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Are the effects induced by increased temperature enhanced in *Mytilus galloprovincialis* submitted to air exposure?

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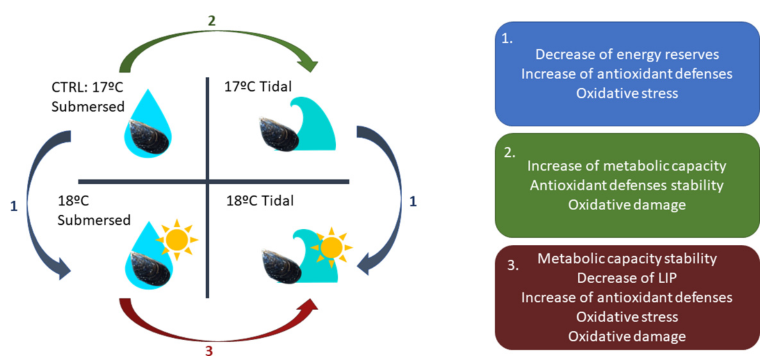
HIGHLIGHTS

- Temperature induced antioxidant defenses avoiding oxidative damage.
- Air exposure increased metabolic capacity and cellular damages.
- The combination of temperature and air exposure caused energy reserves expenditure.
- The combination of stressors activated antioxidant defenses but cellular damage occurred.
- Under tidal conditions the oxidative stress generated by warming is enhanced.

GRAPHICAL ABSTRACT



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ABSTRACT

Intertidal mussel species are frequently exposed to changes of environmental parameters related to tidal regimes that include a multitude of stressors that they must avoid or tolerate by developing adaptive strategies. In particular, besides air exposure during low tides, intertidal mussels are also subjected to warming and, consequently, to higher risk of desiccation. However, scarce information is available regarding the responses of mussels to tidal regimes, particularly in the presence of other stressors such as increased temperature. Investigating the impacts of such combination of conditions will allow to understand the possible impacts that both factors interaction may generate to these intertidal organisms. To this end, the present study evaluated the impacts of different temperatures (18 °C and 21 °C) on *Mytilus galloprovincialis* when continuously submersed or exposed to a tidal regime for 14 days. Results showed that in mussels exposed to increased temperature under submersion conditions, the stress induced was enough to activate mussels' antioxidant defenses (namely glutathione peroxidase, GPx), preventing oxidative damage (lipid peroxidation, LPO; protein carbonylation, PC). In mussels exposed to tides at control temperature, metabolic capacity increased (electron transport system activity, ETS), and GPx was induced, despite resulting in increased LPO levels. Moreover, the combination of tides and temperature increase led to a significant decrease of lipid (LIP) content, activation of antioxidant defenses (superoxide dismutase, SOD; GPx) and increase of oxidized glutathione (GSSG), despite these mechanisms were not sufficient to prevent increased cellular damage. Therefore, the combination of increased temperature and air exposure induced higher oxidative stress in mussels. These findings indicate that increasing global warming could be more impacting to intertidal organisms compared to organisms continuously submersed. Furthermore, our results indicate that air exposure can act as a confounding factor when assessing the impacts of different stressors in organisms living in coastal systems.

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1. Introduction

Organisms present in estuaries and coastal lagoons, must thrive and endure one of the harshest environments. Inhabiting these areas frequently exposed to tides, intertidal organisms are subjected to a large variation of abiotic conditions between aquatic and aerial environments, such as temperature, salinity, oxygen availability and high desiccation risk (Davis, 1985; Freire et al., 2011; Horn et al., 1999; Underwood and Kromkamp, 1999). As a consequence of air exposure, intertidal organisms may face prolonged hypoxic and/or anoxic conditions, with bivalves among the most tolerant to hypoxia (Abele et al., 2009; Gray et al., 2002). Accordingly, alterations on the physiological performance of a diversity of bivalve species experiencing air and tidal exposure have already been demonstrated. While some intertidal bivalves, as the mussel *Mytilus galloprovincialis*, may close their valves when exposed to air and face complete anoxia at ebb tides to avoid desiccation, others may periodically open their valves to maintain a more efficient aerobic metabolism with higher risk of desiccation (Dowd and Somero, 2013; Nicastro et al., 2010; Rivera-Ingraham et al., 2013). *Ruditapes philippinarum* clams showed lower survival and growth when exposed to increased duration of air exposure (Yin et al., 2017). Biochemical alterations can also be induced in bivalves exposed to tidal environment. Previous studies demonstrated an increase of antioxidant defenses in the mussel species *Perna perna* and *M. galloprovincialis* as a defense mechanism against oxidative stress generated during reoxygenation (Almeida and Bainy, 2006; Andrade et al., 2018). Similar biochemical response was observed in *R. philippinarum* clams exposed to daily rhythms of air (Yin et al., 2017). After emergence, *M. edulis* mussels demonstrated over-expression of proteins specially involved in cytoskeleton, chaperoning, energetic metabolism and transcription regulation while presenting decreased activity of antioxidant enzyme superoxide dismutase (Letendre et al., 2011). Rivera-Ingraham et al. (2013) further demonstrated that exposure of *M. edulis* to severe anoxia caused an onset of anaerobiosis (succinate accumulation), while the concentrations of reactive oxygen species (ROS) strongly decreased during anoxic exposure and increased upon reoxygenation.

