicotinamide adenine dinucleotide phosphate reduced (NADPH). The role of glutathione *S*-transferase (GST), a Phase II enzyme, is to conjugate tripeptide glutathione with electrophilic and other xenobiotics.

The suitability of antioxidant and Phase II enzyme activities as biomarkers of pollutants in mussels is uncertain (Fitzpatrick, O'halloran, Sheehan & Walsh, 1997; Regoli, Hummel, Amiard-Triquet, Larroux & Sukhotin, 1998). The goal of this study was to investigate the activities of catalase, G6PDH and GST in digestive glands of *P. perna* transplanted from farming areas to contaminated sites.

Sixty mussels (4–5 cm length) were collected from Ratones Grande Island (RGI), the reference site, in November 1997 (spring). Two samples of 20 mussels were transplanted and held for 150 and 180 days, respectively, at the contaminated sites, Itacorubi (ITAC) and Hercílio Luz Bridge (HLB; Fig. 1). A reference group of 20 mussels was kept at RGI. Water temperature, salinity and pH was recorded monthly at the three sites and the rainfall index was obtained at the Centro Integrado de Meteorologia e Recursos Hídricos de Santa Catarina (Climerh-Epagri). After 150 and 180 days of exposure the digestive glands of five to 10 mussels collected, respectively, from each site were excised and processed as described by Livingstone (1988). The activities of catalase (Beutler, 1975), G6PDH (Glock & McLean, 1953) and GST (Keen, Habig & Jakoby, 1976) were measured in the cytosolic fraction (supernatant) of the digestive gland obtained after a differential centrifugation at 10,000 *g* for 20 min and centrifugation of the supernatant at 38,000 *g* for 70 min. The data were compared by one-way analysis of variance (ANOVA) test followed by Duncan's test for multiple comparisons with unequal numbers of samples for 150 and 180 days of exposure at the three sites: $P < 0.05$ was accepted as significant. Results are presented as mean $\pm$ S.D.

Water temperature at the three sites decreased from 30 to 21°C concurrently during the experimental period. The pH and salinity remained nearly constant at 8.23 ± 0.07 and 32.3 ± 1.2‰, respectively.

The activity of catalase, G6PDH and GST in the digestive gland of the mussels collected at the three sites at 150 and 180 days is shown in Fig. 2. No significant changes were observed in catalase activity of digestive gland in mussels at any site at 150 or 180 days of exposure (Fig. 2). However, a slight increase in the catalase activity in all groups was observed at 180 days of exposure (autumn). This result may be due to seasonal variations in catalase activity probably related to changes in metabolic pattern, food availability and/or reproductive status, since *P. perna* has a reproductive peak on the Brazilian coast in autumn (Ferreira & Magalhães, 1997).

