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Contaminant bioaccumulation and biochemical responses of the bivalve *Scrobicularia plana* and the polychaete *Hediste diversicolor* to ecosystem restoration measures using *Zostera noltei*

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ABSTRACT

A Nature-based Solution (NbS) using *Zostera noltei* transplants was implemented to restore an area historically contaminated with metals and enhance local environmental conditions. However, the benefits of this restoration approach on the health of resident benthic communities remained unclear, considering the time span of one year of implementation. This study evaluated the short-term effects of transplantation by evaluating bioaccumulation and biochemical responses in *Scrobicularia plana* and *Hediste diversicolor*. Overall, the transplanted vegetation played a crucial role in controlling contaminant accumulation, with both species exhibiting lower contaminant levels in vegetated sites compared to bare-bottom sites. The study also revealed species-specific responses to oxidative stress, antioxidant defences and energy budgets, with *H. diversicolor* being more vulnerable to the absence of vegetation than *S. plana*. These findings highlight the critical role of vegetation in improving environmental quality and promoting the health of benthic communities, validating the positive effect of the applied restoration measures.

1. Introduction

Over the past few decades, there has been a significant increase in human population and industrial development in the vicinity of estuaries. This unchecked growth has led to an escalation of estuarine contamination and a subsequent increase of impacted areas, particularly with metal(loid)s, resulting in significant environmental disorders (Chakraborty et al., 2023; Freeman et al., 2019; Kennish, 2002). Throughout the 21st century, laws and regulations have been globally enacted to safeguard, restore, and reverse ecosystem degradation. In Europe, notable examples that contribute to achieving these objectives include the European Union's Water Framework Directive (Directive 2000/60/EC), the Habitats Directive (Directive 92/43/EEC), the Directive on the Assessment and Management of Flood Risks (Directive, 2007/60/EC), as well as the Marine Strategy Framework Directive (Directive, 2008/56/EC). In addition, over time, various methods (physical, chemical, and biological) have been developed, including different treatments (e.g., capping, dredging, and use of nature-based solutions) to restore contaminated degraded areas (Oliveira et al., 2024). However, the most accepted and commonly employed method nowadays is bioremediation (Oliveira et al., 2024). Bioremediation involves the use of living organisms, such as salt marsh plants and seagrasses, to remove, degrade, or isolate contaminants in the environment (Pang et al., 2023; Singh et al., 2023; Sousa et al., 2011). These natural solutions offer several advantages, including the ability to reduce contamination, promote biodiversity, and restore ecosystem functionality (European Union, 2020; Pedersen Zari et al., 2019).

Seagrasses stand out as one of the vegetation types used for

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bioremediation due to their crucial role in remediating degraded aquatic ecosystems. They can retain contaminants, while promoting sediment stabilization, and can be allies against climate change (Rifai et al., 2023; Unsworth et al., 2022) by absorbing carbon dioxide from the atmosphere and storing it in their tissues and sediment – blue carbon (Koswara et al., 2023; Nurafni et al., 2022; Sousa et al., 2019).

Various initiatives and strategies have emerged **to highlight the importance of** ecosystem restoration and promote it worldwide, including the United Nations Decade on Ecosystem Restoration (2021–2030) (Resolution A/RES/73/284) and the new EU Nature Restoration Law (Regulation, 2024/1991). While these initiatives aim to protect, recover, and restore a wide range of ecosystems, they also recognize the ecological importance of seagrass and its role in biodiversity conservation and the delivery of essential ecosystem services (Rifai et al., 2023). Based on these premises, a transplantation of the seagrass *Zostera noltei* was used as a restoration method for a historically degraded and metal contaminated inner bay of a Portuguese coastal lagoon. The choice of this seagrass species was based on its historical presence in the contaminated area, as well as the potential ecological benefits of its presence, including promoting local biodiversity and productivity (Crespo et al., 2023; Dolbeth et al., 2011).

Data from a successful *Z. noltei* restoration process, initiated in 2020, demonstrated that *Z. noltei* followed a normal seasonal pattern in biomass and effectively adapted to different contaminant loads. No significant effects were observed on its photosynthetic performance or metabolic response to the adverse effects of metal(loid)s at the restored site within just one year (Oliveira et al., 2025a, 2025c). Over time, the transplanted seagrass expanded and increased in cover, and by 2024, numerous spontaneous patches were observed around the transplanted site, indicating the natural recovery of the area through sexual reproduction (seed production and dispersal) (Oliveira et al., 2025a). Additionally, the seagrass was able to phytostabilize contaminated sediments, reduce the bioavailable fraction of contaminants (Oliveira et al., 2023) and enhance local benthic biodiversity in the historically contaminated areas following transplantation (Crespo et al., 2023). However, the benefits of this restoration approach on the health of resident communities have not been investigated yet.

While the conventional approach to assessing local fauna conditions relies on analysing total contaminant loads (bioaccumulation), this can be complemented by examining biological effects (biomarkers), such as oxidative stress profile and the energy budget (Hook et al., 2014). These biomarkers of effects are valuable tools for environmental risk assessment, as they provide sensitive and early-warning responses at a sub-organismal level that reflect critical molecular processes (van der Oost et al., 2003). Building upon this framework, the present study introduces a novel approach by combining seagrass transplantation with an ecotoxicological assessment of resident benthic organisms, providing a more holistic evaluation of restoration success in historically contaminated sites. Specifically, the short-term effects of seagrass restoration on contaminant bioaccumulation and biochemical responses, including oxidative stress profile and energy budget, are investigated in two key benthic species: *Scrobicularia plana* (a bivalve) and *Hediste diversicolor* (a polychaete). These species, recognized for their ecological significance and high productivity in transitional ecosystems (Dolbeth et al., 2011), serve as bioindicators to assess both pollutant exposure and potential biological recovery in restored habitats.

2. Methodology

2.1. Study area

The Ria de Aveiro, a shallow coastal lagoon in the central region of Portugal, features an artificial tidal inlet and is influenced by tides and wind (Pinheiro et al., 2020). The lagoon has salt marshes and seagrass meadows, offering diverse ecosystem functions protected by laws such as the Habitats Directive (Directive 92/43/EEC) due to their ecological significance. However, during the second half of the 20th century, industrial effluents, notably from the Estarreja Chemical complex, generated a well-defined environmental contamination gradient (e.g., mercury and arsenic) within the 2 km^2 inner bay known as Laranjo Bay (Coelho et al., 2005; Oliveira et al., 2025b, 2025c) and significant environmental degradation throughout the lagoon. Recent studies indicate a slight recovery of this coastal lagoon as a whole, particularly in its seagrass meadows areal extent and cover (Sousa et al., 2019), suggesting a natural restoration process, although Laranjo Bay remains degraded. This recovery is attributed to measures such as the European Union's Water Framework Directive (Directive, 2000/60/EC) and other environmental protection directives, as well as classification of the coastal lagoon under the Natura 2000 network (code PTZPE0004) and its designation as a Special Protection Area (Portuguese Decree-Law No. 384-B/99).

2.2. Study site description and sampling method

To enhance the recovery rate of the most impacted area and increase local biodiversity, a nature-based restoration technique involving *Z. noltei* seagrass transplants was carried out in the Laranjo Bay in 2020 (Crespo et al., 2023; Oliveira et al., 2023, 2025a). The transplant method involved the collection of compact patches of *Z. noltei* and sediments in a single operation, ensuring no damage to the rhizomes, roots, leaves and rhizosphere (Costa et al., 2022), and allows the seagrass time to adapt to its new environment. These patches were gathered from a Donor Meadow (DM) located 3 km downstream from Laranjo Bay (Cardoso et al., 2013; Fonte et al., 2023; Oliveira et al., 2025b), an area considered non-contaminated, as its contamination levels fall within the system's natural baseline (Cachada et al., 2019). The transplanted patches were arranged in a mosaic structure within the target area. After one year, the meadows grew through the empty spaces of the mosaic, and a compact meadow was visible at the Transplant Meadow (TM) site (Oliveira et al., 2025a).

Hediste diversicolor and *Scrobicularia plana* were chosen as bioindicators of the short-term effects of restoration on the overall health status of resident benthic communities due to their low mobility and continuous exposure to local conditions, as well as for their presence across the different areas under study. For that, the biochemical responses of these species were evaluated between the Transplant (T – contaminated) and the Donor (D – non-contaminated) areas, as well as between vegetated (M – meadow) and non-vegetated (B – bare-bottom) habitats at two distinct time points, during the summer of 2021 (1st campaign) and 2022 (2nd campaign) (Fig. 1) to evaluate the evolution of the overall condition of these species over time. Specifically, comparisons over time were made between different habitats within the same area (DM vs. DB and TM vs. TB) to examine the effects of vegetation, and between equivalent habitats in the Donor and Transplant areas (DM vs. TM and DB vs. TB) to evaluate the impact of the historical contamination.

