

Oxidative stress and neurotoxicity in *Scolelepis goodbodyi* (Polychaeta, Spionidae) after an experimental oil spill in a dissipative sandy beach

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ABSTRACT

Biomarkers of environmental contamination have been frequently used in the assessment of marine ecosystem quality because they provide quantitative measures of biological changes in organisms exposed to pollutants such as hydrocarbons from oil spills. Polychaetes have been tested as sentinel organisms of marine environmental health because they are abundant taxa of benthic assemblages and their sedentary lifestyle ensures chronic exposure to toxins in impacted areas. In this study, we evaluated whether the polychaete *Scolelepis goodbodyi* can be used as a reliable sentinel species for exposure to polycyclic aromatic hydrocarbons (PAHs) from an experimental diesel spill in a dissipative sand beach in the Southern Atlantic. The design used in this study comprised replicated control and diesel impact sites sampled four times (one, two, four and seven days) before and after the impact. Total PAH levels reached 114.0 ng g⁻¹ in the impact site one day after the diesel spill; however, the pattern of biomarker responses in *S. goodbodyi* was primarily influenced by spatial variation rather than being attributed to the simulated diesel spill. The apparent absence of a contaminating effect may be linked to the low levels of the toxins retained in the sediment after the spill was simulated to elicit a response or to the presence of an efficient repair system within the organism. Furthermore, the sediments tested in this study, composed of sandy fractions, had a low capacity to concentrate PAHs after the simulated diesel spill, which may have contributed to the low significant changes in their biomarker activities. Based on our results, further studies testing other Polychaeta species and simulating oil spills in sedimentary environments composed of fine sediments such as salt marshes and mangroves may help produce evidence on more effective biomarker responses in these organisms.

Keywords: Biomarkers, Polycyclic aromatic hydrocarbons, Field experiment, MBACI, Polychaetes

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INTRODUCTION

Biomarkers of environmental contamination are considered important tools for assessing marine environmental conditions and health (Lesser, 2006; Sureda et al., 2013). They are defined as



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quantitative measures of biological changes that result from exposure to xenobiotic substances that lead to biological effects (Lam and Grey, 2003). Biological changes can occur at the cellular, biochemical, molecular, or physiological levels and can be measured in cells, body fluids, tissues, or organs within an organism.

The main reason for using biochemical and cellular responses to pollutants relies on the fact that these suborganismic changes tend to precede those that occur at higher levels of biological organization (Lam and Gray, 2003; Vasseur and Cossu-Leguille, 2003). Because of this, several biomarkers are considered early and effective warnings of the harmful effects of contaminant exposure (Sarkar et al., 2006; Gomes et al., 2014). In addition, biomarkers make it easier to identify whether the contaminant is biologically available, as they provide a link between chemical residues in tissues and the consequences for the health of the individual organism (Moore, 1993).

Antioxidant biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) activities are frequently applied in environmental quality assessments (Vidal-Liñán and Bellas, 2013; Sandrini-Neto et al., 2016; Sardi et al., 2016a, 2016b). SOD is responsible for breaking down superoxide radicals ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). After this process, enzymes such as CAT and GPx play a role in breaking down H_2O_2 . These enzymes have been widely used as biomarkers of oxidative stress in marine organisms. GST, an enzyme involved in phase II detoxification processes, including the conjugation and detoxification of organic compounds, has been widely used as a biomarker to assess exposure to polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and trace metals in invertebrates (e.g., Vidal-Liñán and Bellas, 2013; Rubio-Vargas et al., 2021). Among nonenzymatic defenses, glutathione (GSH) binds to toxic substances and neutralizes their effects, making them less harmful or facilitating their elimination from the body (Lüchmann et al., 2011). Cellular antioxidant defense failure can lead to elevated levels of lipid peroxides (LPO), a significant mechanism by which reactive oxygen

species can harm the lipids of the cell membrane (Turja et al., 2013; Zanette et al., 2015).

Acetylcholinesterase (AChE) is an essential enzyme for the transmission of nerve impulses and is responsible for degrading acetylcholine to choline and acetic acid in the synaptic gap of cholinergic synapses and neuromuscular junctions. Its activity can be inhibited by various toxicants, which leads to severe physiological impairment in marine organisms (Vidal-Liñán and Bellas, 2013). While the inhibition of AChE activity is commonly used as a response to pesticide exposure, this enzyme is considered a nonspecific biomarker (Dean, 2008).

The use of biomarkers to assess marine environmental quality is closely related to the use of sentinel species, since exposure effects largely depend on the ecological lifestyle and diet of organisms (Moukrim et al., 2004). Polychaetes can be an excellent sentinel species because they are abundant taxa of benthic assemblages, and their sedentary lifestyle ensures chronic exposure to toxicants in the environment (Dean, 2008). Spionidae polychaetes are abundant in intertidal zones and shallow marine waters (Pardo and Amaral, 2004). They are infaunal organisms that exhibit discrete motility and reside in tubes or galleries lined with mucus. Spionids are known to be both suspension feeders and deposit feeders (Jumars et al., 2015). These characteristics make them excellent candidates for marine environmental assessment studies (MacCord and Amaral, 2005). The genus *Scolelepis* is commonly found in sandy beaches along the Brazilian coast (Rocha and Paiva, 2012) and monitoring its population has been recommended to assess the effects of global climate change on these environments (Turra and Denadai, 2015).

