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Sediment contamination in two German estuaries: A biomarker-based toxicity test with the ragworm *Hediste diversicolor* under intermittent oxygenation

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ABSTRACT

Toxicity testing is an important tool for risk assessment of sediment contamination in estuaries. However, there has been a predominant focus on fitness parameters as toxic endpoints and on crustaceans as test organisms, while effects at the sub-organismal level and on other benthic taxa have received less attention. Also, interactions between sediment contamination and natural stressors such as oxygen are often neglected in traditional toxicity tests. Here we conducted a toxicity test of sediments from the Elbe and Oder (Odra) estuaries under three weeks of continuous and intermittent oxygenation, using biomarkers in an annelid, the ragworm *Hediste diversicolor*. Contaminated sediments affected worm survival and some biomarkers of antioxidant defense, electrophilic stress, and energy status with response ratios of above 20%. Toxic effects were most pronounced in sediments from the upper Elbe estuary, which contained high levels of heavy metals and organic chemicals. Oxygen regimes hardly changed the sediment effects, suggesting the robustness of the biomarker-based toxicity test with ragworms.

1. Introduction

More than 50% of the world's population lives in river basins and coastal areas (Hongtao and Ting, 2021; Kummu et al., 2011). Increased development in these regions puts immense pressure on aquatic ecosystems, including estuaries (Freeman et al., 2019). Among various anthropogenic stressors, chemical contamination is often considered the greatest threat (Borgwardt et al., 2019). Many heavy metals and organic chemicals released into the water tend to accumulate and persist in estuarine sediments (Burton, 2002; Chapman and Wang, 2001), potentially affecting the health and functioning of local biota, particularly benthic organisms (Pinto et al., 2009). The effects of contaminated sediments can also extend beyond their original location, for example, due to dredging and disposal of dredged material, or by extreme flood events (Crawford et al., 2022; Roberts, 2012). Therefore, the risk assessment of sediment contamination in estuaries is an important task (Apitz, 2011).

An experimental approach to sediment pollution assessment is toxicity testing, which aims to determine the causal effects of contaminated sediments on test organisms under controlled conditions (Chapman, 1990; Simpson et al., 2016). Traditional toxicity tests (bioassays) typically focus on fitness endpoints such as survival, growth, and reproduction (Heise et al., 2020; Leppanen et al., 2024). However, adverse effects on fitness often take time to manifest, and in these cases, sub-organismal changes (biomarkers) can be useful early warning signs (Hagger et al., 2006; Newman, 2015). In terms of test species, current toxicity tests are biased towards crustaceans (e.g., amphipods and copepods), while other important estuarine taxa such as mollusks and annelids are underrepresented (Chapman et al., 2013; Simpson et al., 2016). Also, not many tests have examined the effects of contaminated sediments in combination with natural stressors such as salinity, temperature, and oxygen (Elliott and Quintino, 2007; Pham et al., 2024), despite concerns about their potential interactions (Burton and Johnston, 2010; Sokolova and Lannig, 2008). For example, oxygen depletion may

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limit the energy available for detoxification and damage repair, thereby enhancing contaminant toxicity (Sokolova, 2013, 2021).

To fill these gaps, we conducted a toxicity test of sediments under two oxygen regimes with a focus on biomarker responses of an estuarine annelid. We collected sediments from two German estuaries, the Elbe and the Oder, both contaminated by multiple urban, industrial, and agricultural sources in their catchments (Förstner et al., 2004; Kowalewska et al., 2003; Müller et al., 2002; Wetzel et al., 2013). The sediment toxicity test was set up for three weeks under continuous oxygenation, which is standard practice for laboratory exposures (Simpson et al., 2016), and intermittent oxygenation, which simulates more realistically oxygen fluctuations in the field (Fusi et al., 2023; Jarvis et al., 2023). The ragworm *Hediste diversicolor*, a common benthic organism in European estuaries (Beyer and Sundt, 2006; Scaps, 2002), was used as the test species. By building complex galleries in the sediment, aerating, and feeding, ragworms significantly influence the fluxes of nutrients, organic matter, oxygen, and contaminants across the sediment-water interface, making them important ecosystem engineers (Laing et al., 2022; Porter et al., 2023; Zhu et al., 2016). To assess the toxic effects on ragworms, we measured two fitness endpoints (survival and mass) and a suite of biomarkers related to detoxification and antioxidant defense, electrophilic stress, and energy status (Hampel et al., 2016; Pham et al., 2022; Sokolova et al., 2012).

2. Materials and methods

2.1. Estuarine regions and sediment collection

The Elbe and Oder rivers originate in the Czech Republic and flow into the North Sea and the Baltic Sea, respectively (Fig. 1). Geologically, the Elbe river mouth is a tidal, coastal plain estuary (Amann et al., 2012; Rewrie et al., 2023). It is often divided into three regions based on salinity, the Limnic Elbe (salinity <1), the Transitional Elbe (salinity 1–20), and the Coastal Elbe (salinity >20) (Carstens et al., 2004; Geerts et al., 2012). In contrast, the Oder (Odra) river mouth is a non-tidal, bar-built estuary (Osadczuk et al., 2007). It consists of three geographic regions, the Szczecin Lagoon (salinity <2), connecting straits such as the Peenestrom (salinity 2–8), and the Oder Bay (salinity 8) (Mohrholz and Lass, 1998; Schernewski et al., 2012).

Contamination in the coastal regions of both estuaries is of minor concern due to the dilution effect of seawater and marine sediments (Pham et al., 2024). Therefore, we only investigated sediments in four regions, the Transitional Elbe, Limnic Elbe, Peenestrom, and Szczecin Lagoon (Fig. 1). In each region, surface sediments (top 10 cm) were sampled with Van Veen grabs or spades at two or three sites in October 2021 (one sediment sample per site). The sediments were transported to the laboratory in sealed plastic buckets and stored in a cold, dark chamber at 4 °C.

2.2. Sediment characterization

Total organic matter content and C:N mass ratio were measured in-house following established protocols (Davies, 1974; Verardo et al., 1990). Analyses of dry matter content (total solids), grain size distribution, and contaminants were conducted by an accredited laboratory (GBA Gesellschaft für Bioanalytik, Germany) using standard methods (Otte et al., 2013; Wetzel et al., 2013). A large number of persistent contaminants commonly included in national monitoring programs were analyzed (German Environment Agency, 2024). These were eight heavy metals (Hg, Pb, Cd, As, Cr, Ni, Cu, and Zn), 16 polycyclic aromatic hydrocarbons (PAHs), two chlorobenzenes (CLBs), seven polychlorinated biphenyl congeners (PCBs), five hexachlorocyclohexane isomers (HCH), and dichlorodiphenyltrichloroethane (DDT) along with its metabolites (collectively referred to as DDX). Heavy metals were measured in the fine fraction (<20 μm) of the sediments, while organic contaminants were measured in the gravel-excluded fraction (<2 mm). A list of organic contaminants and details of the standard methods and measurement uncertainties are provided in the Supplementary material.