At each site, fifteen similarly sized individuals of *S. plana* and *H. diversicolor* ($n = 10$ for biochemical analysis and $n = 5$ for bioaccumulation analysis), were collected and stored in a cooled container until reaching the laboratory. At the laboratory, *S. plana* shells were removed, and both *S. plana* and *H. diversicolor* were washed with distilled water to remove sediment. Samples for biochemical analysis were immediately frozen in liquid nitrogen and stored at −80 °C until further processing, while samples for bioaccumulation analysis were freeze-dried for at least 48 h using a Unicryo MC-4 L-60 C freeze-dryer.

Additionally, at these four sites (DM, DB, TM, and TB), physicochemical parameters (salinity, temperature, pH and dissolved oxygen) were measured, and five sediment samples ($n = 5$) were collected in the summer of 2021 to measure the concentrations of metal(loid)s (arsenic [As], cadmium [Cd], copper [Cu], mercury [Hg], nickel [Ni], lead [Pb], tin [Sn], titanium [Ti], uranium [U], and zinc [Zn]), organic matter and

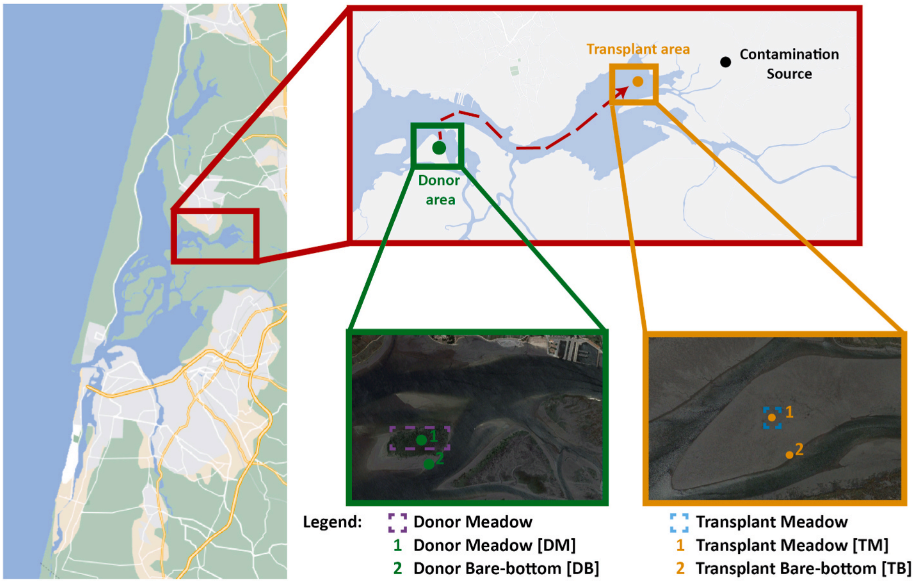

Fig. 1. Schematic plan of the Ria de Aveiro (Portugal) and Laranjo Bay, with the respective sampling sites (Donor Meadow [DM], Donor Bare-bottom [DB], Transplant Meadow [TM], and Transplant Bare-bottom [TB]) in Donor and Transplant areas.

fine fraction (<63 μm). Sediment quantification was only performed in summer 2021 samples, as the concentrations are known to remain stable over time (Oliveira et al., 2023, 2025b). The sediments were collected from the top 5 cm layer and stored in bags. In the laboratory, the sediments were divided into two aliquots. One aliquot was freeze-dried, and subsamples were taken to quantify metal(loid) concentrations. The other aliquot was dried, disaggregated and homogenized to analyse organic matter content and fine fraction.

2.3. Sediment characterization

The organic matter content was determined by loss on ignition (4 h at 500 °C), and the fine fraction analysis of the sediments was determined by wet sieving following the methodology described by Oliveira et al. (2018). Total Hg concentrations in sediments at each site were determined using a LECO AMA-254 (Advanced Mercury Analyzer) following the method described by Costley et al. (2000), with daily performance assessments through Certified Reference Materials (CRM) analysis at the beginning and end of each day. For total Hg in sediments, BCR-277R reference material was used, yielding a recovery rate of 95–112 % (n = 10) within the corresponding confidence intervals. All samples were analysed in triplicate, and the coefficient of variation was always less than 10 %. The remaining metal(loid)s (As, Cd, Cu, Ni, Pb, Sn, Ti, U, and Zn) were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo X Series) following the US EPA 3051 protocol, with daily performance verification similar to the LECO AMA-254. To ensure the accuracy and reliability of the results, parallel blank samples and certified reference materials (ERM-CC141 and BCR-143R) were digested to monitor and control for potential contamination, yielding recovery rates of 96–116 %.

2.4. Contaminant bioaccumulation

The bioaccumulation of contaminants in the benthic species was quantified only for Hg. This decision was based on Oliveira et al. (2025a), who found that Hg was the only metal(loid) in the study area that significantly exceeded the reference values set by the Canadian Interim Sediment Quality Guidelines ($Hg = 0.70\ mg\ kg^{-1}$ dry weight) (CCME, 2002), potentially causing adverse effects on biota. The methodology used for total Hg quantification was the same as for the sediment samples (LECO AMA-254). The reference material used for the benthic organisms was TORT-3 CRM, with recovery rates of 90–98 % (n = 10), within the respective confidence interval. All samples were analysed in triplicate, with a coefficient of variation consistently below 10 %.

2.5. Biochemical analysis

For biochemical analysis, the organisms (whole-body) were divided into three aliquots (≈100 mg fresh weight): for the evaluation of the energy available, the energy consumption, and the oxidative stress profile (antioxidant defences and membrane damage). Subsequently, aliquots were homogenized in specific buffers tailored for each analysis, using a Potter–Elvehjem homogenizer. For the energy available (proteins (PROT), total content of lipids (LIP) and carbohydrates (CH)), homogenization (1:2 ratio) was performed with distilled water. The resulting mixture was then centrifuged at 1000×*g* for 5 min at 4 °C, followed by quick freezing in liquid nitrogen and storage at −80 °C for later evaluation. For the assessment of energy consumption (Electron Transport System (ETS)), homogenization (1:5 ratio) was performed in 0.1M Tris-HCl pH 8.5 buffer with 15 % (w/v) Poly Vinyl Pyrrolidone, 153 μM $MgSO_4$, and 0.2 % (w/v) Triton X-100. The homogenate was subsequently centrifuged at 4 °C for 10 min at 3000×*g*, frozen in liquid nitrogen, and stored at −80 °C until ETS analysis.

Regarding the oxidative stress profile, the organisms were homogenized (1:6 ratio) in a chilled potassium phosphate buffer (0.1M, pH 7.4). Afterwards, the homogenate was divided in two aliquots: for lipid peroxidation (LPO) and to obtain the post mitochondrial supernatant (PMS). For LPO, 100 μL of homogenate was stored with 10 μL of 4 % butylatedhydroxytoluene and frozen in liquid nitrogen. PMS was obtained by centrifugation in a refrigerated centrifuge at 4 °C for 20 min at 12000×*g*. Aliquots of PMS for the assessment of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione s-transferases (GST), and total glutathione (GSHt) were collected, promptly frozen in liquid nitrogen, and then stored at −80 °C until they underwent analysis. Additionally, PMS for GSHt was prepared by precipitating the non-soluble PMS protein in 12 % TCA (1:2 dilution). Briefly, PMS tubes were incubated at 4 °C for 60 min, then centrifuged at 12000×*g* for 5 min at 4 °C. The supernatant was collected, frozen in liquid nitrogen and stored at −80 °C until GSHt analysis.

2.5.1. Energetic budget

The available energy reserves were quantified following the methodology outlined by De Coen and Janssen (2003, 1997), by converting them to energetic equivalents using enthalpy combustion (24 kJ g^{-1} for proteins, 39.5 kJ g^{-1} for lipids, and 17.5 kJ g^{-1} for carbohydrates). Protein content was determined utilizing the Biuret method, following the methodology of Gornall et al. (1949). Bovine serum albumin (BSA) was used as a standard and measured at 540 nm. Total lipids were extracted according to the protocols of Bligh and Dyer (1959) and quantified at 370 nm using tripalmitin as a standard (Novais et al., 2013). Total carbohydrate content extraction was carried out with 5 % phenol and concentrated sulfuric acid, with quantification at 492 nm using glucose as the standard. The results of energy reserves were expressed in millijoules (mJ) per milligram of fresh weight (FW).

