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The effects of arsenic and seawater acidification on antioxidant and biomineralization responses in two closely related *Crassostrea* species

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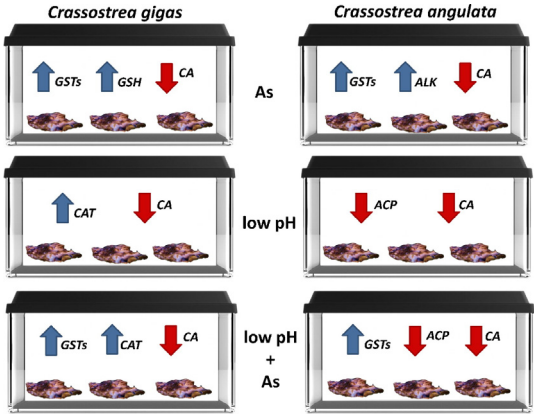
HIGHLIGHTS

- Arsenic and low pH induced oxidative stress in *Crassostrea gigas* and *C. angulata*.
- Arsenic exposure induced greater biochemical alterations than low pH in both species.
- *C. gigas* showed higher response capacity towards tested conditions than *C. angulata*.
- Biomineralization enzyme CA activity decreased in oysters subjected to low pH.
- GST activity significantly increased in oysters exposed to arsenic and pH + As.

GRAPHICAL ABSTRACT



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ABSTRACT

Ocean acidification processes are major threats to marine calcifying organisms, mostly affecting biomineralization related processes. Abiotic stressors acting on marine systems do not act alone, rather in a combination of multiple stressors, especially in coastal habitats such as estuaries, where anthropogenic and environmental pressures are high. Arsenic (As) is a widely distributed contaminant worldwide and its toxicity has been studied on a variety of organisms. However, the effect of low pH on the toxicity of As on marine organisms is unknown. Here, we studied the combined effects of ocean acidification and As exposure on two closely related oyster species (*Crassostrea angulata* and *Crassostrea gigas*), by use of a biochemical approach. Oxidative stress related parameters were studied along with the assessment of biomineralization enzymes activity after 28 days of exposure. Results showed that both species were sensitive to all tested conditions (low pH, As and pH + As), showing enhancement of antioxidant and biotransformation defenses and impairment of biomineralization processes. Glutathione S-transferases (GSTs) activity were significantly higher in oysters exposed to As, showing activation of detoxification mechanisms, and a lower GSTs activity was observed in low pH + As condition, indicating an impact on the oysters capacity to detoxify As in a low pH scenario. Carbonic anhydrase (CA) activity was significantly lower in all tested conditions, showing to be affected by both As and low pH, whereas the combined effect of low pH + As was not different from the effect of low pH alone. Multivariate analysis of biochemical data allowed for the comparison of both species performance, showing a clear distinction of response in both species. *C. gigas* presented overall higher enzymatic activity (GSTs; superoxide dismutase; catalase; CA and acid

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phosphatase) and higher cytosolic GSH content in As exposed oysters than *C. angulata*. Results obtained indicate a higher tolerance capacity of the Pacific oyster *C. gigas* towards the tested conditions.

1. Introduction

Since preindustrial time, atmospheric carbon dioxide (CO_2) concentrations have increased from 280 ppm (parts per million) to 390.5 ppm in 2011 (Ciais et al., 2013) mainly due to human activities such as fossil fuel combustion and deforestation (Forster et al., 2007). A significant amount of anthropogenic CO_2 released into the atmosphere is uptaken by the oceans, causing changes in seawater chemistry and pH decrease, the latter known as ocean acidification (Caldeira and Wickett, 2003; Sabine and Feely, 2007). When CO_2 dissolves in seawater the concentrations of carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-) and hydrogen ion (H^+) increase, whereas the concentration of carbonate ion (CO_3^{2-}) decreases (Millero and Pierrot, 2002). The decrease in CO_3^{2-} ion concentrations reduce the saturation state (Ω) of calcium carbonate ($CaCO_3$), affecting the capacity of calcifying organisms to produce their shells and skeletons (Feely et al., 2009), and thus organisms such as corals, molluscs, echinoderms and crustaceans are expected to be vulnerable to low CO_3^{2-} availability (Fabry et al., 2008). The worlds oceans surface waters have already experienced a 0.1 pH units decrease since the beginning of the industrial revolution, and a further 0.3 to 0.5 pH units fall is predicted to occur up to year 2100 (Caldeira and Wickett, 2003; IPCC, 2013; Orr et al., 2005; Raven et al., 2005). Due to these predictions, an increasing body of research has been published on the effects of seawater acidification on a diversity of marine taxa, overall showing alterations in calcification, shell and skeleton dissolution rates, metabolism and larvae survival (Andersson et al., 2011; Gazeau et al., 2013). Most of these studies however, have focused on fully marine environmental pH conditions (Havenhand and Schlegel, 2009), while less attention has been given to estuarine ecosystems (e.g.: Ivanina et al., 2014). However, estuaries are more susceptible to environmental change including CO_2 enrichment, since they are naturally more subjected to higher CO_2 concentrations and variation (Tomanek et al., 2011). Estuaries pH can decrease lower than 7.0, where seasonal and diurnal pH fluctuation cycles are mainly due to respiration of resident biota, lower buffering capacity, eutrophication, land runoff and upwelling of CO_2 enriched waters (Lockwood, 1976; Rabalais et al., 2009; Ringwood and Keppler, 2002). Therefore, expected atmospheric CO_2 increase may further enhance the impacts of low pH in these aquatic ecosystems (Miller et al., 2009).

