

# The effects of thermal and high-CO<sub>2</sub> stresses on the metabolism and surrounding microenvironment of the coral *Galaxea fascicularis*

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**The effects of thermal and high-CO<sub>2</sub> stresses on the metabolism and surrounding micro-environment of the coral *Galaxea fascicularis***

5 **Effets d'une élévation de la température et du CO<sub>2</sub> dissous sur le métabolisme du corail *Galaxea fascicularis* et son micro-environnement.**

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## **Abstract**

The effects of elevated temperature and high pCO<sub>2</sub> on the metabolism of *Galaxea fascicularis* were studied with oxygen and pH microsensors. Photosynthesis and  
30 respiration rates were evaluated from the oxygen fluxes from and to the coral polyps. High temperature alone lowered both photosynthetic and respiration rates. High pCO<sub>2</sub> alone did not significantly affect either photosynthesis or respiration rates. Under a combination of high temperature and high CO<sub>2</sub> the photosynthetic rate increased to values close to those of the controls. The same pH in the diffusion boundary layer was  
35 observed under light in both CO<sub>2</sub> treatments but decreased significantly in the dark as a result of increased CO<sub>2</sub>. The ATP contents decreased with increased temperature. The effects of temperature on the metabolism of corals were stronger than the effects of increased CO<sub>2</sub>. The effects of acidification were minimal without combined temperature stress. However, acidification combined with higher temperature may  
40 affect coral metabolism due to the amplification of diel variations in the micro-environment surrounding the coral and the decrease in ATP contents.

## **Résumé**

Les effets d'une élévation de la température et de la pression partielle en CO<sub>2</sub> sur le corail *Galaxea fascicularis* ont été étudiés à l'aide de micro-sondes pour l'oxygène et  
45 le pH. Les taux de photosynthèse sont inférieurs à 32°C par rapport à 27°C. Lorsque le CO<sub>2</sub> est aussi augmenté, une augmentation des taux de photosynthèse à 32°C a été observée. Les taux de respirations sont réduits par une augmentation de la température. Le pH dans la couche limite de diffusion est identique sous illumination dans les deux traitements en CO<sub>2</sub> mais est réduit dans le noir par une augmentation en

50 CO<sub>2</sub>. Le contenu en ATP du tissu est réduit par augmentation de la température. Une  
réduction du pH combinée avec une augmentation de la température peut donc  
sévèrement affecte le métabolisme du corail et ses taux de calcification à cause de  
l'augmentation des variations journalières du pH dans le micro-environnement autour  
du corail et de la réduction de la respiration qui entraîne une diminution de l'énergie  
55 produite.

## **Introduction**

As a result of the release of anthropogenic CO<sub>2</sub> in the atmosphere, temperatures worldwide have been increasing, and ocean pH has been decreasing [1]. These two stressors may have dramatic effects on coral reefs [2,3]. The seawater temperature in the tropics has already increased almost 1°C over the past hundred years, and the increase is still continuing [4]. High seawater temperatures have been reported as the principal factor inducing coral bleaching, the loss of the coral photosymbionts [5]. The increase of CO<sub>2</sub> in the atmosphere results in an increased absorption of CO<sub>2</sub> by the ocean. The associated chemistry, the hydrolysis of CO<sub>2</sub>, causes the pH of the water to decrease. The pH of seawater is already 0.1 pH unit lower than pre-industrial values and may decrease another 0.3-0.4 unit by the end of the century if the present trend in CO<sub>2</sub> emissions continues [1]. The change in pH and the increase in dissolved inorganic carbon shift the carbon balance, resulting in a lower aragonite saturation state ( $\Omega_{\text{aragonite}}$ ). Calcifying organisms may be impacted by this change. Decreases in  $\Omega_{\text{aragonite}}$  have been shown to decrease the calcification rate of certain organisms [6–8]. It has been predicted that a doubling of the pre-industrial atmospheric CO<sub>2</sub> concentration may result in a 10–50% decrease of the calcification rate of reef-building corals [9]. However, the effect of acidification on the calcification process is still unclear. Calcification in scleractinian corals occurs in an extracellular calcifying fluid located between the calciblastic cell layer and the skeleton [10]. Corals maintain a high  $\Omega_{\text{aragonite}}$  at the calcification site, and this process requires energy [11–13]. A lower pH in the surrounding water could therefore increase the consumption of energy used to maintain an elevated pH in the sub-calciblast space [14].

