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The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*

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Abstract

The rise in atmospheric CO_2 has caused significant decrease in sea surface pH and carbonate ion (CO_3^{2-}) concentration. This decrease has a negative effect on calcification in hermatypic corals and other calcifying organisms. We report the results of three laboratory experiments designed specifically to separate the effects of the different carbonate chemistry parameters (pH, CO_3^{2-}, CO_2 [aq], total alkalinity [A_T], and total inorganic carbon [C_T]) on the calcification, photosynthesis, and respiration of the hermatypic coral *Acropora eurystoma*. The carbonate system was varied to change pH (7.9–8.5), without changing C_T; C_T was changed keeping the pH constant, and C_T was changed keeping the pCO_2 constant. In all of these experiments, calcification (both light and dark) was positively correlated with CO_3^{2-} concentration, suggesting that the corals are not sensitive to pH or C_T but to the CO_3^{2-} concentration. A decrease of ~30% in the CO_3^{2-} concentration (which is equivalent to a decrease of about 0.2 pH units in seawater) caused a calcification decrease of about 50%. These results suggest that calcification in today's ocean (pCO_2 = 370 ppm) is lower by ~20% compared with preindustrial time (pCO_2 = 280 ppm). An additional decrease of ~35% is expected if atmospheric CO_2 concentration doubles (pCO_2 = 560 ppm). In all of these experiments, photosynthesis and respiration did not show any significant response to changes in the carbonate chemistry of seawater. Based on this observation, we propose a mechanism by which the photosynthesis of symbionts is enhanced by coral calcification at high pH when CO_2(aq) is low. Overall it seems that photosynthesis and calcification support each other mainly through internal pH regulation, which provides CO_3^{2-} ions for calcification and CO_2(aq) for photosynthesis.

The increase in atmospheric CO_2 is associated with global warming, rising sea level, and surface ocean acidification (Brewer 1997; Feely et al. 2004). Atmospheric CO_2 is expected to double relative to its preindustrial level sometime between 2050 and 2100 according to different IPCC scenarios (Houghton et al. 2001). Under such conditions the calculated surface ocean pH will be 7.9 by the year 2060 compared with a value of ~8.2 during the preindustrial time (Brewer 1997). This decrease will cause a significant decline in the carbonate ion concentration (CO_3^{2-}) and, therefore, to a decrease in the saturation state of $CaCO_3$ (Ω) in seawater as defined in Equation 1:

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

where $[Ca^{2+}]$ and $[CO_3^{2-}]$ are the concentrations of calcium and carbonate ions, respectively, and K'_{sp} is the apparent solubility constant for $CaCO_3$ (with different values for calcite or aragonite). For atmospheric CO_2 doubling, the saturation state for mineral aragonite (Ω_{arag}), which is deposited by corals, will change from a preindustrial value of ~4.6 to ~3.1 (Kleypas et al. 1999).

Calcium carbonate precipitation in the ocean is an important part of the oceanic and global carbon cycle. Most of the $CaCO_3$ precipitation in today's ocean is by planktonic calcareous algae (Coccolithophores; Zondervan et al. 2001) and planktonic protozoa (Foraminifera; Barker and Elderfield 2002). However, the majority of their skeletons that sink to the deep ocean readily dissolve; therefore pelagic $CaCO_3$ net accumulation is significantly reduced (e.g., Broecker and Peng 1982). As a result, roughly 40% of the net oceanic $CaCO_3$ precipitation occurs in coastal zones of the tropical seas where coral reefs are a major component (Gattuso et al. 1998*b*). Because in most reefs net primary production and respiration are more or less equal, the dominant net process that affects the carbonate system in corals reefs is $CaCO_3$ precipitation (Kinsey 1985). During $CaCO_3$ precipitation in shallow water, alkalinity is reduced at a rate that is double that of the inorganic carbon (C_T); hence the pH drops and CO_2 is emitted to the atmosphere. Coral reefs are therefore a net source of CO_2 to the atmosphere (Gattuso et al. 1993). The decrease in surface ocean Ω_{arag} may cause a decrease in the calcification of coral reefs worldwide (Gattuso et al. 1999; Kleypas et al. 1999) and hence reduce this CO_2 source to the atmosphere. Reduced calcification will also cause an increase in the alkalinity of the surface ocean, which can increase the ocean's capacity to absorb atmospheric CO_2. However, this negative feedback mechanism is at the