2.3. Test species and control sediment collection

The ragworm *Hediste diversicolor* inhabits all regions of the Elbe and Oder estuaries (Gosselck and Schabelon, 2007; Krieg, 2007; Zauke, 1977) and is therefore a highly representative test species (US EPA, 1994). However, to ensure the sensitivity of the test subjects, we obtained ragworms from a nearby estuary with a presumably lower

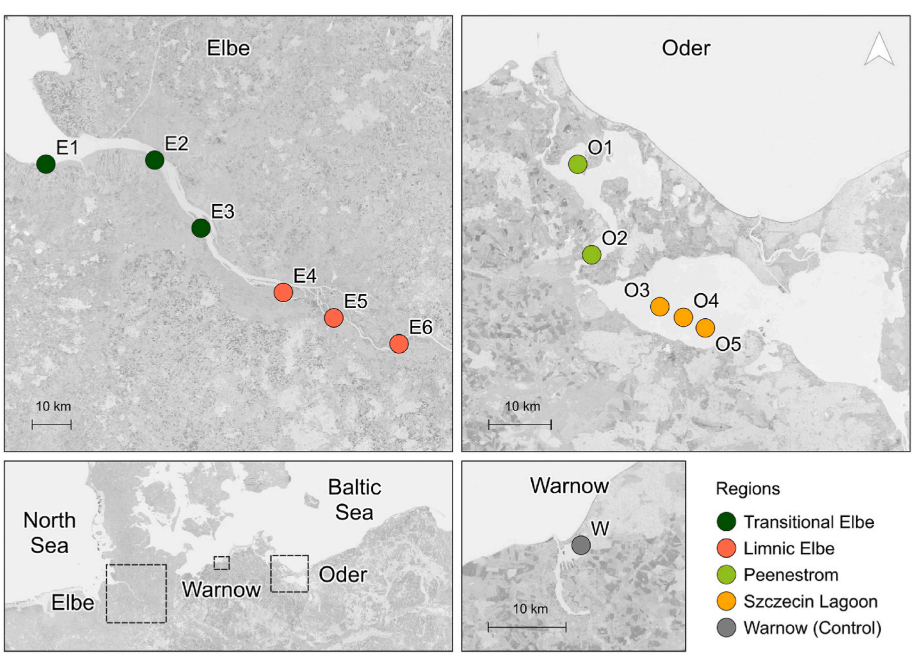

Fig. 1. Sediment sampling sites in the Elbe and Oder estuaries. Six sites (E1 to E6) were selected in two regions of the Elbe estuary and five sites (O1 to O5) were selected in two regions of the Oder estuary. Ragworms *Hediste diversicolor* and control sediment were collected from a site (W) in the Warnow estuary. The coordinates of the sampling sites are given in Table S1. Basemap from Microsoft Bing.

contamination level (Fisch et al., 2017), the Warnow in the Baltic Sea (Fig. 1). The worms were collected by sediment sieving and transported to the laboratory in plastic drums together with field sediments and seawater. The drums were maintained for one week in an environmental room at 17.5 °C and with a 16:8 h light-dark cycle. The overlying water was continuously aerated, supplemented with fish food (Marine Flakes, Tetra, Germany), and renewed daily with artificial seawater at salinity 10 (Pro-Reef, Tropic Marin, Germany). These temperature, light, and salinity conditions approximated those in the field on the day of collection and were also used during the sediment incubation and exposure described later. To examine the temporal changes in test endpoints, a pre-exposure group of 21 worms was collected, shock-frozen in liquid nitrogen, and stored at −80 °C for biomarker measurements.

Sediment was also collected from the same site in the Warnow estuary to serve as a control in the toxicity test (Fig. 1). Its collection and characterization followed the same procedures as the sediments from the Elbe and Oder estuaries.

2.4. Sediment exposure and oxygen regimes

A two-factor experiment was conducted with a 12 × 2 design, corresponding to 12 sediment samples (Fig. 1) and two oxygen regimes (continuous and intermittent oxygenation). For each of the 24 experimental groups, three 1-L glass bottles (ROTILABO, Carl Roth, Germany) were prepared and incubated for one month, each containing 400 mL of sediment and 600 mL of artificial seawater at salinity 10. The overlying water was continuously aerated with an air diffuser, and seawater or deionized water was added daily to compensate for evaporation while maintaining the salinity.

The three-week exposure began with the random assignment of seven ragworms to each bottle ($n = 21$ worms per experimental group). Bottles in the continuous oxygenation groups were left as they were, with saturated oxygen in the overlying water at ~8.9 mg L^{-1}. In contrast, bottles in the intermittent oxygenation groups were aerated and then closed with airtight plastic caps for a total of 12 cycles (Fig. 2). In each closed period, three bottles were randomly selected for oxygen depletion monitoring using modified caps with embedded probes (LDO101 with HQ40d portable meter, Hach, USA). The closed periods were started arbitrarily but ended when the experimenter noticed that at least one of the selected bottles had reached the hypoxia with oxygen in the overlying water at <2 mg L^{-1}. After the exposure, surviving worms were retrieved, shock-frozen in liquid nitrogen, and stored at −80 °C for biomarker measurements.

2.5. Biomarker measurements

After recording the wet mass of each worm, multiple biomarkers were measured using established protocols. Detoxification and antioxidant defense were assessed by the activities of carboxylesterase (CES, Hosokawa and Satoh, 2001), glutathione *S*-transferase (GST, Habig et al., 1974), and glutathione reductase (GR, Mannervik, 1999) and total antioxidant capacity (TAC, Re et al., 1999). Electrophilic stress was evaluated by the levels of methylglyoxal (MGO, Mitchel and Birnboim, 1977), malondialdehyde (MDA, Buege and Aust, 1978), and protein carbonyls (PC, Levine et al., 1990). Energy status was examined by the contents of carbohydrates (CAR, Masuko et al., 2005), lipids (LIP, Van Handel, 1985), and proteins (PRO, Bradford, 1976), mitochondrial electron transport system activity (ETS, De Coen and Janssen, 1997), and the adenylate levels (ATP, ADP, and AMP, Crouch et al., 1993; Jaworek et al., 1974). ATP, ADP, and AMP were measured in a set of ~6 worms per experimental group, while the other biomarkers were measured in another set of ~10 worms per group. All measurements were performed spectrophotometrically on a microplate reader (SpectraMax iD3, Molecular Devices, Germany) at 25 °C.