80 Corals and other benthic communities change the carbon chemistry of the  
seawater flowing through coral reef ecosystems [15]. Moreover, corals are complex  
organisms, within which micro-environments with different chemical characteristics  
can be described [16,17]. The micro-environment surrounding corals can differ  
greatly from the environment seen at a larger scale in the surrounding waters [18,19].  
85 Diffusion limitations in the layer around an organism create a diffusive boundary  
layer (DBL) on the surface of the organism. The chemistry of the DBL is highly  
influenced by the organism's metabolism and shows diurnal variations according to  
changes in mainly photosynthetic and respiratory activity. The oxygen concentration  
varies from undersaturated during the night to hypersaturated during the day. The pH  
90 also shows strong variations between day and night, with values clearly differing from  
the surrounding water [20]. To our knowledge, changes in the chemistry of the DBL  
under different stresses have only been the subject of one study, and only high  
temperature was tested [21]. Up-regulation of the pH at the interface between tissue  
and skeleton leading to higher pHs compared to the surrounding seawater even under  
95 acidified conditions [12,22], highlights the need to study the effect of stressors on the  
micro-environment within corals to understand the effects that changes in the  
surrounding environment will have on corals. In this study the effects of high-  
temperature stress, acidification and a combination of both factors on coral  
metabolism and on the chemistry of the DBL was investigated using microsensor  
100 techniques.

## ***Materials and methods***

### **Biological materials**

Individual polyps were obtained from one parent colony of *Galaxea fascicularis* kept

for several years in an outdoor aquarium with running seawater. The seawater is  
105 pumped from the reef in front of the Sesoko marine station, Tropical Biosphere  
Research Center, Okinawa Japan. The average temperature, salinity, pH and alkalinity  
for the month preceding the experiment were: 24.8°C, 33.2, 8.07 and 2.28 mmol kg<sup>-1</sup>  
respectively. The maximum recorded temperature was 27.1°C (record of coastal  
observation at Sesoko station, Tropical Biosphere Research Center, the University of  
110 the Ryukyus). The individual polyps were allowed to recover for one week, and 32  
polyps were transferred to four separate indoor aquarium with running seawater and a  
12/12 light cycle, with a light level of 200  $\mu\text{mole s}^{-1} \text{m}^{-2}$ . The polyps were fixed on a  
rubber stand to place their body wall at an angle of 45°.

### **Incubation conditions**

115 The polyps were incubated under four different conditions: 27°C / 400 ppm CO<sub>2</sub>,  
27°C / 750 ppm CO<sub>2</sub>, 32°C / 400 ppm CO<sub>2</sub> and 32°C / 750 ppm CO<sub>2</sub> (n = 8 for each  
treatment). The polyps at 27°C, which was the maximum temperature at which corals  
were exposed during the month preceding the experiment, were incubated for 5 days  
at both pCO<sub>2</sub> values, and the polyps at 32°C were incubated for 1 day at both pCO<sub>2</sub>  
120 values in combination with the high temperature. Polyps at both pCO<sub>2</sub> showed no  
signs of stress at 27°C and were fully expanded throughout the incubation and  
measurement period (5 days). However, the polyps incubated at 32°C showed signs of  
bleaching (discoloration) and stress (retraction of the tentacles) after one day. For this  
reason, the measurements at this temperature were performed after only 24 h of  
125 incubation. Each aquarium had a volume of 9 l and was supplied with unfiltered  
seawater at a flow rate of 150 ml min<sup>-1</sup>. pCO<sub>2</sub> levels were established by injecting  
CO<sub>2</sub>-saturated seawater (100%) [23] with a peristaltic pump until the desired pH was

obtained. The flow rates were adjusted to reach a pH of approximately 7.975 and 7.995 at 27°C and 32°C, respectively, corresponding to 750 ppm in each treatment.

130 CO<sub>2</sub>-saturated seawater was prepared by bubbling pure CO<sub>2</sub> gas into natural seawater for 1 h. The pH was measured at the beginning of the incubation with an Orion 4 stars pH sensor equipped with an 8156BNUWP pH electrode (Thermo scientific, Inc., USA) calibrated with standard National Bureau of Standards buffer solution (Mettler Toledo, Inc). The 400 ppm treatment corresponded to the ambient CO<sub>2</sub> levels. The

135 variation in pH of the incubation water was checked on a daily basis in the morning. The pH in the aquarium varied within 0.05 pH unit. Samples for total alkalinity in each aquariums were taken in the morning on day 5 for 27°C and day 1 for the 32°C treatment. Seawater was filtered with a syringe filter (0.45 µm) and kept cool until analysis. Total alkalinity was measured by potentiometric titration with HCl at 0.1

