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Effect of pH and temperature on antioxidant responses of the thick shell mussel *Mytilus coruscus*

Menghong Hu [a, b, c, d, 1], Lisha Li [a, 1], Yanming Sui [a], Jiale Li [a, b, c], Youji Wang [a, b, c, d, *], Weiqun Lu [a, b, c, **], Sam Dupont [e]

[a] College of Fisheries and Life Science, Shanghai Ocean University, 999 Huchenghuan Road, Shanghai 201306, China
[b] Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai 201306, China
[c] Shanghai University Knowledge Service Platform, Shanghai Ocean University Aquatic Animal Breeding Center (ZF1206), Shanghai 201306, China
[d] Department of Integrative Ecophysiology, Alfred-Wegener-Institute Helmholtz Center for Polar- and Marine Research, 27570 Bremerhaven, Germany
[e] Department of Biological and Environmental Sciences, University of Gothenburg, The Sven Lovén Centre for Marine Sciences, Kristineberg, Fiskebäckskil 45178, Sweden

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ABSTRACT

This study evaluated the combined effects of seawater pH decrease and temperature increase on the activity of antioxidant enzymes in the thick shell mussel *Mytilus coruscus*, an ecological and economic bivalve species widely distributed along the East China Sea. Mussels were exposed to three pH levels (8.1, 7.7 and 7.3) and two temperatures (25 °C and 30 °C) for 14 days. Activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione (GSH), acid phosphatase (ACP), alkaline phosphatase (AKP) and glutamic-pyruvic transaminase (GPT) were measured in gills and digestive glands after 1, 3, 7 and 14 days of exposure. All enzymatic activities were significantly impacted by pH, temperature. Enzymatic activities at the high temperature were significantly higher than those at the low temperature, and the mussels exposed to pH 7.3 showed significantly higher activities than those under higher pH condition for all enzymes except ACP. There was no interaction between temperature and pH in two third of the measured activities suggesting similar mode of action for both drivers. Interaction was only consistently significant for GPX. PCA revealed positive relationships between the measured biochemical indicators in both gills and digestive glands. Overall, our results suggest that decreased pH and increased temperature induce a similar anti-oxidative response in the thick shell mussel.

1. Introduction

Intensive fossil-fuel burning and deforestation over the last two centuries has increased atmospheric CO_2 to almost 40% above preindustrial values. These CO_2 emissions have led to ocean acidification and warming [1]. pH and temperature are key drivers of biological response and it is critical to understand the biological response to changing pH and temperature both in the context of present natural variability and future global changes.

For marine ectothermic organisms, habitat temperature is driving all biological processes, from molecular to physiological to behavioural [2–4]. High temperatures are known to enhance reactive oxygen species (ROS) production in the cells [5,6] thereby increasing the risk of oxidative alteration. pH is also playing a key role in many marine animals. For example, we found that pH and temperature affected physiological processes in mussels with severe consequences for their energy budget [7]. Simultaneous changes in multiple drivers can lead to complex interactions [8]. Although both ocean acidification and warming are recognized as serious threats to marine life, little information exists on their combined effect on mussels' physiology [9].

Mytilus coruscus is a mussel widely distributed in the coastal waters of Yellow Sea and East China Sea, and extensively cultured as an important shellfish species in Zhoushan Islands, Zhejiang, China [10,11]. It lives attached to hard substrates and forms subtidal beds playing an important ecological role and affecting the coastal

* Corresponding author. College of Fisheries and Life Science, Shanghai Ocean University, 999 Huchenghuan Road, Shanghai 201306, China.
** Corresponding author. Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai 201306, China.
E-mail addresses: mhhu@shou.edu.cn (M. Hu), youjiwang2@gmail.com (Y. Wang), wqlv@shou.edu.cn (W. Lu).
[1] These authors contributed equally to this work.

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community structure. Despite its economical and ecological importance, information available on the impact of environmental stressors is limited to biomarker responses and immune-related activities [12–14].

The aim of this study was to evaluate the antioxidant response of *M. coruscus* exposed to different pH and temperature. Matozzo et al. (2013) [15] demonstrated that the combination of decreased pH and increased temperature strongly affects immune parameters in the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*. They showed that low pH and high temperature can induce oxidative stress, although the pattern varied between species and tissues. In the oyster *Crassostrea Virginica*, Tomanek et al. (2011) [16] showed that exposure to pH 7.5 induced oxidative stress. They suggested that low pH caused oxidative stress by increasing the production of ROS either indirectly by lowering internal pH, which may enhance the Fenton reaction, and/or directly by CO_2 interacting with other ROS to form more free radicals.

All organisms have their own cellular antioxidant defence system, composed of both enzymatic and non-enzymatic components. In bivalves, enzymatic pathway consists of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which provide cellular defence against endogenous and exogenous ROS [17]. To reduce the damage of ROS, O_2^- are dismutated by SOD to H_2O_2 which is reduced to water and molecular oxygen by CAT or is neutralized by GPX that catalyses the reduction of H_2O_2 to water and organic peroxide to alcohols using glutathione (GSH) as a source of reducing equivalent [6]. The antioxidant system protects cells by maintaining ROS at low levels and by attenuating damages related to their high reactivity. Phosphatases remove phosphate groups from their substrates by hydrolysing phosphoric acid monoesters into phosphate ions and molecules with free hydroxyl groups. ACP and AKP are two important phosphatases in marine organisms, participating in degradation of foreign protein, carbohydrates and lipids [18]. Glutamate-pyruvate transaminase (GPT) catalyses the reversible transamination between alanine and 2-oxoglutarate to generate pyruvate and glutamate and, therefore, plays a key role in the intermediary metabolism of glucose and amino acids. These parameters are classically used as stress indicators in animals [54].