Several composite biomarkers of energy status were also constructed. Carbohydrate, lipid, and protein contents were converted to energy equivalents using the specific enthalpies of 17.5, 39.5, and 24 J mg^{-1} (Gnaiger, 1983) and summed to obtain the total energy available (Ea). ETS activity was transformed to the energy consumption rate (Ecr) using the oxyenthalpic equivalent of 484 kJ mol^{-1} O_2 (De Coen and Janssen, 1997). Cellular energy allocation (CEA, Verslycke et al., 2004) was then calculated as Ea/Ecr. Also, adenylate energy charge (AEC, Atkinson and Walton, 1967) was computed as (ATP + 0.5 ADP)/(ATP + ADP + AMP), where the denominator represents the total adenylates (collectively referred to as AXP).

For the sediment toxicity assessment based on biomarkers, we considered increases in CES, GST, GR, and ETS activities and increases in MGO, MDA, and PC levels as adverse effects on ragworms. Decreases in TAC, Ea, CEA, AXP, and AEC were also considered adverse effects.

2.6. Data analyses

Survival, mass, and biomarker responses of post-exposure worms were analyzed using (generalized) linear mixed models (Bates et al., 2015), with sediment and oxygen as interacting fixed effects and experimental bottle as a random effect. Model predicted means (Lenth et al., 2024) were calculated for 12 sediment samples and two oxygen regimes, as well as 24 combined experimental groups. The response

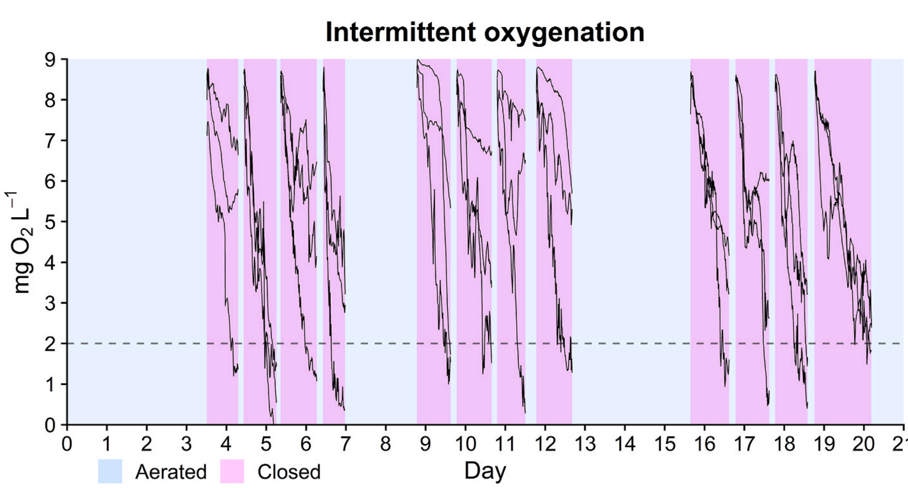

Fig. 2. Effects of intermittent oxygenation on dissolved oxygen in the overlying water of test bottles. The bottles were aerated and closed, and this process was repeated for 12 cycles. Due to the limited number of oxygen probes, only three random bottles were monitored during each closed period. The dashed line marks the hypoxic level that triggered the reoxygenation of the bottles.

ratio (RR, *Friedrich* et al., *2008*; *Pham and Sokolova, 2023*) was also calculated with the Warnow estuary sediment and continuous oxygenation as controls. Two-factor permutational analysis of variance (*Ernst, 2004*; *Luo and Koolaard, 2024*) was applied to the fitted models to test for the effects of sediment, oxygen, and their interaction. Evidence against the null hypotheses was considered very strong, strong, or moderate if the permutation p-values $\leq$0.001, 0.01, or 0.05 (*Muff* et al., *2022*). In these cases of statistical significance, we focused on the means and RR for interpretation instead of performing post-hoc tests (*Kozak and Powers, 2017*). Specifically, we considered a relative change of at least 20% compared with the controls (RR $\leq$ 0.8 or RR $\geq$ 1.2) to be of toxicological significance (*Chapman, 2007*, *2016*; *Neumann-Hensel and Melbye, 2006*; *Simpson* et al., *2016*).

Correlations between all test endpoints and between significant endpoints and contaminant levels were assessed using Pearson's and Spearman's coefficients. When $|r|$ or $|r_S| \geq 0.7$, the relationship was considered strong (*Akoglu, 2018*). Temporal changes in test endpoints were also examined by comparing the pre-exposure worms with the worms exposed to the Warnow estuary sediment under continuous oxygenation. All analyses were conducted in R v4.3.3 (*R Core Team, 2024*) with codes adapted from *Pham et al. (2024)*.

3. Results

3.1. Sediment characteristics

Sediments in the Warnow estuary and the Transitional Elbe had high dry matter contents (~70%), low amounts of fine grains, and little organic matter (Fig. 3). In contrast, the Szczecin Lagoon sediments had low dry matter contents (<20%) and were rich in fine fraction (~70%) and organic matter (~15%). Sediments in the Limnic Elbe and the Peenestrom both contained medium contents of dry matter and medium amounts of organic matter (except for site E6), but the Limnic Elbe sediments had more fine grains. The C:N ratio of organic matter in all sediment samples varied between 8 and 12 (Fig. S1).

Most heavy metals were found with the highest contents in sediments E5 and E6 from the Limnic Elbe (Fig. 4 and S1). The Limnic Elbe sediments (E4, E5, and E6) also contained the highest levels of most organic contaminants, including total CLBs, PCBs, HCH, and DDX. An exception was total PAHs, which were highest in the Szczecin Lagoon sediments (O3, O4, and O5). Contents of contaminants were generally lower in the Transitional Elbe compared with the Limnic Elbe. Similarly, contaminant levels were often lower in the Peenestrom compared with the Szczecin Lagoon. For most contaminants, the Warnow estuary sediment had lower contents than those from the Elbe and Oder estuaries.

