Estuarine, Coastal and Shelf Science
December 2016, Volume 182, Part B, Pages 261-269
http://dx.doi.org/10.1016/j.ecss.2016.04.011
http://archimer.ifremer.fr/doc/00332/44364/

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

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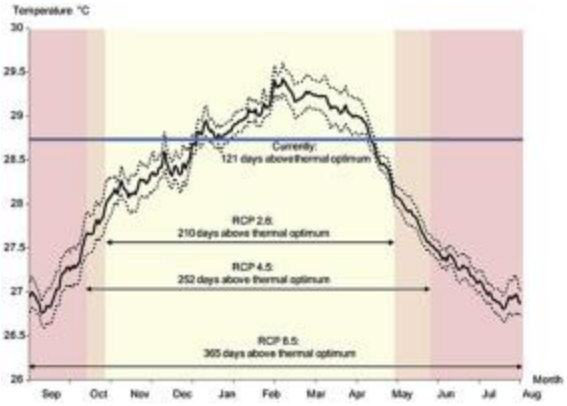
Abstract :

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.

Graphical abstract

Under the current temperature regime (black line; dotted line is the confidence interval at 5%), pearl oysters spends 121 days above their thermal optimum (blue line). Under the Representative Concentration Pathway (RCP) scenarios 2.6 (+1 °C), this would be 210 days (yellow), 252 days under the RCP 4.5 (+1.5 °C; orange) and 365 days under the RCP8.5 (+2.5 °C; red).



Highlights

► At the end of the century, the pearl oyster will be stressed 200 + days a year. ► High temperatures induce metabolic deficits in the pearl oyster. ► Optimal temperatures for shell growth and somatic growth are different. ► Short exposures to low pH levels did not affect the pearl oyster's bioenergetics.

Keywords : Global change, Pearl oyster, Bioenergetic, Biomineralization, Pacific ocean, French Polynesia

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 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

98 negative effect on the energetic balance of marine invertebrates (Stumpp et al., 2011;
99 Zhang et al., 2015), which would directly affect populations through various biological and
100 ecological processes such as a the reduction of reproduction efficiency (Kurihara, 2008).
101 However, counter examples exist (Thomsen et al., 2013; Zhang et al., 2015). Addressed
102 from various methodologies and organisation scales, the main answer to the question
103 resulting from ocean acidification was that the physiological and molecular responses
104 could not be generalised to all phyla or functional groups, and thus, were species-and even
105 life stage-specific.

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

116 In this environmental context, many human activities supported by marine
117 calcifiers could be considered endangered. Among these marine calcifiers is the pearl
118 oyster *Pinctada margaritifera*. This marine bivalve has a significant aesthetic, patrimonial
119 and commercial value, particularly in relation to pearl production, tourism and
120 international standing. In this context, the aim of the present study is to characterise, at the
121 bioenergetic and biomineralization levels, the impacts of climate change (global warming
122 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 500L 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

147 Total alkalinity (TA) was measured via titration with 0.01 N of HCl containing 40.7 g
148 NaCl L^{-1} using a Titrator (Schott Titroline Easy). Parameters of carbonate seawater chemistry
149 were calculated from pH, mean TA, temperature, and salinity using the free access CO_2 Systat
150 package (van Heuven et al., 2009). Targeted values were pH 7.4 (3,667 µatm CO_2), pH 7.8
151 (1,198 µatm CO_2) and the control at pH 8.2 (426 µatm CO_2). Parameters of carbonate
152 seawater chemistry are given in Table 1.
153
154 **2.3 Experimental designs and biological material**
155 The pearl oysters used in this experiment were reared at the Ifremer hatchery. They
156 were obtained from a hatchery batch constituted by 8 wild parents originated from Takaroa
157 atoll (North Tuamotu archipelago). Twelve experimental conditions were tested by applying
158 four temperatures (22, 26, 30 and 34°C) and three different pH levels (pH 8.2, 426 µatm
159 pCO_2 ; pH 7.8, 1,198 µatm pCO_2 ; pH 7.4, 3,667 µatm pCO_2). First, 48 individuals (110.3 ±
160 9.3 mm shell height) were randomly distributed in the 12 tanks one week before starting the
161 experimental exposure period. During this acclimatization step to the controlled conditions,
162 temperature and pH were linearly modified in order to reach the attended value for the
163 beginning of the experimental exposure. After seven days of exposure to the targeted
164 conditions, four pearl oysters were subjected to metabolic measurements for an additional 48h
165 exposure to the treatments. They were then dissected to withdraw a piece of mantle for the
166 gene expression analysis.
167
168 **2.4 Bioenergetic measurements of *P. margaritifera***
169 Once the exposures were finished, four oysters from each treatment were
170 transferred to the ecophysiological measurement system (EMS) where they were
171 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 experimental chambers. RR = V(O1 − O2), where O1 is the oxygen concentration in the control chamber, O2 is the oxygen concentration in the experimental chamber, and V is the water flow rate.

