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Article

Biochemical and Behavioural Alterations Induced by Arsenic and Temperature in *Hediste Diversicolor* of Different Growth Stages

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Abstract: Contamination with Arsenic, a toxic metalloid, is increasing in the marine environment. Additionally, global warming can alter metalloids toxicity. Polychaetes are key species in marine environments. By mobilizing sediments, they play vital roles in nutrient and element (including contaminants) cycles. Most studies with marine invertebrates focused on the effects of metalloids on either adults or larvae. Here we bring information on the effects of temperature increase and arsenic contamination on the polychaete *Hediste diversicolor* in different growth stages and water temperatures. Feeding activity and biochemical responses – neurotransmission, indicators of cell damage, antioxidant and biotransformation enzymes and metabolic capacity - were evaluated. Temperature rise combined with As imposed alterations on feeding activity and biochemical endpoints at different growth stages. Small organisms have their antioxidant enzymes increased, avoiding lipid damage. However, larger organisms are the most affected class due to inhibition of superoxide dismutase, which resulted in protein damage. Oxidative damage was observed on smaller and larger organisms exposed to As and 21 °C, demonstrating higher sensibility to the combination of temperature rise and As. The observed alterations may have ecological consequences, affecting the cycle of nutrients, sediment oxygenation and the food chain that depend on the bioturbation of this polychaete.

Keywords: Arsenic; global warming; invertebrates; behavior; oxidative stress

1. Introduction

Coastal systems often serve as sinks for pollutants arising from aquaculture, shipping, agriculture, industry, mining and sewage treatment plants [1], causing environmental decay of natural conditions [2-3]. Many chemicals and elements have become essential to ensure the quality of products used on a daily basis [4]. In fact, their need keeps growing. However, their production and use often result to contamination of the environment. Fortunately, policy commitments are continuously working to reduce discharges, emissions and losses of contaminants, showing positive results on contaminant reduction [1]. Despite the reduction of contamination, European seas (Baltic Sea, Black Sea, Mediterranean Sea and North-East Atlantic Ocean) still present a variety of contaminants, such as metals and metalloids, organobromines, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) [1], which can induce problems for human health and loss of ecosystem functions and their services [1][5-7].

Metals and metalloids toxicity varies according to the element but usually are toxic at sufficiently high levels. The difference between optimal and toxic levels depends on the

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physiological needs of organisms [8]. Some metals are naturally occurring and are biologically essential (e.g., copper, chromium, manganese and zinc, which are essential for organisms' growth and life cycles). However, high concentrations of these elements can cause toxicity [8-9]. Others are non-essential metals (e.g., cadmium, lead and mercury), since they do not have any function for organisms whose accumulation is dangerous for their living being [10-12]. Metalloids are semimetals that have a physical appearance with metals but acts chemically like nonmetal [11] and are dangerous even in lower concentrations [11][13]. Natural sources of metals and metalloids include minerals by disintegration and alteration of minerals and rocks by physico-biogeochemical processes, volcanic eruptions, forest fires and biogenic sources [14] [9][15]. However, they also have anthropogenic sources like coal and oil combustion, metal mining, smelting and refining, fertilizers, pesticides and waste incineration [15]. Urbanization of marine coastal areas is also contributing to the increase of these contaminants in estuarine coastal systems [16].

Arsenic (As) is a toxic metalloid that is among the most found inorganic contaminant and often occurs from natural sources (e.g., earth crust) as arsenates, sulfides, sulfosalts, arsenides, arsenites, oxides, silicates and elemental As [17]. Regarding this, all organisms are constantly in contact with this element by air, water consumption and food ingestion, which at natural occurring, humans do not accumulate more than 4 mg of As and marine animals 0.3 mg [17][18]. However, as a result of human activities like fertilizer industry and mining activity, As concentration on aquatic ecosystems has increased [19-20]. Previous studies demonstrated that this contaminant tends to bioaccumulate more on producers (e.g., algae) and then to biomagnify to first consumers (e.g., crustaceans, bivalves, annelids) [20]. However, due to the detoxification mechanisms of some organisms (e.g., phytoplankton, bacteria, etc.) that transforms arsenic inorganic forms to methylated and organic forms, decreasing this biomagnification with the increase of trophic level [20]. Exposure to As can induce blood pressure alteration on pregnant women [21], cause cancer on urinary bladder, lung and skin on humans [22], can reduce infertility by reducing sperm production, number and quality on rats [23]. Moreover, it was also demonstrated that As can accumulate in tissues and along with the trophic webs [24-26]. Arsenic has also been shown to affect the regenerative capacity of polychaetes [27-28], in which organisms regenerated fewer segments and took longer to regenerate, as well as alter their behaviour [29-34], which can have serious effects on the ecosystem.

Alongside pollution, global warming is also a worldwide threat, contributing to water acidification, and temperature and seawater salinity increases [35]. Temperature rising is being experienced almost entire globe. In the decade 2006-2015 was observed an increase of global mean surface temperature by 0.87 °C, comparing to the last century [35]. By the end of the century, it is estimated that global mean surface temperature will increase between 0.95 and 2.0 °C depending on how successful is reduction of CO_2 emission [35]. Previous studies demonstrated that temperature rise impacts marine organisms, by increasing diseases and mortality in shrimps [36], redistribution of species, specially fish, to higher latitudes, contributing to homonegenization of marine fauna in lower latitudes [37-39], decreasing burrowing activity and delay on the regenerative capacity of polychaetes [40-41], larval increase, otolith and somatic growth on northern pike [42], increasing of cannibalism behaviour at early age due to length difference between on *Esox lucius* L. [43]. In addition, it is also expected that temperature increase modulates the susceptibility of organisms to pollutants through alterations in the rate of biochemical and physiological processes, but may also change pollutants bioavailability and toxicity [44-45].

Polychaetes are usually the most abundant taxonomic group in estuarine environments [46-47] and play an important role in food chains being a valuable food source to crustaceans, birds and fish populations [48-49];[34]. Additionally, these organisms can accumulate large amounts of contaminants, as they live in close contact with the sediment

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and pore water [50], and by their various feeding strategies like suspension-feeding, deposit-feeding and omnivore [51]. Several studies demonstrated that these marine species are good bioindicators of contaminants, such as metals and metalloids [50], nanoplastics [52] and polycyclic aromatic hydrocarbons [48], among others. Moreover, the activities of macrobenthic polychaetes have significant impacts on sediments mobilization and organisms, like the polychaeta *Hediste diversicolor* (O.F. Müller, 1776), In fact, the deposition of these contaminants in sediments is a natural remediation that removes them from circulating from the environment, accumulating on the sediments [53]. Environmental studies conducted in the Ria de Aveiro Lagoon (Portugal), demonstrated the capacity of the polychaete *Diopatra neapolitana* to accumulate various metals (Cr, Ni, Cu, Pb, Cd, Hg) and As, leading to cellular damage and increased antioxidant and biotransformation enzymes activity [28]. Moreover, the same study demonstrated that the accumulation of the studies metals and As affected the regenerative capacity of *D. neapolitana*, in which organisms regenerated fewer segments and took longer to regenerate. Under laboratory conditions exposure of *Perinereis aibuhitensis* to Cd to for 8 days suggested that Cd interfered with the antioxidant defense system of this polychaete species [54]. Bouraoui et al. [55] also observed the induction of oxidative stress biomarkers in different body regions of the polychaete *H. diversicolor* exposed to Cu.

H. diversicolor, known as common ragworm, is a polychaete species belonging to the phylum Annelida, family Nereididae. Polychaetes nereidids are characterized by the presence of paragnaths, known as chitinous denticles, distributed in groups on maxillary belts and oral belts of the pharynx [56]. The species *H. diversicolor* is characterized by an eversible proboscis with paragnaths on oral and maxillary belts, a subtriangular prostomium with four small eyes, two large palpi and two short frontal antennae, a peristomium with four pairs of tentacular cirri and a length of 45-92 segments [57-58]. The paragnaths located on maxillary belts have the function to grab the food and transfer it towards the gut by pharynx retraction [59]. The paragnaths located on oral belts have the function to burrow and browse on the sediment [60]. This species inhabits the shallow marine and brackish waters, in mud sand but also gravels, clays and turf, distributed in the North temperature zone from both the European and the north American coast of the Atlantic [58]. *H. diversicolor* has great ecological tolerance, being found in European estuaries, where it plays an important role in these ecosystems [58]. It creates borrows causing bioturbation, which potentializes sediment oxygenation, affecting the cycle of nutrients and the availability of contaminants [61][58]. The common ragworm has a commercial interest because it is used for recreational fishing and food in aquaculture [58].

Although several studies on the toxicological effect of As and temperature increase on marine invertebrates have been reported in the literature, there is no data in the literature that allow us to conclude that they may have an effect according to organism's size, particularly on polychaetes. Therefore, the aim of this study is to evaluate the chronic effects of As on *H. diversicolor* with different sizes and at different temperatures (16°C and 21°C). The selected parameters used to evaluate these effects were the activities of the enzymes cholinesterase, glutathione S-transferases, catalase and superoxide dismutase, lipid peroxidation and protein carbonylation to quantify the extent of oxidative damage, and energy related parameters and feeding activity.

2. Materials and Methods

2.1. Test Organisms

Specimens of *H. diversicolor* were born on laboratory and maintained in glass aquaria with artificial seawater (salinity 28 and pH 7.8) and sediment (at a ratio 3:1) at temperature-

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controlled room (16±1°C), under continuous aeration. During this nursery time, organisms were fed *ad libitum* with commercial fish food every 3/4 day (Protein 46.2%, Fat 8.9%). Water was renewed twice a month.

2.2. Experimental Design

Organisms were separated by size (1-2.5 cm (small, with an age of 3 months), 3-5 cm (medium, corresponding to an age of 5 months), 6-9 cm (large, organisms with 7 months)) and then, organisms of each size (20 small, 10 medium and 5 large x 3 replicates), were exposed for 28 days to As (0, 0.05, 0.25 mg/L) at two temperatures :16 ºC and 21 °C, under continuous aeration. For each condition, aquariums were filled with sand and artificial sea water (1:2). To avoid a temperature shock that would happen if the worms were distributed in the first day in each aquarium with the desired temperature, every aquarium started with the same temperature, at the control 16 °C. Every day, the temperature was increased 1 °C. After reached the desired temperature, polychaetes were exposed to the selected conditions for 28 days. Arsenic concentrations were chosen according to the literature available for Ria de Aveiro, Portugal [62][28]. Temperature conditions tested were based on values recorded at Ria de Aveiro and possible climate change scenarios predicted to occur in the near future [35][63][41]. Water was renewed every week, to remove products of metabolism, and organisms were fed with 10 mg of dry fish food per organism every 3/4 days [64][65]. At the end of experiment exposure period, organisms were frozen at -20 ºC for biochemical analysis.

2.3. Quantification of Arsenic

The concentration of Arsenic (As) was determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7700x) after acid digestion. In Teflon vessels was added 1.5 mL of HCl and 4.5 mL of HNO3 to a 500 mg of homogenized tissue. After 24 h, the Teflon vessels were placed on a heating plate at 115 °C and after 6 h the contents were transferred to a falcon tube. After adding 45 mL of ultrapure water, tubes were centrifuged and then read. A rigorous quality control was performed during these analyses, which included the analysis of blanks, duplicate samples, and certified reference materials. The precision and bias error of the chemical analysis was less than 10%.