140 mol l<sup>-1</sup> with a Metrohm titrator (785 DMP titrino), computation was done using the Gran plot method [24]. Reproducibility of the measurement was ± 2 µmol kg<sup>-1</sup> (1σ, n = 10). A working seawater standard was used for the calibration of the measurement. This standard was analyzed precisely for total alkalinity with a certified reference material for oceanic CO<sub>2</sub> measurement (CRM Batch # 50) distributed by A. Dickson

145 of the Marine Physical Laboratory, University of California, San Diego. The conditions obtained in each treatment are summarized in Table 1. The characteristics of the carbonate system in the seawater were calculated using the apparent equilibrium constants K'<sub>0</sub> from Weiss (1974) [25] and K'<sub>1</sub> and K'<sub>2</sub> from Mehrbach and Culberson (1973) [26], as described by Millero (1979) [27] and Casareto et al.

150 [28]. The aragonite saturation state ( $\Omega_{\text{aragonite}}$ ) was calculated from the calcium (Ca<sup>2+</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>) concentrations as  $\Omega_{\text{aragonite}} = [\text{Ca}^{2+}] [\text{CO}_3^{2-}] / K'_{\text{sp}}$ . K'<sub>sp</sub> is the

stoichiometric solubility product of aragonite, derived from a function of salinity and temperature [29]. Calcium was fixed as a function of salinity (33.2) at a value of 10 mmol kg<sup>-1</sup>.

155 *Table 1: Incubation conditions characterizing the carbonate system in the different treatments. Alkalinity (n = 1), pH (n = 5 at 27°C and n= 3 at 32°C) and temperature (n = 5 at 27°C and n= 3 at 32°C) were measured. The other parameters were calculated from the average values.*

	pH	Alkalinity (mmol kg <sup>-1</sup> )	Temp (°C)	[CO <sub>3</sub> <sup>2-</sup> ] (mmol kg <sup>-1</sup> )	[HCO <sub>3</sub> <sup>-</sup> ] (mmol kg <sup>-1</sup> )	[CO <sub>2</sub> (aq)] (mmol kg <sup>-1</sup> )	TIC (mmol kg <sup>-1</sup> )	PCO <sub>2</sub> (ppm)	Ω <sub>aragonite</sub>
<i>Low temperature</i>									
<i>Low pCO<sub>2</sub></i>	8.149 ±0.036	2.21	27.4 ± 0.3	0.20	1.71	0.01	1.93	446	3.138
<i>High pCO<sub>2</sub></i>	7.962 ±0.035	2.21	27.2 ± 0.4	0.14	1.86	0.02	2.02	741	2.192
<i>High temperature</i>									
<i>Low pCO<sub>2</sub></i>	8.146 ±0.042	2.22	32.0 ± 0.5	0.22	1.69	0.01	1.91	474	3.518
<i>High pCO<sub>2</sub></i>	7.972 ±0.028	2.23	32.0 ± 0.5	0.16	1.83	0.02	2.01	769	2.555

