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# Effects of temperature and p CO<sub>2</sub> on the respiration, biomineralization and photophysiology of the giant clam *Tridacna maxima*

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1 Effects of temperature and  $p\text{CO}_2$  on the respiration, biomineralization  
2 and photophysiology of the giant clam *Tridacna maxima*

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5 Running title: Climate change effects on *T. maxima*

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22 **Abstract**

23 Such as many other reef organisms, giant clams are today confronted to global change effects and can  
24 suffer mass bleaching or mortality events mainly related to abnormally high seawater temperatures.  
25 Despite its strong ecological and socio-economical importance, its responses to the two most alarming  
26 threats linked to global change (i.e., ocean warming and acidification) still need to be explored. We  
27 investigated physiological responses of 4-years-old *Tridacna maxima* specimens to realistic levels of  
28 temperature and partial pressure of carbon dioxide ( $p\text{CO}_2$ ) (+1.5°C and +800  $\mu\text{atm}$  of  $\text{CO}_2$ ) predicted  
29 for 2100 in French Polynesian lagoons during the warmer season. During a 65-days crossed-factor  
30 experiment, individuals were exposed to two temperatures (29.2°C; 30.7°C) and two  $p\text{CO}_2$  (430  $\mu\text{atm}$ ;  
31 1212  $\mu\text{atm}$ ) conditions. Impact of each parameter and their potential synergetic effect were evaluated  
32 on respiration, biomineralization and photophysiology. Kinetics of thermal and acidification stress were  
33 evaluated by performing measurements at different times of exposure (29, 41, 53, 65 days). At 30.7°C,  
34 the holobiont  $\text{O}_2$  production, symbiont photosynthetic yield, and density were negatively impacted. High  
35  $p\text{CO}_2$  had a significant negative effect on shell growth rate, symbiont photosynthetic yield and density.  
36 Shell microstructural modifications were observed from 41 days in all temperature and  $p\text{CO}_2$  conditions.  
37 No significant synergetic effect was found. Today thermal conditions (29.2°C) appeared to be sufficiently  
38 stressful to induce a host acclimatization process. All these observations indicate that temperature and  
39  $p\text{CO}_2$  are both forcing variables affecting *T. maxima* physiology and jeopardize its survival under  
40 environmental conditions predicted for the end of this century.

41

42 **Keywords:** Thermal stress, ocean acidification, respiration, photosynthetic yield, giant clam, symbionts

## 43 1. Introduction

44 Since the industrial revolution, human activities released gigatons of CO<sub>2</sub> in the atmosphere, which  
45 participate to global change (Broecker et al., 1979; Caldeira and Wickett, 2003; Sabine et al., 2004; Zeebe  
46 et al., 2008; IPCC, 2014). Due to the “greenhouse gas” property of CO<sub>2</sub>, terrestrial and sea surface  
47 temperature rise constantly (i.e., global warming). According to the last Intergovernmental Panel on  
48 Climate Change report (IPCC, 2018), human-induced warming had already reached about +1°C above  
49 pre-industrial levels and the panel predicts +1.5°C around 2040 if the current warming rate continues.  
50 Enrichment of dissolved CO<sub>2</sub> in the ocean modifies the carbonate chemistry by depressing carbonate  
51 ion concentration ([CO<sub>3</sub><sup>2-</sup>]) and releasing protons which respectively decrease calcium carbonate  
52 saturation state (Ω) and seawater pH (Kleypas et al., 1999; Caldeira and Wickett, 2003) (i.e., ocean  
53 acidification). Indeed, pH had already decreased by 0.1 pH unit since the pre-industrial revolution and  
54 the decrease may reach -0.3 to -0.4 pH unit by 2100 (according to the median scenario RCP 4.5, IPCC,  
55 2014).

56 Since CO<sub>3</sub><sup>2-</sup> is a carbonate ion form involved in biologically controlled calcification process (i.e.,  
57 biomineralization) (Orr et al., 2005), marine organisms which form calcium carbonate structures (e.g.,  
58 exoskeleton, shell, test, spicule), such as scleractinian corals, mollusks, and echinoderms are  
59 particularly vulnerable to ocean acidification (Hoegh-Guldberg et al., 2007; Ries et al., 2009; Kroeker et  
60 al., 2013; Gazeau et al., 2013). Among marine calcifying mollusks, negative impacts of temperature and  
61 pCO<sub>2</sub> have been demonstrated on the survival, growth, biomineralization processes, and others key  
62 physiological functions on different stages of their life cycle (Bougrier et al., 1995; Kurihara, 2008;  
63 Gazeau et al., 2010, 2011; Schwartzmann et al., 2011; Rodolfo-Metalpa et al., 2011; Talmage and  
64 Gobler, 2011; Watson et al., 2012; Liu et al., 2012; Kuhihara et al., 2013; Fitzer et al., 2014; Le Moullac  
65 et al., 2016a,b; Wessel et al., 2018). However, effects of each stressor and their potential synergetic  
66 effect on giant clam physiology are still poorly known.

67 Giant clams live in obligatory symbiosis with photosynthetic dinoflagellates of the family  
68 Symbiodiniaceae (Holt et al., 2014; LaJeunesse et al., 2018). Different giant clam species, i.e., *Tridacna*  
69 *maxima*, *Tridacna crocea*, *Tridacna noae* and *Tridacna squamosa*, have been found to associate with  
70 different Symbiodiniaceae genera, i.e., *Symbiodinium* (formerly clade A, LaJeunesse et al., 2018),  
71 *Cladocopium* (formerly clade C, LaJeunesse et al., 2018) and *Durusdinium* (formerly clade D,  
72 LaJeunesse et al., 2018) (Pinzón et al., 2011; DeBoer et al., 2012; Ikeda et al., 2017; Lim et al., 2019).  
73 Hereafter, the symbiotic Symbiodiniaceae are named “symbionts”. In giant clams, symbionts are located  
74 extracellularly inside a tubular system called “Z-tubules” found in the outer epithelium of the mantle and  
75 connected to the stomach (Norton et al., 1992; Holt et al., 2014). These mixotrophic organisms acquire  
76 nutrients from heterotrophic (via seawater filtration) and symbionts photo-autotrophic pathways (Klumpp  
77 et al., 1992; Hawkins and Klumpp, 1995). Nutrients provided by symbionts (such as glucose, Ishikura et  
78 al., 1999) may account for a major part of the clam energy needs depending on the species and life  
79 stage (Trench et al., 1981; Klumpp et al., 1992; Klumpp and Lucas, 1994; Klumpp and Griffiths, 1994;  
80 Hawkins and Klumpp, 1995; Elfving et al., 2002; Yau and Fan, 2012; Holt et al., 2014; Soo and Todd,  
81 2014). Like symbiotic coral species, bleaching events affecting giant clam have been recorded several  
82 times in the Indo-Pacific Ocean (Adessi, 2001; Buck et al., 2002; Leggat et al., 2003; Andréfouët et al.,

83 2013; Junchompoo et al., 2013) mainly due to seawater temperature increase by a few degrees above  
84 the seasonal maximum (Adessi, 2001; Andréfouët et al., 2013). Bleaching process results from the  
85 symbiosis breakdown via the loose of symbiotic Symbiodiniaceae (Buck et al., 2002; Leggat et al.,  
86 2003).

87 In French Polynesia, *Tridacna maxima* species (Röding, 1798) is one of the most emblematic  
88 and patrimonial organisms. They represent an important food resource for inhabitants of remote atolls  
89 and giant clam fishery and sustainable aquaculture activities generate substantial incomes for local  
90 fishermen and farmers (Van Wysberge et al., 2016; Andréfouët et al., 2017). However, wild and  
91 cultivated giant clam stocks are largely threatened by environmental disturbances such as abnormally  
92 high sea surface temperature inducing mass mortality events as reported in Tuamotu atolls by Adessi  
93 (2001) and Andréfouët et al. (2013). More recently, two mass bleaching events occurring in Reao and  
94 Tatakoto (Tuamotu islands) were linked to a prolonged high lagoonal temperature ( $\geq 30^{\circ}\text{C}$  over several  
95 weeks) of these semi-closed atolls (Andréfouët et al., 2017; Fig. S1). All these observations strongly  
96 indicate that temperature is an important stressor for giant clams. Besides the ecological consequences,  
97 giant clam mass bleaching and mortality cause a significant loss of incomes for the islanders.