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expense of existing coral reef ecosystems that may reduce significantly their growth and may even start to dissolve (Langdon et al. 2003). Therefore it is important to study the direct effects of carbonate chemistry on the metabolism of hermatypic corals, which are the main frame builders of coral reefs.

Laboratory experiments in mesocosms and for single coral colonies tested the effects of CO_2 levels, pH, C_T, and Ca^{2+} concentrations on coral calcification (Gattuso et al. 1998*a*; Leclercq et al. 2002; Marubini et al. 2003). However, these studies did not separate the effects of pH and pCO_2, and they obtained somewhat conflicting results with respect to the effects of Ca^{2+} (Chalker 1976; Gattuso et al. 1998*a*; Marshall and Clode 2002). In these laboratory experiments, photosynthesis of the symbiotic algae did not change as a function of changes in CO_2 and pH (Leclercq et al. 2002; Langdon et al. 2003). The primary carbon source supporting the photosynthesis of coral algal symbionts was shown to be the dissolved inorganic carbon in seawater, mainly the bicarbonate ion (HCO_3^-) and CO_2 (aq) (Al-Moghrabi et al. 1996; Goiran et al. 1996; de Beer et al. 2000). However, HCO_3^- uptake requires special adaptations in order to provide CO_2 for the Rubisco of the symbiotic algae (Al-Moghrabi et al. 1996; Goiran et al. 1996), and the well-known coupling between photosynthesis and calcification in corals and other calcifying symbiotic associations may be part of this adaptation (McConnaughey and Whelan 1997).

The goal of the present study was to isolate the primary constituent of the seawater carbonate system that influences calcification, photosynthesis, and respiration in hermatypic corals. We chose to perform the experiments on the branching coral *Acropora eurystoma*, a representative of the Acroporidae family, which is an important reef-building family in coral reefs.

Material and methods

Laboratory experiments—Colonies of the coral *A. eurystoma* were collected by scuba diving from a depth of 5–8 m near the Inter-University Institute (IUI) in Eilat, Gulf of Eilat, Red Sea. The colonies were glued to a PVC base using epoxy glue (propoxy 20, Hercules Chemicals) and were left in situ for at least 3 months to recover. Coral colonies that were kept in situ at a depth of 6 m (near the collection site) were retrieved for short (several hours) experiments in a laboratory located next to the waterfront. Experiments included light and dark incubations of about 2 and 1 h, respectively, in a sealed, temperature-controlled chamber (respirometer) equipped with a temperature sensor, oxygen, and pH electrodes, allowing continuous monitoring of these parameters. The electrodes data were collected using Labview version 3 software (National Instruments) and a data acquisition card (AT-MIO-16L-9, National Instruments). Light was provided by an optical fiber (Schott, KL 1500 electronic) or a metal halide lamp 250W (Arcadia). All experiments were carried out at constant irradiance of $\sim 350 \pm 10$ μmol photons m^{-2} s^{-1} and temperature of 24 ± 0.5°C, using a Heto thermostatic circulator (13DT-1). Each experiment was repeated on three branches of the same parent colony using an alternating schedule, which allowed the corals at least 2–3 d of in situ recovery between experiments. Keeping the corals in situ guaranteed that they were well fed in their natural environment.

Seawater preparation—Fresh seawater of the northern Gulf of Eilat ($S = 40.7$) was obtained for each experiment from the pier at the IUI (water depth of 5 m), and the carbonate system was manipulated as described in the following.