In the present study, we compared enzymatic activities in two tissues: the digestive gland and the gill. They are active sites for oxyradical-generation as well as enzyme biotransformation process [19]. Digestive gland is the major tissue involved in metabolic functions in bivalves. Gills are located in the ventilated mantle cavity of the mollusc and directly interact with the marine environment for gas exchange between the hemolymph and mantle fluid [20].

2. Materials and methods

2.1. Animals collection

Samples of the thick shell mussel *M. coruscus* (30 ± 2 mm shell length, 75.0 ± 5.5 mg dry tissue weight) were sampled from a mussel raft at Shengsi island of Zhejiang Province (30° 33′ 00.945″ N, 121° 49′ 59.757″ E), China in September 2013. Mussels with no shell damage were selected and epibionts (i.e., barnacles) were gently removed from the shell. Mussels were acclimated to laboratory conditions in open-flow tanks (500 L) with seawater mimicking the Shengsi island environment at time of sampling: 25 °C, salinity 25.0, pH 8.1 and oxygen concentration 7–8 mg O_2 l^{-1}. They were fed twice daily with the microalgae algae *Chlorella* spp (25,000 cells ml^{-1}, ~3% of the mussel dry weight).

2.2. Experimental design

Two different parameters were tested in a fully crossed design: (i) Two temperatures (25 °C and 30 °C) within the range of present variability (20–30 °C [21,22]); and (ii) three pHs: pH 8.1 as the present average pH, pH 7.7 as the predicted average pH for the year 2100 (IPCC 2007 [23]) and extreme of present natural variability at the sampling site (8.2–7.7 [24]), and pH 7.4 as extreme pH expected by the year 2100. The experiment was conducted over 14 days and in triplicates for each treatment (3 × 6 treatments = 18, 30 L aquarium) with 30 mussels per replicate. A flow-through system with a header tank design was used to minimize any interference from the metabolic waste products of the mussels, and tanks were covered with acrylic plates to reduce or prevent external disturbance.

Measurements on biochemical parameters were conducted at 1, 3, 7 and 14 day of exposure. .Mussels were fed with *Chlorella* spp. as described above. Mussels were slowly acclimatized to the new experimental conditions through a gradual increase in seawater temperatures (1 °C/day) from 25 °C to 30 °C and a gradual decrease in pH values from 8.1 to 7.7 to 7.3.

2.3. Temperature and carbonate chemistry

The water temperatures in the experimental tanks were maintained constant using electronic thermostats. pH was maintained using CapCTRL software (Loligo Systems Inc, Tjele, Denmark) connected to CO_2/pH controller (DAQ-M, 4 Channel, Loligo® Systems Inc., Tjele, Denmark). Low pH conditions in the seawater were achieved using pCO_2/pH feedback STAT systems connected with WTW pH 3310 m and SenTix 41 pH electrodes (Loligo Systems Inc, Tjele, Denmark). pH was also measured daily using a portable pH meter (pH-201, MSITECH (Asia–Pacific) Pte. Ltd., Singapore) calibrated with the NBS scale. Temperature and pH of seawater were continuously monitored during the experiment using STAT systems. Salinity was measured using a salinity refractometer (Hand Refractometer S/Mill-E, Atago, Itabashi-Ku, Tokyo, Japan). Total alkalinity (TA) was determined by titration. Dissolved inorganic carbon (DIC), partial pressure of CO_2 in seawater (pCO_2) and the $CaCO_3$ saturation state for calcite (Ωcal) and aragonite (Ωara) were calculated from temperature, salinity, pH and TA, following the procedures described by Wang et al. (2015) [7].

2.4. Tissue sampling and preparation

At each time point, three mussels per replicate were dissected. Digestive glands and gills were carefully excised, surface dried with tissue paper, thoroughly washed with phosphate buffer (50 mM; pH 7.4), pooled per replicate and then placed in tubes on ice. Aliquots from each pooled tissue were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. The gills and digestive glands were thawed on ice and homogenised (1:4, w:v) in 0.1 M Tris–HCl buffer (pH 7.5) containing 0.15 M KCl, 0.5 M sucrose, 1 mM EDTA, 1 mM Dithiothreitol (DTT, Sigma) and 40 μg/ml Aprotinin (Sigma). Homogenization was performed at 4 °C using 12–15 strokes of a motor driven Teflon Potter-Elvehjem homogenizer. Homogenized samples were sonicated for 2 min at 0 °C with a Braun Labsonic U sonifier at 50% duty cycles and were then centrifuged at 12,000g for 45 min at 4 °C. Supernatants were collected for biochemical analysis.

2.5. Biochemical assays

Assays were performed using substrates from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and following