98 Despite their ecological and socio-economic importance, effects of thermal stress and  
99 acidification on giant clams still need to be investigated. Thermal stress has shown to decrease  
100 fertilization success (Amstrong, 2017) in *Tridacna maxima* and oxygen production and respiration rates  
101 in *T. gigas* and *T. deresa* species (Blidberg et al., 2000). In contrast, an increase of respiration with a  
102 high photosynthetic rate in the holobiont was reported for *T. squamosa* (Elfving et al., 2001). Thermal  
103 stress also reduces the abundance of symbionts in *T. gigas* (Leggat et al., 2003) even after only 12h of  
104 exposure in *T. crocea* (Zhou et al., 2018) and a decrease of photosynthate export from symbionts to the  
105 host (Leggat et al., 2003). In *T. maxima*, heat stress induced changes of fatty acid composition and lipid  
106 pathways and ROS (Reactive Oxygen Species) scavenger overexpression (Dubousquet et al., 2016).  
107 Combined to high light intensities, heat stress caused a decrease of the cell size of remaining symbionts  
108 and of their chlorophyll content (Buck et al., 2002). Recent studies demonstrated that low pH seawater  
109 with high nutrient concentration could have variable effects on growth on four species of *Tridacna* genus  
110 (Toonen et al., 2012). Moreover, Watson (2015) showed that high light irradiance condition limits the  
111 impact of  $p\text{CO}_2$  on shell growth. Regarding the potential synergetic effect of temperature and  $p\text{CO}_2$   
112 parameters, the only study ever carried out showed that ocean warming and acidification may reduce  
113 survival in *T. squamosa* juveniles (Waston et al., 2012). To our knowledge, no study had already  
114 investigated impact of both parameters, and their potential synergetic effect, on several key  
115 physiological parameters of both *Tridacna maxima* host and its symbionts. To fill this gap, our  
116 experimental approach was designed to better understand the physiological mechanisms underlying the  
117 response of giant clams to global climate changes.

118 Based on a 65 days crossed-factor experiment, we investigated physiological responses of 4-years-old  
119 *Tridacna maxima* specimens to temperature and  $p\text{CO}_2$  conditions in the French Polynesian lagoons  
120 during the today warmer season and those predicted for 2100 by the IPCC 2014:  $+1.5^{\circ}\text{C}$  (RCP 4.5  
121 scenario) and  $+800 \mu\text{atm}$  of  $\text{CO}_2$  (RCP 8.5 scenario). Effect of each parameter and their potential  
122 synergetic effect were evaluated on respiration, biomineralization, and photophysiology by analyzing

123 the holobiont O<sub>2</sub> production and respiration, growth rate and ultrastructure of the shell and symbiont  
124 density and photosynthetic yield. In addition, the kinetics of thermal and acidification stress were  
125 accessed by performing analyses at different time of exposure (i.e., 29, 41, 53, 65 days).

126

## 127 **2. Materials and Methods**

### 128 *2.1. Biological material*

129 *Tridacna maxima* specimens of 4-years-old, 5-6 cm height and brownish/dark-green color, were  
130 collected in early January 2016 from the cultivated stock in Reao lagoon (Tuamotu islands) and exported  
131 to the Centre Ifremer du Pacifique in Tahiti where they were acclimatized in outdoor tank with running  
132 seawater. Each individual was placed onto a petri dish on which byssal gland further developed for  
133 fixation. All individuals directly opened up right after their transfer into the outdoor tank. No visual sign  
134 of stress was observed during the acclimation period except for 4 individuals which died within the first  
135 3 days (corresponding to a 2% mortality rate). Specimens used in this study were collected and held  
136 under a special permit (MEI #284) delivered by the French Polynesian government.

137

### 138 *2.2. Experimental design and rearing system*

139 To study the impact of temperature, pCO<sub>2</sub>, and their putative synergetic effect on the physiology of the  
140 giant clams and their symbionts, four experimental conditions were set up by applying two temperatures  
141 (29.2°C and 30.7°C) and two levels of pCO<sub>2</sub> (430 ±22 μatm and 1212 ±35 μatm). The tested conditions  
142 were: (1) control: 29.2°C 430 μatm, (2) acidification stress: 29.2°C 1212 μatm), (3) thermal stress:  
143 30.7°C 430 μatm, (4) acidification and thermal stress: 30.7°C 1212 μatm (Table 1).

144 After a 3 weeks acclimation period in an outdoor tank, 96 clams were randomly distributed in  
145 experimental tanks one week before starting the experiment. For each condition, we used a 500 L tank  
146 containing 4 tanks of 30 L (ecological replicates) renewed at a flow rate of 50 L/h (seawater pumped in  
147 the lagoon). Each 30 L tank contained 6 clams (biological replicates). To avoid physiological shock,  
148 targeted temperature and pCO<sub>2</sub> values were linearly achieved over 7 days. The light was set to obtain  
149 a Photosynthetically Active Radiation (PAR) of 200 ± 20 μmol of photons. m<sup>-2</sup>.s<sup>-1</sup> on a 12 h:12 h light/dark  
150 photoperiod. To evaluate the kinetics of the thermal and/or acidification stress, analyses were performed  
151 at 4 different times of exposure, i.e., 29, 41, 53 and 65 days. In total, 64 clams were used for data  
152 acquisition corresponding to 4 individuals per condition (1 individual per 30 L tank) and per time of  
153 exposure.

154

### 155 *2.3. Monitoring of temperature, pH and water quality*

156 To insure the stability of experimental conditions, temperature and pH parameters were measured twice  
157 a day for each tank at 8:00 am and 4:00 pm using a mercury thermometer (certified ISO 9001, ±0.1°C  
158 accuracy) and a pH-meter Consort P603 (±0.01 accuracy). Total alkalinity (TA) was weekly titrated using  
159 a 0.01 N HCl solution and a titrator (Schott Titroline Easy). Levels of pCO<sub>2</sub> and aragonite saturation  
160 state were calculated from temperature, pH (NBS scale), salinity and mean TA using the CO<sub>2</sub> Systat  
161 software (van Heuven et al., 2009). All parameters including seawater carbonate chemistry are reported  
162 in Table 1.

163

164 *2.4. Holobiont O<sub>2</sub> consumption and production measurements*

165 Giant clams were placed in an ecophysiological measurement system (EMS) to monitor O<sub>2</sub> consumption  
166 and production. The EMS consisted in five open-flow chambers. Four giant clams were individually  
167 placed into 4 chambers while an empty shell was placed into a fifth chamber used as a control. EMS  
168 chambers contained water at the same temperature and pCO<sub>2</sub> conditions as in the experimental tanks.  
169 The light energy and photoperiod conditions were the same as for the acclimation tanks. Flow rate in all  
170 chambers was constantly maintained at 12 L.h<sup>-1</sup>. Each chamber was equipped with a two-way  
171 electromagnetic valve activated by an automaton (FieldPoint National Instruments). When the electro-  
172 valve was opened, the water released from the chamber was analyzed for 3 min using an oxygen sensor  
173 (OXI 538, Cellox 325, WTW, Weilheim, Germany) to quantify dissolved oxygen. Oxygen measurements  
174 were performed over 48 h. The first 8 hours of measurement were discarded due to the animal  
175 acclimatization to the chamber. In each chamber, the cycle was completed within 3 min: the first 2 min  
176 served to stabilize the measurement and an average of oxygen data was performed on the last minute  
177 of acquisition. This cycle was followed by another time frame of 3 min in the control chamber following  
178 the sequence: specimen #1, control, specimen #2, control, specimen #3, control, specimen #4, control.  
179 Respiration rate (RR) and production rate (PR) were calculated from data obtained during night- and  
180 day-time, respectively, using differences in oxygen concentrations between the control and experimental  
181 chambers. RR and PR = V(O<sub>1</sub> - O<sub>2</sub>), with O<sub>1</sub> the oxygen concentration in the control chamber, O<sub>2</sub> the  
182 oxygen concentration in the experimental chamber, and V the water flow rate. RR and PR data were  
183 normalized to tissue dry weight. Once normalized, the terminology becomes O<sub>2</sub> consumption for RR  
184 and O<sub>2</sub> production for PR, both expressed in mg O<sub>2</sub>.h<sup>-1</sup>.g<sup>-1</sup> dry weight.  
185 After O<sub>2</sub> production and O<sub>2</sub> consumption analyses were completed, a piece of the mantle was dissected  
186 for further symbiont fluorescence and density analyses (see section 2.5). The remaining soft tissues  
187 were frozen and lyophilized for RR and PR data normalization.

188

189 *2.5. Fluorescence and density measurements of symbionts*

190 Potential effect of temperature and pCO<sub>2</sub> conditions on the photophysiology of the symbionts was  
191 studied by comparing fluorescence yield of photosystem II between all experimental conditions. After  
192 clams were sacrificed, a 1 cm x 2 cm mantle fragment was dissected. The tissue fragment was gently  
193 swiped using tissue paper to remove a maximum of mucus and symbionts were collected by doing 5  
194 smears using a sterilized razor blade. Collected symbionts were diluted into 5 mL of 0.2 µm filtered-  
195 seawater and placed at the obscurity for 10 min to inactivate the photosystem II before light excitation.  
196 Samples were homogenized and 3 ml of the homogenate were collected, placed into a quartz-glass  
197 cuvette and analyzed with AquaPen fluorometer (APC-100, Photon System Instruments®, Czech  
198 Republic) at a 450 nm wavelength. The fluorescence at the steady state (F<sub>0</sub>) and the maximal  
199 fluorescence in the light (F<sub>m</sub>) were measured. The apparent quantum yield of photosynthesis (Y)  
200 reflecting the efficiency of photosystem II in the light acclimated state (Hoogenboom et al., 2006) was  
201 calculated according to equation (Eqn 1).

