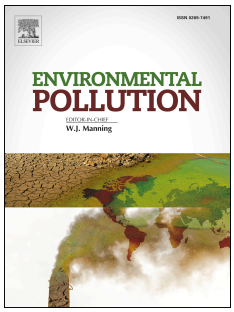

New insights on the impacts of e-waste towards marine bivalves: The case of the rare earth element Dysprosium

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New insights on the impacts of e-waste towards marine bivalves: the case of the rare earth element Dysprosium

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ABSTRACT

With the technological advance and economic development, the multiplicity and wide variety of applications of electrical and electronic equipment have increased, as well as the amount of end-of-life products (waste of electrical and electronic equipment, WEEE). Accompanying their increasing application there is an increasing risk to aquatic ecosystems and inhabiting organisms. Among the most common elements present in WEEE are rare earth elements (REE) such as Dysprosium (Dy). The present study evaluated the metabolic and oxidative stress responses of *mussels Mytilus galloprovincialis* exposed to an increasing range of Dy concentrations, after a 28 days experimental period. The results obtained highlighted that Dy was responsible for mussel's metabolic increase associated with glycogen expenditure, activation of antioxidant and biotransformation defenses and cellular damages, with a clear loss of redox balance. Such effects may greatly impact mussel's physiological functions, including reproduction capacity and growth, with implications to population conservation. Overall the present study pointed out the need for more research on the toxic impacts resulting from these emerging pollutants, specially towards marine and estuarine invertebrate species.

Keywords:

Toxicity; mussels; e-waste; bioaccumulation; oxidative stress; metabolism.

Capsule: Dysprosium induced metabolic and oxidative stress alterations in *Mytilus galloprovincialis*, which may impair mussels physiological mechanisms.

1. INTRODUCTION

The growing demand and use of rare earth elements (REEs) by the industries to produce electric and electronic equipment (EEE) led to enormous amount of e-waste generated annually (49.8 Mt in 2018) and consequently to its discharge into the environment. The disposal of e-waste into the environment is mainly due to the lack of government measures to raise public awareness about e-waste recycling and how to recycle it, to the lack of efficient recycling methodologies, by low yields rates obtained and the tedious and costly steps involved in the separation of the elements to further use (Baldé et al., 2017; European Rare Earths Competency Network (ERECON), 2014; Dutta et al., 2016). E-waste is not biodegradable and accumulates in the environment, in the soil, air, water and aquatic organisms (UNEP et al., 2019; ou, 2013). Although it is estimated that e-waste can represent only 2% of solid waste streams, it can represent up to 70% of the hazardous waste that ends up in landfill (UNEP et al., 2019). There is already an increase of REEs concentration in the superficial waters from the mining activities as a result of increased EEEs production demand. Recent studies revealed an increase of REEs concentration from 1 μg L^{-1} (Ouyang et al., 2006) to 3007 μg L^{-1} (He et al., 2010) in Pearl River, China – an extensive river system that across several REEs producing zones.

Dysprosium (Dy, Z = 66) is a REE with high economic importance but also with high supply risk (Batinic et al., 2018; Critical Raw Materials - European Commission Report, 2018). For this reason, Dy is identified as a critical element by the EU Commission (Rabe et al., 2017). According to Abrahami et al. (2015), neodymium (Nd), Dy and terbium (Tb) are among the REEs at most supply risk within the next few years. Almost 95% of the total Dy demand is related to its use in magnets (Zapp et al., 2018), due to its high resistance to demagnetization at high temperatures (Kim et al., 2017). Nevertheless, this element is also used in compounds like Dy iodide to be applied in commercial lighting, to produce an intense white light. Moreover, Dy oxide-nickel cermet (composite material made of ceramic and sintered metal) is used in nuclear reactor control rods, to absorb neutrons for a long period without contracting or expanding. Lastly, Dy, when combined with vanadium and other REEs, is used in the production of laser materials (Antić et al., 2016).

The concentration of Dy in natural waters varies from a few ng/L in seawater (0.90 ng/L) (Johannesson et al., 1994; Tai et al., 2010), rain water (1.48 – 1.8 ng/L), throughfall (3.62 ng/L), soil solution (19.0 ng/L) and stream water (46.8 ng/L) (Kabata-Pendias and Mukherjee, 2007) to a few µg/L in surface waters (such as Terengganu River Basin, in Malaysia, with 0.0038-1.93 µg/L) (Sultan and Shazili, 2009) and groundwaters (13.5 µg/L) (Johannesson et al., 1994). Furthermore, the concentration of Dy in contaminated environments can achieve 172 – 186 µg/L (Berkeley Pit lake, a large acidic mining lake in Butte, Montana) (Gammons et al., 2003). A recent review on the spatial concentration of REEs, including Dy, in various water matrices around several continents (including 35 countries) showed that Dy concentrations in groundwater and surface water (both freshwater and seawater) vary from few ng to more than 100 µg/L (Adeel et al., 2019). Nevertheless, it has been estimated that the e-waste generated will increase to 52 Mt in 2021 and to 120 Mt in 2050 (Baldé et al., 2017; UNEP et al., 2019), which will lead to an increase in the concentration of REEs, namely Dy, in the aquatic systems.

Although it is reported the increasing presence of REEs in marine coastal areas, their toxicological understanding, an in particular for Dy, in such aquatic systems is still almost unknown but of increasing concern. Nevertheless, the emergence of Dy in the aquatic systems has raised attention into the scientific community related to its effects in the living organisms. Oral et al. (2017) investigated the effect of REEs on early life cycle stages in *Paracentrotus lividus* sea urchins, exposing the embryos and sperm of these species to trichloride salts of five REEs, including Dy. The results obtained showed that *P. lividus* embryos had a decreased mitotic activity and an increased aberration rate. Sperm exposed to these elements showed decrease in fertilization success along with increase in offspring damage. These authors concluded that REE-associated toxicity affected embryogenesis, fertilization, cytogenetic and redox endpoints. In another study, Anaya et al. (2016) evaluated the effect of Dy oxide nanoparticles (nDy_2O_3) on the bacteria *Escherichia coli*. This nDy_2O_3 has several biomedical applications due to its fluorescence and paramagnetic properties contributing to the location, diagnosis and treatment of diseases. During this study fluorescent dyes (Live/Dead) were used to measure the undisturbed cell membrane (UCM) and respirometric assays allowing the measure of remaining respiration percentage (RRP). After bacteria exposure to nDy_2O_3, the UCM and RRP decreased to 88% and 43%, respectively, evidencing Dy toxicity, with Dy(III) as

the main contributor to the overall toxicity. Vukov et al. (2016) compared the toxicological effect of the Dy to the freshwater invertebrates *Daphnia pulex* and *Hyalella azteca*. The results revealed that *H. azteca* is 1.4 times more sensitive than *D. pulex*. In this study, it was also verified the toxicity modifying influence of Ca, Na, Mg, pH and dissolved organic matter (DOM) in the presence of Dy with a more sensitive organism, *H. azteca*. It was concluded that additions of Ca and Na, low pH and DOM provided protection of the organisms against Dy toxicity, while on the contrary the addition of Mg increase the toxicity of Dy.

From the literature available it is possible to recognize that no knowledge exists on the toxic effects of Dy towards marine or estuarine bivalves, namely on species with high ecological and economic relevance. Nevertheless, marine coastal systems are frequently final destination of these pollutants putting at risk inhabiting animals and public health in the case of bivalves associated with human consumption. Therefore, the present study aimed to investigate the biochemical alterations induced in the mussel species *Mytilus galloprovincialis*, when exposed to an increasing exposure gradient of Dy, resembling low to highly contaminated areas. Although no studies are known on the impacts of Dy in bivalves, and in particular in mussels, recent studies demonstrated the negative impacts of other REEs (e.g. Neodymium, Lanthanum, Gadolinium) towards *M. galloprovincialis*, including impairments on their metabolic capacity and occurrence of oxidative stress, with alterations on mussels antioxidant capacity and redox balance (Freitas et al., 2020; Henriques et al., 2019; Pinto et al., 2019). For this reason the present study measured biochemical parameters related with alterations on mussel's metabolic capacity (electron transport system activity), energy reserves content (glycogen content, GLY; total protein content, PROT), oxidative stress (activity of antioxidant and biotransformation enzymes), cellular damage (lipid peroxidation and protein carbonyl levels) and redox balance (ratio between reduced glutathione and oxidized glutathione, GSH/GSSG), factors that may compromise the normal physiological functioning of mussels such as filtration and respiration rates, growth and reproductive capacity.

2. MATERIALS AND METHODS

2.1 Experimental conditions

The Mediterranean mussel *Mytilus galloprovincialis* was selected as biological model for the present study. Among the most widely used mussel species identified as good bioindicator is *Mytilus galloprovincialis*, with several studies demonstrating the capacity of this species to respond to pollutants accumulation with physiological and biochemical alterations (among others, Andrade et al., 2019; Burgos-Aceves and Faggio, 2017; Coppola et al., 2018a; Henriques et al., 2019; Monteiro et al, 2019; Munari et al., 2018; Pirone et al., 2019; Renault, 2015).

Animals were collected in September 2018, at the Ria de Aveiro lagoon (Portugal). Mussels with similar size (5.7±0.7 cm length; 3.0±0.4 cm width) were selected to avoid differences in biological responses.

Bivalves were transported from the field to the laboratory where they were placed in aquaria for depuration and acclimation to laboratory conditions for two weeks. During this period, mussels were maintained under constant aeration in different aquaria with artificial seawater (Tropic Marin® SEA SALT) at temperature, pH and salinity values resembling the sampling site conditions (18.0 ± 1.0 ºC; 8.0 ± 0.1, 30 ± 1, respectively). Seawater was renewed every day during the first seven days and then every three days until the end of the acclimation period.

After this period, mussels were distributed in different aquaria (with four aquaria per condition with 3 L of seawater each) and exposed to the following conditions for twenty-eight days: control (CTL, 0 µg L^{-1}), 2.5, 5, 10, 20, 40 µg L^{-1} of Dy (Dy^{3+}). A total of twenty mussels were used per tested concentration (five mussels per aquarium). The selection of the Dy exposure concentrations was based on the levels identified in low to highly contaminated environments (see for review Adeel et al., 2019).