In this study, we evaluated whether the polychaete *Scolelepis goodbodyi* can be used as a reliable sentinel species of exposure to PAHs in a dissipative sand beach in the Southern Atlantic. We hypothesized that biomarkers of oxidative stress (SOD, CAT, GPx and GST activities, and GSH and LPO levels) and neurotoxicity (AChE activity) in oil-exposed areas would be significantly different (i.e., induced or inhibited) than in control areas before and after the experimental diesel spill.

METHODS

STUDY SITE AND EXPERIMENTAL DESIGN

The field experiment was performed on a dissipative sandy beach in Pontal do Sul, southern Brazil (Figure 1a), with the permission of the Chico Mendes Institute of Biodiversity Conservation (ICMbio) of the Brazilian Ministry of the Environment (licence no. 47415-2). The experiment was conducted from April 9 to April 23, 2015 and the simulated single acute impact took place on April 16, 2015.

The experimental design was based on the MBACI (Multiple Before-After-Control-Impact) model, which is recommended 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 (Keough and Mapstone, 1997; Downes et al., 2002). The design used in this study comprised replicated control and diesel impact sites (one control and one impact site in two experimental areas on the same beach), sampled four times (one, two, four and seven days) before and after the impact.

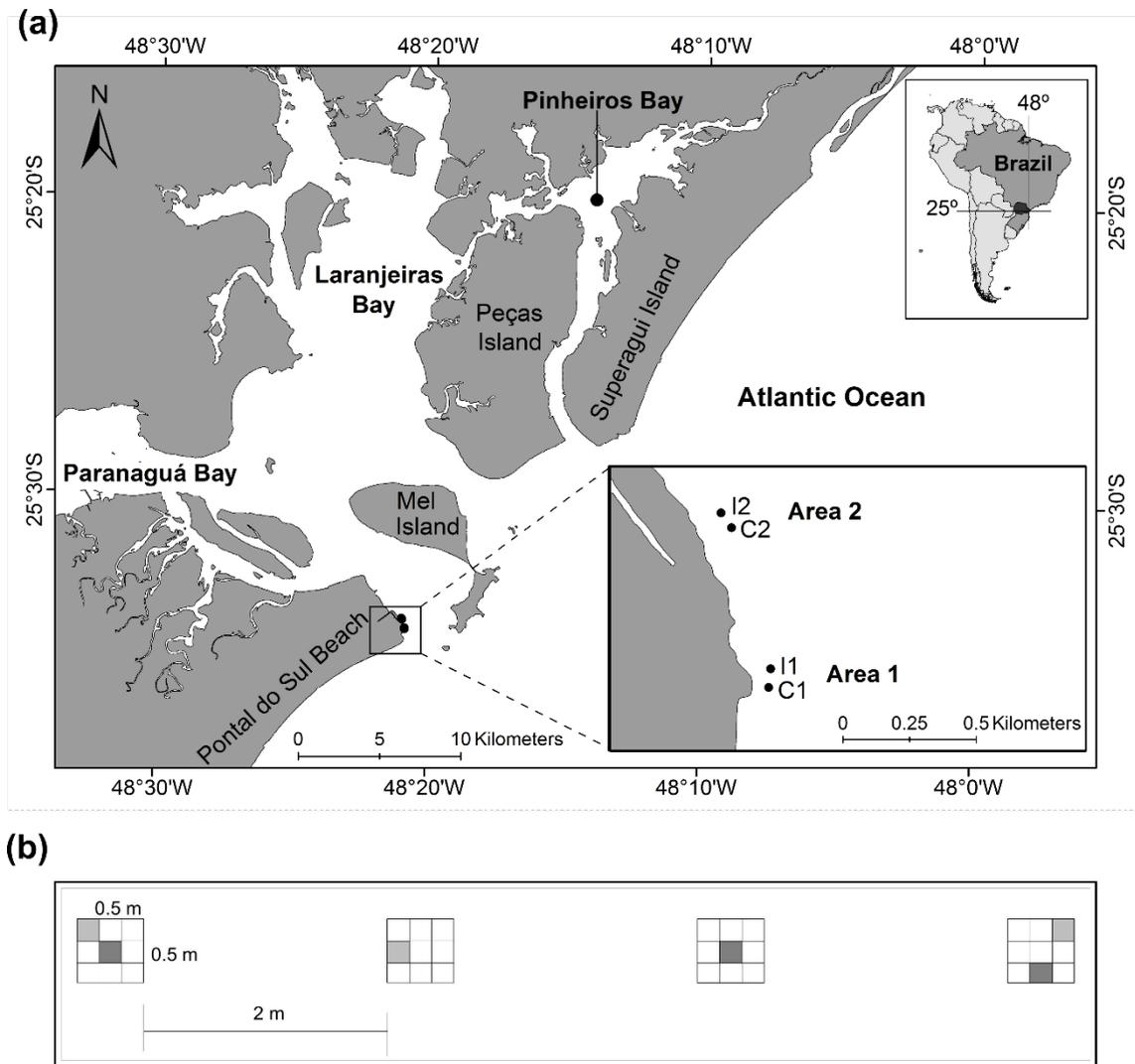


Figure 1. (a) Location of experimental areas 1 and 2 on Pontal do Sul beach, southern Brazil. The design consisted of two control (C1 and C2) and two impact (I1 and I2) sites. (b) Schematic representation of the four 0.5×0.5 m plots within each site. Within each plot, there were nine quadrats. Colored quadrats represent three random replicates taken on two different dates.

