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Assessing the Impact of Arsenic on Benthic Estuarine Fauna Behavior: Implications for Ecosystem Sustainability

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Abstract: Estuaries are dynamic ecosystems exposed to a wide range of stressors, including metal (loid) contamination. The assessment of the behavioral characteristics of the species inhabiting these ecosystems may provide a new point of view on chemical contamination since these behaviors generally regulate population dynamics and ecosystem stability. Therefore, this study aimed to investigate the changes in behavioral patterns of three estuarine benthonic species (the native polychaete *Hediste diversicolor*, the non-native polychaete *Arenicola marina*, and the native clam *Scrobicularia plana*) when exposed to different concentrations of the metalloid arsenic (0, 0.5, 1.5, 4.5, 13.5, 40.5 mg/kg sediment). Behavioral assessment included bioturbation activity (measured by fluorescent particle remobilization) and determination of the maximum penetration depth by each species, both after 1 and 21 days of exposure. After 21 days of exposure, the ability of each species to burrow was evaluated. Results showed that the bioturbation activity of *S. plana* was immediately reduced by exposure to As (day 1) but disappeared with exposure time (day 21), whereas *A. marina* bioturbation activity was significantly increased from day 1 to day 21, expressing their highest values in sediments of 4.5, 13.5, and 40.5 mg of As/kg on day 21. For *H. diversicolor*, no changes were observed within each time or between the times. Results of the burrowing assay showed that *A. marina* nearly doubled its burrowing time, as well as increased in double its maximum penetration depth at As concentrations ≥ 1.5 mg/kg sediment. These results suggest that native species can be quite resilient to chemical contamination over time. However, the greater particle remobilization by the non-native species *A. marina* when exposed to As may cause displacement of the native fauna, disrupting the natural mutualism created in these environments, and possibly decreasing estuary functionality and biodiversity. Behavioral assessments under chemical exposure may improve the establishment of more feasible protection goals for more sustainable estuaries.

Keywords: polychaetes; clams; non-native; bioturbation; re-burrowing



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1. Introduction

Arsenic (As) appears first on a list of 275 chemicals according to the Agency for Toxic Substances and Disease Registry's 2022 list and is ranked as one of the 10 chemicals of greatest public health according to the World Health Organization. This metalloid occurs naturally throughout the world (e.g., by disintegration and alteration of minerals and rocks by physical and biogeochemical processes, volcanic activity, forest fires, and biogenic sources [1,2]); however, its environmental levels have been widely exceeded due to various anthropogenic activities such as mining, industrial, wastewater, and/or agricultural discharges [3,4]. Considering the destination of a series of contaminants, estuaries are most likely sinks for As, as this metalloid can easily bind to sediments. Depending on the composition of the sediments, along with other environmental variables such as salinity and degree of anthropogenic stress, As levels in estuarine sediments have been reported in the milligram range. For example, in Bangladesh, India, Nickson et al. [5] reported As concentrations between 10 and 196 mg/kg of sediment, while Chakraborti et al. [6] found

values ranging from 9.0 to 28.0 mg/kg in sediments from the Ganges delta, India. Bai et al. [7] estimated sediment As concentrations of 8.79–13.73 mg/kg for the Yellow River Estuary (China), while Ereira et al. [8] estimated values of 2.8 to 94 mg/kg for sediments from the Ria de Aveiro estuary. All values are well above the permitted levels and are therefore capable of inducing effects on biota, potentially disrupting the communities that inhabit these ecosystems [9].

The accumulation of As in estuarine sediments poses additional risks to the benthic fauna. Studies on the effects of As on estuarine biota have classified the effects as ranging from mortality after an 11-day exposure to 1.25 mg/L [10], changes in biochemical status up to concentrations of 25 mg/L [11], and the impact of long-term exposure [12], or bioaccumulation and cell partitioning of metalloids [11–14], alone or in combination with scenarios that mimic climate change [10]. For example, Coppola et al. [12] reported total mortality for *Diopatra neapolitana* at the highest tested concentration of 1.25 mg/L after a long exposure of 28 days, while the lowest tested concentrations of 0.05 and 0.25 mg/L significantly reduced the regenerative capacity of this species. From the 18th day of exposure, the control organisms differed significantly from the two As concentrations in the number of newly regenerated segments, chaetigers, and total body length [12]. Furthermore, there was significant evidence of increased lipid peroxidation and oxidative stress damage (superoxide dismutase and catalase) in polychaetes exposed to As compared with control organisms. Cuccaro et al. [15] found no evidence of compromised sperm viability of the tube worm *Ficopomatus enigmaticus* after exposure to 1 mg/L of As. However, Cuccaro et al. [15] found that all As concentrations (0.05, 0.5, and 1 mg/L) induced significant DNA damage at salinities of 20 and 30.

