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# Cytotoxicity and enzymatic biomarkers as early indicators of benthic responses to the soluble-fraction of diesel oil

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A B S T R A C T

Xenobiotics from oil tanker leaks and industrial discharges are amongst the main human impacts to confined coastal areas. We assessed the genotoxic responses to the water-soluble fraction of diesel oil in the polychaete *Laeonereis culveri* and the bivalve *Anomalocardia flexuosa*, two widespread benthic species in subtropical estuaries from the Southwestern Atlantic. We hypothesized that the highest responsiveness would be expressed by significantly different biomarkers responses between control and oil-impacted treatments. Responsiveness to diesel oil was investigated using an experimental design with two fixed factors (contaminant percentages and times of exposure). After exposure, we monitored the responses of the oxidative stress enzymes and performed micronuclei tests. Results were congruent for both species. Antioxidant defense of glutathione S-transferase and the induction of micronuclei and nuclear buds, the latter just for the bivalve, were significantly affected by polycyclic aromatic hydrocarbons, with significant increases on the seventh day of exposure and in the higher concentrations, compared to controls groups. We assessed the benefits and drawbacks of using each biomarker in laboratory experiments. Both species are indicators of early, and rapid responses to genotoxic contaminants in subtropical estuarine habitats. We suggest that the micronuclei frequency in *A. flexuosa* is a simple, fast and cheap test for genotoxicity in oil-impacted areas. Such early biomarkers are needed to develop better protocols for impact assessment and monitoring under real field conditions.

## 1. Introduction

Marine confined habitats, such as estuaries, are amongst the most impacted by xenobiotics derived from coastal human activities. The acute or chronic release of pollutants like diesel oil into the water requires monitoring and impact assessment strategies (Farrington, 2014). The crude oil-related contaminants of primary concern, because of their potential carcinogenic, genotoxic and teratogenic effects on the marine organisms, are the polycyclic aromatic hydrocarbons, hereafter PAHs (Boehm and Page, 2007). PAHs are among the most stable, persistent, and harmful xenobiotics in aquatic environments due to their low water solubility and particulate apprehension deposited in sediments (Bacosa and Inoue, 2015). Once released into the sea, oil suffers subsequent chemical transformations after weathering processes. Marine diesel oil carries volatile compounds highly dispersible due to its low viscosity, which results in faster evaporation, dispersion and dissolution processes (Hansen et al., 2013). Most of these compounds evaporate fastly after spills, but remaining fractions can still harm organisms (Neff et al., 2000).

Estuaries are transitional areas between seawater and freshwater and may act as terrestrial pollutants reservoirs and sources of pollutants to the ocean (Jiang et al., 2013). Estuarine-coastal regions are affected by PAHs contamination from the atmosphere and land, by sedimentary processes and by river discharge (Wang et al., 2016). Benthic estuarine animals may accumulate and retain organic and inorganic contaminants due to their bottom-living strategies. They are frequently used to assess the damage caused by oil spills, mainly because they respond to changes in physical and chemical parameters very precisely and quickly (Dauvin et al., 2010).

Polychaetes numerically dominate the estuarine communities and

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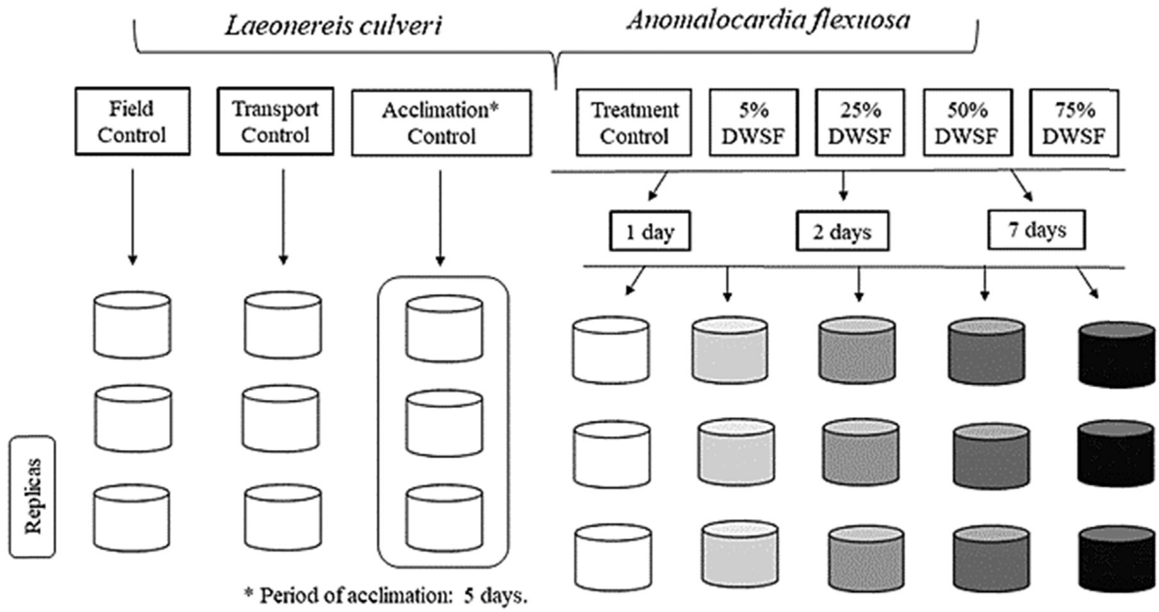