Constant C_T—C_T was kept constant at a natural level of $\sim 2{,}056 \pm 13$ μmol kg^{-1}, whereas pH and total alkalinity (A_T) were altered by adding 0.1 mol L^{-1} NaOH (NaOH solutions were always freshly prepared prior to use) or 0.1 mol L^{-1} HCl. In this set of experiments the range of pH was 7.9 to 8.5, obtaining CO_2(aq), HCO_3^-, and CO_3^{2-} concentrations as shown in Table 1. The HCO_3^- concentration changed by 15%, whereas those of CO_3^{2-} and CO_2(aq) changed by about 400% and 500%, respectively. As a result, the Ω_{arag} varied between 1.5 and 6.4. The solutions were used immediately after preparation and were not allowed to exchange with the atmosphere.

Constant pH—pH was kept constant at 8.28 ± 0.02, whereas C_T and A_T were changed—hence the relative proportions of the carbonate species were constant while their concentrations varied. To obtain constant pH, seawater was stripped of most of its C_T by acidifying with 1 mol L^{-1} HCl to a pH below 4 and by strong stirring for at least 2 h. $NaHCO_3$ was added to the seawater to obtain different C_T values, and then the pH was adjusted with HCl or with freshly prepared NaOH. The solutions were immediately used for a closed-system experiment. The C_T range was $\sim$1,100–3,100 μmol kg^{-1}, whereas the CO_3^{2-}, HCO_3^-, CO_2(aq), and A_T ranged 100–410 μmol kg^{-1}, 1610–1920 μmol kg^{-1}, 5–27.5 μmol kg^{-1}, and 1.425–3.400 meqv kg^{-1}, respectively (Table 1).

Constant CO_2(aq)—Constant CO_2(aq) was obtained by equilibration with the atmospheric level, whereas pH, A_T, and C_T varied. Seawater was acidified with 0.1 mol L^{-1} HCl or alkalized with 0.1 mol L^{-1} NaOH to obtain a pH range of $\sim$7–9. These solutions were allowed to exchange with ambient fresh air (using an aquarium pump) for at least 12 h. The CO_2(aq) value obtained was 8.29 ± 0.5 μmol kg^{-1}, whereas changes in C_T, A_T, and pH ranged 1,600–2,700 μmol kg^{-1}, 1.85–3.36 meqv kg^{-1}, and 8.22–8.39, respectively (Table 1).

In all of these experiments, the accurate initial and final carbonate chemistry was determined using the techniques described below.

Analytical techniques and calculations—Photosynthesis and respiration: Net photosynthesis (P_N) and dark respiration (R) rates were calculated using the difference in dissolved oxygen (ΔO_2) between the end and the beginning of light or dark incubations, respectively (Eq. 2). Dissolved O_2 (μmol L^{-1}) was measured using the modified Winkler

Table 1. Constant C_T, constant pH, and constant pCO_2 experiments: pH, C_T, and A_T were measured while the inorganic carbon speciation was calculated. Numbers in bold characters represents the parameter that was kept constant in each experiment.

