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Effect of $CaCO_3$ (aragonite) saturation state of seawater on calcification of *Porites* coral

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Using living corals collected from Okinawan coral reefs, laboratory experiments were performed to investigate the relationship between coral calcification and aragonite saturation state (Ω) of seawater at 25°C. Calcification rate of a massive coral *Porites lutea* cultured in a beaker showed a linear increase with increasing $\Omega_{aragonite}$ values (1.08–7.77) of seawater. The increasing trend of calcification rate (c) for Ω is expressed as an equation, $c = a\Omega + b$ (a, b: constants). When Ω was larger than ~4, the coral samples calcified during nighttime, indicating an evidence of dark calcification. This study strongly suggests that calcification of *Porites lutea* depends on Ω of ambient seawater. A decrease in saturation state of seawater due to increased $p\mathrm{CO}_2$ may decrease reef-building capacity of corals through reducing calcification rate of corals.

Keywords: coral, calcification, aragonite, saturation state, cultured experiment

INTRODUCTION

Aragonite ($CaCO_3$) is accumulated in coral reefs by calcareous organisms like corals through $CaCO_3$ production (calcification). Such coral calcification plays an important role in constructing reefs in warm shallow-water in which many organisms grow. Since a decrease in coral calcification leads to a decrease in reef-building capacity of corals, it is important to elucidate factors controlling coral calcification. As a chemical factor controlling coral calcification, Ohde and van Woesik (1999) suggested that coral calcification depends on the saturation state of surface seawater with respect to aragonite. The saturation degree of seawater ($\Omega_{aragonite}$) is defined as:

$$\Omega = [Ca^{2+}][CO_3^{2-}]/K'_{sp} \qquad (1)$$

where K'_{sp} is the stoichiometric solubility product for aragonite. Calcium is uniformly distributed throughout the world's oceans due to its long residence time (1.1×10^6 years) (Broecker and Peng, 1982), whereas carbonate concentration $[CO_3^{2-}]$ is subjected to change due to chemical and biological processes in seawater and possibly affects Ω of seawater. Corals are assumed to release CO_2 through calcification as follows:

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3\downarrow + H_2O + CO_2\uparrow. \qquad (2)$$

As a result of coral calcification (Eq. (2)), the $\Omega_{aragonite}$ of seawater is altered through releasing CO_2 in surrounding environments. The elevated CO_2 in marine environments lowers pH through decreasing CO_3^{2-} concentration and leads to a decrease in Ω of seawater.

The relationship between coral-reef calcification and Ω of seawater has been reported through field observations. Broecker and Takahashi (1966) observed that precipitation rate of $CaCO_3$ on the Bahama Bank was proportional to the degree of supersaturation, as a second-order reaction. Smith and Pesret (1974) pointed out that coral calcification in the lagoon of Fanning Island was affected by the carbonate mineral saturation state. On a coral reef flat of Okinawa, Ohde and van Woesik (1999) also observed that calcification rate increased with the increase in $\Omega_{aragonite}$ of seawater during daytime. From a global viewpoint associated with atmospheric CO_2 budget, Kleypas *et al*. (1999) strongly pointed out that coral calcification responds to Ω of seawater.

On the other hand, the relationship between coral calcification and $\Omega_{aragonite}$ of seawater was also studied through laboratory experiments under controlled conditions. Gattuso *et al*. (1998) observed a nonlinear increase in calcification rate of *Stylophora pistillata* with increasing Ω of seawater with a plateau in the rate when saturation state was larger than 3.90. Marubini and Atkinson (1999) also observed that calcification rate of *Porites compressa* decreased with decreasing $\Omega_{aragonite}$ of seawater. However, only a few studies have provided clear relationship between coral calcification and seawater Ω.

In order to elucidate the relationship between coral

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*Corresponding author (e-mail: sohde@sci.u-ryukyu.ac.jp) calcification and $\Omega_{aragonite}$ of seawater, we performed laboratory experiments using *Porites lutea* cultured in a small habitat at 25°C. The reasons why we selected *Porites lutea* are: (1) this coral species is widely distributed on coral reefs in the world (Veron, 2000) due to its strong survival activity, and (2) its skeleton is often used as a recorder of past sea-surface temperature (Smith *et al*., 1979; Shen *et al*., 1996; Mitsuguchi *et al*., 1996; Gagan *et al*., 1998). Here we present observations on coral calcification at 25°C and various $\Omega_{aragonite}$ values of seawater in the laboratory.