3.2. Sediment-oxygen interactions

There was no evidence for the interactive effects of sediment and oxygen on most of the test endpoints. The exceptions were protein content and Ea, which are described later. Given the lack of interaction, the figures in the next sections show the effects of sediment and oxygen separately.

3.3. Survival and mass

Moderate evidence was found for the effects of sediment and oxygen on worm survival (Fig. 5). Worms exposed to sediments E1, E5, and E6 had lower survival probabilities than worms in the control sediment (RR = 0.76–0.78), while worms in sediments O1 and O2 had higher survival probabilities (RR = 1.18–1.21). Worms kept under intermittent oxygenation also had a higher survival rate than worms in continuous oxygenation (RR = 1.13).

While there was no evidence for the effect of sediment on worm mass, very strong evidence was found for the effect of oxygen, with worms exposed to intermittent oxygenation having higher mass than worms under continuous oxygenation (RR = 1.21, Fig. 5).

3.4. Detoxification and antioxidant defense

There was no evidence for the effect of sediment on CES activity but very strong evidence for the effect of oxygen, with worms exposed to intermittent oxygenation having lower CES activity (RR = 0.86, Fig. 6). For GST and GR activities, no evidence was found for the effects of sediment and oxygen (Fig. S2).

Strong and very strong evidence was found for the effects of sediment and oxygen on TAC (Fig. 6). Worms exposed to sediments from the Elbe and Oder estuaries often had lower TAC than those in the control sediment, with the lowest TAC in sediment O5 (RR = 0.77). Worms kept under intermittent oxygenation also had a lower TAC than worms under continuous oxygenation (RR = 0.9).

3.5. Electrophilic stress

There was very strong evidence for the effect of sediment on MGO levels, but no evidence for the effect of oxygen (Fig. 6). Compared with the control sediment, all sediments from the Elbe and Oder estuaries resulted in higher MGO levels. Notably, the MGO levels were highest in

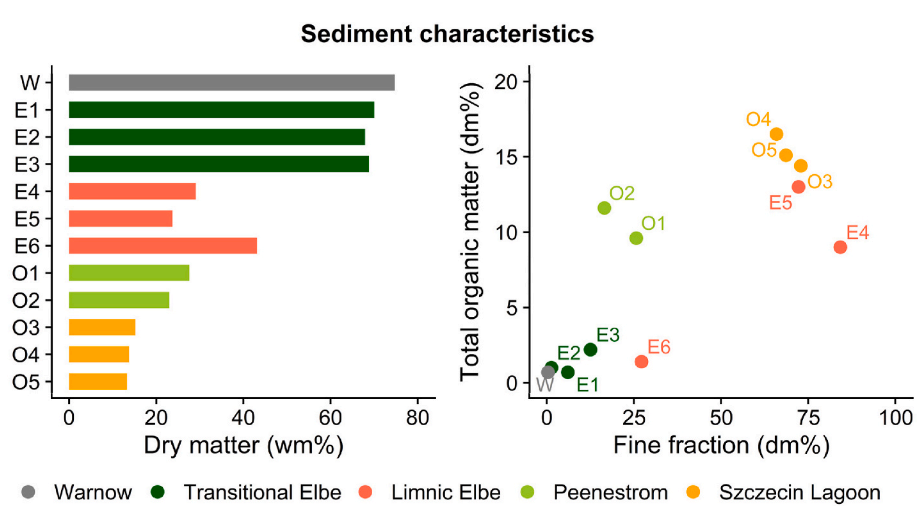

Fig. 3. Physicochemical properties of sediments collected at 12 sites in the Warnow, Elbe, and Oder estuaries. Units are wm% - percent wet mass or dm% - percent dry mass. C:N ratio is shown in Fig. S1.

