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Sex differences in oxidative stress responses of tropical topshells (*Trochus histrio*) to increased temperature and high pCO_2

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ABSTRACT

Given scarcity of knowledge on gender ecophysiological responses of tropical marine organisms to global climate change, the major aim of this research was to investigate potential sex differences in oxidative status of topshell *Trochus histrio*, after a combined exposure to increased temperature and pCO_2. Lipid peroxidation, heat-shock response and antioxidant enzymatic activities were evaluated. Lipid peroxidation varied differently between sexes, with males undergoing cellular damage under high pCO_2, which was elevated temperature-counteracted. Heat shock response was thermo- and sex-regulated, with males exhibiting significantly higher heat shock proteins production than females. Catalase activity increased with temperature and was exacerbated in combination with hypercapnia, being highest in females, while glutathione S-transferases activity peaked in males. These results clearly support the existence of distinct physiological strategies to cope oxidative stress between sexes, apparently more efficient in females, and also reinforce for the need of encompassing sex as meaningful variable in future biomarker studies.

1. Introduction

The increase in atmospheric carbon dioxide (CO_2) levels has been anticipated as the primary cause of ocean acidification, which is now considered one of the most pervasive human impacts on global marine biodiversity. The absorption of increasingly higher CO_2 concentrations into the ocean translates into a decline in the mean ocean pH, predicted to vary between 0.13 and 0.42 units by the end of the century (IPCC, 2014). In parallel with this phenomenon a substantial increase in the global average temperature (0.3–4.8 °C) is predicted to occur until 2100 (IPCC, 2014). Ocean warming and acidification have severe impacts for calcifying marine organisms, particularly molluscs, the major producers of calcium carbonate. Recent meta-analyses have identified shelled molluscs as one of the most vulnerable invertebrate taxa under changing ocean conditions (Byrne and Przeslawski, 2013; Kroeker et al., 2013; Wittmann and Pörtner, 2013), thereby becoming prime models for climate change experimentation (Parker et al., 2013).

Climate and toxic-related environmental changes can often disrupt metabolic homeostasis of the organisms. Such disruption can occur through direct mechanisms including temperature effects on enzyme properties, protein conformation, and lipid fluidity of biological membranes, as well as through indirect pathways, such as the overproduction of reactive oxygen species [ROS; e.g. superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-)] (Santoro and Thiele, 1997; Pannunzio and Storey, 1998). These radicals can damage DNA, proteins and lipids and thereby endanger cellular and organism fitness and functions, compelling them to invest more energy into cell repair (Pöhlmann et al., 2011). Peroxidation, the most common cellular damage caused by oxidative stress, is the reaction of ROS with lipids, constituents of cell membranes (Lesser, 2011), resulting in malondialdehyde (MDA) production, among other reactive end-products (Uchiyama and Mihara, 1978; Pannunzio and Storey, 1998; Lopes et al., 2013). Aerobic organisms have developed cytoprotective mechanisms including antioxidant enzymes to cope with hostile environmental conditions (Lopes et al., 2013; Troschinski et al., 2014). These biomarkers have been widely used in several studies proving to be valuable tools to understand biochemical responses of the organisms to both environmental contamination by anthropogenic pollutants (Sampaio et al., 2018) or climate related-changes (Lopes et al., 2013; Matoo et al., 2013; Rosa et al., 2014) and ultimately to evaluate ecosystems' health. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are critically important to minimize detrimental effects caused by ROS produced in cells under physiological strain (Abele and Puntarulo, 2004; Lopes et al., 2013). Glutathione S-

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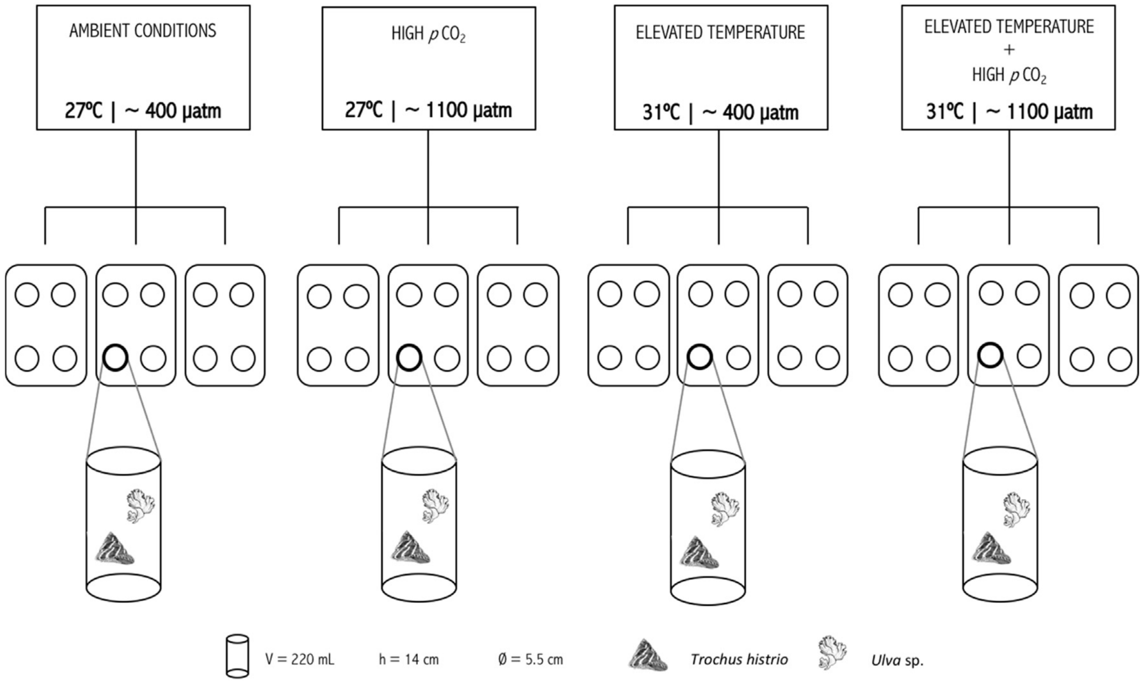

