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Comparison of the antioxidant defense system in *Crassostrea rhizophorae* and *Crassostrea gigas* exposed to domestic sewage discharges

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Abstract

Oysters *C. rhizophorae* and *C. gigas* were kept for fourteen days at four sites in São José, SC, Brazil, chosen according to a sewage discharge contamination gradient. Enzymatic activities of CAT, GST, G6PDH and GR were evaluated in gills and digestive glands. Higher CAT activity was observed in tissues of *C. rhizophorae* in response to sewage contamination possibly indicating peroxisome proliferation

induction. *C. gigas* showed elevated G6PDH activity in digestive gland, and GR in gills, after domestic sewage exposure. In conclusion, *C. rhizophorae* and *C. gigas* showed different biochemical responses after *in situ* exposure to domestic sewage. However, *C. rhizophorae* showed more significant changes in CAT suggesting that this organism could be a better monitor to this kind of effluent.

Keywords: Pollution; Antioxidant defenses; Biomarkers; Oyster; Domestic sewage

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Domestic sewage discharges are frequently observed in Brazilian coast, where only 20 % of the cities possess wastewater treatment plant (IBGE, 2000). In the present study, a suite of antioxidant and ancillary enzymes were evaluated in two potential monitor species, the oysters *Crassostrea rhizophorae* and *Crassostrea gigas*. *C. rhizophorae* is distributed from Southern Brazil to the Caribbean and the Pacific oyster *C. gigas* is worldwide distributed being the most cultivated oyster in the world. Specimens of *C. rhizophorae* and *C. gigas*, six-months old (2-3 cm and 4-6 cm, respectively) were transplanted from a farming area, in September 2006, to four sites in São José, SC, Brazil, where they were kept for fourteen days. These stations were chosen according to a contamination gradient starting at 0.01, 0.4, 1.5 and 4.5 Km far from the mouth of Bücheler River, the most contaminated site (stations 1, 2, 3 and 4, respectively). Gill and digestive gland from each individual were excised, homogenized, centrifuged and the obtained S9 fraction was prepared to quantify the activity of

catalase (CAT), glutathione *S*-transferase (GST), glucose-6-phosphate dehydrogenase (G6PDH) and glutathione reductase (GR), as previously described (Carlberg and Mannervik, 1985; Silva et al., 2005). One-way ANOVA, HSD for unequal sample size, $P < 0.05$, was used to compare the data of stations from each species and tissue, considering the animals from site 4 as the reference group. Fecal coliforms (FC), used as indicator of the presence of domestic sewage discharges were analyzed by multiple-tube fermentation technique according to Standard Methods (1999). The FC average values recorded during the experiment (1, 7 and 14 days) at stations 1, 2, 3 and 4 were 14,100; 1,083; 103; and 46 MPN per 100 ml, evidencing the presence of a gradient of the sewage discharge in the study area. Likewise, the average turbidity and dissolved phosphate levels at the station 1 was about 4 to 10-fold higher than the other sites (data not shown). Correlations between the FC and enzymatic activities of the organisms from each station were performed by Pearson's coefficient ($P < 0.05$).

Higher CAT activity was observed in both tissues of *C. rhizophorae* kept at station 1, when compared to the other stations (Figure 1A). Oysters from the station 2 showed higher CAT activity in gills, when compared to the reference site organisms (Figure 1A). Positive correlation between water FC levels and CAT activity was observed in both tissues of *C. rhizophorae*, suggesting a possible association between this enzyme and chemicals discharged in the untreated domestic sewage (Table 1). Elevated CAT activity has been associated with the presence of peroxisome proliferators (PP) in mussel digestive gland (Cancio et al., 1999). CAT activity copes the increase in hydrogen peroxide produced by peroxisomal enzymes (eg.: acyl-CoA oxidases, poly amine oxidase and xanthine oxidase) (Schrader and Fahimi, 2006). In the present study, we could hypothesize that compounds present in the domestic sewage might have induced peroxisome proliferation, with associated increase in the CAT

activity. Zanette et al. (2006) also reported increases in CAT activity in the gills of *C. rhizophorae* sampled at sites contaminated by sewage discharges. Further studies are required to investigate which chemical could be responsible for the increase in CAT activity observed in the present report.

Higher G6PDH activity in the digestive gland of *C. gigas* from station 1 was observed (Figure 1C). The G6PDH is the main regulatory enzyme in the pentose phosphate shunt which produces NADPH, required for the maintenance of the cellular antioxidant capacity, fatty acid biosynthesis, and xenobiotic biotransformation (Silva et al., 2005). NADPH donates electrons to reduce oxidized glutathione (GSSG) through the enzyme glutathione reductase (GR), recycling GSH. Higher GR activity was observed in gills of *C. gigas* from station 1 (Fig. 1D), possibly to compensate a decrease in GSH levels. Lower levels of GSH were found in gills of *C. gigas* from station 1 (data not show). These results suggest that the increase of G6PDH and GR activity in *C. gigas* exposed to domestic sewage occurs in a tissue distinct manner to cope the increase in cellular pro-oxidants and/or depletion of NADPH/GSH employed as substrate by antioxidant and biotransformation enzymes (eg.: GPx and CYP, respectively).