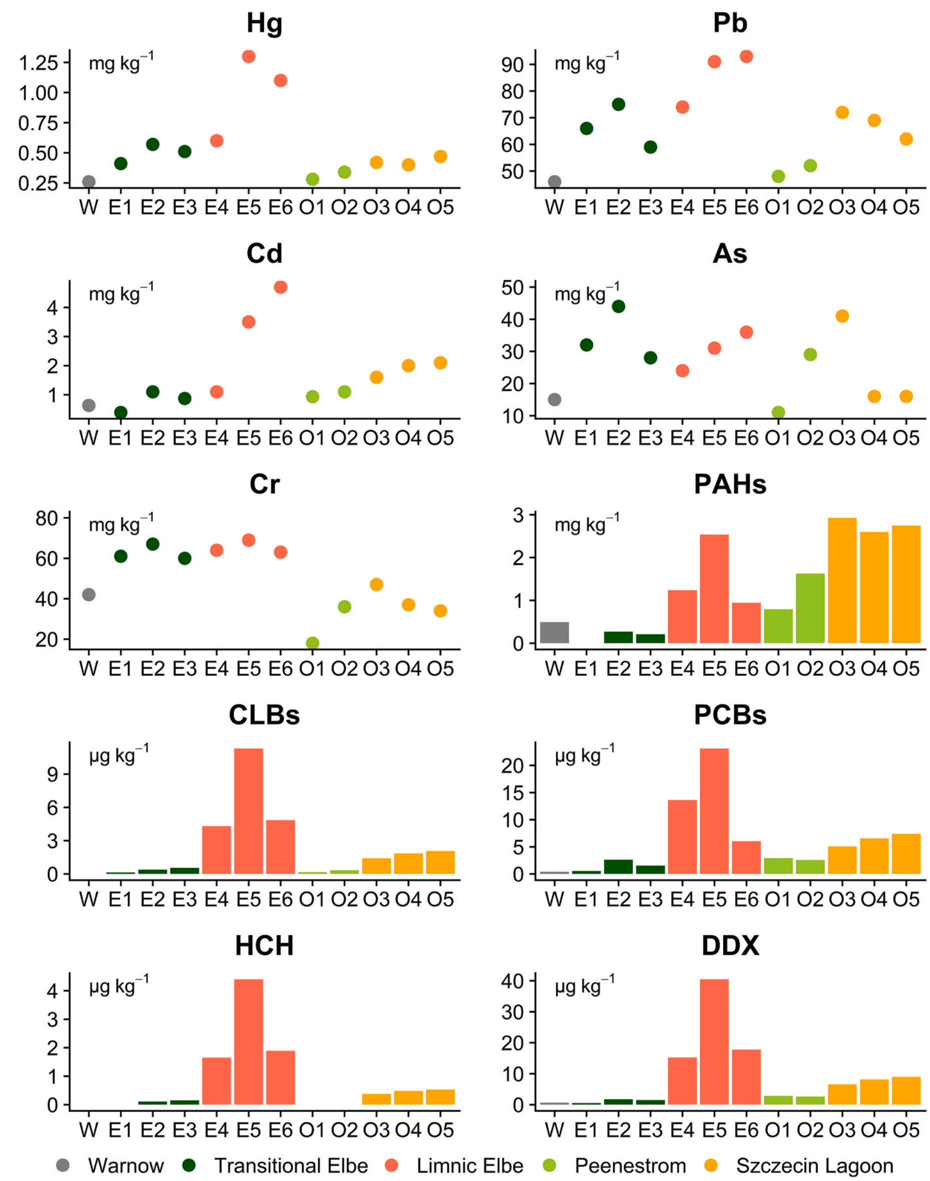

Fig. 4. Contents of heavy metals and organic contaminants in sediments collected at 12 sites in the Warnow, Elbe, and Oder estuaries. Total contents are reported for five groups of organic contaminants, PAHs - polycyclic aromatic hydrocarbons, CLBs - chlorobenzenes, PCBs - polychlorinated biphenyls, HCH - hexachlorocyclohexane, and DDX - dichlorodiphenyltrichloroethane (DDT) and metabolites. Ni, Cu, and Zn are shown in Fig. S1. Because heavy metals have a geological background, dot plots are used instead of bar plots to highlight relative differences between sites.

worms exposed to the Limnic Elbe sediments (RR = 1.33–1.44).

No evidence was found for the effects of sediment and oxygen on MDA and PC levels (Fig. S3).

3.6. Energy status

There was no evidence for the effects of sediment and oxygen on carbohydrate and lipid contents (Fig. S4). Very strong evidence was found for the interactive effect of sediment and oxygen on protein content (Fig. S4). In most sediments, worms exposed to intermittent oxygenation had higher protein contents than those under continuous oxygenation. The exceptions were sediments E1, E5, and E6, in which worms under continuous oxygenation had higher protein levels.

Very strong evidence was also found for the interactive effect of sediment and oxygen on Ea (Fig. 7). In most sediments, worms exposed to continuous and intermittent oxygenation had similar Ea values. Some exceptions were sediments E2 and E6, where the differences between two oxygen regimes were large. However, worms in sediments from the Elbe and Oder estuaries generally had higher Ea than those in the control sediment.

There was very strong evidence for the effects of sediment and oxygen on ETS activity (Fig. 7). Most sediments from the Elbe and Oder estuaries resulted in higher ETS activity compared with the control sediment. Notably, the ETS activity was highest in worms exposed to sediments E5 and E6 (RR = 1.21–1.29). Worms exposed to intermittent oxygenation had lower ETS activity than those in continuous oxygenation (RR = 0.91). There was no evidence for the effect of sediment on CEA, but moderate evidence was found for the effect of oxygen, in which worms exposed to intermittent oxygenation had higher CEA (RR = 1.07, Fig. 7).

No evidence was found for the effects of sediment and oxygen on AMP levels, but there was moderate to strong evidence for the effects of sediment and oxygen on ATP and ADP levels (Fig. S5). Most sediments from the Elbe and Oder estuaries resulted in elevated ATP levels compared with the control sediment (RR up to 1.44), while ADP levels were more variable (RR = 0.84–1.26). Both ATP and ADP levels of

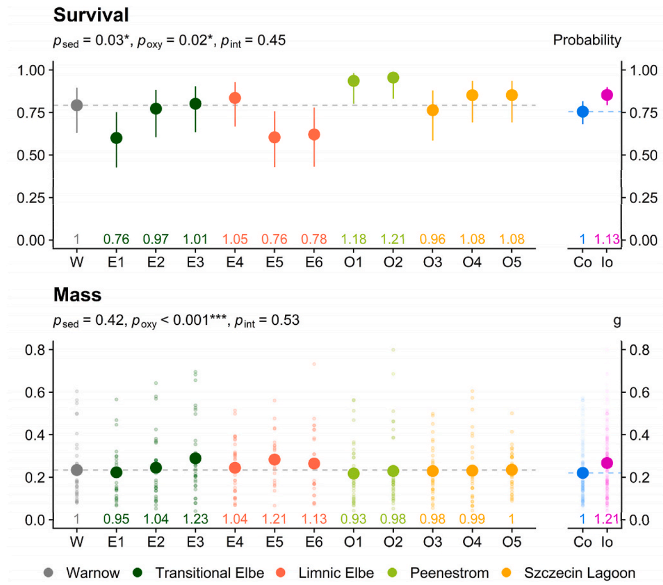

Fig. 5. Effects of the Elbe and Oder estuary sediments (E1-E6 and O1-O5) and intermittent oxygenation (Io) on survival and mass of ragworms, with the Warnow estuary sediment (W) and continuous oxygenation (Co) as controls. Individual observations are shown as small dots and predicted means are shown as large dots. For survival, 95% confidence intervals are shown ($n = 42$ worms per sediment group and 252 worms per oxygen group). Dashed lines indicate the control means. Response ratios (RR) are given for 12 sediment samples and two oxygen regimes. Permutation *p*-values are given for sediment (sed), oxygen (oxy), and their interaction (int).

worms under intermittent oxygenation were lower than those in continuous oxygenation (RR = 0.89 and 0.92).

There was very strong evidence for the effects of sediment and oxygen on total adenylates, with elevated levels in most sediments (RR up to 1.22) but a reduced level under intermittent oxygenation (RR = 0.9, Fig. 8). However, no evidence was found for the effects of sediment and oxygen on AEC (Fig. 8).

3.7. Endpoint correlations and temporal changes

Strong correlations ($|r| \geq 0.7$) occurred only between composite biomarkers and their constituent biomarkers (Fig. S6). In particular, Ea was positively correlated with lipid content ($r = 0.78$). AXP was also positively correlated with ATP content ($r = 0.87$), while AEC was positively correlated with ATP content ($r = 0.79$) but negatively correlated with AMP content ($r = -0.88$).

There was no evidence for the differences in mass and biomarker responses between pre-exposure worms and post-exposure control worms (Table S2).

3.8. Toxicity assessment of sediments

In summary, evidence was found for the effects of sediment on worm survival, TAC, MGO content, and ETS activity. Based on these endpoints, sediment samples that were considered toxic are listed in Table 1. Toxic effects were most frequently observed in sediments E5 and E6.