For the assessment of energy consumption, the ETS activity was determined following the protocol of De Coen and Janssen (2003, 1997). The reaction was initiated by introducing INT (p-IodoNitroTetrazolium), leading to the production of formazan. Formazan formation was measured at 490 nm every 25 s for 10 min at 20 °C. The quantity of formazan generated was calculated using an extinction coefficient (ε) of 15900 M^{-1} cm^{-1}. Electron transport system activity provides an average of the maximum oxygen uptake rate (Fanslow et al., 2001) and is based on the theoretical stoichiometric ratio, where 2 μmol of formazan formed corresponds to 1 μmol of oxygen consumed in the ETS (De Coen and Janssen, 1997). The oxygen (O_2) consumed was then converted into energy equivalents utilizing the specific oxygenthalpic equivalents for an average lipid, protein and carbohydrate mixture of 484 kJ mol^{-1} O_2 (Gnaiger, 1983). The results were expressed as mJ h^{-1} mg^{-1} of FW.

2.5.2. Oxidative stress profile

Catalase activity was determined according to the methodology of Claiborne (1985) and Giri et al. (1996). The consumption of hydrogen peroxide (H_2O_2) was measured at 240 nm for 3 min, at 10 s intervals at 25 °C, calculated using an extinction coefficient of 43.5 M^{-1} cm^{-1}, and expressed as mmol of H_2O_2 consumed min^{-1} mg^{-1} of protein. The activity of the GPx followed the method described by Mohandas et al. (1984) modified by Athar (1998), while GR was determined according to the Cribb et al. (1989) method. Both activities measured the oxidation of NADPH at 340 nm, during 5 min at intervals of 30 s at 25 °C and were expressed as nmol of $NADP^+$ min^{-1} mg^{-1} protein ($\varepsilon = 6.22 \times 10^3$ M^{-1} cm^{-1}). The activity of the enzyme GSTs was assessed following Habig et al. (1974) methodology using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Absorbance was registered at 340 nm every 30 s for 5 min and activity was expressed as nmol of GS-DNB formed min^{-1} mg^{-1} protein ($\varepsilon = 9.6 \times 10^3$ M^{-1} cm^{-1}). Total GSH was determined according to the methodology of Vandeputte et al. (1994). The formation of TNB resulting from this methodology is directly proportional to the sum of the concentrations of reduced glutathione (GSH) and oxidized glutathione (GSSG) present in the sample. The absorbance was read at 415 nm for 7 min at 30 s intervals at 25 °C. The formation of TNB concentration was expressed as nmol TNB conjugated min^{-1} mg^{-1} protein ($\varepsilon = 14.1 \times 10^3$ M^{-1} cm^{-1}). Lipid peroxidation was determined spectrophotometrically using the methodology of Bird and Draper (1984) and adapted by Wilhelm Filho et al. (2001). The thiobarbituric acid reactive substances (TBARS) are the equivalents of the malondialdeyde (MDA) concentration and were measured at 535 nm. MDA concentration was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as μmol of TBARS formed mg^{-1} of protein. Protein content was determined using the Biuret method, as previously described in the energetic budget section (2.5.1).

2.6. Statistical analysis

All results were statistically tested using the permutation multivariate analysis of variance (PERMANOVA). A two-way crossed design was employed to evaluate differences in the sediment characteristics, considering the area (Donor and Transplant) and habitat (vegetated and non-vegetated) as the factors. For all other results, a three-way crossed design was applied, with area (Donor and Transplant), habitat (vegetated and non-vegetated), and time (1st and 2nd campaign) as the factors. Prior to the PERMANOVA, each factor was tested for its dispersion around the centroid using the PERMDISP analysis. All analyses were conducted with the PRIMER v6 software with PERMANOVA + add-on (Anderson et al., 2008). Significant differences were assigned for a p-value ≤ 0.05. Spearman correlations were calculated in SPSS V29 software between total Hg in sediments, total Hg in *S. plana* and *H. diversicolor*.

3. Results

3.1. Site characterization

Physicochemical parameters showed significant differences across areas, habitats, and times. Lower salinity ($F = 518.14$, $p = 0.001$), temperature ($F = 2464$, $p = 0.001$), pH ($F = 147.19$, $p = 0.001$), and dissolved oxygen ($F = 20.568$, $p = 0.001$ in percentage; $F = 39.061$, $p = 0.001$ in mg/L) were observed in bare-bottom sites compared to their respective meadows in both campaigns (Supplementary Tables 1 and 2). However, in the second campaign, salinity, temperature, and dissolved oxygen levels were higher than those recorded in the first campaign.

Higher percentages and significant differences ($F = 77.436$, $p = 0.001$) of organic matter were observed in the Donor area (DM and DB sites), ranging from 8.5 to 10.6 %, compared to the Transplant area (TM and TB sites), where organic matter percentages ranged between 4.8 and 7.7 % (Table 1). The percentage of fine fraction varied randomly; however, when comparing the sites within each area (DM vs. DB and TM vs. TB), significant differences were observed ($F = 17.991$, $p = 0.002$), with lower fine fraction observed in bare-bottom sites. For the metal(loid)s As, Cd, Cu, Hg, Sn, and Zn, higher concentrations were found in the Transplant area (TM and TB sites) compared to the Donor area (DM and DB sites), and within the Transplant area (TM vs. TB), the bare site (TB) had higher concentrations of these metal(loid)s. The remaining metals (Ni, Pb, Ti, U) varied randomly across the four sites. Significant area-habitat interactions ($p \leq 0.05$) were observed for all metal(loid)s.

3.2. Total Hg in *S. plana* and *H. diversicolor*

Total Hg concentrations in *S. plana* ranged from 0.21 to 0.75 mg kg^{-1}

Table 1
Organic matter (%), fine fraction (%) and metal(loid) concentrations (mg kg^{-1}) in surface sediments from the Donor sites (DM, DB) and Transplant sites (TM, TB). Values are presented as means ± standard deviation of 5 replicates. Superscript letters indicate statistically significant differences between sites at the 95 % confidence level.

		DM	DB	TM	TB
Organic matter (%)		$10 \pm 0^{(a)}$	$9.5 \pm 0.7^{(a)}$	$5.9 \pm 1.2^{(b)}$	$6.8 \pm 1.0^{(b)}$
Fine fraction (%)		$23 \pm 2^{(a)}$	$19 \pm 1^{(b,c)}$	$21 \pm 2^{(a,c)}$	$18 \pm 2^{(b)}$
Metal (loid)s (mg kg^{-1})	**[As]**	$20 \pm 1^{(a)}$	$18 \pm 1^{(b)}$	$24 \pm 0^{(c)}$	$51 \pm 5^{(d)}$
	[Cd]	$0.34 \pm 0.01^{(a)}$	$0.33 \pm 0.04^{(b)}$	$0.40 \pm 0.06^{(c)}$	$0.82 \pm 0.19^{(d)}$
	[Cu]	$18 \pm 0^{(a)}$	$15 \pm 0^{(b)}$	$19 \pm 1^{(c)}$	$48 \pm 11^{(d)}$
	[Hg]	$0.55 \pm 0.21^{(a)}$	$0.57 \pm 0.01^{(a)}$	$1.9 \pm 0.4^{(b)}$	$9.1 \pm 1.8^{(c)}$
	[Ni]	$16 \pm 0^{(a)}$	$15 \pm 0^{(b)}$	$14 \pm 1^{(b)}$	$18 \pm 1^{(c)}$
	[Pb]	$23 \pm 0^{(a)}$	$21 \pm 1^{(b)}$	$20 \pm 1^{(c)}$	$33 \pm 4^{(d)}$
	[Sn]	$2.8 \pm 0.3^{(a)}$	$2.6 \pm 0.3^{(b)}$	$3.1 \pm 0.2^{(a,c)}$	$3.4 \pm 0.3^{(c)}$
	[Ti]	$1186 \pm 164^{(a)}$	$1150 \pm 29^{(a)}$	$916 \pm 122^{(b)}$	$1033 \pm 75^{(a,b)}$
	[U]	$3.5 \pm 0.2^{(a)}$	$2.8 \pm 0.1^{(b)}$	$2.7 \pm 0.4^{(b)}$	$3.2 \pm 0.1^{(c)}$
	[Zn]	$136 \pm 3^{(a)}$	$123 \pm 4^{(b)}$	$143 \pm 8^{(a)}$	$234 \pm 24^{(c)}$

