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Research Article

Effect of Elevated Carbon Dioxide on Two Scleractinian Corals: *Porites cylindrica* (Dana, 1846) and *Galaxea fascicularis* (Linnaeus, 1767)

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This study reveals the effect of elevated pCO_2 on *Porites cylindrica* and *Galaxea fascicularis*. The corals responded differently under elevated pCO_2. Zooxanthellae cell density, cell mitotic index, and photosynthesis rate of *P. cylindrica* decreased drastically under the elevated pCO_2. At the end of the experiment, *P. cylindrica* suffered from a declining calcium carbonate precipitation rate. *G. fascicularis* increased its respiration rate and expelled 71% of its symbiotic zooxanthellae algae under elevated pCO_2. Photosynthetic pigments in the remaining zooxanthellae algae increased from 1.85 to 11.5 times to sustain its photosynthetic outputs. At the end of the experiment, *G. fascicularis* managed to increase the rate of its calcium carbonate precipitation. Increase pCO_2 in the atmosphere may affect species diversity of coral reefs.

1. Introduction

Coral reefs are among the most productive marine ecosystems serving as a feeding and breeding ground for a wide spectrum of marine organisms. Corals precipitate calcium carbonate from the seawater onto its skeletal organic matrix (SOM) and form the coral skeleton [1, 2]. The calcium carbonate saturation state in seawater (Ω) regulates the supply of ions for the calcification process. Any changes on the seawater carbonate system would impact the precipitation process and hence threaten the corals' health.

Intensified anthropogenic CO_2 emission from land use change, fossil fuels, cement, coal and oil combustion has caused the average partial pressure of carbon dioxide (pCO_2) increased from 180 to 300 ppmv (part per million by volume) in the 1800s [3] to the current 380 ppmv [4] and is expected to reach 700 ppmv or more towards the end of the 21st century [5]. When Caldeira and Wickett [6] reported the ocean acidification in 2003, the impacts of the elevated pCO_2 on marine organisms were widely investigated [7–12]. Nevertheless, there is no unanimous agreement on the degree of impacts among the reports. In one hand, Langdon et al. [13], Leclercq et al. [8], and Marubini et al. [14] reported declining calcification rate of reef organisms under elevated CO_2. In the other hand, McNeil et al. [11] predicted an increase in the annual calcification rate of the coral reef in the future global warming. Lough and Barnes [12] also reported a continuous increment in the calcification of the *Porites* corals in the Great Barrier Reef despites of the increasing pCO_2 over the last century. Reynaud et al. [9] reported that calcification of *Stylophora pistillata* did not change in response to an increased pCO_2 under normal temperature. Basically, most of the reports suggested more studies are needed to reveal the effect of elevated pCO_2 on corals. Hence, this study aims to extend the understanding on the impact of elevated pCO_2 on the scrleractinian corals, *Porites cylindrica* (Dana 1846) and *Galaxea fascicularis* (Linnaeus, 1767).

2. Materials and Methods

2.1. Test Organism. Six colonies of each scleractinian coral, *Porites cylindrica* and *Galaxea fascicularis*, were collected from Pulau Bidong, Malaysia (05°37′17″N, 103°02′27″E)

at an average water depth of 10 m. The coral colonies were broken into smaller coral nubbins (~50 polyps per nubbin) and acclimatized in a 500 l aquarium supplied with running seawater for one month. The coral nubbins were supplied with 12-h light: 12-h dark cycle illumination by a metal-halide lamp at 392 ± 5 μmol m^{-2} s^{-1}. During the acclimatization, the corals nubbins were fed with newly hatched *Artemia salina* (Royal Artemia, 99% hatching) once for every three days, according to the recommendation made by Hii et al. [15]. Twelve coral nubbins for each species were randomly selected for the experiments (six for each elevated pCO_2 and control experiment).

