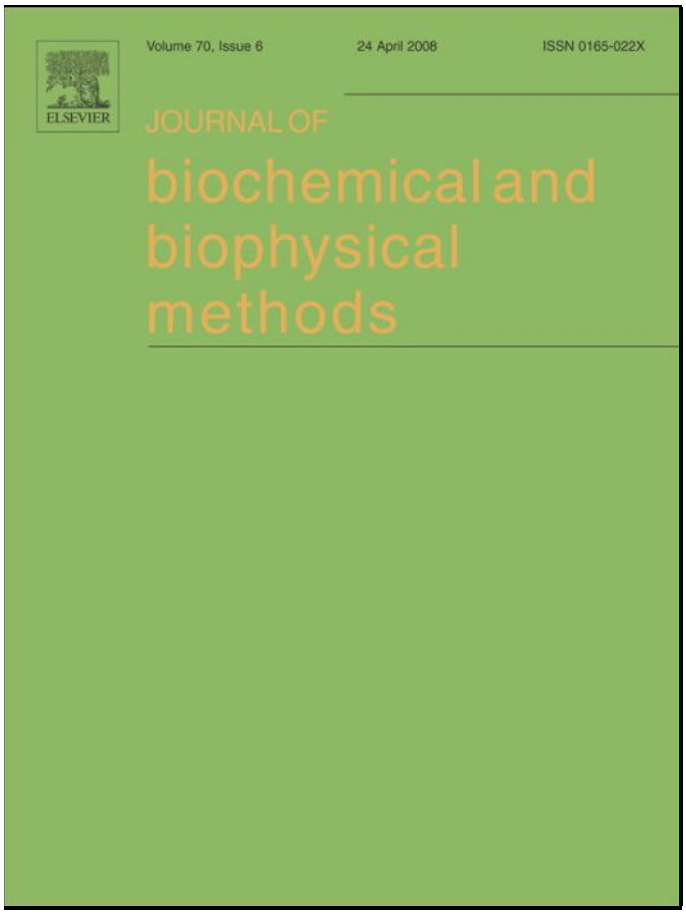
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Continuous-flow complete-mixing system for assessing the effects of environmental factors on colony-level coral metabolism

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Received 19 September 2007; accepted 30 January 2008

Abstract

A small-scale chamber experimental system was designed to study the effects of temperature on colony-level coral metabolism. The system continuously supplies fresh seawater to the chamber, where it is mixed immediately and completely with the seawater already present. This continuous-flow complete-mixing system (CFCM system), in conjunction with theoretical equations, allows quantitative determination of chemical uptake and release rates by coral under controlled environmental conditions. We used the massive hermatypic coral *Goniastrea aspera* to examine variations in pH, total alkalinity, and total inorganic carbon for 16 days at 27 °C under controlled light intensities (300 and 0 μmol m^{-2} s^{-1}). We confirmed the stability of the CFCM system with respect to coral photosynthetic and calcification fluxes. In addition, we obtained daily photosynthetic and calcification rates at different temperatures (27 °C, 29 °C, 31 °C, and 33 °C). When seawater temperature was raised from 31 °C to 33 °C, the gross primary production rate (P_{gross}) decreased 29.5%, and the calcification rate (G) decreased 85.7% within 2 days. The CFCM system allows quantitative evaluation of coral colony chemical release and uptake rates, and metabolism.
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Keywords: Calcification; Chamber study; Continuous-flow complete-mixing model; Coral bleaching; Metabolism; Photosynthesis; Temperature effect

1. Introduction

Large-scale coral bleaching has been observed in many coral reefs worldwide over the past several decades. Coral bleaching is characterized by the loss of symbiotic algae, or zooxanthellae, or their pigments, and is caused by unusual environmental conditions, such as high seawater temperature, strong UV light, low salinity or sedimentation [1]. The most important factor in coral bleaching is high seawater temperature, which affects the symbiotic association between the animal host and the algae. The possible mechanism of the bleaching phenomenon is photosystem-II damage in zooxanthellae, followed by the shedding of these symbionts from the coral host [2]. Because coral bleaching influences both zooxanthellae and the coral host, calcification and photosynthesis can be used as measures of bleaching stress.

Photosynthesis and calcification are frequently measured by enclosing coral colonies in a small seawater chamber for a period and monitoring changes in the chemical composition of the seawater, such as pH, alkalinity, oxygen, and calcium levels. However, enclosure within a small chamber significantly stresses corals, because of the lack of fresh seawater. Furthermore, changes in the chemical constituents of the seawater cannot be monitored over a long period, as the water quality degrades quickly to levels outside the range found in the natural environment. Eventually, stressed coral releases mucus, and metabolism becomes abnormal.