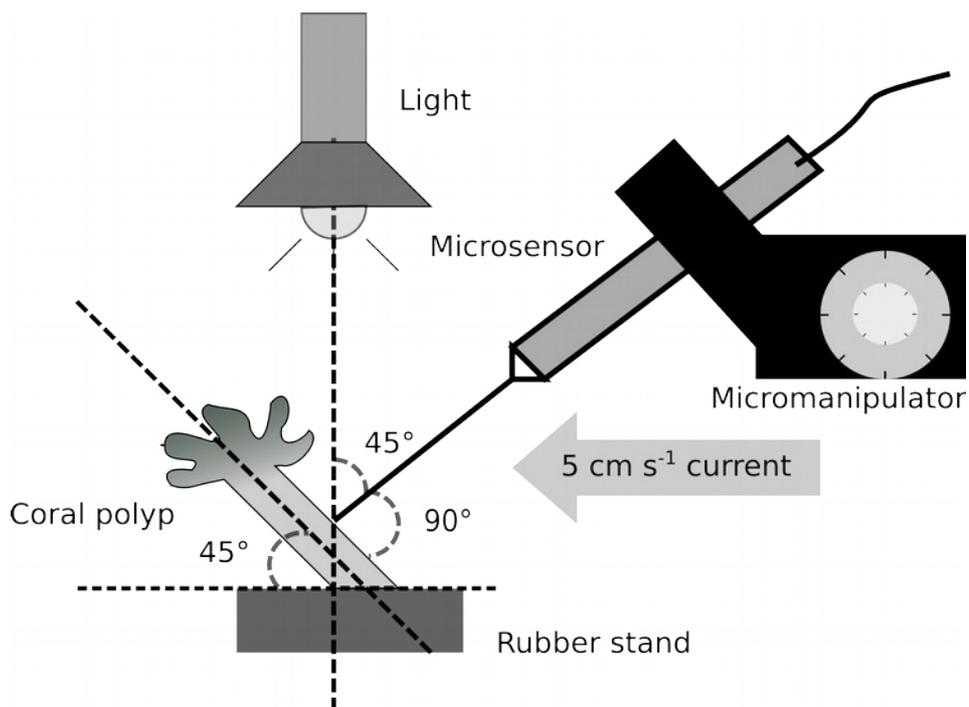
### Microsensor measurements

160 The microsensor measurements were performed in a custom flume with a laminar flow of 5 cm s<sup>-1</sup> (Figure 1). The total volume of the flume and reservoir was 3 l. Water was changed after each measurements. Water from the respective incubation aquariums was used to fill the flume. For the measurement at 32°C, the flume was placed in a heated water bath (32 ± 0.5°C). Oxygen, pH and temperature were

165 monitored in the flowing water during the measurements using an Orion 4 star pH/DO meter placed at the end of the flume. No substantial changes occurred in the oxygen and pH levels in the flowing water during metabolic rates measurements of each polyps. Light at 200 μmole s<sup>-1</sup> m<sup>-2</sup> was provided by a metal halide lamp with an angle of incidence of 45° to the wall of the polyp. Oxygen microsensor (oxygen micro

170 optode, Type PSt1, Presens Gmbh, Regensburg, Germany) and a pH microsensor (pH micro optode, Presens Gmbh, Regensburg, Germany) were mounted on a manually

170 operated Märzhäuser micro-manipulator (MM33, Sutter Instruments, 10  $\mu\text{m}$   
 resolution). Microsensors were calibrated as indicated by the manufacturer. Briefly,  
 oxygen microsensors were calibrated using a 0% oxygen solution made by dissolving  
 1g of sodium sulfite in 100 ml of distilled water and a 100% oxygen solution made by  
 bubbling air in 100 ml of distilled water. pH microsensors were calibrated using the  
 175 multipoint calibration method, the different pH used were 5.0, 6.0, 7.0, 8.0 and 9.0.  
 The solutions at different pH were prepared just before calibration by adjusting the  
 pH of seawater with HCl or NaOH. The measurements were performed on the body  
 wall of the polyps at an angle of  $90^\circ$ .



*Figure 1 Schema showing the set-up used for microsensor measurements. Individual coral polyps were fixed on rubber stands and placed in a custom flume under a  $5 \text{ cm s}^{-1}$  current. Light at  $200 \mu\text{mol s}^{-1} \text{ m}^{-2}$  was provided by a metal halide lamp.*

180 The thickness of the DBL in the flume conditions was measured on 5 polyps,  
 with 3 measurements per polyp. The polyps were maintained at  $27^\circ\text{C}$  / 400 ppm. The

variations in oxygen concentration as a function of the distance from the polyps were used for estimation of the DBL thickness. The net photosynthetic rates and dark respiration rates were calculated by measuring the flux of oxygen under an electrode placed at the surface of the corals ( $n = 3$  polyps, with 10 measurements per polyp). Fick's law of diffusion was used to calculate the oxygen fluxes [30]. The parameters used were the oxygen concentrations at the surface of the polyp and outside the DBL, the average DBL thickness and the temperature-corrected diffusion coefficients. The oxygen diffusion coefficients used were  $-D_s = 2.3525 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  at  $27^\circ\text{C}$  and  $-D_s = 2.6318 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  at  $32^\circ\text{C}$  [31,32]. For each polyp, the oxygen at the surface of the polyp was measured at ten different locations on the up-front body wall. The oxygen fluxes were calculated for each location. The measurements were performed under light and dark conditions after a minimum of 5 min of adaptation after the change in luminosity. The results were analyzed using a 2 way ANOVA mixed model with the polyp factor used as a random factor. The pH in the DBL in light and dark condition was measured for the polyps at  $27^\circ\text{C}$  and at both  $\text{pCO}_2$  values by placing the microsensor tip at the surface of the polyps. Minimum and maximum values of pH were recorded once the pH stabilized. Significance of the difference of the pH values in the DBL under the two different  $\text{pCO}_2$  were evaluated using a pairwise t-test.