202

$$Y = \frac{(F_0 - F_m)}{F_m} \quad (1)$$



203 In addition, symbiont densities were evaluated from mantle fragments. For each individual, a circular (5  
204 mm diameter) piece of mantle was collected using a punch. The piece was weighted, grounded in 0.2  
205  $\mu\text{m}$  filtered seawater and homogenized. Then, 20  $\mu\text{L}$  of the tissue extract were immediately collected  
206 and placed into Mallassez cells for symbiont counting under optical microscope. For each sample,  
207 counting was performed on four replicates (3 columns per replicate). Data are expressed in number of  
208 symbionts/mg of mantle tissue.

209

#### 210 *2.6. Calcein labelling for evaluating daily shell extension rate*

211 To study the impact of temperature and  $p\text{CO}_2$  on shell growth rate, the mineralization front of giant clams  
212 was marked using calcein fluorochrome which is irreversibly precipitated at the  $\text{CaCO}_3$  mineralization  
213 site. Before the experiment starts, giant clams were immersed in a 100 mg/L calcein solution (Sigma  
214 Aldrich) (calcein diluted in 1  $\mu\text{m}$  filtered-seawater) for 8 h in the dark. During the labelling procedure, the  
215 bath of calcein solution was aerated using bubblers and a water current was created via pumps. Calcein-  
216 labelled specimens were then placed into the experimental tanks. At the end of the experiment, for each  
217 individual, a 5 mm thick section was cut along the maximal shell growth axis through the right valve  
218 using a Swap Top Inland<sup>®</sup> diamond saw. All sections obtained were polished and observed under  
219 epifluorescence ( $\lambda_{\text{excitation}} = 495 \text{ nm}$ ,  $\lambda_{\text{emission}} = 515 \text{ nm}$ ) with a Leitz Dialux<sup>®</sup> 22 microscope. The distance  
220 between the fluorescent calcein mark and the edge of the shell formed during the experiment was  
221 measured following the maximal growth direction. Daily shell extension rate (expressed in  $\mu\text{m}/\text{day}$ ) was  
222 obtained by dividing the measured distance by the number of days of incubation in experimental  
223 conditions.

224

#### 225 *2.7. Shell scanning electron microscopy study*

226 To characterize temperature and  $p\text{CO}_2$  effect on the shell ultrastructure, a scanning electron microscopy  
227 (SEM) study was carried out. For each individual, a 10 mm thick shell section was cut facing the section  
228 used for calcein observations. The section was then fractured along the width, 2 cm below the growing  
229 part of the shell (marginal part), using a small chisel and a hammer. Then, the apical fragment was  
230 longitudinally fractured and one piece was sonicated in tap water for 10 s, air-dried and an additional  
231 drying was done overnight at 35°C. Sample was placed on a stub covered with carbon tape, gold-coated  
232 and observed at 15 kV using a Hitachi TM3030 SEM at the Université de la Polynésie française. For  
233 each condition, and for each time of exposure, 2 samples were selected based on their daily extension  
234 rate, i.e., samples showing the lowest and the highest rate. To evaluate the impact of temperature and/or  
235  $p\text{CO}_2$  on the shell ultrastructure, SEM observations were performed for each specimen in two different  
236 zones of the crossed-lamellar outer layer (Pätzold et al., 1991). Zone 1 corresponds to the shell formed  
237 *in situ* (i.e., in the shell region located before the calcein mark) while zone 2 corresponds to the shell  
238 formed during the experiment. Observations were made at the ultrastructural level and focused on the  
239 aspect and integrity of the lamellae of the crossed-lamellar outer layer.

240



## 241 2.8. Statistical analyses and data processing

242 Normality of data distribution and homogeneity of variance were tested with the Shapiro-Wilk test and  
243 the Bartlett test, respectively. Production and consumption of O<sub>2</sub> data followed the conditions of  
244 application of parametric tests, but photosynthetic yields and symbiont densities were transformed using  
245 Box Cox transformation while shell extension rates were square root to meet these conditions.  
246 Comparisons were done using a three-way ANOVA with interaction where factors were time of  
247 exposure, temperature and pCO<sub>2</sub>. Tukey post hoc comparisons were done at  $\alpha=0.05$  for all analyses.  
248 Correlations between physiological parameters were tested using Pearson method with a threshold of  
249  $r=0.25$  ( $\alpha=0.05$ ).

250 For all physiological parameters, i.e., O<sub>2</sub> respiration, O<sub>2</sub> consumption, symbiont photosynthetic yield and  
251 density, means ( $\pm$ s.d.) were calculated based on the 4 biological replicates for each condition and each  
252 time of exposure. For the daily shell extension rate, means ( $\pm$ s.d.) were calculated for each condition  
253 and each time of exposure.

254

## 255 3. Results

### 256 3.1. Effect of temperature and pCO<sub>2</sub> on the holobiont oxygen balance

257 For all sampling time (i.e., 29, 41, 53, 65 days), a cyclic pattern of O<sub>2</sub> production and consumption is  
258 observed following the circadian cycle (Fig. 1). This pattern corresponds to oxygen photosynthetically  
259 produced and heterotrophically consumed by the holobiont during the day and the night, respectively.  
260 Mean values of normalized O<sub>2</sub> production and consumption are shown in Fig. 2. These values were  
261 analyzed using a three-way ANOVA with 3 fixed factors: temperature, pCO<sub>2</sub>, and the time of exposure  
262 (Table 2). Tukey post hoc test results are reported in Table 3. The ANOVA indicates that oxygen  
263 production of the holobiont during day-time is significantly altered at 30.7°C ( $P=0.009$ ) but not at 1212  
264  $\mu$ atm of CO<sub>2</sub> ( $P=0.361$ ) (Table 2). In addition, O<sub>2</sub> production is higher at 29.2°C than at 30.7°C for all  
265 times of exposure (Fig. 2A) and decreases along the experiment ( $P=0.030$ ) (Table 2). The night-time O<sub>2</sub>  
266 consumption, however, is not significantly influenced by temperature ( $P=0.590$ ) neither by pCO<sub>2</sub>  
267 ( $P=0.361$ ) nor by time of exposure ( $P=0.533$ ) and remains stable along the whole experiment (Fig.  
268 2A,B). No interaction effect between the 3 tested factors is found to affect the holobiont oxygen balance  
269 (Table 2).

270

### 271 3.2. Symbiont density and photosynthesis under different T°/pCO<sub>2</sub> conditions

272 After two months of exposure, the symbiont photosynthetic yield and density are significantly impacted  
273 at 30.7°C ( $P<0.0001$ ), high pCO<sub>2</sub> (1212  $\mu$ atm) ( $P<0.0001$  and  $P=0.05$ , respectively), and by the time of  
274 exposure ( $P=0.021$  and  $P=0.001$ , respectively). During the whole experiment and for both pCO<sub>2</sub>  
275 conditions, the means of photosynthetic yield (Fig. 2C) and symbiont density (Fig. 2D) tend to be higher  
276 at 29.2°C than at 30.7°C. However, an inter-individual variability is observed.

277 Concerning the interaction parameters, only interaction between temperature and time of exposure  
278 parameters has a significant effect on the symbiont photosynthetic yield ( $P=0.025$ ) (Table 2). No  
279 parameter interaction is found to affect the symbiont density (Table 2).

280

281 *3.3. Negative effect of pCO<sub>2</sub> on daily shell extension rate*

282 Calcein mark was detectable in 55 over 64 shell sections. Statistical analyses on shell extension rate  
283 data indicate that pCO<sub>2</sub> and the time of exposure have a significant effect on the shell extension rate  
284 ( $P=0.010$  and  $P=0.007$ , respectively) (Table 2). In the Fig. 2E, the mean values of shell extension rates  
285 tend to be lower at high pCO<sub>2</sub>.

286

287 *3.4. Relationship between physiological parameters*

288 To establish if relationships exist between the various physiological parameters measured, a correlation  
289 matrix was generated (Table 4). The photosynthetic yield of symbionts is strongly correlated to their  
290 density ( $r=0.667$ ). O<sub>2</sub> production is also strongly correlated to symbiont density ( $r=0.482$ ) and  
291 photosynthetic yield ( $r=0.331$ ). Concerning the circadian functioning of the holobiont, its nocturnal  
292 oxygen need is strongly correlated to the diurnal O<sub>2</sub> production ( $r=0.629$ ). No significant correlation is  
293 found between shell extension rate and other physiological parameters.