Micro-sensors have been used under water-flow conditions for metabolic studies of tissues and polyps when chemical

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doi:10.1016/j.jprot.2008.01.014

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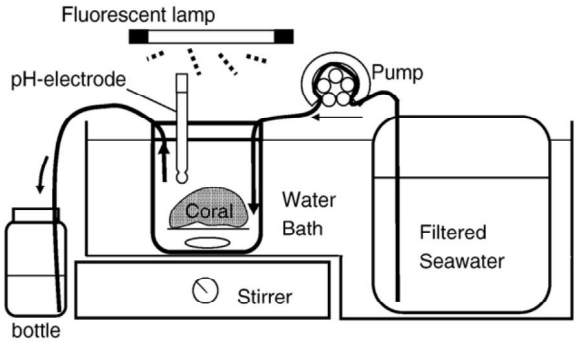


Fig. 1. Schematic of the continuous-flow complete-mixing (CFCM) system.

concentrations and changes in them are relatively high compared to the surrounding seawater. Gardella and Edmunds [3] used a fresh seawater flow rate of 75 L h^{-1} and estimated cellular-level photosynthesis in *Dichocoenia stokesii* using an oxygen micro-sensor. Using a Ca^{2+} micro-sensor and circulated seawater to measure calcification in *Galaxea fascicularis*, Al-Horani et al. [4] observed a decrease in Ca^{2+} concentration at three different locations on a coral polyp. Micro-sensors are advantageous for elucidating the mechanisms of photosynthesis and calcification at specific locations on a tissue or polyp. However, the conversion and extrapolation of micro-sensor data to various chemical uptake and release rates at the coral colony level require knowledge of the concentration gradients and diffusion coefficients of each chemical constituent [5]. Examining the long-term effects of environmental changes by determining the flux of chemical constituents during coral colony metabolism requires a theoretically and mathematically considered flow-through system.

We designed a small-scale chamber continuous-flow complete-mixing (CFCM) system and conducted experiments under well-controlled light and temperature conditions to examine the effects of seawater temperature on coral metabolism. The CFCM system allowed us to quantify the chemical uptake and release rates of the coral and to quantitatively assess the effects of environmental changes that proceed gradually over a longer time period. Here, we describe the CFCM system and how we used it to demonstrate the effects of increased seawater temperature on *Goniastrea aspera* metabolism.

2. Materials and methods

2.1. Continuous-flow complete-mixing (CFCM) system

We measured metabolic activity by observing changes in the chemical concentrations in seawater surrounding a coral colony enclosed in a glass chamber (diameter, 9.5 cm; height, 12.0 cm; volume, 850 mL; Fig. 1). The chamber was closed with a transparent polyethylene terephthalate resin (PET) lid and had an inlet and outlet for seawater flow. It was connected to a 20-L tank containing filtered fresh seawater, which a peristaltic pump supplied to the glass chamber continuously at 2.0 mL min^{-1}. The seawater outflow from the glass chamber was collected in a sampling bottle, and a constant volume of seawater was maintained in the glass chamber. The chamber was placed on a magnetic stirrer to ensure immediate and complete mixing of incoming and existing seawater. A fluorescent lamp provided controlled light intensities. Thus, the system had a continuous flow of incoming seawater that was completely mixed with the existing seawater.

A major advantage of the CFCM system is that it allows the measurement of uptake and release rates of chemicals by the coral during the course of an experiment (see Fig. 2 for a model of the CFCM system). The rate of change of a chemical in the chamber at any time is equal to its rate of entry into the chamber (inflow) minus its rate of exit from the chamber (outflow), plus its release rate (or minus its uptake rate) by the coral in the chamber (coral activity). This is described by the following equation:

$$\frac{d(CV)}{dt} = rC_{in} - rC + E \tag{1}$$

where C_{in} is the concentration of the chemical in the incoming seawater (mol L^{-1}), C is the concentration of the chemical in the chamber and in the outgoing seawater (mol L^{-1}), r is the flow rate (L min^{-1}), V is the chamber volume (L), t is time (min), and E is the uptake (−) or release (+) rate of the chemical by the coral (mol min^{-1}).

Because seawater entering the chamber is mixed immediately and completely, the concentration of a chemical in the chamber is the same as that in the outflow (Fig. 2). We assumed that coral uptake and release rates of a chemical remain constant under fixed light and temperature conditions, regardless of the chemical constituent concentrations. The good fit of the measured data with data calculated based on our model confirmed this as a reasonable assumption for the chemical species considered here.

Integrating Eq. (1) results in the following equation, which provides the concentration of a chemical species at time t:

$$C(t) = C_{in} + \frac{E}{r} + \left(C_o - C_{in} - \frac{E}{r}\right) \cdot e^{-r/V \cdot t} \tag{2}$$

where C_o is the initial concentration of the chemical at the beginning of the experiment. A detailed derivation of Eq. (2) is described in the Appendix.

