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Pinctada margaritifera responses to temperature and pH: Acclimation capabilities and physiological limits

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ABSTRACT

The pearl culture is one of the most lucrative aquacultures worldwide. In many South Pacific areas, it depends on the exploitation of the pearl oyster *Pinctada margaritifera* and relies entirely on the environmental conditions encountered in the lagoon. In this context, assessing the impact of climatic stressors, such as global warming and ocean acidification, on the functionality of the resource in terms of renewal and exploitation is fundamental. In this study, we experimentally addressed the impact of temperature (22, 26, 30 and 34 °C) and partial pressure of carbon dioxide pCO_2 (294, 763 and 2485 μatm) on the biomineralization and metabolic capabilities of pearl oysters. While the energy metabolism was strongly dependent on temperature, results showed its independence from pCO_2 levels; no interaction between temperature and pCO_2 was revealed. The energy metabolism, ingestion, oxygen consumption and, hence, the scope for growth (SFG) were maximised at 30 °C and dramatically fell at 34 °C. Biomineralization was examined through the expression measurement of nine mantle's genes coding for shell matrix proteins involved in the formation of calcitic prisms and/or nacreous shell structures; significant changes were recorded for four of the nine (*Pmarg*-Nacrein *A1*, *Pmarg*-MRNP34, *Pmarg*-Prismalin 14 and *Pmarg*-Aspein). These changes showed that the maximum and minimum expression of these genes was at 26 and 34 °C, respectively. Surprisingly, the modelled thermal optimum for biomineralization (ranging between 21.5 and 26.5 °C) and somatic growth and reproduction (28.7 °C) appeared to be significantly different. Finally, the responses to high temperatures were contextualised with the Intergovernmental Panel on Climate Change (IPCC) projections, which highlighted that pearl oyster stocks and cultures would be severely threatened in the next decade.

1. Introduction

Since the industrial revolution, the use of fossil energy has been constantly increasing, and has already led to the emission of gigatons of greenhouse gases into the atmosphere, inducing global climate changes. This phenomenon drives significant environmental pressures through global warming and ocean acidification. The former has already been materialised by a global ocean temperature increase of 0.7 °C, and the second through the loss of 0.1 pH units (Hoegh-Guldberg et al., 2007). The latest Intergovernmental Panel on Climate Change (IPCC) report highlights that, under all scenarios of greenhouse gas emission for the next century, the sea surface temperature will continue to increase from about +1 °C to +2.5 °C by the horizon 2081–2100 in tropical areas (IPCC, 2014). Concomitantly to the worsening of global warming, ocean surface water will lose an additional 0.1 pH units under the most optimistic scenario and 0.4 pH units under the most pessimistic one (IPCC, 2014).

The main scientific concerns about the effect of ocean acidification were about its putative negative effect on the ability of marine calcifiers to maintain the processes of biomineralization. Indeed, several experimental, ecophysiological and molecular

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studies have shown that a low pH can decrease the calcification rate and skeletal growth of these organisms (Kroeker et al., 2010; Ries et al., 2009). However, others have reported the absence of effects, or even an increase in biomineralization activity (Kroeker et al., 2010; Ries et al., 2009). These contrasting results were also confirmed in ecosystems naturally subjected to low pH levels because of CO_2 vents (Fabricius et al., 2011; Rodolfo-Metalpa et al., 2011). Another important concern linked to the increase of partial pressure of carbon dioxide (pCO_2) is the induction of hypercapnia and its subsequent metabolic deregulation. While its effects on marine vertebrates have been studied to some extent (Ishimatsu et al., 2005; Pörtner et al., 2005), little is known about the ecophysiological impacts of pCO_2 increase on invertebrates. Some recent studies suggest that ocean acidification exerts a negative effect on the energetic balance of marine invertebrates (Stumpp et al., 2011; Zhang et al., 2015), which would directly affect populations through various biological and ecological processes such as a the reduction of reproduction efficiency (Kurihara, 2008). However, counter examples exist (Thomsen et al., 2013; Zhang et al., 2015). Addressed from various methodologies and organisation scales, the main answer to the question resulting from ocean acidification was that the physiological and molecular responses could not be generalised to all phyla or functional groups, and thus, were species-and even life stage-specific.

In ectotherms, many biological processes, such as development and survival, are subject to temperature. All species have an optimal thermal window with both upper and lower limits of tolerance, which allows them to acquire energy for growth and reproduction. Beyond this thermal window, the conditions are not met for proper development. At low temperatures, the energy acquisition is low; at high temperatures, energy consumption is higher than the energy gained. Temperature directly regulates the metabolism of ectotherms, with increasing growth rates as temperatures rises; however, warming directly affects individuals that struggle to maintain cardiac function and respiration in the face of increased metabolic demand (Neuheimer et al., 2011; Pörtner et al., 2007).