To evaluate the stability of Dy in the water medium a parallel experiment was conducted, in the absence of mussels. For this, glass containers with 500 mL of artificial seawater were spiked with 2.5 and 40 µg L^{-1} of Dy (10 containers per concentration) and, during seven days (corresponding to the period between water renewals along the twenty-eight days experimental assay), aliquots of 5 mL were daily collected to assess concentrations of Dy in the water.

During the experimental period (twenty-eight days), water was changed every week and the medium conditions re-established, including Dy concentrations and seawater parameters (temperature 17 ± 1.0 ºC, pH 8.0± 0.1 and salinity 30 ± 1). During the exposure period water medium in each aquarium was continuously aerated with a photoperiod of 12h light:12h dark. Every week, immediately after water renewal, water samples were collected from each aquarium for Dy quantification, to assess real exposure concentrations. During this period, mussels were fed with Algamac protein plus (150,000 cells/animal) three times per week. Mortality was also daily checked, with 100% of survival recorded during all the experimental period.

At the end of the exposure period, mussels were frozen individually with liquid nitrogen and stored at -80ºC, until homogenization of each individual soft tissue under liquid nitrogen. Each homogenized organism was divided into aliquots (each with 0.5 g fresh weigh, FW) for biomarkers analyses and Dy quantification.

2.2 Dysprosium quantification in water and in mussel tissues

To guarantee that nominal and real concentrations were similar, Dy concentrations in water samples, collected every week from each aquaria immediately after water contamination, were quantified using inductively coupled plasma mass spectrometry (ICP-MS), on a Thermo ICP-MS X Series equipped with a Burgener nebulizer after adequate sample dilution and acification to pH <2. Water samples collected daily to evaluate the stability of Dy in seawater (in the absence of mussels), along seven days experimental period, were analysed following the same procedure.

Total Dy concentrations in *M. galloprovincialis* whole soft tissues (2 individuals per replicate (8 individuals per condition) were also quantified by ICP-MS, after microwave assisted acid digestion. After freeze-drying, mussel samples with 100–200 mg were digested in a microwave, firstly with 2 mL of HNO_3 (70%) at 170 ℃ for 15 min, followed by a second i dentical microwave cycle with 0.5 mL of H_2O_2 (30%). After addition of H_2O_2, the mixture was allowed to stand for 15 min so that the microwave reaction was not as violent. The obtained digests were transferred into 25 mL polyethylene vessels and the volume made up with ultrapure water.

204

205 2.3 Biochemical markers

206 The whole tissue of mussels was used for biomarkers determination. For each
207 biochemical parameter, 0.5 g of FW tissue per organism was used, with 2 individuals per
208 replicate (8 individuals per condition, the same used for Dy quantification). For each condition,
209 metabolic capacity (electron transport system activity, ETS), energy reserves (glycogen content,
210 GLY; total protein content, PROT), antioxidant and biotransformation defences (activities of
211 superoxide dismutase, SOD; catalase, CAT; glutathione S-transferases, GSTs), cellular
212 damage (lipid peroxidation levels, LPO; protein carbonyl levels, PC) and redox balance (ratio
213 between reduced glutathione and oxidized glutathione, GSH/GSSG) markers were assessed.
214 Each sample was performed at least in duplicate (2 sub-samples from each organism), for
215 operator quality control. All measurements were done using a microplate reader. The extraction
216 for each biomarker was performed with specific buffers: phosphate buffer for SOD, CAT, GSTs,
217 PROT, GLY and PC; magnesium sulphate buffer for ETS; trichloroacetic acid buffer for LPO
218 and KPE buffer for GSH/GSSG. Each sample was sonicated for 15 s at 4 ºC and centrifuged for
219 25 min (or 15 min for GSH/GSSG) at 10,000 *g* (or 3,000 *g* for ETS). Supernatants were stored
220 at -80 ºC. Biomarkers quantifications were performed as described previously by Carregosa et
221 al. (2014), Andrade et al. (2019), Coppola et al. (2019a) and Freitas et al. (2018).

222

223 *Antioxidant defences*

224 SOD activity was determined by the Beauchamp and Fridovich (1971) method after
225 adaptations performed by Carregosa et al. (2014). The standard curve was formed using SOD
226 standards (0.25-60 U/mL). Samples' absorbance was read at 560 nm after 20 min of incubation
227 at room temperature. Results were expressed in U per g FW where one unit (U) represents the
228 quantity of the enzyme that catalyzes the conversion of 1 µmol of substrate per min.

229 CAT activity was quantified according to the Johansson and Borg (1988) method and the
230 modifications performed by Carregosa et al. (2014). The standard curve was determined using
231 formaldehyde standards (0–150 µM). Absorbance was measured at 540 nm. The enzymatic
232 activity was expressed in U per g of FW, where U represents the amount of enzyme that caused
233 the formation of 1.0 nmol formaldehyde per min at 25 ℃.

GPx activity was quantified following Paglia and Valentine (1967). The absorbance was measured at 340 nm in 10 sec intervals during 5 min and the enzymatic activity was determined using ε = 6.22 $mM^{-1}cm^{-1}$. The results were expressed as U per g FW, where U represents the amount of enzyme that caused the formation of 1.0 µmol NADPH oxidized per min.

Biotransformation defences

GSTs activity was quantified following Habig et al. (1974) protocol with some adaptations performed by Carregosa et al. (2014). GSTs activity was measured spectrophotometrically at 340nm (ε = 9.6 mM^{-1} cm^{-1}). The enzymatic activity was expressed in U per g of FW where U is defined as the amount of enzyme that catalysis the formation of 1 µmol of dinitrophenyl thioether per min.

Redox balance

GSH and GSSG glutathione contents were measured at 412 nm (Rahman et al., 2007). The ratio GSH/GSSG was determined taking in account the number of thiol equivalents (GSH / 2*GSSG).

Cellular damage

LPO determination was done following the method described by Ohkawa et al. (1979). LPO levels were measured trough the quantification of malondialdehyde (MDA), a by-product of lipid peroxidation. Absorbance was measured at 535 nm and the extinction coefficient of 156 mM^{-1} cm^{-1} was used to calculate LPO levels, expressed in nmol of MDA formed per g of FW.

The quantification of carbonyl groups in oxidized proteins (PC) was done following the 2,4-dinitrophenylhydrazina (DNPH) alkaline method, described by Mesquita et al. (2014). Absorbance was measured at 450 nm and the extinction coefficient of 22,308 M^{-1} cm^{-1} was used to calculated PC levels, which were expressed in nmol per g of FW.

Metabolic capacity and energy reserves

The ETS activity was measured based on King and Packard (1975) and the modifications performed by De Coen and Janssen (1997). Absorbance was measured during 10 min at 490

nm with intervals of 25 s and the extinction coefficient of 15,900 $M^{-1}cm^{-1}$ was used to calculate the amount of formazan formed. Results were expressed in nmol per min per g of FW.

For GLY quantification the sulphuric acid method was used, as described by Dubois et al. (1956). Glucose standards were used (0–10 mg/ mL) to produce a calibration curve. Absorbance was measured at 492 nm after incubation during 30 min at room temperature. Results were expressed in mg per g FW.

The PROT content was determined according to the spectrophotometric Biuret method (Robinson and Hogden, 1940). Bovine serum albumin (BSA) was used as standard calibration curve (0–40 mg/mL). Absorbance was read at 540 nm. The results were expressed in mg per g FW.

2.4 Integrated biomarker response

The integrated biomarker response (IBR) was calculated according to Beliaeff and Burgeot (2002) aiming to evaluate the general mussel's biochemical response among tested concentrations. All biomarkers determined were used in the calculation of the IBR and they were arranged clockwise in the following order: ETS, GLY, PROT, LPO, PC, GSH/GSSG, SOD, CAT, GPx and GSTs. Values were discussed in terms of a general response given by the final IBR value, where higher values correspond to higher mussels' response.

2.5 Statistical analyses

Results from biochemical analyses and Dy concentrations in mussel's tissues, obtained from each condition, were submitted to statistical hypothesis testing using permutational analysis of variance (PERMANOVA+add-on in PRIMER v6, Anderson et al., 2008). When significant differences were observed in the main test, pairwise comparisons were performed among conditions. Values of *p* lower than 0.05 were considered as significantly different and identified in the figures with different lowercase letters and *p*-values are presented in a Table format.

The matrix gathering the Dy concentrations in mussels soft tissues and biochemical results per condition were used to calculate the Euclidean distance similarity matrix, which was .

294 simplified through the calculation of the distance among centroids matrix based on the
295 concentration and submitted to ordination analysis (Principal Coordinates, PCO). Pearson
296 correlation vectors of biochemical descriptors (correlation >0.75) were provided as
297 supplementary variables, whcih were superimposed on the top of the PCO graph.
298

3. RESULTS

3.1 Dysprosium concentrations in seawater and mussel tissues

Results concerning the stability of Dy in seawater medium showed that, in the absence of mussels, concentrations were maintained along seven days' exposure period, with results showing that the mean±STDEV values after exposure to 2.5 and 40 µg/L of Dy were, respectively, 2.5±0.1 and 44±3.2 µg/L of Dy. These results clearly demonstrate the stability of Dy during the seven days' exposure period, the interval used between water renewal along the experimental assay.

In what regards to Dy concentrations in seawater from the experimental exposure assay, values obtained in water samples collected immediately after spiking revealed that measured and nominal concentrations were similar, for all the conditions and weeks, validating the Dy spiking process (Table 1).

The results obtained from Dy quantification in mussel's tissues showed significant difference among animals exposed to tested conditions, with increasing Dy levels along the increasing exposure concentration (Table 1).

3.2 Biochemical markers

Antioxidant defences

The activity of SOD was significantly lower at control and at 2.5 µg/L of Dy in comparison to mussels exposed to higher concentrations (Figure 1A, Table 2). The activity of CAT was significantly higher in mussels exposed to 20 and 40 µg/L of Dy in comparison to the remaining conditions (Figure 1B, Table 2). The activity of GPx was significantly higher in mussels exposed to 40 µg/L of Dy in comparison to non-contaminated mussels and the ones exposed to 5.0 and 10 µg/L of Dy (Figure 1C, Table 2).

Biotransformation defences

The activity of GSTs increased with the increase of Dy exposure concentration, with significantly higher values in mussels exposed to 20 and 40 µg/L of Dy in comparison to animals under control and exposed to the lowest Dy concentration (Figure 2, Table 2).