294

295 *3.5. Effect of long-term exposure to temperature and pCO<sub>2</sub> on the shell microstructure*

296 Ultrastructural observations of shell fractures were done in two zones: zone 1 corresponding to the shell  
297 formed *in situ* (i.e., before the experiment) and zone 2 corresponding to the shell formed in the  
298 experimental conditions. The lamellae formed before the experiment (zone 1) are well-cohesive and  
299 display an elongated shape, a smooth surface, and sharp or slightly rounded outlines (Fig. 3A,B,C). For  
300 all experimental conditions, no difference between zone 1 and 2 is observed for all samples exposed for  
301 29 days (Fig. 3A,D). From 41 days, the lamellae observed in the majority of shells formed during the  
302 experiment (zone 2), in all temperature/pCO<sub>2</sub> conditions, appeared less cohesive with a pronounced  
303 granular aspect of the surface (Fig. 3E,H) and/or less cohesive lamellae with a crazed-like aspect (Fig.  
304 3F,I). Two states classifying ultrastructural differences between the shell formed before and during the  
305 experiment were defined as follow: *i*) ND; no difference observed between zone 1 and 2, *ii*) D; difference  
306 observed between zone 1 and 2. In total, 19 samples over 24 displayed differences in lamellae aspect  
307 between both zones. Data are reported in Table 5.

308

309 *3.6. Effect of time of exposure on oxygen balance, biomineralization and symbionts photophysiology*

310 Time of exposure to thermal and acidification stress was shown to have a significant impact on O<sub>2</sub>  
311 production ( $P=0.030$ ), photosynthetic yield ( $P=0.021$ ), symbiont density ( $P=0.001$ ) and shell growth rate  
312 ( $P=0.007$ ) (Table 2). Post hoc tests showed that the kinetics of thermal or acidification stress varies  
313 depending on the physiological parameter measured: O<sub>2</sub> production and photosynthetic yield are  
314 significantly different between 29 and 65 days while symbiont density and shell growth rate are both  
315 impacted from 53 days of exposure (Table 3).

316

#### 317 4. Discussion

318 In this present study, we investigated the physiological responses of *Tridacna maxima* (i.e., 4-years-old  
319 specimens) to temperature and  $p\text{CO}_2$  conditions in the French Polynesian lagoons under nowadays  
320 warmer season conditions and those predicted for 2100 by the IPCC 2014 (+1.5°C and +800  $\mu\text{atm}$  of  
321  $\text{CO}_2$ ). Today thermal conditions are already and sufficiently stressful to induce an acclimatization  
322 process pictured by a slight regulation of the symbiont density without the collapse of the photosynthesis.  
323 However, if in the today conditions the giant clams are able to mount this acclimatory and efficient  
324 response that enable to maintain their key physiological characteristics, the thermal and  $p\text{CO}_2$  conditions  
325 of tomorrow appear to be much more stressful and sufficient to induce strong disturbances of the two  
326 key functional compartments of the giant clam, its phototrophic symbiosis and its biomineralization.  
327 Indeed, the 30.7°C treatments have significantly impacted the holobiont by inducing a strong bleaching  
328 response illustrated by the reduction of its  $\text{O}_2$  production, symbiont density and photosynthetic yield.  
329 High  $p\text{CO}_2$  (+800  $\mu\text{atm}$  of  $\text{CO}_2$ ) was shown to alter the symbiont photosynthetic yield and density and to  
330 affect its biomineralization process by decreasing the shell growth rate.

331

##### 332 4.1. No synergetic effect between temperature and $p\text{CO}_2$ on giant clam and symbiont physiological 333 parameters

334 Increase of atmospheric  $\text{CO}_2$  impacts both the seawater temperature and pH inducing ocean warming  
335 and acidification (Sabine et al., 2004, IPCC, 2014). Therefore, the analysis of the synergetic effect of  
336 both stressors on marine bivalve physiology is crucial. Watson et al. (2012) investigated the synergetic  
337 effect of ocean acidification and warming on the survival of *T. squamosa* juveniles over a 60 days  
338 crossed-factor experiment. They reported that the combination of the highest  $p\text{CO}_2$  (+600  $\mu\text{atm}$  of  $\text{CO}_2$ )  
339 and both moderate and highest seawater temperatures (30.0°C and 31.5°C) resulted in the lowest  
340 survival rate of giant clams (<20%). They concluded that increased ocean  $p\text{CO}_2$  and temperature are  
341 likely to reduce the survival of *T. squamosa* juveniles. In our study, no synergetic effect was detected  
342 for all measured physiological parameters on *T. maxima*. Moreover, no mortality was observed in all  
343 tested temperature/ $p\text{CO}_2$  conditions during the whole experiment which lead us to suggest that a  
344 temperature of 30.7°C and a  $p\text{CO}_2$  of 1212  $\mu\text{atm}$  seem to be non-lethal, at least over 65 days of  
345 exposure. However, we cannot exclude that some specimens, especially the totally bleached  
346 individuals, may have died after a longer exposure to the experimental conditions.

347

##### 348 4.2. Effect of temperature on holobiont oxygen balance, symbiont photophysiology and shell 349 ultrastructure

350 Temperature is a factor well-known to influence several physiological parameters in bivalves as it  
351 regulates the metabolism of ectotherm organisms (Aldridge et al., 1995, Bougrier et al., 1995, Watson  
352 et al., 2012, Gazeau et al., 2013; Le Moullac et al., 2016a; Latchère et al., 2017). However, since giant  
353 clams live in symbiosis with photosynthetic symbionts (Klumpp et al., 1992; Klumpp and Griffiths, 1994;  
354 Soo and Todd, 2014), the comparison at the metabolic level with non-symbiotic bivalves can be  
355 audacious. Discussion on elevated temperature effect on the holobiont oxygen balance should therefore  
356 integrate the effect on both giant clam and its symbionts (Jones and Hoegh-Guldberg, 2001). Even

357 though physiological and molecular response to thermal stress may differ, the comparison with  
358 scleractinian corals, which are also symbiotic and calcifying organisms, makes sense to better  
359 understand the impact of temperature on the holobiont physiology.

360 We observed that high temperature (+1.5°C) significantly reduces the holobiont O<sub>2</sub> production, the  
361 density and the photosynthetic yield of symbionts from 29 days of exposure. Additionally, partial or total  
362 bleaching was observed for the majority of individuals exposed to 30.7°C (at both ambient and high  
363 pCO<sub>2</sub>, Fig. S2). This suggests that thermal stress has a significant impact on the holobiont  
364 photophysiology. The reduction of the symbiont photosynthetic yield could reflect a photoinhibition.  
365 Photoinhibition was previously linked to the degradation of the D1 protein of the reaction center of the  
366 photosystem II (PSII) altering the photosynthetic apparatus functioning in symbiotic corals and sea  
367 anemone subjected to thermal stress (Warner et al., 1999; Smith et al., 2005; Richier et al., 2006;  
368 Ferrier-Pagès et al., 2007). Diminution of photosynthetic activity reduces the symbiont O<sub>2</sub> production  
369 and consequently, impacts the holobiont O<sub>2</sub> production. Comparable results were obtained from a 24-  
370 hour experiment showing that an increase of 3°C affects the oxygen production of *Tridacna gigas* and  
371 *Tridacna deresa* (Blidberg et al., 2000). Photoinhibition led to the production of reactive oxygen species  
372 (ROS) by symbionts which are known to pass through cellular membranes, cause oxidative damages  
373 (Lesser et al., 1990; Lesser, 1996; Downs et al., 2002) and impact the photosystem II (Richter et al.,  
374 1990). Considering corals, a strong positive correlation between accumulation of oxidative damage and  
375 coral bleaching was shown (Downs et al., 2002). Indeed, ROS such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) may  
376 play a role of signaling molecule activating the symbiosis dissociation (Fitt et al., 2001; Smith et al.,  
377 2005; Perez and Weis, 2006). Expulsion of symbionts by the host may be a strategy to limit oxidative  
378 stress and damage to ultimately survive environmental stress (Downs et al., 2002; Perez and Weis,  
379 2006). Two recent works support this hypothesis. One of Zhou et al. (2018) which related a decrease in  
380 symbiont density in response to an excess of oxidative stress in thermally stressed *T. crocea*. A second  
381 of Dubousquet et al. (2016) which showed that *T. maxima* overexpressed genes encoding ROS  
382 scavengers in response to thermal stress. Interestingly, the cellular and molecular mechanisms  
383 enhanced upstream the bleaching response seem similar between giant clams and corals and conduct  
384 to the same phenomenon of symbiosis dissociation. However, since giant clams and corals display an  
385 extra- and an intra-cellular symbiosis respectively, the mechanisms of the symbiosis dissociation must  
386 differ. In every instance, this constitutes an interesting example of evolutionary convergence of a stress  
387 response in two very distant organisms. In this context, it would be interesting to test if the bleaching  
388 response in giant clam can be adaptive as it was proposed for corals (Fautin and Buddemeier, 2004)  
389 and if variations in giant clam thermo-tolerance are correlated to the composition of the symbiotic  
390 population (Baker, 2003).