in the Donor area and 0.60–2.68 mg kg^{-1} in the Transplant area, while *H. diversicolor* showed concentrations from 0.21 to 0.39 mg kg^{-1} in the Donor area and 0.84–1.95 mg kg^{-1} in the Transplant area (Table 2). *Scrobicularia plana* exhibited Hg levels 2–2.5 times higher at the contaminated vegetated site (TM) compared to the reference site (DM), whereas *H. diversicolor* had concentrations 3.5–4 times higher. In bare-bottom sites, *S. plana* showed a 3-fold increase and *H. diversicolor* a 4–5-fold increase at TB, where both species recorded the highest Hg concentrations. These findings resulted in significant differences in Hg levels for the area-habitat interaction in both species (Table 3 and pairwise comparisons detailed in Supplementary Table 3). Additionally, a strong positive correlation was observed between total Hg levels in the sediment and the body burdens of *S. plana* (r = 0.75) and *H. diversicolor* (r = 0.89), reflecting the gradient found in the sediments (Table 1).

3.3. *Biochemical status*

3.3.1. *Energetic budget*

For *S. plana*, PROT levels showed differences only between sampling times (Table 4), with higher levels observed during the first campaign (Fig. 2). Total lipid content had high dispersion, and no significant trends were observed across areas, habitats, or sampling times. Carbohydrate content had generally higher levels in Donor compared to Transplant (Fig. 2). However, their differences depended on the habitat (significant interaction, Table 4), being only significant for the non-vegetated areas (Supplementary Table 4). Energy consumption varied significantly by area, with higher activity observed in the Transplant area. There was also a significant interaction between time and habitat (Table 4), with evidence of higher energy consumption in non-vegetated habitats (DB and TB) compared to vegetated ones (DM and TM), particularly during the first campaign (Supplementary Table 4).

In *H. diversicolor*, the area always influenced the energy budget (Fig. 3). The significance of this result could either be due to the area effect alone, as seen for LIP and ETS with generally higher values for the Transplant area (Fig. 3) or dependent on habitat for CH and even on habitat and time for PROT (Table 5, significant interactions). PROT levels showed a complex variation pattern and significance of their pairwise comparisons (Supplementary Table 5), with generally higher levels in the Donor area, yet with higher PROT levels at DB during the first campaign and at TB during the second (Fig. 3). Additionally, in the second campaign, DM had higher PROT levels than DB, and TB had higher levels than TM. Carbohydrate content showed higher levels observed in the Transplant area, specifically in the non-vegetated habitat (TB site). Along with the energy consumption for the Transplant area, as mentioned above, differences per habitat were also found (Table 5), with greater activity in non-vegetated habitats (Fig. 3).

3.3.2. *Oxidative stress profile*

For *S. plana*, CAT activity and LPO levels were significantly higher in the Transplant area (Fig. 4, Table 4). For all other parameters, all other factors, e.g., area, time and habitat, had an effect that could be combined (significant interactions) or in isolation (Table 4). For instance, both GPx and GSHt activities showed a significant 3-factor interaction. For GPx, large dispersion was observed, with greater variation seen in the second campaign. Notably, higher activity was evident in vegetated sites (DM and TM) during the second campaign, and DM showed higher activity compared to DB in this campaign (Fig. 4, Supplementary Table 4). For GSHt, higher values were observed at DM and DB sites during the second campaign, and DB displayed higher levels than DM. Additionally, TM had the highest values in the first campaign compared to DM, while DB showed the highest levels in the second campaign among the non-vegetated sites. GR activity exhibited higher activity at DM and DB sites during the second campaign (Fig. 4, Supplementary Table 4), whereas these sites showed lower activity during the first campaign compared to TM and TB. Additionally, in non-vegetated habitats, especially at DB, GR activity was significantly higher in the second campaign than in the first. Furthermore, non-vegetated habitats consistently showed greater GR activity than vegetated habitats, particularly during the second campaign. For GST, a higher activity was observed in the first campaign (Fig. 4). There was also a significant area-habitat interaction, with higher activity in the DB site compared to TB and DM (Supplementary Table 4).

Table 2
Total mercury (Hg) concentrations in whole bodies of *Scrobicularia plana* and *Hediste diversicolor* at Donor sites (DM, DB) and Transplant sites (TM, TB). Values represent the mean ± standard deviation for 10 benthic species replicates.

Sites	Scrobicularia plana (mg kg^{-1})		Hediste diversicolor (mg kg^{-1})	
	1st campaign	2nd campaign	1st campaign	2nd campaign
DM	0.32 ± 0.11	0.42 ± 0.13	0.25 ± 0.04	0.28 ± 0.05
DB	0.57 ± 0.18	0.66 ± 0.08	0.26 ± 0.02	0.34 ± 0.05
TM	0.80 ± 0.20	0.86 ± 0.08	0.91 ± 0.07	1.1 ± 0.1
TB	1.8 ± 0.9	1.9 ± 0.8	1.1 ± 0.1	1.7 ± 0.3

Table 3
Summary of significant terms using Total Hg in *Scrobicularia plana* and *Hediste diversicolor* as dependent variables, with area, habitat, and time as explanatory variables.

Dependent variable	Significant terms	d.f.	Pseudo-F	P (perm)
Total Hg in *S. plana*	Area X Habitat	1	6.364	0.017
Total Hg in *H. diversicolor*	Time X Area X Habitat	1	4.754	0.032

Table 4
Summary of significant terms for *Scrobicularia plana* using all biochemical parameters as dependent variables and area, habitat, and time as explanatory variables. PROT - Total protein content; LIP - Total lipid content; CH - Total carbohydrate content; ETS - Electron Transport System Activity; CAT - Catalase activity; GPx - Glutathione peroxidase activity; GR - Glutathione reductase activity; GST - Glutathione-S-transferase activity; GSHt - Total glutathione content; LPO - Lipid peroxidation.

Parameters	Dependent variable	Significant terms	d.f.	Pseudo-F	P (perm)
Energy budget	**PROT**	Time	1	17.889	0.002
	LIP	Non-significant			>0.050
	CH	Area X Habitat	1	3.886	0.046
	ETS	Area	1	47.810	0.001
		Time x Habitat	1	7.975	0.005
Oxidative stress profile	**CAT**	Area	1	32.884	0.001
	GPx	Time X Area X Habitat	1	5.350	0.019
	GR	Time x Area	1	19.234	0.001
		Time x Habitat	1	6.356	0.011
	GST	Time	1	11.094	0.002
		Area X Habitat	1	6.640	0.014
	GSHt	Time X Area X Habitat	1	4.522	0.038
	LPO	Area	1	17.134	0.001

For *H. diversicolor,* significant interactions were found for all oxidative stress parameters, indicating that differences depended on the combination of at least 2 factors (Table 5, pairwise comparisons in Supplementary Table 5). CAT, GSHt and LPO had a time-area-habitat significant interaction, while for all others there was a 2-factor interaction. For CAT, higher activity occurred at the DM site in the second campaign. Additionally, differences were observed between Donor and Transplant areas during the first campaign for both habitats, and in the second campaign for the non-vegetated habitat (Fig. 5). CAT activity was also higher at the DB site compared to DM in the first campaign and higher at TB compared to TM in the second campaign. For GPx, higher activity was observed across all sites during the first campaign.