The two experimental areas, located 600 meters apart, were chosen based on their similar morphodynamic states and abundance of *S. goodbodyi*. The impact and control sites in each area were established 60 m away from one another and positioned parallel to the coast at the same tidal level. Both the control site and impact site were composed of four 0.5×0.5 m plots subdivided into nine quadrats (Figure 1b). Three quadrats from these plots were taken randomly each time before and after the simulated spill, resulting in a total of 96 replicates. The sediment within the quadrats was removed with a shovel, stored in plastic containers, and kept cold until being processed in the laboratory. The sediment was carefully washed through a 0.5 mm mesh sieve, and 30 *S. goodbodyi* individuals with similar sizes were retrieved. The polychaetes were transferred to 1.5 mL microtubes and stored at -80 °C until biomarker analysis.

EXPERIMENTAL DIESEL OIL SPILL

The experimental spill was conducted during low tide, when the areas were exposed, which optimized the time for the oil to penetrate the sediment. Before the oil spill, square metal artefacts were carefully placed in the impact plots to prevent dispersion and cross-contamination between sites. Subsequently, an equivalent volume of 1.5 L m⁻² diesel oil was spilled in each impacted plot. The square metal artefacts were removed after the oil had fully percolated into the sediment. The total amount of diesel to be used was calculated based on an experiment conducted by Sandrini-Neto et al. (2016), which investigated the impact of varying frequencies and intensities of diesel spills on the antioxidant defense response of macrofaunal species.

SEDIMENT ANALYSIS

Sediment grain size and PAH concentrations, both abiotic measures, were determined. Grain size was measured from two replicates at the control and impact sites before the start of the experiment. Sediment analysis was conducted by pipetting and sieving, and granulometric parameters (i.e., sediment mean grain size and sorting) were obtained in accordance with Suguio (1973).

PAHs were measured from a sediment sample from the control and impact sites one day before, one day after, and seven days after the diesel spill. The analysis of PAHs was based on the method described by UNEP (1992), incorporating minor modifications presented by Wisniewski et al. (2016). Approximately 20 g of sediment from each site were extracted using a Soxhlet apparatus for 8 h with 80 mL of a mixture of (1:1, v/v) *n*-hexane and dichloromethane (DCM). The organic extract was reduced to approximately 2 mL by rotoevaporation, and the concentrated extract was subjected to a purification step via alumina and silica column adsorption chromatography after the elution of different solvents according to the organic marker group (specifically, 15 mL of a (3:7, v/v) DCM/*n*-hexane mixture to fraction containing the PAHs). The instrumental analyses were performed using gas chromatography with an Agilent HP 7890A coupled to an Agilent 5975C inert MSD with a triple-axis detector mass spectrometer. More details on laboratory procedures and instrumental and calibration details, such as the quality assurance procedures of the PAH analyses, were fully described by Martins et al. (2015) and Dauner et al. (2016).

BIOMARKER ANALYSIS

A pool of 30 *S. goodbodyi* individuals was homogenized in phosphate buffer (0.10 mol L⁻¹) at pH 7.0 and centrifuged at 10,000 × *g* for 20 min (at 4 °C). Supernatants were then collected and stored at -80 °C until analysis. These supernatants were used to estimate protein contents, activities of SOD, CAT, GST, GPx, and AChE, and levels of GSH and LPO.

CAT activity was assayed by direct measurement of H₂O₂ degradation (Aebi, 1984). The supernatant (5 µL) was mixed with a reaction medium (295 µL, 20 mmol L⁻¹ H₂O₂, 50 mmol L⁻¹ Tris-base, 0.25 mmol L⁻¹ EDTA, pH 8.0) in a microplate, and the absorbance decrease was measured at 240 nm for 1 min at 27 °C. The activity was expressed as µmol min⁻¹ mg protein⁻¹.

SOD activity was measured using the method proposed by Gao et al. (1998), which is based on the ability of SOD to inhibit the autoxidation of pyrogallol. The supernatant was diluted 1:4

(v/v) in 0.1 mol L⁻¹ potassium phosphate buffer (pH 7.0). In a microtube, 885 µL of buffer (1 mol L⁻¹ Tris-base and 5 mmol L⁻¹ EDTA, both pH 8.0) and 40 µL of sample were added. After agitation, 50 µL of 15 mmol L⁻¹ pyrogallol were added, and the solution was incubated for 30 min. The reaction was stopped with 25 µL of 1 mol L⁻¹ HCl. Absorbance was read at 440 nm, and the activity was expressed as U mg protein⁻¹.