In addition to the natural environmental stress associated to estuaries (e.g.: salinity, temperature, O_2 and pH fluctuations), these ecosystems are also characterized by anthropogenic pollution, including inorganic contaminants such as metals and metalloids (Riba et al., 2004; Schropp et al., 1990). Arsenic (As), is one of the most widely distributed pollutants worldwide, and is highly mobile in nature (Mandal and Suzuki, 2002). Anthropogenic activities (e.g.: mining, agricultural pesticides, coal burning) have been increasing environmental As concentrations worldwide (Leermakers et al., 2006; Pacyna et al., 1995), raising public concern due to its high toxicity and carcinogenic properties (Aposhian et al., 2003). In the marine environment, in both seawater and sediment (Fattorini et al., 2006; Neff, 1997), As occurs mainly in its inorganic and more toxic forms (arsenite and arsenate) (Fattorini and Regoli, 2004). Marine organisms are capable of biotransforming inorganic arsenicals into less toxic organic forms, and to accumulate As (Zhang et al., 2015, 2012a). Arsenic induced toxicity has generally been attributed to alterations in cellular homeostatic imbalance between prooxidant and antioxidant status, leading to oxidative stress (Samuel et al., 2005). However, impacts of other stressors, such as seawater acidification, may alter the toxicity of As and at the same time change the sensitivity of organisms to this element. Nevertheless, to our knowledge no studies are published on the combined effects of high pCO_2 and As exposure. Even so, other researchers have recently studied the combined effect of ocean acidification and other pollutants, such as metals (Ivanina et al., 2014; Ivanina and Sokolova, 2013), and even pharmaceuticals (Freitas et al., 2015). These studies have generally demonstrated the enhancement of negative effects caused by common pollutants, when combined with low pH conditions.

Given this context, the present study assessed the effect of As acting alone and in combination with high pCO_2 concentrations, in two closely related oyster species: the Portuguese oyster *Crassostrea angulata* (Lamarck, 1819) and the Pacific oyster *Crassostrea gigas* (Thunberg, 1973). These species are currently considered as distinct taxa (www.marinespecies.org), and despite closely related, present differences in growth rate, survival, reproduction, and ecophysiology (Goulletquer, 1999; Haure et al., 2003). Given the socio economic importance of oysters (FAO, 2012), their wide spatial distribution and environmental relevance, it is important to evaluate how these species will respond towards a changing environment.

To evaluate the effects induced by high pCO_2 and As a biochemical approach was used, including oxidative stress related parameters as well as enzymes related to biomineralization processes. The use of enzymatic and non-enzymatic biomarkers as indicators of organisms response to pollutants has been extensively used, namely through the assessment of oxidative stress (Monserrat et al., 2007). Recent studies have also assessed the oxidative stress response of marine invertebrates exposed to low pH (high pCO_2) conditions (Freitas et al., 2016; Matozzo et al., 2013).

2. Materials and methods

2.1. Study organisms and experimental setup

Oysters were collected in November 2014, with an estimated age of seven months. Year recruits were chosen for this study, to avoid gonadal maturation. Specimens were collected in two separate locations, since pristine *C. angulata* populations are restricted to few estuarine systems in the Southern Iberian Peninsula. *C. gigas* were obtained from an aquaculture exploration in the Ria de Aveiro estuary, NW Portugal (40°36′N, 8°44′W); while *C. angulata* specimens were collected in the Sado estuary, SW Portugal, from natural oyster banks (38°22′N, 8°31′W).

After collection oysters were transferred to the laboratory, and acclimated for one month prior to exposure. Species were kept separately and distributed in 110 L tanks, at a stocking density of 0.4 ind·L^{-1}, with constant filtration and providing water circulation over 1200 L·h^{-1} in artificial seawater (Tropic Marin Reef Mix) prepared at salinity 29 ± 1, and temperature maintained at 20 ± 1 °C.

After the acclimation period, specimens were randomly distributed in 20 L aquaria, stocking density of 3 individuals per tank, maintaining species separation, salinity and temperature conditions as during the acclimation period (salinity 29 ± 1, temp. 20 ± 1 °C). Water flow and filtration for each tank was obtained by individual power filter units, giving a flow rate of 400 L·h^{-1}. Three exposure conditions were tested plus control: Arsenic (As), low pH (pH), low pH plus Arsenic (pH + As), and control (CTL). Each condition was three fold replicated.

Arsenic was dosed at a sublethal concentration of 4 mg·L^{-1} (Zhang et al., 2015), using Sodium Arsenate (Sigma-Aldrich), which enabled oysters to accumulate between 3.25 and 3.93 μg·g^{-1} FW. These concentrations are in the range of As reported in bivalves from the environment (e.g.: 9.0 μg·g^{-1} dry weight (DW) in *C. gigas* from China (Liu et al., 2006), 26.7 μg·g^{-1} (DW) in *C. gigas* from France (Kohlmeyer et al., 2002) and 13.9 μg.·L^{-1} in clams *Ruditapes philippinarum*