Strong correlations ($|r_S| \geq 0.7$) were found between these endpoints and several contaminants (Fig. S7). Notably, survival was negatively correlated with Cr and Ni. MGO was positively correlated with Hg, Pb, Ni, Zn, CLBs, HCH, and DDX. Also, ETS was positively correlated with Pb, As, and Zn.

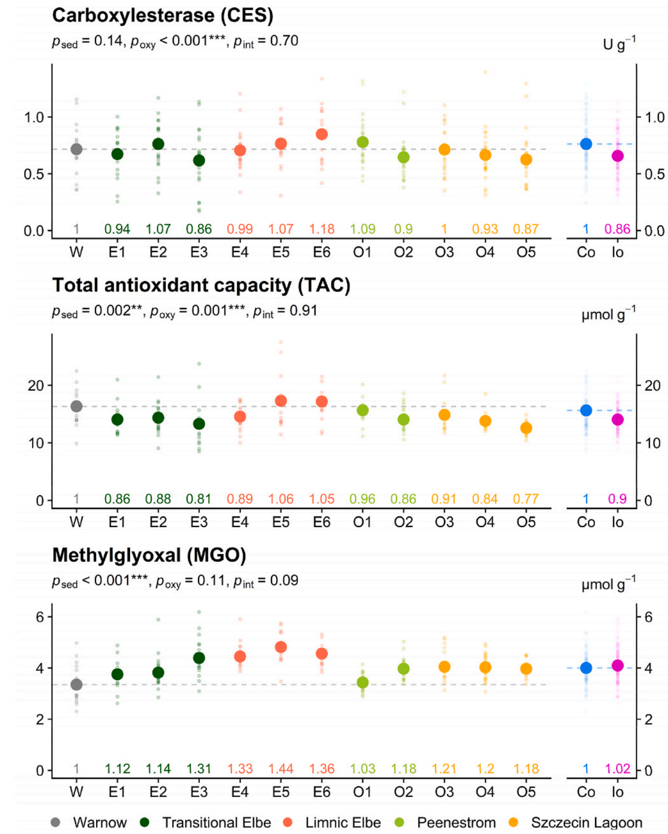

Fig. 6. Effects of the Elbe and Oder estuary sediments (E1-E6 and O1-O5) and intermittent oxygenation (Io) on carboxylesterase (CES) activity, total antioxidant capacity (TAC), and methylglyoxal (MGO) content of ragworms, with the Warnow estuary sediment (W) and continuous oxygenation (Co) as controls. Individual observations are shown as small dots and predicted means are shown as large dots. Dashed lines indicate the control means. Response ratios (RR) are given for 12 sediment samples and two oxygen regimes. Permutation *p*-values are given for sediment (sed), oxygen (oxy), and their interaction (int).

4. Discussion

4.1. Sediment contamination in the Elbe and Oder estuaries

In this study, we considered only certain classes of persistent contaminants that are commonly used in monitoring programs (*German Environment Agency, 2024*). However, we expect that the observed patterns shared by the analyzed chemicals could be generalized to many other unmeasured substances.

Our data suggest that contamination gradients exist in both estuaries, with upstream sediments typically being more contaminated. This could be explained by the low-energy environment in the upper estuarine regions (*Kowalewska* et al., *2003*; *Wetzel* et al., *2013*), which favored the deposition of fine grains and organic matter. Due to the affinity of these sediment fractions for heavy metals and organic chemicals (Burton, 2002), the Limnic Elbe and the Szczecin Lagoon became effective sinks for contaminants. Towards the North Sea and the Baltic Sea, these contaminants were diluted by seawater and marine sediments (*Pham* et al., *2024*), resulting in lower contamination status in the Transitional Elbe and the Peenestrom. Notably, the influence of the marine environment can also be found in upstream sampling sites, as indicated by the C:N ratios in sediments between 8 and 12, typical for organic matter of mixed marine and terrestrial origins (*Tang* et al., *2020*).

In general, the Elbe estuary had higher levels of contaminants than the Oder. Several factors may contribute to this trend, such as differences in contaminant loads from their catchment areas or simply the

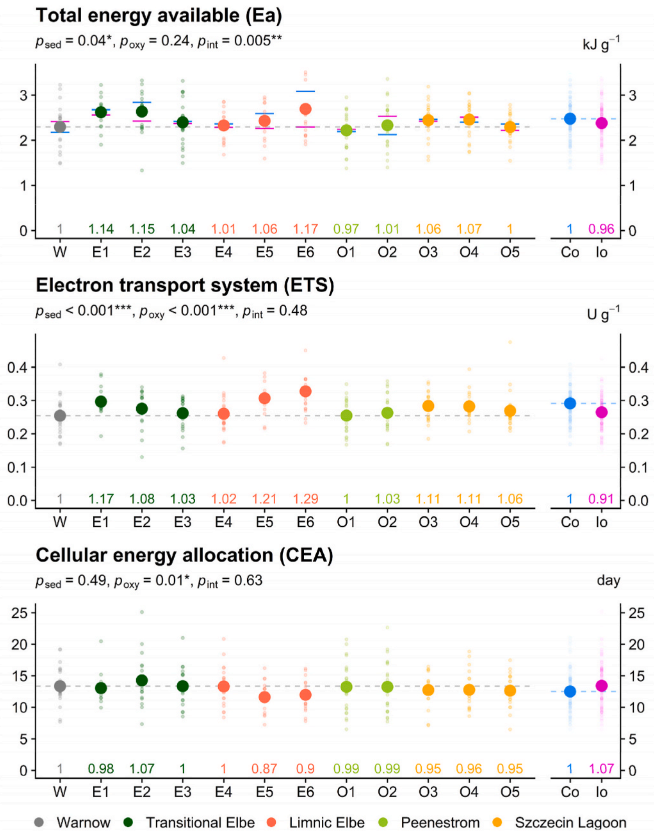

Fig. 7. Effects of the Elbe and Oder estuary sediments (E1-E6 and O1-O5) and intermittent oxygenation (Io) on total energy available (Ea), electron transport system (ETS) activity, and cellular energy allocation (CEA) of ragworms, with the Warnow estuary sediment (W) and continuous oxygenation (Co) as controls. Individual observations are shown as small dots and predicted means are shown as large dots. Dashed lines indicate the control means. Response ratios (RR) are given for 12 sediment samples and two oxygen regimes. Permutation *p*-values are given for sediment (sed), oxygen (oxy), and their interaction (int). To illustrate the interactive effect on Ea, two short segments indicating predicted means of oxygen regimes are additionally shown for each sediment sample.