For each specific chemical constituent, a positive E value indicates the release of that chemical by a coral colony, whereas

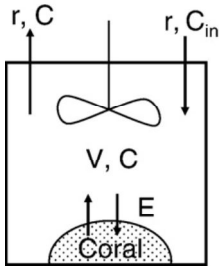


Fig. 2. CFCM system model. C_{in}, chemical species concentration in the seawater inflow (mol L^{-1}); C, concentration of chemical constituents in the chamber and outflow (mol L^{-1}); r, flow rate (L min^{-1}); V, chamber volume (L); E, coral uptake (−) or release (+) rate (mol min^{-1}) of a chemical constituent. Because seawater entering the chamber is mixed immediately and completely, the chemical concentrations in the chamber are identical to those in the outflow.

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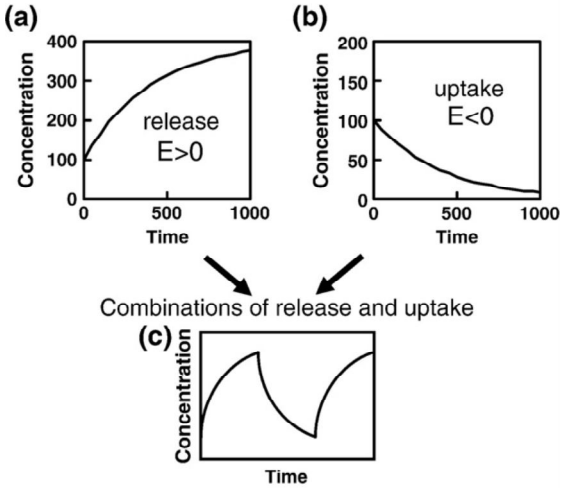


Fig. 3. Variation in chemical constituent concentrations in the CFCM system. (a) $E>0$ (release); (b) $E<0$ (uptake); (c) release and uptake combined. The (a) and (b) curves show Eq. (2) calculated at $E=0.4$ and $E=-0.4$, respectively, with the other parameters at $C_0=100$, $C_{in}=200$, $r=0.002$, and $V=0.8$.

a negative E value indicates its uptake. Fig. 3a and b show plots of C over time with positive and negative E values, respectively. The actual variation of a chemical species over time may be a combination of these two curves (Fig. 3c).

2.2. Calculation of E

To calculate the uptake or release rate, E, we integrated both sides of Eq. (2) over a period of time, T, during which the light and temperature conditions were fixed. Integrating the left side from time 0 to time T ([0,T]) equals the concentration of the chemical in the seawater collected in the outflow sampling bottles (C_{bottle}) multiplied by time T, i.e.,

$$\int_0^T C(t)dt = C_{bottle}\cdot T. \tag{3}$$

Integrating the right side of Eq. (2) over the time period ([0,T]), we get:

$$\begin{aligned}\int_0^T & C_{in}+\frac{E}{r}+\left(C_o-C_{in}-\frac{E}{r}\right)\cdot e^{-r/V\cdot t}dt\\ &=\left[C_{in}\cdot t+\frac{E}{r}\cdot t-\left(C_o-C_{in}-\frac{E}{r}\right)\cdot\frac{V}{r}\cdot e^{-r/V\cdot t}\right]_0^T\\ &=C_{in}\cdot T+\frac{E}{r}\cdot T-\left(C_o-C_{in}-\frac{E}{r}\right)\cdot\frac{V}{r}\\ &\quad\times\left(e^{-r/V\cdot T}-1\right).\end{aligned} \tag{4}$$

Thus, E is obtained from Eqs. (3) and (4) as follows:

$$\begin{aligned}E=&\left\{\left(C_{bottle}-C_{in}\right)\cdot T+\left(C_o-C_{in}\right)\cdot\frac{V}{r}\cdot\left(e^{-r/V\cdot T}-1\right)\right\}\\ &\Big/\left\{\frac{T}{r}+\frac{V}{r^2}\cdot\left(e^{-r/V\cdot T}-1\right)\right\}.\end{aligned} \tag{5}$$

To calculate E of a chemical, we measured the initial concentration of the chemical in the chamber (C_0) and in the incoming seawater (C_{in}). The system ran for a period of time, T, with fresh seawater pumped into the chamber and all exiting seawater collected at the outflow. At time T, we measured the concentration of the chemical in the collected seawater (C_{bottle}). As the values of r and V were known, we could calculate E.