391 Concerning the effect of temperature on the ultrastructure of the crossed-lamellar structure, the lamellae  
392 formed in all experimental conditions in the first 29 days are well-cohesive with elongated shape and a  
393 granular aspect which is consistent with the description made by Agbaje et al. (2017) in *T. deresa*.  
394 However, after 29 days, the lamellae observed in the majority of shells formed in both temperature  
395 conditions (whatever the pCO<sub>2</sub> condition) appeared to be less-cohesive with pronounced granular  
396 aspect. We suggest that these features are due to a lack of organic matrix between the lamellae (i.e.,

397 inter-lamellar organic matrix) and embedding the nano-grains forming the first-order lamellae in the  
398 crossed-lamellae structure (as described by Agbaje et al., 2017). Indeed, the biomineralization of  
399 calcium carbonate structures involved the transport of ions (calcium, bicarbonates and protons) to the  
400 mineralization site and the synthesis of macromolecules referred to as “organic matrix” (Allemand et al.,  
401 2004). The organic matrix, consisting of 0.9% weight of *T. deresa* shell, and is mainly composed of  
402 assemblage of macromolecules such as polysaccharides, glycosylated, and unglycosylated proteins  
403 and lipids (Agbaje et al., 2017). These macromolecules are commonly found in molluscan shell organic  
404 matrix (Marin et al., 2012) present as inter-crystalline envelope and around individual crystal units  
405 (Harper, 2000). The formation of these macromolecules is energetically costly for mollusks such as  
406 marine gastropods (Palmer, 1992). According to the conclusion made by Milano et al. (2016) in the non-  
407 symbiotic bivalve *Cerastoderma edule*, we suggest that in our tested experimental temperature, while  
408 respiration function is significantly altered, less energy is available and allocated to the synthesis of  
409 macromolecules involved in biomineralization which may explained the lack of organic matrix embedded  
410 into the giant clam shell.

411

#### 412 4.3. Effect of $p\text{CO}_2$ on giant clam biomineralization and symbiont photophysiology

413 Ocean acidification is related to an increase of dissolved  $\text{CO}_2$  in the seawater resulting in a modification  
414 of carbonate chemistry equilibrium and a decrease of seawater pH. This phenomenon is known to affect  
415 biomineralization process of marine calcifiers since the production of biocarbonate structures depends  
416 on both calcium carbonate saturation state and on the ability to express proteins involved in  
417 biomineralization (Fitzer et al., 2014). The  $p\text{CO}_2$  is a well-known parameter to influence mollusk  
418 calcification process in terms of growth rate, expression of gene encoding proteins involved in  
419 biomineralization, and to alter the integrity of biocarbonate structures (Welladsen et al., 2010; Melzner  
420 et al., 2011; Fitzer et al., 2014; Le Moullac et al., 2016b), even if counter examples exist (Ries et al.,  
421 2009; Kroeker et al., 2010).

422 Concerning giant clams, our results indicate that in high  $p\text{CO}_2$  condition (+800  $\mu\text{atm}$ ), shell extension  
423 rate decreases significantly. This observation is in accordance with those obtained for giant clams in  
424 three different experiments using various  $\text{CO}_2$  enrichment level and exposure duration. Waters (2008)  
425 and Watson (2015) have exposed juveniles *T. squamosa* to +200  $\mu\text{atm}$  of  $\text{CO}_2$  for 13 weeks and to +250  
426 and +550  $\mu\text{atm}$  of  $\text{CO}_2$  for 8 weeks, respectively. Toonen et al. (2012) demonstrated that young  
427 specimens of *T. maxima* and *T. squamosa* have lower shell growth rates, compared to those reported  
428 in the literature under natural  $p\text{CO}_2$ /pH conditions, when kept 1 year in +350 to +1000  $\mu\text{atm}$   $p\text{CO}_2$   
429 conditions.

430 As mentioned above, high  $p\text{CO}_2$  condition (+800  $\mu\text{atm}$ ) affects giant clam shell growth rate but also  
431 symbiont photosynthetic yield and density. Negative effect of  $p\text{CO}_2$  on the shell growth rate may be  
432 linked to (1) the aragonite saturation state which is lower at 1212  $\mu\text{atm}$  than at 430  $\mu\text{atm}$  and/or (2) an  
433 alteration of symbiont photophysiology leading to a potential reduction of the ‘light-enhanced  
434 calcification’ (LEC). Concerning the former, decline of coral calcification rate has been related to



435 modifications of carbonate chemistry due to a limitation of  $\text{CO}_3^{2-}$  ions available for calcification. Even if  
436 corals may upregulate the pH at their mineralization site (Venn et al., 2013; Holcomb et al., 2014), the  
437 decrease of coral calcification was also linked to a decline in pH in the calcifying fluid due to a lower  
438 seawater pH (Ries et al., 2011; McCulloch et al., 2012). In our case, we suggest that giant clam *T.*  
439 *maxima* exposed to high  $p\text{CO}_2$  may allocate more energy to maintain a proper pH of the fluid in the  
440 extra-pallial space for nucleation and deposition of aragonite since the  $\Omega_{\text{aragonite}}$  at high  $p\text{CO}_2$  is twice  
441 lower than at ambient  $p\text{CO}_2$  ( $\Omega_{\text{aragonite}, 1212 \mu\text{atm}} = 2$ ,  $\Omega_{\text{aragonite}, 430 \mu\text{atm}} = 4$ ). Regarding the light-enhanced  
442 calcification (LEC) (Vandermeulen et al., 1972), this phenomenon observed in Symbiodiniaceae-host  
443 symbiosis has been extensively described in corals and represents the capacity of symbionts to  
444 stimulate the host calcification (Allemand et al., 2004). The symbionts stimulate the host metabolism  
445 and calcification by providing energy resources and/or  $\text{O}_2$  (Chalker and Taylor, 1975). Moreover,  
446 symbionts may promote the aragonite precipitation by providing inorganic carbon, nitrogen and  
447 phosphorus and by synthesizing molecules used as precursor for the synthesis of skeletal organic matrix  
448 (Pearse and Muscatine, 1971; Cuif et al., 1999; Furla et al., 2000; Muscatine et al., 2005). They also  
449 facilitate  $\text{CaCO}_3$  precipitation by influencing the Dissolved Inorganic Carbon (DIC) equilibrium by  
450 removing the  $\text{CO}_2$  via photosynthesis (Goreau, 1959). Such phenomenon has been also described in  
451 giant clams (Ip et al., 2006; Ip et al., 2017). In *T. squamosa*, LEC increases the pH and reduces the  
452 ammonia concentration at the interface between the inner mantle and the shell in the extra-pallial fluid,  
453 where the biomineralization occurs (Ip et al., 2006). Recently, Chew et al. (2019) reported a light-  
454 enhanced expression of a carbonic anhydrase (i.e., CA4-like) in the inner mantle of *T. squamosa* and  
455 suggested that this enzyme is involved in giant clam biomineralization by catalyzing the conversion of  
456  $\text{HCO}_3^-$  to  $\text{CO}_2$ . In this context, an altered photophysiology of the symbiont can rationally alter LEC and  
457 consequently results in a decrease of the shell growth rate. Finally, one can suggest that under  
458 acidification stress, giant clam may reduce or stop some physiological functions such as  
459 biomineralization and allocate more energy to essential functions to its survival. In our study,  
460 temperature also altered photophysiology and holobiont  $\text{O}_2$  production, but did not significantly affect  
461 shell growth rate. Therefore, the most plausible hypothesis explaining the negative effect of  $p\text{CO}_2$  on  
462 shell growth rate may be related to the low aragonite saturation state at high  $p\text{CO}_2$ .

463 Concerning the effect of  $p\text{CO}_2$  on the shell microstructural integrity, in the temperate bivalve *Mytilus*  
464 *edulis*, an exposition to +150, +350 and +600  $\mu\text{atm}$  of  $\text{CO}_2$  for 6 months induced a disorientation in the  
465 shell of the newly formed calcite crystals of the prismatic layer (Fitzer et al., 2014). In the giant clam *T.*  
466 *maxima*, high  $p\text{CO}_2$  had no negative effect on the integrity of the aragonitic lamellae of the crossed-  
467 lamellar layer during the first 29 days of exposure. From 41 days of exposure, its potential impact  
468 remains unresolved as differences were noticed in the shells formed under future high temperature, high  
469  $p\text{CO}_2$  and even under today high temperature/ $p\text{CO}_2$  conditions. The fact that differences are reported  
470 for shells formed in all experimental conditions from 41 days suggests that they may be due to a long-  
471 term exposure to 29.2°C and 30.7°C.