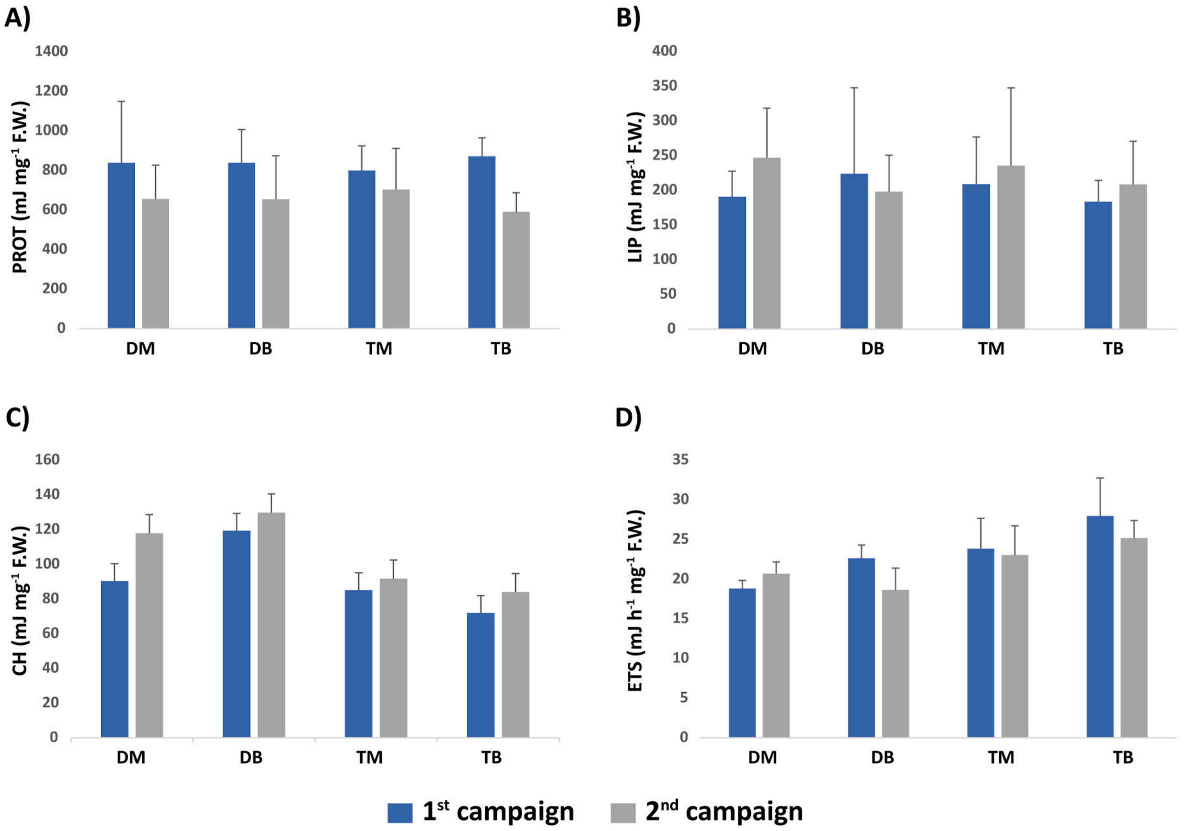

Fig. 2. Energy-related parameters (energy available and energy consumed) *in Scrobicularia plana* at Donor (D) and Transplant (T) areas in vegetated (M) and non-vegetated (B) habitats across two sampling campaigns. Columns represent the mean (n = 10), and error bars indicate the standard error. DM - Donor Meadow; DB - Donor Bare-bottom; TM - Transplant Meadow; TB - Transplant Bare-bottom; PROT - Total protein content; LIP - Total lipid content; CH - Total carbohydrate content; ETS - Electron Transport System Activity. Significant terms and pairwise comparisons among factors are available in Table 4 and Supplementary Table 4.

However, during this campaign, the DM and DB sites had lower activity compared to the TM and TB sites. A higher activity was also found in TM compared to DM and TB sites. GR activity was higher at the Transplant than at Donor sites, especially during the first campaign, due to the low activity at the DM site. A higher activity was also observed in DB and TM compared to DM sites. GST showed higher activity in the second campaign at non-vegetated sites compared to vegetated ones. TB also showed higher activity than TM and DB, DB higher than DM, and DM higher than TM. GSHt in non-vegetated sites (DB and TB) had lower values in the first campaign, while vegetated sites (DM and TM) showed higher values during the same period. In the second campaign, differences between the Donor and Transplant areas were observed only in non-vegetated sites, with TB showing lower values than DB. Additionally, within the Donor area, DM had higher values in the first campaign, whereas DB had higher values in the second campaign. For Transplant sites (TM and TB), differences appeared only in the second campaign, with TB showing higher values. For LPO, the DB site exhibited higher levels in the second campaign and TM in the first. Additionally, differences between the Donor and Transplant areas were only observed in the first campaign for both habitats, with higher levels in TM and TB compared to DM and DB, respectively. Furthermore, when comparing DM with DB and TM with TB, non-vegetated areas consistently displayed higher levels.

4. Discussion

4.1. Site characterization

Metal(loid)s' concentrations in sediments varied across sites, with As, Cd, Cu, Hg, Sn, and Zn showing a clear upward trend toward the contamination historical source at Laranjo Bay. Despite this persistent gradient, a comparative analysis with the findings of Ereira et al. (2015), García-Seoane et al. (2016), and Oliveira et al. (2018) reveals a decreasing trend in the concentrations of various metal(loid)s in surface sediment layers over the years since the cessation of discharges. This suggests a natural attenuation process, as no active human remediation interventions have been implemented in the area. Geochemical variables such as organic matter and fine fraction also influenced metal(loid)s concentrations. However, according to Stoichev et al. (2020, 2019), the proximity to the contamination source still plays a significant role in this concentration gradient in Laranjo Bay for most of the metal(loid)s under study.

Among the metal(loid)s analysed, total Hg concentrations in sediments from the Transplant sites (TM and TB) significantly exceed the reference values set by the Canadian Interim Sediment Quality Guidelines for Hg (0.70 mg kg^{-1} dry weight) (CCME, 2002), corroborating previous findings (Oliveira et al., 2025a). At the Transplant area, the habitat with vegetation (TM) had 2.5 times higher concentrations, while the bare one exhibited levels 13 times higher than the reference values. This indicates that the sediments remain heavily contaminated, potentially leading to adverse effects on biota, especially in bare mud sites.

Despite the high Hg concentrations found in Transplant sites, recent studies have shown that the introduction of *Z. noltei* in this contaminated area has significantly reduced bioavailable/labile Hg levels in interstitial waters shortly after transplantation (Oliveira et al., 2023). Additionally, the presence of salt marsh plants in nearby regions can decrease Hg levels in sediments by accumulating it in their tissues, such as roots (Figueira et al., 2012; Oliveira et al., 2022; Sousa et al., 2008), making it less available to benthic species and across the trophic chain. This is a positive outcome for the benthic species, as it reduces potential adverse