329 *Redox balance*

330 The GSH/GSSG values were significantly lower in contaminated mussels compared to
331 control ones, with the lowest values in animals exposed to concentrations 2.5 and 5.0 µg/L of
332 Dy (Figure 3, Table 2).

333

334 *Cellular damage*

335 Levels of LPO were significantly higher in contaminated mussels compared to control
336 ones, with the highest values in mussels exposed to 20 µg/L of Dy. No significant differences
337 were observed among mussels exposed to 2.5, 5.0 and 10 µg/L of Dy (Figure 4A, Table 2). The
338 PC levels increased in mussels exposed to Dy, with significant differences between control and
339 Dy exposed mussels. Although higher PC levels were obtained in animals exposed to 10 µg/L
340 of Dy, no significant differences were observed among contaminated mussels (Figure 4B, Table
341 2).

342

343 *Metabolic capacity and energy reserves*

344 The ETS activity showed no significant differences among conditions, although higher
345 values were observed at the highest Dy exposure concentrations (Figure 5A, Table 2). The GLY
346 content decreased in Dy exposed mussels, with significant differences between the control and
347 mussels exposed to 5, 10, 20 and 40 µg/L of Dy (Figure 5B, Table 2). As for the ETS activity, no
348 significant differences were observed among tested conditions in terms of PROT content,
349 although higher values were noticed at higher Dy concentrations (Figure 5C, Table 2).

350

351 ## 3.3 Integrated Biomarker Response

352 Integrated Biomarker Response (IBR) values showed the highest score (3.4) for
353 mussels exposed to the highest Dy exposure concentration. The lowest IBR values were
354 observed for mussels exposed to the concentrations 2.5 and 5.0 µg/L of Dy (Table 3).

355

356 ## 3.4 Principal Coordinates Analysis

357 The Principal Coordinates Analysis (PCO) representation revealed that PCO1 explained
358 64.2% of the total variation among the data, separating mussels exposed to control and to the

two lowest Dy exposure concentrations (2.5 and 5 µg/L of Dy) in the positive side from the mussels exposed to higher concentrations (10, 20 and 40 µg/L of Dy) in the negative side. PCO2 explained 16.5% of the total variation, separating control (CTL) and the two highest concentrations in the positive side from the remaining conditions in the negative side. LPO, GLY and GSH/GSSG ratio presented a correlation higher than 0.6 with PCO1 positive side, with Dy, ETS, PROT, GSTs, SOD and PC being the factors that best correlate with PCO1 negative side ($r>0.7$).

4. DISCUSSION

The present study evaluated the toxic impacts of Dy in the mussel *M. galloprovincialis*, evaluating the changes induced by this element in mussels oxidative stress status, metabolic capacity and energetic reserves content.

It has been reported that when in the presence of pollutants bivalves may increase the production of reactive oxygen species (ROS, the singlet oxygen $^1O_2$, the superoxide anion O_2^-, the hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO)), that are naturally produced during several cellular pathways of aerobic metabolism including oxidative phosphorylation, electron transport chains in mitochondria and microsomes, or during the activation of immune mechanisms (Halliwell and Gutteridge, 2007). Under basal conditions the adverse effects of ROS are prevented by a series of antioxidant defence mechanisms, including Phase I antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). While SOD is responsible for the removal of O_2^- with formation H_2O_2, CAT and GPx are involved in the reduction of H_2O_2 to H_2O (Regoli and Giulianni, 2014). The present study revealed that *M. galloprovincialis* increased the activity of SOD, CAT and GPx enzymes along with Dy increasing exposure gradient, with the highest activities at the highest exposure concentrations. As previously demonstrated, in the presence of pollutants *M. galloprovincialis* has de capacity to activate antioxidant defence mechanisms, with published data revealing the ability of this species to increase the activity of antioxidant enzymes in the presence of metal(oid)s (Coppola et al., 2018a, 2018b; Freitas et al., 2019b; Monteiro et al, 2019), pharmaceuticals and personal care products (Balbi et al., 2018; Freitas et al., 2019a; Mezzelani et al., 2018; Munari et al., 2018; Pirone et al., 2019), and nanoparticles (Andrade et al., 2019; Barmo et al., 2013; Hull et al., 2013). Most recently is was also shown the capacity of *M. galloprovincilais* to increase the activity of antioxidant enzymes when exposed to the REEs Gadolinium (Gd) and Lanthanum (La) (Henriques et a., 2019; Pinto et al., 2019). Thus, the present results highlight that mussels exposed to Dy were able to develop a defence strategy to eliminate the excess of ROS produced, in a tentative to avoid cellular damages and loss of redox balance.

Glutathione S-transferases (GSTs) are a superfamily of Phase II detoxification enzymes that detoxify both ROS and toxic xenobiotics, through conjugation to reduced glutathione (GSH)

with compounds that contain an electrophilic centre through the formation of a thioether bond between the sulfur atom of GSH and a broad range of substrates. Both GSH content and GSTs enzyme activities are under tight homeostatic control, with higher GSTs activity often associated with low GSH content, indicating loss of cellular redox balance. Under stressful conditions, GSTs activity are induced to achieve efficient protection. In this way, since xenobiotics are a primary source of oxidative stress, GSTs also play an important albeit indirect role in antioxidant defense, by eliminating toxic substances and preventing subsequent deleterious effects. The present study demonstrated the capacity of GSTs to detoxify Dy, with higher activity at higher exposure concentrations. Also previous studies already demonstrated the increased activity of GSTs in mussels exposed to different pollutants, including metal(oid)s (Coppola et al., 2018a, 2018b; Freitas et al., 2019b; Peric and Buric, 2019), drugs (Gonzalez-Rey and Bebianno, 2013; Martin-Diaz et al., 2009) and nanoparticles (Canesi et al., 2010; Ciacci et al., 2012; Huang et al., 2018) and most recently Pinto et al. (2019) and Henriques et al. (2019b) also demonstrated the capacity of this group of enzymes to detoxify other REEs, namely La and Gd, respectively. Thus, previous studies and the presents findings highlight the efficiency of this group of enzymes to detoxify mussels from REE.

Besides antioxidant and biotransformation enzymes, low molecular weight scavengers are also able to neutralize ROS by direct reaction with them. The most abundant cytosolic scavenger is GSH. In particular, GSH can be oxidized to GSSG (oxidized glutathione) by GPx. For this, H_2O_2 acts also as substrate for GPx, using GSH as electron donor to catalyse the reduction of H_2O_2 to H_2O. Thus, when under a stressful condition GSSG content is enhanced above the reducing capacity of glutathione reductase (GRed) and the ratio GSH/GSSG is altered, decreasing along the increasing stress level. For this reason, the ratio GSH/GSSG has been frequently used as an indicator of cellular redox status after exposure to pollutants. The results obtained in the present study also demonstrated that mussels exposed to Dy strongly decreased the ratio GSH/GSSG, a clear sign of cells loss redox homeostasis when in the presence of this REE. Similarly, Coppola et al. (2017) showed a decrease of GSH/GSSG values along the exposure to metals. Other authors showed a similar pattern in bivalves exposed to pharmaceuticals and personal care products (Almeida et al. 2014, 2015; Freitas et al., 2019a). Recently, studies published by Pinto et al. (2019) and Henriques et al. (2019b) also highlighted

426 the use of GSH/GSSG ratio as an indicator of redox balance in bivalves exposed to REEs (La
427 and Gd, respectively), with significantly lower values in contaminated animals in comparison to
428 control ones.

429 When enzymatic and non-enzymatic antioxidant defences are not sufficient to eliminate
430 the excess of ROS these free radicals can easily react with organism's membrane lipids,
431 inducing an alteration of membrane permeability, as well as with proteins, causing oxidative
432 modifications which might result in catalytically less active enzymes or proteins more
433 susceptible to proteolytic degradation. Such events are normally assessed by measuring lipid
434 peroxidation (LPO) and protein carbonylation (PC) in animals. Lipid peroxidation is the oxidative
435 degradation of lipids in cell membranes, resulting in cell damage, being commonly measured by
436 the content of malondialdehyde (MDA), one of the most abundant aldehydes generated during
437 lipid oxidation and also probably the most commonly used as an oxidative stress marker. In the
438 present study *M. galloprovincialis* showed increased LPO levels in Dy contaminated specimens.
439 Similarly, several other studies used LPO as a marker of the oxidative stress generated by the
440 exposure of bivalves to different pollutants, including in mussels, exposed to classical pollutants
441 as metal(oid)s (among others, Vlahogianni and Valavanidis, 2006; Coppola et al., 2018a), and
442 emerging pollutants, such as pharmaceuticals and personal care products (Gonzalez-Rey and
443 Bebianno, 2011, 2014; Quinn et al., 2011; Teixeira et al., 2017), nanoparticles (Gornati et al.,
444 2016; Gomes et al., 2011, 2014) and the REEs La and Gd (Henriques et al., 2019b; Pinto et al.,
445 2019). Malondialdehyde (MDA) levels also increased in the sea urchin *Paracentrotus lividus*
446 larvae exposed to Dy (Oral et al., 2017). Protein carbonylation is also one biomarker of
447 oxidative stress, resulting from the oxidation of proteins by ROS and corresponding to the
448 presence of carbonyl groups, such as aldehyde and ketone, in specific amino acid side chains
449 such as lysine, proline, arginine and threonine. The present findings also highlighted the
450 capacity of Dy to induce oxidation of proteins, with higher PC levels in contaminated mussels.
451 Although less used as oxidative stress biomarker in bivalves, and especially in clams and
452 mussels (Andreade et al., 2019; Matozzo et al., 2016; Merad et al., 2016; Parolini et al., 2016),
453 few studies revealed increased PC levels in *M. galloprovincialis* exposed to the drugs triclosan
454 and diclofenac (Freitas et al., 2019a), cadmium (Dailianis et al., 2009), the pesticides
455 chlorpyrifos and penoxsulam (Patetsini et al., 2012).