2.4. Feeding Activity

21 days after the exposition, it was recorded the time that polychaetes needed to feed. For this assay, commercial fish food was added to the aquariums, and the time needed to polychaetes detect and catch the food in the sediment surface was recorded.

2.5. Biochemical Analysis

For biochemical analysis, the samples were homogenized in 0.1M Potassium Phosphate Buffer (pH 7.4). Homogenates were separated into 3 fractions: one for Lipid Peroxidation assessment; other for Cholinesterase and Electron Transport System, which was centrifuged for 3 minutes 3300 *g*, at 4 °C, and the rest of the samples were centrifuged for 20 minutes at 10000g at 4 °C, for Post-Mitochondrial Fraction (PMS) isolation [66].

2.5.1. Neurotransmission

Cholinesterase (ChE) activity was determined according to [67] adapted to microplate [68]. The rate of acetylthiocholine degradation was assessed at 412 nm by measuring the increase in the yellow color due to the binding of the thiocholine with 5,5-dithio-bis (2-

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nitrobenzoic acid). The results were expressed as nmol of thiocholine formed per minute per g of FW ($\varepsilon = 1.36 \times 104\ M^{-1}\ cm^{-1}$), using acetylthiocholine as substrate [66].

2.5.2. Energy Related Parameters

Total protein content from PMS fraction content was determined with Biuret method [69], as performed by [70], using bovine serum albumin (BSA) as standards (0-40 mg/mL). Absorbance was read at 540 nm. Results were expressed in mg per Fresh Weight (FW). The Electron Transport System (ETS) activity was measured according to the [71] methodology with modifications from [72], performed by [65]. Absorbance was measured at 490nm in microplate reader every 25 seconds for 10 minutes. The amount of formazan formed was calculated using $\varepsilon = 15.900\ M^{-1}\ cm^{-1}$ and the results expressed in nmol/min per g FW. Sugars were quantified using the phenol-sulphuric acid method, following procedure described by [73] and performed by [28]. Absorbance was measured at 462 nm and results were expressed in mg per g FW.

2.5.3. Antioxidant Enzymes

For superoxide dismutase (SOD) activity, it was used the method described by [74], with modifications [65]. Absorbance was measured spectrophotometrically in a microplate reader at 560 nm, after 20 minutes of incubation, at 25ºC. Results were expressed as U per g of FW.

Catalase (CAT) activity was assessed by the method of [75], with modifications described by [76]. Formaldehyde (0-150µM) standards were used and samples were incubated at room temperature. Absorbance was measured at 540nm. Results were expressed as U per g of FW.

2.5.4. Biotransformation Enzymes

Glutathione S-Transferases (GSTs) activity was determined based [77] method, with adaptions described by [78]. Absorbance was measured spectrophotometrically in a microplate reader at 340 nm ($\varepsilon = 9.6 \times 103\ mM^{-1}\ cm^{-1}$). Results were expressed in U/g of FW.

2.5.5. Indicators of Oxidative Damage

Lipid Peroxidation (LPO) was measured according to the method of [79] with modifications performed by [28], by quantifying thiobarbituric acid reactive substances (TBARS), reacting malondialdehyde (MDA) with 2-thiobarbituric acid (TBA). The absorbance was measured at 532 nm. The calculations of the concentration of MDA were made using the molar extinction coefficient ($\varepsilon = 1.56 \times 105\ M^{-1}\ cm^{-1}$) and expressed in nmol per FW.

Protein carbonylation (PC) levels were analyzed by the quantification of carbonyl groups through the 2,4-Dinitrophenylhydrazine (DNPH) alkaline method described by [80] with modifications described by [81]. Absorbance was read at 450 nm and results were expressed in nmol per FW, using 22,308 $M^{-1}\ cm^{-1}$ as molar absorptivity of the carbonyl-dinitrophenylhydrazine adduct [80].

2.6. Statistical Analysis

For each condition, the previous parameters (Feeding activity, LPO, PC, ETS, Sugars, SOD, CAT, GST, ChE) were submitted to hypothesis testing using permutational multivariate analysis of variance, with PERMANOVA+ add-on in PRIMER v6 [82]. Data was analyzed following a one-way hierarchical design, with exposure concentration as the main fixed factor. The null hypothesis tested were: 1) no significant differences existed

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among As concentrations (0, 0.05 and 0.25 mg/L) for each size (Small, Medium or Large) and for each temperature (16°C or 21°C); 2) no significant differences existed between sizes at same concentration of exposure and for each temperature; 3) no significant differences existed between organisms of the same sizes, exposed at the same concentration, among temperatures. The pseudo-F values in the PERMANOVA main tests were evaluated in terms of significance among different concentrations, sizes and temperature. When the main test revealed statistically significant differences ($p \leq 0.05$), pairwise comparisons were performed. Significance levels ($p \leq 0.05$) among concentrations and sizes were presented with different letters and significance differences among temperatures were presented with asterisk.

3. Results

3.1. Accumulation of Arsenic

Total As accumulated in *H. diversicolor* tissues from each class size exposed to As at 16 °C and As at 21 °C is represented in Fig 1A and Fig 1B, respectively. Exposed organisms, independently of the size, showed significantly higher total As concentrations compared to control for both temperatures (Fig 1A, B). An increasing trend in accumulated total As with increasing class size was observed in organisms exposed to 0.05 mg/L of As at 16 °C with significant differences among small and large sizes. On the other hand, a decreasing trend in accumulated total As with increasing class size was observed in organisms exposed to 0.25 mg/L of As at 16 °C with significant differences among small and large sizes.

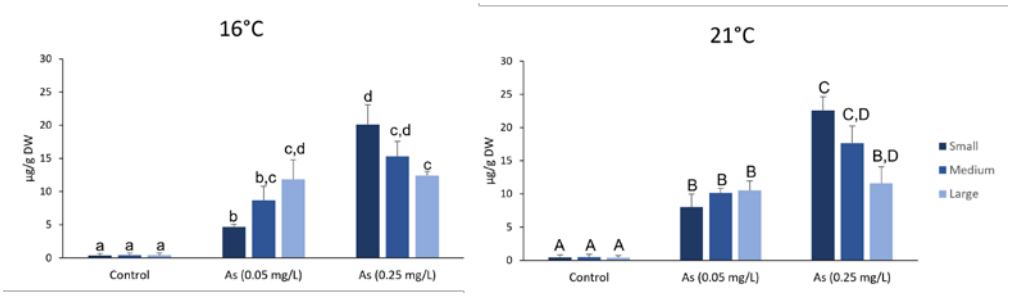

Figure 1. Total Arsenic in *Hediste diversicolor* tissues from different class sizes after exposure to Arsenic at 16 °C (A) and at 21 °C (B). Different letters represent significant differences ($p \leq 0.05$) between conditions (lowercase letters for 16 °C; uppercase letters for 21 °C).

The same trend was observed in organisms exposed at the same concentration and at 21 ºC. No significant differences were observed among small, medium and large size organisms exposed at 0.05 mg/L of As at 21 ºC. Small and medium organisms exposed at the highest As concentration at 21 ºC presented significantly higher As accumulation than organisms from the same class size exposed at 0.05 mg/L of As. Comparing both temperatures, no significant differences were observed for each class size exposed at different temperatures at the same As concentration.

3.2. Feeding Activity

Feeding activity of *H. diversicolor* organisms from each class size is represented in Fig 2, exposed at As and 16ºC (Fig 2A) and As and 21ºC (Fig 2B). Large and medium size organisms exposed at As and 16ºC significantly needed more time to detect the food, compared to the control (Fig 2A). The feeding time was especially long at the highest concentration (0.25 mg/L), being 3-fold longer than the control (Fig 2A). No significant changes were

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observed for small size organisms along with concentration increase. Large size organisms exposed at As and 21ºC also significantly increased their feeding time with concentration increase (Fig 2B). Additionally, medium size organisms exposed at As 0.25 mg/L and 21ºC have significantly increased time to take food compared to the medium size organisms from the others conditions.

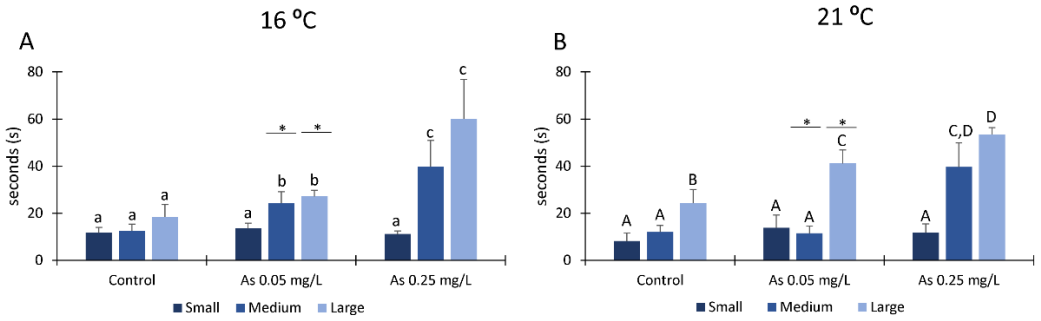

Figure 2. Feeding activity (time needed to detect the food) 21 days after exposure to Arsenic and 16 °C (A) and Arsenic and 21 °C (B) of *Hediste diversicolor* from different class sizes. Different letters represent significant differences (p≤0.05) between conditions (lowercase letters for 16 °C; uppercase letters for 21 °C). Asterisk (*) represent significant differences (p≤0.05) among temperatures for the same class size.

No significant differences were observed for small organisms (Fig 2B). Comparing both temperatures, significant differences were only observed in medium and large size organisms exposed at 0.05 mg/L of As. Medium size organisms took longer to detect food when exposed at 16 °C than organisms exposed at the same concentration exposed at 21 °C. On the other hand, large organisms exposed at the same As concentration needed more time to detect the food when were exposed at 21 °C.

3.3. Neurotransmission (ChE)

Large size organisms exposed at As 0.05 mg/L and 16 °C showed a significant decrease ChE activity compared to control. Moreover, at the control condition at 16 °C, large size organisms have higher ChE activity than small and medium size organisms. Additionally, at As 0.25 mg/L and 16 °C, small organisms have lower ChE activity than large polychaetas, being observed significant differences among sizes (Fig 3A). Regarding exposure to 21 °C, significant differences were only detected in small size organisms exposed at the highest As concentration, with organisms presenting significant lower activity than small size organisms from the remaining conditions (Fig 3B).