METHODS

Coral samples of *Porites lutea* were collected at ~2 m depth from surface water in Mizugama estuary and Rukansho, Okinawa. Fresh seawater was collected from Ginowan and Mizugama estuary to culture the corals in the laboratory. The samples were cultured for a few months prior to experiments putting into individual plastic box with fresh seawater. Seawater was usually renewed every 5 days during coral culturing. Boxes with coral samples were placed in a big plastic tub wherein water temperature was maintained at 25 ± 0.5°C using a cool-water circulator (CL-80F, Taitec, Japan). Aeration was supplied continuously to every coral habitat during the culture. During daytime a white-cool fluorescent light (~18 W) was placed over the coral samples for a photoperiod of 12 hours a day. The light intensity during culture experiments was ~13 μmol photon/m^2/s on average. The light intensity for coral culturing was very low compared with the natural sunlight, because such a light condition was one of the best conditions for small coral samples surviving in a small area (plastic box).

Laboratory experiments were carried out using 4-type seawaters of various $\Omega_{aragonite}$ values, prepared freshly prior to the experiments. The seawater Ω value was controlled by mixing fresh seawater and NaOH solution or HCl solution, shown in Table 1. After preparing each solution, salinity, pH (NBS) and total alkalinity (TA) were measured in order to calculate $\Omega_{aragonite}$ values of the seawater as described below.

Four samples of *Porites lutea* (samples A, B, C and D) incubated at 25°C, were used in this study. The samples were small massive corals shaped as a half sphere to full sphere. The colony sizes are listed in Table 2. A series of laboratory experiments was carried out during January 19–24, March 6–9 and May 9–11, 2002 (Table 2). The experiments were performed for 6 and 3 hours during daytime and nighttime, respectively. For an interval between daytime and nighttime experiments, the coral samples received a rest period of ~2 h, immersing into fresh seawater with aeration.

Depending upon the coral size, 1.0 kg seawater of various saturation states ($\Omega_{aragonite}$) was taken weighing by a balance and poured into a pyrex beaker (1 L). Then one of the selected coral samples was taken from its incubation habitat and was immersed in the beaker. The beaker's opening was sealed by a transparent plastic film (Saran wrap) to avoid air exchange and evaporation. With these arrangements beakers were placed in the water bath of 25°C. During daytime experiments, a fluorescent light was placed over the beakers as an artificial light. The light intensity (μmol photon/m^2/s) was measured by a light meter (LI-250, LI-COR, USA) at every sampling time (0, 3 and 6 h) and the average value was calculated for the respective daytime experiment (Table 2). No light was provided during nighttime experiments and the water tub was covered by black polyethylene bags which prevented penetration of light from outside.

Initial and final values of pH (NBS), total alkalinity (TA) and salinity of seawater were measured for both daytime and nighttime experiments. In addition, pH and TA of experimental seawaters were measured every 3 h during daytime experiments. Soon after collecting experimental seawaters, pH was measured at 25°C using a pH meter (HM-60S, TOA Electronic, Japan) after calibrating it with NBS buffer solutions (pH 6.865 and 9.180). Within a few hours, TA was measured by potentiometric titration with ~49 mM HCl in 0.65M NaCl solution at 25°C (Butler, 1982). The concentration of HCl solution was determined using the CO_2 standard seawater of the

Table 1. Preparation of experimental seawaters. In order to change saturation state ($\Omega_{aragonite}$) of seawater, 12M HCl solution or 10M NaOH solution was added into 10 kg of fresh seawater (salinity = 34.6 psu)

Type of seawater	Chemical addition	pH	TA (μmol kg^{-1})	$\Omega_{aragonite}$
Low Ω	12M-HCl, 0.2 ml	~7.9	2064	~1.6
Fresh	—	~8.2	2273	~3.6
High Ω	10M-NaOH, 0.2 ml	~8.4	2452	~5.5
Higher Ω	10M-NaOH, 0.4 ml	~8.6	2622	~7.2

—: denotes no chemical addition.

Scripps Institute. The equivalence point for each titration was determined by the Gran plot (Gran, 1952). Experimental seawaters preserved for TA measurement were used to measure salinity. Salinity was measured using a salinometer (601 MK III, YEO-KAL, Australia) after calibrating it with an IAPSO standard seawater (salinity = 34.996 psu, K_{15} = 0.99990).

We used 5 data, pH, TA, salinity, temperature (25°C) and pressure (1 atm) to calculate an $\Omega_{\mathrm{aragonite}}$ value of experimental seawaters (Morse and Mackenzie, 1990). The alkalinity anomaly technique (Smith and Kinsey, 1978) was applied to estimate calcification rates of coral samples. Net hourly calcification rate (c) during daytime and nighttime experiments was calculated using the equation:

$$c = 0.5(\Delta \mathrm{TA}) \bullet W / \Delta T \tag{3}$$

where ΔTA is the change of total alkalinity (μmol/kg), W is weight of experimental seawater (kg) and ΔT is the experimental period (h).