Apart from alterations induced by Dy in oxidative stress related biomarkers, the impacts induced in *M. galloprovincialis* by this REE can be also assessed by evaluating mussel metabolism. For this, the electron transport system (ETS) activity is commonly used as a measure of the potential respiration that could be supported by the enzymatic machinery activity (Cammen et al., 1990). The results obtained in the present study indicate that mitochondrial respiration tended to increase at higher Dy concentrations, probably to fuel up mussels defence mechanisms, with higher ETS activity at higher exposure concentrations (especially at 20 and 40 µg/L of Dy). Accompanying this metabolic enhance the obtained results further demonstrated an increase of the total protein (PROT) content at higher exposure concentrations, which may result from higher production of enzymes, namely antioxidant enzymes, to fight against the stress induced by Dy. As a consequence of increased metabolic capacity and activation of defence mechanisms, bivalves tended to decrease their glycogen (GLY) concentration, evidencing that under Dy exposure conditions mussels may use this energy reserve. In aquatic species, including bivalves, ETS activity has been used also to assess the oxygen demand to evaluate environmental changes (Sokolova et al., 2012), including the ones related with seasonal alterations (Fanslow et al., 2001), pH decrease (De Marchi et al., 2017; Simcic and Brancelj, 2006), and the presence of pollutants (De Marchi et al., 2018; Bielen et al., 2016; Gagné et al., 2016; Duquesne et al., 2004; Hamza-Chaffai et al., 2003). A similar response was already demonstrated by other authors when exposing mussels to different pollutants, with increasing ETS activity and decreasing GLY content along the increasing exposure gradient of different pollutants (Coppola et al., 2017; Freitas et al., 2019a; Monteiro et al., 2019a, 2019b; Pinto et al., 2019).

Overall the present study clearly demonstrated a dose-dependent response, with mussels showing higher biological impacts at to higher exposure concentrations. Nevertheless, at intermediate exposure concentrations, namely at 5 µg/L of Dy, it seems that mussels were able to avoid injuries by efficiently activating their defence mechanisms (including increase of antioxidant enzymes activities), while at the lowest concentration these strategies were not activated due to low stress levels. At higher concentrations, although enzymes activities increased mussels these defence mechanisms were not enough to avoid injuries, leading to

higher impacts. Such effects were corroborated by IBR values, based on measured biochemical markers, with the highest values at the highest exposure concentration and the lowest value at 5.0 µg/L of Dy. Such results are in accordance with previous studies that already highlighted IBR as a useful tool for quantitative assessment of stress levels in mussels exposed to different pollutants (Pinto et al., 2019; Yuan et al., 2017; Beliaeff and Burgeot, 2002). The lowest IBR values obtained at 5 µg/L of Dy may corroborate the hypothesis of an hormesis response, indicating a certain adaptive response of organisms to a moderate stress. Furthermore, PCO analysis applied to Dy concentration levels and biochemical responses, demonstrated a clear distinction between: i) mussels exposed to control and the two lowest Dy concentrations (PCO axis 1, positive side), associated to lower cellular damage and the maintenance of redox balance; ii) and mussels exposed to the three higher Dy concentrations (PCO axis 1, negative side), close associated with higher metabolic capacity, higher antioxidant and biotransformation defences, and higher protein content.

5. CONCLUSIONS

The present study clearly demonstrated the impacts of Dy in *M. galloprovincialis*, with increasing antioxidant defences, cellular damages and oxidative stress in Dy contaminated mussels. The results obtained further demonstrated that mussels exposed to Dy increased their metabolic capacity, with expenditure of GLY, indicating higher metabolic requirements under Dy contamination. With the increasing use of REE and the associated risks due to the increasing e-wastes resulting from electronic and electric devices, aquatic environments are increasingly endangered by the presence of these hazardous elements. Therefore, the present findings highlight the potential toxic impacts of REEs in marine animals, with oxidative stress and metabolic changes that might compromise mussel's physiological functions, such as respiration and filtration rates, growth, and reproduction. Considering that even at the lowest tested concentrations (2.5 and 5.0 µg/L of Dy) significant biochemical impairments were observed, the present study highlights the risk of toxic effects under actual concentration levels, identifying Dy as an hazardous element towards mussel's populations, and potential other bivalve species.

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REFERENCES

Abrahami, S. T., Xiao, Y., Yang, Y., 2015. Rare-earth elements recovery from post-consumer hard-disc drives. *Mineral Processing and Extractive Metallurgy*, *124*(2), 106-115.

Adeel, M., Lee, J. Y., Zain, M., Rizwan, M., Nawab, A., Ahmad, M. A., Shafiq, M., Yi, H., Jilani, G., Javed, R., Horton, R., Rui, Y., Tsang, D.C.W., Xing, B., 2019. Cryptic footprints of rare earth elements on natural resources and living organisms. *Environment international*, *127*, 785-800.

Almeida, Â., Calisto, V., Esteves, V. I., Schneider, R. J., Soares, A. M., Figueira, E., Freitas, R., 2014. Presence of the pharmaceutical drug carbamazepine in coastal systems: effects on bivalves. *Aquatic Toxicology*, *156*, 74-87.

Almeida, Â., Freitas, R., Calisto, V., Esteves, V. I., Schneider, R. J., Soares, A. M., Figueira, E., 2015. Chronic toxicity of the antiepileptic carbamazepine on the clam *Ruditapes philippinarum*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *172*, 26-35.

Anaya, N. M., Solomon, F., Oyanedel-Craver, V., 2016. Effects of dysprosium oxide nanoparticles on *Escherichia coli*. *Environmental Science: Nano*, *3*(1), 67-73.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA for PRIMER: Guide to software and statistical methods. University of Auckland and PRIMER-E, Plymouth.

Andrade, M., De Marchi, L., Pretti, C., Chiellini, F., Morelli, A., Figueira E., Rocha R.J.M., Soares, A.M.V.M., Freitas, R., 2019. The impacts of warming on the toxicity of carbon nanotubes in mussels. *Marine Environmental Research* 145, 11-21.

Antić, Ž.,* Dramićanin,M.D., Prashanthi, K., Jovanović, D., Kuzman, S., Thundat, T., 2016. Pulsed Laser Deposited Dysprosium-Doped Gadolinium–Vanadate Thin Films for Noncontact, Self-Referencing Luminescence Thermometry. Advanced Materials. DOI: 10.1002/adma.201601176.

Balbi, T., Montagna, M., Fabbri, R., Carbone, C., Franzellitti, S., Fabbri, E. and Canesi, L., 2018. Diclofenac affects early embryo development in the marine bivalve *Mytilus galloprovincialis*. *Science Total Environment* 642, 601-609.

Baldé CP, Forti V, Gray V, Kuehr R, Stegmann P. The Global E-Waste Monitor - 2017. Bonn/Geneva/Vienna: 2017)

559 Barmo, C., Ciacci, C., Canonico, B., Fabbri, R., Cortese, K., Balbi, T., Marcomini, A.,
560 Pojana, G., Gallo G., Canesi, L., 2013. In vivo effects of n-TiO2 on digestive gland and immune
561 function of the marine bivalve *Mytilus galloprovincialis*. *Aquatic Toxicology* 132, 9-18.
562 Batinic, B., Vaccari, M., Savvilotidou, V., Kousaiti, A., Gidarakos, E., Marinkovic, T., Fiore,
563 S., 2018. Applied WEEE pre-treatment methods: Opportunities to maximizing the recovery of
564 critical metals. *Global Nest Journal*, *20*(4), 706-711.
565 Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an
566 assay applicable to acrylamide gels. *Analytical Biochemistry* 44, 276–287.
567 Beliaeff B., Burgeot T., 2002. Integrated biomarker response: a useful tool for ecological
568 risk assessment. *Environmental Toxicology Chemistry*, 21, 1316–1322.
569 Bielen, A., Bošnjak, I., Sepčić, K., Jaklič, M., Cvitanić, M., Lušić, J., Lajtner, J., Simčič, T.,
570 Hudina, S., 2016. Differences in tolerance to anthropogenic stress between invasive and native
571 bivalves. *Science Total Environment* 543, 449-459.
572 Burgos-Aceves, M. A., Faggio, C., 2017. An approach to the study of the immunity
573 functions of bivalve haemocytes: physiology and molecular aspects. *Fish Shellfish Immunology*
574 67, 513-517.
575 Cammen, L. M., Corwin, S., Christensen, J. P., 1990. Electron transport system (ETS)
576 activity as a measure of benthic macrofaunal metabolism. *Marine Ecology Progress Series*,
577 *65*(1), 171-182.
578 Canesi, L., Fabbri, R., Gallo, G., Vallotto, D., Marcomini, A., Pojana, G., 2010.
579 Biomarkers in *Mytilus galloprovincialis* exposed to suspensions of selected nanoparticles (Nano
580 carbon black, C60 fullerene, Nano-TiO2, Nano-SiO2). *Aquatic Toxicology* 100(2), 168-177.
581 Carregosa, V., Velez, C., Soares, A.M.V.M., Figueira, E., Freitas, R., 2014. Physiological
582 and biochemical responses of three Veneridae clams exposed to salinity changes. *Comparative*
583 *Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 177–178, 1–9.
584 Ciacci, C., Canonico, B., Bilaniĉovă, D., Fabbri, R., Cortese, K., Gallo, G., Marcomini, A.,
585 Pojana, G., Canesi, L., 2012. Immunomodulation by different types of N-oxides in the
586 hemocytes of the marine bivalve *Mytilus galloprovincialis*. *PLoS One* 7(5), e36937.
587 Coppola, F., Almeida, Â., Henriques, B., Soares, A.M.V.M., Figueira, E., Pereira, E.,
588 Freitas, R., 2018a. Biochemical responses and accumulation patterns of *Mytilus*

589 *galloprovincialis* exposed to thermal stress and Arsenic contamination. *Ecotoxicology and*
590 *Environmental Safety* 147, 954–962.

591 Coppola, F., Almeida, Â., Henriques, B., Soares, A.M.V.M., Figueira, E., Pereira, E.,
592 Freitas, R., 2017. Biochemical impacts of Hg in *Mytilus galloprovincialis* under present and
593 predicted warming scenarios. *Science Total Environment* 601–602, 1129–1138.

594 Coppola, F., Henriques, B., Soares, A.M., Figueira, E., Pereira, E., Freitas, R., 2018b.
595 Influence of temperature rise on the recovery capacity of *Mytilus galloprovincialis* exposed to
596 mercury pollution. *Ecological Indicators* 93, 1060-1069.

597 Dailianis, S., Patetsini, E., Kaloyianni, M., 2009. The role of signalling molecules on actin
598 glutathionylation and protein carbonylation induced by cadmium in haemocytes of mussel
599 *Mytilus galloprovincialis* (Lmk). *Journal of Experimental Biology*, *212*(22), 3612-3620.