2.2. Experimental Setup. Two 160 l aquaria (25 cm × 50 cm × 83 cm) were used for the experiment. One of these aquaria was used for the elevated pCO_2 and the other one as control. Six similar sizes *P. cylindrica* nubbin (~50 polyps; 10 × 10 cm) were randomly selected and placed in each aquarium. Water temperature was maintained at 26 ± 1°C by using a water chillers. The aquaria were supplied with 392 ± 5 μmol m^{-2} s^{-1} illumination by using metal-halide light on a 12-h light: 12-h dark cycle. The experiment was conducted under laboratory condition at 27°C, salinity of 35 psu, pH_T 8 and dissolved oxygen greater than 5 mg l^{-1}. Wave simulators were used to generate water movement in the aquarium. The elevated CO_2 aquarium was simulated by a constant pressure flow of carbon dioxide into the fed water tank. Check valves were used to prevent seawater backflow and bubble counter was used to estimate CO_2 diffusion. The control experiment was conducted under similar condition but without CO_2 stimulation. Instead of CO_2, constant compress airflow was used in the control experiment. The CO_2 and compress air for the elevated CO_2 and control experiment was adjusted until constant seawater pH at 7.90 ± 0.05 and 8.19 ± 0.02 was obtained, respectively. Table 1 shows carbon dioxide, pH, dissolved oxygen, temperature, and salinity in the experimental chambers. The experiment was conducted for 15 days. The aquaria were cleaned once a week to remove microalgae grew on the glass. Seawater alkalinity was determined daily, photosynthesis rate of the corals was determined once every three days, while the zooxanthellae density, mitotic index, chlorophyll pigments, and surface area of the specimens were analyzed at the end of the experiment. Similar experimental setup was repeated for *G. fascicularis*.

2.3. Determination of Net Photosynthesis Rate. Coral nubbins were carefully transferred into six (3 transparent and 3 opaque) 1.5 L airtight chambers (one nubbin in each chamber). The chambers were filled with seawater at the desired pCO_2, tighten without headspace and air bubble, and incubated in the experimental aquarium for two hours. Dissolved oxygen (DO) in the glass chamber was measured before and after the incubation by using Winkler's method [16]. Gross primary production rate was assessed by summation of oxygen produced by net photosynthesis and respiration rate of the corals [17]. Six empty chambers (3 transparent and 3 opaque) were also incubated in the experimental aquaria to serve as controls. Data were standardized using surface area of the specimens. Surface area of the specimens was measured using paraffin wax technique [18]. Briefly, paraffin wax was used to coat the specimen. Surface area of the specimens could be obtained by referring the weight of the paraffin wax coated on the specimen to the standard curve of paraffin wax versus surface area. The standard curve was generated by regressing weight of the paraffin wax to known surface area density blocks.

2.4. Zooxanthellae Density and Mitotic Index. Coral tissue was extracted by using modified Water Pik method [19] by brushing off the coral surfaces and flushed with filtered seawater (0.2 μm milipore). The process was repeated until the coral specimen turned white. The volume of the extracts was recorded and later determined for the zooxanthellae density. The zooxanthellae cell was counted using an improved Neubauer haemacytometer. Zooxanthellae density was determined from the total zooxanthellae cell extracted from the coral over the total surface area of the specimen. Mitotic Index (MI %) was determined from the number of cell division over the population of the cell density [20]. Dividing cell is defined as any cell between the points of initial appearance of cell walls in the mother cells to the formation of their own cell envelope in the daughter cells [21].

2.5. Chlorophyll Analysis. Chlorophyll pigment was analysed by using spectrophotometric method [16]. Three aliquots of coral tissue extracts were used to determine the chlorophylls content. The extract was first filtered through a GFC filter paper (Milipore) at half the atmospheric pressure (0.5 atm); a few drops of magnesium carbonate (Merck) were added during the filtration to prevent the formation of phaeopigments. The GFC filter paper was then transferred into a 15 mL centrifuge tube; 10 mL of 90% acetone (Merck) was added into the tube and shaken thoroughly. The sample was stored at −20°C in the dark for 24 hours before they were centrifuged at 875 g for 10 minutes. The supernatant was decanted into a 1 cm glass cuvette and measured at 664 nm, 647 nm, and 630 nm by using a double beam UV-Visible spectrophotometer (Shimadzu 1601). The chlorophyll a, b, and c content were determined by using the equations reported by Parsons et al. [16].

2.6. Coral Calcification, Surface Area, and CO_2 Speciation. Coral calcification rate was measured by using method developed by Smith and Kinsey [22] based on alkalinity anomaly. This technique assumed that each mole of $CaCO_3$ precipitated the total alkalinity was lowered by two equivalents. Alkalinity was determined by using titration method as described by APHA [23]. Concentration of bicarbonate ion, carbonate ion, and dissolved carbon dioxide was calculated based on the total alkalinity and pH to examine the dynamics of the entire carbonate system for the experiment. Seawater pH was measured in total scale. Measurement and preparation of the calibration buffer for total scale pH was based on the procedures described by Wedborg et al. [24].