RESULTS AND DISCUSSION

The laboratory experiments were performed to elucidate the relationship between coral calcification and $\Omega_{\mathrm{aragonite}}$ of seawater. We prepared seawaters that gave an initial Ω value between 1.08 and 7.77. Initial and final $\Omega_{\mathrm{aragonite}}$ values are calculated by above method (see Table 2). The saturation states are considered to be changed during experimental running for 6 or 3 hours. As shown in Table 2, 15 values of Ω except 8 decreased from their initial values during daytime experiments, whereas 22 values except 1 decreased during nighttime. Because of small changes in alkalinity and seawater equilibria, decreases of Ω values coincide with decreases of pH data as listed in Table 2. Such decreases of Ω and pH are mainly controlled by CO_2 input from coral respiration and calcification. Using such free drift experiments, we measured coral calcification rates and Ω values.

Using Eq. (3), calcification rates of coral samples during daytime and nighttime experiments were calculated as listed in Table 2 and the data versus $\Omega_{\mathrm{aragonite}}$ of seawater for coral A are plotted in Fig. 1. Figure 2 shows the data for coral B. Using data in Table 2, the average value of Ω is plotted including a bar in the figures. Seawater $\Omega_{\mathrm{aragonite}}$ values prepared for the experiments ranged from 1.08 to 7.77 (Table 2). As shown in Table 2, calcification rate of *Porites* coral (c) grossly increases from –2.05 to 33.5 μmol/h during daytime experiments. Figures 1 and 2 clearly indicate that coral samples calcified at 25°C during daytime (see open plots and above lines in the figure)

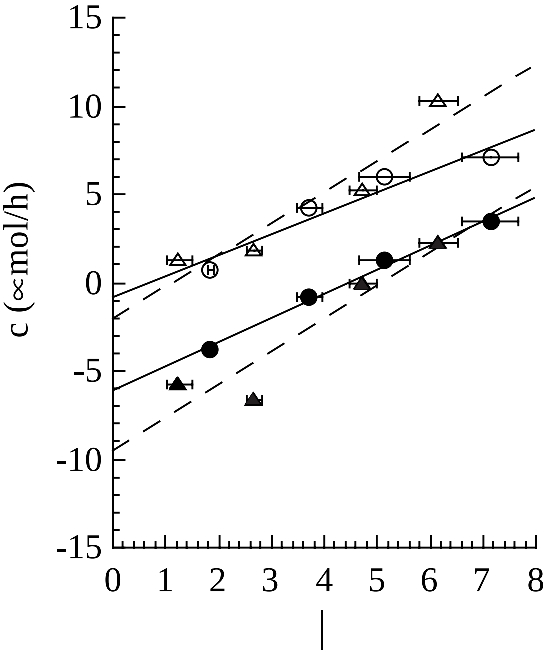

Fig. 1. Relationship between calcification rate (c) of coral sample A and aragonite saturation state (Ω). Open and filled symbols are plotted for daytime and nighttime experiments (see Table 2). Lines in the figure are listed in Table 3. Bars show range of seawater Ω during the experiments.

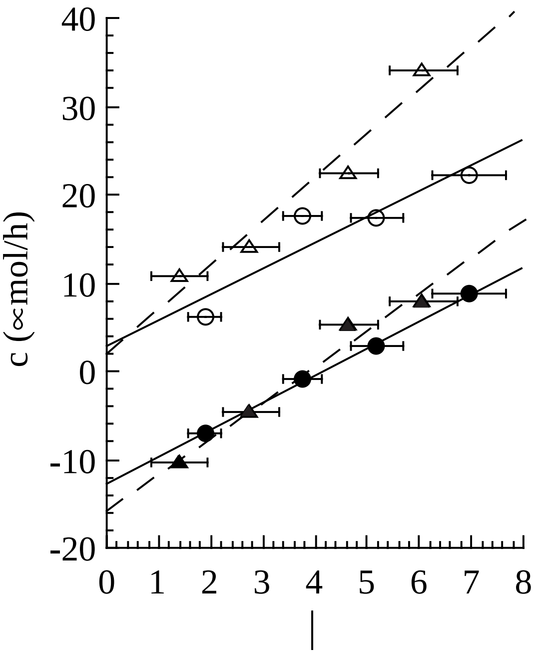

Fig. 2. Relationship between calcification rate (c) of coral sample B and aragonite saturation state (Ω). Open and filled symbols are plotted for daytime and nighttime experiments (see Table 2). Lines in the figure are listed in Table 3. Bars show range of seawater Ω during the experiments.