600 De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in Daphnia magna toxicity
601 testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of
602 toxicant-stressed Daphnia populations. *Journal of Aquatic Ecosystem Stress and Recovery* 6,
603 43–55.

604 De Marchi, L., Neto, V., Pretti, C., Figueira, E., Chiellini, F., Morelli, A., Soares,
605 A.M.V.M., Freitas, R., 2018. Toxic effects of multi-walled carbon nanotubes on bivalves:
606 Comparison between functionalized and nonfunctionalized nanoparticles. *Science Total*
607 *Environment* 622–623, 1532–1542.

608 De Marchi, L., Neto, V., Pretti, C., Figueira, E., Chiellini, F., Morelli, A., Soares, A.M.V.M.,
609 Freitas, R., 2017. The impacts of seawater acidification on *Ruditapes philippinarum* sensitivity to
610 carbon nanoparticles. *Environmental Science: Nano* 4(8), 1692-1704.

611 Dia et al., 2000

612 Duquesne, S., Liess, M. and Bird, D.J., 2004. Sub-lethal effects of metal exposure:
613 physiological and behavioural responses of the estuarine bivalve *Macoma balthica*. *Marine*
614 *Envrionmental Research* 58(2-5), 245-250.

615 Dutta, T., Kim, K. H., Uchimiya, M., Kwon, E. E., Jeon, B. H., Deep, A., Yun, S. T., 2016.
616 Global demand for rare earth resources and strategies for green mining. *Environmental*
617 *Research*, *150*, 182-190.

Fanslow, D. L., Nalepa, T. F., & Johengen, T. H., 2001. Seasonal changes in the respiratory electron transport system (ETS) and respiration of the zebra mussel, *Dreissena polymorpha* in Saginaw Bay, Lake Huron. *Hydrobiologia*, *448*(1-3), 61-70.

Freitas, R., Coppola, F., De Marchi, L., Codella, V., Pretti, C., Chiellini, F., Morelli, A., Polese, G., Soares, A.M.V.M., Figueira, E., 2018. The influence of Arsenic on the toxicity of carbon nanoparticles in bivalves. *Journal of Hazardous Materials* 358, 484–493.

Freitas R., Coppola F., Costa S., Pretti C., Intorre L., Meucci V., Soares A.M.V.M., Sole M., 2019a. The influence of temperature on the effects induced by Triclosan and Diclofenac in mussels. *Science of Total Environment* 663, 992-999.

Freitas, R., Leite, C., Pinto, J., Costa, M., Monteiro, R., Henriques, B., Di Martino, F., Coppola, F., Soares, A.M.V.M., Solé, M., Pereira, E., 2019b. The influence of temperature and salinity on the impacts of Lead in *Mytilus galloprovincialis*. *Science of Total Environment* 601, 1129-1138.

Freitas, R., Costa, S., Cardoso, C., Morais, T., Moleiro, P., Matias, A.C., Pereira, A.F., Machado, J., Correia, B., Pinheiro D., Rodrigues, A., Colónia J., Soares, A.M.V.M., Pereira, E., 2020. Toxicological effects of the rare earth element Neodymium in *Mytilus galloprovincialis. Chemosphere.* 244, 125457

Gagné, F., Auclair, J., Trépanier, S., Turcotte, P., Pilote, M., Gagnon, C., 2016. The impact of zinc oxide nanoparticles in freshwater mussels exposed to municipal effluents. *Invertebrate Survival Journal* 13(1), 281-290.

Gammons, C.H., Wood, S.A., Jonas, J.P., Madison, J.P., 2003. Geochemistry of the rare-earth elements and uranium in the acidic Berkeley Pit lake, Butte, Montana. *Chemical Geology* 198, 269–288.

Gomes, T., Pereira, C.G., Cardoso, C., Sousa, V.S., Teixeira, M.R., Pinheiro, J.P., Bebianno, M.J., 2014. Effects of silver nanoparticles exposure in the mussel *Mytilus galloprovincialis*. *Marine Environmental Research* 101, 208-214.

Gomes, T., Pinheiro, J.P., Cancio, I., Pereira, C.G., Cardoso, C., Bebianno, M.J., 2011. Effects of copper nanoparticles exposure in the mussel *Mytilus galloprovincialis*. *Environmental Science and Technology* 45(21), 9356-9362.

Gomes, T., Pinheiro, J.P., Cancio, I., Pereira, C.G., Cardoso, C., Bebianno, M.J., 2011. Effects of copper nanoparticles exposure in the mussel *Mytilus galloprovincialis*. *Environmental Science and Technology* 45(21), 9356-9362.

Gonzalez-Rey, M., Bebianno, M.J., 2011. Non-steroidal anti-inflammatory drug (NSAID) ibuprofen distresses antioxidant defense system in mussel *Mytilus galloprovincialis* gills. *Aquatic Toxicology* 105(3-4), 264-269.

Gonzalez-Rey, M., Bebianno, M. J., 2013. Does selective serotonin reuptake inhibitor (SSRI) fluoxetine affects mussel *Mytilus galloprovincialis*? *Environmental Pollution* 173, 200-209.

Gonzalez-Rey, M., Bebianno, M.J., 2014. Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel *Mytilus galloprovincialis*. *Aquatic Toxicology* 148, 221-230.

Gornati, R., Longo, A., Rossi, F., Maisano, M., Sabatino, G., Mauceri, A., Bernardini, G., Fasulo, S., 2016. Effects of titanium dioxide nanoparticle exposure in *Mytilus galloprovincialis* gills and digestive gland. *Nanotoxicology*, 10(6), 807-817.

Gornati, R., Longo, A., Rossi, F., Maisano, M., Sabatino, G., Mauceri, A., Bernardini, G., Fasulo, S., 2016. Effects of titanium dioxide nanoparticle exposure in *Mytilus galloprovincialis* gills and digestive gland. *Nanotoxicology*, 10(6), 807-817.

Grant, K., 2013Health consequences of exposure to e-waste: a systematic review. *The Lancet Global Health* 1, 350–361.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry* 249, 7130–7139.

Halliwell, B., & Gutteridge, J. M. C., 2007. Antioxidant defences: endogenous and diet derived. *Free radicals in biology and medicine*, *4*, 79-186.

Hamza-Chaffai, A., Pellerin, J. and Amiard, J.C., 2003. Health assessment of a marine bivalve *Ruditapes decussatus* from the Gulf of Gabès (Tunisia). *Environment International* 28(7), pp.609-617.

He J., Lü C., Xue H., Liang Y., Bai S., Sun Y., Shen, L.L., Mi, N., Fan, Q.-Y., 2010. Species and distribution of rare earth elements in the Baotou section of the Yellow River in China. *Environmental Geochemistry and Health* 32, 45–58.

Henriques, B., Coppola, F., Monteiro, R., Pinto, J., Viana, T., Pretti, C., Soares, A., Freitas, R., Pereira, E., 2019. Toxicological assessment of anthropogenic Gadolinium in seawater: Biochemical effects in mussels *Mytilus galloprovincialis*. *Science of the Total Environment* 664, 626-634.

Huang, X., Liu, Z., Xie, Z., Dupont, S., Huang, W., Wu, F., Kong, H., Liu, L., Sui, Y., Lin, D., Lu, W., 2018. Oxidative stress induced by titanium dioxide nanoparticles increases under seawater acidification in the thick shell mussel *Mytilus coruscus*. *Marine Environmental Research* 137, 49-59.

Hull, M. S., Vikesland, P. J., Schultz, I. R., 2013. Uptake and retention of metallic nanoparticles in the Mediterranean mussel (*Mytilus galloprovincialis*). *Aquatic Toxicology* 140, 89-97.

Johannesson, K. H., Lyons, W. B., Fee, J. H., Gaudette, H. E., McArthur, J. M., 1994. Geochemical processes affecting the acidic groundwaters of Lake Gilmore, Yilgarn Block, Western Australia: a preliminary study using neodymium, samarium, and dysprosium. *Journal of Hydrology*, *154*(1-4), 271-289.

Johansson, L. H., Borg, L. H., 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. *Analytical biochemistry*, *174*(1), 331-336.

Kabata-Pendias, A., & Mukherjee, A. B., 2007. *Trace elements from soil to human*. Springer Science & Business Media.

Kim, C. J., Yoon, H. S., Chung, K. W., 2017. Precipitation Properties of Neodymium and Dysprosium Double Salt in Sulfuric Acid Leaching Solution from Permanent Magnet Scrap. In *2017-Sustainable Industrial Processing Summit* (Vol. 1, pp. 259-260). Flogen Star Outreach.

King, F.D., Packard, T.T., 1975. Respiration and the respiratory electron transport in marine zooplankton. *Limnology and Oceanography* 2849–2854.

Martin-Diaz, L., Franzellitti, S., Buratti, S., Valbonesi, P., Capuzzo, A., Fabbri, E., 2009. Effects of environmental concentrations of the antiepilectic drug carbamazepine on biomarkers

and cAMP-mediated cell signaling in the mussel *Mytilus galloprovincialis*. *Aquatic Toxicology* 94(3), 177-185.

Matozzo, V., Battistara, M., Marisa, I., Bertin, V., Orsetti, A., 2016. Assessing the effects of amoxicillin on antioxidant enzyme activities, lipid peroxidation and protein carbonyl content in the clam *Ruditapes philippinarum* and the mussel *Mytilus galloprovincialis*. *Bulletin of environmental contamination and toxicology*, *97*(4), 521-527.

Merad, I., Bairi, Y., Sifi, K., Soltani, N., 2016. Protein carbonyls as biomarkers of oxidative stress induced by cadmium in *Donax trunculus*: gonad contents during exposure and recovery. *Fresenius Environmental Bulletin*, *25*, 5889-5895.

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.

Mezzelani, M., Gorbi, S., Fattorini, D., d'Errico, G., Consolandi, G., Milan, M., Bargelloni, L., Regoli, F., 2018. Long-term exposure of *Mytilus galloprovincialis* to diclofenac, Ibuprofen and Ketoprofen: Insights into bioavailability, biomarkers and transcriptomic changes. *Chemosphere* 198, 238-248.

Monteiro, R., Costa, S., Coppola, F., Freitas R., Vale, C., Pereira E., 2019. Evidences of metabolic alterations and cellular damages in mussels after short pulses of Ti contamination. *Science of the Total Environment* 650, 987-995.