To compare ingestion and RRs, it was necessary to correct for differences in specimen weights. Values of the ecophysiological activities were converted to a standard animal basis (1 g, dry weight), using the formula: Ys = (Ws/We)b × Ye, where Ys is the physiological activity of a standard oyster, Ws is the dry weight of a standard oyster (1 g), We is the dry weight of the specimen, Ye is the measured physiological activity, and b is the allometric coefficient of a given activity. The average b allometric coefficients were 0.66 for IR and 0.75 for oxygen consumption rate (Savina and Pouvreau, 2004).

Assimilation efficiency (AE) of OM was assessed by analysing microalgae, faeces and pseudofaeces according to (Conover, 1966) and described by Chavez et al. (2013). The pearl oysters were laid out in a collector, in which the deposits were collected on a 10-µm

sieve. Biodeposits were centrifuged for 15 min at 4,500 t min^{-1}. The supernatant was removed, and the pellet was washed twice with ammonium formate (37% in distilled water). The pellet was then put in a pre-weighed aluminium cup to be dried at 70°C for 36h before being burnt at 450°C for 4 h. Microalgae OM was obtained by the centrifugation of 5 L of the microalgae mixture, and the pellet was treated with the same procedure used for the biodeposits. The AE was then calculated according to the following equation:

$$AE = \frac{\%\mathrm{OM\mu alg} - \%\mathrm{OMbiodeposit}}{(100 - \%\mathrm{OMbiodeposit})\mathrm{x}\ \%\mathrm{OM\mu alg}}$$

Ecophysiological data were converted into energetic values to define the Scope For Growth (SFG) for each oyster: SFG = (IR × AE) − RR, where IR is the ingestion rate, AE is the assimilation efficiency, and RR is the respiration rate. We used 20.3 J for 1 mg of particulate OM (Bayne et al., 1987) and 14.1 J for 1 mg O_2 (Bayne and Newell, 1983; Gnaiger, 1983).

In order to model the optimal temperature for somatic growth and reproduction, RR was used according to the following polynomial equation:

$$RR = -0.02615T^2 + 1.49875T - 18.84308$$

where RR is oxygen consumption and T is temperature. The temperature corresponding to the maximum value of RR is given by the following equation:

$$\mathrm{T}optRR = \frac{-b}{2a}$$

where T_{optRR} is the optimal temperature, a = -0.02615 and b = 1.49875.

2.5 Gene expression in mantle of *P. margaritifera*

Once the ecophysiological measures were complete, the pearl oysters were sacrificed, and a strip of mantle tissue, measuring approximately 0.5 cm in width, was dissected from the

mantle edge to the adductor muscle. The mantle strip was dissected on the right valve along the maximum shell height. Gene expression in the calcifying mantle of four individuals per temperature treatment were analysed (n=16 *P. margaritifera*). Total RNA was extracted from each sample using TRIZOL®Reagent (Life Technologies), according to the manufacturer's recommendations. RNA was quantified using a NanoDrop®ND-1000 spectrophotometer (NanoDrop®TechnologiesInc). Three thousand ng of total RNA were treated for each sample with DNAse (Ambion) to degrade any potential contaminating DNA in the samples. First strand cDNA was synthesised from 500 ng of total RNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche), using 2 µL of anchored-oligo (dT) and 1 µL of random hexamer primers.

The expression levels of nine genes, four encoding proteins specific to the nacreous layer (*Pmarg-Pif 177, MSI60, Pmarg-Pearlin* and *Pmarg-MRNP34*), four encoding proteins of the prismatic layers (*Pmarg-Shematrin 9, Pmarg-Prismalin14, Pmarg-PUSP6* and *Pmarg-Aspein*) and one involved in the organic matrix of both layers (*Pmarg-Nacrein A1*) (Marie et al., 2012a; Marie et al., 2012b; Montagnani et al., 2011), were quantified to characterise the response of pearl oysters to treatments at the biomineralization level. These expressions were analysed by quantitative RT-PCR analysis using a set of forward and reverse primers provided in Table 2. Three genes, commonly used as reference genes for the comparison of gene expression data, were chosen based on their ubiquitous and constitutive expression pattern in bivalves: universal primers for the 18S rRNA gene (Larsen et al., 2005), GAPDH (Dheilly et al., 2011) and specific to *P. margaritifera* tissue: REF1 (Joubert et al., 2014). Quantitative-RT-PCR amplifications were carried out on a Stratagene MX3000P (Agilent Technologies) using 12.5 µL of Brilliant II SYBR® Green QPCR Master Mix (Stratagene) with 400 nM of each primer and 10 µL of a 1:100 cDNA template.

The following amplification protocol was used: initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 30 s, primers annealing at 60°C for 30 s and extension at 72°C for 1 min. Lastly, to verify the specificity of the product, a melting curve analysis was performed from 55 to 95°C increasing at increments of 0.5°C. All q-RT-PCR reactions were made in duplicate. The comparative Ct (threshold cycle) method was used to analyse the expression levels of the candidate's genes. The relative expression ratio was calculated based on the delta-delta method, normalised with three reference genes to compare the relative expression results, which is defined as: ratio = $2^{-[\Delta Ct\ sample-\Delta Ct\ calibrator]} = 2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). Here, the ΔCt calibrator represents the mean of the ΔCt values obtained for all tested genes in all conditions.