Table 2. List of samples, experimental dates, light, changes in pH, alkalinity and $\Omega_{aragonite}$ *of seawater, and coral calcification rates (c) at 25 °C. Calcification rate (c) is normalized to surface area of the coral sample.*

Coral sample, Covered area (cm^2)**, Radius (cm)**, Collection location (depth, m), Date	Date (time)	Light (μmol/m^2/s)	pH (NBS)*	Alkalinity* (μmol/kg)	$\Omega_{aragonite}$*	Calcification rate	
						c (μmol/h)	Normalized (μmol/cm^2/h)
A							
123	19 Jan. 2002	11.5	7.930–7.944	2064–2059	1.85–1.90	0.775	0.0063
4.4	20 ″	″	8.296–8.243	2274–2223	4.00–3.57	4.28	0.035
Mizugama (2 m)	23 ″	″	8.473–8.365	2452–2377	5.71–4.69	6.05	0.049
20 Apr. 2001	24 ″	″	8.656–8.542	2622–2535	7.77–6.52	7.09	0.058
	(11:00–17:00)						
	19 Jan. 2002	0	7.930–7.889	2081–2104	1.87–1.77	–3.81	–0.031
	20 ″	″	8.277–8.205	2273–2278	3.87–3.43	–0.863	–0.0070
	23 ″	″	8.445–8.343	2455–2447	5.49–4.67	1.26	0.010
	24 ″	″	8.633–8.539	2624–2603	7.58–6.68	3.43	0.028
	(19:00–22:00)						
	6 Mar. 2002	14.6	7.664–7.885	2069–2054	1.08–1.69	1.31	0.011
	7 ″	″	8.076–8.111	2304–2285	2.75–2.91	1.78	0.014
	8 ″	″	8.371–8.385	2480–2419	4.96–4.94	5.23	0.042
	9 ″	″	8.521–8.488	2642–2519	6.63–6.02	10.3	0.084
	(11:30–17:30)						
	6 Mar. 2002	0	7.679–7.696	2084–2119	1.12–1.18	–5.81	–0.047
	7 ″	″	8.060–8.016	2296–2336	2.66–2.49	–6.65	–0.054
	8 ″	″	8.353–8.283	2485–2485	4.83–4.31	–0.030	–0.00024
	9 ″	″	8.504–8.423	2647–2633	6.49–5.73	2.23	0.018
	(19:30–22:30)						
B							
211	19 Jan. 2002	11.5	7.930–8.066	2064–1994	1.85–2.32	6.13	0.029
3.8	20 ″	″	8.296–8.364	2274–2060	4.00–4.02	17.7	0.084
Rukan-sho (2 m)	23 ″	″	8.473–8.474	2452–2240	5.71–5.19	17.3	0.082
17 Sep. 2001	24 ″	″	8.656–8.591	2622–2346	7.77–6.39	22.3	0.106
	(11:00–17:00)						
	19 Jan. 2002	0	7.930–7.804	2081–2123	1.87–1.48	–7.00	–0.033
	20 ″	″	8.277–8.152	2273–2279	3.87–3.12	–0.892	–0.0042
	23 ″	″	8.445–8.307	2455–2438	5.49–4.38	2.82	0.013
	24 ″	″	8.633–8.483	2624–2571	7.58–6.10	8.76	0.041
	(19:00–22:00)						
	6 Mar. 2002	14.6	7.664–8.075	2069–1943	1.08–2.30	10.4	0.049
	7 ″	″	8.076–8.253	2304–2140	2.75–3.49	13.8	0.065
	8 ″	″	8.371–8.449	2480–2215	4.96–4.96	22.0	0.104
	9 ″	″	8.521–8.561	2642–2237	6.63–5.85	33.5	0.159
	(11:30–17:30)						
	6 Mar. 2002	0	7.679–7.623	2084–2147	1.12–1.03	–10.5	–0.049
	7 ″	″	8.060–7.903	2296–2324	2.66–1.98	–4.78	–0.023
	8 ″	″	8.353–8.191	2485–2456	4.83–3.63	4.92	0.023
	9 ″	″	8.504–8.345	2647–2600	6.49–5.00	7.71	0.036
	(19:30–22:30)						

**Initial – final values.*

***Coral size is denoted as both covered surface area (cm^2) and radius (cm) as assumed sphere.*

Table 2. *(continued)*

Coral sample, Covered area (cm^2)**, Radius (cm)**, Collection location (depth, m), Date	Date (time)	Light (μmol/m^2/s)	pH (NBS)*	Alkalinity* (μmol/kg)	$\Omega_{aragonite}$*	Calcification rate	
						c (μmol/h)	Normalized (μmol/cm^2/h)
C							
87	19 Jan. 2002	11.5	7.930–7.970	2064–2064	1.85–2.00	0.314	0.0036
3.9	20 ″	″	8.296–8.300	2274–2215	4.00–3.91	4.91	0.056
Rukan-sho (2 m)	23 ″	″	8.473–8.445	2452–2378	5.71–5.30	6.27	0.072
17 Sep. 2001	24 ″	″	8.656–8.600	2622–2539	7.77–7.03	6.62	0.076
	(11:00–17:00)						
	19 Jan. 2002	0	7.930–7.890	2081–2092	1.87–1.73	–1.79	–0.021
	20 ″	″	8.277–8.222	2273–2270	3.87–3.52	0.465	0.0053
	23 ″	″	8.445–8.384	2455–2449	5.49–4.99	0.983	0.011
	24 ″	″	8.633–8.562	2624–2598	7.58–6.87	4.28	0.049
	(19:00–22:00)						
D							
48	9 May 2002	13.0	7.956–7.863	2051–2076	1.93–1.60	–2.05	–0.043
4.5	10 ″	″	8.301–8.211	2267–2246	4.00–3.40	1.70	0.035
Mizugama (2 m)	11 ″	″	8.483–8.376	2434–2374	5.75–4.76	5.02	0.104
11 Apr. 2002	(11:45–17:45)						
	9 May 2002	0	7.962–7.857	2071–2083	1.97–1.60	–2.01	–0.042
	10 ″	″	8.286–8.186	2257–2259	3.89–3.30	–0.233	–0.0048
	11 ″	″	8.468–8.383	2414–2392	5.58–4.90	3.70	0.077
	(20:30–23:30)						