Munari, M., Matozzo, V., Gagné, F., Chemello, G., Riedl, V., Finos, L., Pastore, P., Badocco, D., Marin, M.G., 2018. Does exposure to reduced pH and diclofenac induce oxidative stress in marine bivalves? A comparative study with the mussel *Mytilus galloprovincialis* and the clam *Ruditapes philippinarum*. *Environmental Pollution* 240, 925-937.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95, 351–358.

Oral, R., Pagano, G., Siciliano, A., Gravina, M., Palumbo, A., Castellano, I., Trifuoggi, M., 2017. Heavy rare earth elements affect early life stages in *Paracentrotus lividus* and *Arbacia lixula* sea urchins. *Environmental research*, *154*, 240-246.

Ouyang, T.P., Zhu, Z.Y., Kuang, Y.Q., Huang, N.S., Tan, J.J., Guo, G.Z., Gu, L.S., Sun, B., 2006. Dissolved trace elements in river water: spatial distribution and the influencing factor, a study for the Pearl River Delta Economic Zone, China. *Environmental Geology* 49, 733–742.

Paglia D.E., Valentine W.N., 1967. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*. 70, 158-169.

Parolini, M., Magni, S., Castiglioni, S., Binelli, A., 2016. Amphetamine exposure imbalanced antioxidant activity in the bivalve *Dreissena polymorpha* causing oxidative and genetic damage. *Chemosphere*, *144*, 207-213.

Patetsini, E., Dimitriadis, B. K., Kaloyianni, M., 2012. Exposure of mussels Mytilus galloprovinciallis to environmental pesticides. Study of LMS, ROS, DNA damage, protein carbonylation and antioxidant capacity for their use as biomarkers. *Comparative Biochemistry and Physiology, Part A*, (163), S26.

Perić, L., Burić, P., 2019. The effect of copper and chlorpyrifos co-exposure on biomarkers in the marine mussel Mytilus galloprovincialis. *Chemosphere*, *225*, 126-134.

Pinto, J., Costa, M., Leite, C., Borges, C., Coppola, F., Henriques, B., Monteiro, R., Russo, T., Di Cosmo, A., Soares, A.M.V.M., Polese, G., Pereira, M., Freitas, R., 2019. Ecotoxicological effects of lanthanum in Mytilus galloprovincialis: Biochemical and histopathological impacts. *Aquatic Toxicology*, *211*, 181-192.

Pirone G., Coppola F., Pretti C., Soares A.M.V.M., Sole M., Freitas R., 2019. The effect of temperature on Triclosan and Lead exposed mussels. *Comparative Biochemistry and Physiology Part B* 232, 42-50.

Quinn, B., Schmidt, W., O'Rourke, K., Hernan, R., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on biomarker expression in the zebra mussel (*Dreissena polymorpha*) and their comparison with standardised toxicity tests. *Chemosphere* 84(5), 657-663.

Rabe, W., Kostka, G., Stegen, K. S., 2017. China's supply of critical raw materials: Risks for Europe's solar and wind industries? *Energy Policy*, *101*, 692-699.

760 Rahman, I., Kode, A., Biswas, S.K., 2007. Assay for quantitative determination of
761 glutathione and glutathione disulfide levels using enzymatic recycling method. *Nature Protocols*
762 1, 3159–3165.
763 Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative
764 stress biomarkers in marine organisms. *Marine Environmental Research* 93, 106–117.
765 Robinson, H.W., Hogden, C.G., 1940. The biuret reaction in the determination of serum
766 proteins. *The Journal of Biological Chemistry* 135, 707–725.
767 Simčič, T., Brancelj, A., 2006. Effects of pH on electron transport system (ETS) activity
768 and oxygen consumption in *Gammarus fossarum*, *Asellus aquaticus* and *Niphargus*
769 *sphagnicolus*. *Freshwater Biology*, *51*(4), 686-694.
770 Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy
771 homeostasis as an integrative tool for assessing limits of environmental stress tolerance in
772 aquatic invertebrates. *Marine Environmental Research* 79, 1-15.
773 Sultan, K., Shazili, N. A., 2009. Distribution and geochemical baselines of major, minor
774 and trace elements in tropical topsoils of the Terengganu River basin, Malaysia. *Journal of*
775 *geochemical exploration*, *103*(2-3), 57-68.
776 Tai, P., Zhao, Q., Su, D., Li, P., Stagnitti, F., 2010. Biological toxicity of lanthanide
777 elements on algae. *Chemosphere*, *80*(9), 1031-1035.
778 Teixeira, M., Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Wrona, F.J., Soares,
779 A.M., Figueira, E., Freitas, R., 2017. Toxic effects of the antihistamine cetirizine in mussel
780 *Mytilus galloprovincialis*. *Water Research* 114, 316-326.
781 UNEP, PACE, ITU, ILO, UNIDO, UNU, et al. A New Circular Vision for Electronics Time
782 for a Global Reboot. 2019.
783 Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular
784 biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants.
785 *Ecotoxicology and environmental safety*, *64*(2), 178-189.
786 Vukov, O., Smith, D. S., McGeer, J. C., 2016. Acute dysprosium toxicity to Daphnia pulex
787 and Hyalella azteca and development of the biotic ligand approach. *Aquatic Toxicology*, *170*,
788 142-151.

Yuan M., Wang Y., Zhou B., Jian X., Dong W., Tang X., 2017. An integrated biomarker response index for the mussel *Mytilus edulis* based on laboratory exposure to anthracene and field transplantation experiments. C*hinese Journal of Oceanology and Limnology* 35, 1165-1178.

Zapp, P., Marx, J., Schreiber, A., Friedrich, B., Voßenkaul, D., 2018. Comparison of dysprosium production from different resources by life cycle assessment. *Resources, Conservation and Recycling*, *130*, 248-259.

FIGURE CAPTIONS

Figure 1. A: Superoxide dismutase activity (SOD); B: Catalase activity (CAT); and C: Glutathione peroxidase activity (GPx), in *Mytilus galloprovincialis* exposed to different Dysprosium concentrations (CTL-0, 2.5, 5.0, 10, 20 and 40 µg/L). Values are mean + standard deviation. Significant differences among concentrations are represented with different letters.

Figure 2. Glutathione S-transferases activity (GSTs), in *Mytilus galloprovincialis* exposed to different Dysprosium concentrations (CTL-0, 2.5, 5.0, 10, 20 and 40 µg/L). Values are mean + standard deviation. Significant differences among concentrations are represented with different letters.

Figure 3. Ratio between reduced (GSH) and oxidized (GSSG) glutathione (GSH/GSSG), in *Mytilus galloprovincialis* exposed to different Dysprosium concentrations (CTL-0, 2.5, 5.0, 10, 20 and 40 µg/L). Values are mean + standard deviation. Significant differences among concentrations are represented with different letters.

Figure 4. Lipid peroxidation levels (LPO); and B: Protein Carbonylation (PC), in *Mytilus galloprovincialis* exposed to different Dysprosium concentrations (CTL-0, 2.5, 5.0, 10, 20 and 40 µg/L). Values are mean + standard deviation. Significant differences among concentrations are represented with different letters.

Figure 5. A: Electron transport system activity (ETS), B: Glycogen content (GLY) and C: Total Protein content (PROT), in *Mytilus galloprovincialis* exposed to different Dysprosium concentrations (CTL-0, 2.5, 5.0, 10, 20 and 40 µg/L). Values are mean + standard deviation. Significant differences among concentrations are represented with different letters.

Figure 6. Centroids ordination diagram (PCO) based on Dy concentrations and biochemical parameters, measured in *Mytilus galloprovincialis* exposed to different Dy concentrations (CTL: control, C1: 2.5; C2: 5; C3: 10; C4: 20; C5: 40 µg L^{-1} of Dy). Pearson correlation vectors

are superimposed as supplementary variables, namely biochemical data ($r > 0.75$): Dy, ETS, GLY, PROT, SOD, GSTs, LPO, PC, GSH/GSSG.

Table 1- Dysprosium (Dy) concentrations in water (µg/L), collected immediately after spiking at the 1st, 2nd, 3rd and 4th weeks of exposure (mean values for the four weeks ± STDEV) and in mussel's tissues (µg/g dry weight) collected at the end of the experimental period (4th week) (mean values of 8 individuals per condition ± STDEV), from each condition (CTL-0, 2.5, 5.0, 10, 20, 40 µg/L of Dy). Different letters denote statistical significance among tested concentrations. Limit of quantification (LOQ) for water samples 10 ng/L; LOQ for tissue samples 0.00125 µg/g.

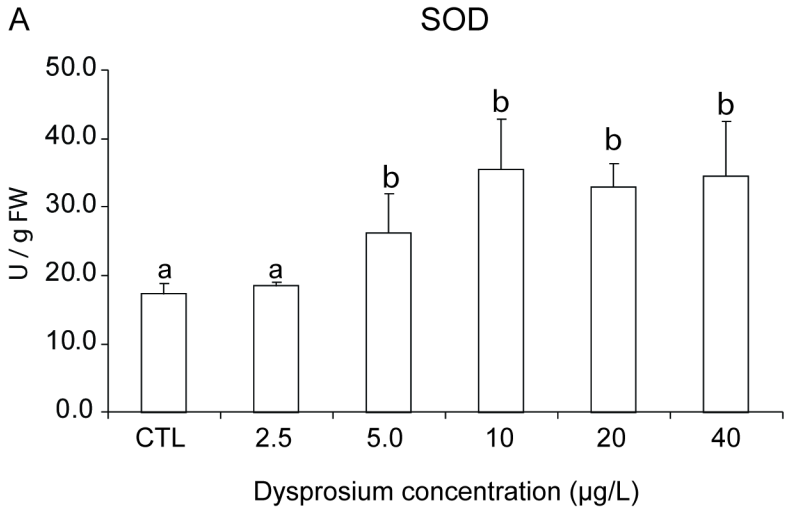
Dy concentrations	Seawater medium	Mussels tissues
	Weekly, after spiking	End of the 4th week
CTL	<LOQ	0.013±0.002[a]
2.5	2.6±0.18	0.036[b]
5.0	5.3±0.27	0.080±0.004[c]
10	10±1.8	0.147±0.001[d]
20	22±4.6	0.226±0.002[e]
40	44±7.0	0.484±0.009[f]

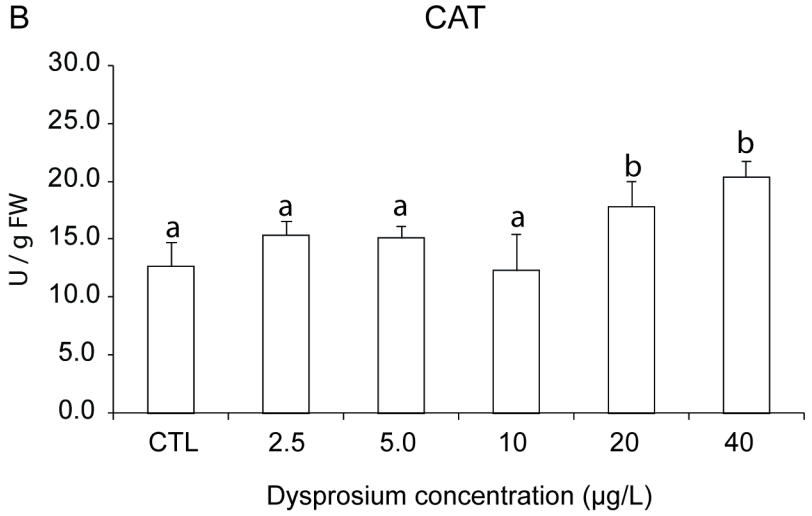
Table 2- p-values obtained through pairwise comparisons performed on biochemical results using PERMANOVA routine from the software PRIMER (PERMANOVA+add-on in PRIMER v6. Anderson et al., 2008).