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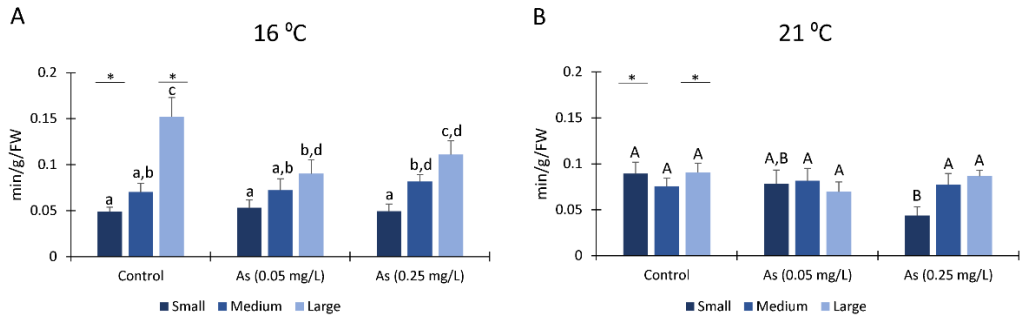

Figure 3. Cholinesterase (ChE) activity measured in *Hediste diversicolor* from different class sizes after 28 days of exposure to Arsenic at 16 °C (A) and 16 °C (B). Different letters represent significant differences ($p \leq 0.05$) between conditions (lowercase letters for 16 °C; uppercase letters for 21 °C). Asterisk (*) represent significant differences ($p \leq 0.05$) among temperatures for the same class size.

Comparing both temperatures, large organisms from all conditions exposed at 21ºC presented lower ChE activity than large organisms exposed at 16 °C (Fig 3A,B), however significant differences were only detected in organisms from the control (As 0.0 mg/L). No significant differences were observed among medium organisms between temperatures. Small size organisms not exposed to As (control) presented significantly higher ChE activity at 21 °C than organisms from the same class size at 16 °C for the same conditions (Fig 3A,B).

3.4. Energy-related Parameters

3.4.1. ETS

Medium size organisms exposed to As 0.05 mg/L at 16 °C presented significantly lower ETS levels compared to medium size organisms from the remaining conditions (Fig 4.1A). Additionally, medium size organisms exposed to As 0.25 mg/L at 21 °C have significantly higher ETS levels than same size organisms exposed to As 0.05mg/L at the same temperature (Fig 4.1B).

Small size organisms not exposed to As at 16 °C and exposed to As 0.25 mg/L at 21 °C have significantly lower ETS levels when compared to the other class size organisms from the same condition (Fig 4.1A,B). Comparing exposure among temperatures, large size organisms exposed to As showed significantly higher ETS levels when exposed to 21 °C (Fig 4.1A,B).

3.4.2. Sugars

Sugars content showed a decreasing tendency when the organisms from all class sizes were exposed to As at 16 °C (Fig 4.2A). However, when organisms were exposed at 21 °C, sugars content increased on large size organisms when exposed to 0.25mg/L As (Fig 4.2B). For the same condition, sugars levels were significantly higher on large size organisms and lowest on small size organisms.

Comparing exposure among temperatures, medium class size organisms exposed at 21 °C and exposed to 0.25 mg/L of As showed significant higher sugars levels than organisms from the same class size exposed at 16 °C. On the other hand, large size organisms exposed

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to As and at 21 °C presented significantly higher sugars levels than organisms exposed at 16 °C (Fig 4.2A, B).

3.4.3. Protein Content

Small size organisms exposed at As and 16 °C have significantly higher protein content compared to control (Fig 4.3A). Protein levels in organisms exposed at 16 °C and without As (control) vary significantly among sizes, with small size organisms presenting lower levels and large organisms having higher protein levels. No significant differences were observed for the remaining organisms exposed at 16 °C (Fig 4.3A). For organisms exposed at 21 °C, significant differences among class sizes were observed only in small organisms not exposed to As and exposed at As at 0.25 mg/L that had significantly lower protein content than medium and large organisms exposed at the same conditions (Fig 4.3B). Moreover, all organisms from each class size exposed at 16 °C have higher protein content than organisms exposed at 21 °C, being significantly different in large organisms from control, medium organisms exposed at 0.05 mg/L and small and medium polychaetes exposed at 0.25 mg/L (Fig 4.3A,B).

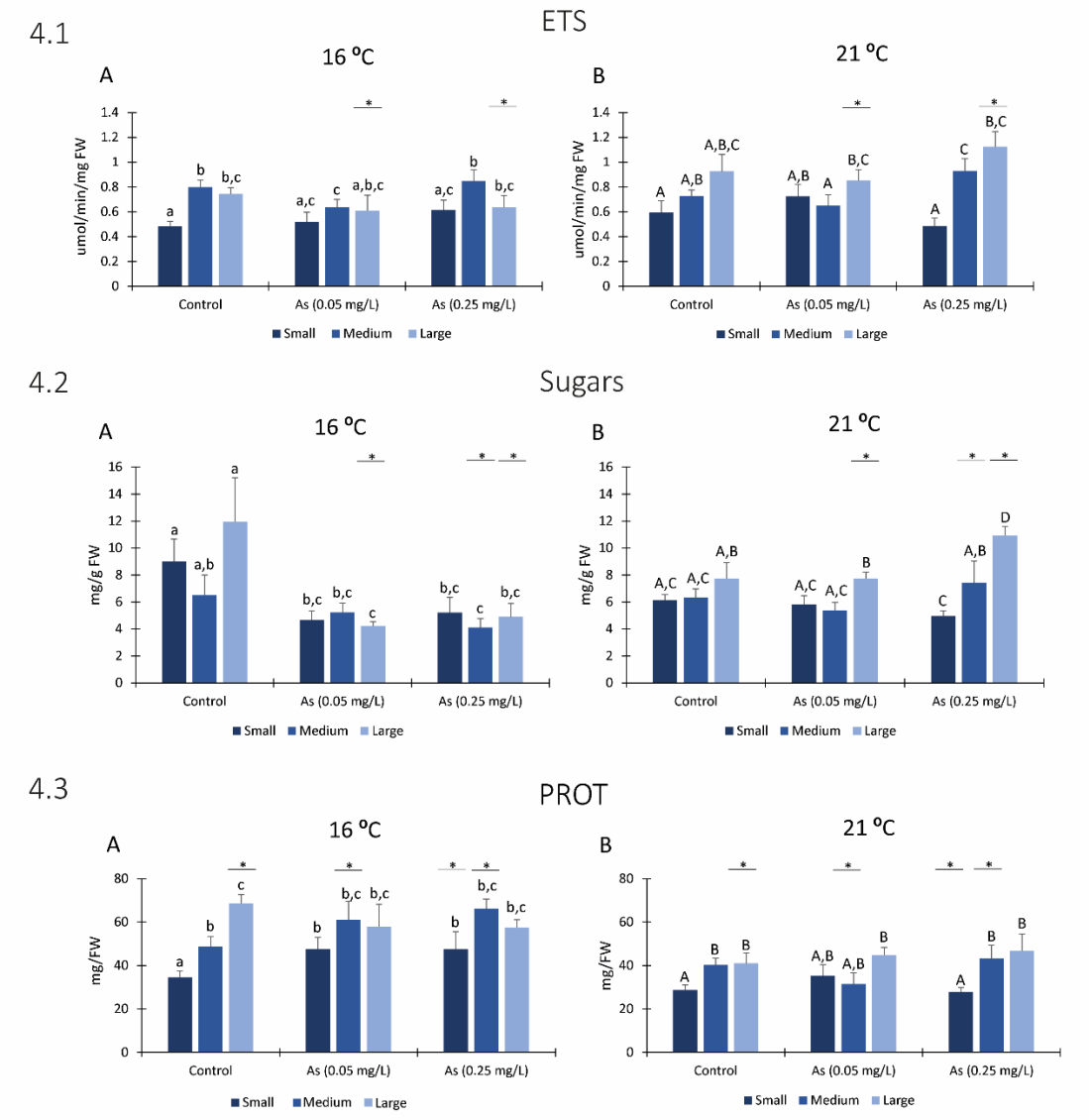

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Figure 4. Metabolism related parameters. (4.1) Electron transport system (ETS) on temperature 16 °C (A) and 21 °C (B) of *Hediste diversicolor* after 28 days of exposure to Arsenic. (4.2) Sugars content measured in *H. diversicolor* from different class sizes after 28 days of exposure to Arsenic at 16oC (A) and at 21oC (B). (4.3) Protein content 28 days after exposure to Arsenic and 16 °C (A) and Arsenic and 21 °C (B) of *Hediste diversicolor* from different class sizes. Different letters represent significant differences ($p \leq 0.05$) between conditions (lowercase letters for 16 °C; uppercase letters for 21 °C). Asterisk (*) represent significant differences ($p \leq 0.05$) among temperatures for the same class size.

3.5. Antioxidant Enzymes

3.5.1. SOD

At 16 °C, small size exposed organisms at 0.05 mg/L As significantly decrease the SOD activity comparing to the same size organisms from control. Moreover, large size organisms exposed at the highest As concentration (0.25 mg/L) presented significantly lower SOD activity than polychaetas of the same size from the others conditions (Fig 5.1A).

Regarding *H. diversicolor* exposed to 21 °C, small size organisms exposed at As 0.05 mg/L have significantly lower SOD activity than non-exposed organisms from the same size. On the other hand, large size organisms exposed at As 0.25 mg/L have significantly lower SOD activity than control organisms of the same size (Fig 5.1B).

Comparing both temperatures, all organisms from all conditions exposed at 21 °C presented higher SOD activity than organisms exposed at 16 °C, with significant differences in small size organisms of all conditions, medium size organisms exposed at 0.02 mg/L and large size organisms exposed at 0.25 mg/L (Fig 5.1A,B).