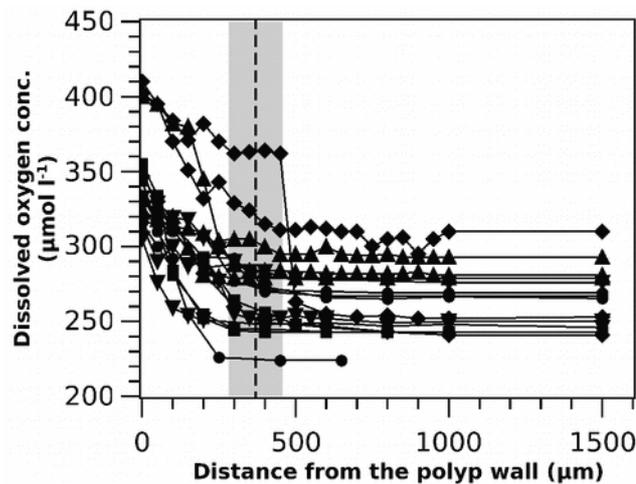
#### 200 **Tissue ATP concentration analysis**

Tissue homogenates were prepared with the air-jet method, in which MilliQ NaCl ( $30 \text{ g l}^{-1}$ ) with formaldehyde at a final concentration of 5% was sprayed using a paint sprayer ST (Asahi pen, Japan) to strip the tissue from the coral skeleton. The tissue slurry was then centrifuged at  $3000 \text{ g}$  for 10 min to separate host and zooxanthellae, and ATP was measured in the supernatant (host fraction) using an ATP

auto-analyzer (TOA DKK, AF-100) after 1000 times dilution, as described by the manufacturer. The protein content was also determined in the supernatant by the Bradford method [33] after dilution. Results were analyzed using a 2 way ANOVA.

## Results

The DBL thickness was found to be  $370 \pm 90 \mu\text{m}$  (average  $\pm$  SD,  $n = 15$ ) and ranged from 250 to 500  $\mu\text{m}$  (Figure 2).



*Figure 2 Dissolved  $\text{O}_2$  profiles used for determination of the thickness of the DBL. Each symbol represents the measurement for a different polyp. The vertical dashed line represents the average thickness of the DBL in this experiment, the gray area its standard deviation.*

The net photosynthetic rates (Figure 3, A) decreased significantly in the high-  
 215 temperature stress treatment (DF = 8,  $p < 0.05$ ) but did not change significantly in  
 response to the increased partial pressure of  $\text{CO}_2$  (DF = 8  $p = 0.31$ ). No significant  
 interaction was observed between temperature and  $p\text{CO}_2$  treatments (DF = 8,  $p =$   
 0.096). The net photosynthetic rates were  $0.25 \pm 0.08 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$  and  $0.18 \pm 0.10$   
 220  $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$  (average  $\pm$  standard deviation,  $n = 6$ , 10 measurements each) at  $27^\circ\text{C}$   
 and  $32^\circ\text{C}$  respectively.

The respiration rates (Figure 3, B) were low for all corals and close to zero for  
 the polyps incubated at high temperature. These low rates made the measurements  
 difficult. The averages  $\pm$  standard deviation respiration rates ( $n = 6$ , 10 measurements

each) for the different temperature treatments were:  $0.08 \pm 0.07$  and  $0.00 \pm 0.04$  for  
 225 27°C and 32°C respectively. The respiration rates decreased significantly in response  
 to temperature stress (DF = 8,  $p < 0.05$ ). CO<sub>2</sub> did not significantly affect respiration  
 rates (DF = 8,  $p = 0.41$ ) and no interactions were observed (DF = 8,  $p = 0.60$ ).

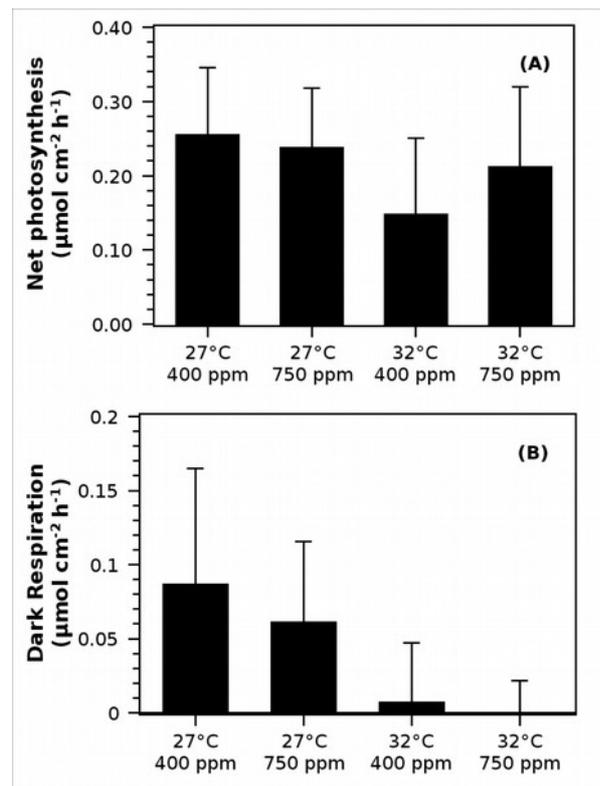


Figure 3: Net photosynthetic rates (A) and dark respiration rates (B) as measured by oxygen fluxes in the light and dark, respectively. Respiration rates were corrected to positive values. The error bars indicate standard deviations among all measurements,  $n = 3$ , 10 measurements each.

