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 pCO₂ on *Porites cylindrica* and *Galaxea fascicularis*. The corals responded differently under elevated pCO₂. *Zooxanthellae* cell density, cell mitotic index, and photosynthesis rate of *P. cylindrica* decreased drastically under the elevated pCO₂. 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 pCO₂. 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 pCO₂ in the atmosphere may affect species diversity of coral reefs.

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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 CO₂ emission from land use change, fossil fuels, cement, coal and oil combustion has caused the average partial pressure of carbon dioxide (pCO₂) 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 pCO₂ 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 CO₂. 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 despite of the increasing pCO₂ over the last century. Reynaud et al. [9] reported that calcification of *Stylophora pistillata* did not change in response to an increased pCO₂ under normal temperature. Basically, most of the reports suggested more studies are needed to reveal the effect of elevated pCO₂ on corals. Hence, this study aims to extend the understanding on the impact of elevated pCO₂ on the scleractinian 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 $392 \pm 5\mu mol \text{m}^{-2} \text{s}^{-1}$. 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 $p$CO$_2$ 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 aquarium was used for the elevated $p$CO$_2$ and the other one as control. Six similar sizes *P. cylindrica* nubbins (~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 $392 \pm 5\mu mol \text{m}^{-2} \text{s}^{-1}$ 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 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 macroalgae 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 $p$CO$_2$, tightened 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].
2.7. Data Analysis. The effect of elevated CO2 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 CO2 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 pCO2. On average, the net photosynthesis rate of P. cylindrica under elevated pCO2 and control experiment was 0.156 ± 0.07 μmol O2 cm−2 h−1 and 0.307 ± 0.07 μmol O2 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 pCO2 and control experiment was 0.77 ± 0.06 μmol O2 cm−2 h−1 and 0.97 ± 0.06 μmol O2 cm−2 h−1, respectively.

Elevated pCO2 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 CO2 was 1.18 ± 0.13 and 1.17 ± 0.11 μmol O2 cm−2 h−1, respectively. Unlike P. cylindrica, respiration rate of G. fascicularis under the elevated CO2 environment was significantly higher than the control (P = .004). The respiration rate of G. fascicularis in normal and the elevated pCO2 was 0.66 ± 0.11 and 1.10 ± 0.19 μmol O2 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 pCO2 was 2.27 ± 0.25 and 1.83 ± 0.14 μmol O2 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 pCO2 (Table 2). Photosynthetic pigments of the zooxanthellae algae in P. cylindrica seem to be increased under elevated pCO2; however the increment was not significant (chlorophyll-a, P = .088; chlorophyll-b, P = .386; chlorophyll-c, P = .109). Nevertheless, elevated pCO2 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 pCO2 (chlorophyll-a, P = .016; chlorophyll-b, P = .004; chlorophyll-c, P = .006). Under the elevated pCO2, 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 pCO2 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 ± 0.137 μmol CaCO3 cm−2 d−1 (control) down to −0.150 ± 0.196 μmol CaCO3 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 pCO2 stress.

G. fascicularis sustained a higher calcium carbonate precipitation rate under the elevated pCO2. On average, the CaCO3 precipitation rate of G. fascicularis in the control and elevated CO2 environment was 0.112 ± 0.099 and 0.256 ± 0.209 μmol CaCO3 cm−2 d−1, respectively. The calcium carbonate precipitation rate of G. fascicularis under elevated CO2 environment overtook the control after four days.