The current lack of understanding of the deeper and more global effects of As is highlighted. Lower As concentrations may be detrimental to certain behaviors that are an integral part of the life cycle of estuarine sedimentary biota (for instance, burrowing). Because of the lower sensitivity of the parameters tested to date (e.g., mortality and regeneration), these effects may continue to go unnoticed, preventing adequate management of the risks of this metalloid as well as the delivery of more robust conservation plans for estuarine ecosystems. As scientific research evolves and new challenges arise, there is a need to improve the assessment of chemical contamination in ecosystems. Behavioral assessment can fill this gap by presenting itself as an early sign of stress [16]; that is, behavioral changes can manifest at chemical concentrations much lower than those necessary to induce biochemical or physiological changes, e.g., [17–19]. Behavioral assessment has stood out in the ecotoxicology field and has gained ground in the regulatory and risk assessment fields, as it allows the assessment of a wide range of responses with high ecological relevance, such as foraging for food or refuge, growth, or reproduction [20,21], and ultimately links individual fitness to long-term effects at the population, community, and ecosystem levels. Accordingly, this study hypothesis is to confirm that environmental relevant concentrations of As in estuarine sediments significantly decrease the behavioral patterns of three estuarine benthic species (the polychaetes *Hediste diversicolor* and *Arenicola marina* and the clam *Scrobicularia plana*). All selected species are bioturbators, contributing to the remobilization of sediments in estuaries and promoting oxygenation and circulation of elements and nutrients in these ecosystems, so the two selected behavioral tests were those resembling biota natural behavior: the redistribution of sediments assessed using fluorescent sand and the re-burial after exposure to the contaminant. Furthermore, it is worth highlighting the socioeconomic importance of *H. diversicolor* and *S. plana* as well as the invasive potential of *A. marina* in these coastal ecosystems.

2. Materials and Methods

2.1. Test Organisms: Collection and Acclimation

All species, *H. diversicolor*, *A. marina*, and *S. plana*, were harvested from Ria de Aveiro, a shallow coastal lagoon on the northwest coast of Portugal (40°38' N–08°45' W). After collection, the organisms were acclimated to the laboratory conditions for at least two

weeks. For this purpose, the organisms were placed in glass aquaria prepared in advance, containing 28 ppt saltwater (Ocean Fish, Prodac, Italy) and sand (mixture of fine and medium sand), supplied with continuous aeration at a room temperature of 18 ± 1 °C. The water:sand ratios in the aquaria were 3:1 for *H. diversicolor* and 2:1 for *A. marina* and *S. plana*, respectively. During this period, polychaetes were fed three times a week with commercial Vipar Nature tropical fish food flakes at a proportion of 10 mg per organism [22,23], while the clams were fed with a plankton substitute (Tropic Marin®, Tropic Marin Company, Wartenberg, Germany) [24]. Rearing water was renewed every week. In the first week, special care was given to the appearance of dying and/or injured animals, which were immediately removed to avoid degrading the water quality for other organisms. For the assays, attention was paid to the size of the organisms, so that variability during the assay associated with this factor was minimal. Accordingly, for polychaetes, only adult organisms with no visible injuries or fragmentation were selected.