Table 1: Water quality parameters (mean ± standard deviation) in the control and experimental aquaria. The reading was based on day 0 and subsequent daily measurements for fifteen days ($n = 16$).

	P. cylindrica		*G. fascicularis*	
	Control	Elevated CO_2	Control	Elevated CO_2
CO_2, μmol·kg^{-1}	23.34 ± 3.46	70.07 ± 7.36	28.65 ± 2.67	64.77 ± 9.59
CO_3^{-2}, μmol·kg^{-1}	27.45 ±1.34	15.17 ±1.18	28.91 ±2.32	15.57 ±1.91
HCO_3^-, μmol·kg^{-1}	1016 ±8	1072 ±7	1018 ±5	1066 ±9
Salinity, %	35 ± 1	35 ± 1	35 ± 1	35 ± 1
Temperature, °C	27 ± 1	27 ± 1	27 ± 1	27 ± 1
pH	8.19 ± 0.02	7.90 ± 0.05	8.18 ± 0.01	7.89 ± 0.03
Dissolved oxygen, mg O_2L^{-1}	6.31 ± 0.03	6.71 ± 0.09	6.37 ± 0.05	6.47 ± 0.08

2.7. Data Analysis. The effect of elevated CO_2 on photosynthesis, calcification, chlorophyll, mitotic index, and the zooxanthellae cell density of the scleractinian corals was tested by using paired sample *t*-test. Paired sample *t*-test was conducted to analyze data collected in day 0 and day 14. Coral calcification against time was tested using regression analysis. SAS and Microsoft Excel with analysis tool-pack module were used for the statistical analysis.

3. Results

3.1. Effect of Elevated CO_2 on Net Photosynthesis and Respiration. The effect of elevated carbon dioxide in seawater on photosynthesis and respiration rate of the corals was dependent on species (Figure 1). Photosynthesis ($P = .02$) and respiration rate ($P = .04$) of *P. cylindrica* decreased significantly under the elevated pCO_2. On average, the net photosynthesis rate of *P. cylindrica* under elevated pCO_2 and control experiment was $0.156 \pm 0.07\,\mu$mol O_2 cm^{-2} h^{-1} and $0.307 \pm 0.07\,\mu$mol O_2 cm^{-2} h^{-1}, respectively. Oxygen consumption rate of *P. cylindrica* was also significantly lower ($P = .04$). On average, the oxygen consumption rate of *P. cylindrica* under elevated pCO_2 and control experiment was $0.77 \pm 0.06\,\mu$mol O_2 cm^{-2} h^{-1} and $0.97 \pm 0.06\,\mu$mol O_2 cm^{-2} h^{-1}, respectively.

Elevated pCO_2 in the seawater did not lead to a significant change in the net photosynthesis rate of *G. fascicularis* ($P = .48$). Average net photosynthesis rate of the coral under normal and elevated CO_2 was 1.18 ± 0.13 and $1.17 \pm 0.11\,\mu$mol O_2 cm^{-2} h^{-1}, respectively. Unlike *P. cylindrica*, respiration rate of *G. fascicularis* in the elevated CO_2 environment was significantly higher than the control ($P = .004$). The respiration rate of *G. fascicularis* in normal and the elevated pCO_2 was 0.66 ± 0.11 and $1.10 \pm 0.19\,\mu$mol O_2 cm^{-2} h^{-1}, respectively. Gross photosynthesis rate of *G. fascicularis* was also significantly higher than that of the control ($P = .001$). On average, gross photosynthesis rate of *G. fascicularis* under normal and the elevated pCO_2 was 2.27 ± 0.25 and $1.83 \pm 0.14\,\mu$mol O_2 cm^{-2} h^{-1}, respectively.

3.2. Chlorophyll Pigments, Zooxanthellae Cell Density, and Mitotic Index. Chlorophyll pigments, zooxanthellae cell density, and mitotic index of *P. cylindrica* and *G. fascicularis* responded differently under elevated pCO_2 (Table 2). Photosynthetic pigments of the zooxanthellae algae in *P. cylindrica* seem to be increased under elevated pCO_2; however the increment was not significant (chlorophyll-a, $P = .088$; chlorophyll-b, $P = .386$; chlorophyll-c, $P = .109$). Nevertheless, elevated pCO_2 reduced the zooxanthellae cell density ($P = .043$) and the mitotic index ($P = .010$) significantly in *P. cylindrica*.