Experiment	pH	C_T (μmol kg^{-1})	A_T (μeqv kg^{-1})	pCO_2 (μatm)	CO_2(aq) (μmol kg^{-1})	HCO_3^- (μmol kg^{-1})	CO_3^{-2} (μmol kg^{-1})
Constant C_T	7.87 ± 0.05	**2056 ± 13**	2260 ± 18	889.43 ± 60.42	24.41 ± 3.39	1904 ± 15	117 ± 16
	8.08 ± 0.02	**2056 ± 13**	2364 ± 13	527.47 ± 1.88	14.99 ± 0.97	1852 ± 19	179 ± 9
	8.26 ± 0.02	**2056 ± 13**	2504 ± 30	315.17 ± 9.02	9.29 ± 0.27	1769 ± 21	262 ± 3
	8.41 ± 0.01	**2056 ± 13**	2591 ± 47	227.01 ± 12.26	6.69 ± 0.36	1705 ± 9	339 ± 15
	8.5 ± 0.001	**2056 ± 13**	2731 ± 35	175.65 ± 9.9	5.18 ± 0.29	1633 ± 21	402 ± 12
Constant pH	**8.28 ± 0.02**	1524 ± 57	1836 ± 63	227.97 ± 0.91	6.72 ± 0.03	1310 ± 44	207 ± 13
	8.28 ± 0.02	1751 ± 12	1919 ± 248	259.04 ± 3.14	7.64 ± 0.09	1503 ± 11	240 ± 1
	8.28 ± 0.02	2088 ± 15	2493 ± 51	280.29 ± 10.66	8.71 ± 0.92	1781 ± 26	298 ± 22
	8.28 ± 0.02	2127 ± 23	2533 ± 4	315.13 ± 17.32	9.29 ± 0.51	1826 ± 29	292 ± 7
	8.28 ± 0.02	2400 ± 37	2810 ± 20	348.97 ± 1.06	10.29 ± 0.03	2056 ± 27	334 ± 10
Constant pCO_2	8.22 ± 0	1640 ± 3	1878 ± 25	**281.25 ± 7.53**	**8.29 ± 0.53**	1428 ± 3	204 ± 0
	8.25 ± 0.01	1758 ± 33	2054 ± 37	**281.25 ± 7.53**	**8.29 ± 0.53**	1518 ± 31	233 ± 1
	8.28 ± 0.02	1932 ± 2	2269 ± 1	**281.25 ± 7.53**	**8.29 ± 0.53**	1653 ± 7	271 ± 9
	8.34 ± 0.07	2255 ± 54	2673 ± 103	**281.25 ± 7.53**	**8.29 ± 0.53**	1895 ± 1	351 ± 57
	8.39 ± 0.01	2714 ± 15	3311 ± 72	**281.25 ± 7.53**	**8.29 ± 0.53**	2234 ± 17	471 ± 2

titration (Grasshoff et al. 1983), with a precision of ± 0.7%. Gross photosynthesis (P_G) was calculated as the difference between P_N and R, assuming that the light and dark respirations are equal (Eq. 3).

$$P_N \text{ or } R\left(\mu\text{mol } O_2 \text{ cm}^{-2} \text{ h}^{-1}\right) = \frac{\Delta[O_2]\cdot(V_{\text{chamber}} - V_{\text{coral}})}{T\cdot SA} \quad (2)$$

V_{chamber} is the volume of the experimental chamber (mL), V_{coral} is the displacement volume of the coral (mL), T is time in hours, and SA is the surface area of the coral (cm^2) estimated by measuring the length and diameter of all branches of each colony assuming a cylindrical geometry. The volume of the chamber was 700 mL and the volume of the colonies ranged between 35 and 70 mL.

$$P_G = P_N - R \quad (3)$$

Calcification rate: Calcification rate was calculated from the difference in A_T measured at the beginning and the end of each incubation period. A_T was measured using an automatic potentiometric titration to the second end point (Grasshoff et al. 1983) of roughly 12 g seawater sample accurately weighed. The acid used was 0.025 mol L^{-1} HCl (BDH ConvoL), and the titrator used was Radiometer, Copenhagen (TitraLab, TIM90, Titration Manager). The average difference between duplicate samples was better than ± 0.4%. Calcification rates were calculated according to Equation 4 and are reported in units of μmol $CaCO_3$ cm^{-2} h^{-1}.

$$\text{Calcification}\left(\mu\text{mol } CaCO_3 \text{ cm}^{-2} \text{ h}^{-1}\right) = \frac{\frac{\Delta A_T}{2}\cdot(V_{\text{chamber}} - V_{\text{coral}})\cdot 1000\cdot 1.028}{T\cdot SA} \quad (4)$$

where 1.028 g mL^{-1} is the density of seawater in the northern Gulf of Eilat, and the symbols are as defined in Equation 3.