Fig. 1. Schematics of experimental design. Main treatments: ambient condition, high pCO_2, elevated temperature, high temperature and pCO_2.

transferases (GSTs) play an essential role in the transformation of xenobiotics into other conjugates as part of a detoxification pathway (Lesser, 2011; Lopes et al., 2013).

Moreover, marine organisms also produce chaperones (heat shock proteins, HSP), in order to stabilize and restore denatured proteins and thereby preventing formation of cytotoxic aggregates (Hartl, 1996; Fink, 1999). The thresholds of heat shock response in marine organisms are correlated with habitat temperature and the stress levels at which the organisms are usually exposed to (Feder and Hofmann, 1999). Considering that tropical species already live closer to their thermal tolerance limit (Rosa et al., 2014), the latest official reports (IPCC, 2014) highlight for their high vulnerability facing this rate of ongoing changes.

Topshells or banded *Trochus* spp. snails live on intertidal and shallow subtidal in the tropical Indo-West Pacific coral reefs (Lee and Lynch, 1997). They are nocturnal grazing herbivores and detritivores (Jolivet et al., 2015) and are among the most economically valuable marine snails in the tropical Pacific, constituting a highly consumed traditional food and an important leading export item to Asia and Europe as their aragonite shells are primary raw material for mother-of-pearl buttons. Each year, 3000–6000 tons of *Trochus* spp. are estimated to be harvested for subsistence and commercial purposes (Shokita et al., 1991; Ramakrishna and Sivaperuman, 2010). Nevertheless, their ecophysiology remains poorly understood and in the face of upcoming global changes, it becomes crucial to understand how increasing ocean temperature and high pCO_2 may or may not affect the biochemistry of *Trochus histrio*.

Whilst the ecophysiological impacts of medium and long-term changes in temperature and oceanic uptake of atmospheric CO_2 have been increasingly studied, the gender ecophysiological effects on oxidative status of tropical marine organisms have received much less attention. Some studies have demonstrated that response to thermal shifts is influenced by sex and commonly attributed to differences in the physiology and energy allocations between males and females (Madeira et al., 2012; Bedulina et al., 2017). Taking this evidence into consideration, the main goal of the present work was to evaluate, for the first time, the short-term effects of combined thermal and hypercapnia stress on the biochemical responses of different *T. histrio* genders. Can different sexes have distinct strategies to cope with the same climate stressors? This is the main question addressed along the manuscript. For that purpose, we performed a 15-day experiment to investigate potential sex differences in lipid peroxidation, heat shock response, and antioxidant enzymes (e.g. CAT and GSTs) machinery in economically relevant topshell *T. histrio* under a realistic scenario of increased temperature ($\Delta T = 4\,°C$) and high pCO_2 ($\Delta pCO_2 \approx 700$ µatm).

2. Materials and methods

2.1. Organism collection and laboratory acclimation

Topshells (*T. histrio*) were collected during low tide by local fishermen from the Indo-West Pacific region, near Bali (Indonesia) coastline, during summer (July 2015). The transport to aquaculture facilities of Laboratório Marítimo da Guia (Cascais, Portugal) was ensured by the Tropical Marine Centre UK, a marine aquarium wholesaler recognized for its efforts on the sustainable fishing of reef organisms and promotion of animal welfare. Topshells were 1-week laboratory acclimated under seawater conditions mimicking those at collection site: salinity = 35 ± 0.93 (V2 refractometer, TMC Iberia, Portugal); water temperature = 27 ± 0.81 °C (TFX 430 Precision Thermometer, WTW GmbH, Germany) and pH = 8.0 ± 0.2 / pCO_2 ~ 400 µatm (SG8 – SevenGo pro™ pH/Ion meter, Mettler-Toledo International Inc., Switzerland). During initial acclimation to laboratory conditions, topshells were randomly placed in twelve 50 L tanks, each part of individual recirculating aquaculture systems (RAS) with mechanical and biological filtration (bio-balls and skimmers), as well as additional UV disinfection. During laboratory acclimation and exposure to climate change conditions, topshells were fed ad libitum with green algae *Ulva* sp. from the same site of animals' collection.

After the acclimation period, temperature and pCO_2 were gradually regulated to mimic scenarios of ocean warming ($\Delta T = +\ 4\,°C$) and acidification ($\Delta pH = -0.4$ units, i.e. $\Delta pCO_2 \sim +700$ µatm) predicted for the end of the century (IPCC, 2014), in a full factorial design. In more detail, and before starting the 15 day-exposure period, the pH, as a proxy of pCO_2, was slowly decreased, at a rate of 0.1 units per day until reaching the desired pH (from 8.0 to 7.6). In parallel, temperature