2.2. Experimental Conditions for the Behavioral Assays

2.2.1. Particle Reworking Activity

The sediments used in all assays were prepared 24 h before the assays. Sediments were spiked with a stock solution of sodium arsenate (Na_3AsO_4) (CAS no. 10048-95-0, Sigma-Aldrich, St. Louis, MO, USA) prepared in Milli-Q water to obtain the concentrations 0.5, 1.5, 4.5, 13.5, and 40.5 mg of As/kg dry weight (dw) of sediment. The sand was vigorously mixed to ensure homogenization and left to rest the following 24 h. For the sand of the control group, the same procedure was also applied 24 h earlier, but the sand was moistened only with water. The particle reworking activity was adapted from the work of Lopes et al. [24]. Briefly, square glass vessels (1.5 dm³ capacity) were filled with sand previously prepared according to the desired As concentration or with clean sand (in the case of the control group) in a water (salinity 28): sand ratio of 2:1. Five replicates were set up for each treatment for each species. After selecting healthy organisms, four specimens of *H. diversicolor*, one specimen of *A. marina*, and three specimens of *S. plana* were added to each vessel [density of organisms observed under field conditions at Ria de Aveiro ([25], personal observations)]. After complete burrowing of the organisms, a layer of fluorescently stained sediment particles (luminophores, ~30 g) was added and distributed on the top of the test sand. Aeration was introduced into the vessels, ensuring that oxygen bubbles did not cause luminophore resuspension. The assays were performed for 21 days at 18 ± 1 °C, with observation points at 0, 1, and 21 days. The determination of the luminophore area in the sediment (used as a surrogate measure of the bioturbation activity of the organism) and luminophores' maximum depth of mixing (reflecting the maximum extent of mixing over the exposure period) followed the method described by Dolbeth et al. [26] and Lopes et al. [24]. To this end, all four walls of each glass vessel were photographed [Xiaomi Redmi Note 8 (48 MP, aperture $f = 1.8$) camera, Xiaomi Inc., Beijing, China] against a black background illuminated with a fluorescent lamp [15 W ($\lambda = 395$ nm), Pocketman, Amazon.com, Seattle, WA, USA] on days 0, 1, and 21.

2.2.2. Post-Exposure Burrowing

Following the particle reworking assay, the collected organisms were evaluated for their ability to burrow. This procedure followed the methodologies described by Bonnard et al. [27]. Surviving polychaetes and clams were placed individually in round bowls filled with 5 cm of clean sediment and 1 cm of artificial seawater. Subsequently, with the help of a chronometer, the time taken to complete the burrow was recorded.

2.3. Data Analysis

For sediment particle reworking activity, photographs taken were first saved with JPEG compression (Joint Photographic Experts Group) and analyzed using a custom-made plugin that runs within ImageJ (Version 1.53t), a java-based public domain program developed at the US National Institutes of Health (available at <https://imagej.net/>, accessed on

16 September 2024) (Dolbeth et al. [26] and Lopes et al. [24]). Examples are given at Supplementary Data File Figure S1. The area (cm^2) of remobilized sediment, the maximum depth of mixing, and the burrowing time of each As treatment for each species at the end of the assays were compared to control organisms using a one-factorial ANOVA for the three species. All analyses were performed using Sigmaplot Software® 15.0 for Windows (Grafiti LLC, St Palo Alto, CA, USA). Significant differences were considered for p -values lower than 0.05.

3. Results

The results obtained from the particle remobilization behavioral assays as an indirect measure of bioturbation activity during exposure to increased concentrations of As and the post-exposure burial assay for three estuarine species are shown in Figures 1–3 and in Table 1.