4. Discussion

The very first effect of the elevated pCO2 was on the thermodynamic equilibrium of the seawater carbonate. The seawater pH declined from pH7 8.19 ± 0.02 in the control experiment down to pH7 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 $p$CO$_2$. Open bar (□) indicates elevated $p$CO$_2$ 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 pCO₂. 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 pCO₂. Bolded figures indicate a significant different and vice versa.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Elevated pCO₂</th>
<th>P</th>
<th>Control</th>
<th>Elevated pCO₂</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>P. cylindrica</strong></td>
<td></td>
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</tr>
<tr>
<td>Chlorophyll-a (µg 10⁶ cell⁻¹)</td>
<td>8.25 ± 2.37</td>
<td>12.47 ± 6.28</td>
<td>.088</td>
<td>6.75 ± 1.61</td>
<td>12.51 ± 0.59</td>
<td>.016</td>
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<tr>
<td>Chlorophyll-b (µg 10⁶ cell⁻¹)</td>
<td>10.65 ± 4.25</td>
<td>11.58 ± 5.95</td>
<td>.386</td>
<td>3.50 ± 1.11</td>
<td>13.08 ± 0.63</td>
<td>.004</td>
</tr>
<tr>
<td>Chlorophyll-c (µg 10⁶ cell⁻¹)</td>
<td>9.70 ± 3.96</td>
<td>14.25 ± 7.77</td>
<td>.109</td>
<td>1.64 ± 0.62</td>
<td>18.79 ± 8.89</td>
<td>.006</td>
</tr>
<tr>
<td>Zooxanthellae cell density (× 10⁶ cells cm⁻²)</td>
<td>1.58 ± 0.18</td>
<td>1.09 ± 0.56</td>
<td>.043</td>
<td>2.90 ± 1.08</td>
<td>0.87 ± 0.21</td>
<td>.002</td>
</tr>
<tr>
<td>Mitotic index (%)</td>
<td>2.69 ± 1.30</td>
<td>0.95 ± 0.68</td>
<td>.010</td>
<td>5.18 ± 1.46</td>
<td>6.47 ± 3.01</td>
<td>.322</td>
</tr>
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|                      |                  |               |        |                  |               |        |
| **G. fascicularis**  |                  |               |        |                  |               |        |
| Chlorophyll-a (µg 10⁶ cell⁻¹) | 3.01 ± 0.32     | 1.46 ± 6.47   | .002   | 0.21 ± 0.00      | 8.89 ± 0.006  | .006   |
| Chlorophyll-b (µg 10⁶ cell⁻¹) | 1.11 ± 13.08    | 0.63 ± 0.004  | .016   | 8.89 ± 0.006     | 0.59 ± 0.016  | .016   |
| Chlorophyll-c (µg 10⁶ cell⁻¹) | 0.59 ± 1.66     | 0.59 ± 1.66   | .004   | 0.59 ± 1.66      | 0.59 ± 1.66   | .004   |
| Zooxanthellae cell density (× 10⁶ cells cm⁻²) | 3.01 ± 0.32 | 1.46 ± 6.47 | .002 | 0.21 ± 0.00 | 8.89 ± 0.006 | .006 |
| Mitotic index (%)     | 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 pCO₂ experiment. Dashed line and solid circle indicate control while solid line and open circle indicate elevated pCO₂.

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 (Ω_{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 pCO₂.

When incubated under high pCO₂, *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 pCO₂. The calcium carbonate precipitation rate of the coral’s declined significantly under the elevated pCO₂. At the end of the experiment, the corals were dying and produced substantial amount of mucous. *G. fascicularis* reacted differently under elevated pCO₂. The zooxanthellae cells contained in the coral were expelled by 71% under the elevated pCO₂. There is no significant change in the mitotic index of the zooxanthellae cell incubated under elevated pCO₂ and control experiment. The photosynthetic pigments of the remaining zooxanthellae cells increased significant under the elevated pCO₂. Under the elevated pCO₂, 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 pCO₂ experiment. The *G. fascicularis* remained healthy under the elevated pCO₂ condition until the end of the experiments.

In terms of corals’ calcification rate, *G. fascicularis* was able to withstand the pCO₂ stress while *P. cylindrica* failed to tolerate elevated pCO₂. 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 calciblastic ectoderm [2]. The elevated pCO₂ depressed the respiration rate of *P. cylindrica*. As a result, lower energy supply retarded the calcium carbonate precipitation in the coral. Under elevated pCO₂, *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 pCO2 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 calcification process. 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 CO2 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 pCO2 while *G. fascicularis* was able to deal with the stress. The impact of elevated pCO2 on corals is species specific. Different species of scleractinian corals in the reefs may have their own physiological adaptation to overcome the pCO2 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 pCO2 in seawater stressed *P. cylindrica* and *G. fascicularis*. Degree of the CO2 stress on the corals was species dependent. High pCO2 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 pCO2. Photosynthetic pigments contained in the remaining symbiotic zooxanthellae increased to sustain the photosynthesis. High pCO2 also increased respiration rate of *G. fascicularis*. At the end of the experiment, *G. fascicularis* incubated under high pCO2 increased its calcium carbonate precipitation rate. *G. fascicularis* showed better tolerance towards high pCO2 as compared to *P. cylindrica*. The species-dependent reaction of the corals suggested that increase atmospheric pCO2 would affect species diversity of the coral reefs.

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References