Photosynthetic pigments of the zooxanthellae algae in *G. fascicularis* increased significantly under elevated pCO_2 (chlorophyll-a, $P = .016$; chlorophyll-b, $P = .004$; chlorophyll-c, $P = .006$). Under the elevated pCO_2, zooxanthellae cell density in *G. fascicularis* declined significantly ($P = .002$) as compared to the control. However, The mitotic cell division index was remained as that in the control experiment.

3.3. Calcification. Figure 2 showed the effect of elevated pCO_2 on the calcium carbonate precipitation rate of *P. cylindrica* and *G. fascicularis*. *P. cylindrica* suffered from a significant decline in the calcium carbonate precipitation rate. On average, the calcium carbonate precipitation rate of *P. cylindrica* declined from $0.203 \pm 0.137\,\mu$mol $CaCO_3$ cm^{-2}d^{-1} (control) down to $-0.150 \pm 0.196\,\mu$mol $CaCO_3$ cm^{-2}d^{-1}. At the end of the experiment, *P. cylindrica* suffered from a net dissolution of calcium carbonate from its skeleton. *G. fascicularis* was able to tolerate the pCO_2 stress.

G. fascicularis sustained a higher calcium carbonate precipitation rate under the elevated pCO_2. On average, the $CaCO_3$ precipitation rate of *G. fascicularis* in the control and elevated CO_2 environment was 0.112 ± 0.099 and $0.256 \pm 0.209\,\mu$mol $CaCO_3$ cm^{-2} d^{-1}, respectively. The calcium carbonate precipitation rate of *G. fascicularis* under elevated CO_2 environment overtook the control after four days.

4. Discussion

The very first effect of the elevated pCO_2 was on the thermodynamic equilibrium of the seawater carbonate. The seawater pH_T declined from pH_T 8.19 ± 0.02 in the control experiment down to pH_T 7.90 ± 0.04 in the experimental tank. Decrease seawater pH would have an effect on the intracellular pH of the corals [25]. Changes in seawater pH