GPx activity was measured based on the decrease in NADPH absorbance (Paglia and Valentine, 1967). A volume of 10 µL of supernatant and 130 µL of the reaction medium (3.08 mmol L⁻¹ sodium azide; 0.308 mmol L⁻¹ reduced nicotinamide-adenine dinucleotide phosphate - β-NADPH, 1.54 U mL⁻¹ glutathione reductase, and 3.08 mmol L⁻¹ reduced glutathione in 0.1 mol L⁻¹ sodium phosphate buffer, pH 7.0) were mixed. After two minutes, 60 µL of 1.5 mmol L⁻¹ H₂O₂ were added. Absorbance was monitored at 340 nm, and the activity was expressed as nmol min⁻¹ mg protein⁻¹.

GST activity was measured using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates (Keen et al., 1976). The supernatant (20 µL) was placed in a microplate, then 180 µL of the reaction medium (3 mmol L⁻¹ GSH, 3 mmol L⁻¹ CDNB, 0.1 mol L⁻¹ potassium phosphate buffer, pH 6.5) were immediately added. The absorbance increase was measured at 340 nm, and the activity was expressed as µmol min⁻¹ mg protein⁻¹.

GSH was measured in accordance with Sedlak and Lindsay (1968). A volume of 25 µL of supernatant (after protein precipitation by 10% trichloroacetic acid and centrifugation at 10,000 × g for 10 min at 4 °C) and 115 µL of Tris-base (0.4 mol L⁻¹, pH 8.9) were placed in a microplate, and 20 µL of 2.5 mmol L⁻¹ DTNB in 25% methanol were subsequently added. The absorbance was determined at 415 nm, and the GSH concentration was calculated by comparison with the standard curve for GSH. The activity was expressed as µg mg protein⁻¹.

The analysis of LPO was carried out using the ferrous oxidation–xylenol assay (Jiang et al., 1992). A volume of 100 µL of supernatant resuspended in methanol (1:1, v/v) was mixed with 900 µL of reaction solution (0.1 mmol L⁻¹ xylenol

orange, 25 mmol L⁻¹ H₂SO₄, 4.0 mmol L⁻¹ butylated hydroxytoluene, BHT, and 0.25 mmol L⁻¹ ammonium ferrous sulfate, NH₄FeSO₄, added in this specific order into 90% grade methanol). After 30 min of reaction at room temperature, the absorbance was measured at 570 nm, and the LPO was expressed as nmol hydroperoxides mg protein⁻¹.

AChE activity was measured at 405 nm, in accordance with the method described by Ellman et al. (1961) and modified by Assis (1998). In the microplate, 25 µL of the sample, 200 µL of (5,5-dithio-bis-2-nitrobenzoate, DTNB) at 0.75 mmol L⁻¹, and 50 µL of acetylthiocholine iodide at 10 mmol L⁻¹ were added. The activity was expressed as nmol min⁻¹ mg protein⁻¹.

The protein concentration was determined using Bradford's method (1976), with bovine serum albumin as the standard. In total, 10 µL of supernatant (diluted 1:20) and 250 µL of Bradford reagent (Bio-Rad) was placed in a microplate, and the absorbance was measured at 595 nm. The absorbance measurements were carried out on a TECAN A-5082 absorbance reader (Tecan, Austria).

DATA ANALYSIS

Biomarker responses were analyzed separately, using a permutational analysis of variance. The linear model consisted of four factors: stress (two levels, fixed: control and impact), period (two levels, fixed and crossed with stress: before and after), days (four levels, fixed and nested within period), and areas (two levels, random and nested within stress). The impact was given by the change in response between the control and impact treatments from before to after the spill and expressed by the period × stress interaction in the analysis (Keough and Mapstone, 1997).

The normality and skewness of the data were assessed using graphical diagnostics, including the examination of histograms and quantile-quantile plots (QQ plots). Because most of the resulting data distribution was asymmetrical and did not fulfil the assumptions of normality for a parametric ANOVA, PERMANOVA (Anderson, 2001, 2005), a distance-based permutation procedure, was used for the factorial analysis. Euclidean distance was used for univariate analyses, as recommended (Anderson, 2001), followed by the

corresponding *a posteriori* pairwise comparison tests for main effects and high-order interactions. The statistical analyses were performed using the PERMANOVA+ add-on package for PRIMER v.6 (Clarke and Gorley, 2006; Anderson et al., 2008). Graphs were generated in R 4.1.0 (R Core Team, 2022), combined with the sciplot package (Morales, 2020).

Table 1. Mean grain size and sorting (μm) of sediments in control and impact sites on Pontal do Sul beach, Southern Brazil. Control 1 and Impact 1 are treatment sites of the experimental area 1; Control 2 and Impact 2 are treatment sites of the experimental area 2.

Replicate	Control 1		Control 2		Impact 1		Impact 2	
	1	2	1	2	1	2	1	2
Mean grain size	167.2	171.9	154.9	155.2	156.9	165.1	159.5	156.7
Sorting	40.63	42.23	34.70	34.39	35.98	41.61	40.15	36.29

The PAH results are presented in Figures 2 and 3. All sites presented similar PAH concentrations 24 hours before the diesel spill and were independent of treatment. According

RESULTS

GRAIN SIZE AND PAH LEVELS

The sediment of the experimental areas was composed of well-selected fine sands. There was a minor difference in sediment grain size among areas, and Area 1 presented relatively large variability between samples and sites (Table 1).

to the diagnostic PAH ratios, these compounds had the same origin, i.e., petrogenic input and residues of oil and byproduct combustion (Figure 3).