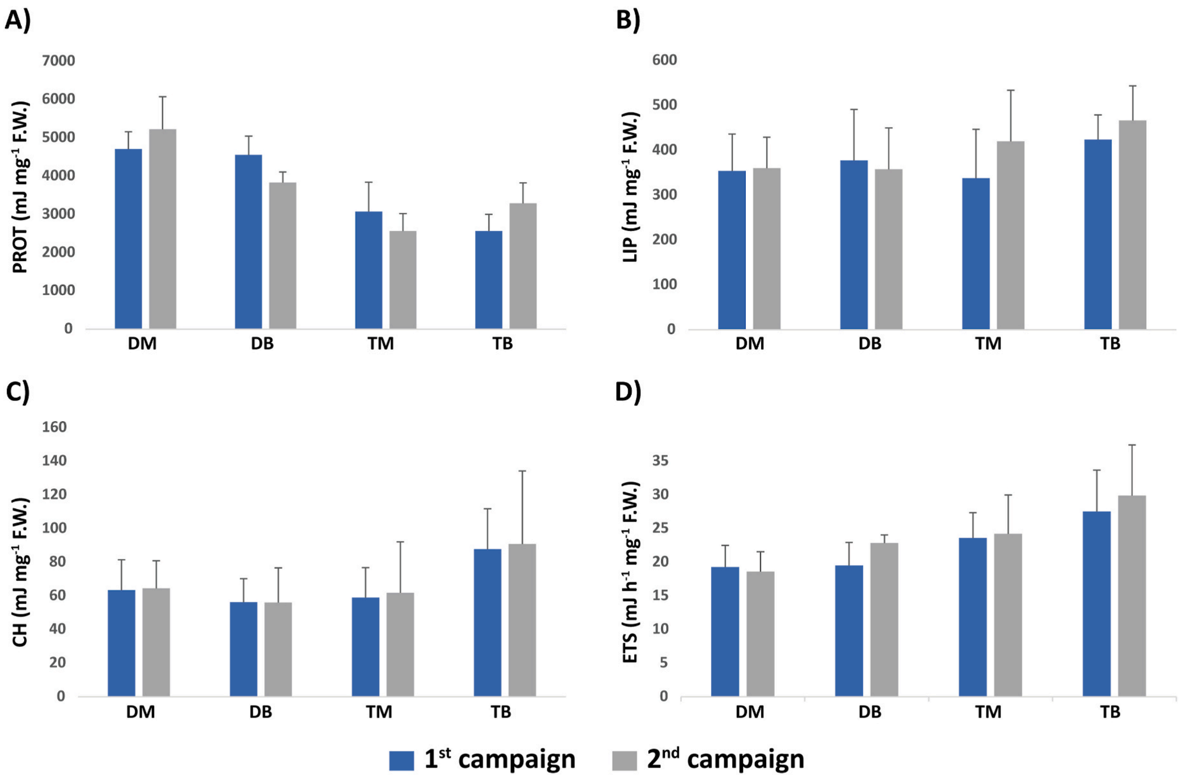

Fig. 3. Energy-related parameters (energy available and energy consumed) in *Hediste diversicolor* at Donor (D) and Transplant (T) areas in vegetated (M) and non-vegetated (B) habitats across two sampling campaigns. Columns represent the mean (n = 10), and error bars indicate the standard error. DM - Donor Meadow; DB - Donor Bare-bottom; TM - Transplant Meadow; TB - Transplant Bare-bottom; PROT - Total protein content; LIP - Total lipid content; CH - Total carbohydrate content; ETS - Electron Transport System Activity. Significant terms and pairwise comparisons among factors are available in Table 5 and Supplementary Table 5.

Table 5
Summary of significant terms for *Hediste diversicolor* using all biochemical parameters as dependent variables and area, habitat, and time as explanatory variables. PROT - Total protein content; LIP - Total lipid content; CH - Total carbohydrate content; ETS - Electron Transport System Activity; CAT - Catalase activity; GPx - Glutathione peroxidase activity; GR - Glutathione reductase activity; GST - Glutathione-S-transferase activity; GSHt - Total glutathione content; LPO - Lipid peroxidation.

Parameters	Dependent variable	Significant terms	d.f.	Pseudo-F	P (perm)
Energy budget	**PROT**	Time X Area X Habitat	1	21.973	0.001
	LIP	Area	1	5.350	0.016
	CH	Area X Habitat	1	9.906	0.004
	ETS	Area	1	32.587	0.001
		Habitat	1	10.282	0.003
Oxidative stress profile	**CAT**	Time X Area X Habitat	1	4.905	0.033
	GPx	Time x Area	1	4.500	0.036
		Area X Habitat	1	5.839	0.025
	GR	Time x Area	1	5.823	0.017
		Area X Habitat	1	16.941	0.001
	GST	Time x Habitat	1	180.390	0.001
		Area X Habitat	1	120.980	0.001
	GSHt	Time X Area X Habitat	1	18.741	0.001
	LPO	Time X Area X Habitat	1	5.908	0.021

effects by lowering Hg accumulation in their tissues. Over time, this reduction in bioavailable/labile Hg may help mitigate the stress that the sediment contaminant historical load could impose on benthic species in the area.

4.2. *Total Hg in S. plana and H. diversicolor*

The observed Hg body burdens clearly reflected an increasing gradient toward the contamination source, as also observed by Cardoso et al. (2012), consistent with the sediment contamination gradient observed and highlighting the persistent effect of historical contamination on faunal bioaccumulation patterns. The Hg bioaccumulation in *S. plana* was higher at all sites compared to *H. diversicolor* (except at the TM site), consistent with findings from Cardoso et al. (2009) and Coelho et al. (2013). Additionally, the presence of vegetation had a positive effect on benthic accumulation patterns by reducing Hg accumulation, particularly after the second campaign. This Hg reduction in vegetated sites indicates an improvement in the environmental conditions, especially after two years of successful transplantation. Prior to transplanting, the species were buried in non-vegetated environments and thus had similar initial Hg concentrations. However, since the 2020 transplant, a decrease in Hg body burdens was observed at the meadow site compared to the bare one (TM to TB), likely linked to the rapid reduction of labile Hg in interstitial waters following the establishment of seagrass in this contaminated area (Oliveira et al., 2023). Beyond the improvements mediated by the presence of seagrasses in contaminated areas, species-specific characteristics such as lifespan, feeding behaviour, morphology (e.g., presence or absence of shell) (Cardoso et al., 2009; Coelho et al., 2013; Nunes et al., 2008), metabolic efficiency, mobility (Piló et al., 2016), and dietary habits (Cardoso et al., 2012; Coelho et al., 2008) also seemed to be influencing the higher Hg accumulation in these species.

Given that both species serve as an important trophic link within the ecosystem, the observed decline in tissue Hg levels is particularly relevant. Lower contaminant levels in these key species are expected to reduce the trophic transfer of Hg, thereby benefiting predators and enhancing overall food web health.

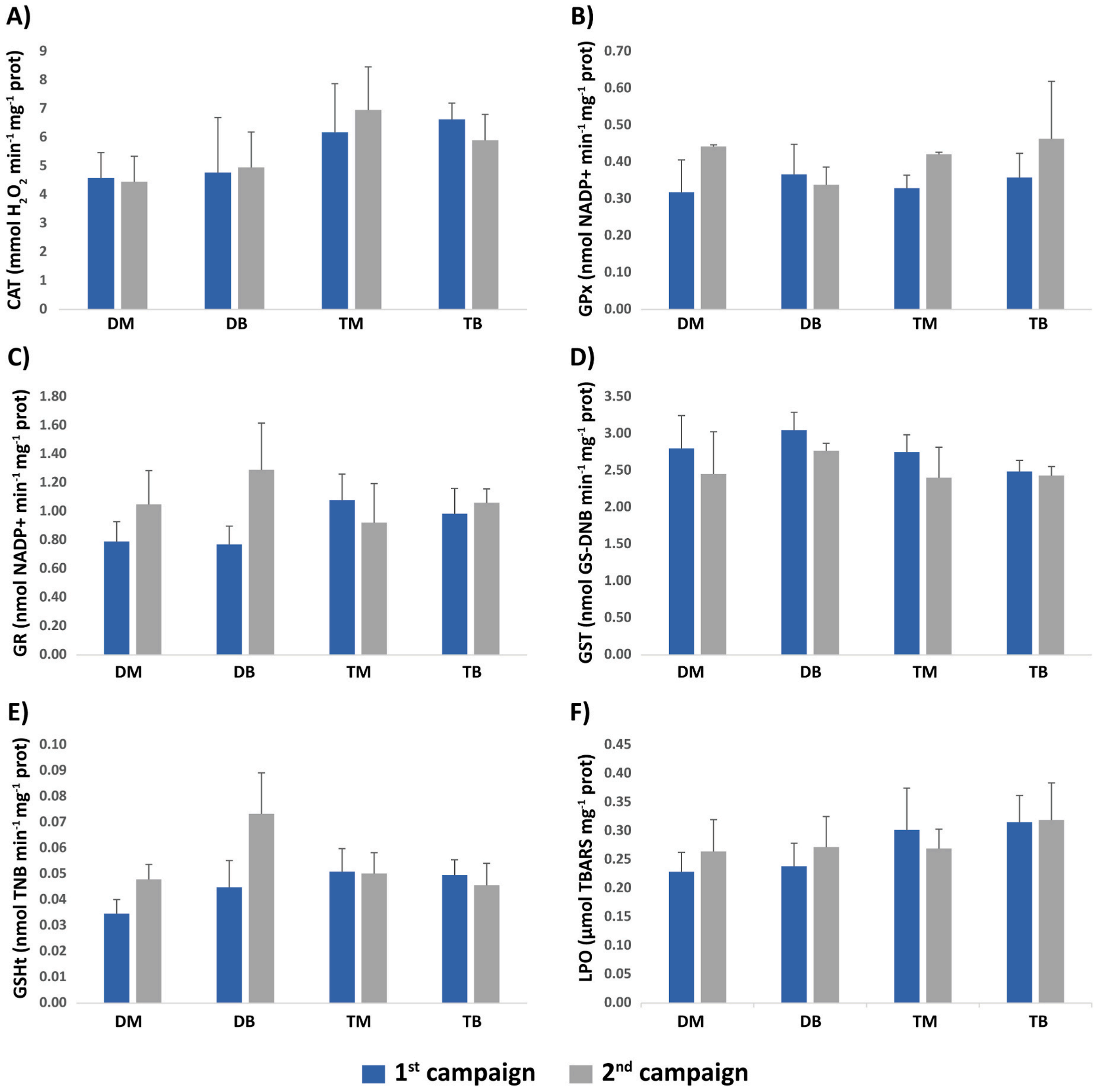

Fig. 4. Oxidative stress profile in *Scrobicularia plana* at Donor (D) and Transplant (T) areas in vegetated (M) and non-vegetated (B) habitats across two sampling campaigns. Columns represent the mean (n = 10), and error bars indicate the standard error. DM - Donor Meadow; DB - Donor Bare-bottom; TM - Transplant Meadow; TB - Transplant Bare-bottom; CAT - Catalase activity; GPx - Glutathione peroxidase activity; GR - Glutathione reductase activity; GST - Glutathione-S-transferase activity; GSHt - Total glutathione content; LPO - Lipid peroxidation. Significant terms and pairwise comparisons among factors are available in Table 4 and Supplementary Table 4.