C_T measurement: Duplicate C_T samples were taken prior to and immediately following the incubation without contact with the atmosphere. Water samples were stored in 60 mL brown glass bottles with gas-tight screw. The samples were poisoned with 0.5 mL of saturated $HgCl_2$ solution immediately after sampling to avoid any microbial activity. Analysis proceeded by acidifying the samples with H_3PO_4 (85%) and collection of the CO_2 (g) in a vacuum line. Initially the CO_2 (g) was collected in a large trap cooled with liquid N_2 (−196°C), after which it was distilled in a series of liquid N_2 and cooled alcohol (−90°C) traps. The C_T was measured manometrically using an electronic pressure gauge with a precision of 0.2–0.4%. C_T and pH were used to calculate the carbonate species concentration and their relative proportions using the apparent thermodynamic dissociation constants for activity scale (Mehrbach et al. 1973) in each of the experiments. Ω_{arag} was calculated according to Equation 1, and the K′sp for aragonite was calculated using the equations in Mucci (1983).

pH measurements were made using a Radiometer Copenhagen pH meter (PHM64 Research pH Meter) and Radiometer Copenhagen combination electrode (GK2401c). The electrode was calibrated using NBS scale standard buffers (Radiometer analytical) of 7.000 and 10.012 and was soaked in seawater for at least 1 h before measurement.

Results

Constant C_T experiments—The results of the constant C_T experiments show a significant positive relation between calcification and pH with a similar slope for both light and dark incubations (Fig. 1a; $p < 0.002$ and $p = 0.0002$, respectively). The constant offset between light and dark calcification (~0.2 μmol $CaCO_3$ cm^{-2} h^{-1}) is apparent

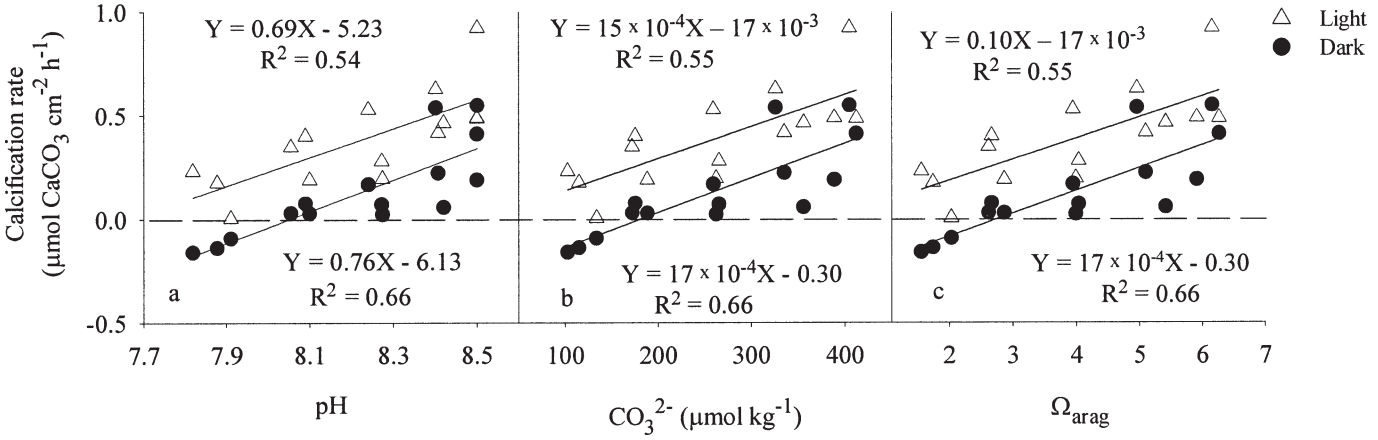

Fig. 1. Calcification as a function of (a) pH, (b) CO_3^{-2}, and (c) Ω, under light and dark conditions and constant total inorganic carbon (C_T).