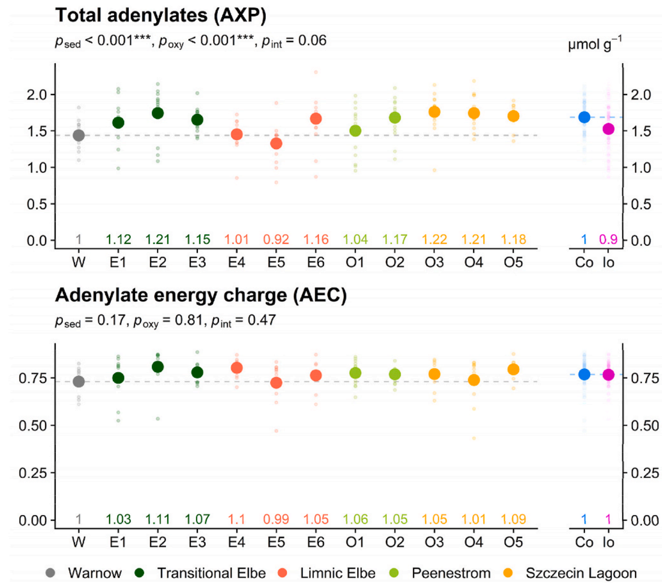

Fig. 8. Effects of the Elbe and Oder estuary sediments (E1-E6 and O1-O5) and intermittent oxygenation (Io) on total adenylates (AXP) and adenylate energy charge (AEC) of ragworms, with the Warnow estuary sediment (W) and continuous oxygenation (Co) as controls. Individual observations are shown as small dots and predicted means are shown as large dots. Dashed lines indicate the control means. Response ratios (RR) are given for 12 sediment samples and two oxygen regimes. Permutation *p*-values are given for sediment (sed), oxygen (oxy), and their interaction (int).

proximity of sampling sites to the upstream contamination sources. An exception among the analyzed chemicals was PAHs, which were more abundant in the Oder estuary sediments. As by-products of the incomplete combustion of organic matter, elevated PAH contents possibly reflect the intensive industrial activities such as coal mining and metallurgy in the Oder headwaters in the past (Kowalewska et al., 2003; Müller et al., 2002).

Compared with the 1980s, water and sediment quality in the Elbe and Oder rivers have reportedly improved as a result of European and national environmental measures, such as emission reductions and the construction of wastewater treatment plants (Ciszewski, 2003; Netzband et al., 2002). Although we did not investigate the temporal changes in sediment contamination, our study found similar ranges of heavy metals and organic contaminants as previous surveys in the Elbe and Oder estuaries (Kowalewska et al., 2003; Wetzel et al., 2013). This may signal a slowdown in the sediment quality improvement in recent years.

The presence of synthetic organic chemicals or the elevated contents of naturally occurring substances such as heavy metals in sediments only indicates contamination. Pollution, on the other hand, is the extent to which contamination causes adverse biological effects (Chapman, 2007). For sediment pollution assessment, chemical data are often compared with regulatory benchmarks, such as Sediment Quality Guidelines (SQG) or European Environmental Quality Standards (EQS). However, these benchmarks are only provided for a limited number of chemicals (Birch, 2018; Burton, 2002) and usually not tailored to estuarine sediments (Chapman, 2002; Chapman and Wang, 2001). Among the analyzed contaminants in our study, EQS are only available for As, Cr, Cu, and Zn (40, 640, 160, 800 mg kg^{-1} of the <63 μm fraction, respectively), and PCBs (20 μg kg^{-1} of the <2 mm fraction for each congener) (German Environment Agency, 2017). While the PCBs content in all sediment samples did not exceed the threshold value, a comparison of heavy metal contents with EQS is unfortunately not possible because our measurements were made in the <20 μm fraction. Given the limitations of chemical-based pollution assessment, it is often used in combination with other approaches such as sediment toxicity testing (Chapman, 1990), as was done in our study.

4.2. Sediment toxicity to ragworms

The most ecologically relevant endpoint in our toxicity test was survival. A significant decrease in worm survival was found in sediments E5 and E6 from the Limnic Elbe, which is not surprising as these sediments were the most contaminated. However, survival was also low in sediment E1 from the Transitional Elbe, which did not show high levels of the analyzed contaminants. It is possible that the observed toxic effect was caused by unmeasured chemicals in the sediment. In this case, Toxicity Identification Evaluation (TIE) procedures could be performed to find the causative chemicals (Ho and Burgess, 2009). Notably, worm survival was high in the Peenestrom sediments, which could be the combined result of low contamination and adequate organic matter supply in the samples. Given the differences in worm survival between sediment samples, survival bias may be present in other endpoints.

We did not observe increased activities of the biotransformation enzymes CES, GST, and GR in worms. CES and GST detoxify contaminants by hydrolysis and glutathione conjugation, respectively (Parkinson et al., 2022), while GR is responsible for glutathione regeneration (Couto et al., 2016). TAC, which measures the amount of enzymatic and non-enzymatic antioxidants to neutralize reactive oxygen species (ROS) (Regoli et al., 2002), was significantly reduced in only one sample (sediment O5 in the Szczecin Lagoon). These results may indicate that

Table 1
Sediment toxicity assessment based on test endpoints. Only endpoints showing the sediment effect with *p*-value ≤0.05 were included. Sediment samples with RR ≤ 0.8 or RR ≥ 1.2 were identified as toxic and are marked with "+".

	E1	E2	E3	E4	E5	E6	O1	O2	O3	O4	O5
Survival	+				+	+					
TAC											+
MGO			+	+	+	+			+	+	
ETS					+	+					

the contamination levels of most sediments from the Elbe and Oder estuaries were not high enough to stimulate detoxification and antioxidant defense in ragworms.

We also examined three biomarkers of electrophilic stress, MGO, MDA, and PC. MGO is formed as a by-product of glycolysis and many other metabolic pathways (Lai et al., 2022), while MDA is an end product of ROS-induced lipid peroxidation (Ayala et al., 2014). MGO and MDA are both reactive carbonyl species (RCS) that irreversibly modify proteins and lead to the formation of PC (Rodríguez-García et al., 2020). Here we found elevated levels of MGO in worms in more than half of the sediment samples, but no changes in MDA and PC levels, suggesting substantial, but not very high, electrophilic stress.