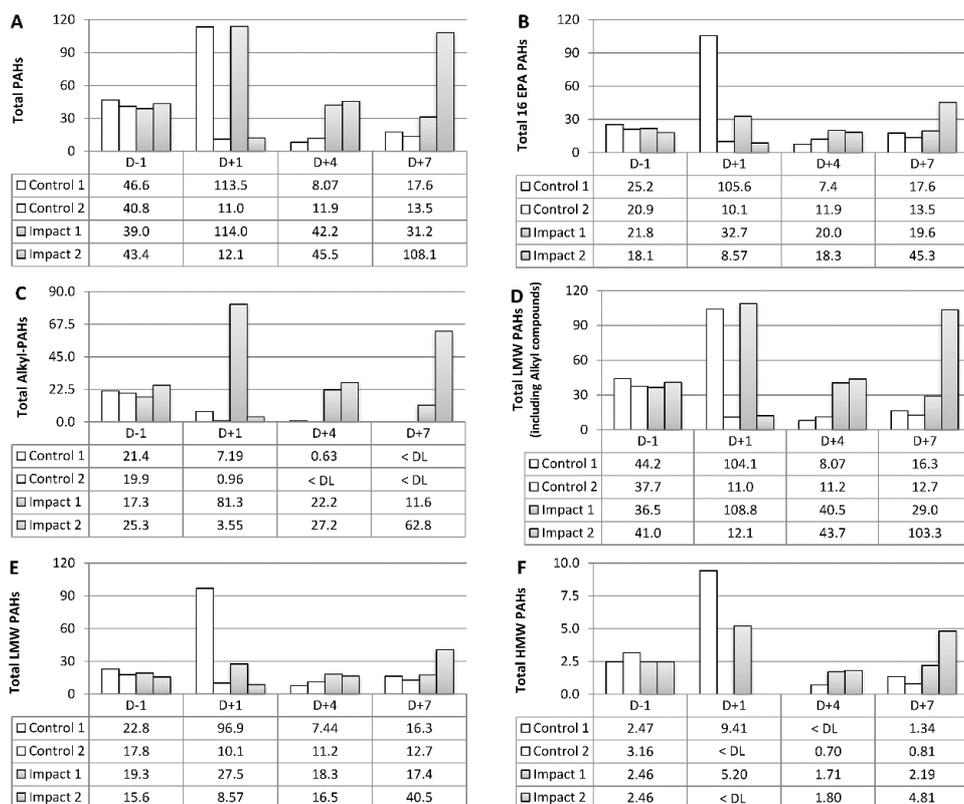


Figure 2. PAHs concentrations (in ng g^{-1} dry sediment) in control and impact sites located at Area 1 (Control 1 and Impact 1) and Area 2 (Control 2 and Impact 2). D-1 represents one day before the experimental oil spill; D+1, D+4 and D+7 indicate, respectively, one, four and seven days after the experimental oil spill. < DL: below detection limit (0.50 ng g^{-1} dry sediment).

The highest PAH concentrations were observed in Area 1, at both the control and impact sites, 24 h after the diesel spill. However, the PAH origin was attributed to different sources. At the control site, the largest contributions were unsubstituted low-molecular-weight (LMW) PAHs and high-molecular-weight (HMW) PAHs, while at the impact site, alkylated PAHs were the largest contributors (Figure 2). This means that biomass and coal

combustion were the main source for the PAHs at the control site and petrogenic input was the main source for the PAHs at the impact site (Figure 3).

Four and seven days after the spill, PAH concentrations were lower at the control sites than at the impact sites, in both experimental areas. Additionally, it was found that impact site 2 (D+4 to D+7) had increased concentrations of PAH, which is related to its petrogenic origin (Figures 2 and 3).