2.6 Temperature data

In order to contextualise the results obtained for the different thermal treatments with the current and predicted temperatures, data measured in 10 lagoons located in the north of the Tuamotu Archipelago were used. The current temperatures correspond to daily mean temperatures recorded from 1 January 1999 to 31 December 2007 in the lagoons of Ahe, Apataki, Arutua, Fakarava, Manihi, Rangiroa, Raroia, Takapoto, Takaroa and Takume (Bissery and Nicet, 2008). Natural temperature variations were estimated by the calculation of the confidence interval at the 5% level. The temperatures predicted for 2081–2100 were calculated according to the geographic localisation of the Tuamotu combined with the IPCC scenarios (IPCC, 2014); RCP 2.6 (+1°C), RCP 4.5 (+1.5°C) and RCP 8.5 (+2.5°C).

2.7 Statistical analysis

Normality of data distribution and homogeneity of variance were tested using the Shapiro-Wilk test and the Bartlett test, respectively. RR data followed the conditions of

application of parametric tests, but IR and SFG data were subjected to the Box-Cox transformation to satisfy these conditions. AE was analysed using the arcsine square root AE/100 value. The impact of temperature and the pCO_2 level was tested using a two-way ANOVA followed by PLSD Fisher post hoc tests. Alpha was set at 0.05 for all analyses. The expression values of the nine candidate genes met the condition for a parametric ANOVA after normalisation by the Box-Cox transformation. PLSD Fisher tests were used to determine significant differences.

3 Results

The aim of the present study was to characterise, at the bioenergetic and biomineralization levels, the impact of a seven-day exposure to an acidification (pH 8.2, 7.8 and 7.4) cross temperature (22, 26, 30 and 34°C) experiment.

3.1 Bioenergetics

The two-way ANOVA did not reveal significant differences between AE in response to the temperature (p=0.94; Table 3) or the pCO_2 (p=0.20; Table 3); nor did it reveal an interaction between both treatments (p=0.10; Table 3). Conversely, the same analysis showed that the IRs were significantly different between temperature treatments (p<0.0001; Table 3) but not according to the pCO_2 (p=0.83; Table 3). No significant interaction was revealed (p=0.28; Table 3). The PLSD Fisher test showed that the IR increased significantly with the temperature until it reached a maximum at 30°C; finally, at 34°C, it significantly dropped to the value measured at 22°C (Figure 1A). The oxygen consumption rate (OC) was significantly affected by temperature (p<0.0001; Table 3), but not by pCO_2 (p=0.52; Table 3), and no significant interaction between treatments was revealed (p=0.94; Table 3). The PLSD Fisher test showed that OC was lowest at 22°C, highest at 26°C and 30°C, and started to decrease at

34°C (Figure 1B). SFG increased significantly with temperature (p=0.0003; Table 3), but it was not affected by the pCO_2 level (p=0.38; Table 3), and no significant interaction was revealed (p=0.59; Table 3). The PLSD Fisher test showed a significant increase at 30°C in comparison to the three other temperatures tested (Figure 1C). According to the polynomial equation provided above, the optimal temperature for somatic growth and reproduction was T_{optRR} = 28.7°C.

3.2 Mantle gene expression

Gene expression measurements were focused on the effect of temperature because of the absence of a pCO_2 effect at the bioenergetic level. Among the nine candidate genes tested, the expression of four were significantly affected by temperature treatments (Figure 2). *Pmarg-MRNP34* gene expression decreased significantly at 30 and 34°C in comparison to 26°C (p=0.05; Table 4, Figure 2D). A significant change of the *Pmarg-Prismalin14* gene expression was recorded (p=0.01; Table 4,Figure 2F) between 26 and 30°C. *Pmarg-Aspein* expression was significantly higher at 26°C and decreased significantly at 30°C and 34°C (p=0.01; Table 4, Figure 2G). *Pmarg-Nacrein A1* gene expression was maximal at 26°C and decreased significantly at 30 and 34°C (p=0.01; Table 4, Figure 2I). According to the polynomial equation provided above, the optimal temperatures for *Pmarg-Nacrein A1*, *Pmarg-MRNP34*, *Pmarg-Prismalin14* and *Pmarg-Aspein* expression were 24.8°C, 24.6°C, 21.5°C and 26.5°C, respectively.

3.3 Actual and predicted temperature in the lagoon of the north Tuamotu Archipelago

The temperature data measured in 10 lagoons of the North Tuamotu Archipelago and the downstream descriptive statistical analysis showed that the annual average temperature between 1999 and 2007 was 27.88°C (+/- 1.00°C). The warmest month was March (29.14 +/-

0.05°C), and the coldest was August (26.35 +/- 0.05°C). With this regime, pearl oysters were experiencing temperatures above their physiological optimal temperature threshold 121 days per year. In perspective with the IPCC scenarios (RCP 2.6, RCP 4.5 and RCP 8.5), this threshold would be exceeded, 210, 252 and 365 days per year, respectively (Figure 3).