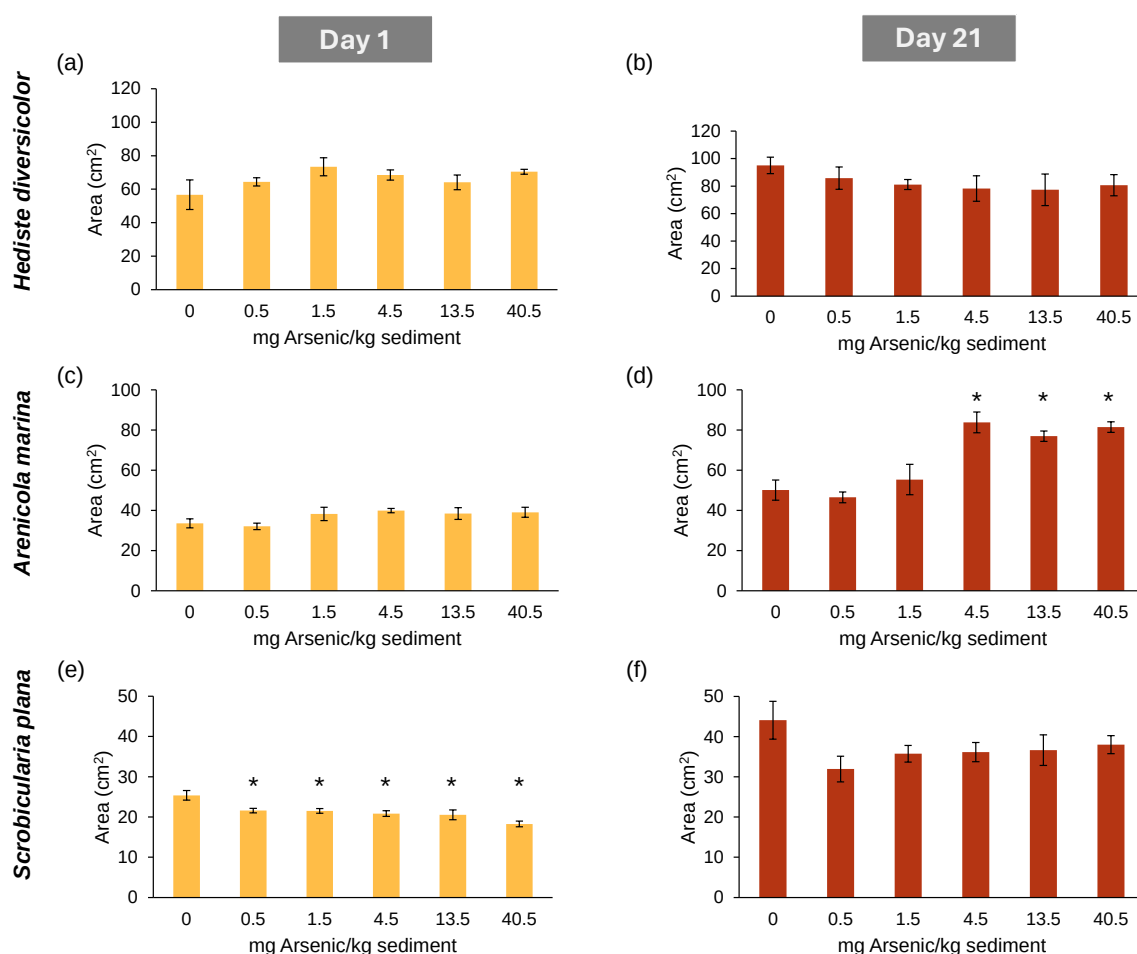


Figure 1. Bioturbation activity measured through the distributional area (cm^2) of fluorescent-sand luminophores detected by UV-light after 1 day (left) and 21 days (right) of exposure to increased concentrations of the metalloid arsenic (mg/kg sediment) for three estuarine species: (a,b) the native polychaete *Hediste diversicolor*, (c,d) the non-native polychaete *Arenicola marina*, and (e,f) the native clam *Scrobicularia plana*. Data are represented as the mean $\pm$ standard deviation ($n = 15, 5$, and 15 , respectively). The * indicate statistical differences between the As treatment and the respective control condition within each period (Dunnett's, $p < 0.05$).

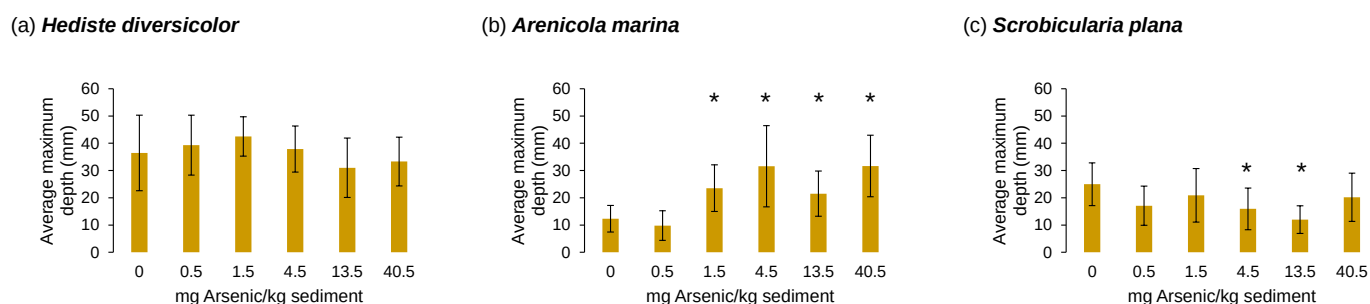


Figure 2. Average maximum depth (mm; determined as the difference between the initial luminophores layer at the vessel surface and the position of the luminophores in direction to the bottom of the vessel) obtained for three estuarine species, (a) the native polychaete *Hediste diversicolor* ($n = 5$), (b) the non-native polychaete *Arenicola marina* ($n = 5$), and (c) the native clam *Scrobicularia plana* ($n = 5$), after 21 days of exposure to increased concentrations of the metalloid arsenic. The * indicate statistical differences between the As treatment and the respective control condition after outlier retrieving (Dunnett's, $p < 0.05$).