472

473 This study enables the evaluation of *Tridacna maxima* physiological responses to temperature  
474 and  $p\text{CO}_2$  thresholds predicted at the end of this century. We demonstrated that high temperature has  
475 a significant negative impact on symbiont densities and photosynthetic capacities, which induce a  
476 decrease in holobiont  $\text{O}_2$  production rate. We suggest that the observed decrease of holobiont  $\text{O}_2$   
477 production rate results from a decrease of symbiont  $\text{O}_2$  production. Therefore, by influencing symbiont  
478 physiology, the temperature may affect the energetic needs of the giant clam host. The high  $p\text{CO}_2$  has  
479 a negative impact on shell growth rate, symbiont densities and photosynthetic capacities. Shell  
480 microstructure is affected neither by temperature nor by the  $p\text{CO}_2$  in the first 29 days of exposure.  
481 However, for all temperature/ $p\text{CO}_2$  conditions, a longer exposition ( $\geq 41$  days) modified the shell  
482 ultrastructure. These observations support our hypothesis that  $29.2^\circ\text{C}$  is a temperature that already  
483 affects giant clam metabolism, at least over a long-term exposition. However, no synergetic effect was  
484 found between temperature and  $p\text{CO}_2$  parameters. All these observations suggest that temperature and  
485  $p\text{CO}_2$  influence different physiological functions and that giant clam populations may dramatically suffer  
486 from the temperature and  $p\text{CO}_2$  conditions predicted for the next decades. To complement these results  
487 on the effects of temperature on the holobiont, it is now essential to conduct further and integrative  
488 analyses. This may include the definition of thermal optimum of *T. maxima* and the study of the  
489 transcriptomic response of the giant clam and its symbiotic Symbiodiniaceae to thermal stress for  
490 instance. All these analyses will allow a better understanding of the fundamental physiological processes  
491 of the holobiont and its response to future changes. Attempted results may also help in adapting local  
492 policies and management to maintain a sustainable exploitation of giant clam resource, especially in the  
493 Eastern Tuamotu islands where bleaching events have been observed at an increasing and alarming  
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495

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504

#### 505 **Competing interests**

506 The authors declare no competing interests.

507

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511



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782 **Figure and table legends**

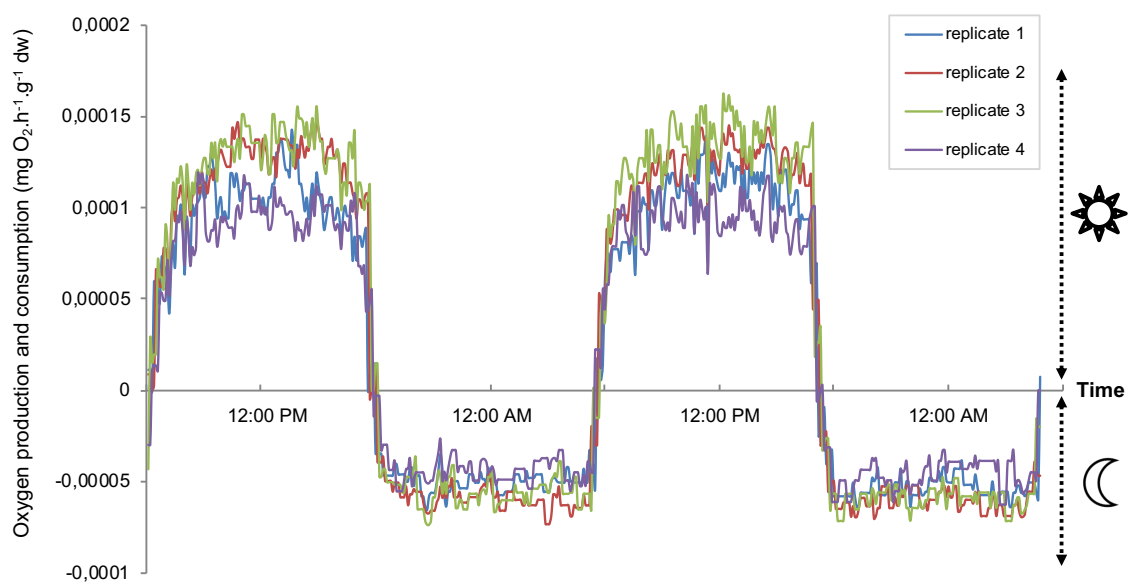
783

784 **Table 1. Measured and calculated parameters of seawater for all treatments.** Total alkalinity is  
785 given in mean ( $\pm$ s.d.) based on weekly measurements for each experimental tank corresponding to  
786  $n=36$  for each condition.  $p\text{CO}_2$  and  $\Omega_{\text{aragonite}}$  were calculated using the CO<sub>2</sub> Systat software.

787

Treatments	Temperature (°C)	$p\text{CO}_2$ ( $\mu\text{atm}$ )	$\text{pH}_{\text{NBS}}$	Salinity (‰)	Total alkalinity ( $\mu\text{mol/kg SW}$ )	$\Omega_{\text{aragonite}}$
Control	29.2 ( $\pm 0.1$ )	428 ( $\pm 21$ )	8.19 ( $\pm 0.01$ )	35	2422 ( $\pm 170$ )	4.17
Thermal stress	30.7 ( $\pm 0.1$ )	431 ( $\pm 25$ )	8.19 ( $\pm 0.01$ )	35	2409 ( $\pm 157$ )	4.14
Acidification stress	29.2 ( $\pm 0.1$ )	1210 ( $\pm 41$ )	7.81 ( $\pm 0.03$ )	35	2466 ( $\pm 90$ )	1.99
Acidification and thermal stress	30.7 ( $\pm 0.1$ )	1213 ( $\pm 29$ )	7.81 ( $\pm 0.03$ )	35	2423 ( $\pm 110$ )	2.02

788



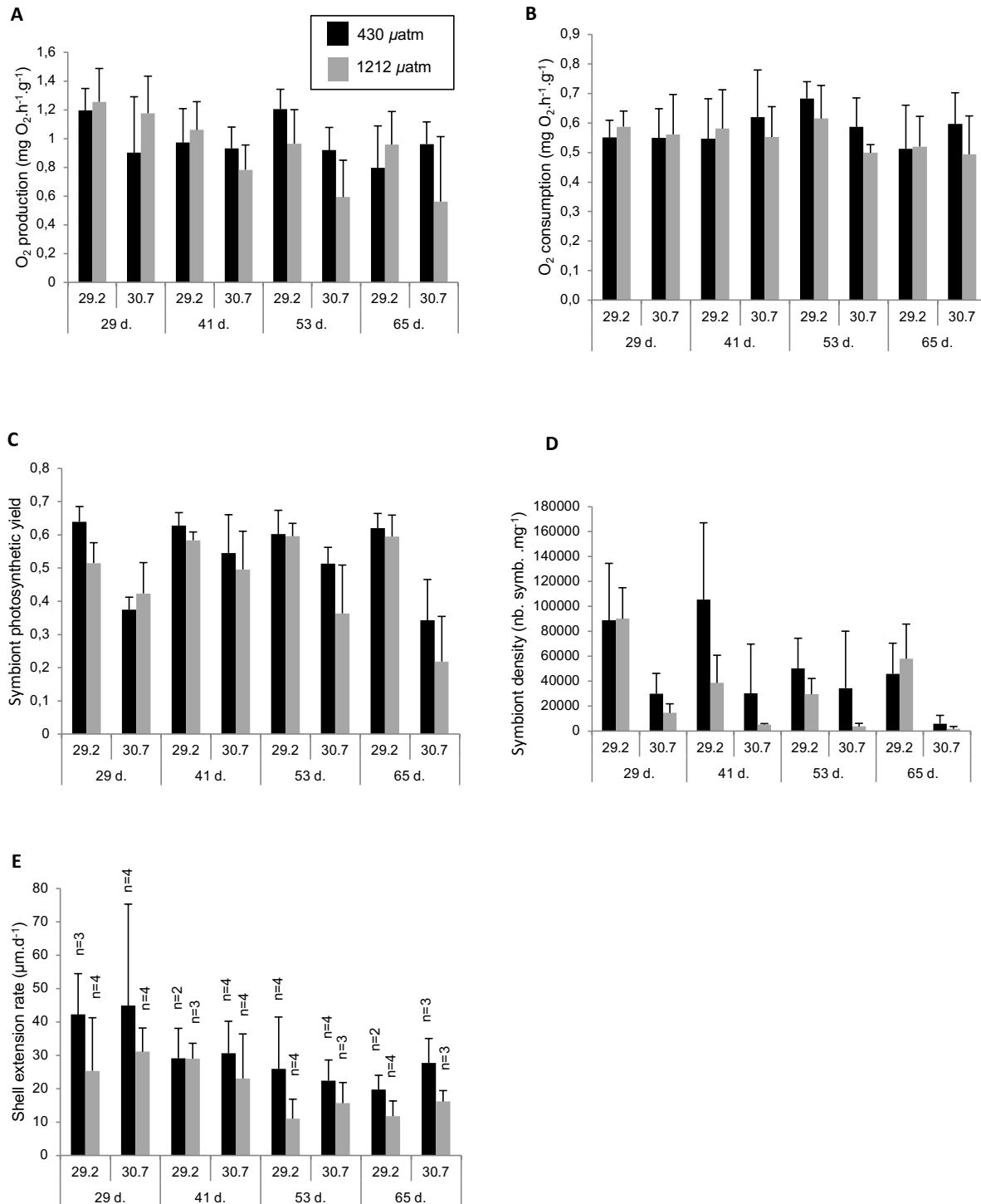
789

790 **Figure 1. Variations of oxygen consumption and oxygen production acquired over a 48 h period**