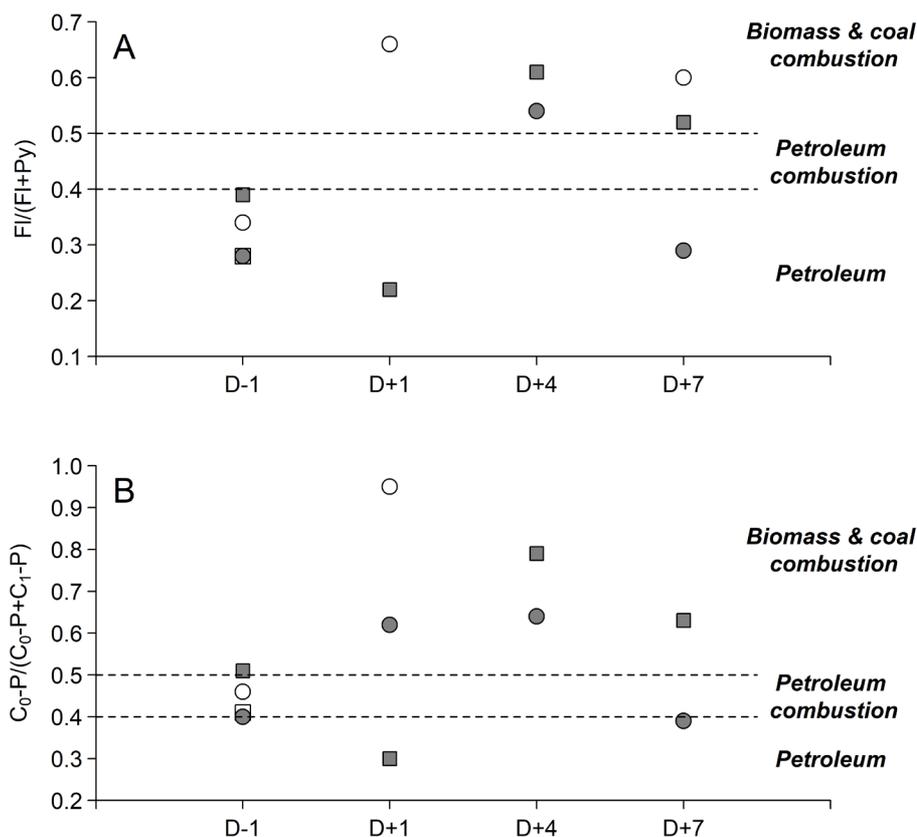


Figure 3. PAHs diagnostic ratios in sediments of control and impact sites located at Area 1 (Control 1 = ○ and Impact 1 = ●) and Area 2 (Control 2 = □ and Impact 2 = ■). D-1 represents one day before the experimental oil spill; D+1, D+4 and D+7 indicate, respectively, one, four and seven days after the experimental oil spill. FI/(FI + Py): ratio between fluoranthene (FI) and pyrene concentrations (Py) (A). $C_0\text{-P}/(C_0\text{-P} + C_1\text{-P})$: ratio between phenanthrene ($C_0\text{-P}$) and alkyl-phenanthrenes ($C_1\text{-P}$) (B). Source of PAHs according to range values to ratios: >0.00–0.40: petrogenic; 0.40–0.50: residues from combustion of petroleum and byproducts; > 0.50: residues from biomass and coal combustion (based on Yunker et al., 2002).

BIOMARKER RESPONSES

Figure 4 shows the means and standard errors of biomarker responses in *S. goodbodyi* individuals in control and impact treatments, from before to after the experimental exposure to diesel oil. Among the biomarkers examined, GPx exhibited

a discernible pattern that suggests its sensitivity to diesel exposure. GPx activity was consistently higher after the spill, particularly at the impact site. CAT activity levels were higher in the control site of experimental Area 1 one day after the spill, which had the highest observed concentrations of total PAH in the sediment (Figure 2).

The activities of SOD, GST, and AChE, as well as the levels of GSH and LPO, varied greatly on different days, both before and after the spill, and did not show a distinct pattern in the control and impact treatments, which means that no pattern could be attributed to exposure (Figure 4).

The permutational analysis of variance (Table 2) showed no significant differences for the main effects and high-order interactions. The main source of variability of most biomarkers (i.e., SOD, CAT, GPx

and LPO) was associated with spatial heterogeneity, and significant differences were observed among areas. Additionally, SOD and GST activities varied according to the interactions between period and area, which indicates that differences between areas vary on a day-to-day basis and are not related to diesel exposure. Additionally, significant complex interactions between days and areas were observed for AChE activity and GSH levels, indicating a short-term, small-scale variability.

