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Antioxidant defense responses in *Mytella guyanensis* (Lamarck, 1819) exposed to an experimental diesel oil spill in Paranaguá Bay (Paraná, Brazil)

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ABSTRACT

We evaluated the effects of diesel oil on the bivalve *Mytella guyanensis* using biomarkers of oxidative stress (glutathione S-transferase, glutathione peroxidase, and reduced glutathione) after an experimental in situ spill in a mangrove area in southern Brazil. A linear model was developed for the Multiple Before–After Control–Impact (MBACI) experimental design to assess the significance of biological responses. Control and impacted sites were sampled seven and two days before as well as two and seven days after the spill. With the exception of a late response of reduced glutathione (GSH) levels on day seven, none of the biomarkers were significantly altered by the impact. This result was attributed to the high environmental variability of the experimental sites combined with a low sensitivity of *Mytella guyanensis* to diesel oil at short time-scales. The high resistance of *M. guyanensis* suggests that its antioxidant response is triggered only after a medium- to long-term exposure to contaminants.

1. Introduction

The production of reactive oxygen species (ROS) results from cellular processes related to basal metabolism, and is highly influenced by environmental factors, such as variations in salinity and temperature, or exposure to pollutants. Antioxidant defenses play a crucial role in maintaining homeostasis within cells when the ROS production increases, and its induction reflects the organism's response to pollutants (Doyotte et al., 1997; Vidal-Liñán and Bellas, 2013). When these defenses are not sufficient, oxidative stress can cause DNA damage, enzyme inactivation, and peroxidation of cell membranes, primarily of lipids (Lushchak, 2011). Therefore, oxidative stress-related parameters, such as the activity of antioxidant defenses and lipid peroxidation, may be regarded as biomarkers of environmental stress.

The metabolism of polycyclic aromatic hydrocarbons (PAHs) by the cythochrome P450 system produces oxyradicals (Lushchak, 2011). Thus, the detoxification processes of PAHs can lead to formation of ROS and, consequently, a change in antioxidant defense levels. The antioxidant responses, such as reduced glutathione (GSH), and the enzymes glutathione peroxidase (GPx) and glutathione-S-transferase (GST), have been used as biochemical markers of estuarine pollution. GST plays an important role in phase II of biotransformation. Besides presenting peroxidase activity (Sheehan et al., 2001), it is considered an indirect antioxidant, since it can eliminate byproducts of ROS (Luchmann et al., 2011; Bebianno and Barreira, 2009; Boutet et al., 2004).

Many studies have reported changes in these biomarkers associated with bivalve exposure to hydrocarbons (Cheung et al., 2001; Vidal-Liñán et al., 2010). However, most of the experiments evaluating biomarker responses in aquatic organisms have been carried out under laboratory conditions (Alves et al., 2002; López-Galindo et al., 2010; Silva et al., 2005). There is a growing need to associate laboratory information with expected responses in organisms exposed to contaminants in situ, which demands field experiments (Martín-Díaz et al., 2007; Reid and Macfarlane, 2003). Experimental approaches with simulated oil spills in the field enable more robust inferences about the effects of hydrocarbons than the usual descriptive approaches, or laboratory bioassays. Laboratory bioassays do not reflect natural variability and their interactions with xenobiotic toxicity, and thus they lack ecologically relevant information. Moreover, the use of in situ bioassays reduces problems with collection, culturing, and handling of organisms (Soares et al., 2005; Giesy and Hoke, 1989). The advantage of planned impacts on descriptive approaches (e.g., collecting in

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impacted and non-impacted sites) is that the detected responses can be assigned to a known, and relatively bounded, contamination source in space and time (Downes et al., 2002; Glasby and Underwood, 1996).

Diesel oil is one of the most common fuels used in boats, and one of the most common sources of hydrocarbon contamination in estuaries (Rosner, 1996). It has acute toxic effects on bivalves, which can be detected either at biochemical or community levels (Zanette et al., 2011; Boldina-Cosqueric et al., 2010; Ansaldo et al., 2005; Carman et al., 2000). Benthic macrofauna is considered tolerant or resilient to in situ planned oil impacts in Brazilian subtropical environments at short spatial scales (Egres et al., 2012; Faraco and Lana, 2003), with low rates of mortality and rapid recovery of species densities. However, it is not known whether these macrofaunal assemblages suffered sublethal effects from these planned impacts, which were not large or consistent enough to produce long-term effects at higher levels of biological organization.

Biomarkers, which may provide an early warning of contamination before detectable effects at the population/community levels, are useful tools to assess marine contamination and to predict ecological effects (Monserrat et al., 2007; Zagatto and Bertoletti, 2006). The scant knowledge of sublethal effects on marine organisms exposed to contaminants along subtropical coasts justifies the identification of effective biomarkers of hydrocarbon exposure. Bivalves, especially mussels, have been widely used as sentinels in monitoring programs due to their widespread distribution, well-known physiology, sessile condition, bioaccumulation capacity, and filter-feeding behavior (Fossi Tankoua et al., 2013; Cajaraville et al., 2000; Goldberg, 1984). The mussel *Mytella guyanensis* is one of the most important economic resources in artisanal fisheries, mostly in subtropical mangroves (Nishida et al., 2008). Since it is a species that is closely related to *Mytillus edulis*, a bioindicator that is used worldwide, it may be putatively considered an effective bioindicator for estuarine/mangrove ecosystems.