In this environmental context, many human activities supported by marine calcifiers could be considered endangered. Among these marine calcifiers is the pearl oyster *Pinctada margaritifera*. This marine bivalve has a significant aesthetic, patrimonial and commercial value, particularly in relation to pearl production, tourism and international standing. In this context, the aim of the present study is to characterise, at the bioenergetic and biomineralization levels, the impacts of climate change (global warming and ocean acidification) on the pearl oyster (*P. margaritifera*). To address this aim, oysters were subjected to an acidification (pH 8.2, 7.8 and 7.4) cross-temperature (22, 26, 30 and 34 °C) experiment. The impacts of treatments were quantified at the bioenergetic and the biomineralization levels. Finally, the results obtained were contextualised with the prediction of environmental changes to lay the foundation for the first projection of the future of *P. margaritifera* in the northern lagoons of French Polynesia.

2. Material and methods

2.1. Rearing system, temperature and pH control

The rearing system was set up in an experimental bivalve hatchery operated by Ifremer in Vairao, Tahiti, French Polynesia. The facility is supplied with filtered seawater from the Vairao lagoon. The pearl oysters were placed in 500 L tanks with controlled flow-through. Seawater was renewed at the rate of 100 L h^{-1} for all the experiments. The pearl oysters were fed with the microalgae *Isochrysis galbana* supplied continuously using Blackstone dosing pumps (Hanna). A constant concentration of 25,000-cell mL^{-1} was maintained throughout the experiment. Temperature and algae concentration were controlled continuously by a fluorescence probe (Seapoint Sensor Inc.) and a temperature sensor (PT 100). Seawater was heated by an electric heater or cooled with a heat exchanger (calorie exchange with cold freshwater) plugged into a sensor. Both apparatuses were operated by a temperature controller. The pH was manipulated in flow-through tanks by bubbling CO_2 until the pH target was reached. This was operated by pH electrodes and temperature sensors connected to a pH-stat system (Dennerle) that continuously monitored pH (calibrated to NIST scale) and temperature to control CO_2 bubbling.

2.2. Carbonate chemistry

Total alkalinity (TA) was measured via titration with 0.01 N of HCl containing 40.7 g NaCl L^{-1} using a Titrator (Schott Titroline Easy). Parameters of carbonate seawater chemistry were calculated from pH, mean TA, temperature, and salinity using the free access CO_2 Systat package (van Heuven et al., 2009). Targeted values were pH 7.4 (3667 μatm CO_2), pH 7.8 (1198 μatm CO_2) and the control at pH 8.2 (426 μatm CO_2). Parameters of carbonate seawater chemistry are given in Table 1.

2.3. Experimental designs and biological material

The pearl oysters used in this experiment were reared at the Ifremer hatchery. They were obtained from a hatchery batch constituted by 8 wild parents originated from Takaroa atoll (North Tuamotu archipelago). Twelve experimental conditions were tested by applying four temperatures (22, 26, 30 and 34 °C) and three different pH levels (pH 8.2, 426 μatm pCO_2; pH 7.8, 1198 μatm pCO_2; pH 7.4, 3667 μatm pCO_2). First, 48 individuals (110.3 ± 9.3 mm shell height) were randomly distributed in the 12 tanks one week before starting the experimental exposure period. During this acclimatization step to the controlled conditions, temperature and pH were linearly modified in order to reach the attended value for the beginning of the experimental exposure. After seven days of exposure to the targeted conditions, four pearl oysters were subjected to metabolic measurements for an additional 48 h exposure to the treatments. They were then dissected to withdraw a piece of mantle for the gene expression analysis.

2.4. Bioenergetic measurements of P. margaritifera

Once the exposures were finished, four oysters from each treatment were transferred to the ecophysiological measurement system (EMS) where they were individually placed in a metabolic chamber to monitor ingestion and respiration rates (RRs). During these 48hs period, the pearl oysters were placed on biodeposition collectors to quantify the assimilation of organic matter (OM). The EMS consisted of five open-flow chambers. For each treatment, each of the four oysters was placed, simultaneously, in one of the chambers, and the fifth chamber remained empty as a control (Chávez-Villalba et al., 2013). The experimental conditions applied during treatments (temperature, pH) were replicated in the EMS during measurements.

Ingestion rate (IR, cell. h^{-1}), an indicator of feeding activity, was defined as the quantity of microalgae cleared per unit of time. IR was estimated using fluorescence measurements and calculated as: $IR = V(C1 - C2)$, where C1 is the fluorescence level of the control chamber, C2 is the fluorescence of the experimental chamber containing an oyster, and V is the constant water flow rate (10 L h^{-1}).

Respiration rate (RR, mg O_2 h^{-1}) was calculated using differences in oxygen concentrations between the control and

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