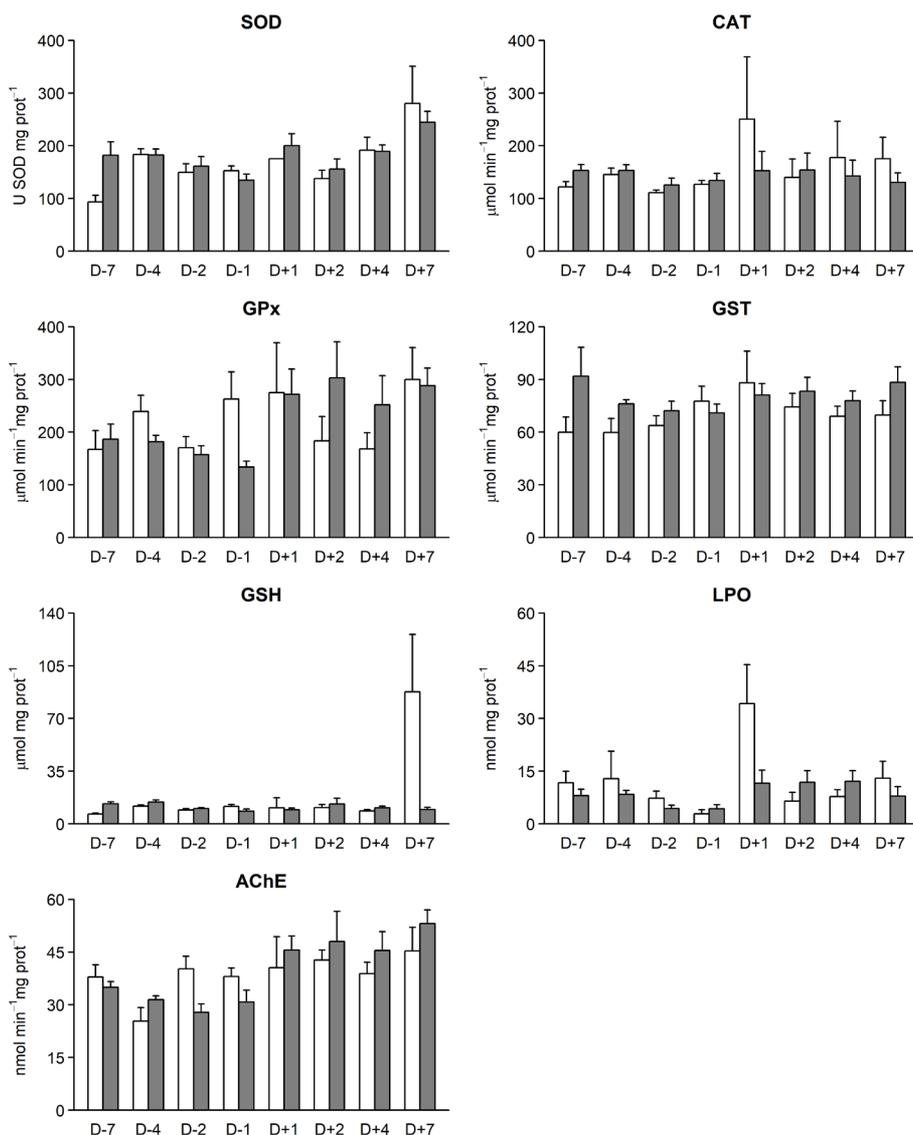


Figure 4. Mean (+Standard Error) activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), acetylcholinesterase (AChE) and levels of reduced glutathione (GSH) and lipid peroxidation (LPO) in *S. goodbodyi* during an experimental spill of diesel. White bars: Control; Grey bars: Impact; D-7, D-4, D-2, and D-1: seven, four, two and one day before of the simulated spill; D+1, D+2, D+4 and D+7: one, four and seven days after of the simulated spill.

Table 2. Permutational analysis of variance (PERMANOVA) of the MBACI model for activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and acetylcholinesterase (AChE), and levels of reduced glutathione (GSH) and lipid peroxidation (LPO).

		SOD		CAT		GPx	
Source	df	MS	<i>Pseudo-F</i>	MS	<i>Pseudo-F</i>	MS	<i>Pseudo-F</i>
Period = P	1	0.9517	3.1906	0.1561	0.3868	1.4482	9.1142
Stress = S	1	0.0888	0.5167	0.0332	0.0287	0.0017	0.0020
Day(P) = D(P)	6	0.2670	4.3247*	0.1570	2.0865	0.2291	2.4530
Area(S) = A(S)	2	0.1900	3.3424	1.1631	6.1058**	0.8456	5.5039**
P × S	1	0.0586	0.1965	0.4531	1.1226	0.9128	5.7445
P × A(S)	2	0.3366	5.9208**	0.4047	2.1246	0.1589	1.0345
D(P) × S	6	0.1452	2.3510	0.0972	1.2912	0.1946	2.0833
D(P) × A(S)	12	0.0621	1.0931	0.0737	0.3868	0.0920	0.5985
		GST		GSH		LPO	
Source	df	MS	<i>Pseudo-F</i>	MS	<i>Pseudo-F</i>	MS	<i>Pseudo-F</i>
Period = P	1	0.2849	0.7669	0.6383	0.4090	4.1979	6.8181
Stress = S	1	0.4257	18.443	0.3159	0.2985	0.2326	0.0451
Day(P) = D(P)	6	0.0299	1.4789	0.5371	0.9402	1.6327	3.0028
Area(S) = A(S)	2	0.0229	0.4367	1.1446	10.820	5.1975	16.018***
P × S	1	0.0464	0.1250	1.5413	0.9876	0.0835	0.1356
P × A(S)	2	0.3739	7.1393**	1.6927	16.002	0.6179	1.9041
D(P) × S	6	0.0864	4.2798*	0.8648	1.5137	1.1205	2.0609
D(P) × A(S)	12	0.0197	0.3762	0.5915	5.5913***	0.5470	1.6858
		AChE					
Source	df	MS	<i>Pseudo-F</i>				
Period = P	1	1.8759	20.1510				
Stress = S	1	0.0001	0.0010				
Day(P) = D(P)	6	0.1081	0.7319				
Area(S) = A(S)	2	0.0787	1.8736				
P × S	1	0.1790	1.9228				
P × A(S)	2	0.0967	2.3029				
D(P) × S	6	0.1166	0.7897				
D(P) × A(S)	12	0.1514	3.6059***				

Significance codes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

DISCUSSION

The pattern of biomarker responses in *S. goodbodyi* was primarily influenced by spatial variation rather than being attributed to the simulated diesel spill. The apparent absence of a contaminating effect may be linked to the low levels of the toxic compounds retained in the sediment after the spill was simulated to elicit a response or to the presence of an efficient repair

system within the organism. Hence, it seems that antioxidant biomarkers and AChE activity in *S. goodbodyi* should not be deemed suitable for oil spill monitoring purposes. However, it should be emphasized that the power of the MBACI model to detect consistent long-term impacts, as evaluated by the interaction between treatments (impact vs. control) and periods (before vs. after), depends on the replication within Control and Impact groups

(Downes et al., 2002). Increasing the number of control and oil-exposed areas in our study would strengthen our conclusions. Nonetheless, there are many challenges in simulating oil spills in several replicated areas in an intertidal environment with limited emerged time.