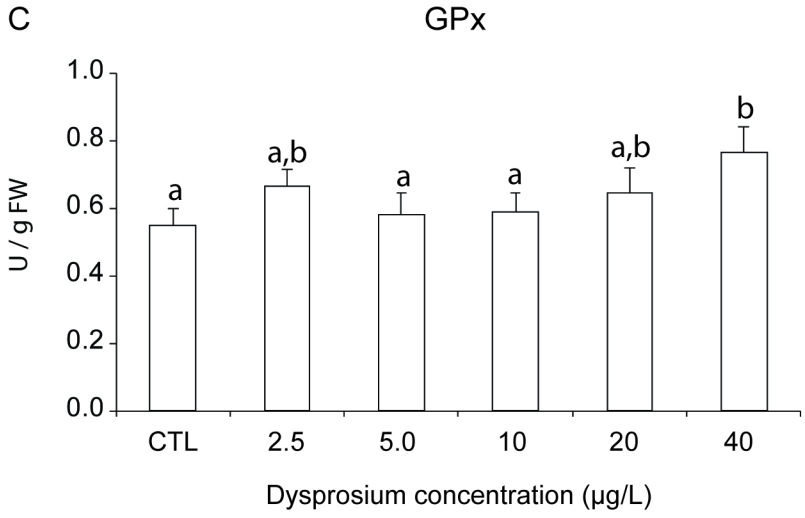
	SOD	CAT	GPx	GSTs	GSH/GSSG	LPO	PC	ETS	GLY	PROT
Main test	0.0496	0.0023	0.0581	0.0005	0.0001	0.0002	0.0023	0.3306	0.0082	0.2168
	Pairwise									
CTL vs 2.5	0.3789	0.9562	0.0814	0.235	**0.0001**	**0.0026**	**0.0187**		0.0834	
CTL vs 5.0	**0.0345**	0.9299	0.4525	0.2132	**0.0002**	**0.0035**	**0.0488**		**0.0285**	
CTL vs 10	**0.0252**	0.3879	0.2624	0.0902	**0.0002**	**0.023**	**0.008**		**0.0209**	
CTL vs 20	**0.0013**	**0.0063**	0.0994	**0.0022**	**0.0001**	**0.0077**	**0.0338**		**0.0369**	
CTL vs 40	**0.0079**	**0.019**	**0.0198**	**0.0006**	**0.0002**	**0.0013**	**0.0102**		**0.0016**	
2.5 vs 5.0	**0.0286**	0.8765	0.0699	0.9127	0.2654	0.4141	0.4931		0.9972	
2.5 vs 10	**0.0426**	0.4258	0.2498	0.1462	0.1502	0.5984	0.0883		0.6492	
2.5 vs 20	**0.0004**	**0.0025**	0.2712	**0.0201**	0.0895	**0.0023**	0.2534		0.1588	
2.5 vs 40	**0.0269**	**0.0228**	0.4814	**0.0119**	0.1129	**0.0158**	0.6932		0.4415	
5.0 vs 10	0.4207	0.3843	0.6752	0.1857	0.2893	0.4532	0.0962		0.6397	
5.0 vs 20	0.198	**0.0016**	0.4728	0.1271	**0.0467**	**0.0005**	0.1428		0.138	
5.0 vs 40	0.2083	**0.0155**	**0.0497**	0.1143	0.1172	**0.027**	0.2207		0.4174	
10 vs 20	0.8913	**0.0359**	0.8013	0.2559	0.1355	**0.0386**	0.0743		0.097	
10 vs 40	0.7579	**0.0008**	**0.0224**	0.3963	0.083	**0.001**	0.0639		0.9353	
20 vs 40	0.7438	0.3842	0.2256	0.4877	0.3451	**0.0317**	0.3408		0.0912	

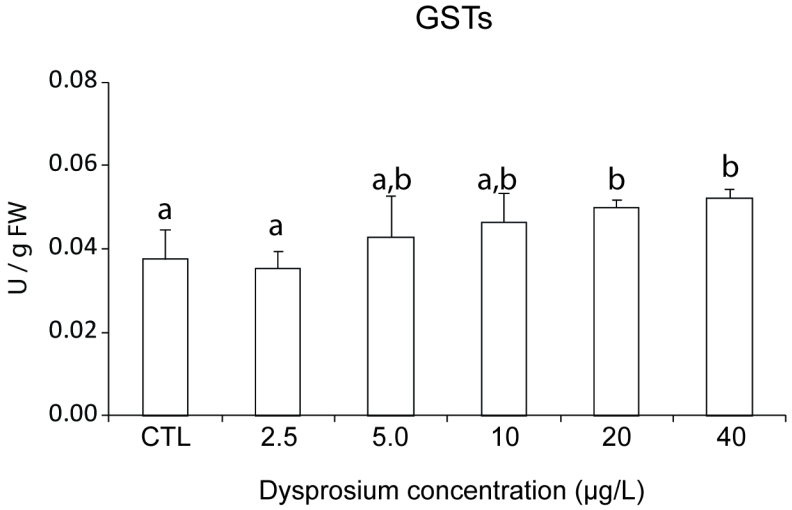
Table 3- Integrated Biomarker Response (IBR) obtained for each condition (CTL-0, 2.5, 5.0, 10, 20, 40 µg/L of Dy).

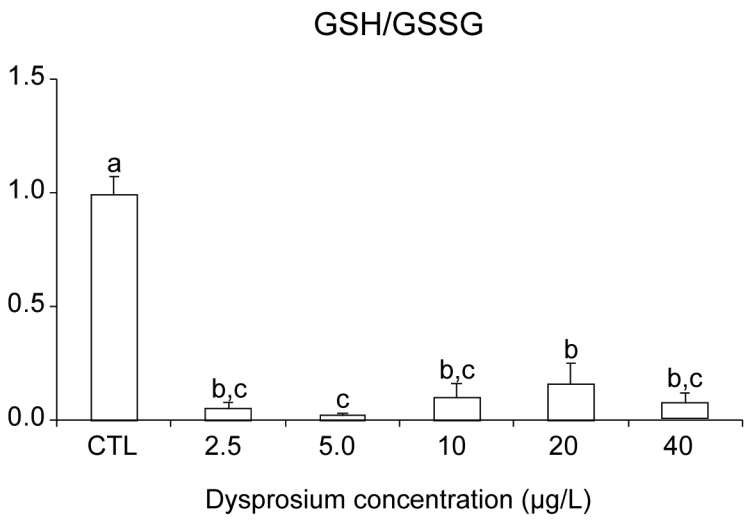
Dy exposure conditions µg/L	***IBR values***
CTL	-
2.5	1.1
5.0	0.22
10	2.1
20	1.9
40	3.4

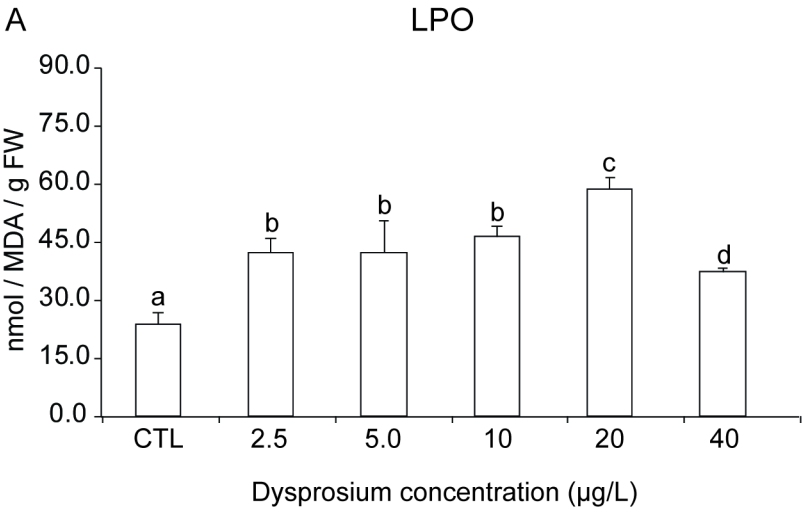

A
SOD
U / g FW
50.0
40.0
30.0
20.0
10.0
0.0
a
a
b
b
b
b
CTL
2.5
5.0
10
20
40
Dysprosium concentration (µg/L)

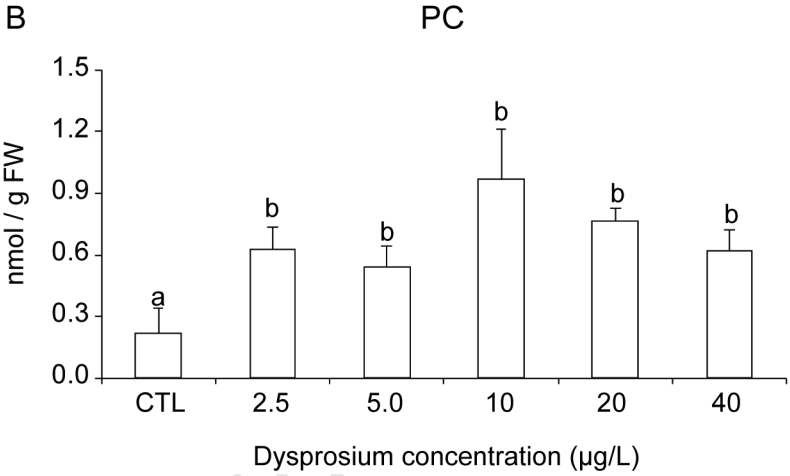

B
CAT
U / g FW
30.0
25.0
20.0
15.0
10.0
5.0
0.0
a
a
a
a
b
b
CTL
2.5
5.0
10
20
40
Dysprosium concentration (µg/L)