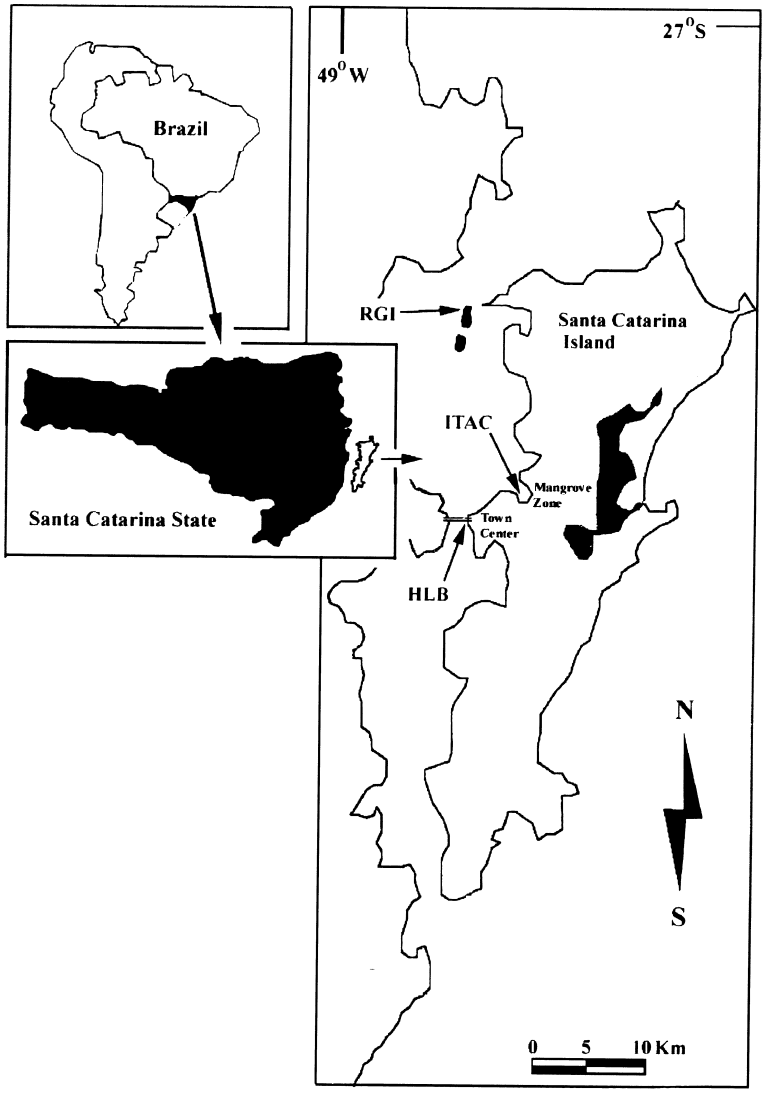

Fig. 1. Map of Brazil showing Santa Catarina State and the positions of the reference and transplanting sites on Santa Catarina Island. RGI, Ratones Grande Island (Reference); ITAC, Itacorubi; HLB, Hercílio Luz Bridge.

A significantly lower G6PDH activity was observed in mussels kept for 150 days at ITAC (Fig. 2). A similar, but not significant trend, was observed in mussels kept for 180 days at this site. Low G6PDH activity may compromise cellular NADPH production, required for maintaining the antioxidant power, biotransformational reactions and lipid biosynthesis pathway. Bainy, Saito, Carvalho and Junqueira (1996) observed reduced G6PDH activity in gill, liver and kidney of tilapia from a