Because pH was monitored inside the incubation chamber, pH E was calculated directly from Eq. (2) as follows:

$$E=\left\{C-C_{in}+\left(C_{in}-C_o\right)\cdot e^{-r/V\cdot T}\right\}\cdot r\Big/\left(1-e^{-r/V\cdot T}\right). \tag{6}$$

2.3. Calculation of carbon metabolism

Total dissolved inorganic carbon (TIC) was calculated using pH, total alkalinity (TA), and equilibrium constants for the carbonate system as follows [6]:

$$\begin{aligned}\mathrm{TIC}=&\frac{a_{H^+}^2+K'_1a_{H^+}+K'_1K'_2}{K'_1(a_{H^+}+2K'_2)}\\ &\times\left(\mathrm{TA}-\frac{K'_B\cdot 1.212\times10^{-5}\cdot S}{a_{H^+}+K'_B}\right)\end{aligned} \tag{7}$$

where TIC is the total dissolved inorganic carbon (μmol kg^{-1}), TA is the total alkalinity (μmol kg^{-1}), a_{H^+} is the activity of hydrogen ions ($=10^{-pH}$), K'_1 is the apparent first dissociation constant of carbonate ions (9.914×10^{-7} at 25 °C), K'_2 is the apparent second dissociation constant of carbonate ions (7.718×10^{-10} at 25 °C), K'_B is the apparent dissociation constant of boric acid (2.020×10^{-9} at 25 °C), and S is the salinity (34.642‰).

The rates of calcification (G), dissolution of carbonate (D), gross primary production (P_{gross}), net primary production (P_{net}), and 24-h respiration (R_{24h}) of the incubated coral were estimated from inorganic and organic carbon production, using a pH-alkalinity technique [7–9]. Inorganic carbon production rate per unit area (IP) was expressed by dividing the E of TA (E_{TA}) by two times the colony surface area (A) as follows:

$$\mathrm{IP}=-E_{TA}/(2\cdot A). \tag{8}$$

To measure daily coral metabolism at a fixed temperature, the chamber containing the coral was exposed to 12 h light/12 h dark. Because coral calcification is stimulated by photosynthesis in light [10], and dissolution may occur in the dark [6], the calcification (G) and dissolution (D) rates were calculated for each light and dark period, respectively, using Eq. (8). G and D were estimated by multiplying IP in light and dark conditions by the duration of each condition.

$$G=-E_{TA}^{light}/(2\cdot A)\cdot T^{light} \tag{9}$$

$$D=E_{TA}^{dark}/(2\cdot A)\cdot T^{dark} \tag{10}$$

where T^{light} and T^{dark} are the durations of light and dark conditions, respectively. Both calcification and dissolution rates are expressed as positive values.

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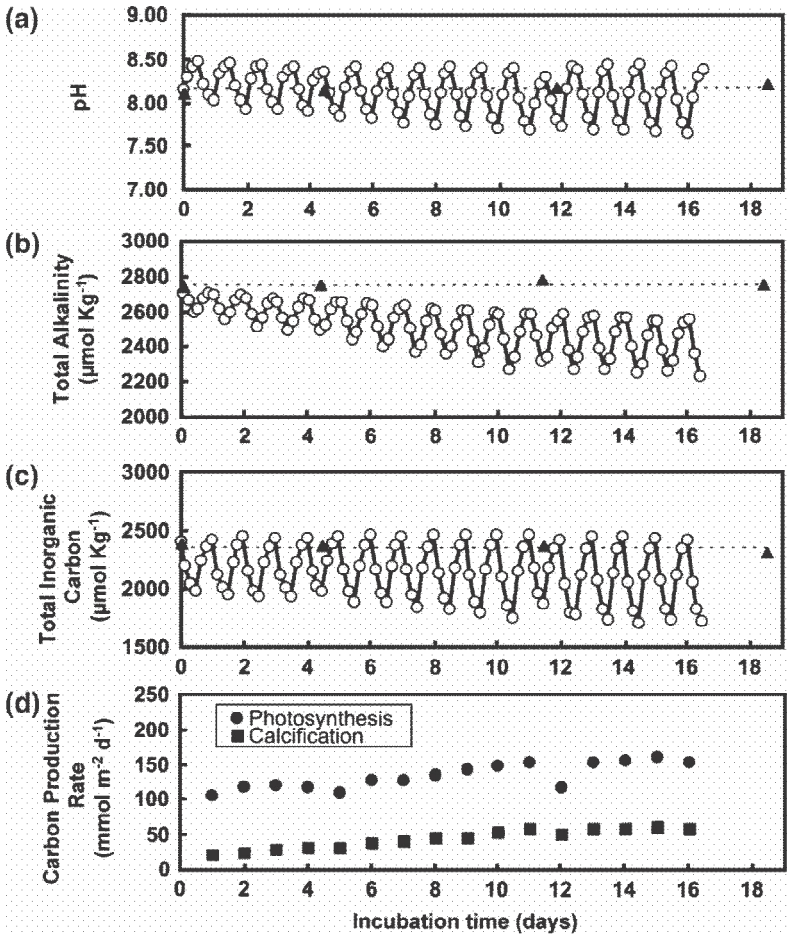


Fig. 4. Calculated (solid line) and measured (open circle) variation in pH (a), TA (b), and TIC (c), and variation in daily carbon production rate (d) for 16 days. Measured values of supplied tank water are shown as solid triangles, while averaged levels are shown as dotted lines.

