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Physiological and biochemical impacts induced by mercury pollution and seawater acidification in *Hediste diversicolor*

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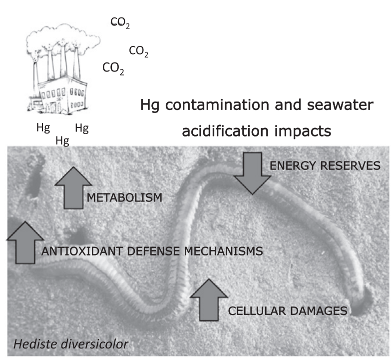
HIGHLIGHTS

- Hg contaminated polychaetes (pH control and pH 7.5) increased their metabolism.
- *H. diversicolor* used GLY and PROT when under stressful conditions.
- Polychaetes activated defence mechanisms when exposed stressful conditions.
- *H. diversicolor* showed oxidative stress when exposed to Hg, low pH and pH 7.5 + Hg.
- The combination of stressors did not induce higher impacts than single stressors.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study evaluated the impacts of predicted seawater acidification and Hg pollution, when stressors were acting alone and in combination, on the polychaete *Hediste diversicolor*. Polychaetes were exposed during 28 days to low pH (7.5), Hg (5 μg/L) and pH 7.5 + Hg, and physiological alterations (respiration rate), biochemical markers related to metabolic potential (glycogen and protein content, electron transport system activity) and oxidative status (activity of antioxidant and biotransformation enzymes, lipid peroxidation) were evaluated. The results obtained clearly showed that polychaetes were sensitive to low pH and Hg contamination, both acting alone or in combination. Organisms used their energy reserves under stressful conditions, which decreased by up to half of the control content, probably to fuel defence mechanisms. Our findings further demonstrated that polychaetes exposed to these stressors presented increased antioxidant defence mechanisms (3 fold compared to control). However, organisms were not able to prevent cellular damage, especially noticed at Hg exposure and pH 7.5. Overall, although all the tested conditions induced oxidative stress in *Hediste diversicolor*, the combined effect of seawater acidification and Hg contamination did not induce higher impacts in polychaetes than single stressor exposures. These findings may indicate that predicted climate change scenarios may not increase *Hediste diversicolor* sensitivity towards Hg and may not significantly change the toxicity of this contaminant to this polychaete species.

1. Introduction

Pollution levels in aquatic systems and the impacts induced in inhabiting organisms have been topics of research for a long time. In

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these ecosystems, and especially in estuaries, metals are among the most common pollutants, with several studies demonstrating high concentrations in sediment, water and organisms and their deleterious effects at diverse biological levels. Mercury (Hg) is one of the most toxic elements due to its high persistence, potential for bioaccumulation, and toxicity (Ahmad et al., 2012; Driscoll et al., 2013; Rahman et al., 2014; Rice et al., 2014; Taylor et al., 2012), and is listed as priority hazardous substance (ATSDR, 2015). Inputs of Hg to ecosystems are expected to increase substantially in the future with projected impacts on aquatic organisms, including species with economic relevance. As an example, seawater Hg concentration trajectories in areas such as the North Pacific Ocean that supply large quantities of marine fish to the global seafood market are projected to increase by >50% by 2050 (Sunderland and Selin, 2013; Sunderland et al., 2009). Nevertheless, up to now, numerous studies demonstrated that even at low concentrations in the environment, Hg may be accumulated by aquatic organisms, namely benthic invertebrates, causing negative effects on the organisms oxidative status, energy metabolism, physiological mechanisms, and reproduction (Ahmad et al., 2012; Boening, 2000; Dean, 2008; Freitas et al., 2012; Ramos-Gómez et al., 2011; Spada et al., 2012; Taylor et al., 2012). Under laboratory conditions, recent studies also demonstrated that a wide range of Hg concentrations can cause negative impacts on invertebrates, from physiological to biochemical impairments (Brock, 1993; Liu et al., 2011; Muhaya et al., 1997; Pytharopoulou et al., 2013; Singaram et al., 2013; Velez et al., 2016a, 2016b; Verlecar et al., 2007).

Nowadays anthropogenic pollution, including by Hg, do not occur isolated. Due to human activities, the atmospheric CO_2 concentration is increasing, and is predicted to reach between 500 and 1000 μatm by the end of this century (Caldeira and Wickett, 2003, 2005; IPCC, 2013; Orr et al., 2005; Raven et al., 2005). This increase has led to a decrease in both pH and the availability of carbonate ions in seawater (defined as ocean acidification, OA) and reductions from 0.3 to 0.5 pH units are predicted to occur up to year 2100 (Caldeira and Wickett, 2003, 2005; Feely et al., 2004; IPCC, 2013; Orr et al., 2005; Raven et al., 2005; Solomon et al., 2007), with greater impacts in coastal systems, such as estuaries (Tomanek et al., 2011; Melzner et al., 2013). Recent studies have already demonstrated the negative impacts of predicted seawater pH decrease including alterations in survival, regenerative capacity, filtration, respiration and metabolic rates, osmoregulation and oxidative status in several invertebrate species, most of them calcifying organisms but also in non-calcifying organisms such as polychaetes (e.g., Basallote et al., 2012; Calosi et al., 2013; Campbell et al., 2014; Freitas et al., 2016a, 2016b; Garrard et al., 2013; Gazeau et al., 2013; Lane et al., 2013; Navarro et al., 2013; Matoo et al., 2013; Matozzo et al., 2013; Michaelidis et al., 2005; Pires et al., 2015; Ricevuto et al., 2015a, 2015b).