4.3. Biochemical status

The presence or absence of vegetation, along with the presence or absence of contaminants and variations in physicochemical parameters, can impact bivalves and polychaetes in terms of energetic budgets and oxidative stress profiles, given their unique species-specific characteristics (Buffet et al., 2011; Freitas et al., 2017; Silva et al., 2020). Considering the lack of available information on the effect of vegetation on the studied species, it was important to assess whether the vegetation in the non-contaminated area induced changes in the energetic budget and oxidative stress profile of the species. Therefore, a comparison was made between the biochemical status of fauna in DM and DB. Additionally, to investigate whether contaminants present in the sediments of the Transplant area cause alterations in the biochemical parameters of these species, comparisons were made between vegetated (DM vs. TM) and non-vegetated (DB vs. TB) habitats. Furthermore, to evaluate whether the transplantation of *Z. noltei* provided benefits to the studied species in the contaminated area, comparisons between TM and TB were analysed.

4.3.1. Energetic budget

In the non-contaminated Donor area, the observed variations in the energetic profiles of both species can be attributed to a combination of environmental stressors (e.g., temperature, salinity, and oxygen levels) (Cyronak et al., 2016; Fitzer et al., 2016; Sokolova et al., 2012), species-specific characteristics such as mobility (Piló et al., 2016) and fluctuations in yearly food availability (Cardoso et al., 2009; Coelho et al., 2007, 2013). Additionally, the presence of vegetation, appears to play a crucial role in shaping these differences. Seagrass meadows are known to enhance habitat quality by reducing predation rates (González-Ortiz et al., 2014b), promoting juvenile settlement within the canopy (Barbier et al., 2017; Hovel and Fonseca, 2005), and improving environmental conditions such as hydrodynamics (Peralta et al., 2008; Tagliapietra et al., 2016). Furthermore, these vegetated habitats

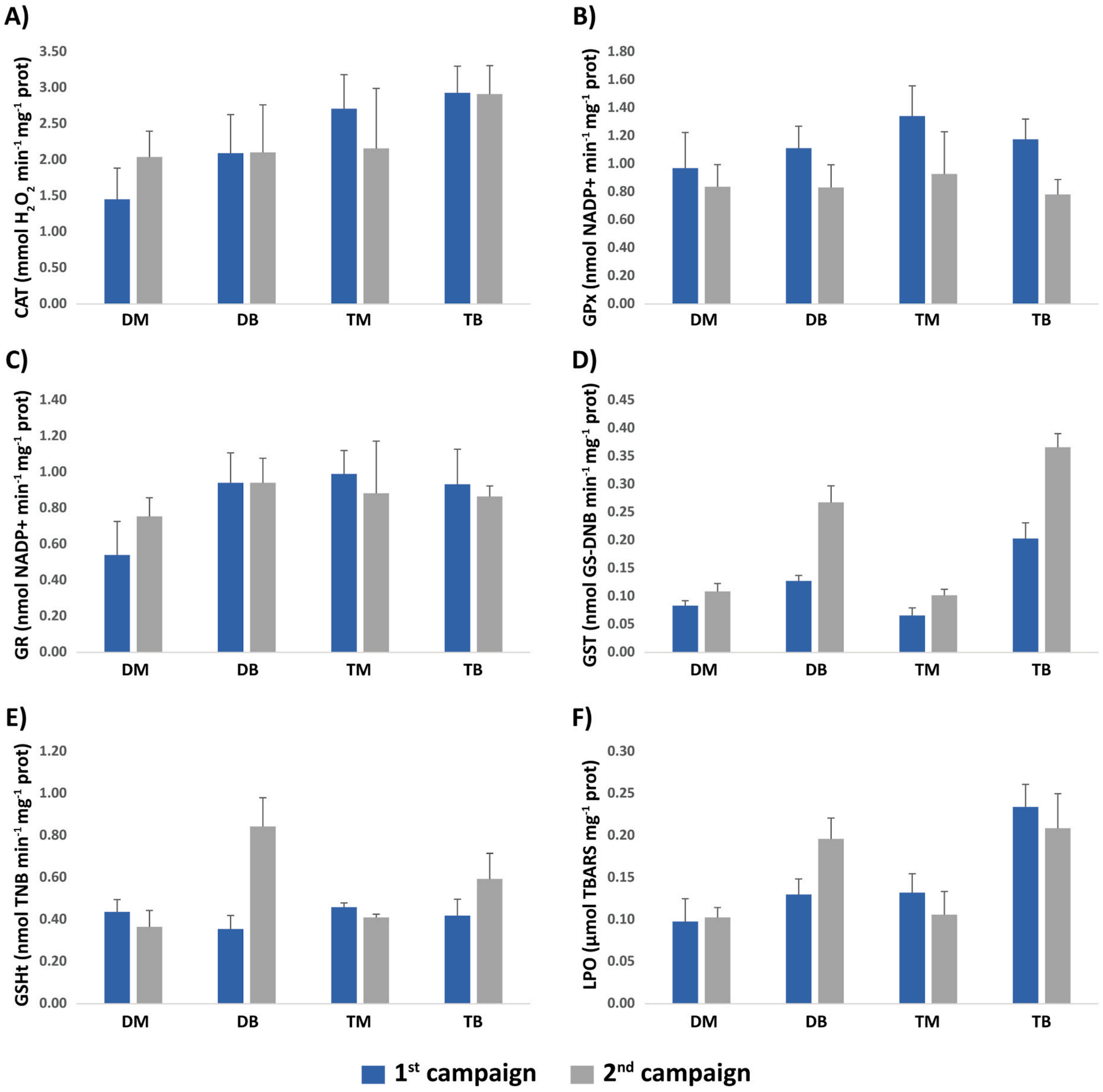

Fig. 5. Oxidative stress profile in *Hediste diversicolor* at Donor (D) and Transplant (T) areas in vegetated (M) and non-vegetated (B) habitats across two sampling campaigns. Columns represent the mean (n = 10), and error bars indicate the standard error. DM - Donor Meadow; DB - Donor Bare-bottom; TM - Transplant Meadow; TB - Transplant Bare-bottom; CAT - Catalase activity; GPx - Glutathione peroxidase activity; GR - Glutathione reductase activity; GST - Glutathione-S-transferase activity; GSHt - Total glutathione content; LPO - Lipid peroxidation. Significant terms and pairwise comparisons among factors are available in Table 5 and Supplementary Table 5.

increase food availability (González-Ortiz et al., 2014a), which likely explains the observed differences in energy budgets between vegetated and non-vegetated sites. In non-vegetated site, the fluctuating energy reserves and sporadic increases in ETS activity suggest that species may be responding to temporary environmental stressors, such as rapid variations in temperature and salinity caused by tides (Freitas et al., 2016; Madeira et al., 2021). This response could also indicate an active search for more suitable conditions (Brun et al., 2021; Román et al., 2022) or be linked to physiological mechanisms such as restoring cellular homeostasis, stress protection, and damage repair (Sokolova, 2021).

When considering the impact of metal(loid)s contamination on the energetic profiles of the studied species, a reduction in energy reserves and an increase in ETS activity were observed, suggesting that both species were experiencing physiological stress. Although Hg poses the most significant adverse effects on benthic species due to its elevated levels in sediments—exceeding PEL values, as discussed in section 4.1—other metal(loid)s present at lower concentrations, below PEL reference values, may also contribute synergistically to metabolic alterations. This response is a typical physiological adaptation in marine invertebrates exposed to contaminants, as energy is redirected toward defence mechanisms (Freitas et al., 2017; Mouneyrac et al., 2008; Stomperudhaugen et al., 2009). Variations in energy reserves due to exposure to metal(loid)s have been previously documented. Freitas et al. (2018, 2017) reported metabolic shifts in *H. diversicolor* following exposure to Hg and As, while *S. plana* exhibited similar responses when exposed to Cd, As, Cu and Zn (Boldina-Cosqueric et al., 2010; Rodríguez-Moro et al., 2018; Tankoua et al., 2013).