4 Discussion

Bivalve growth is known to be strongly influenced by environmental conditions such as food supply and water temperature. The aim of this study was to simultaneously evaluate *P. margaritifera's* bioenergetic and biomineralization abilities as a function of environmental conditions, temperature and pCO_2 in the context of global warming and ocean acidification.

4.1 Acidification did not influence energy management in *Pinctada margaritifera*

This first study on the impact of acidification on energy metabolism of *P. margaritifera* indicated that, after a short-term exposure (9 days), no significant change occurred; *P. margaritifera* seems tolerant to acidification given that the metabolic index did not vary. This is not the case for other species of bivalves living in temperate areas where different levels of metabolic adaptation have been observed. The energy input is reduced in the clam *Ruditapes decussatus* when exposed to high pCO_2 levels, due to a general metabolic depression (Fernández-Reiriz et al., 2011). When the mussel *Mytilus galloprovincialis* was exposed to high pCO_2, the SFG was better, thus promoting better growth and reproduction; this is based on better absorption efficiency and a lower ammonium excretion rate (Fernández-Reiriz et al., 2012). The metabolic rate of the wild oyster *Saccostrea glomerata* was not impacted by low pCO_2, while, for selected oysters (for growth and disease resistance) it increased oxygen needs (Parker et al., 2012). In

addition, the response to acidification seems sometimes contradictory. Indeed, within the same species, *Ruditapes decussatus*, at similar sizes and in similar experimental conditions, conclusions on the impacts of acidification are not the same. At the metabolic level, Fernandez-Reiriz et al. (2011) observed a depression, while Range et al. (2011) measured no difference in terms of net calcification, size or weight. They argue that the local response was not extrapolated to the overall response of the species. In any case, the accumulation of data obtained from an intraspecific to an interspecific level in bivalves over several years argues in favour of a high genetic and phylogenetic effect on the response to ocean acidification.

In this study, we used a short term exposure to the high pCO_2 treatments. This short-term period did not aim to induce a response of an adaptive type but to study the acclimation stage. In Le Moullac et al. (in this ECSS issue) authors have shown that, after 100 days of exposure, the metabolic response did not vary regardless of the tested level of pCO_2. This suggests that in response to high pCO_2, the adjustment of the energy metabolism in the pearl oyster is fast which confirms that this species seems to be tolerant to such a disturbance.

4.2 The effects of temperature on *Pinctada margaritifera*

As previously shown in numerous bivalves (Aldridge et al., 1995; Hicks and McMahon, 2002; Le Moullac et al., 2007; Marsden and Weatherhead, 1998) including sister species of the genus *Pinctada* sp. (Saucedo et al., 2004; Yukihira et al., 2000), our study confirms that temperature influences metabolic rates (MR) in the pearl oyster *P. margaritifera*. Indeed, the linear relationship between temperature and MR shows an increase of energy gain from 22 to 30°C, which is the temperature where MRs were maximised.

Otherwise, our study revealed a non-lethal thermal-maximum, at 34°C, that caused a severe metabolic depression where the individual could no longer acquire energy. Indeed, the RR at 34°C still represent 70% of the RR at 30°C, which represent a high-energy expenditure, while the concomitant food intake represent a lack of energy acquisition. This metabolic situation is akin to fasting, and may not last for a long time since pearl oysters would rely on their energy reserves which will lead to an energy deficit and thus, to the death by exhaustion (Patterson et al., 1999). To date, this is the first evidence about the putative consequences of warming on the physiology of the pearl oyster. This phenomenon must be studied further given that it would result in a strong population disorder induced by an energy depletion and/or a decrease of the reproduction capacity.

The general metabolism of an organism can be evaluated by measuring RR, which is a good biomarker of health and energetic balance. Experimental approaches had confirmed in many bivalves that RR increases with increasing temperatures (Bougrier et al., 1995). However, the relationship between RR and the temperature is only valid in a range of temperatures corresponding to the thermal limits of the species. Concerning *P. margaritifera*, the RR is maximal at 30°C. However, the polynomial modelling of the relationship with the temperature highlights 28.7°C as the thermal optimum. This value can be considered as a reference value for *P. margaritifera* pointing out the temperature threshold where above the organism is stressed by the temperature inducing an energy deficit.