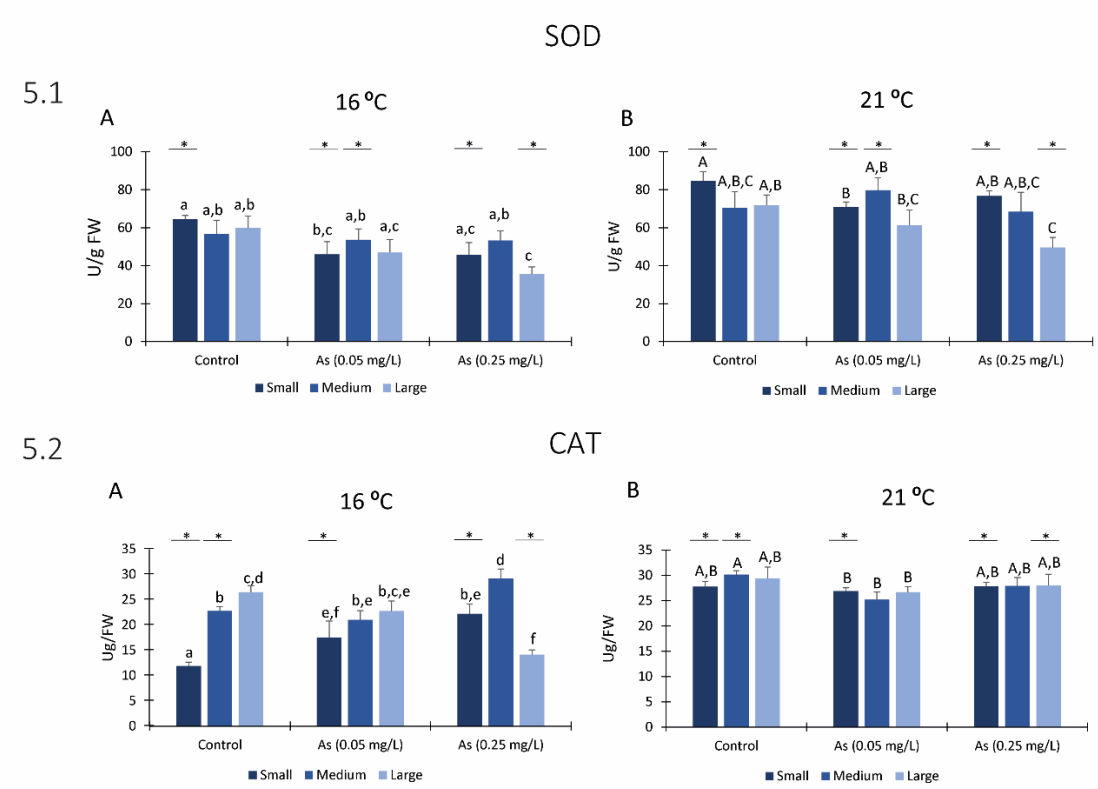

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Figure 5. Antioxidant enzymes: (5.1) Superoxide dismutase activity (SOD) measured in *H. diversicolor* from different class sizes after 28 days of exposure to Arsenic at 16 °C (A) and 21 °C (B). (5.2) Catalase (CAT) activity measured in *H. diversicolor* from different class sizes after 28 days of exposure to Arsenic at 16 °C (A) and 21 °C (B). Different letters represent significant differences ($p \leq 0.05$) between conditions (lowercase letters for 16 °C; uppercase letters for 21 °C). Asterisk (*) represent significant differences ($p \leq 0.05$) among temperatures for the same class size.

3.5.2. CAT

Small size organisms exposed to As significantly increased CAT activity when exposed at 16 °C (Fig 5.2A), while medium size organisms only significantly increased CAT activity when exposed to As 0.25 mg/L comparing to organisms from the same size from remaining conditions. Additionally, large size organisms exposed at As 0.25 mg/L significantly decreased CAT activity comparing to same size of other conditions (Fig 5.2A). Comparing among conditions, large size organisms exposed at 16 °C without As presented significantly higher CAT activity and small size organisms' lower activity. On the other hand, small and medium organisms exposed at As 0.25 mg/L showed higher CAT activity than large ones, being observed significant differences among sizes.

Regarding organisms exposed at 21 °C, only medium size organisms exposed at 0.05 mg/L of As presented significant lower CAT activity when compared to organisms from control with the same size (Fig 5.2B).

Small size organisms exposed at 21 °C presented higher CAT activity than organisms exposed at 16 °C for all conditions. Medium organisms presented significantly higher CAT levels when exposed at 21 °C without As and at the highest As condition, than medium organisms exposed at the same concentrations at 16 °C. Large polychaetes only presented significantly higher CAT levels when exposed at 21 °C at 0.25 mg/L AS. (Fig 5.2A,B).

3.6. Biotransformation Enzymes (GSTs)

Small size organisms exposed at As 0.25 mg/L and 16 °C showed significantly higher GSTs activity than small size control organisms (Fig 6A). Medium size organisms exposed at As 0.25 mg/L and 16 °C have significantly higher CAT activity than medium size organisms exposed at control and at As 0.05 mg/L. Furthermore, large size organisms of As 0.25 mg/L and 16 °C showed significantly higher activity than the others of the same size conditions. Additionally, in control condition, medium size organisms have significantly higher activity than organisms from the remaining sizes. Nevertheless, small size organisms exposed at As 0.25 mg/L at 16 °C have significantly lower activity from the condition (Fig 6A).

Small size organisms exposed at As 0.25 mg/L at 21 °C presented significantly lower activity than small size of As 0.05 mg/L. Additionally, large size organisms from control at 21 °C presented significantly higher activity than small and medium size organisms (Fig 6B).

Comparing both temperatures, almost all organisms from all conditions exposed at 21 °C presented significantly higher GSTs activity than organisms exposed at 16 °C, except medium size organisms not exposed to As (Fig 6A,B).

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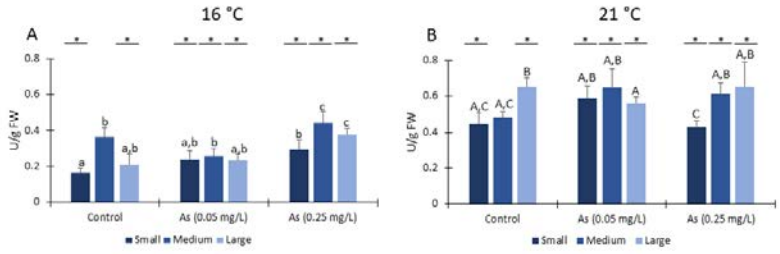

Figure 6. Gluthathione-S-transferases (GSTs) activity measured in *H. diversicolor* from different class sizes after 28 days of exposure to Arsenic at 16 °C (A) and 21 °C (B) of *Hediste diversicolor* after 28 days of exposure. Different letters represent significant differences (p≤0.05) between conditions (lowercase letters for 16 °C; uppercase letters for 21 °C). Asterisk (*) represent significant differences (p≤0.05) among temperatures for the same class size.

3.7. Indicators of Oxidative Damage

3.7.1. LPO

No significant differences in lipid peroxidation levels were observed on organisms exposed at 16 °C (Fig 7.1A), independently of the class size or the exposure to As.

Lipid peroxidation levels significantly increased for large size organisms exposed at the highest As concentration and at 21 °C (Fig 7.1B). Moreover, large organisms exposed at 21 °C for all As conditions and medium size organisms exposed at As 0.05 mg/L at 21 °C have significant higher lipid peroxidation levels compared to *H. diversicolor* organisms from the same class sizes exposed at 16 °C (Fig 7.1A,B).

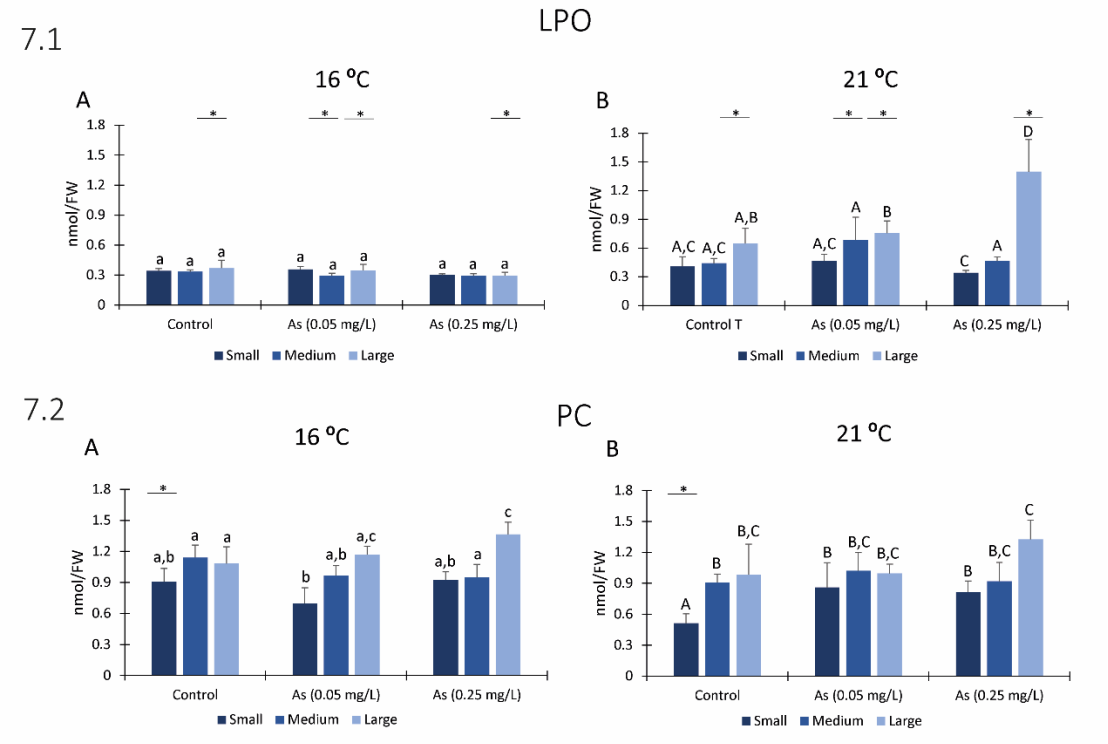

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Figure 7. Indicators of oxidative damage: (7.1) Lipid peroxidation (LPO) measured in *Hediste diversicolor* from different class sizes after 28 days of exposure to Arsenic and at 16 °C (A) and 21 °C (B). (7.2) Protein carbonylation (PC) measured in *Hediste diversicolor* from different class sizes after 28 days of exposure to Arsenic and 16 °C (A) and 21 °C (B). Different letters represent significant differences ($p \leq 0.05$) between conditions (lowercase letters for 16 °C; uppercase letters for 21 °C). Asterisk (*) represent significant differences ($p \leq 0.05$) among temperatures for the same class size.

3.7.2. PC

Protein carbonylation levels significantly increased in large organisms compared to control conditions in large organisms exposed to the highest As concentration and at 16 °C (Fig 7.2A). Regarding exposure to 21 °C, small size organisms exposed at As presented significantly higher protein carbonylation than organisms not exposed to As (Fig 7.2B). Moreover, small organisms exposed at 21 °C without As (0.0 mg/L) presented significantly lower protein carbonylation when compared to the same class size exposed to 16 °C and not exposed to As (Fig 7.2A,B).

4. Discussion

Polychaetes are being widely used as bioindicators in ecotoxicological assays to evaluate behavioural and biochemical alterations caused by contaminants, as metalloids and climate change [40][83-85]. The species *H. diversicolor* is one of the most used species, usually showing high sensitivity to low levels of these alterations, being translated in alterations of behaviour, energy status, oxidative stress, neurotoxicity, oxidative damage [40][52]. Studies usually use adult organisms, not existing information about how contaminants and climate alterations impact organisms from different ages. Thus, this study aimed to investigate how contamination by As impacts the polychaete *H. diversicolor* from different sizes (small, medium and large organisms) exposed at two different temperatures (16 °C and 21 °C).

Total As accumulation in polychaetes tissues showed an accumulation pattern related to As concentration. Small organisms presented significant higher accumulation levels in the highest As concentration of exposure, compared to large organisms. Previous studies demonstrated that elements concentration in polychaetes tissues might also be influenced by their weight [86]. However, comparing As accumulation among studied temperatures, besides some increasing trend in As accumulation in small and medium organisms exposed at 21°C, no significant differences were observed. However, a previous study indicated that As concentrations in *Mytillus galloprovinciallis* were significantly higher after 28 days of exposure to As (1.0 mg/L) at 21°C than at 17°C [87].