Significantly elevated ETS activity was observed in worms exposed to sediments E5 and E6 from the Limnic Elbe, indicating increased cellular maintenance costs to cope with contaminants (Fanslow et al., 2001; Sokolova, 2021). However, worms in most sediment samples were able to maintain high total energy available in their carbohydrate, lipid, and protein reserves, as well as high levels of ATP and total adenylates. Consequently, both CEA and AEC were maintained at the normal levels. CEA can be thought of as the time that worms can survive on their energy reserves after the energy input is stopped (Pham et al., 2023), while AEC indicates the amount of useable metabolic energy stored in the adenylate pool (Fuente et al., 2014). Worm mass, as a proxy for growth, also did not differ between sediment samples. Taken together, these results suggest that the Elbe and Oder estuary sediments did not cause significant impairment of worm energy status, probably due to the fact that more contaminated sediments often have more food supply in the form of organic matter (Mouneyrac et al., 2010).

Among the multiple biomarkers measured in our study, TAC, MGO content, and ETS activity were more sensitive to contaminated sediments. In particular, MGO and ETS levels were strongly associated with multiple contaminants in sediments, suggesting potential causal relationships. Similar results were obtained in previous toxicity tests with ragworms (Pham et al., 2023, 2024). These findings suggest that the testing effort could be reduced by focusing on a small set of sensitive biomarkers in this species (Falfushynska et al., 2024).

Our current testing approach with ragworms also has several limitations. First, ragworms used for toxicity testing must be collected from relatively uncontaminated areas, which are becoming rarer due to widespread global contamination (Williams et al., 2022). This challenge could be overcome by breeding and rearing worms under laboratory conditions or indoor systems (Nesto et al., 2018). Secondly, we focused only on a pre-selected set of biomarkers, and the small mass of ragworms necessitates a large sample size to perform all traditional biomarker assays. Future studies could use omics approaches to evaluate a broader range of biomarkers in smaller sample sizes (Simpson et al., 2016).

4.3. Effects of oxygen on test endpoints

Worms under intermittent oxygenation had lower ETS activity and ATP levels. These results are expected because the lack of oxygen as a terminal electron acceptor slows down the mitochondrial electron transport chain, which in turn suppresses ATP production by chemiosmosis (Sokolova et al., 2019). Since ETS activity decreased but the total energy available remained stable, intermittent oxygenation led to an increase in CEA (Pham et al., 2023). The metabolic depression under intermittent oxygenation probably also led to the observed decreases in ADP and total adenylates, which helped maintain the AEC within its narrow range (Fuente et al., 2014; Sokolova, 2013).

We found the reduced TAC in worms under intermittent oxygenation, indicating a depleted amount of antioxidants. This could be explained by the excessive production of ROS during both deoxygenation and reoxygenation (Dröse et al., 2016). We also observed a decrease in CES activity during intermittent oxygenation, which could be due to enzyme damage by ROS or low enzyme production under low ATP conditions.

Notably, intermittent oxygenation led to higher worm survival and mass. We postulate that a lower metabolic rate at reduced oxygen levels probably prolonged the lifespan as well as preserved the standing stock of energy reserves. In this regard, continuous oxygenation, often used in laboratory settings, might be considered a stressor for ragworms.

It is a common assumption that defense against contaminants is energetically costly and therefore cannot work at full capacity under limited energy conditions, such as deoxygenation (Sokolova, 2013). This is the basis for the concerns about the interaction between contaminants and oxygen. In our study, we found interaction effects only on protein content and consequently on total energy available, while all other endpoints showed additive effects. The lack of interaction could be attributed to the relatively weak effects of intermittent oxygenation, as indicated by small effect sizes with little toxicological significance. This could be explained by the tolerance of ragworms to oxygen fluctuations, including prolonged hypoxia as suggested by previous studies (Santos et al., 2016; Vismann, 1990). Our result implies the robustness of the biomarker-based toxicity test using ragworms, as reported in other studies (Pham et al., 2023, 2024), in which the deviations of controlled conditions such as temperature and oxygen do not influence the conclusion about sediment toxicity (Simpson et al., 2016).

4.4. Ecological and regulatory implications

The high levels of heavy metals and organic chemicals in sediments from the upper region of the Elbe estuary and their observed toxic effects on ragworms indicate ecological risks to estuarine ecosystems. These contaminants may not only affect local benthic communities but may also affect estuarine organisms in the coastal region, especially if disturbance events such as dredging or flooding cause the transport of contaminated suspended particles downstream (Crawford et al., 2022; Roberts, 2012). Therefore, careful management of dredging, disposal, and flood control practices is important to limit adverse ecological effects.

An early proposal for sediment pollution assessment is the Sediment Quality Triad (SQT), which integrates three pillars: sediment chemical analysis, sediment toxicity testing, and benthic community surveys (Chapman, 1990). While the first and third pillars have been incorporated into European regulatory frameworks, the adoption of sediment toxicity testing has been slower (Ausili et al., 2022; Leppanen et al., 2024; Tarazona et al., 2014). This delay can be attributed to the stakeholder skepticism about the utility of current toxicity tests, including concerns about the ecological relevance of test conditions, test species, and endpoints such as biomarkers (Hagger et al., 2006; Heise et al., 2020). These concerns could be mitigated by conducting toxicity tests on contaminated sediments under more relevant climatic scenarios, with more ecologically important species, and using multiple biomarkers to provide a more reliable assessment (*Pham* et al., *2024*; *Pham and Sokolova, 2023*), as exemplified by our study.

4.5. Conclusions

Sediments from the Elbe and Oder estuaries were contaminated with various heavy metals and organic chemicals. Based on survival and multiple biomarkers, we identified several sediment samples as toxic to ragworms. The most problematic sediments were from the upper region of the Elbe estuary, which contained high levels of contaminants. The effects of sediments on ragworms were generally not modified by oxygen regimes, suggesting the robustness of the sediment toxicity test. Our study demonstrates the utility of biomarker-based toxicity testing for estuarine sediment pollution assessment and its potential for incorporation into regulatory frameworks.

CRediT authorship contribution statement

Duy Nghia Pham: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Safia El Toum:** Writing – review & editing, Investigation. **Raphaëlle Martineau:** Writing – review & editing, Investigation. **Susanne Heise:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Data curation. **Inna M. Sokolova:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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.2024.120451.

Data availability

Research data are publicly available on Zenodo at https://doi.org/10.5281/zenodo.14037268.

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