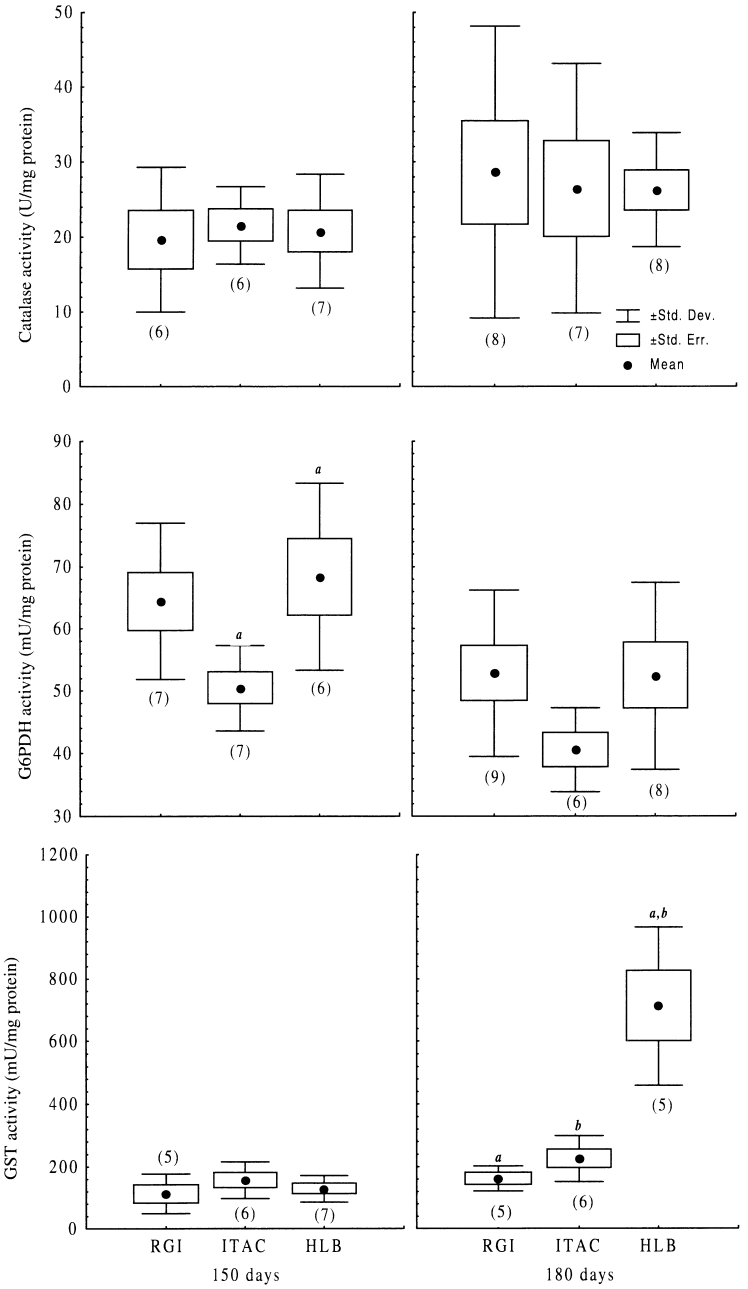

Fig. 2. Analysis of catalase, glucose-6-phosphate dehydrogenase (G6PDH) and glutathione *S*-transferase (GST) in digestive gland of *Perna perna* kept at Ratones Grande Island (RGI), Itacorubi (ITAC) and Hercílio Luz Bridge (HLB) sites for 150 and 180 days. Number of samples are indicated in parentheses. Same letters indicate significant differences for $P < 0.05$, by one-way analysis of variance (ANOVA) test, followed by Duncan's test for multiple comparisons.

contaminated site which was associated with the oxidative stress in these tissues. Kohler, Bahns and Van Noorden (1998) recently observed that prolonged pollutant exposure partially inhibits or inactivates G6PDH in flounder liver tissue which is compensated by a reduction in K_m. The kinetic properties of G6PDH in the digestive gland of *P. perna* are unknown and the differences in G6PDH activity between mussels from the two contaminated sites in this study are not clearly understood.

The mussels held for 150 days at the three sites showed no significant differences in GST activity (Fig. 2). However, mussels held for 180 days at HLB and ITAC sites showed significant increase of 4.4- and 1.4-fold, respectively. The high GST activity at HLB and ITAC sites could be associated with the elevated rainfall index observed in the period preceding mussel collection at 180 days. According to Climerh-Epagri (1998) the accumulated rainfall index recorded 8 days before the mussel collection at 180 days was 3.4-fold higher than that observed at the same period preceding the collection at 150 days (137.7 and 39.9 mm, respectively). Rain runs untreated from Santa Catarina Island directly into the ocean, causing greater xenobiotic input which could induce biological responses in the exposed organisms, such as the observed increase in GST activity.

Studies on the inducibility of GST activity in mussels from contaminated sites have yielded inconsistent results. Fitzpatrick et al. (1997) observed high GST activity in mussels from industrial sites but no changes were observed in mussels held near to leather tannery effluents. To the contrary, GST inhibition was observed by Regoli et al. (1998) on studies with antarctic scallop *Adamussium colbecki* contaminated with Cu and Hg. Our studies further support GST as useful biomarker of aquatic contamination. However, data analysis must be done with caution, since different chemicals can promote distinct effects on the GST activity. Additional studies to determine agonistic or antagonistic effects of chemicals isolated or administrated together on the GST activity of digestive gland of *P. perna* are needed to validate the use of this parameter as a biomarker of aquatic contamination in the natural environment.

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References

Bainy, A. C. D., Saito, E., Carvalho, P. S. M., & Junqueira, V. B. C. (1996). *Aquatic Toxicology, 34,* 151–162.

Beutler, E. (1975). *Red cell metabolism: a manual of biochemical methods*. New York: Grune & Straton.

Costa, S. W., Grumman, A., Neto, F. M. O., & Rockanski, M. (1998). *Productive chains of Santa Catarina State: aquaculture and fisheries* (Technical Report No. 97). Florianópolis: Epagri (in Portuguese).

Ferreira, J. F. & Magalhães, A. R. M. (1997). *Mussels, biology and farming*. Florianópolis: UFSC, (in Portuguese).
Fitzpatrick, P. J., O'halloran, J., Sheehan, D., & Walsh, A. R. (1997). *Biomarkers, 2,* 51–56.
Glock, G. E., & McLean, P. (1953). *Biochemical Journal, 55,* 400–408.
Keen, J. H., Habig, W. H., & Jakoby, W. B. (1976). *Journal of Biological Chemistry, 251,* 6183–6188.
Kohler, A., Bahns, S., & Van Noorden, C. J. F. (1998). *Marine Environmental Research, 46,* 179–183.
Livingstone, D. R. (1988). *Marine Ecology Progress Series, 64,* 37–43.
Regoli, F., Hummel, H., Amiard-Triquet, C., Larroux, C., & Sukhotin, A. (1998). *Archives of Environmental Contamination and Toxicology, 35,* 594–601.