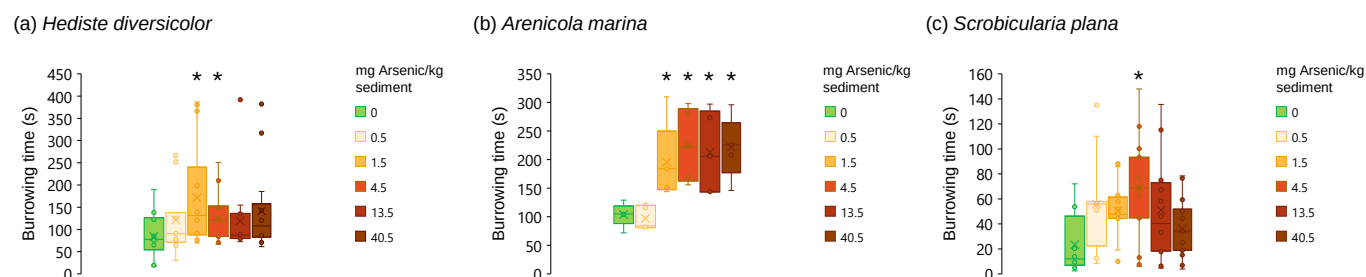


Figure 3. Burrowing time (expressed in seconds) obtained for three estuarine species, (a) the native polychaete *Hediste diversicolor* ($n = 15$), (b) the non-native polychaete *Arenicola marina* ($n = 5$), and (c) the native clam *Scrobicularia plana* ($n = 15$), after a 21-day exposure period to increased concentrations of the metalloid arsenic. The * indicate statistical differences between the As treatment and the respective control condition after outlier retrieving (Dunnett's, $p < 0.05$).

Table 1. Repeated measures ANOVA results for bioturbation activity between Day 1 and Day 21 of three estuarine species exposed to increased concentrations of arsenic (mg/kg sediment). Significant p -values are represented in bold and italic (after Bonferroni correction).

Comparison	As (mg/kg Sediment) Treatment	DF	Difference of Means	t	p Value
<i>Hediste diversicolor</i> Day 1 vs. Day 21	0	4	-	-	0.057
	0.5	4	-	-	0.100
	1.5	4	-	-	0.091
	4.5	4	-	-	0.377
	13.5	4	-	-	0.266
	40.5	4	-	-	0.229
<i>Arenicola marina</i> Day 1 vs. Day 21	0	4	16.55	3.16	0.030
	0.5	4	14.42	4.52	0.010
	1.5	4	17.12	2.78	0.049
	4.5	4	2.824	8.90	<0.001
	13.5	4	38.50	41.6	<0.001
	40.5	4	2.784	14.4	<0.001
<i>Scrobicularia plana</i> Day 1 vs. Day 21	0	4	18.73	3.65	0.022
	0.5	4	0.977	3.82	0.019
	1.5	4	1.335	7.57	0.002
	4.5	4	15.33	6.94	0.002
	13.5	4	16.14	5.12	0.007
	40.5	4	19.75	8.61	0.001

3.1. Particle Reworking Activity

3.1.1. Remobilized Area

The results from the particle reworking activity, measured through the area (cm²) of remobilized sediment, have shown two patterns of response in both periods (day 1 and day 21; Figure 1). After 1 day of exposure to increased concentrations of As, it was possible to see that both polychaetes (*H. diversicolor* and *A. marina*) presented a tendency for increased particle reworking with increasing As concentrations, although this was not significant ($p > 0.05$; Figure 1a,c and S2). However, *S. plana* particle reworking activity was found to be significantly reduced at concentrations higher than or equal to 0.5 mg/kg of sediment just after 24 h of exposure ($p < 0.05$; Figure 1c and S2). After 21 days of exposure to As, the two polychaetes have shown distinct patterns of response (unlike at day 1), with *H. diversicolor* presenting a non-significant decreasing trend in its particle reworking activity ($p > 0.05$; Figure 1b and S2), whereas *A. marina* has shown to significantly increase its activity at concentrations higher than or equal to 4.5 mg/kg sediment ($p < 0.05$; Figure 1d and S2). As for the clam, *S. plana*, no significant differences were found between As-exposed clams and control clams ($p > 0.05$; Figure 1f and S2).