In molluscs, the biomineralization of the shell is a costly function (Palmer, 1992), which suggests an intimate link between the bioenergetic balance and temperature. To address these links over the range of temperatures that *P. margaritifera* can experience, the expression levels of nine gene-encoding proteins of the shell organic matrix were quantified on mantle samples taken off at 22, 26, 30 and 34°C. Among these genes were four encoded for proteins

specific to the nacreous layer (*Pmarg-Pif 177, Pmarg-MSI60, Pmarg-Pearlin* and *Pmarg-MRNP34*), four encoded for proteins of the prismatic layer (*Pmarg-Shematrin 9, Pmarg-Prismalin14, Pmarg-PUSP6* and *Pmarg-Aspein*) and one involved in the organic matrix of both layers (*Nacrein A1*) (Marie et al., 2012a; Marie et al., 2012b; Montagnani et al., 2011). Among these genes, four were significantly regulated by temperature (*Pmarg-Prismalin14*, *Pmarg-Aspein*, *Pmarg-MRNP34* and *Pmarg-Nacrein A1*) and displayed a maximum expression between 21.5 and 26.5°C. Surprisingly, these maximums were all below the optimal temperature for somatic growth and reproduction. However, these regulations are in agreement with those previously reported for *P. margaritifera* (Joubert et al., 2014) and a closely related species, *Pinctada fucata* (Liu et al., 2012).

The differences observed between the bioenergetic thermal optimum and the one calculated for the biomineralization argue in favour of the presence of an antagonistic biological function that is highly thermal-dependent. Among these processes, reproduction in bivalves is well known to be highly correlated with temperature (Moal et al., 2007). This function requires much energy to ensure an optimal gametogenesis resulting in the so-called trade-off mechanism, “the reproductive cost” (Calow, 1979). In *P. margaritifera*, reproduction occurs throughout the year, but presents maximal activity during the warm season (Pouvreau et al., 2000a); the season where shells grow at the slowest rate (Pouvreau et al., 2000b). This correlation would explain the differences observed between the optimum temperature for somatic growth/reproduction and the optimum temperature for biomineralization. Further works will be needed to disentangle all putative confounding effects and to confirm this hypothesis.

4.3 The pearl oyster in front of global warming

One of the most direct effects of global change is the sea surface temperature increase. Indeed, since the beginning of the 20th century, the global ocean temperature has already increased by 0.7°C (Hoegh-Guldberg et al., 2007). The last IPPC report highlighted that, under all scenarios of greenhouse gas emissions for the next century, temperatures will continue to increase, with a higher intensity for tropical areas (IPCC, 2014). The results of our work show that the optimal temperature for *P. margaritifera* somatic growth and reproduction is 28.7°C under our experimental conditions. This result is in agreement with previous work highlighting an optimal temperature range for growth between 23–28°C and 26–29°C for adult and larvae of Australian population of *P. margaritifera*, respectively (Doroudi et al., 1999; Yukihira et al., 2000). The threshold of 28. 7°C is already exceeded annually during the warmer months (121 days per year), during which the growth rate of *P. margaritifera* was shown to decrease (Pouvreau et al., 2000b). Indeed, it is well documented that thermal optimums of tropical marine ectotherms are usually very close to their critical thermal maximums, which explains how close to the edge they are in front of global warming (Somero, 2012). All these data taken together let us hypothesize that major biological functions, such as somatic growth, reproduction and biomineralization, will be annually compromised, or at least significantly slowed, during the next decades. Under the optimistic scenario, RCP2.6 (+1°C), and the medium scenario, RCP4.2 (+1.5°C), pearl oysters in the lagoons of the North Tuamotu archipelago will be confronted to temperatures above their thermal optimum for 210 and 252 days per year, respectively. Alarmingly, this threshold will be exceeded throughout the year in the most pessimistic scenario, the RCP8.5 (+2.5°C).

In the socio-economical context of French Polynesia, these hypotheses and predictions suggest that major scientific works will be needed to sustain pearl production. Future research should be developed to better calibrate the critical thermal maximums of different pearl oyster populations. The battery of “Omics” and physiological tools in association with the power of

next-generation sequencing will be useful to characterise and quantify pearl oyster adaptability throughout the Polynesian archipelago. All these fundamental approaches would enable the identification of the mechanisms of thermotolerance in *P. margaritifera.* This research would provide new management tools such as biomarkers of thermal tolerance that would be used in the emerging genetic selection plan (Ky et al., 2013). In parallel some actions should be undertaken to significantly enhance the chances of natural adaptation in *P. margaritifera* populations. As an example among others, we can mention the needs in management effort aiming to conserve the genetic diversity of *P. margaritifera*, a diversity that had already suffered from the pearl culture activity (Arnaud-Haond et al., 2004; Lemer and Planes, 2012). In every instance, the Austral archipelago, an area identified as a temporary thermal refuge in French Polynesia (Van Hooidonk et al., 2013) would be used to maintain this activity if the temperature becomes a too strong environmental pressure.

5 Acknowledgments

This work was financially supported by the ANR funding POLYPERL project (reference ANR-11-AGRO-006-01-POLYPERL).

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608 # Figures and figure legend

609

610 **Figure 1: Bioenergetic responses**

611 Bioenergetic behaviour after one week exposure to temperature (22, 26, 30 and 34°C) and
612 pCO_2 level (3667 µatm (light grey), 1198 µatm (grey), 426 µatm (dark grey); (A) ingestion
613 rate (IR), (B) respiration rate (RR) (C) scope for growth (SFG)) of the black-lip pearl oyster
614 *Pinctada margaritifera*. Means are presented with standard error (n=4). Lowercases illustrate
615 significant differences between temperatures.