H. diversicolor are important organisms in the ecosystems they inhabit since their functional traits, such as burrowing and feeding behaviour provide sediment irrigation, oxygenation and particle mixing [61]; [58] and alterations on these bioturbation activities can provide ecological consequences [88]. In this study, it was detected that exposure to As induced negative effects on medium and large organisms behaviour, since exposed organisms needed more time to detect the food with increasing concentration, independently of temperature. Previous studies, also reported negative impacts on burrowing behaviour and distance travelled by *H. diversicolor* chronically exposed to diazepam [89]. Additionally, [52] also demonstrated that *H. diversicolor* also took longer to borrow in the sediment when exposed to nanoplastic particles. Additionally, [90] demonstrated that *H.*

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diversicolor exposed to graphene nanoflakes resided deeper in the sediment than controls, suggesting an escape response to graphene. Thus, besides this parameter had not been measured in our study, if organisms are deeper in the sediment, it could explain the increased time that polychaetes needed to detect food. Additionally, the reduction of feeding activity will conduct to a decrease on sediment oxygenation, which is essential for maintaining infauna diversity [61]. Some studies demonstrated that temperature increase have an important role on organisms' activity, as feeding rate or swimming performance [91-92]. In our study, significant differences among temperatures were detected only in medium and large size organisms exposed at 0.05 mg/L of As, with medium size organisms needing less time to detect food when exposed at 21 °C than organisms exposed at the same concentration exposed at 16 °C. On the other hand, large organisms exposed at the same As concentration needed more time to detect the food when were exposed at 21 °C. A study carried out with *Dicentrarchus labrax* juveniles demonstrate that these species have their swimming performance improved when water increase to maximum to 25 °C [91]. Additionally, an increase of feeding rate with temperature increase was observed for *Amphiprion clarkia* post-larval stage [92]. On the other hand, juveniles of *Salmo trutta* L. demonstrated that temperature increase receded their food intake, activity and swimming endurance when exposed at temperatures higher than 18 °C [93].

In this work, the activity of the enzyme ChE, an enzyme responsible for normal muscular and behavioural functions [94-96] seems not to compromise small and medium organisms exposed at 16 °C. However, large organisms significantly diminished their ChE activity when exposed to arsenic, which may indicate that the mobility of this organism could be compromised, and thus, needed more time to leave the sediments for feeding, and also revealed the ability of As to inhibit neurotransmission. Though, at 21 °C, only small size organisms have their neurotransmission compromised when exposed to As 0.25 mg/L at 21 °C. Previous studies demonstrated that *H. diversicolor* have their ChE activity reduced when exposed to contaminants, as polystyrene nanoplastics [52] and inorganic mercury [84]. This pattern of negative effects of ChE activity was also observed on *Diopatra neapolitana* when exposed to multi-walled carbon nanotubes[97], *Donax trunculus* exposed to microplastics [98] and *Tegillarca granosa* exposed to bisphenol A and microplastics [99]. On the other hand, the bivalves *Mytilus galloprovincialis* and *Corbicula fluminea* have their ChE activity increased proportionally with temperature increase, achieving the maximum activity at 38 and 45 °C [100], indicating that temperature increase may contributes to the increase of ChE activity, as observed in small organisms in our study.

Energy metabolism has an important function in organisms' survival and function, as well as in stress tolerance and adaptation [101]. Moreover, under environmental disturbances, organisms can increase energy expenditure, being considered a mechanism of cellular protection [102]. The parameter ETS activity has been used as a measure of metabolic capacity on invertebrates in order to response to environmental disturbances, as chemical and metal stress [97]; [83]; [103]; [104]; [65][105] [52]. In fact, in this study, the obtained results suggested that temperature increase contributed to an increase of *H. diversicolor* metabolism on large size organisms exposed to As. Some previous studies demonstrated that this species have their ETS activity increased when exposed to climate alterations, as pH decrease [103] or when exposed to others contaminants, as polystyrene nanoplastics [52] and multi-walled carbon nanotubes [97]. This similar behaviour was also demonstrated on *Diopatra neapolitana* and *Ruditapes philippinarum* when exposed to carbon nanoparticles [106][97]. On the other hand, in stressful situations, to avoid energy expenditure which may prevent them from greater damages, a decrease on ETS activity may occur, as observed in medium organisms exposed to As 0.05 mg/L at 16 °C from our study and as detected in *H. diversicolo*r organisms exposed to 6.0 - 9.0 μg/L of carbamazepine [65].

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Sugars and protein (PROT) act as major sources of energy in polychaetes on stressful environment [83]. In this study, organisms at 16 °C when exposed to As, uses sugar as main font of energy, lowering their sugar reserves. Previous studies also showed a decrease of sugar content when exposed to mercury and acidification [83] carbamazepine and caffeine [65], indicating that at this conditions polychaetes used sugars to obtain energy. In other hand, large size organisms increased their sugar content when exposed As 0.25 mg/L at 21 °C, indicating that this species, under stressful conditions, as As contamination and temperature increase, may prevent energy expenditure in specific processes (e.g., limiting their use for polychaetes burrowing activity) or, to fuel up defense mechanisms were using other energy sources, as lipids.

Regarding PROT content, it was observed a pattern of protein content among temperatures, with higher protein levels at 16 °C than at 21 °C. Moreover, small size organisms at 16 °C have PROT content increased when exposed to As. Other studies also revealed that energy reserves of *H. diversicolor* exposed to mercury and/or seawater acidification diminished [83]; [103], demonstrating that *H. diversicolor* polychaetes tried to prevent oxidative stress by increasing organisms' antioxidant defenses (namely increasing SOD and CAT activity), leading to energy expenditure.

Previous studies showed that contamination and climate change induced several harmful biochemical effects, such as oxidative stress in exposed organisms [40][85], which result from the overproduction and accumulation of reactive oxygen species (ROS). To prevent these injuries, organisms are able to activate their defences, as antioxidant (SOD and CAT) and biotransformation (GSTs) enzymes to eliminate ROS and toxic compounds formed from the metabolism of oxidized molecules such as lipid hydroperoxides, avoiding cell and protein damage [107]. In this study it was detected an increased antioxidant activity of the enzymes SOD and CAT when *H. diversicolor* was exposed at 21°C. Besides that, small and large size organisms have their superoxide dismutase (SOD) activity lower when exposed to arsenic, indicating that the activity of this enzyme was inhibited. However, previous studies, showed that usually SOD activity of *H. diversicolor* usually increased when exposed to contaminants, as polystyrene nanoplastics, carbamazepine, caffeine, mercury, acidification and multi-walled carbon nanotubes [97]; [83]; [103]; [65]; [52]. Same pattern was observed with *Diopatra neapolitana* when exposed to arsenic and multi-walled carbon nanotubes [27]; [97].

Regarding catalase (CAT) activity, in small and medium organisms' at 16 °C the activity of this enzyme increased in As exposed organisms, but it was inhibited in large size organisms. Previous studies also showed an activity reduction of this enzyme when exposed to contaminants, as polystyrene nanoplastics, indicating an adaptative mechanism as a possible explanation for this inhibition [52]. However, CAT inhibition will cause an inefficiency of defense, contributing for the increasing of LPO levels, as observed when *H. diversicolor* was exposed to carbamazepine and caffeine [65].

GSTs are responsible for detoxifying the xenobiotics and metabolites produced from oxidative stress, as lipid peroxides by-products and is widely used to evaluate the detoxification capacity of organisms. Previous studies demonstrated that As induces GSTs activity [108][27][109]. In fact, in this work, *H. diversicolor* increased GSTs activity when exposed to As 0.25 mg/L at 16 °C. Previous studies also reported that *H. diversicolor* increased GSTs activity when exposed to others contaminants, as carbamazepine, caffeine, mercury and multi-walled carbon nanotubes, confirming their detoxification capacity [97]; [83]; [65]. The highest GSTs activity in medium and large organisms when exposed to 0.25 mg/L may had contributed for the lower As concentrations were observed in polychaetes of this class size exposed at this concentration. In our study, organisms exposed to 21 °C presented significantly higher GSTs activity than organisms exposed to 16 °ºC for almost

all class sizes and As conditions. A study with *Mytilus galloprovincialis* with similar conditions showed that the combination of As and 21 °C stimulates GSTs activity [87]. This increase of GSTs activity, also occurs when *Tigriopus japonicus* is exposed to 35 °C for 60-120 minutes due to ROS increase [110].

In this study it was observed that the combination of As and temperature rise (21 °C), contributed to the increased of lipid peroxidation (LPO). However, no cellular damage was observed in all size organisms exposed to normal temperature condition (16 °C) with same As concentration (0.05 and 0.25 mg/L). [111] demonstrated that when antioxidant mechanism fails to manage the stress LPO increase occurs cellular damage, suggesting that antioxidant enzymes, as SOD and CAT were not able to eliminate ROS. In fact, SOD activity was inhibited at large size organisms from As 0.25 mg/L at 21 °C, being not able to eliminate ROS and conducing to cellular damage despite of higher CAT activity. Furthermore, some studies also demonstrated that the polychaete *D. neapolitana* always presented higher LPO levels when exposed to stress [27] [112].

Regarding protein carbonylation (PC), in this study it was observed that large organisms from As 0.25 mg/L at 16 °C and small organisms when exposed to As at 21 °C have an increased PC levels, suggesting potential protein damage. PC is one of the most harmful and irreversible oxidative protein modifications. Carbonyl stress is related to biomolecules malfunction, immunogenicity, inflammation, cell toxicity and apoptosis [113]. Thus, under stressful conditions, as exposure to contaminants, is expected to observe an increase of PC levels in exposed organisms, as observed in *H. diversicolor* when exposed to polystyrene nanoplastics [52].

5. Conclusions

The present study demonstrated that *H. diversicolor* from different sizes behave differently among As concentrations and temperatures. These results are helpful to understand the effects of contaminants and climate change at different ages, and how it could affect *H. diversicolor* population. Moreover, in this work it was possible to observe that, for some parameters (as ETS, SOD, GSTs and LPO) temperature increase combined with As have a higher impact on *H. diversicolor* at almost every different sizes.

Small size organisms, have SOD activity inhibited but CAT and GSTs activity increased which may had contributed to avoid lipid damage. Large size organisms, in general, are the most affected size class among the observed ages since they needed more time to detect the food, and also had higher cellular damage, probably due SOD inhibition. Additionally, medium size organisms seem to be the most well adapted group class, since this group of organisms presented less alterations in the tested parameters. However, the most concerning factor observed in this study was the increased protein damage (PC) at early-stage and late-stage of development (small and large size organisms) of *H. diversicolor*, meaning the antioxidant and biotransformation defenses activated by the organisms under stressful conditions were not enough to avoid oxidative damages.

Furthermore, this study highlighted the importance of integrating behavioural and biochemical responses, as an approach to understand the mechanistic bases of stress responses and interpret their ecological consequences. As a deposit feeder, *H. diversicolor* bury sediments and cause bioturbation, greatly affecting the biogeochemical cycle of nutrients and contaminants, a reduction in its feeding activity can conduct to reductions in detritus processing and in organic matter decomposition rates in the ecosystem. These disturbances may affect benthic fauna usually associated and dependent of *H. diversicolor* activity.