In comparison of bioturbation activity between day 1 and day 21 (Table 1; Figure S2), it is possible to highlight three response patterns. The bioturbation activity of *A. marina* and *S. plana* at the beginning of As exposure was significantly different from that of day 21 of exposure at all concentrations tested ($p < 0.05$; Table 1; Figure S2). However, whereas for *A. marina* the bioturbation activity increased significantly from day 1 to day 21 at all tested concentrations of As tested ($p < 0.05$; Table 1), for *S. plana* the significant reduction in its activity at day 1 compared with control disappeared at day 21, remaining similar along the control and the As concentration range ($p < 0.05$; Table 1; Figure S2). For *H. diversicolor*, the pattern of response in this endpoint remained unchanged between day 1 and day 21, therefore not marking significant differences between the two timepoints ($p > 0.05$; Table 1; Figure S2).

3.1.2. Maximum Depth Penetration

The results of the average maximum penetration depth of each species at the end of the 21-day experiment are depicted in Figure 2. For *H. diversicolor*, there was a tendency for a decrease in this parameter at the concentrations of 13.5 and 40.5 mg/kg, though this was not statistically different from the control (Figure 2a). Contrarily, *A. marina* presented a significant increase in the average maximum depth penetration at As conditions higher than or equal to 1.5 mg/kg, with on average a two-fold increment from the control to these As treatments (Figure 2b). As for the clam *S. plana*, the maximum depth penetration was found to be significantly reduced compared to the control conditions at the concentrations of 4.5 and 13.5 mg/kg (Figure 2c).

3.2. Post-Exposure Burrowing

As for the ability of the estuarine species to burrow after a 21-day exposure period to As, results have shown that *H. diversicolor* specimens exposed to intermediate concentrations of As (1.5 and 4.5 mg/kg) took on average significantly more time to burrow than control organisms (average burrowing time of 172, 125, and 84 s in 1.5, 4.5, and 0 mg/kg treatments, respectively; $p < 0.05$; Figure 3a). *Arenicola marina* appeared to be the most affected estuarine species, with a significant increase in burrowing times at concentrations ≥ 1.5 mg/kg compared to control conditions: As-exposed *A. marina* almost doubled its burrowing time (average burrowing time ≥ 196 s) compared to the control (104 s; $p < 0.05$; Figure 3b). As for clams, only organisms exposed to 4.5 mg/kg were found to take significantly more time to burrow than control clams ($p < 0.05$; Figure 3c).

3.3. Principal Coordinate Ordination (PCO)

The principal coordinate ordination (PCO) is shown in Figure S3. The PCO1 axis explained 64.6% of the total variation, while the PCO2 axis accounted for 26.7% of the total variation. According to PCO1, the control and the lowest concentration (0.5 mg/kg) were

found on the positive side of axis 1, while the remaining concentrations were separated on the negative side of axis 1 (Figure S3). This separation is associated with the remobilized area, maximum depth, and burrowing time of *A. marina*, as well as the burrowing time of *S. plana*, which showed the highest correlation values with the negative side of this axis (−0.848, −0.837, −0.888, −0.803, respectively). It is also associated with the remobilized area of *H. diversicolor* and the maximum depth of *S. plana*, which showed the highest correlation values with the positive side of this axis (0.944, 0.668, respectively) (Figure S3). Regarding the PCO₂ axis, the concentrations 0.5, 1.5, and 4.5 mg/kg were separated on the negative side of the axis. This separation was mainly related to the remobilized area of *S. plana*, which showed a higher correlation with the positive side of PCO₂ (0.797), and the burrowing time of *S. plana*, which had a higher correlation with the negative side of this axis (−0.601) (Figure S3).

4. Discussion

The understanding about ecosystem sustainability has evolved greatly, striving to include responses that were previously not very in-depth, like behavioral analysis. These efforts to align relevant natural responses of the organisms with realistic management and conservation efforts are paramount for biodiversity and ecosystem resilience and health. Attempting to promote future research on this subject, this work aimed at exploring different behavioral responses of three estuarine species (two native and one invasive) to chemical contamination induced by arsenic (As).