when Ω is larger than ~1. The calcification rate of coral samples increased with increasing Ω values of seawater, suggesting that seawater $\Omega_{aragonite}$ influenced calcification rates of *Porites lutea*.

In order to find a clear relationship between calcification rate of *Porites lutea* and $\Omega_{aragonite}$ of seawater, we compare our experimental results with that of Zhong and Mucci (1989). They demonstrated the following empirical equation on inorganic $CaCO_3$ precipitation from seawater:

$$R = k(\Omega_{calcite/aragonite} - 1)^n \tag{4}$$

where R is the inorganic $CaCO_3$ precipitation rate, k is the rate constant, n is the empirical reaction order and $(\Omega_{calcite/aragonite} - 1)$ is the degree of supersaturation with respect to calcite/aragonite. We fit the data in Table 2 to Eq. (4), although negative data of calcification rates are not available for Eq. (4). Calculated n values range from 0.4 to 1.7 using daytime data in Table 2. Therefore, we infer that the n value is possibly ~1 assuming a first-order reaction of coral calcification. As lines shown in Figs. 1 and 2, calcification rate (c) shows good correlation with seawater $\Omega_{aragonite}$ as equation, $c = a\Omega + b$, where a and b are constants. The constant values and r^2 are listed in Table 3. The r^2 values range from 0.820 to 0.999. Such good correlations strongly suggest that coral calcification depends on seawater Ω. Our results also suggest that the coral calcification is inferred to be a first-order reaction of aragonite formation.

In order to generalize c values to coral sizes, calcification rates (c) are normalized to surface covered area of the coral colony. Calculated rates ranged from –0.043 to 0.159 μmol/cm^2/h during daytime (Table 2) and the data are plotted in Fig. 3(a). The rates ranged from –0.054 to 0.077 μmol/cm^2/h during nighttime (Table 2) and the data are plotted in Fig. 3(b). Because normalized rates are calculated from only surface covered area, the rates are unbiased from other biological activities. However, Fig. 3 shows that calcification rates increase with increasing seawater Ω. The intercept of Ω (where $c = 0$) to the line in Fig. 3(a), gives 0.83. A value of 0.83 is close to 1.0 shown in Eq. (4). Inorganic aragonite is theoretically precipitated when Ω is larger than 1. Our finding suggests that coral calcification during daytime is possibly controlled by an inorganic precipitation law.

Coral calcification rates during daytime have been measured through a few laboratory experiments. Gattuso

Table 3. A linear correlation between coral calcification rate (c) and seawater Ω including correlation co-efficient (r^2)

Coral sample, Date	Relationship ($c = a\Omega + b$)					
	Daytime			Nighttime		
	a	b	r^2	a	b	r^2
A						
Jan. 19–24, 2002	1.19	–0.769	0.925	1.37	–6.13	0.991
Mar. 6–9, 2002	1.80	–2.06	0.899	1.86	–9.52	0.878
B						
Jan. 19–24, 2002	2.94	2.80	0.849	3.06	–12.7	0.998
Mar. 6–9, 2002	4.93	1.82	0.950	4.12	–15.8	0.977
C						
Mar. 6–9, 2002	1.14	–0.692	0.820	1.05	–3.86	0.949
D						
May 9–11, 2002	2.05	–5.72	0.999	1.63	–5.32	0.932

et al. (1998) reported that calcification rates of *Stylophora pistillata* (colony size: 3–5 cm, wt.: 7.2–25.7 g) increased ~3-fold with increasing $\Omega_{\text{aragonite}}$ from 0.98 to 3.90. Using artificial seawater, halide lamp (450 μmol/m^2/s) and 27°C conditions, they changed [Ca^{2+}] in seawater, maintained a constant pH and [CO_3^{2-}], and observed a nonlinear increase in calcification rate with increasing $\Omega_{\text{aragonite}}$ of seawater. They also observed a plateau in calcification rates when $\Omega_{\text{aragonite}}$ was between 3.90 and 5.85. Marubini and Thake (1999) also observed using halide lamp and 27°C conditions, that calcification rate of *Porites porites* increased with increasing $\Omega_{\text{aragonite}}$ of seawater. After culturing tips of *Porites compressa* in ambient seawater at pH 8.0, Marubini and Atkinson (1999) reduced seawater pH from 8.0 to 7.2 through addition of HCl. They observed that calcification rate (8.4 mg $CaCO_3$/d) of the coral tips (1–3 g) decreased a half at pH 7.2 than that (16.4 mg $CaCO_3$/d) at pH 8.0. Summarized the laboratory studies, they demonstrated that calcification rates of corals increase with increasing Ω values of seawater. Our experimental results (Table 3) are similar to their results, and suggest that calcification rates of coral samples are clearly related with $\Omega_{\text{aragonite}}$ of seawater.