Future studies should assess the effects of combining the alterations of abiotic factors, as temperature rise, salinity changes with other metalloid/metals, which usually are present in coastal/estuarine sediments. Furthermore, there is a need to understand how these environmental alterations will impact organisms in different life stages, to check a possible compromised generation of *H. diversicolor*. Additionally, should be also considered to perform prolonged or a life study of *H. diversicolor*, at laboratorial conditions, to track growth, survival, behaviour, reproduction success in order to understand how this species answer under stressful conditions. Since this species have an important role on environment, any alterations can unbalance the ecosystem which this species inhabits.

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References

1. Andersen, J. H., Harvey, T., Murray, C., Reker, J., & Jesper H. Andersen, Therese Harvey, Ciarán Murray, J. R. (2018). Contaminants in Europe' s seas. Retrieved from https://www.eea.europa.eu/publications/contaminants-in-europes-seas
2. Fan, W., Xu, Z., & Wang, W. X. (2014). Metal pollution in a contaminated bay: Relationship between metal geochemical fractionation in sediments and accumulation in a polychaete. Environmental Pollution, 191, 50–57. https://doi.org/10.1016/j.envpol.2014.04.014
3. Wu, B., Li, X., Song, J., Hu, L., & Shi, X. (2016). Impact of extreme metal contamination at the supra-individual level in a contaminated bay ecosystem. Science of the Total Environment, 557–558, 102–109. https://doi.org/10.1016/j.scitotenv.2016.03.047
4. Milieu Ltd, Ökopol, (RPA), R. & P. A., & RIVM. (2017). Study for the strategy for a non-toxic environment of the 7th Environment Action Programme Final Report. https://doi.org/10.2779/025
5. Chen, Y. ping, Zhao, Y., Zhao, M. M., Wu, J. H., & Wang, K. bo. (2021). Potential health risk assessment of HFRs, PCBs, and OCPs in the Yellow River basin. Environmental Pollution, 275, 116648. https://doi.org/10.1016/j.envpol.2021.116648
6. Hsieh, H. Y., Huang, K. C., Cheng, J. O., Lo, W. T., Meng, P. J., & Ko, F. C. (2019). Environmental effects on the bioaccumulation of PAHs in marine zooplankton in Gaoping coastal waters, Taiwan: Concentration, distribution, profile, and sources. Marine Pollution Bulletin, 144(May), 68–78. https://doi.org/10.1016/j.marpolbul.2019.04.048
7. Mao, S., Liu, S., Zhou, Y., An, Q., Zhou, X., Mao, Z., … Liu, W. (2021). The occurrence and sources of polychlorinated biphenyls (PCBs) in agricultural soils across China with an emphasis on unintentionally produced PCBs. Environmental Pollution, 271. https://doi.org/10.1016/j.envpol.2020.116171
8. Health, N. R. C. (US) C. on D. and. (1989). Diet and Health: Implications for Reducing Chronic Disease Risk. https://doi.org/10.17226/1222
9. Adriano, Domy C. (2001). Bioavailability of Trace Metals. Trace Elements in Terrestrial Environments, 61–89. https://doi.org/10.1007/978-0-387-21510-5_3
10. Bolan, N. S., Choppala, G., Kunhikrishnan, A., Park, J., & Naidu, R. (2013). Microbial Transformation of Trace Elements in Soils in Relation to Bioavailability and Remediation. https://doi.org/10.1007/978-1-4614-6470-9
11. Duffus, J. H. (2002). "Heavy metals "— a meaningless term ? (IUPAC technical report). 74(5), 793–807.
12. Vareda, J. P., Valente, A. J. M., & Durães, L. (2019). Assessment of heavy metal pollution from anthropogenic activities and remediation strategies : A review. Journal of Environmental Management, 246 (May), 101–118. https://doi.org/10.1016/j.jenvman.2019.05.126

13. Chai, M., Li, R., Gong, Y., Shen, X., & Yu, L. (2021). Bioaccessibility-corrected health risk of heavy metal exposure via shell fish consumption in coastal region of China *. Environmental Pollution, 273, 116529. https://doi.org/10.1016/j.envpol.2021.116529
14. Adriano, Domy C. (2001). Biogeochemical Processes Regulating Metal Behavior. Trace Elements in Terrestrial Environments, 29–59. https://doi.org/10.1007/978-0-387-21510-5_2
15. Nriagu, J. O., & Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature Publishing Group, 333, 1341–139. https://doi.org/10.1038/33313440
16. Yadav, I. C., Devi, N. L., Singh, V. K., Li, J., & Zhang, G. (2019). Spatial distribution, source analysis, and health risk assessment of heavy metals contamination in house dust and surface soil from four major cities of Nepal. Chemosphere, 218, 1100–1113. https://doi.org/10.1016/j.chemosphere.2018.11.202
17. Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world : a review. 58, 201–235.
18. WHO. (2011). Arsenic in drinking water. WHO Press, 27(9), 358–359. https://doi.org/10.1016/j.kjms.2011.05.002
19. Mirlean, N., Andrus, V. E., Baisch, P., Griep, G., & Casartelli, M. R. (2003). Arsenic pollution in Patos Lagoon estuarine sediments, Brazil. Marine Pollution Bulletin, 46(11), 1480–1484. https://doi.org/10.1016/S0025-326X(03)00257-1
20. Rahman, M. A., Hasegawa, H., & Peter, R. (2012). Bioaccumulation , biotransformation and trophic transfer of arsenic in the aquatic food chain. Environmental Research, 116, 118–135. https://doi.org/10.1016/j.envres.2012.03.014
21. Wang, X., Wu, Y., Sun, X., Guo, Q., Xia, W., Wu, Y., … Li, Y. (2021). Arsenic exposure and metabolism in relation to blood pressure changes in pregnant women. Ecotoxicology and Environmental Safety, 222(March). https://doi.org/10.1016/j.ecoenv.2021.112527
22. Cohen, S. M., Arnold, L. L., Beck, B. D., Lewis, A. S., & Eldan, M. (2013). Evaluation of the carcinogenicity of inorganic arsenic. Critical Reviews in Toxicology, 43(9), 711–752. https://doi.org/10.3109/10408444.2013.827152
23. Couto-Santos, F., Viana, A. G. de A., Souza, A. C. F., Dutra, A. A. de A., Mendes, T. A. de O., Ferreira, A. T. da S., … Machado-Neves, M. (2021). Prepubertal arsenic exposure alters phosphoproteins profile, quality, and fertility of epididymal spermatozoa in sexually mature rats. Toxicology, 460(July). https://doi.org/10.1016/j.tox.2021.152886
24. Golovanova, I. L. (2008). Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. Inland Water Biology, 1(1), 93–101. https://doi.org/10.1007/s12212-008-1014-1
25. Has-Schön, E., Bogut, I., Vuković, R., Galović, D., Bogut, A., & Horvatić, J. (2015). Distribution and age-related bioaccumulation of lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) in tissues of common carp (*Cyprinus carpio*) and European catfish (*Sylurus glanis*) from the Buško Blato reservoir (Bosnia and Herzegovina). Chemosphere, 135, 289–296. https://doi.org/10.1016/j.chemosphere.2015.04.015
26. Kiser, T., Hansen, J., & Kennedy, B. (2010). Impacts and pathways of mine contaminants to bull trout (*Salvelinus confluentus*) in an Idaho watershed. Archives of Environmental Contamination and Toxicology, 59(2), 301–311. https://doi.org/10.1007/s00244-009-9457-x
27. Coppola, F., Pires, A., Velez, C., Soares, A. M. V. M., Pereira, E., Figueira, E., & Freitas, R. (2016). Biochemical and physiological alterations induced in *Diopatra neapolitana* after a long-term exposure to Arsenic. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology, 189. https://doi.org/10.1016/j.cbpc.2016.06.006
28. Pires, A., Velez, C., Figueira, E., Soares, A. M. V. M., & Freitas, R. (2017). Effects of sediment contamination on physiological and biochemical responses of the polychaete *Diopatra neapolitana*, an exploited natural resource. Marine Pollution Bulletin, 119(1), 119–131. https://doi.org/10.1016/j.marpolbul.2017.03.014
29. Buffet, P. E., Amiard-Triquet, C., Dybowska, A., Risso-de Faverney, C., Guibbolini, M., Valsami-Jones, E., & Mouneyrac, C. (2012). Fate of isotopically labeled zinc oxide nanoparticles in sediment and effects on two endobenthic species, the clam *Scrobicularia plana* and the ragworm *Hediste diversicolor*. Ecotoxicology and Environmental Safety, 84, 191–198. https://doi.org/10.1016/j.ecoenv.2012.07.010
30. Buffet, P. E., Poirier, L., Zalouk-Vergnoux, A., Lopes, C., Amiard, J. C., Gaudin, P., … Mouneyrac, C. (2014). Biochemical and behavioural responses of the marine polychaete *Hediste diversicolor* to cadmium sulfide quantum dots (CdS QDs): Waterborne and dietary exposure. Chemosphere, 100, 63–70. https://doi.org/10.1016/j.chemosphere.2013.12.069
31. Buffet, P. E., Richard, M., Caupos, F., Vergnoux, A., Perrein-Ettajani, H., Luna-Acosta, A., … Mouneyrac, C. (2012). A mesocosm study of fate and effects of CuO nanoparticles on endobenthic species (*Scrobicularia plana*, *Hediste diversicolor*). Environmental Science and Technology, 47(3), 1620–1628. https://doi.org/10.1021/es303513r
32. Buffet, P. E., Tankoua, O. F., Pan, J. F., Berhanu, D., Herrenknecht, C., Poirier, L., … Mouneyrac, C. (2011). Behavioural and biochemical responses of two marine invertebrates *Scrobicularia plana* and *Hediste diversicolor* to copper oxide nanoparticles. Chemosphere, 84(1), 166–174. https://doi.org/10.1016/j.chemosphere.2011.02.003
33. Buffet, P. E., Zalouk-Vergnoux, A., Châtel, A., Berthet, B., Métais, I., Perrein-Ettajani, H., … Mouneyrac, C. (2014). A marine mesocosm study on the environmental fate of silver nanoparticles and toxicity effects on two endobenthic species: The ragworm *Hediste diversicolor* and the bivalve mollusc *Scrobicularia plana*. Science of the Total Environment, 470–471, 1151–1159. https://doi.org/10.1016/j.scitotenv.2013.10.114
34. Thit, A., Dybowska, A., Købler, C., Kennaway, G., & Selck, H. (2015). Influence of copper oxide nanoparticle shape on bioaccumulation, cellular internalization and effects in the estuarine sediment-dwelling polychaete, *Nereis diversicolor*. Marine Environmental Research, 111, 89–98. https://doi.org/10.1016/j.marenvres.2015.06.009