Bioturbation in estuaries plays a critical role in maintaining ecosystem health and functionality, for which all the tested species contribute to different extents but complementarily [28]. Between polychaetes and the clam, the differential responses were therefore somehow expected due to their lifestyle, morphological, and physiological traits [29]. The two groups (bivalves and annelids) have been shown previously to have not only different requirements in terms of sediment and depth at which they can be found, but also in terms of response timing when burrowing. For instance, in Wisebron et al. [30], clams (*S. plana* and *Cerastoderma edule*) were observed to prefer soft, sandy sediments and generally burrowing deeper, while polychaetes (*H. diversicolor* and *A. marina*) preferred soft, muddy sediments staying at lower depth layers. However, in this study, the polychaetes have also shown quite distinct responses between them. Initially, after 24 h, neither species showed differences in sediment reworking between control and arsenic conditions. This trend continued for *H. diversicolor*, while *A. marina* displayed increased sediment reworking by day 21. The limited changes observed in *H. diversicolor*, with no alterations for the majority of the parameters conducted, may be partially due to its environmental origin, since As is a metalloid naturally occurring in various states in the sediments of estuarine ecosystems, and thus long-term and certainly generational exposure dictates the response of this polychaete to metal contamination. This hypothesis is supported by other work carried out with metals, in which the authors found that regardless the places of origin (uncontaminated versus contaminated), specimens of *H. diversicolor* contained both active and, in high proportions, cellular components responsible for detoxification of metals [31]. Samples from the clean site contained a higher proportion of very specific cytosolic components in which metallothioneins are included, while worms from contaminated sites were rich in extracellular granules, another important detoxification process [32], thus suggesting that mechanisms of metal(loid) tolerance may be available even in the absence of the stressor, allowing this species to have greater environmental and chemical plasticity. Moreover, the lack of agreement between tolerance on the short- and on the long-term (day 1 vs. day 21) has been reported elsewhere and is believed to be related to organisms' response to immediate chemical contamination being regulated by general and less specific mechanisms, while long-term responses trigger more specific pathways of response [33]. Despite other studies having indicated a potential escape response from *H. diversicolor* by residing deeper in the sediment (observed by higher particle reworking [32,34]), it is to highlight that these works have dealt with the study of the effects of manufactured nanomaterials (e.g., graphene), additionally suggesting that behavioral responses in *H. diversicolor* may be chemical-specific as

well. Unlike *H. diversicolor*, *A. marina* showed many signs of As-induced stress, namely in the reworking of particles after 21 days, greater difficulty in burrowing after exposure, and in being able to move deeper into the sand layer. Taking advantage of the results of Urban-Malinga et al. [32], the average maximum depth may be interpreted as a potential fleeing response to chemical contamination. The work carried out by Figueiredo et al. [34] conveyed the idea that a closed configuration similar to the one used here (e.g., glass vessels) combined with the use of an unconfined, linear, and multi-compartmented system (which allows free choice by the organism) confirms the answer of avoidance of *A. marina* upon exposure to graphene oxide flakes [32,34]. If the opportunity to move away from contamination is offered, *A. marina* flees [34]. This vertical avoidance may this way relate to the differences observed in the other behavioral parameters. By excavating deeper to potentially escape As-contamination, there is higher particle remobilization (luminophores extended by a greater vessel area), but there may also be great energy expenditure in organisms', potential impairing or delayed *A. marina* ability to burrow. Closed settings have their own limitations, but they are still needed to increase our understanding of behavioral responses and to complement other emerging approaches such as unconfined and free-choice devices [16,17].