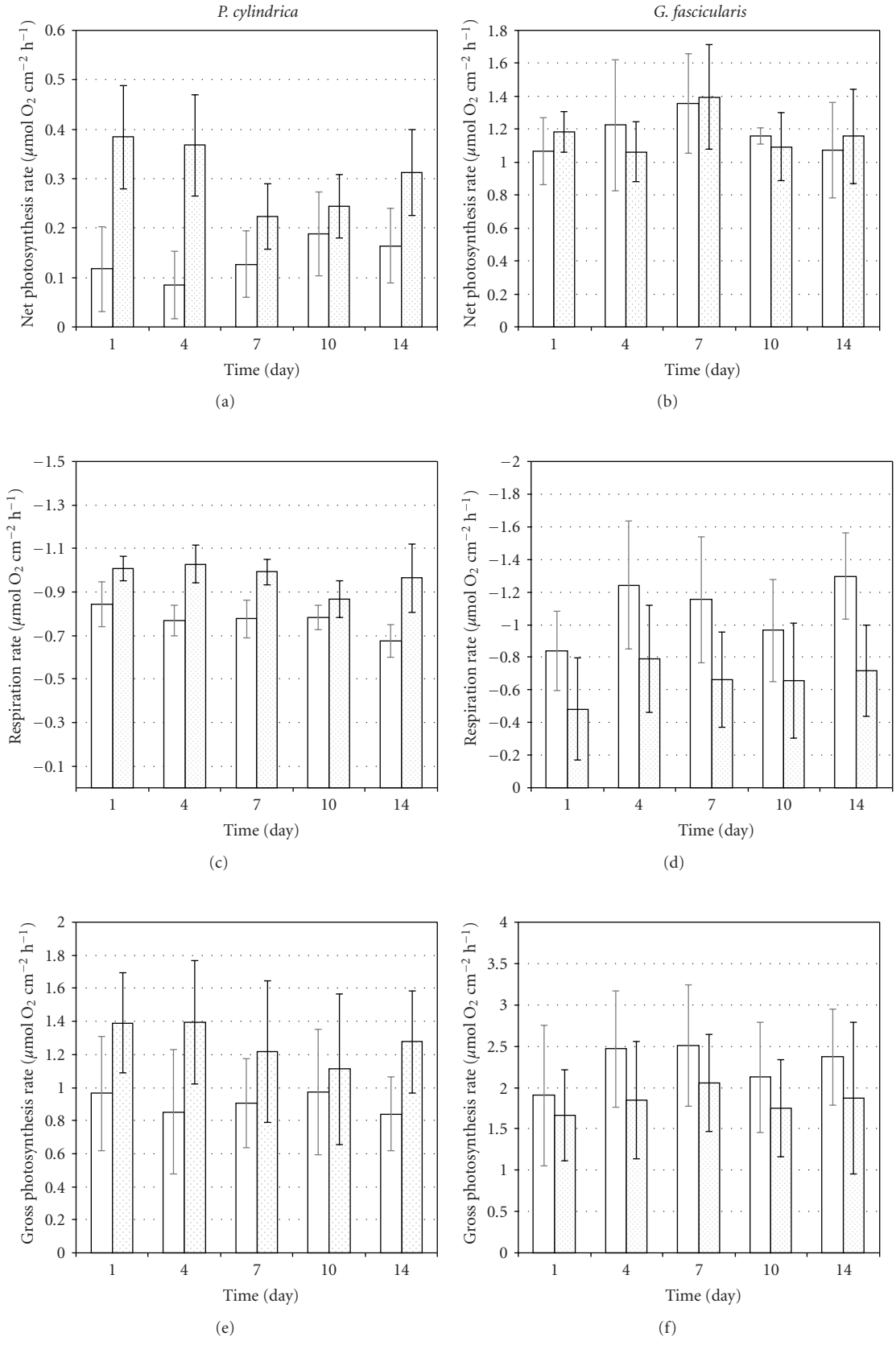

Figure 1: Changes in net photosynthesis, respiration, and gross photosynthesis in *P. cylindrica* and *G. fascicularis* under normal and elevated pCO_2. Open bar (□) indicates elevated pCO_2 while dotted bar (□) indicates control condition.

Table 2: Chlorophyll pigments, zooxanthellae cell density, and mitotic index of *P. cylindrica* and *G. fascicularis* (mean ± standard deviation) under control and elevated pCO_2. Each reading was based on six coral nubbins analysed at the end of the experiment. Pair *t*-test was conducted to compare the parameters under control and elevated pCO_2. Bolded figures indicate a significant different and vice versa.

	P. cylindrica			*G. fascicularis*		
	Control	Elevated pCO_2	*P*	Control	Elevated pCO_2	*P*
Chlorophyll-*a* (μg 10^6 cell^{-1})	8.25 ± 2.37	12.47 ± 6.28	.088	6.75 ± 1.61	12.51 ± 0.59	.016
Chlorophyll-*b* (μg 10^6 cell^{-1})	10.65 ± 4.25	11.58 ± 5.95	.386	3.50 ± 1.11	13.08 ± 0.63	.004
Chlorophyll-*c* (μg 10^6 cell^{-1})	9.70 ± 3.96	14.25 ± 7.77	.109	1.64 ± 0.62	18.79 ± 8.89	.006
Zooxanthellae cell density ($\times$ 10^6 cells cm^{-2})	1.58 ± 0.18	1.09 ± 0.56	.043	2.90 ± 1.08	0.87 ± 0.21	.002
Mitotic index (%)	2.69 ± 1.30	0.95 ± 0.68	.010	5.18 ± 1.46	6.47 ± 3.01	.322