However, Gattuso *et al*. (1998) reported nonlinear relation between calcification rates of *Stylophora pistillata* and $\Omega_{\text{aragonite}}$ of seawater. The rates showed a plateau when saturation state was between 3.90 to 5.85. As described above, our results show linear relation and disagree with that of Gattuso *et al*. (1998) (nonlinearity with a plateau). Our coral samples differ in size, mode and species from those of the Gattuso *et al*. experiment. Our samples of *P. lutea* were larger in size (see Table 2) compared with those of *S. pistillata* (colony size: 3–5 cm, wt.: 7.2–25.7 g). Coral calcification probably depends on coral conditions such as size, mode, health and species. In addition, Gattuso *et al*. (1998) altered Ω of artificially prepared seawater through changing [Ca^{2+}] without changing its carbonate system. Gattuso *et al*. (1999) suggested that due to such [Ca^{2+}] change, [Ca^{2+}] transportation possibly hampered during their experiments. Leclercq *et al*. (2000) also pointed out that the coral samples used by Gattuso *et al*. (1998) showed less response to changes in CO_2 system and provided a plateau in calcification rate when Ω reached over 3.90.

As many coral researchers have studied, light is considered to be one of important factors for the metabolism of an autotrophic community. Our experiments were performed under lower light intensity (~13 μmol/m^2/s) compared with that of Okinawan coral reefs (~300–1000 μmol/m^2/s) observed during 2001–2002. Considered coral metabolism in a small water bath, the low light condition was assumed to be suitable for corals surviving in limited environments. Although we expected very low coral metabolisms under low light conditions, the corals had survived more than two years. Using water data of pH and alkalinity, rates of net hourly organic production (y) during daytime and hourly respiration (r) during nighttime are calculated in order to estimate P/R ratios ($12(y + r)/24r$) (Ohde and van Woesik, 1999). Estimated P/R ratio for the coral colony ranged from 0.22 to 2.35. Almost all P/R values showed less than 1 (except two data), suggesting that the coral samples may behave as heterotorph with low metabolisms. Such low metabolisms could be resulted from low light conditions. To calculate a P/R ratio, we used several assumptions such as carbonate equilibrium and a small data set. Further studies are needed

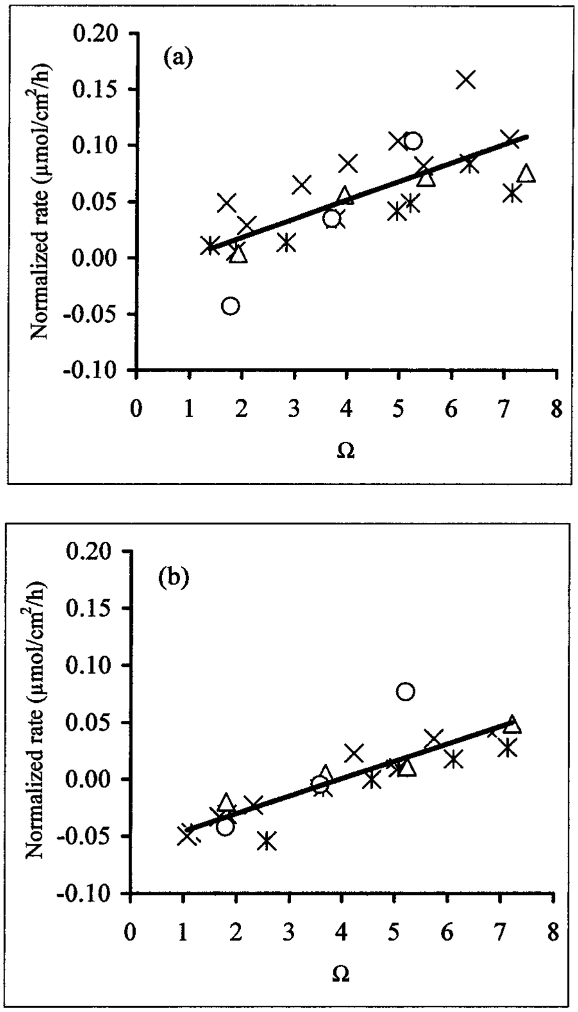

Fig. 3. Normalized coral calcification rate (R) versus seawater Ω. (a) Daytime experimental data with a fitted line according to linear equation ($R = 0.0166\Omega - 0.015$, $r^2 = 0.538$). (b) Nighttime data with a fitted line ($R = 0.0154\Omega - 0.061$, $r^2 = 0.762$). Same symbol plots represent data of a series of experiments using the same sample (see Table 2).

to confirm the results and to expand for understanding coral calcification associated with coral metabolisms.