Many polychaete species have demonstrated a relatively good ability to regulate organic contaminants, including PAHs and pesticides (Dean, 2008). These resistant polychaetes can inhabit contaminated substrates without accumulating similar concentrations of contaminants in their tissues, due to specific regulatory pathways. For example, studies by Rust et al. (2004) showed that *Alitta succinea* and *Alitta virens* are capable of converting PAHs into less toxic metabolites. Driscoll and McElroy (1996) exposed the nereidid *Hediste diversicolor* and the spionid *Marenzelleria viridis* to the same pollutant and found that these species are able to rapidly metabolize hydrocarbons. Similarly, the spionid *Streblospio benedicti* has demonstrated a high tolerance to PAHs in laboratory experiments, not undergoing significant increases in tissue concentrations or apparent mortality effects (Chandler et al., 1997). Therefore, if *S. goodbodyi* has the ability to rapidly regulate hydrocarbon body burdens, it may not be suitable for use as an indicator of early PAH contamination.

Nevertheless, it is important to note that the highest Σ PAH concentration observed in our study, which was 114 ng g^{-1} , is significantly lower (10 to 500 times) than the concentrations reported by Chandler et al. (1997) and Driscoll and McElroy (1996), respectively. Even though our findings indicate that effective diesel contamination occurred in the oil-exposed plots, our results remain well below the threshold concentration of 500 ng g^{-1} , suggested by Notar et al. (2001). The low PAH concentrations observed in the oil-exposed plots can probably be attributed to the sediment granulometric (well-selected fine sand) and dynamics of the studied area, which favored the dispersion of pollutants by intense tidal currents.

Assessing the effects of pollution on benthic organisms is a complex task that cannot be oversimplified. The involvement of multiple molecules and pathways, particularly in oxidative

metabolism, adds a significant complexity to this process, which must be duly considered when utilizing antioxidant responses in ecotoxicology (Benedetti et al., 2015). Enzymatic activities often exhibit considerable fluctuations, which can be attributed to various simultaneous factors beyond exposure to xenobiotic substances. According to Vidal-Liñán and Bellas (2013), the use of biomarkers is limited by its inherent natural variability, which is strongly influenced by environmental and biological factors. Environmental factors include temperature, salinity, food availability, and dissolved oxygen levels, while intrinsic factors encompass the organism's reproductive status. Vasseur and Cossu-Leguille (2003) emphasize that a comprehensive understanding of the biological function of biomarkers is necessary to accurately analyze environmental impact.

The differences observed between most biomarkers in the experimental areas can be attributed to the effect of natural stressors on *S. goodbodyi* individuals. Even slight differences in sea levels among the areas can lead to significant variations in temperature, salinity, dissolved oxygen levels, and food availability, thereby affecting the physiology of *S. goodbodyi* individuals, which primarily inhabit the intertidal zone with limited mobility. According to Vidal-Liñán and Bellas (2013), sessile intertidal organisms are highly susceptible to the effects of air exposure and sudden temperature changes. In their study, these authors highlighted the importance of considering tidal height as a significant factor when assessing basal enzymatic activities, particularly those of GST and CAT.

The MBACI experimental setting was not able to detect a significant response of antioxidant and neurotoxicity biomarkers in *S. goodbodyi* to diesel spills in field conditions. Despite the widespread use of these biomarkers in ecotoxicological studies, we did not observe any significant changes in their activities and levels that could be directly linked to restricted diesel exposure and low hydrocarbon concentrations, as the sediments tested in this study, composed of sandy fractions, had a low capacity to concentrate PAHs after the simulated diesel spill. The observed significant differences primarily arose from spatial variability between the experimental areas. The use of bioassays may reveal relevant

underlying mechanisms of toxicity, isolating specific effects of oil exposure from other confounding factors and confirming potential causal relationships.

We hypothesized that a diesel spill would result in biochemical alterations (induction or inhibition of biomarkers) in *S. goodbodyi*, which would show that this species could be used as a sentinel to monitor PAH contamination in sand beaches. However, our results did not support this hypothesis. Further studies should test other Polychaeta species and simulate oil spills in sedimentary environments composed of fine sediments such as salt marshes and mangroves.

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AUTHOR CONTRIBUTIONS

C.P.O.: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing;

I.C.G.; H.C.S.A.: Formal analysis, Writing - Review & Editing;

C.C.M.: Writing - Review & Editing;

L.S.-N.: Visualization, Writing - Review & Editing;

P.C.L.: Conceptualization, Resources, Supervision, Project administration, Funding acquisition.

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