Although several studies demonstrated that climate change may affect invertebrates, less is known on the influence of climate change related factors on the bioavailability and toxicity of pollutants (Macdonald et al., 2005; Noyes et al., 2009) and on the sensitivity of invertebrates when exposed to pollutants. Regarding Hg it is known that in sediment and water, inorganic Hg can be converted into toxic organic forms mainly by microbial mediated processes but also by abiotic factors such as pH, salinity, and temperature, which is favourable in acidic pH, low salinity, high dissolved organic carbon concentrations as well as high temperatures (Celo et al., 2006). It has been demonstrated that toxic free-ion concentration of metals such as copper (Cu) may increase by as much as 115% in coastal waters in the next 100 years due to decreased pH (Pascal et al., 2010; Richards et al., 2011), whereas the free-ion concentration of other metals including cadmium (Cd) may decrease or be unaffected (Lacoue-Labarthe et al., 2009, 2011; Pascal et al., 2010). Increased accumulation of various trace elements has been observed in the clam *R. philippinarum* (López et al., 2010; Rodríguez-Romero et al., 2014a) and in the polychaete *Hediste diversicolor* (Rodríguez-Romero et al., 2014b) due to low pH that increased the bioavailability of trace metals that were bound to sediment. Regarding the impacts of climate change on pollutants toxicity Pascal et al. (2010) showed increased toxicity of Cu to the copepod *Amphiascoides atopus* under conditions of elevated CO_2. Roberts et al. (2012) showed that low pH can affect sediment toxicity and bioavailability of metals. It was also demonstrated that short term acute hypercapnia indirectly modulated the bioavailability of trace metals in a metal-specific way (Ivanina and Sokolova, 2013) whereas chronic exposure to some metals (e.g. Cu) can alter the susceptibility to oxidative stress conditions (Geracitano et al., 2004) and the sensitivity to OA in some species, as seen in polychaete *Pomatoceros lamarckii* larvae (Lewis et al., 2013). Campbell et al. (2014) demonstrated that *Arenicola marina* larval survival decreased by 24% when exposed to both OA and Cu combined, compared to single OA or Cu exposures. These authors also revealed that sperm motility was negatively affected by both OA and Cu singularly, with additive toxicity effects of the two stressors when combined. Recently Nardi et al. (2017) demonstrated that while temperature and Cd contamination enhanced glutathione-dependent antioxidant protection and capability to neutralize peroxyl radicals, the co-exposure to acidification and Cd increased the accumulation of lipid peroxidation products, enhanced metal genotoxicity and the onset of permanent DNA damage in haemocytes of *Mytilus galloprovincialis*.

Although scarce information is available on the impacts of climate change related factors on polychaetes (Batten and Bamber, 1996; Basallote et al., 2012; Campbell et al., 2014; Freitas et al., 2016a, 2016b; Lane et al., 2013; Lewis and Watson, 2012; Ricevuto et al., 2016), this group of organisms is commonly used as bioindicator species of pollution in aquatic systems, with known effects on their biochemical and physiological performance, including changes on polychaetes oxidative status, metallothionein content, energy reserves, osmoregulation and regenerative capacity (e.g., Bouraoui et al., 2015; Coppola et al., 2016; Díaz-Jaramillo et al., 2013; Pini et al., 2015; Pires et al., 2016a, 2016b, 2016c; Tian et al., 2014). Among polychaetes *Hediste diversicolor* (O.F. Müller, 1776) is one of the most commonly used species as bioindicator of environmental pollution (Bouraoui et al., 2009, 2015; Buffet et al., 2014a, 2014b; Burlinson and Lawrence, 2007; Catalano et al., 2012; Cong et al., 2014; Idardare et al., 2008; Pook et al., 2009; Maranho et al., 2014; Thit et al., 2015). This species already showed a high physiological tolerance to variation of environmental parameters such as temperature, salinity and dissolved oxygen (Abele-Oeschger et al., 1994; Ait Alla et al., 2006; Bartels-Hardege and Zeeck, 1990; Neuhoff, 1979; Scaps and Borot, 2000) but to our knowledge, scarce information is available on the effects of seawater pH decrease on this species (Freitas et al., 2016a) and no information is available on the combined effects of metals and pH decrease, namely on biochemical and physiological performance.

Since we will face increased uncertainties in chemical risk assessment under climate change predictions, the present study aimed to acquire knowledge on the impacts caused by the combination of environmental factors (such as pollution and seawater acidification), resembling present and future environmental scenarios. Thus, to understand how Hg contaminated polychaetes will act under seawater acidification, we compared the physiological and biochemical alterations induced in *H. diversicolor* by Hg contamination under current and predicted acidification scenarios, exposing *H. diversicolor* specimens to Hg (5 μg/L), low pH (7.5) and the combination of both stressors (pH 7.5 + Hg) during 28 days. The impacts induced were measured in terms of organisms respiration rate, metabolic and energy related parameters, and oxidative stress markers, as previous studies demonstrated impacts of both stressors on invertebrates oxidative stress and metabolic capacity.

2. Materials and methods

2.1. Study organisms and experimental setup

Hediste diversicolor specimens were collected in the Mira channel, a low contaminated area located within the Ria de Aveiro lagoon