616

617 **Figure 2: Biomineralization response**

618 Effects of seven days exposure to 22, 26, 30 and 34°C on the expression of nine candidate
619 genes involved in biomineralization. *Pmar-Pif-177*, *Pmar-pearlin*, *Pmar-MS160* and *Pmar-*
620 *MRNP34* are involved in nacre. *Pmar-Shematrin 9*, *Pmar-Prismalin14*, *Pmar-Aspein*, and
621 *Pmar-PUSP6* are involved in prism. *Pmar-Nacrein A1* is involved in both minerals.

622

623 **Figure 3: Thermal optimum and future temperature at the horizon of 22th century**

624 Under the current temperature regime (black line; dotted line is the confidence interval at
625 5%), the pearl oyster spends 121 days above its thermal optimum (blue line). Under the RCP
626 scenarios 2.6 (+1°C), this would be 210 days (yellow), 252 days under the RCP4.5 (+1.5°C;
627 orange) and 365 days under the RCP8.5 (+2.5°C; red).

628

Tables

Table 1: Water parameters calculated from samples taken from all experimental trays. The carbonate parameters were calculated using CO_2systat software.

Temperature (°C)	pH	Salinity (‰)	Alkalinity (µmol/kg-SW)	pCO_2 (µatm)	Ωca.	Ωar.
34	7.4	35	2660	3712	1.78	1.21
34	7.8	35	1980	996	3.01	2.05
34	8.2	35	2950	489	9.32	6.33
30	7.4	35	2760	3767	1.62	1.09
30	7.8	35	2730	1366	3.72	2.49
30	8.2	35	1940	317	5.43	3.64
26	7.4	35	2770	3679	1.43	0.94
26	7.8	35	2340	1326	3.25	2.15
26	8.2	36	2870	473	7.44	4.93
22	7.4	35	2730	3510	1.23	0.80
22	7.8	35	2310	1105	2.44	1.60
22	8.2	35	2600	426	6.00	3.92

656 Table 2: Set of forward and reverse primers used for the gene expression analysis.

Gene	GenBank Accession Number	Forward primer	Reverse primer
Pmarg-PIF 177	HE610401	5'-AGATTGAGGGCATAGCATGG-3'	5'-TGAGGCCGACTTTCTTGG-3'
Pmarg-Pearlin	DQ665305	5'-TACCGGCTGTGTTGCTACTG-3'	5'-CACAGGGTGTAATATCTGGAACC-3'
Pmarg-MRNP34	HQ625028	5'-GTATGATGGGAGGCTTTGGA-3'	5'-TTGTGCGTACAGCTGAGGAG-3'
Pmarg-MSI60	SRX022139*	5'-TCAAGAGCAATGGTGCTAGG-3'	5'-GCAGAGCCCTTCAATAGACC-3'
Pmarg-Shematrin	ABO92761	5'-TGGTGGCGTAAGTACAGGTG-3'	5'-GGAAACTAAGGCACGTCCAC-3'
Pmarg-Prismalin	HE610393	5'-CCGATACTTCCCTATCTACAATCG-3'	5'-CCTCCATAACCGAAAATTGG-3'
Pmarg-PUSP6	SRX022139*	5'-TTCATTTTGGTGGTTATGGAATG-3'	5'-CCGTTTCCACCTCCGTTAC-3'
Pmarg-Aspein	SRX022139*	5'- TGAAGGGGATAGCCATTCTTC -3'	5'- ACTCGGTTCGGAAACAACTG -3'
Pmarg-Nacrein A1	HQ654770	5'-CTCCATGCACAGACATGACC-3'	5'-GCCAGTAATACGGACCTTGG-3'

657 *SRA accession number ; EST library published in Joubert et al. 2011

658

659

660 Table 3**:** Two-way ANOVA results for bioenergetic values of seven day exposure to
661 temperature and pCO_2 level (absorption efficiency (AE), ingestion rate (IR), respiration rate
662 (RR), scope for growth (SFG)).

Sources of variation	ddl	AE* (arcsinsqr)		IR (Box Cox)		RR		SFG (Box Cox)	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Temperature	3	0.14	0.94	11.03	**<0.0001**	16.21	**<0.0001**	8.13	**0.0003**
pCO_2	2	1.68	0.20	0.18	0.83	0.67	0.52	0.98	0.38
Temperature x pCO_2	6	3.40	0.10	1.31	0.28	0.28	0.94	0.78	0.59