The organic carbon production rate per unit area (OP) was calculated by subtracting IP from the E of TIC (E_{TIC}) and dividing by the colony surface area (A):

$$\mathrm{OP} = -E_{\mathrm{TIC}}/A - \mathrm{IP}. \tag{11}$$

Organic carbon production rates under light ($\mathrm{OP}^{\mathrm{light}}$) and dark ($\mathrm{OP}^{\mathrm{dark}}$) conditions were calculated as follows:

$$\mathrm{OP}^{\mathrm{light}} = -E_{\mathrm{TIC}}^{\mathrm{light}}/A \cdot T^{\mathrm{light}} - G \tag{12}$$

$$\mathrm{OP}^{\mathrm{dark}} = -E_{\mathrm{TIC}}^{\mathrm{dark}}/A \cdot T^{\mathrm{dark}} + D. \tag{13}$$

Because $T^{\mathrm{light}} = T^{\mathrm{dark}} = 12$ h in our experiment, daily gross primary production (P_{gross}) was estimated as:

$$P_{\mathrm{gross}} = \mathrm{OP}^{\mathrm{light}} + \left(-\mathrm{OP}^{\mathrm{dark}}\right). \tag{14}$$

The 24-h respiration ($R_{24\mathrm{h}}$) rate was estimated as:

$$R_{24\mathrm{h}} = 2 \cdot \left(-\mathrm{OP}^{\mathrm{dark}}\right) \tag{15}$$

and net primary production (P_{net}) was calculated by:

$$P_{\mathrm{net}} = P_{\mathrm{gross}} - R_{24\mathrm{h}}. \tag{16}$$

2.4. Coral specimens

We sampled *G. aspera* corals from the intertidal zone near Sesoko Station, of the Tropical Biosphere Research Center, University of the Ryukyus, with the permission of the government of Okinawa Prefecture (No. 14–36). Round corals approximately 5 cm in diameter were collected during low tide and immediately transferred to an outdoor tank at Sesoko Station. After 1 month, the corals were moved to an indoor tank in our laboratory for 2 months of acclimation prior to the experiments. After the incubation experiment, coral colony surface area was measured using the aluminum foil method [11].

2.5. Chemical analyses

Seawater flowing out of the glass chamber was collected in high-density polyethylene sample bottles (500 mL). Each bottle collected seawater for a 4-h period. Seawater samples were stored in the refrigerator at 4 °C until all chemical analyses had been performed (within 1 week of sampling). TA was measured using the method of Smith and Kinsey [12]. A pH electrode was

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Table 1
Uptake and release rates (*E* values) derived from the CFCM system at constant temperature of 27 °C for 16 days

Conditions		*E* value		
		pH (pH min^{-1})	TA (μmol min^{-1})	TIC (μmol min^{-1})
Day 1	Light	0.0009	−0.464	−0.945
	Dark	−0.0003	−0.022	0.322
Day 2	Light	0.0008	−0.519	−0.993
	Dark	−0.0004	−0.033	0.407
Day 3	Light	0.0008	−0.614	−1.080
	Dark	−0.0005	−0.059	0.382
Day 4	Light	0.0008	−0.664	−1.052
	Dark	−0.0005	−0.061	0.412
Day 5	Light	0.0006	−0.661	−0.957
	Dark	−0.0007	−0.098	0.411
Day 6	Light	0.0006	−0.777	−1.153
	Dark	−0.0007	−0.105	0.449
Day 7	Light	0.0005	−0.863	−1.176
	Dark	−0.0008	−0.151	0.445
Day 8	Light	0.0005	−0.928	−1.247
	Dark	−0.0009	−0.152	0.473
Day 9	Light	0.0006	−0.963	−1.318
	Dark	−0.0009	−0.159	0.492
Day 10	Light	0.0006	−1.084	−1.410
	Dark	−0.0010	−0.151	0.518
Day 11	Light	0.0006	−1.180	−1.496
	Dark	−0.0010	−0.166	0.524
Day 12	Light	0.0003	−1.036	−1.185
	Dark	−0.0010	−0.246	0.381
Day 13	Light	0.0007	−1.169	−1.531
	Dark	−0.0009	−0.203	0.457
Day 14	Light	0.0007	−1.169	−1.535
	Dark	−0.0011	−0.215	0.496
Day 15	Light	0.0007	−1.230	−1.583
	Dark	−0.0011	−0.241	0.494
Day 16	Light	0.0007	−1.191	−1.528
	Dark	−0.0011	−0.267	0.460

Note: a positive value of *E* indicates release of the chemical species by the coral, and a negative one indicates uptake of the chemical species by the coral.

installed on the transparent lid of the incubation chamber to monitor pH continuously during the experiment (Fig. 1), with pH readings logged to a personal computer every minute.