**Fig. 1.** Experimental design. The three preliminary controls, with three replicates each, are displayed on the left side. The experimental treatments, with the corresponding concentrations of diesel water-soluble fraction (DWSF) (Control, 5%, 25%, 50% and 75%) and time of exposure (1d, 2d, and 7d) are shown on the right side. For each exposure time and treatment, three replicates were distributed, totaling 45. For each time, specimens of three beakers from each treatment were sacrificed.

are considered potential sentinels of xenobiotic impacts, due to their physiological plasticity and sensitivity (Bat, 2005). Populations of the nereidid polychaete *Laeonereis culveri*, a common detritivore species found in tropical and subtropical estuaries in South America, are often exposed to oil pollution, absorbing several types of pollutants through the epidermis and digestive tube (Durou et al., 2005).

Filter-feeding bivalves, on the other hand, are more sessile and may accumulate chemical compounds present in the water column (Gowland et al., 2002). They display limited abilities to metabolize PAHs and may accumulate high levels of these compounds in their tissues (Dyrynda et al., 1997). *Anomalocardia flexuosa* is a common bivalve species present along the subtropical and tropical Atlantic coasts of South America (Colonese et al., 2017). Their populations can withstand significant contamination caused by diesel oil due to the protection conferred by their shells (Sandrini-Neto et al., 2016). They are suitable for impact assessment and monitoring (Sandrini-Neto et al., 2016; Sardi et al., 2017, 2016), but still need to be tested in ecotoxicological experiments.

Molecular and cellular responses are in general the earliest signals after environmental disturbance, and they are commonly used as biomarkers in ecotoxicological investigations (Regoli et al., 2004). The use of a multi-biomarker approach can provide a consistent combination of evidence on the mechanisms that define the appearance of biological alterations (Guidi et al., 2010). In general, genotoxic responses are driven by multiple factors such as time of exposure, uptake, metabolic activation, defense mechanisms and repair efficiency, and may differ significantly for different cell types (Lewis and Galloway, 2008). At sub-organism levels, one of the most investigated pathways after exposure to xenobiotics is the induction of oxidative stress. Oxidative stress has received increasing attention from aquatic toxicologists because it reflects perturbations of oxyradical metabolism, in which environmental pollutants can change the natural balance between prooxidant forces and antioxidant defenses (Benedetti et al., 2015). The measurement of marine invertebrates is extensively used as a pollution index (Regoli and Giuliani, 2014).

PAHs can also affect the integrity of DNA due to DNA strand breaks, loss of methylation and the formation of DNA adducts (Pisoni et al., 2004). Micronuclei appear when cells fail to incorporate complete or fragmented chromosomes into the daughter nuclei during cell division. Genetic fragments are instead incorporated in small additional nuclei, where they remain throughout the life of the cell. The presence of micronuclei (MN) is an indicator of chromatin breakage which may be caused by clastogens or spindle dysfunctions, ultimately caused by toxic compounds (Carrano and Natarajan, 1988). The micronucleus test has been widely applied in the field and cultivated marine invertebrates (Bolognesi et al., 2004; Siu et al., 2004; Viarengo et al., 2007). It is a cytogenetic technique commonly used for the assessment of genotoxic effects caused by environmental stressors.

Recent studies have been integrating MN tests in bivalves to evaluate the presence of xenobiotics in the environment (D'Agata et al., 2014; D'costa et al., 2018; Falfushynska et al., 2018) and also attributed as the biomarker of PAHs' toxicity (Baršienė et al., 2010; Farhadi et al., 2011; Vincent-Hubert et al., 2011). The MN frequency detected in these bivalves is a guide to accumulated genetic damage during the cell lifespan, providing a time-integrated response of an organism's exposure to contaminant mixtures (Gomiero et al., 2015).

Experimental evaluations of the response of subtropical benthic species combined with the use of multi-biomarker approaches are still scant. In this sense, ecotoxicological tests were carried out to assess the responsiveness of the polychaete *L. culveri* and the bivalve *A. flexuosa* to the soluble fraction of diesel oil. We evaluated: i) the activity of oxidative stress enzymes (i.e., superoxide dismutase, SOD; catalase, CAT, and glutathione S-transferase, GST) along with levels of lipid peroxides (LPO), and ii) the frequency of cytogenetic abnormalities. Considering that both species display different life strategies and external body protection, our hypothesis was that the highest responsiveness will be associated with the most vulnerable species and will be expressed by significantly different biomarkers responses between control and oil-impacted treatments as a function of concentrations and times of exposure to the contaminant.

Comparisons among different species and biomarkers allied to the use of robust experimental designs are much needed to develop cheap and fast protocols in evaluations of impacts produced by diesel oil in subtropical estuaries.