In Okinawan coral reefs, the growth rates of *Porites* coral colonies were observed at ~10–16 mm/y as annual skeletal extension (Mitsuguchi *et al.*, 1996; Sato and Ohde, 2001). Assuming average skeletal density of 1.3 g/cm^3, the growth rate (10–16 mm/y) is calculated to be 1.5–2.4 μmol/cm^2/h (3.6–5.8 mg/cm^2/d) as the coral calcification rate. As listed in Table 2, the normalized calcification rates during daytime are one to two orders smaller compared with those observed in Okinawan coral reefs. The extremely low level of calcification rate may be attributed to low light intensity which probably caused a suppressed metabolic condition used in the laboratory experiments. Before the experimental run, the corals were cultured in a closed water bath for several months. Such cultured conditions possibly made the corals low metabolic activities. Although laboratory experiments could not reproduce the natural occurrence in coral reef communities, further experiments under controlled conditions such as high light and water flow would help to understand coral calcification.

In turn, the coral samples calcified during daytime and nighttime except a few experiments at night, and calcification rates during daytime experiments were comparatively higher than those of nighttime (Table 2). Through field studies, Goreau and Goreau (1959) found that $CaCO_3$ deposition by corals was the fastest in sunlight, less during cloudy weather and the least in darkness. They suggested that light intensity has a great effect on coral calcification. According to Barnes and Chalker (1990), hermatypic corals could provide a 2–3 times faster calcification in light than in darkness. Chalker *et al.* (1985) performed a laboratory experiment using *Acropora formosa* under light and dark conditions and observed that the light to dark calcification ratio was 2.87. Gattuso *et al.* (1999) summarized that calcification rates of scleractinian corals and coral reefs could be 3 times higher in light than that in darkness on average. We also observed that daytime calcification rates of *Porites lutea* were comparatively higher than those of nighttime (Table 2 and Fig. 3). In summary, light could accelerate coral calcification. However, we observed that the coral samples calcified during nighttime.

In order to evaluate dark calcification, we measured calcification rate under dark conditions. As shown in Figs. 1, 2 and 3(b), we observed that calcification rates at night were negative when Ω was less than ~3. Under low Ω conditions (<3), $CaCO_3$ dissolution possibly occurred at night, but there was no evidence for $CaCO_3$ dissolution except alkalinity increased during the experiments. On the other hand, dark calcification could occur at night when Ω values exceeded ~4. The intercept of Ω (where $c = 0$) to the line in Fig. 3(b) is calculated at 4.0 for the nighttime experiments. Theoretically, this value must be 1.0 when the coral calcification obeys to inorganic reaction (see Eq. (4)). But this dark calcification occurs through biological processes after Ω exceeds 4. In turn, dark calcification is considered to occur in Okinawan coral reefs, where seawater saturation states were observed at ~4–5 (Ohde and van Woesik, 1999). It is assumed that the coral samples could response to dark calcification when Ω exceeds ~4 as one of memory effects. The finding suggests that higher saturation state ($\Omega > 4$) was needed for dark calcification. Such saturation state possibly caused extremely low calcification under dark conditions. Mechanisms for dark calcification are obscured in this study.

Experiments under limited conditions used in this study may inform biological phenomena of corals, but new designed studies are needed to elucidate factors controlling coral calcification associated with environmental conditions in coral reefs. Such studies could provide information for understanding the oceanic carbon cycle and its impact on global climate.

CONCLUSIONS

The gradual increase in atmospheric CO_2 is a big problem at present for the world's environments. The elevated CO_2 leads to a decrease in $\Omega_{\text{aragonite}}$ value of seawater which possibly decreases calcification rates of corals. The reduction in calcification rate will cause a decrease in the reef-building activity of corals. In order to elucidate the relationship between coral calcification and $CaCO_3$ (aragonite) saturation state of seawater, the present study was performed using a massive coral *Porites lutea*. Calcification rates in light and dark conditions at 25°C were observed under various saturation states of seawater ($1 < \Omega < 7.8$) with respect to aragonite. The following tentative conclusions are derived from the laboratory experiments.

(1) The laboratory experiments clearly indicate that calcification rate of *Porites lutea* increased with increasing $\Omega_{\text{aragonite}}$ of seawater during daytime. A linear correlation was found between calcification rate (c) and $\Omega_{\text{aragonite}}$ of seawater inferred as a first-order reaction. The observed relationship was as, $c = a\Omega + b$ (a and b are constants). Our experimental results suggest that coral calcification depends on $\Omega_{\text{aragonite}}$ of seawater.