Intertidal organisms may not only face air exposure during tidal regimes but may, at the same time, be exposed to environmental changes derived from climate change. In particular, as a consequence of global climate change, daily and seasonal environmental variations can be enhanced, such as the increase of temperature. The projected increase of atmospheric CO_2 until the end of the 21st century is considered one of the most important factors contributing to global warming and, consequently, to an increase of global mean air and ocean temperatures (IPCC, 2014). Consequently, intertidal organisms that are exposed to increased temperatures associated with aerial exposure may be subjected to deleterious effects. Different studies have demonstrated that temperatures exceeding the organisms' thermal tolerance range can cause physiological perturbations, namely concerning individuals' growth and reproduction (Pörtner and Knust, 2007; Boukadida et al., 2016), adding to the decrease of aerobic capacity, metabolic rate and respiratory capacity (Jansen et al., 2009; Pörtner, 2005, 2010; Velez et al., 2017). Furthermore, warming can also enhance reactive oxygen species (ROS) production in the cells (Kefaloyianni et al., 2005; Verlecar et al., 2007), leading to oxidative stress. In particular, transcriptomic and biochemical alterations have been observed in different bivalve species in response to temperature rise. *M. galloprovincialis* mussels showed an increase of antioxidant enzymes and metallothionein gene expression levels when exposed to heat stress (Banni et al., 2014). In the same species, significant variations in the immune system were also observed due to increased temperature (Nardi et al., 2017). *M. coruscus* mussels displayed an increase of antioxidant enzymatic activity with increased temperature (Hu et al., 2015).

M. galloprovincialis (Lamarck, 1819) is a common mussel species present in infra littoral areas across the globe (Mitchelmore et al., 1998; FAO, 2016; Vazzana et al., 2016), in rocky areas, cliffs, boulders or other substrates to which it adheres (FAO, 2016; Vazzana et al., 2016). Along coastal areas *M. galloprovincialis* presents a wide spatial distribution and abundance, sedentary and filter feeding behavior and high tolerance to a wide range of environmental conditions, and for these reasons is considered a good sentinel and bioindicator species (Banni et al., 2014; Coppola et al., 2017, 2018a, 2018b; Faggio et al., 2016; Freitas et al., 2018; Kristan et al., 2014; Sureda et al., 2011; Viarengo et al., 2007). Several studies have also demonstrated the ecological relevance of this species. In particular, *M. galloprovincialis* has shown to be able to improve water quality thought the filtration of particles and excess of nitrogen in aquatic environment (Shumway et al., 2003). The expansion of *M. galloprovincialis* into new habitats has also benefited a near-threatened bird species, the African black oystercatcher *Haemotopus moquini*, which switched its diet to this mussel species thus increasing its food availability (Hockey and van Erkom Schurink, 1992). Mussel beds can also provide refuge to fish or act as nurseries for juvenile fish and crustaceans (Shumway et al., 2003).

Considering that scarce information is available regarding marine organisms' responses to tidal regime in the presence of other abiotic stressors, the investigation of such scenarios will generate knowledge that will contribute to better understand the impacts resulting from the interaction of factors such as air exposure and temperature rise. Within this context, the present study investigated the possible interactions of air exposure and warming in *M. galloprovincialis* performance, by evaluating the physiological and biochemical alterations induced in organisms exposed to different tidal regimes and temperature conditions, testing the hypothesis: tidal exposure changes the physiological and biochemical performance of *M. galloprovincialis* submitted to warming conditions.

2. Methodology

2.1. Sampling and experimental conditions

Mytilus galloprovincialis specimens were collected in September 2017 during low tide in an intertidal area at the Mira Channel (Ria de Aveiro, a coastal lagoon, northwest of Portugal). After sampling mussels were transported to the laboratory, where they were placed in aquaria for depuration and acclimation to laboratory conditions for 7 days. Acclimation system operated with synthetic saltwater, prepared by mixing a commercially available salt mixture (Tropic Marin Pro Reef salt; Tropic Marine, Germany) with freshwater purified by reverse osmosis (four stage unit, Aqua-win RO-6080, Thailand). During this acclimation period, organisms were maintained at 18 $\pm$ 1.0 °C (control temperature), pH 8.0 $\pm$ 0.1 (control pH) and salinity 35, resembling estuarine conditions while being kept under continuous aeration during a 12 h light: 12 h dark photoperiod.

For the laboratory experiment, mussels were distributed into different 20 L aquaria (with synthetic saltwater, salinity 35), with 6 individuals *per* aquarium and 3 aquaria *per* treatment. The treatments tested were: submersion under control temperature (Sub); submersion under increased temperature (Sub + Temp); exposure to tides simulation under control temperature (Tide); exposure to tides simulation under increased temperature (Tide + Temp). Aquaria were placed in two different climatic rooms to maintain the temperature levels at 18 $\pm$ 1.0 °C (control temperature) and 21 $\pm$ 1.0 °C (increased temperature). For the tidal simulation, an automatic system that mimicked estuarine tidal regime typical of this species habitat (5 h of low tide and 7 h of high tide cycles) was developed and used.

The control temperature of 18 $\pm$ 1.0 °C was chosen considering the average temperature of the sampling area during September (IPMA, 2017). To simulate warming conditions, temperature of 21 °C, was selected taking in account the annual range of average temperatures (13.4–22.9 °C) for *M. galloprovincialis* habitats in Ria de Aveiro (Coelho et al., 2014; Santos et al., 2009; Velez et al., 2015) and considering the projected global temperature change to the year 2100 (up to 4.0 °C,