663

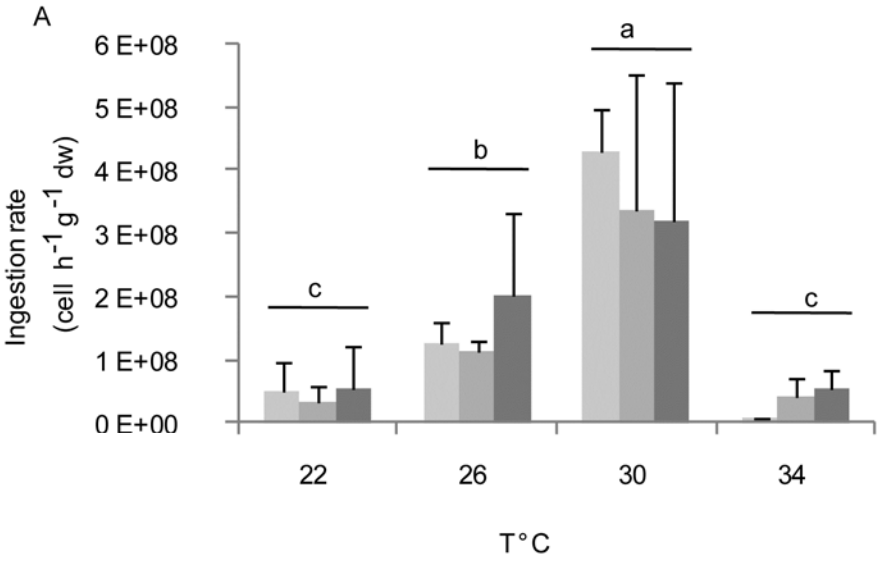
664 Table 4: Significance level of ANOVA and Kruskal Wallis (*) test of biomineralization related gene expression levels according to temperature

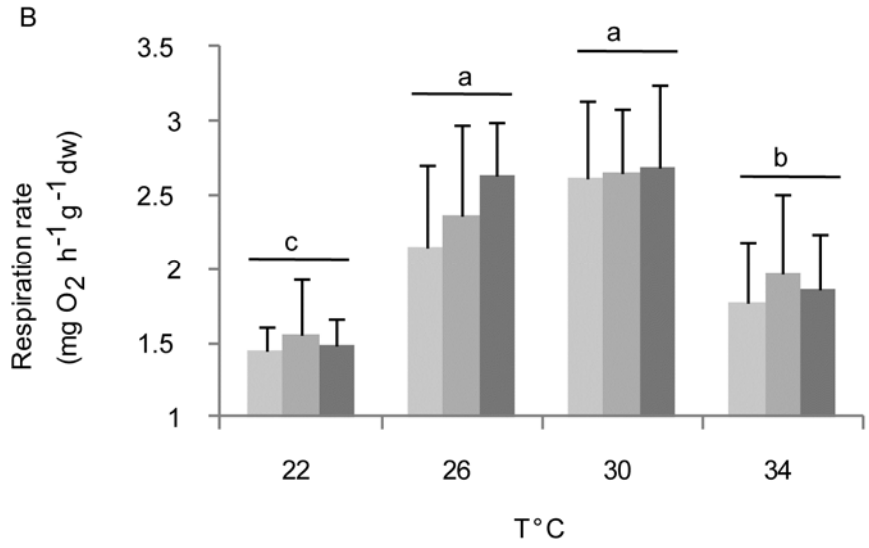
665 levels.