2.6. Incubation experiment

To check the stability of the CFCM system with respect to coral photosynthesis and calcification, we ran the system for 16 days under 12-h alternating light and dark conditions. A fluorescent lamp placed above the chamber provided a light intensity at the coral head of ~300 μmol quanta m^{-2} s^{-1}. The incubation chamber and seawater reservoir tank temperatures were controlled at 27±0.1 °C using a chiller (Coolnics CTE42A, Yamato-Komatsu Co., Tokyo, Japan). Filtered seawater (0.2-μm filter) in the reservoir tank was bubbled with ambient air for 1 h before use and sealed with Parafilm during the experiment. Seawater in the reservoir tank was analyzed several times during the experiment to confirm that it underwent virtually no change in chemical composition after the experimental period.

After confirming its stability, we applied the CFCM system to observation of temperature effects on coral metabolism. The temperature was set to 27 °C for days 1–2, 29 °C for day 3, 31 °C for day 4, and 33 °C for days 5–6. An identical system was also run at 27 °C throughout the experimental period as a control. Each experiment was performed twice, using a different *G. aspera* colony.

3. Results

3.1. Long-term stability of coral carbon production in the CFCM system

Fig. 4a–c shows variations in pH, total alkalinity, and TIC calculated using Eq. (7), respectively. The solid lines indicate the values calculated using Eq. (2) and derived *E*. The symbols in Fig. 4a–c indicate measured values, which fit very well with the calculated lines, thus verifying the model described in Eq. (1) and the combined variation described in Fig. 3c. Table 1 shows pH, TA, and TIC *E* values. Because of CO_2 uptake by photosynthesis and CO_2 release by respiration, pH *E* values are positive under light and negative under dark conditions. TA is negative throughout, with its absolute value greater in light than in the dark, indicating that uptake of HCO_3^- or CO_3^{2-} ions during calcification was higher under light than under dark conditions. The *E* value of TIC is negative (i.e., CO_2, HCO_3^- and/or CO_3^{2-} uptake) in light and positive in the dark, due to both calcification and photosynthesis–respiration. Fig. 4d shows the photosynthesis

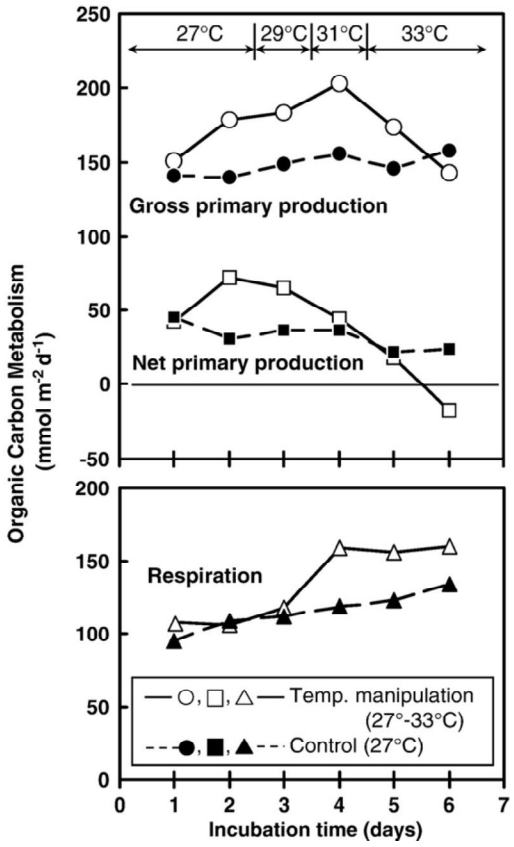


Fig. 5. Relation between seawater temperature and organic carbon metabolism.

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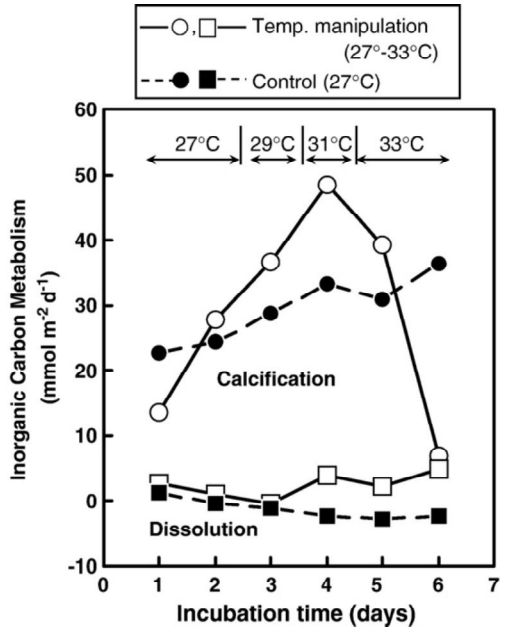


Fig. 6. Relation between seawater temperature and inorganic carbon metabolism.

and calcification carbon production rates derived from Eqs. (14) and (9), respectively, using the daily light–dark E values for pH, TA, and TIC (Table 1).