In the Transplant area, the introduction of *Z. noltei* to the contaminated site (site TM) provided measurable benefits to the studied species, with the vegetated habitat inducing a reduction in ETS activity. This reduction was anticipated, as seagrasses typically provide more favourable environmental conditions for fauna development (Brun et al., 2021; Crespo et al., 2023; Peterson and Heck, 2001). However, despite the overall decrease in ETS activity, energy reserves did not differ significantly for *S. plana* and only showed minor variations for *H. diversicolor* (e.g., in PROT and CH) between contaminated habitats. This may be attributed to the study being conducted within the first two years post-transplant—a period potentially too short to observe substantial changes in the energy budget.

4.3.2. Oxidative stress profile

In the non-contaminated Donor area, *S. plana* exhibited an increase in GSHt content at the bare site (DB) in both campaigns, suggesting an enhanced ability to supply reduced glutathione (GSH) as a substrate for glutathione-dependent enzymes like GPx and GST. However, a decrease in GPx activity was noted during the second campaign at DB, suggesting an increased susceptibility to elevated H_2O_2 levels. This reduction in GPx activity might be due to less favourable conditions for bivalves in non-vegetated sites, such as lower food availability (Brun et al., 2021; Román et al., 2022) and the inherently unstable and unfavourable physicochemical conditions, such as lower dissolved oxygen levels (Silva et al., 2012), in bare-bottom habitats. Moreover, without the protective seagrass cover that helps retain sediment moisture even during low tides, bivalves are likely exposed to greater desiccation stress, which can compromise their antioxidant defence mechanisms (Folmer et al., 2012; Powell and Schaffner, 1991). In addition, environmental fluctuations (e.g., significant temperature increase from the first to the second campaign), may further undermine the bivalves' antioxidant defences (Amorim et al., 2020; Park et al., 2008; Rahman et al., 2019). Despite these challenges and the observed variations in antioxidant enzymes activity, *S. plana* demonstrated adaptability to the absence of vegetation and fluctuations in physicochemical parameters, as evidenced by the lack of differences in lipid peroxidation between habitats across both campaigns.

Similarly, in *H. diversicolor*, the increase in GR activity at the DB site in both campaigns may be linked to elevated GST activity, as GR plays a crucial role in maintaining the balance between reduced and oxidized glutathione (Regoli and Giuliani, 2014), providing sufficient GSH for GPx and GST. The increased GST activity facilitates the conjugation of electrophilic compounds, acting as an antioxidant defence against oxidative stress (Hellou et al., 2012; van der Oost et al., 2003), thereby preventing oxidative damage. Additionally, *H. diversicolor* showed higher CAT activity in the first campaign at DB, which aids in breaking down H_2O_2 into water and oxygen. Nevertheless, despite these adaptive responses, the antioxidant defences in *H. diversicolor* were insufficient, as indicated by increased LPO levels in both campaigns. This suggests that *H. diversicolor* is more susceptible to fluctuations in physicochemical parameters in parallel with the absence of vegetation compared to *S. plana*, potentially due to species-specific traits such as differences in metabolic efficiency, mobility, and food availability (Cardoso et al., 2009, 2012; Coelho et al., 2013). Moreover, the lack of a protective shell in *H. diversicolor* likely renders it more vulnerable to thermal stress and desiccation, as has been documented for soft-bodied marine invertebrates (Whiteley and Mackenzie, 2016).

When considering the impact of metal(loid)s contamination on oxidative stress, both species experienced pro-oxidative effects from contaminants. In contaminated sites (TM and TB), both *S. plana* and *H. diversicolor* exhibited increased CAT activity in both campaigns — a response commonly associated with exposure to metals such as Cd, Cu, Cr, Hg, Pb and Zn (Ahmad et al., 2011; Ghribi et al., 2019; Pedro et al., 2022). Besides CAT, there were random fluctuations in GST and GPx activities, both of which are essential in ROS (reactive oxygen species) degradation and maintaining cellular redox balance (Regoli and Giuliani, 2014; Trombini et al., 2022). These variations may be related to a lack of NADPH, a key cofactor for the regeneration of GSH, or from variations in GR activity, which is essential for maintaining the GSH/GSSG balance. Additionally, glutathione in both species may bind to metals, like Hg via sulfhydryl/thiol groups (-SH) (Ajsuvakova et al., 2020; Erofeeva, 2015; Sharma et al., 2012), reducing the toxic effects and availability of Hg. The formation of GSH-Hg complexes could represent an adaptive strategy, functioning as a mechanism for Hg excretion, similar to processes observed in humans (Ajsuvakova et al., 2020; Endo and Sakata, 1995).

Despite an overall increase in antioxidant responses in *S. plana* and *H. diversicolor* during the first campaign at the Transplant sites, these defences were insufficient to prevent oxidative damage, as evidenced by elevated LPO levels. However, during the second campaign, LPO levels reached identical values to those observed at the respective Donor sites for both species. This convergence, occurring two years post-transplant between DM and TM, may be partially attributed to the ecological benefits of seagrass habitats, which can gradually reduce the bioavailability of metal(loid)s (e.g., Hg) (Oliveira et al., 2023), decrease sediment erosion, and enhance sedimentation processes (Brun et al., 2021; Valdez et al., 2020), thereby mitigating metal-induced oxidative stress over time. Conversely, the similarity in LPO levels between bare-bottom habitats (DB and TB) likely reflects physicochemical factors, such as higher temperatures during the second campaign in DB, that elevated baseline LPO levels, potentially masking differences between sites.

Within the Transplant area, comparing vegetated (TM) and bare (TB) habitats revealed further species-specific responses. For *S. plana*, there were no significant changes in antioxidant defences or LPO between the two habitat types, underscoring once again its robust adaptability to fluctuations in physicochemical parameters and contaminated environments. In contrast and similar to what was observed in the Donor area, *H. diversicolor* showed a notable increase in GST activity at the TB site compared to TM during both campaigns, along with higher CAT activity during the second campaign. Despite these adaptive responses, the persistently elevated LPO levels in the non-vegetated habitat confirm the vulnerability of *H. diversicolor* to the absence of vegetation, regardless of contamination. Furthermore, results from the Transplant area suggest that the introduction of *Z. noltei* has improved environmental conditions, as evidenced by decreased LPO levels for *S. plana* and a significant reduction for *H. diversicolor* during the second campaign. These observations suggest that ecosystem restoration is yielding positive results, although long-term studies are necessary to confirm these trends.

Overall, given the improved conditions observed for both species due to ecological restoration, their populations are likely to increase favourably over time in the restored area, as seen by Crespo et al. (2023).

5. Conclusion

Short-term effects of *Z. noltei* restoration demonstrated positive outcomes for the health of resident benthic communities. A reduction in Hg bioaccumulation in animal tissues was observed in the transplanted area compared to bare-bottom habitats, underscoring the significant role of seagrass meadows in reducing contaminant exposure. Furthermore, biochemical assessments revealed that the presence of seagrasses mitigated the adverse effects of metal(loid) contamination. Both species exhibited adaptive changes in their energetic budgets and oxidative stress profiles, including reduced energy consumption and lower levels of lipid peroxidation in vegetated areas. These findings suggest that seagrass restoration not only improves sediment quality but also reduces physiological stress in benthic organisms, thereby enhancing overall ecosystem health. Integrating faunal ecotoxicological assessment in seagrass transplant monitoring emerges as a promising approach to evaluate restoration success in contaminated sites.

CRediT authorship contribution statement

V.H. Oliveira: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **B. Marques:** Investigation. **A. Carvalhais:** Writing – review & editing, Investigation. **D. Crespo:** Writing –

review & editing, Investigation. **M. Dolbeth:** Writing – review & editing, Investigation, Formal analysis. **A.I. Sousa:** Writing – review & editing, Resources, Investigation, Formal analysis. **A.I. Lillebø:** Writing – review & editing, Resources. **M. Pacheco:** Writing – review & editing, Resources. **M.E. Pereira:** Writing – review & editing, Resources. **S. Díez:** Writing – review & editing, Resources. **J.P. Coelho:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **C.L. Mieiro:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT to improve the language and readability of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121429.

Data availability

Data will be made available on request.

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