The variations in pH in the DBL, measured with the electrode placed in  
 contact with the polyp, was recorded over the light and dark phases for the polyps  
 230 incubated at 27°C (Figure 4). The pH generally stabilized after 5 min, and the  
 stabilized value was recorded. The maximum pH was recorded under light conditions,  
 the minimum pH under dark conditions (Figure 5). The minimum pH (average  $\pm$  SD)

at the surface of the polyp, obtained during dark conditions, was significantly lower at 235 750 ppm,  $7.5 \pm 0.1$ , compared with 400 ppm,  $7.9 \pm 0.1$ , (t-test,  $p = 0.01$ ,  $n = 3$ ). In contrast, the pH in the DBL did not differ significantly between the CO<sub>2</sub> treatments in the light condition, with pH values of  $8.7 \pm 0.3$  and  $8.5 \pm 0.3$  for 400 and 750 ppm CO<sub>2</sub>, respectively (t-test,  $p = 0.66$ ,  $n = 3$ ).

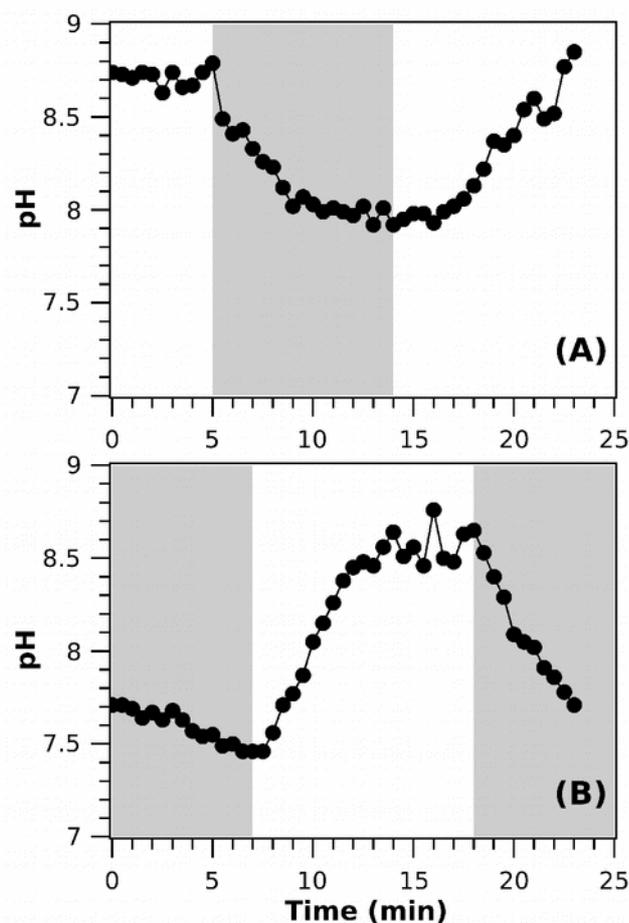


Figure 4: Examples of pH dynamics at the surface of isolated polyps incubated at 27°C under two different pCO<sub>2</sub>: (A) 400 ppm and (B) 750 ppm. Gray and white areas represent dark and light periods, respectively.

High-temperature stress produced a significant decrease in the host ATP 240 content (DF = 1,  $p < 0.05$ ) (Figure 6). High pCO<sub>2</sub> had no significant effect at the 95% interval of confidence on host ATP contents (DF = 1,  $p = 0.06$ ). The average ( $\pm$  standard deviation,  $n = 10$ ) of host ATP contents were  $3.67 \pm 1.49$  nmol mg prot<sup>-1</sup> and