3.2. Photosynthesis and respiration

Fig. 5 shows the effects of temperature on organic carbon metabolism, which consists of gross primary production (P_{gross}), respiration (R_{24h}), and net primary production (P_{net}). P_{gross} increased as the temperature rose from 27 °C to 31 °C, but decreased by 14.6% on day 5 and by 29.5% on day 6, when the temperature rose from 31 °C to 33 °C. R_{24h} showed similar values to those of the control up to 29 °C, but then increased 35.0% at 31 °C. P_{net} decreased steadily from day 4 (31 °C) to day 6 (33 °C).

3.3. Calcification and dissolution of carbonate

The effects of temperature on inorganic carbon metabolism, i.e., calcification and dissolution, are shown in Fig. 6. The calcification rate of *G. aspera* increased continuously with the rise in seawater temperature from 27 °C to 31 °C, but decreased abruptly by 18.9% at day 5 and by 85.7% at day 6 when the temperature rose from 31 °C to 33 °C. The dissolution rate decreased gradually at 29 °C, but rose to 4.1 mmol m^{-2} d^{-1} at 31 °C, whereas the rate of the control sample continued to decrease to −2.4 mmol m^{-2} d^{-1} (negative value indicates calcification in the dark).

4. Discussion

4.1. Long-term stability of coral carbon production in the CFCM system

The photosynthesis and calcification rates increased slightly for the first 7 days as the coral acclimated to the chamber environment. The rates were relatively constant throughout the experimental period until day 16, except for day 12, when the light intensity was decreased from 300 to 50 µmol quanta m^{-2} s^{-1} to check the lamp. Both photosynthesis and calcification returned to the previous level on day 13, when the light intensity was adjusted to 300 µmol quanta m^{-2} s^{-1} again. Thus, we concluded that the CFCM system did not influence coral metabolism if environmental factors were kept stable and that the system could be applied to further experiments.

4.2. Photosynthesis and respiration

The optimum temperature for P_{gross} was 31 °C, which corresponds to the ambient summer seawater temperature at Sesoko Island (30–31 °C). According to Jokiel and Coles [13] and Coles and Jokiel [14], an increase of 2–3 °C above ambient summer temperature results in gradual and moderate bleaching. During our experiment, the brown color of the coral paled somewhat at 33 °C. However, no zooxanthellae were observed in the seawater in either the incubation chamber or the sample bottles. One possible mechanism of coral bleaching is photosystem-II damage and increased active oxygen species in zooxanthellae, followed by oxidative stress of the coral host [2]. Our results indicate that the *G. aspera* host was not damaged by active oxygen from zooxanthellae after 2 days of heat stress at 33 °C, although symbionts were affected by the stress, given that the P_{gross} decrease and pigment fading occurred with no shedding of symbionts.

Coles and Jokiel [13] reported that temperature increases caused more rapid increases in respiration than in photosynthesis rates. We also observed a rapid increase in respiration at 31 °C, which then remained stable until 33 °C. Consequently, P_{net} began to decline at the P_{gross} optimum temperature of 31 °C and decreased steadily to below 0 with the decline of P_{gross}. Thus, a carbon budget deficit emerged at 33 °C (day 6), and the mixotrophic nutrition of the coral colony changed to heterotrophic (P_{gross}/R_{24h}=0.9) (Table 2). Thus, if coral cannot acquire sufficient energy from other sources such as zooplankton, prolonged high seawater temperature could eventually lead to coral death.

4.3. Calcification and dissolution of carbonate

The optimal temperature for maximum calcification coincided with the summer ambient seawater temperature at

Table 2
Gross primary production to 24-h respiration (P_{gross}/R_{24h}) ratio of coral "*Goniastrea aspera*" at different temperatures measured by the CFCM system (averaged values, n=2)

Incubation days	Temperature manipulation		Control	
	Temperature	P_{gross}/R_{24h}	Temperature	P_{gross}/R_{24h}
Day 1	27 °C	1.4	27 °C	1.5
Day 2	27 °C	1.7	27 °C	1.3
Day 3	29 °C	1.6	27 °C	1.3
Day 4	31 °C	1.3	27 °C	1.3
Day 5	33 °C	1.1	27 °C	1.2
Day 6	33 °C	0.9	27 °C	1.2

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Sesoko Island (30–31 °C), which is consistent with the findings by Jokiel and Coles [15] that the optimal temperature for calcification is similar to the maximum summer temperature. In addition, Clausen and Roth [16] found that the calcification rate of *Pocillopora damicornis* in Enewetak, in the Marshall Islands, peaked at 31 °C.

Isotopic analysis of growth bands in *Polites* sp. at the Shiraho reef in Okinawa demonstrated reduced skeletal growth during a 1988 bleaching event [17]. Using the buoyant weight technique, Coles and Jokiel [18] observed reduced skeletal growth at temperatures 1–2 °C higher than the maximum summer temperature of ambient seawater. This traditional method of measuring skeletal growth requires several days or weeks to detect skeletal growth rates [19]. In contrast, the CFCM system allows calculation of daily calcification rates, and it clearly showed a large reduction in the calcification rate at 33 °C, which was probably caused by a reduction in organic carbon translocation to the animal hosts from the symbionts.