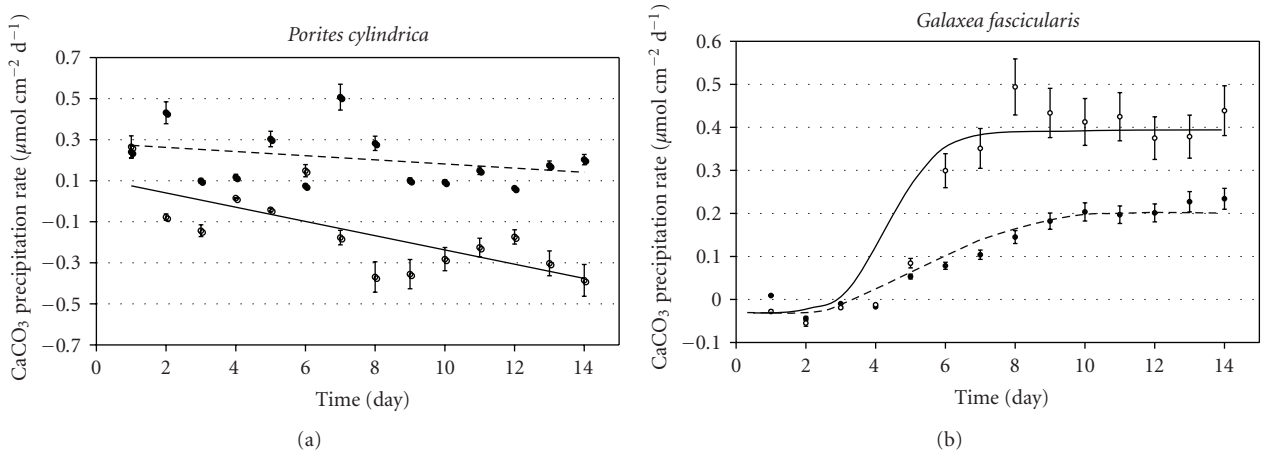

Figure 2: Calcium carbonate precipitation rate for *P. cylindrica* and *G. fascicularis* under control and elevated pCO_2 experiment. Dashed line and solid circle indicate control while solid line and open circle indicate elevated pCO_2.

would subsequently affect protein properties, cell membrane permeability and other biological functions including the calcium carbonate precipitation rate [26, 27]. Although decrease seawater pH would have an effect on the aragonite saturation state ($\Omega_{aragonite}$), Gattuso et al. [28] suggested that calcification of *Stylophora pistillata* and *Acropora* sp. may not be affected significantly at the level corresponding to 560 μatm pCO_2.

When incubated under high pCO_2, *P. cylindrica* showed a lower respiration rate, photosynthesis rate, zooxanthellae density, and the cell mitotic index and there was no significant different in terms of the photosynthesis pigments in the zooxanthellae cells incubated under normal and elevated pCO_2. The calcium carbonate precipitation rate of the coral's declined significantly under the elevated pCO_2. At the end of the experiment, the corals were dying and produced substantial amount of mucous. *G. fascicularis* reacted differently under elevated pCO_2. The zooxanthellae cells contained in the coral were expelled by 71% under the elevated pCO_2. There is no significant change in the mitotic index of the zooxanthellae cell incubated under elevated pCO_2 and control experiment. The photosynthetic pigments of the remaining zooxanthellae cells increased significant under the elevated pCO_2. Under the elevated pCO_2, the corals increased gross photosynthesis rate to offset the increased respiration rate. The net photosynthesis remained no change in the experiments. At the end of the incubation, *G. fascicularis* sustained a positive calcium carbonate precipitation rate in the elevated pCO_2 experiment. The *G. fascicularis* remained healthy under the elevated pCO_2 condition until the end of the experiments.

In terms of corals' calcification rate, *G. fascicularis* was able to withstand the pCO_2 stress while *P. cylindrica* failed to tolerate elevated pCO_2. The mechanisms of how corals precipitate calcium carbonate onto their skeletal organic matrix are not fully understood yet. It is commonly recognized that, calcification is a highly biologically control mechanisms and simultaneous supply of ions and the formation of skeletal organic matrices are essential controls for the coral's calcification. In order to build the skeleton, corals have to supply calcium and inorganic carbon from the seawater to the deposition site and also remove protons produced during the process. The transcellular pathway for transporting ions through the epithelia is an active process which required energy to against the concentration gradient; especially in the calicoblastic ectoderm [2]. The elevated pCO_2 depressed the respiration rate of *P. cylindrica*. As a result, lower energy supply retarded the calcium carbonate precipitation in the coral. Under elevated pCO_2, *G. fascicularis* expelled its zooxanthellae cell in order to counter the stress. Zooxanthellae cell density was significantly declined and the photosynthetic pigments (chlorophyll-a, b and c)

of the remaining zooxanthellae cells increased significantly (1.85–11.5 fold) to compensate the photosynthetic ration of the expelled cells. Higher respiration rate of *G. fascicularis* under the elevated pCO_2 enhanced higher energy supply to the coral and drove higher calcium carbonate precipitation rate for the coral.