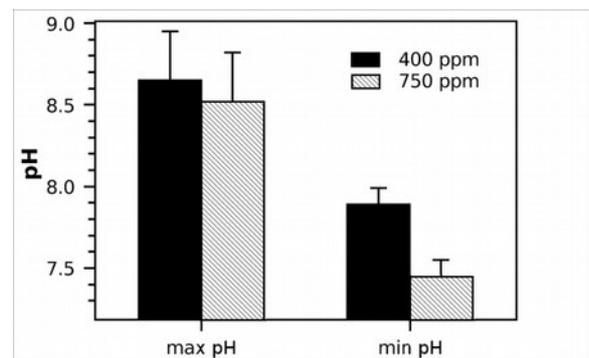
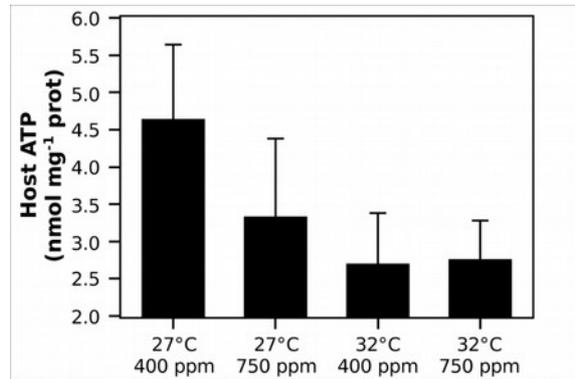


Figure 5: pH measured in the DBL of isolated polyps incubated at 27°C under two different pCO<sub>2</sub>. Error bars indicate standard errors. Max pH indicates the average value for three polyps in each treatment of the maximum stabilized pH reached in the DBL under light conditions. Min pH represents the average value of the minimum stabilized pH reached in the DBL under dark conditions. Examples of the dynamics of the pH in the DBL under light/dark shifts are shown in Fig. 4.

$2.65 \pm 1.08$  nmol mg prot<sup>-1</sup> for 27°C and 32°C respectively. No significant interaction between treatments could be observed (DF = 1, p = 0.18).

245



*Figure 6: Host ATP content in isolated polygs (n = 5) for each treatment. Error bars indicate standard deviation among replicates.*

## **Discussion**

High-temperature stress had a strong negative effect on the metabolism of isolated polyps. Signs of bleaching were visible after 24 h and concurrently; the net photosynthetic rates were reduced. High variability in oxygen measurements was observed but the photosynthetic rates found in the control treatment of this study are in agreement with rates from a previous study [34]. This variability may be due to the difference in the DBL thickness. Thickness of the DBL may vary with the shape of the polyps and with the location on the polyp where the microsensor was placed. Different chlorophyll contents among the polyps in the same treatments may also have influence the rates observed. No significant interaction on photosynthesis rates of temperature and pCO<sub>2</sub> could be shown at the 95% level of confidence however the decrease in photosynthesis seemed of less amplitude under combined high temperature and high pCO<sub>2</sub> compare to high pCO<sub>2</sub> alone. This difference may indicate that higher partial pressure in CO<sub>2</sub> may mitigate the negative effect of high temperature on the photosynthetic rates. It has been reported that zooxanthellae use HCO<sub>3</sub><sup>-</sup> as a source of inorganic carbon [35]. We manipulated the pCO<sub>2</sub> of seawater in the incubation vessels by adding CO<sub>2</sub>-saturated seawater. This addition produced a slight increase in the concentration of bicarbonate ions. The enhanced photosynthetic rates at high CO<sub>2</sub> only occurred at the high temperature. It is possible that the zooxanthellae were only limited by the amount of CO<sub>2</sub> at this temperature and that other factors limit the photosynthetic rate of zooxanthellae at normal temperatures [36]. Zooxanthellae also use CO<sub>2</sub> produced through respiration, as previously shown by Al Horani et al. (2003, [11]) Under high temperature stress, host respiration was decreased and therefore the supply of CO<sub>2</sub> to the zooxanthellae also decreased. Under

270 combined high pCO<sub>2</sub> and high temperature, the increase in CO<sub>2</sub> in the surrounding environment may have compensated in part for the decrease in production of CO<sub>2</sub> through the host respiration. The opposing trends in photosynthesis and respiration may show a disruption of the symbiosis.

The lack of a significant effect of CO<sub>2</sub> on respiration may result from the near-  
275 zero respiration rates found at the higher temperature. The very low respiration rates recorded may be due to the location of the measurements, the body wall, which is not a metabolically active location [20]. Moreover it was reported that photosynthesis results in build up of oxygen in the coral tissue [20]. This increased internal oxygen concentration may have rendered the fluxes of oxygen from the surrounding water  
280 close to zero or even positive as it was observed when the respiration rates were the lowest, i.e. under high temperature stress. A longer adaptation time than the 5 minutes used should be considered to measure accurately low respiration rates by first depleting the internal pool of oxygen. Decreased respiration rates at high temperatures were also reported for other coral species [37]. In a study with microsensor  
285 techniques, a similar but smaller decrease in respiration rates between 29°C and 32°C was observed for *G. fascicularis* [21]. However, some corals also showed an increase or no change in respiration rates under high-temperature stress [38–40]. This discrepancy may be due to the different temperatures used in the experiments. As seen for photosynthesis [40,41], respiration may increase with temperature until a threshold  
290 value, above which it decreases. In our case, the temperature of 32°C may have been overly high and may have caused the threshold value to be reached. A hypothesis to explain the decrease in respiration rates under high temperature stress is that elevated temperature may have damaged the mitochondrial electron transport system, the