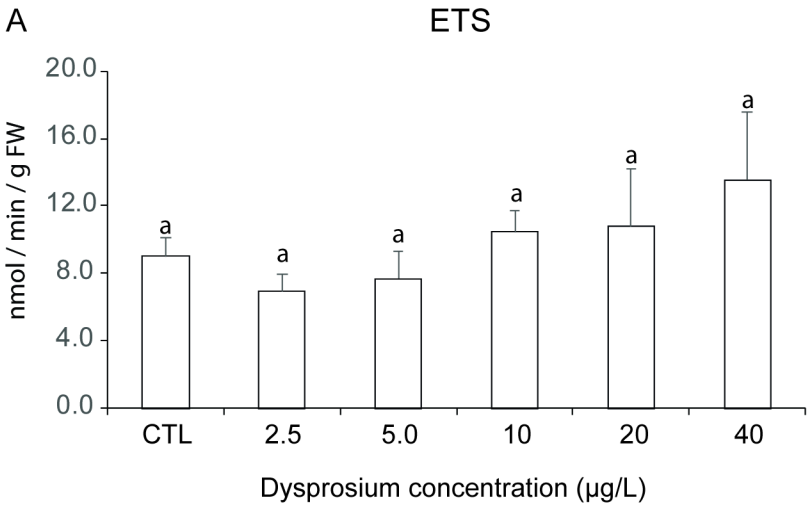

C
GPx
U / g FW
1.0
0.8
0.6
0.4
0.2
0.0
a
a,b
a
a
a,b
b
CTL
2.5
5.0
10
20
40
Dysprosium concentration (µg/L)

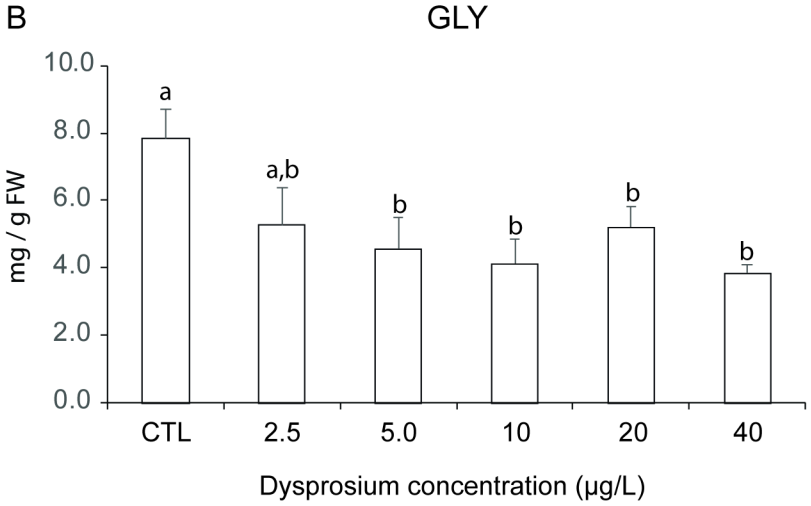


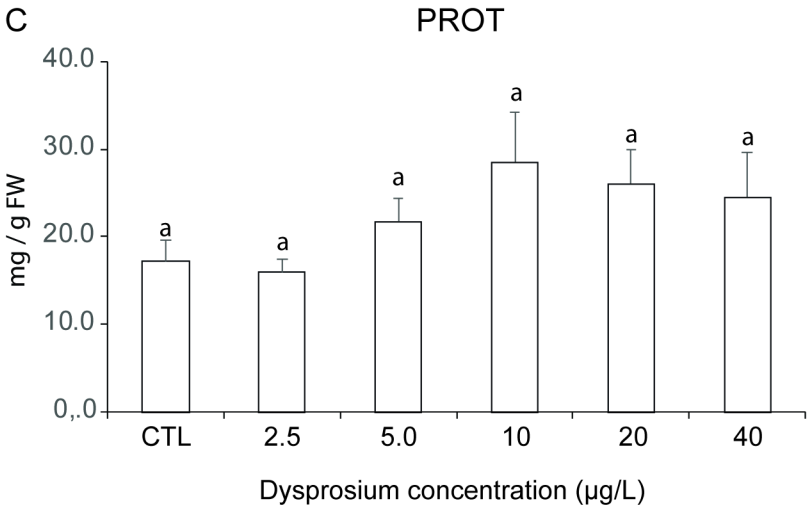


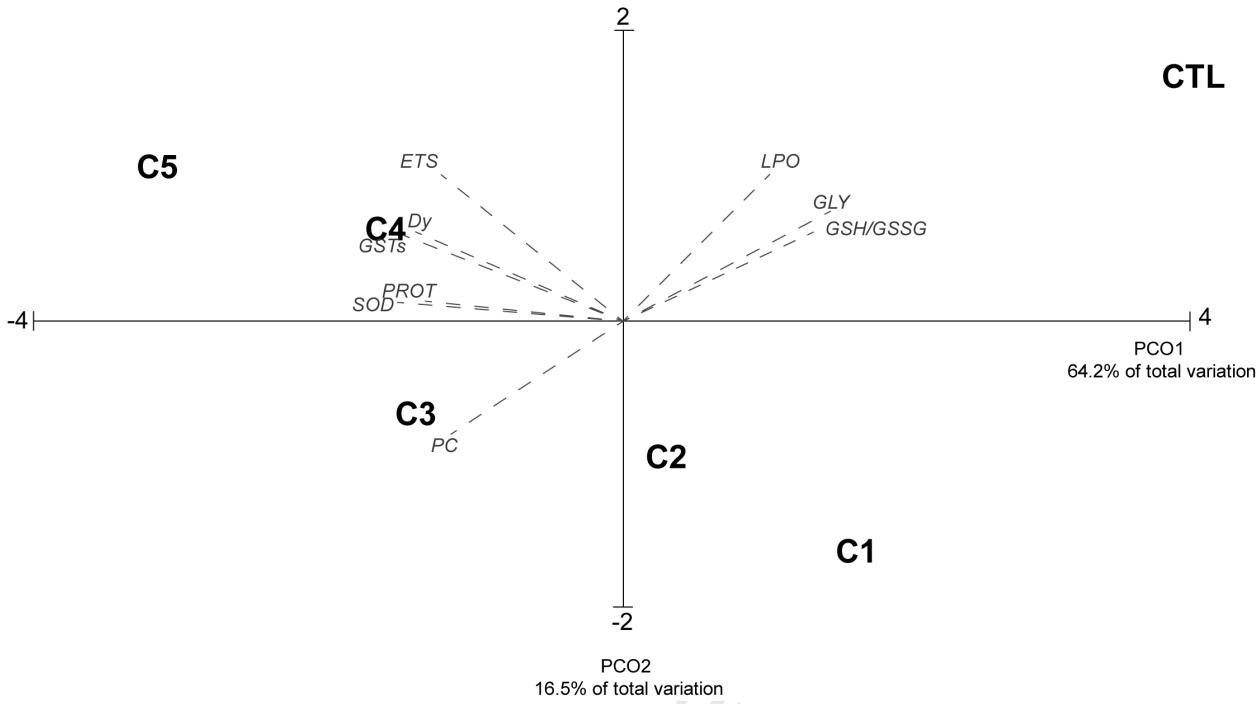

A
LPO
nmol / MDA / g FW
90.0
75.0
60.0
45.0
30.0
15.0
0.0
a
b
b
b
c
d
CTL
2.5
5.0
10
20
40
Dysprosium concentration (μg/L)


B
PC
nmol / g FW
1.5
1.2
0.9
0.6
0.3
0.0
a
b
b
b
b
b
CTL
2.5
5.0
10
20
40
Dysprosium concentration (μg/L)


A
ETS
nmol / min / g FW
20.0
16.0
12.0
8.0
4.0
0.0
a
a
a
a
a
a
CTL
2.5
5.0
10
20
40
Dysprosium concentration (µg/L)


B
GLY
mg / g FW
10.0
8.0
6.0
4.0
2.0
0.0
a
a,b
b
b
b
b
CTL
2.5
5.0
10
20
40
Dysprosium concentration (µg/L)


C
PROT
mg / g FW
40.0
30.0
20.0
10.0
0,.0
a
a
a
a
a
a
CTL
2.5
5.0
10
20
40
Dysprosium concentration (µg/L)


2
CTL
C5
ETS
LPO
GLY
GSH/GSSG
C4
Dy
GSTs
PROT
SOD
-4
4
PCO1
64.2% of total variation
C3
PC
C2
C1
-2
PCO2
16.5% of total variation

- *Mytilus galloprovincialis* bioaccumulated Dysprosium
- Mussels exposed to Dy decreased their metabolic capacity
- Contaminated mussels increased antioxidant and biotransformation defences
- Lipid peroxidation occurred in contaminated mussels
- Oxidative stress was observed in mussels exposed to Dy

Rosa Freitas and Eduarda Pereira are supervisors of the students that co-authored this ms (Silvana Costa, Celso Cardoso, Tiago Morais, Pedro Moleiro, André F. D. Lima, Márcio Soares, Samuel Figueiredo, Tiago L. Águeda, Pedro Rocha, Gonçalo Amador). Students did the exposure assay (for 28 days under controlled conditions), performed all methods and analyses for Nd quantification and biomarkers determination.

Rosa Freitas and Eduarda Pereira gave the idea of this study to the students that accepted this challenge and performed all the analyses during their last year of their bachelor degree. Eduarda Pereira is the responsible for the laboratory where Nd quantification was done. Rosa Freitas and Amadeu Soares are the responsible persons for the labs where biomarkers were determined. Eduarda Pereira, Rosa Freitas and Amadeu Soares funded this study.

Conflict of Interest

The Authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affi liations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.