Effects of seawater temperature increase on economically relevant native and introduced clam species

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ARTICLE INFO

ABSTRACT

As a consequence of climate change, global warming is expected to increase during the 21st century. Taking this into account, the impact of rising temperatures on the native *Ruditapes decussatus* and introduced *R. philippinarum* bivalve species was assessed, through biochemical and mRNA transcription analyses. Our findings showed that at 21 °C the electron transport system and antioxidant enzyme activity, as well as the expression of *Hsp70* gene were induced in *R. decussatus* when compared with 17 °C. On the other hand, at 25 °C results suggested that *R. decussatus* closed their valves during short periods, as a behavioral strategy, down-regulating the expression of genes associated with mitochondrial metabolism (*Cox-1* and *16S*) and chaperone function (*Hsp70*) compared with organisms at 17 °C. In addition, the introduced species (*R. philippinarum*) increased the electron transport system and antioxidant activities, as well as gene expression of antioxidant enzymes and molecular chaperone (*Hsp70*) at 21 °C. However, antioxidant mechanisms were not enough to prevent lipid membrane damages at 21 °C. At 25 °C *R. philippinarum* presented increased electron transport system and antioxidant activity, as well as the expression of genes associated with apoptosis regulation and molecular chaperone. Overall, the present findings indicate that in a global warming scenario both species are able to induce different mechanisms to mitigate the impacts of temperature increase.

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1. Introduction

The continuous increase of atmospheric CO_2 has been predicted to occur up to the end of the 21st century (IPCC, 2007), contributing to global warming (1.0—4.0 °C) and consequently to an increase of global mean ocean temperatures (IPCC, 2007). Among marine environments, coastal areas may be especially affected by this phenomenon, possibly leading to changes in ecosystems structure and function, including loss of breeding areas and aquatic biodiversity, as well as the risk of hypoxia due to eutrophication (Eissa and Zaki, 2011). In the environment, aquatic organisms are able to acclimatize to temperature shifts, up to a certain tolerance limit (Schiedek et al., 2007). This tolerance depends on the natural environmental temperature that each organism is acclimatized (Sorte et al., 2011).

Although marine bivalves are exposed to periodic temperature changes, previous studies have demonstrated that temperature changes will have a strong impact on survival, internal biological processes (e.g. metabolism and oxidative status; gene expression), reproduction patterns, growth, population abundance and bivalves geographic distribution (e.g. Pörtner et al., 2007; Matozzo et al., 2013; Munari et al., 2011; Santos et al., 2011; Sobral and Widdows, 1997; Verdelhos et al., 2011, 2015). Several studies have been focusing on the thermal tolerance of bivalves, often using biochemical markers to assess the effect of temperature increase on these organisms (e.g. Bielen et al., 2016; Matozzo et al., 2013; Matoo et al., 2013). One of the most used biomarkers is the electron transport system (ETS) activity that provides indirect information about the metabolic activity through the respiratory chain (Bielen et al., 2016). Previous works have shown that bivalves exposed to increasing temperatures increased their metabolic capacity (Bielen et al., 2016; Le Moullac et al., 2007; Solan and Whiteley, 2016). The exposure to high temperatures and the consequent increase of the mitochondrial respiratory activity may also increase the activity of the enzymes involved in the detoxification of reactive oxygen species (ROS). Among biochemical markers, the enzyme superoxide dismutase (SOD) is commonly measured since it plays an important role in antioxidant mechanisms, catalyzing the dismutation of superoxide radicals into a less toxic molecule (Buttemer et al., 2010).

However, if antioxidant mechanisms are not able to prevent ROS proliferation, oxyradical perturbation will occur, including lipid peroxidation (LPO) (Taylor and Maher, 2010).

The induction/inhibition of mechanisms involved in oxidative stress is associated to gene expression adjustment performed by organisms in response to environmental changes (Buckley et al., 2001). These mechanisms provide to the organisms a source of physiological plasticity, especially important in ectotherms that are subjected to daily fluctuations of environmental conditions (Buckley et al., 2001). In fact, it is known that gene expression regulation can be one of the most rapid and sensitive responses to environmental stress conditions (Zippay and Hofmann, 2010; Gracey et al., 2008). Gene expression has been used by different authors to assess the effects of abiotic factors (such as temperature) on marine bivalves, revealing its importance in understanding the health status of organisms living in the field under different environmental stressors (e.g. Hamdoun et al., 2003; Liu et al., 2012; Park et al., 2009). Examples of some genes affected by environmental stressors include bcl-2-like protein 4 (*Bax*), cytochrome C oxidase subunit I gene (*Cox-1* or *coi*), *16S*, SOD, heat shock protein (*Hsp*) and *gadd45* (e.g. Binias et al., 2014; Hamdoun et al., 2003; Meistertzheim et al., 2007; Park et al., 2009; Pil et al., 2008). The expression of *Bax* has been assessed in different organisms, highlighting the regulation of apoptosis in mitochondria (Floros et al., 2006). Among the mitochondrial markers, *Cox-1* and *16S* genes are involved in mitochondrial metabolism (Lakra et al., 2009). Other important genes include *MnSOD* (mitochondrial) and *Cu-ZnSOD* (cytosol) involved in the oxidative stress response (Park et al., 2009; Pil et al., 2008). One of the genes that has been used to assess the physiological effects of thermal variations in organisms is the *Hsp70*, showing an important role in thermal stress response (Anestis et al., 2008). Another gene implicated in the signaling of physiological or environmental stress is *gadd45*. This gene provides information about cell cycle arrest, DNA repair, cell survival and senescence, or apoptosis (Liebermann and Hoffman, 2008).