enzymatic chain leading to the reduction of oxygen, due for example, to the formation  
295 of reactive oxygen species in the mitochondria [42,43]. The increase in CO<sub>2</sub> did not  
affect the coral respiration rates. This result differs from the findings of previous  
studies that showed an increase in respiration with increased pCO<sub>2</sub> [40,44]. More  
research is still required to understand the impact of acidification on coral respiration.

The low ATP contents observed for corals incubated at 32°C could be due to  
300 the extreme decrease in respiration rates observed or a decrease in the amount of  
photosynthetates translocated. At 32°C and high pCO<sub>2</sub>, the net photosynthesis did not  
decrease as much as under high temperature stress alone. In this case the low  
respiration rates may indicate that the coral hosts could not use the photosynthetates  
supplied through photosynthesis.

305 Our results are in agreement with previous measurements at the micro scale,  
which showed extreme diel variations of pH in the DBL [45]. Under normal pCO<sub>2</sub> and  
between light and dark conditions, the corals experienced a variation in pH of  
approximately 0.8 pH unit in the DBL. Such variations are greater than -0.3 pH unit,  
the expected change in pH due to global change over 100 years. These diel variations  
310 were amplified by the addition of CO<sub>2</sub>, with a difference between dark and light of  
nearly 1.1 pH unit (Figure 5). The impacts of acidification on corals may vary  
between day and night. During the day, corals may be able to tolerate the predicted  
decrease in the seawater pH of 0.3 unit because the high photosynthetic rates prevent  
a change in the pH in the micro-environment around corals. However, acidification  
315 becomes important during the night and may affect corals. An amplification of diel  
variations in pH in seawater has been predicted due to the lowering of the buffering  
capacity of CO<sub>2</sub>-enriched water [46] and has been confirmed by mesocosm

experimentation [47]. Our results show an attenuation of acidification during the day at the micro-scale, due primarily to photosynthetic activity. A similar attenuation was shown to occur at the reef scale due to the presence of macro-algae with a high photosynthesis:respiration ratio in upstream water on coral reefs during the day [15,48]. The pH in the DBL was reduced in the dark by the addition of CO<sub>2</sub> in combination with the CO<sub>2</sub> produced by respiration. At this low pH, the metabolism of the coral host, energy allocation, may have been more severely affected than during the daytime, when the pH in the DBL was not decreased relative to the control.

Lower ATP contents during the night have been reported for corals and have been attributed to a higher consumption of ATP in the dark compared to under light [11]. In this study ATP was measured in the morning, after a couple of hours of light period, which may have minimized the effect of acidification on the host ATP contents compared to if it had been measured during the night. Even if the effect of an increase of CO<sub>2</sub> on host ATP could not be shown at the 95% level of confidence, it is probable that CO<sub>2</sub> alone may lead to higher consumption, or lower production, of ATP as a small but non significant decrease of ATP contents at high pCO<sub>2</sub> and 27°C was observed ( $p = 0.06$ ).

Relatively few studies have investigated the impact of a combined high temperature and a decrease in the ambient pH on the coral calcification process. The internal pH of corals, especially the pH in the sub-calicoblast space, the site of calcification, needs to be maintained at a high value to precipitate calcium carbonate [10]. Two processes maintain the pH; calcium-ATPase pumps protons from the sub-calicoblast to the coelenteron, and the protons in the coelenteron are titrated by OH<sup>-</sup> produced through photosynthesis [49]. The decrease in respiration rates and ATP

contents of host tissues under high temperature may have lead to a decrease in the pH at the calcification site due to the lack of energy available to the calcium ATPase. Moreover under high temperature and 400 ppm of CO<sub>2</sub> photosynthesis also decreased  
 345 and may have lead to a further decrease of sub calcicoblastic pH due to a decrease in OH<sup>-</sup> produced. These mechanisms may explain the reported decrease in the calcification of bleached corals [21,50].

Micro-scale studies should be considered for the study of the impacts of stresses on coral physiology. Our results confirmed that the water chemistry, including  
 350 pH, in the micro-environment surrounding benthic organisms is highly influenced by their metabolism and may differ from the chemistry of the surrounding environment. This difference may therefore enhance or mitigate the stress due to environmental changes.

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