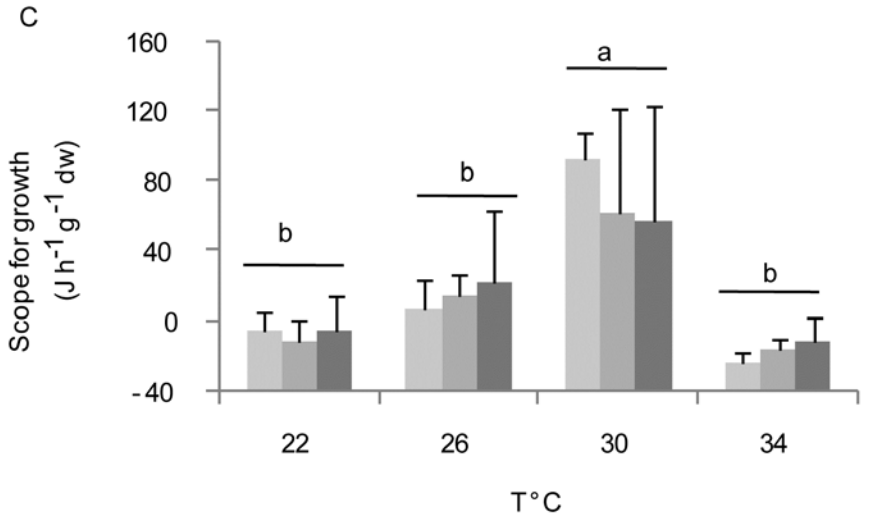
666

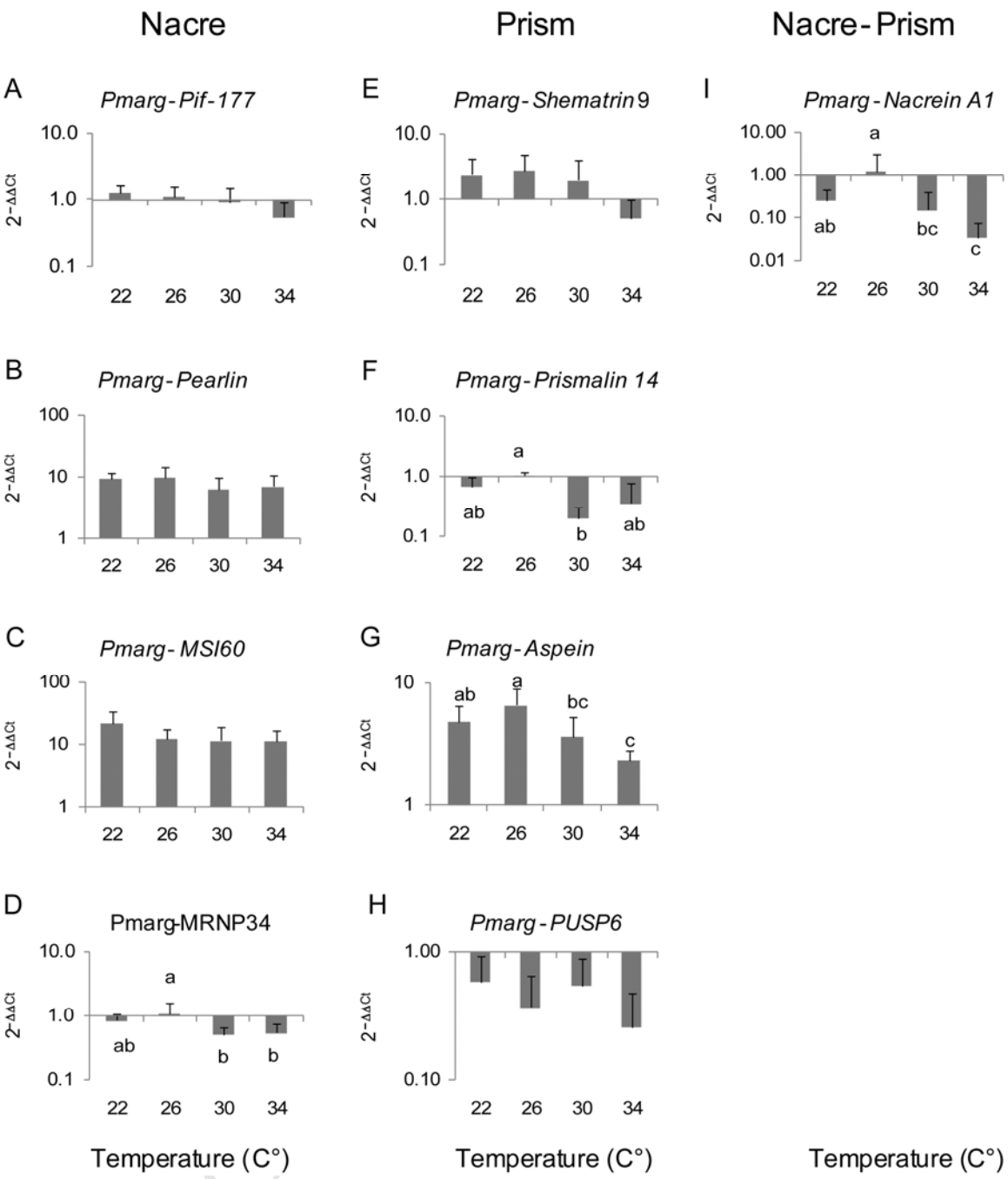
	Pmarg-PIF-177		***Pmarg-Nacrein A1***		***Pmarg-PUSP6***		***Pmarg-Pearlin***		***Pmarg-MRNP34***		***Pmarg-MSI60***		***Pmarg-Shematrin 9***		***Pmarg-Primalin 14***		***Pmarg-Aspein***	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
T°C	1.79	*0.19*	5.15	***0.01***	1.00	*0.42*	0.85	*0.49*	3.16	***0.05***	1.36	*0.29*	1.27	*0.32*	7.28	***0.003***	5.31	***0.01***

667


A
Ingestion rate
(cell h-1 g-1 dw)
6 E+08
5 E+08
4 E+08
3 E+08
2 E+08
1 E+08
0 E+00
a
b
c
c
22
26
30
34
T°C


B
Respiration rate
(mg O2 h-1 g-1 dw)
3.5
3
2.5
2
1.5
1
a
a
b
c
22
26
30
34
T°C


C
Scope for growth
(J h-1 g-1 dw)
160
120
80
40
0
-40
a
b
b
b
22
26
30
34
T°C


Nacre
Prism
Nacre - Prism
A
Pmarg - Pif - 177
E
Pmarg - Shematrin 9
I
Pmarg - Nacrein A1
B
Pmarg - Pearlin
F
Pmarg - Prismalin 14
C
Pmarg - MSI60
G
Pmarg - Aspein
D
Pmarg-MRNP34
H
Pmarg - PUSP6
2-ΔΔCt
Temperature (C°)