Although photosynthesis of the symbiotic algae is highly coupled with the coral calcification, the full mechanisms underlying the interaction are not known [1, 2]. Allemand et al. [2] proposed photosynthesis-induced OH^- secretion that neutralized H+ produced during the calcium carbonate precipitation. By removing the hydrogen ion, the process of the calcification could be driven the precipitation process. *G. fascicularis* in this study was able to sustain their photosynthesis rate and the physiological adaptation of the coral made them to withstand the CO_2 stress. Other than that, thick tissue biomass of *G. fascicularis* may also help the coral's calcification as this process took place in the cell cytoplasm. Loya et al. [29] also hypothesized that under environmentally stressful conditions, thick tissue could protect the underlying zooxanthellae from severe stress. The thin layer tissue and dense skeleton of *P. cylindrica* do not have this advantages and this may be contributed to the skeletal loss of the coral.

This study found that *P. cylindrica* suffered from the elevated pCO_2 while *G. fascicularis* was able to deal with the stress. The impact of elevated pCO_2 on corals is species specific. Different species of scleractinian corals in the reefs may have their own physiological adaptation to overcome the pCO_2 stress. Coral calcification is a biological controlled and energy-demanding mechanism. It is believed that, the status of the coral in terms of its energy reserves and the feeding strategies should have a role in their ability to tolerate external stress.

5. Conclusion

Elevated pCO_2 in seawater stressed *P. cylindrica* and *G. fascicularis*. Degree of the CO_2 stress on the corals was species dependant. High pCO_2 declined zooxanthellae cell density, mitotic cell index, respiration, and photosynthesis rate of *P. cylindrica* and, as a result, reduced calcium carbonate precipitation rate of *P. cylindrica*. *G. fascicularis* expelled its zooxanthellae cell under high pCO_2. Photosynthetic pigments contained in the remaining symbiotic zooxanthellae increased to sustain the photosynthesis. High pCO_2 also increased respiration rate of *G. fascicularis*. At the end of the experiment, *G. fascicularis* incubated under high pCO_2 increased its calcium carbonate precipitation rate. *G. fascicularis* showed better tolerance towards high pCO_2 as compared to *P. cylindrica*. The species-dependent reaction of the corals suggested that increase atmospheric pCO_2 would affect species diversity of the coral reefs.

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References

[1] Y. Helman, F. Natale, R. M. Sherrell, et al., "Extracellular matrix production and calcium carbonate precipitation by coral cells in vitro," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 1, pp. 54–58, 2008.

[2] D. Allemand, C. Ferrier-Pagès, P. Furla, et al., "Biomineralisation in reef-building corals: from molecular mechanisms to environmental control," *Comptes Rendus Palevol*, vol. 3, no. 6-7, pp. 453–467, 2004.

[3] J. R. Petit, J. Jouzel, D. Raynaud, et al., "Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica," *Nature*, vol. 399, no. 6735, pp. 429–436, 1999.

[4] J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. Linden, and D. Xiaosu, *Climate Change 2001: The Scientific Basic*, Cambridge University Press, Cambridge, UK, 2001.

[5] S. Solomon, D. Qin, M. Manning, et al., Eds., *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change*, IPCC (Intergovernmental Panel on Climate Change), Cambridge University Press, Cambridge, UK, 2007.

[6] K. Caldeira and M. E. Wickett, "Oceanography: anthropogenic carbon and ocean pH," *Nature*, vol. 425, no. 6956, p. 365, 2003.

[7] J. C. Orr, V. J. Fabry, O. Aumont, et al., "Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms," *Nature*, vol. 437, no. 7059, pp. 681–686, 2005.

[8] N. Leclercq, J.-P. Gattuso, and J. Jaubert, "CO_2 partial pressure controls the calcification rate of a coral community," *Global Change Biology*, vol. 6, no. 3, pp. 329–334, 2000.

[9] S. Reynaud, N. Leclercq, S. Romaine-Lioud, C. Ferrier-Pagès, J. Jaubert, and J.-P. Gattuso, "Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral," *Global Change Biology*, vol. 9, no. 11, pp. 1660–1668, 2003.

[10] C. Langdon and M. J. Atkinson, "Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment," *Journal of Geophysical Research C*, vol. 110, no. 9, pp. 1–16, 2005.