In conclusion, *C. rhizophorae* and *C. gigas* showed different responses in the activity of antioxidant and ancillary enzymes after *in situ* exposure to domestic sewage. However, the native Atlantic southwestern oyster *C. rhizophorae* showed more significant changes in CAT suggesting that this organism is more adequate monitor in this kind of effluent. Further studies need to be carried out to investigate the presence of peroxisome proliferator-inducing compounds in these discharges.

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Figure captions

Fig. 1. Catalase (CAT), glutathione *S*-transferase (GST), glucose-6-phosphate dehydrogenase (G6PDH) and glutathione reductase (GR) activities in gills and digestive glands (dg) of *C. gigas* and *C. rhizophorae* (rhiz) oysters from different stations (means \pm standard deviation). Equal small letters indicate absence of significant differences between stations for each specie/tissue group ($P>0.05$). Equal capital letters indicate absence of significant difference among reference sites (station 4) from different specie/tissue groups ($P>0.05$). Log transformation was employed if normality and homoscedasticity were not verified. The number in parenthesis indicates the number of oysters analyzed in each group.

Figure 1.

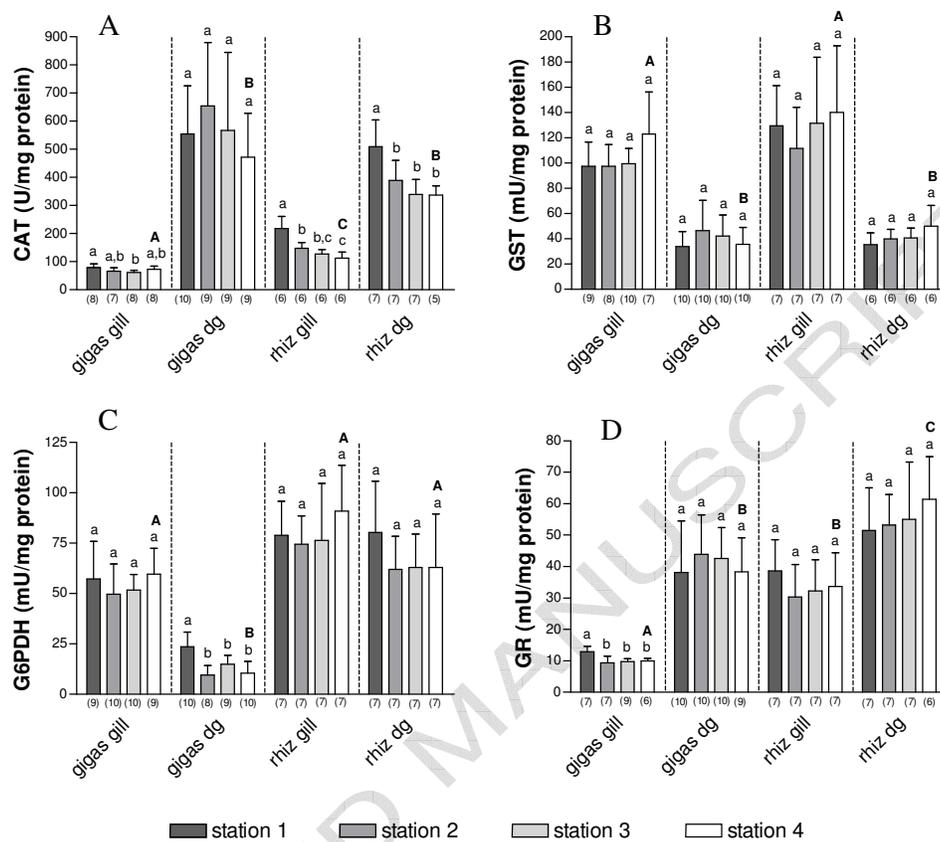


Table 1.

Correlations of faecal coliforms in water (FC) with catalase (CAT), glutathione *S*-transferase (GST), glucose-6-phosphate dehydrogenase (G6PDH) and glutathione reductase (GR) activities in gills and digestive glands (dg) of *C. gigas* and *C. rhizophorae* oysters transplanted to field stations (Pearson correlation coefficients). * Indicates significant correlations. Values of FC and enzyme activities were Log transformed.

Oyster	Tissue	Enzyme	fecal coliform correlation coefficient	<i>P</i> -value of correlation
<i>C. gigas</i>	gill	CAT	0.557	0.443
		GST	-0.678	0.322
		G6PDH	0.825	0.175
		GR	-0.860	0.351
	dg	CAT	0.457	0.542
		GST	-0.222	0.778
		G6PDH	-0.056	0.943
		GR	0.759	0.434
<i>C. rhizophorae</i>	gill	CAT	0.981	0.019 *
		GST	-0.436	0.563
		G6PDH	-0.475	0.524
		GR	0.649	0.140
	dg	CAT	0.976	0.023 *
		GST	-0.879	0.120
		G6PDH	-0.475	0.524
		GR	0.565	0.240