791 **after 29 days of exposure to 29.2°C and 430  $\mu$ atm of CO<sub>2</sub>. Data are expressed in mg O<sub>2</sub>.h<sup>-1</sup>.g<sup>-1</sup> tissue**

792 **dry weight (dw) and correspond to day-time and night-time acquisitions for 4 giant clam replicates.**

793



794

795 **Figure 2. Graphs reporting data of (A) oxygen production, (B) oxygen consumption, (C) symbiont**  
 796 **photosynthetic yield and (D) density, and (E) daily shell extension rate obtained for each**  
 797 **temperature/ $p\text{CO}_2$  experimental condition and time of exposure. Black and grey columns**  
 798 **correspond to today  $p\text{CO}_2$  condition (430  $\mu\text{atm}$ ) and future  $p\text{CO}_2$  condition (1212  $\mu\text{atm}$ ), respectively.**  
 799 **Data are given in mean (+s.d.) calculated from 4 replicates (n=4) for each physiological parameter**  
 800 **excepted for daily shell extension rate. For this latter parameter, numbers of replicates are indicated on**  
 801 **the graph.**

802

803 **Table 2. Results from the three-way ANOVA performed on holobiont O<sub>2</sub> production and**  
 804 **consumption data, symbiont photosynthetic yield, symbiont density, and giant clam shell**  
 805 **extension rate.** The three fixed factors are pCO<sub>2</sub>, temperature and time of exposure.  
 806 Significant differences (*P*<0.05) are highlighted in grey.  
 807

		O <sub>2</sub> production (n=64)	O <sub>2</sub> consumption (n=64)	Photosynthetic yield (n=64)	Symbiont density (n=64)	Shell extension rate (n=55)
<b>Time</b>	<i>F</i>	3.264	0.740	3.566	6.035	4.695
	<i>P</i>	0.030	0.533	0.021	0.001	0.007
<b>Temperature</b>	<i>F</i>	7.551	0.294	65.200	79.158	0.466
	<i>P</i>	0.009	0.590	<0.0001	<0.0001	0.499
<b>pCO<sub>2</sub></b>	<i>F</i>	0.846	0.851	6.125	8.665	7.409
	<i>P</i>	0.362	0.361	0.017	0.005	0.010
<b>Time x Temperature</b>	<i>F</i>	0.413	0.965	3.393	1.528	0.319
	<i>P</i>	0.744	0.417	0.025	0.219	0.812
<b>Time x pCO<sub>2</sub></b>	<i>F</i>	1.765	0.476	0.024	1.033	0.251
	<i>P</i>	0.167	0.700	0.995	0.386	0.860
<b>Temperature x pCO<sub>2</sub></b>	<i>F</i>	1.334	1.029	0.100	1.989	0.026
	<i>P</i>	0.254	0.315	0.753	0.165	0.872
<b>Time x Temperature x pCO<sub>2</sub></b>	<i>F</i>	1.216	0.148	2.153	0.474	0.336
	<i>P</i>	0.314	0.930	0.106	0.702	0.799

808

809 **Table 3. Results from Tukey post hoc tests following the three-way ANOVA performed on**  
 810 **analyzed physiological parameters.** The effects of significant parameters were tested as time of  
 811 exposure alone and combined to the temperature. The letter annotations correspond to the significances  
 812 between conditions ( $P < 0.005$ ).  
 813

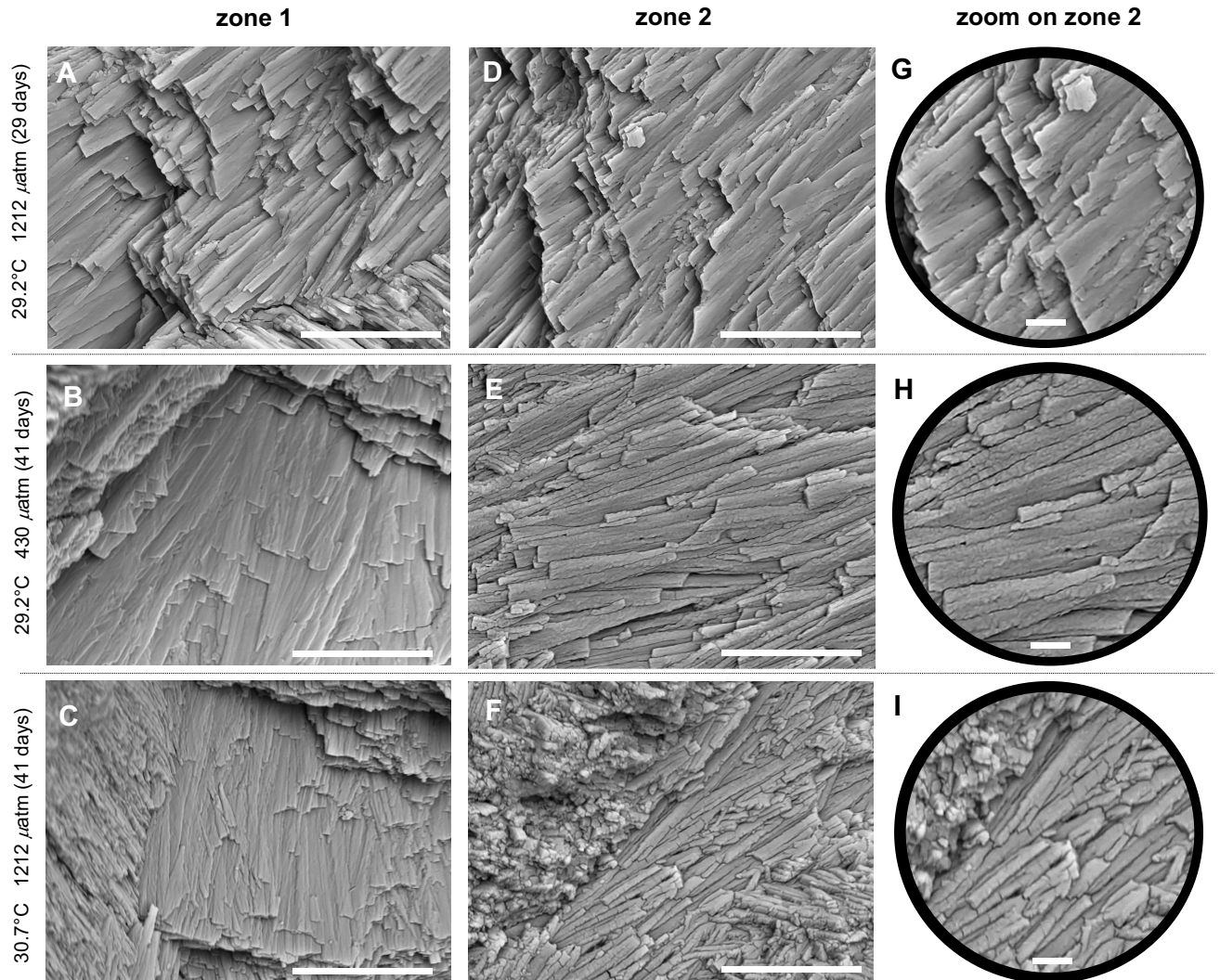
		O <sub>2</sub> production	Photosynthetic yield	Symbiont density	Shell extension rate
<b>Time</b>	29 d.	a	a	a	a
	41 d.	ab	ab	ab	ab
	53 d.	ab	ab	b	b
	65 d.	b	b	b	b
<b>Temperature</b>	29.2°C	a	a	a	-
	30.7°C	b	b	b	-
<b>pCO<sub>2</sub></b>	430 $\mu$ atm	-	a	a	a
	1212 $\mu$ atm	-	b	b	b
<b>Time x Temperature</b>	29 d. x 29.2°C	-	ab	-	-
	41 d. x 29.2°C	-	a	-	-
	53 d. x 29.2°C	-	a	-	-
	65 d. x 29.2°C	-	a	-	-
	29 d. x 30.7°C	-	cd	-	-
	41 d. x 30.7°C	-	abc	-	-
	53 d. x 30.7°C	-	bcd	-	-
	65 d. x 30.7°C	-	d	-	-

814

815 **Table 4. Correlation matrix integrating the different physiological parameters monitored, i.e., O<sub>2</sub>**  
816 **production and consumption, shell extension rate and symbiont photosynthetic yield and**  
817 **density.** Correlation were tested using the Pearson method (threshold of  $r=0.250$ ,  $\alpha=0.05$ ) and  
818 significant relationships between parameters are highlighted in grey.