The dissolution rate decreased gradually at 29 °C, but rose at 31 °C, whereas the rate of the control sample continued to decrease. This suggests that dark calcification was arrested at 31 °C and overwhelmed by dissolution. The dissolution rate remained nearly constant at 4.1–5.0 mmol m^{-2} d^{-1} between 31 °C and 33 °C, although the $CaCO_3$ saturation state of seawater (Ω) with respect to both aragonite and calcite remained oversaturated ($\Omega > 1$) during the course of the experiment. Because the dissolution rate did not change greatly at 33 °C, in contrast to the large reduction in the calcification rate in light, dissolution is probably not linked to coral metabolism.

We suspect that the dissolution was caused by the biological activity of organisms on the reverse side of the coral, which had no coral tissue. Lazar and Loya [20] reported that boring by the bivalve *Lithophaga lessepsiana* chemically redissolved up to 40% of the $CaCO_3$ deposited by the coral *S. pistillata*. Before conducting the incubation experiment, we removed visible living organisms on the reverse side of the coral, but microorganisms and boring organisms living in the carbonate substrata may have contributed to the dissolution of the carbonate substrata.

5. Simplified description of the method and its applications

We designed a continuous-flow, complete-mixing (CFCM) system together with theoretical equations to study colony-level coral metabolism. Our assumption that the release or uptake rate (E) of a chemical is constant under fixed light and temperature conditions was confirmed by the good fit of our calculated data with measured data. The CFCM system allows quantitative evaluation of pH, TA, and TIC release and uptake rates under fixed light and temperature conditions. Thus, day-to-day coral metabolism of photosynthesis–respiration and calcification–dissolution under water-flow conditions can be quantified. The CFCM system does not exert significant stress on corals and thus allows for experiments of relatively long duration (here, 16 days). We demonstrated that gross primary production and calcification of *G. aspera* peaks at 31 °C (the ambient summer seawater temperature at Sesoko Island) and decreases sharply at 33 °C. The CFCM system can be used to examine the effects of other environmental variables (e.g., nutrient levels, salinity, and sunlight) and can be applied to examine the effects of prolonged low-level exposure to toxic chemicals (e.g., organotin, diuron) on coral metabolism and skeletal growth.

Acknowledgments

We thank Dr. H. Wang for valuable comments on the manuscript. We also thank Dr. Kurosawa of Shizuoka University for useful information on uptake/release rate experiments in corals exposed to air. This study was supported by a Grant-in-Aid for Young Scientists (A) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (#14703036), and by the 21st Century COE program of the University of the Ryukyus.

Appendix

Below is a detailed derivation of Eq. (2) from Eq. (1).

Eq. (1) in the text is:

$$\frac{d(CV)}{dt} = rC_{\text{in}} - rC + E. \tag{A1}$$

Because volume V is a constant, $\frac{d(CV)}{dt} = V\frac{dC}{dt}$. This is substituted into (A1), and the terms are rearranged to produce:

$$\frac{dC}{dt} + \frac{r}{V}C = \frac{rC_{\text{in}} + E}{V}. \tag{A2}$$

Let:

$$y = C, x = t, P = \frac{r}{V}, \text{and } Q = \frac{rC_{\text{in}} + E}{V}. \tag{A3}$$

(A2) becomes a typical first-order differential equation, which is solved as follows:

$$\frac{dy}{dx} + Py = Q \tag{A4}$$

$$e^{Px}\frac{dy}{dx} + P \cdot e^{Px} \cdot y = e^{Px} \cdot Q$$

$$\frac{d(e^{Px} \cdot y)}{dx} = e^{Px} \cdot Q$$

$$\int d(e^{Px} \cdot y) = \int e^{Px} \cdot Q dx$$

$$e^{Px} \cdot y = \frac{Q}{P} \cdot e^{Px} + w$$

$$y = \frac{Q}{P} + w \cdot e^{-Px} \tag{A5}$$

where w is a differential constant.

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Substituting x, y, P, and Q, using (A3) gives:

$$C = C_{in} + \frac{E}{r} + w \cdot e^{-r/V \cdot t}. \quad \text{(A6)}$$

Let C_0 be the initial concentration of the particular chemical species of interest in the chamber at time $t=0$. Substituting C_0 and $t=0$ into (A6) gives:

$$C_0 = C_{in} + \frac{E}{r} + w$$

and w is expressed as:

$$w = C_0 - C_{in} - \frac{E}{r}. \quad \text{(A7)}$$

Substituting (A7) into (A6) gives:

$$C = C_{in} + \frac{E}{r} + \left(C_0 - C_{in} - \frac{E}{r}\right) \cdot e^{-r/V \cdot t}. \quad \text{(A8)}$$

(A8) is Eq. (2) in the text.

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