[11] B. I. McNeil, R. J. Matear, and D. J. Barnes, "Coral reef calcification and climate change: the effect of ocean warming," *Geophysical Research Letters*, vol. 31, no. 22, pp. 1–4, 2004.

[12] J. M. Lough and D. J. Barnes, "Environmental controls on growth of the massive coral Porites," *Journal of Experimental Marine Biology and Ecology*, vol. 245, no. 2, pp. 225–243, 2000.

[13] C. Langdon, T. Takahashi, C. Sweeney, et al., "Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef," *Global Biogeochemical Cycles*, vol. 14, no. 2, pp. 639–654, 2000.

[14] F. Marubini, C. Ferrier-Pagès, P. Furla, and D. Allemand, "Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism," *Coral Reefs*, vol. 27, no. 3, pp. 491–499, 2008.

[15] Y.-S. Hii, C.-L. Soo, and H.-C. Liew, "Feeding of scleractinian coral, *Galaxea fascicularis*, on *Artemia salina nauplii* in captivity," *Aquaculture International*, vol. 17, no. 4, pp. 363–376, 2009.

[16] T. R. Parsons, Y. Maita, and C. M. Lalli, *A Manual of Chemical and Biological Methods for Seawater Analysis*, Pergamon, Oxford, UK, 1984.

[17] A. T. Marshall and P. L. Clode, "Effect of increased calcium concentration in sea water on calcification and photosynthesis in the scleractinian coral *Galaxea fascicularis*," *Journal of Experimental Biology*, vol. 205, no. 14, pp. 2107–2113, 2002.

[18] J. Stimson and R. A. Kinzie III, "The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions," *Journal of Experimental Marine Biology and Ecology*, vol. 153, no. 1, pp. 63–74, 1991.

[19] R. E.. Johannes and W. J. Wiebe, "Method for determination of coral tissue biomass and composition," *Limnology and Oceanography*, vol. 15, no. 5, pp. 822–824, 1970.

[20] J. M. Cervino, R. Hayes, T. J. Goreau, and G. W. Smith, "Zooxanthellae regulation in yellow blotch/band and other coral diseases contrasted with temperature related bleaching: in situ destruction vs expulsion," vol. 37, no. 1–3, pp. 63–85.

[21] E. A. Titlyanov, T. V. Titlyanova, V. A. Leletkin, J. Tsukahara, R. van Woesik, and K. Yamazato, "Degradation of zooxanthellae and regulation of their density in hermatypic corals," *Marine Ecology Progress Series*, vol. 139, no. 1–3, pp. 167–178, 1996.

[22] S. V. Smith and K. W. Kinsey, "Calcification and organic carbon metabolism as indicated by carbon dioxide," in *Coral Reefs: Research Methods*, D. R. Stoddart and R. E. Johannes, Eds., pp. 469–484, UNESCO, Paris, France, 1978.

[23] APHA (American Public Health Association), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 20th edition, 2002.

[24] M. Wedborg, D. R. Turner, L. G. Anderson, and D. Dyrssen, "Determination of pH," in *Methods of Seawater Analysis*, K. Grasshoff, K. Kremling, and M. Ehrhardt, Eds., pp. 109–126, Wiley-VCH, Weinheim, Germany, 1999.

[25] F. Marubini and M. J. Atkinson, "Effects of lowered pH and elevated nitrate on coral calcification," *Marine Ecology Progress Series*, vol. 188, pp. 117–121, 1999.

[26] J.-P. Gattuso and R. W. Buddemeier, "Ocean biogeochemistry: calcification and CO_2," *Nature*, vol. 407, no. 6802, pp. 311–313, 2000.

[27] C. Jiang, A. Rojas, R. Wang, and X. Wang, "CO_2 central chemosensitivity: why are there so many sensing molecules?" *Respiratory Physiology and Neurobiology*, vol. 145, no. 2-3, pp. 115–126, 2005.

[28] J.-P. Gattuso, D. Allemand, and M. Frankignoulle, "Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry," *American Zoologist*, vol. 39, no. 1, pp. 160–183, 1999.

[29] Y. Loya, K. Sakai, K. Yamazato, Y. Nakano, H. Sambali, and R. van Woesik, "Coral bleaching: the winners and the losers," *Ecology Letters*, vol. 4, no. 2, pp. 122–131, 2001.