	<b>Photosynthetic yield (n=64)</b>	<b>Symbiont density (n=64)</b>	<b>Shell extension rate (n=55)</b>	<b>O<sub>2</sub> production (n=64)</b>
Symbiont density	0.667			
Shell extension rate	-0.032	0.168		
O <sub>2</sub> production	0.331	0.492	0.221	
O <sub>2</sub> consumption	0.163	0.226	0.129	0.629





819

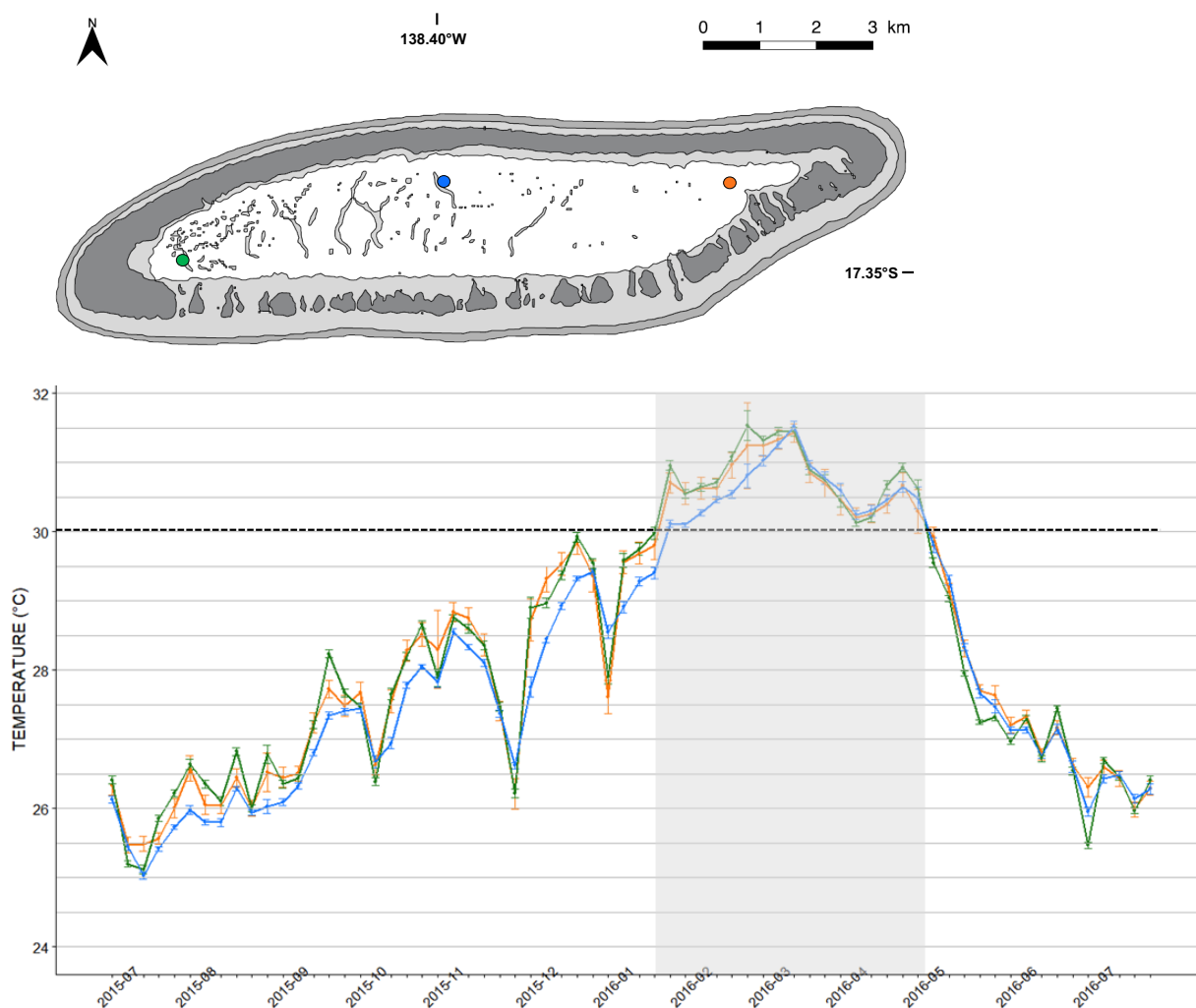
820 **Figure 3. Effects of experimental conditions on the ultrastructure of the *Tridacna maxima* shell**  
821 **outer layer investigated via scanning electron microscopy.** (A-F: scale bar: 10  $\mu\text{m}$ , G-I: scale bar:  
822 2  $\mu\text{m}$ ). Zone 1 and zone 2 correspond to the shell formed *in situ* (i.e., before the experiment) and in the  
823 experimental conditions, respectively. (A, B and G): 29.2°C 1212  $\mu\text{atm}$  (29 days): no difference between  
824 zone 1 and 2. In both zones, lamellae are well-cohesive, displaying elongated shape with a smooth  
825 surface and slightly rounded outlines. (B, E and H) 29.2°C 430  $\mu\text{atm}$  (41 days): lamellae in zone 2 are  
826 less-cohesive, with a pronounced granular aspect. (C, F and I) 30.7°C 430  $\mu\text{atm}$  (41 days): lamellae in  
827 zone 2 are less-cohesive and display crazes.

828

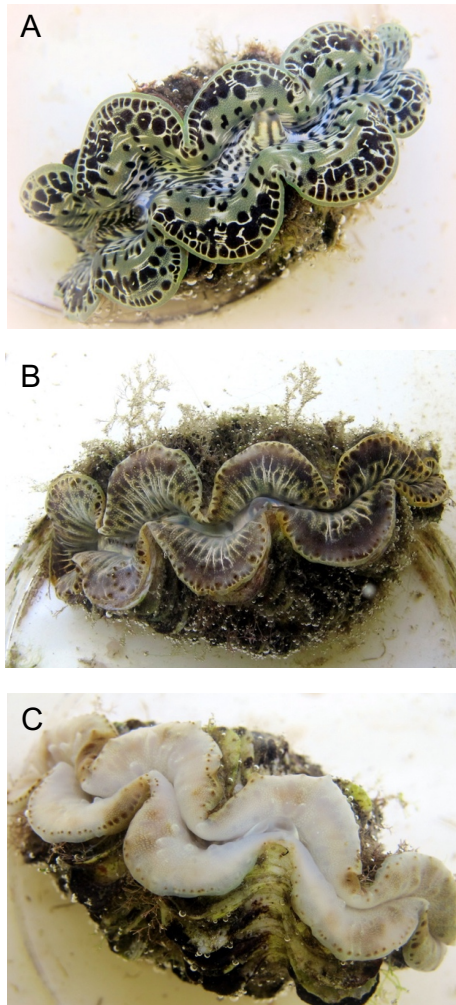
829 **Table 5. Compilation of ultrastructural observations of the lamellae of the shell outer layer using**  
 830 **scanning electron microscopy.** “ND” means that no significant difference is observed between the  
 831 shell formed before and during the experiment. “D” means that a notable difference is observed between  
 832 both parts of the shell. “A” and “B” represent the two samples observed for each treatment and time of  
 833 exposure.  
 834

Time of exposure	Temperature (°C)	pCO <sub>2</sub> (μatm)	Sample	Difference
29 d.	29.2	430	A	ND
			B	ND
	30.7	430	A	ND
			B	ND
	29.2	1212	A	ND
			B	ND
	30.7	1212	A	ND
			B	ND
41 d.	29.2	430	A	D
			B	D
	30.7	430	A	D
			B	D
	29.2	1212	A	D
			B	D
	30.7	1212	A	D
			B	ND
53 d.	29.2	430	A	D
			B	D
	30.7	430	A	D
			B	D
	29.2	1212	A	ND
			B	D
	30.7	1212	A	D
			B	ND
65 d.	29.2	430	A	D
			B	D
	30.7	430	A	D
			B	ND
	29.2	1212	A	D
			B	D
	30.7	1212	A	ND
			B	D

835 **Supplementary Material**  
836



837  
838 **Figure S1. *In situ* seawater temperatures recorded from July 2015 to July 2016 inside the lagoon**  
839 **of Tatakoto atoll (Tuamotu islands) at 2 m depth.** Temperature were recorded using UA-002-64  
840 HOBO Onset data loggers. Curves on the graph report data from 3 sites localized on the map by circles  
841 with corresponding color. Grey rectangle highlights the period when giant clams were exposed to a  
842 temperature  $\geq 30^{\circ}\text{C}$ .



843

844 **Figure S2. Visual observation of bleaching in giant clam *Tridacna maxima* specimens after 65**

845 **days in experimental conditions. (A) Non-bleached specimen exposed to 29.2°C 430  $\mu$ atm of CO<sub>2</sub>,**

846 **(B) partially bleached and (C) completely bleached specimens exposed to 30.7°C 1212  $\mu$ atm of CO<sub>2</sub>.**