It is also worth noting that *A. marina* has invasive potential (as it has already expanded into new estuaries [25]), and it has a greater capacity to excavate sediments deeply, leading to the rise of new concerns. The higher and deeper redistribution of particles may lead to physical and chemical changes in the sediment, which directly impact the benthic fauna and flora composition. For instance, these shifts may lead to the favoring of opportunistic or pollution-tolerant species over more sensitive, local ones, leading to imbalances in natural ecosystems [35]. In the long term, there is a high probability for profound changes in population dynamics across the estuarine ecosystems through the disruption of trophic interactions settled by native species [36]. In addition, it is to highlight also the role of *A. marina* on the potential to remobilize to other sediment layers emergent contaminants that were previously restricted to the most superficial layers, influencing further biogeochemical processes that occur there. For example, Petersen et al. [37] confirmed that *A. marina* was the bioturbating species that most promoted the movement of cadmium metal to uncontaminated layers (around 13 cm), compared to *H. diversicolor* and *Corophium volutator*, in which the metal was restricted to a surface layer never deeper than 4 cm [38]. In another study, through a combination of particle transport by remobilization of sediment through the galleries they create or by waste resulting from digestion, Gebhardt and Forster [38] found that, for example, microplastics could be found up to almost 20 cm deeper in sediment where *A. marina* was present. In both cases, the elements that could be less accessible to be degraded and/or integrated into biogeochemical processes have increased their residence time in the estuarine environment. The clam presented a significant decrease in bioturbated sediment after only 24 h of exposure in all As conditions, but which disappeared with time. This initial stress may be explained by the mechanisms of burrowing and valve closure that seem to require higher energy intake and expenditure in clams [39]. For instance, the soft-shell clam *Mya arenaria* dispends about 7% of its energetic budget for each burrowing cycle (about 30 min) and cannot burrow if that budget drops below 25% [38]. Possibly due to its hard shell that may confer safety to the soft tissues under stressful conditions for some time, clams have been shown to be the least affected species by As [40]. Anyway, valve closure may not truly reflect the physiological state of the organism, and there is cause for concern at the human safety level as As is one of the metal(loid)s bioaccumulated in greater amounts by clams in its edible tissues [41]. It is hypothesized that other meaningful behavioral traits aside from the ones tested here should be considered for clams to benchmark more meaningful As safety levels in estuaries, such as the estimation of feeding, filtration, and respiration rates [42,43].

Natural community assemblies play a well-defined role in ecosystems. For instance, bioturbation performed by native clams may be a weighing factor for total organic carbon (TOC) removal, while native polychaetes may enhance total nitrogen and phosphorous removal. However, when combining both native clams and polychaetes, the process of

TOC removal may be greatly improved [28]. Other changes may relate to the inability to properly feed or reproduce, as some species require burrows to have access to and consume sediment-dwelling microorganisms and organic matter or require specific burrow conditions for spawning; if not able, it may lead to reduced or suppressed reproductive success [30]. The perturbation of natural assemblies through chemically induced disruption or the introduction of non-native species may cause imbalances in ecosystem processes. A deeper understanding of estuarine fauna behavior may help inform conservation strategies, habitat management, and ecosystem restoration, ensuring that natural processes maintain biodiversity and ecosystem services in alignment with human activities. This study also highlighted that As concentrations may affect the behavioral traits of estuarine benthic fauna within the range of As concentrations reported previously as environmentally relevant, e.g., [5–9], emphasizing the need to continuously monitor and analyze As ecological impact [44]. As a legacy contaminant, the delivery and updating of data (as obtained here) may be capable of being framed in directives (such as the Marine Strategy Framework Directive, MSFD) to better address the sustainability and protection of ecosystems or even make public health considerations. The MSFD's current policy contemplates the incorporation of multiple lines of evidence and highlights that ecosystem-based management can only be balanced if it is multidisciplinary. There is currently a consensus that behavior is an early (and therefore more sensitive) signal of disturbance in ecosystems and translates into higher levels of organization (individual, population, community, and ecosystem [20,45]); it can be a key point in multidisciplinary management.

5. Conclusions

This study highlights how behavioral ecology may boost ecosystem sustainability by providing insights into how different species behave under chemical exposure and how they interact in real estuarine environments. The sustainability of estuarine ecosystems is closely linked to the health of their sediment dynamics, which support key habitats, species, and ecological processes. Long-term disturbances that affect sediment quality and contaminant distribution can have profound cascading effects on biodiversity, food webs, and ecosystem services. To maintain ecosystem sustainability, it is essential to carefully manage sediment disturbances and mitigate the introduction and remobilization of contaminants. This behavioral study lists four of the 11 qualitative descriptors for determining good environmental status, namely biodiversity, introduction of exotic species and their impact on native fauna, species of commercial interest and/or exploitation, and changes in the components of natural capital (DIRECTIVE 2008/56/EC [46]). It is therefore highlighted here that the study of behavior is/will be an indispensable tool and complementary to other parameters in future research aimed at the conservation of estuaries.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su16229728/s1>, Figure S1: Example of images taken under UV-light during the experiments and after conversion to black and white in ImageJ® software (Version 1.53t). Figure S2: Example of images representing particle reworking performed by the three estuarine species studied and between two time points. Figure S3: Centroids ordination diagrams (PCO) based on remobilized area, maximum deep and burrowing activity 21 days after exposure to different As concentrations (0–45 mg As/kg of sediment). Pearson correlation vectors are superimposed as supplementary variables ($r > 0.75$).

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