Global warming may also affect the interaction between marine fauna, favoring the introduced species that may thrive in a new environment (IPCC, 2014). Recently, Bielen et al. (2016) demonstrated that the invasive clam *Sinanodonta woodiana* presented higher tolerance to warmer water temperatures than the native species, *Anodonta anatina*, a factor that has been contributing for the replacement of the native clams. The clam *Ruditapes philippinarum* (Adams and Reeve, 1850) is among the most widespread marine invasive species (FAO, 2016a). It has been introduced along the Pacific coast of North America, the Atlantic coast of Europe and the Mediterranean Sea due to their high socio-economic value (FAO, 2016a). The success of *R. philippinarum* is related with its faster growing rates (FAO, 2016a), immune response to bacteria (Moreira et al., 2012) and greater reproductive capacity than native species, namely *R. decussatus* (Delgado and Pérez-Camacho, 2007). The clam *R. decussatus* is a common European species, an important component of infaunal communities (FAO, 2016b). The harvest of this species mainly occurs in the Atlantic coast of France, Spain, Portugal and Ireland, and in the Mediterranean basin, where it is considered an economically important bivalve (FAO, 2016b). Both of these species present similar feed requirements, filtering organic matter and plankton from the seawater through two siphons (FAO, 2016a, b). Usually, the reproduction of both species is during the summer. However, in Spring the reproduction of both species can be artificially conditioned by higher temperatures and abundant food (FAO, 2016a, b).

It is known that *R. philippinarum* has succeeded in the occupation of the native species areas (Otero et al., 2013), coexisting in some areas with closely related clam species, such as *R. decussatus*

and *Venerupis corrugata*, namely in the Ria de Aveiro (Portugal) (Velez et al., 2015), while in other ecosystems *R. philippinarum* replaced the native species (Venice Lagoon, Otero et al., 2013). Due to that, it is important to understand how expected seawater warming will affect native and invasive species. To our knowledge, few studies have assessed and compared the influence of temperature on *R. decussatus* and *R. philippinarum* and no studies used a combined biochemical and molecular approach to assess the effects of temperature on these species. Anacleto et al. (2014a; b) studied the responses of *R. decussatus* and *R. philippinarum* to environmental warming, but in this case organisms were exposed to thermal shock (22 to 46 °C). Furthermore Munari et al. (2011) and Paillard et al. (2004) studied the effects of different temperatures of acclimation just on *R. philippinarum* through mortality, pinocytotic activity, lysozyme activity and hemolymph immune parameters (5, 15 and 30; 8, 14 and 21 °C, respectively). Since the global mean surface temperatures will increase gradually, the present study assessed and compared the effect of warming scenarios (21 and 25 °C) in gills of native (*R. decussatus*) and introduced (*R. philippinarum*) clams, through subcellular (metabolism, antioxidant capacity and membrane damage) and molecular (*Bax*, *Cox-1*, *16S*, *MnSOD*, *Cu-ZnSOD*, *Hsp70* and *gadd45*) biomarkers.

2. Materials and methods

2.1. Experimental conditions

Clams *R. decussatus* and *R. philippinarum* (80 of each species) were collected in the Ria de Aveiro (northwest Atlantic coast of Portugal), in October 2015. In order to minimize the effect of body weight, organisms with similar weight (15—22 g) were selected.

Individuals of both species were maintained in the laboratory for 7 days before testing, in separate aquaria, to release metals and microorganisms (Freitas et al., 2012; Maffei et al., 2009). Both species were maintained at salinity 28 ± 1, 17.0 ± 1.0 °C; pH 7.80 ± 0.10, 12 light: 12 dark photoperiod and continuous aeration, in artificial seawater (Tropic Marin® SEA SALT from Tropic Marine Center). Seawater was renewed every two days.

After this period, 60 specimens of each species were acclimated to experimental conditions (21 and 25 °C; 20 specimens/temperature/species). The seawater temperature was increased one or two degrees per day until 21 and 25 °C. The water temperatures were increased and maintained in different aquaria through electronic thermostats. The acclimatization period finished when all tested temperatures were reached in all testing groups. Higher temperatures (21 and 25 °C) were selected taking into account the range of annual mean temperature (13.4—22.9 °C) for *R. decussatus* and *R. philippinarum* habitats in the Ria de Aveiro (Coelho et al., 2014; Santos et al., 2009; Velez et al., 2015), the mean temperature in October in the sampling area (16-19 °C, IPMA, 2016) and the predicted temperature increase from 1.0 to 4.0 °C (IPCC, 2007).

After acclimatization, both species were submitted to different temperatures (17, 21 and 25 °C), for 28 days. For each temperature and for each species, 18 organisms were used per condition (17, 21 and 25 °C) (3 replicates/condition, with 6 organisms/replicate). Six containers, 3 for each species, with 2.5 L of seawater (salinity = 28) were placed in different water baths at 17, 21 and 25 °C. Posteriorly, 6 specimens of each species were added to each respective container. The seawater in each aquarium was maintained at constant values using electronic thermostats. Containers were continuously aerated. Temperature and salinity were daily checked with a thermometer and refractometer. Temperature and salinity values were adjusted, adding water to the containers, according to the above conditions, whenever necessary. Mortality was daily checked and organisms were considered dead when their shells