This study evaluates the effects of an experimental diesel oil spill on the mangrove mussel *M. guyanensis* by monitoring the responses of oxidative stress biomarkers. The concentrations and distribution of polycyclic aromatic hydrocarbon, as the most potentially toxic fraction of oil hydrocarbons (Wang et al., 1999), were also considered. We hypothesize that if GST and GPx activity and GSH levels in *M. guyanensis* are effective biomarkers of acute toxicity associated to diesel, then biological responses will be more evident in impacted than in control sites, from before to after experimental exposure. Such in situ experimental studies may provide more realistic approaches to assess the sensitivity of estuarine animals to acute oil impacts and their suitability for environmental monitoring purposes.

2. Materials and Methods

2.1. Study area

The Paranaguá Estuarine Complex (PEC), in southern Brazil, presents a great diversity of natural habitats such as marshes, mangroves, rocky shores, and tidal flats. Local mangroves are comprised mainly of *Rhizophora mangle*, *Avicennia schaueriana*, and *Laguncularia racemosa* (Lana et al., 2001), and are home to species of bivalve mollusks of ecological and commercial interest, such as the native oyster *Crassostrea rhizophorae* and the mangrove mussel *M. guyanensis*. The PEC is susceptible to acute and chronic diesel oil spills because of intense harbor activities associated with intense traffic of small tourist and fishing boats, which use marine diesel oil as the most common fuel.

The experiment was performed in two undisturbed mangroves mainly comprised of *A. schaueriana*, *R. mangle and L. racemosa* in Laranjeiras Bay, located on the north-south axis of PEC (Fig. 1), with less maritime traffic due to being away from the main harbor activities.

2.2. Experimental design and field procedures

The sampling design was based on the MBACI (Multiple Before–After Control–Impact) strategy, which is ideal for planned impacts because it considers multiple sampling times, both before and after impact, in multiple control and impacted locations, ensuring appropriate temporal and spatial replication (Downes et al., 2002; Keough and Mapstone, 1997). The MBACI strategy identifies the impact as an interaction between the period (before vs. after) and stress (control vs. impact), i.e., a response that is different in the impacted area from natural changes in the non-impacted area during the same period (Downes et al., 2002; Underwood. 2000). The design used in this work comprised replicated control and impact sites (control and impact sites in two mangroves), sampled at two times before (−seven and −two days) and two times after (+two and +seven days) the impact (Fig. 2).

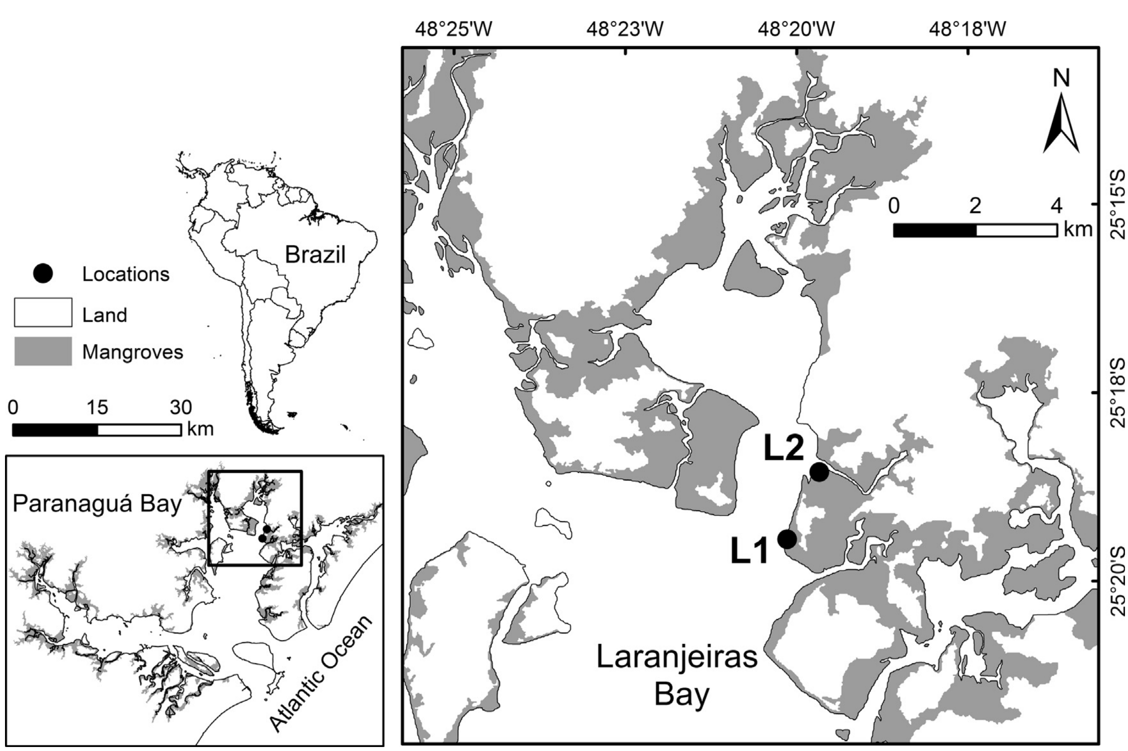

Fig. 1. Map of the Paranaguá Estuarine Complex with the location of experimental sites.