(2) Daytime calcification rates of the coral samples were comparatively higher than those of nighttime, indicating that light is one of controlling factors for coral calcification. When Ω is larger than ~4, the coral samples calcified during nighttime indicating an evidence of dark calcification.

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REFERENCES

Barnes, D. J. and Chalker, B. E. (1990) Calcification and photosynthesis in reef-building corals and algae. *Ecosystems of the World 25, Coral Reefs* (Dubinsky, Z., ed.), 109–131, Elsevier, Amsterdam.

Broecker, W. S. and Peng, T-H. (1982) *Tracers in the Sea*. Eldigio Press, NY, 690 pp.

Broecker, W. S. and Takahashi, T. (1966) Calcium carbonate precipitation on the Bahama Banks. *J. Geophys. Res.* **71**, 1575–1602.

Butler, J. N. (1982) *Carbon Dioxide Equilibria and Their Applications*. Addison-Wesley, Reading, 259 pp.

Chalker, B., Carr, K. and Gill, E. (1985) Measurement of primary production and calcification *in situ* on coral reefs using electrode techniques. *Proceedings of the Fifth International Coral Reef Congress* **6**, 167–172.

Gagan, M. K., Ayliffe, L. K., Hopley, D., Cali, J. A., Mortimer, G. E., Chappell, J., McCulloch, M. T. and Head, M. J. (1998) Temperature and surface-ocean water balance of the Mid-Holocene tropical western Pacific. *Science* **279**, 1014–1018.

Gattuso, J. P., Frankignoulle, M., Bourge, I., Romaine, S. and Buddemeier, R. W. (1998) Effect of calcium carbonate saturation of seawater on coral calcification. *Global Planetary Change* **18**, 37–46.

Gattuso, J. P., Allemand, D. and Frankignoulle, M. (1999) Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: A review on interactions and control by carbonate chemistry. *Amer. Zool.* **39**, 160–183.

Goreau, T. F. and Goreau, N. I. (1959) The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. *Biol. Bull.* **161**, 239–250.

Gran, G. (1952) Determination of equivalence point in potentiometric titration: Part II. *Analyst* **77**, 661–671.

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J. P., Langdon, C. and Opdyke, B. N. (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **284**, 118–120.

Leclercq, N., Gattuso, J. P. and Jaubert, J. (2000) CO_2 partial pressure controls the calcification rate of a coral community. *Global Change Biology* **6**, 329–334.

Marubini, F. and Atkinson, M. J. (1999) Effects of lowered pH and elevated nitrate on coral calcification. *Mar. Ecol. Prog. Ser.* **188**, 117–121.

Marubini, F. and Thake, B. (1999) Bicarbonate addition promotes coral growth. *Limnol. Oceanogr.* **44**, 716–720.

Mitsuguchi, T., Matsumoto, E., Abe, O., Uchida, T. and Isdale, P. J. (1996) Mg/Ca thermometry in coral skeletons. *Science* **274**, 961–963.

Morse, J. W. and Mackenzie, F. T. (1990) *Geochemistry of Sedimentary Carbonates*. Elsevier, Amsterdam, 707 pp.

Ohde, S. and van Woesik, R. (1999) Carbon dioxide flux and metabolic processes of a coral reef, Okinawa. *Bull. Mar. Sci.* **65**, 559–576.

Sato, Y. and Ohde, S. (2001) A Sr/Ca thermometer in coral aragonite. *Chikyukagaku* (*Geochemistry*) **35**, 1–11 (in Japanese).

Shen, C-C., Lee, T., Chen, C-Y., Wang, C-H., Dai, C-F. and Li, L-A. (1996) The calibration of *D* [Sr/Ca] versus sea surface temperature relationship for *Porites* corals. *Geochim. Cosmochim. Acta* **60**, 3849–3858.

Smith, S. V. and Kinsey, D. W. (1978). Calcification and organic carbon metabolism as indicated by carbon dioxide. *Coral Reefs: Research Methods 5* (Stoddart, D. R. and Johannes, R. E., eds.), 469–484, UNESCO, Paris.

Smith, S. V. and Pesret, F. (1974) Processes of carbon dioxide flux in the Fanning Island lagoon. *Pacific Sci*. **28**, 225–245.

Smith, S. V., Buddemeier, R. W., Redalje, R. C. and Houck, J. E. (1979) Strontium-calcium thermometry in coral skeletons. *Science* **204**, 404–407.

Veron, J. E. N. (2000) *Corals of the World*. Australian Inst. Mar. Sci., Qld., 287 pp.

Zhong, S. and Mucci, A. (1989) Calcite and aragonite precipitation from seawater solutions of various salinities: Precipitation rates and overgrowth compositions. *Chem. Geol*. **78**, 283–299.