35. IPCC. (2018). Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change,. In Ipcc - Sr15 (Vol. 2). Retrieved from www.environmentalgraphiti.org
36. Moser, J. R., Galván, D. A., Mendoza, F., Encinas, T., Coronado, D. E., Portillo, G., … Hernández, J. (2012). Water temperature in fl uences viral load and detection of White Spot Syndrome Virus (WSSV) in *Litopenaeus vannamei* and wild crustaceans. Aquaculture, 326–329, 9–14. https://doi.org/10.1016/j.aquaculture.2011.10.033
37. Last, P. R., White, W. T., Gledhill, D. C., Hobday, A. J., Brown, R., Edgar, G. J., & Pecl, G. (2011). Long-term shifts in abundance and distribution of a temperate fish fauna: A response to climate change and fishing practices. Global Ecology and Biogeography, 20(1), 58–72. https://doi.org/10.1111/j.1466-8238.2010.00575.x
38. Mueter, F. J., & Litzow, M. A. (2008). Sea ice retreat alters the biogeography of the Bering Sea continental shelf. Ecological Applications, 18(2), 309–320. https://doi.org/10.1890/07-0564.1
39. Perry, A. L., Low, P. J., Ellis, J. R., & Reynolds, J. D. (2005). Ecology: Climate change and distribution shifts in marine fishes. Science, 308(5730), 1912–1915. https://doi.org/10.1126/science.1111322
40. Bhuiyan, K. A., Rodríguez, B. M., Pires, A., Riba, I., Dellvals, Á., Freitas, R., & Conradi, M. (2021). Experimental evidence of uncertain future of the keystone ragworm *Hediste diversicolor* (O.F. Müller, 1776) under climate change conditions. Science of the Total Environment, 750, 142031. https://doi.org/10.1016/j.scitotenv.2020.142031
41. Pires, A., Figueira, E., Moreira, A., Soares, A. M. V. M., & Freitas, R. (2015). The effects of water acidification, temperature and salinity on the regenerative capacity of the polychaete *Diopatra neapolitana*. Marine Environmental Research, 106(1), 30–41. https://doi.org/10.1016/j.marenvres.2015.03.002
42. Fey, D. P., & Greszkiewicz, M. (2021). Effects of temperature on somatic growth , otolith growth , and uncoupling in the otolith to fish size relationship of larval northern pike , *Esox lucius* L. Fisheries Research, 236(May 2020), 105843. https://doi.org/10.1016/j.fishres.2020.105843
43. Greszkiewicz, M., & Fey, D. P. (2020). Positive temperature effects on the initiation and intensity of cannibalistic behaviour of larval pike, *Esox lucius* L . Is cannibalism reflected in otolith fluctuating asymmetry? Hydrobiologia, 847(14), 3139–3152. https://doi.org/10.1007/s10750-020-04328-5
44. Attig, H., Kamel, N., Sforzini, S., Dagnino, A., Jamel, J., Boussetta, H., … Banni, M. (2014). Effects of thermal stress and nickel exposure on biomarkers responses in *Mytilus galloprovincialis* (Lam). Marine Environmental Research, 94, 65–71. https://doi.org/10.1016/J.MARENVRES.2013.12.006
45. Hoffmann, L. J., Breitbarth, E., Boyd, P. W., & Hunter, K. A. (2012). Influence of ocean warming and acidification on trace metal biogeochemistry. Marine Ecology Progress Series, 470, 191–205. https://doi.org/10.3354/meps10082
46. Lewis, C., & Watson, G. J. (2012). Expanding the ecotoxicological toolbox: The inclusion of polychaete reproductive endpoints. Marine Environmental Research, 75, 10–22. https://doi.org/10.1016/j.marenvres.2011.08.002
47. Rodrigues, A. M., Quintino, V., Sampaio, L., Freitas, R., & Neves, R. (2011). Benthic biodiversity patterns in Ria de Aveiro, Western Portugal: Environmental-biological relationships. Estuarine, Coastal and Shelf Science, 95(2–3), 338–348. https://doi.org/10.1016/j.ecss.2011.05.019
48. Catalano, B., Moltedo, G., Martuccio, G., Gastaldi, L., Virno-Lamberti, C., Lauria, A., & Ausili, A. (2012). Can *Hediste diversicolor* (Nereidae, Polychaete) be considered a good candidate in evaluating PAH contamination? A multimarker approach. Chemosphere, 86(9), 875–882. https://doi.org/10.1016/j.chemosphere.2011.10.040
49. Masero, J. A., Pérez-González, M., Basadre, M., & Otero-Saavedra, M. (1999). Food supply for waders (Aves: Charadrii) in an estuarine area in the Bay of Cadiz (SW Iberian Peninsula). Acta Oecologica, 20(4), 429–434. https://doi.org/10.1016/S1146-609X(99)00125-3
50. Dean, H. K. (2008). The use of polychaetes (Annelida) as indicator species of marine pollution: a review. Revista de Biología Tropical, 56(4), 11–38. https://doi.org/10.15517/rbt.v56i4.27162
51. Costa, P. F. e, Oliveira, R. F., & Fonseca, L. C. da. (2006). Feeding ecology of *Nereis diversicolor* (O.F. Müller) (Annelida, Polychaeta) on estuarine and lagoon environments in the southwest coast of Portugal. Pan-American Journal of Aquatic Sciences, 1, 104–113.
52. Silva, M. S. S., Oliveira, M., Valente, P., Figueira, E., Martins, M., & Pires, A. (2020). Behavior and biochemical responses of the polychaeta *Hediste diversicolor* to polystyrene nanoplastics. Science of the Total Environment, 707, 134434. https://doi.org/10.1016/j.scitotenv.2019.134434
53. Adriano, D C, Wenzel, W. W., Vangronsveld, J., & Bolan, N. S. (2004). Role of assisted natural remediation in environmental cleanup. 122, 121–142. https://doi.org/10.1016/j.geoderma.2004.01.003
54. Yuan, X., Chen, A., Zhou, Y., Liu, H., & Yang, D. (2010). The influence of cadmium on the antioxidant enzyme activities in polychaete *Perinereis aibuhitensis* Grube (Annelida: Polychaeta). Chinese Journal of Oceanology and Limnology, 28(4), 849–855. https://doi.org/10.1007/s00343-010-9127-x
55. Bouraoui, Z., Ghedira, J., & Boussetta, H. (2015). Biomarkers responses in different body regions of the polychaeta *Hediste diversicolor* (Nereidae , Polychaete) exposed to copper. Journal of Integrated Coastal Zone Management, 15(3), 371–376. https://doi.org/10.5894/rgci594
56. Maltagliati, F., Massaro, L., Cossu, P., & Castelli, A. (2007). Morphological differentiation in the ragworm, *Hediste diversicolor* (Polychaeta , Nereididae), as revealed by variation of paragnath number and distribution. 0003. https://doi.org/10.1080/11250000600700052

57. Barnes, R. S. K., & Head, S. M. (1977). Variation in paragnath number in some British populations of the estuarine polychaete *Nereis diversicolor*. Estuarine and Coastal Marine Science, 5(6), 771–781. https://doi.org/10.1016/0302-3524(77)90048-2
58. Scaps, P. (2002). A review of the biology , ecology and potential use of the common ragworm *Hediste diversicolor* (O . F . Müller) (Annelida : Polychaeta). Hydrobiologia, 470(1), 203–218. https://doi.org/10.1023/A:1015681605656
59. Hateley, J. G., Grant, A., Taylor, S. M., & Jones, N. V. (1992). Morphological and other evidence on the degree of genetic differentiation between populations of *Nereis diversicolor*. Journal of the Marine Biological Association of the United Kingdom, 72(2), 365–381. https://doi.org/10.1017/S0025315400037760
60. Garcia-Arberas, L., & Rallo, A. (2000). Variations in paragnath number of *Hediste diversicolor* from the Basque coast, in relation to habitat conditions. Journal of the Marine Biological Association of the United Kingdom, 80(4), 733–734. https://doi.org/10.1017/S002531549900257X
61. Banta, G. ., & Andersen, O. (2003). Bioturbation and the fate of sediment pollutants- Experimental case studies of selected infauna species. (December).
62. Ereira, T., Coelho, J. P., Duarte, A. C., Pardal, M. A., & Pereira, M. E. (2015). Size-Dependent Arsenic Accumulation in Scrobicularia plana in a Temperate Coastal Lagoon (Ria de Aveiro, Portugal). Water, Air, and Soil Pollution, 226(7). https://doi.org/10.1007/s11270-015-2484-5
63. Lopes, J. F., Lopes, C. L., & Dias, J. M. (2019). Climate change impact in the ria de aveiro lagoon ecosystem: A case study. In Journal of Marine Science and Engineering (Vol. 7). https://doi.org/10.3390/jmse7100352
64. Diepens, N. J., Van den Heuvel-Greve, M. J., & Koelmans, A. A. (2015). Modeling of Bioaccumulation in Marine Benthic Invertebrates Using a Multispecies Experimental Approach. Environmental Science & Technology, 49, 13575−13585. https://doi.org/10.1021/acs.est.5b02500
65. Pires, A., Almeida, Â., Calisto, V., Schneider, R. J., Esteves, V. I., Wrona, F. J., … Freitas, R. (2016). *Hediste diversicolor* as bioindicator of pharmaceutical pollution: Results from single and combined exposure to carbamazepine and caffeine. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology, 188, 30–38. https://doi.org/10.1016/j.cbpc.2016.06.003
66. Oliveira, M., Cardoso, D. N., Soares, A. M. V. M., & Loureiro, S. (2015). Effects of short-term exposure to fluoxetine and carbamazepine to the collembolan *Folsomia candida*. Chemosphere, 120, 86–91. https://doi.org/10.1016/j.chemosphere.2014.06.038
67. Ellman, G. L., Courtney, K. D., Valentino, A. jr, & Featherstone, R. M. (1961). A new and rapid colorimetric of acetylcholinesterase determination. 7, 88–95.
68. Guilhermino, L., Lopes, M. C., Carvalho, A. P., & Soares, A. M. V. M. (1996). Acetylcholinesterase activity in juveniles of *Daphnia magna* straus. Bulletin of Environmental Contamination and Toxicology, 57(6), 979–985. https://doi.org/10.1007/s001289900286
69. Robinson, H. W., & Hogden, C. G. (1940). the Biuret Reaction in the Determination of Serum Proteins. Journal of Biological Chemistry, 135(2), 707–725. https://doi.org/10.1016/s0021-9258(18)73134-7
70. Freitas, R., Almeida, Â., Pires, A., Velez, C., Calisto, V., Schneider, R. J. R. J., … Soares, A. M. V. M. (2015). The effects of carbamazepine on macroinvertebrate species: Comparing bivalves and polychaetes biochemical responses. Water Research, 85, 137–147. https://doi.org/10.1016/j.watres.2015.08.003
71. Owens, T. G., & King, F. D. (1975). The measurement of respiratory electron-transport-system activity in marine zooplankton. Marine Biology, 30(1), 27–36. https://doi.org/10.1007/BF00393750
72. Janssen, C. R., & De Coen, W. M. (1997). The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular Energy Allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. Aquatic Ecosystem Stress and Recovery, 6, 43–55.
73. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry, 28(3), 350–356. https://doi.org/10.1021/ac60111a017
74. Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44(1), 276–287. https://doi.org/10.1016/0003-2697(71)90370-8
75. Johansson, L. H., & Håkan Borg, L. A. (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. Analytical Biochemistry, 174(1), 331–336. https://doi.org/10.1016/0003-2697(88)90554-4
76. Freitas, R., Costa, E., Velez, C., Santos, J., Lima, A., Oliveira, C., … Figueira, E. (2012). Looking for suitable biomarkers in benthic macroinvertebrates inhabiting coastal areas with low metal contamination: Comparison between the bivalve *Cerastoderma edule* and the Polychaete *Diopatra neapolitana*. Ecotoxicology and Environmental Safety, 75(1), 109-118. https://doi.org/10.1016/j.ecoenv.2011.08.019
77. Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S transferases. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249(22), 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8
78. Carregosa, V., Velez, C., Soares, A. M. V. M., Figueira, E., & Freitas, R. (2014). Physiological and biochemical responses of three Veneridae clams exposed to salinity changes. Comparative Biochemistry and Physiology Part - B: Biochemistry and Molecular Biology, 177–178, 1–9. https://doi.org/10.1016/j.cbpb.2014.08.001
79. Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. Methods in Enzymology, 52, 302–310. Retrieved from http://www.sciencedirect.com/science/article/pii/S0076687978520326
80. Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., & Marcos, J. C. (2014). Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Analytical Biochemistry, 458, 69–71. https://doi.org/10.1016/j.ab.2014.04.034

81. Udenigwe, C. C., Udechukwu, M. C., Yiridoe, C., Gibson, A., & Gong, M. (2016). Antioxidant mechanism of potato protein hydrolysates against in vitro oxidation of reduced glutathione. Journal of Functional Foods, 20, 195–203. https://doi.org/10.1016/j.jff.2015.11.004
82. Anderson, M., Gorley, R. N., & Clarke, K. (2008). PERMANOVA+ for primer: Guide to software and statistical methods. In Plymouth: Primer-E.
83. Freitas, R., de Marchi, L., Moreira, A., Pestana, J. L. T., Wrona, F. J., Figueira, E., & Soares, A. M. V. M. (2017). Physiological and biochemical impacts induced by mercury pollution and seawater acidification in Hediste diversicolor. Science of the Total Environment, 595, 691–701. https://doi.org/10.1016/j.scitotenv.2017.04.005
84. Moltedo, G., Martuccio, G., Catalano, B., Gastaldi, L., Maggi, C., Virno-Lamberti, C., & Cicero, A. M. (2019). Biological responses of the polychaete Hediste diversicolor (O.F.Müller, 1776) to inorganic mercury exposure: A multimarker approach. Chemosphere, 219, 989–996. https://doi.org/10.1016/j.chemosphere.2018.12.064
85. Sun, F. hong, & Zhou, Q. xing. (2008). Oxidative stress biomarkers of the polychaete *Nereis diversicolor* exposed to cadmium and petroleum hydrocarbons. Ecotoxicology and Environmental Safety, 70(1), 106–114
86. Garcês, J., & Costa, M. H. (2009). Trace metals in populations of *Marphysa sanguinea* (Montagu, 1813) from Sado estuary: Effect of body size on accumulation. Scientia Marina, 73(3), 605–616. https://doi.org/10.3989/scimar.2009.73n3605
87. Coppola, Francesca, Almeida, Â., Henriques, B., Soares, A. M. V. M., Figueira, E., Pereira, E., & Freitas, R. (2018). Biochemical responses and accumulation patterns of *Mytilus galloprovincialis* exposed to thermal stress and Arsenic contamination. Ecotoxicology and Environmental Safety, 147(July 2017), 954–962. https://doi.org/10.1016/j.ecoenv.2017.09.051
88. Bonnard, M., Roméo, M., & Amiard-Triquet, C. (2009). Effects of copper on the burrowing behavior of estuarine and coastal invertebrates, the polychaete *Nereis diversicolor* and the bivalve *Scrobicularia plana*. Human and Ecological Risk Assessment, 15(1), 11–26. https://doi.org/10.1080/10807030802614934
89. Nogueira, A. F., & Nunes, B. (2021). Acute and chronic effects of diazepam on the polychaete *Hediste diversicolor*: Antioxidant, metabolic, pharmacologic, neurotoxic and behavioural mechanistic traits. Environmental Toxicology and Pharmacology, 82(July 2020), 103538. https://doi.org/10.1016/j.etap.2020.103538noge
90. Urban-Malinga, B., Jakubowska, M., Hallmann, A., & Dąbrowska, A. (2021). Do the graphene nanoflakes pose a potential threat to the polychaete *Hediste diversicolor*? Chemosphere, 269. https://doi.org/10.1016/j.chemosphere.2020.128685
91. Koumoundouros, G. (2002). Effect of temperature on swimming performance of sea bass juveniles. Journal of Fish Biology, 60(4), 923–932. https://doi.org/10.1006/jfbi.2002.1902
92. Ye, L., Yang, S.-Y., Zhu, X.-M., Liu, M., Lin, J.-Y., & Wu, K.-C. (2011). Effects of temperature on survival, development, growth and feeding of larvae of Yellowtail clownfish *Amphiprion clarkii* (Pisces: Perciformes). Acta Ecologica Sinica, 31(5), 241–245. https://doi.org/10.1016/j.chnaes.2011.06.003
93. Ojanguren, A. F., Reyes-Gavilán, F. G., & Braña, F. (2001). Thermal sensitivity of growth, food intake and activity of juvenile brown trout. Journal of Thermal Biology, 26(3), 165–170. https://doi.org/10.1016/S0306-4565(00)00038-3
94. Cajaraville, M. P., & Bebianno, M. J. (2000). The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Science of the Total Environment, 247(2–3), 295–311.
95. Fonseca, T. G., Morais, M. B., Rocha, T., Abessa, D. M. S., Aureliano, M., & Bebianno, M. J. (2017). Ecotoxicological assessment of the anticancer drug cisplatin in the polychaete *Nereis diversicolor*. Science of the Total Environment, 575, 162–172. https://doi.org/10.1016/j.scitotenv.2016.09.185
96. Payne, J. F., Mathieu, A., Melvin, W., & Fancey, L. L. (1996). Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in Newfoundland. Marine Pollution Bulletin, 32(2), 225–231. https://doi.org/10.1016/0025-326X(95)00112-Z
97. De Marchi, L., Neto, V., Pretti, C., Figueira, E., Chiellini, F., Soares, A. M. V. M., & Freitas, R. (2017). Physiological and biochemical responses of two keystone polychaete species: *Diopatra neapolitana* and *Hediste diversicolor* to Multi-walled carbon nanotubes. Environmental Research, 154(October 2016), 126–138. https://doi.org/10.1016/j.envres.2016.12.018
98. Tlili, S., Jemai, D., Brinis, S., & Regaya, I. (2020). Microplastics mixture exposure at environmentally relevant conditions induce oxidative stress and neurotoxicity in the wedge clam *Donax trunculus*. Chemosphere, 258. https://doi.org/10.1016/j.chemosphere.2020.127344
99. Tang, Y., Zhou, W., Sun, S., Du, X., Han, Y., Shi, W., & Liu, G. (2020). Immunotoxicity and neurotoxicity of bisphenol A and microplastics alone or in combination to a bivalve species, *Tegillarca granosa*. Environmental Pollution, 265, 115115. https://doi.org/10.1016/j.envpol.2020.115115
100. Mora, P., Michel, X., & Narbonne, J. F. (1999). Cholinesterase activity as potential biomarker in two bivalves. Environmental Toxicology and Pharmacology, 7(4), 253–260. https://doi.org/10.1016/S1382-6689(99)00019-8
101. Sokolova, I. M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integrative and Comparative Biology, 53(4), 597–608. https://doi.org/10.1093/icb/ict028
102. Bielen, A., Bošnjak, I., Sepčić, K., Jaklič, M., Cvitanić, M., Lušić, J., … Hudina, S. (2016). Differences in tolerance to anthropogenic stress between invasive and native bivalves. Science of the Total Environment, 543, 449–459. https://doi.org/10.1016/j.scitotenv.2015.11.049
103. Freitas, R., Pires, A., Moreira, A., Wrona, F. J., Figueira, E., & Soares, A. M. V. M. (2016). Biochemical alterations induced in Hediste diversicolor under seawater acidification conditions. Marine Environmental Research, 117, 75–84. https://doi.org/10.1016/j.marenvres.2016.04.003

104. Pereira, K. C., Costa, P. M., Costa, M. H., Luque, Á., DelValls, T. A., & López, I. R. (2016). Effects of the increase of temperature and CO2 concentration on polychaetae *Nereis diversicolor*: simulating extreme scenarios of climate change in marine sediments. Hydrobiologia, 772(1), 161–174. https://doi.org/10.1007/s10750-016-2656-3
105. Schmidlin, L., von Fumetti, S., & Nagel, P. (2015). Temperature effects on the feeding and electron transport system (ETS) activity of *Gammarus fossarum*. Aquatic Ecology, 49(1), 71–80. https://doi.org/10.1007/s10452-015-9505-8
106. De Marchi, L., Neto, V., Pretti, C., Figueira, E., Chiellini, F., Soares, A. M. V. M., & Freitas, R. (2017). The impacts of emergent pollutants on *Ruditapes philippinarum*: biochemical responses to carbon nanoparticles exposure. Aquatic Toxicology, 187, 38–47. https://doi.org/10.1016/j.aquatox.2017.03.010
107. Regoli, F., & Giuliani, M. E. (2014). Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Marine Environmental Research, 93, 106–117. https://doi.org/10.1016/j.marenvres.2013.07.006
108. Casado-Martinez, M. C., Duncan, E., Smith, B. D., Maher, W. A., & Rainbow, P. S. (2012). Arsenic toxicity in a sediment-dwelling polychaete: Detoxification and arsenic metabolism. Ecotoxicology, 21(2), 576–590. https://doi.org/10.1007/s10646-011-0818-7
109. Geiszinger, A. E., Goessler, W., & Francesconi, K. A. (2002). Biotransformation of arsenate to the tetramethylarsonium ion in the marine polychaetes *Nereis diversicolor* and *Nereis virens*. Environmental Science and Technology, 36(13), 2905–2910. https://doi.org/10.1021/es015808d
110. Han, J., Jeong, C. B., Byeon, E., & Lee, J. S. (2018). Effects of temperature changes on the generation of reactive oxygen species and the expression and activity of glutathione-S transferases in two congeneric copepods *Tigriopus japonicus* and *Tigriopus kingsejongensis*. Fisheries Science, 84(5), 815–823. https://doi.org/10.1007/s12562-018-1224-3
111. Alves de Almeida, E., Celso Dias Bainy, A., Paula de Melo Loureiro, A., Regina Martinez, G., Miyamoto, S., Onuki, J., … Di Mascio, P. (2007). Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 146(4), 588–600. https://doi.org/10.1016/j.cbpa.2006.02.040
112. Freitas, R., Pires, A., Velez, C., Almeida, Â., Moreira, A., Wrona, F. J., … Figueira, E. (2016). Effects of seawater acidification on *Diopatra neapolitana* (Polychaete, Onuphidae): Biochemical and regenerative capacity responses. Ecological Indicators, 60, 152–161. https://doi.org/10.1016/j.ecolind.2015.06.032
113. Fedorova, M., Bollineni, R. C., & Hoffmann, R. (2014). Protein carbonylation as a major hallmark of oxidative damage: Update of analytical strategies. Mass Spectrometry Reviews, 33(2), 79–97. https://doi.org/10.1002/mas.21381