over the entire range of pH values used in the experiment (7.9–8.5). Under dark conditions at pH values below 8.06, calcification becomes negative (i.e., there is net dissolution of $CaCO_3$). For the entire pH range, the CO_3^{2-} decreased by a factor of 4 followed by a threefold decrease in the calcification rates (both in light and dark incubations). This suggests that calcification in *A. eurystoma* under present-day conditions is about 20% lower then that calculated for the preindustrial period. Calcification is positively and linearly correlated with CO_3^{2-} and Ω_{arag} (Fig. 1b,c; $p < 0.002$ and $p = 0.0002$ for light and dark incubation, respectively).

P_N did not display any significant trend with pH and CO_2(aq) concentration, or any other parameter of the carbonate system in this experiment (Fig. 2a,b, respectively). A slight increase in R with pH also caused to a slight increase in P_G, but these trends were not significant ($p > 0.2$). The average R was slightly higher than the average P_N (0.60 ± 0.15 and 0.48 ± 0.12 μmol O_2 cm^{-2} h^{-1}, respectively).

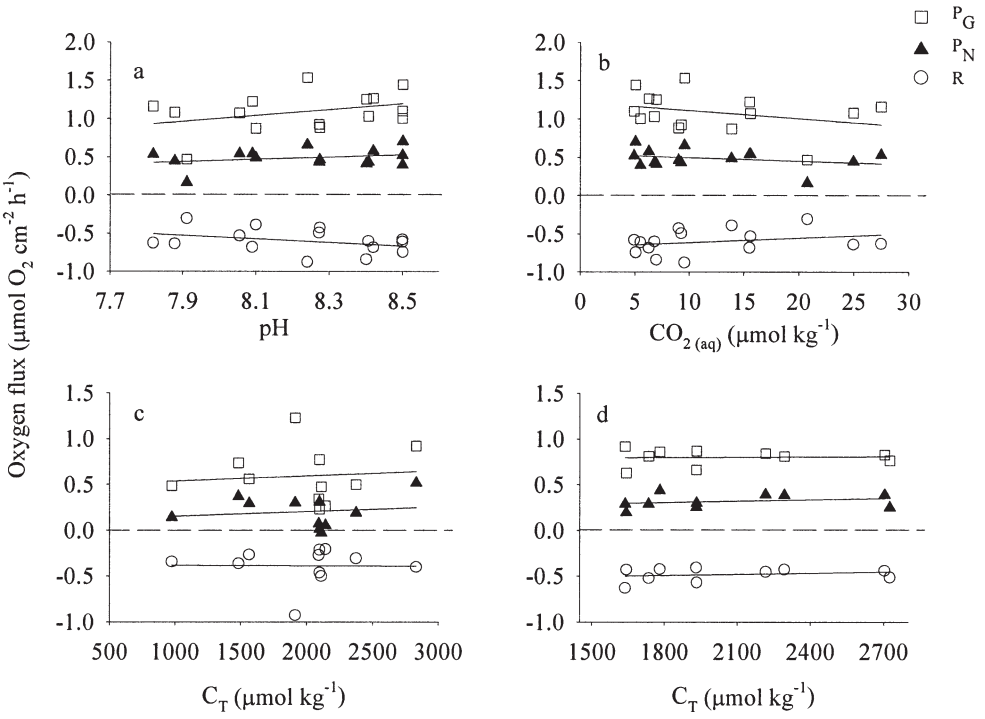

Fig. 2. Net photosynthesis (P_N), gross photosynthesis (P_G), and respiration (R) as a function of (a) pH at constant C_T, (b) CO_2(aq) at constant C_T, (c) C_T at constant pH, and (d) C_T at constant CO_2(aq).

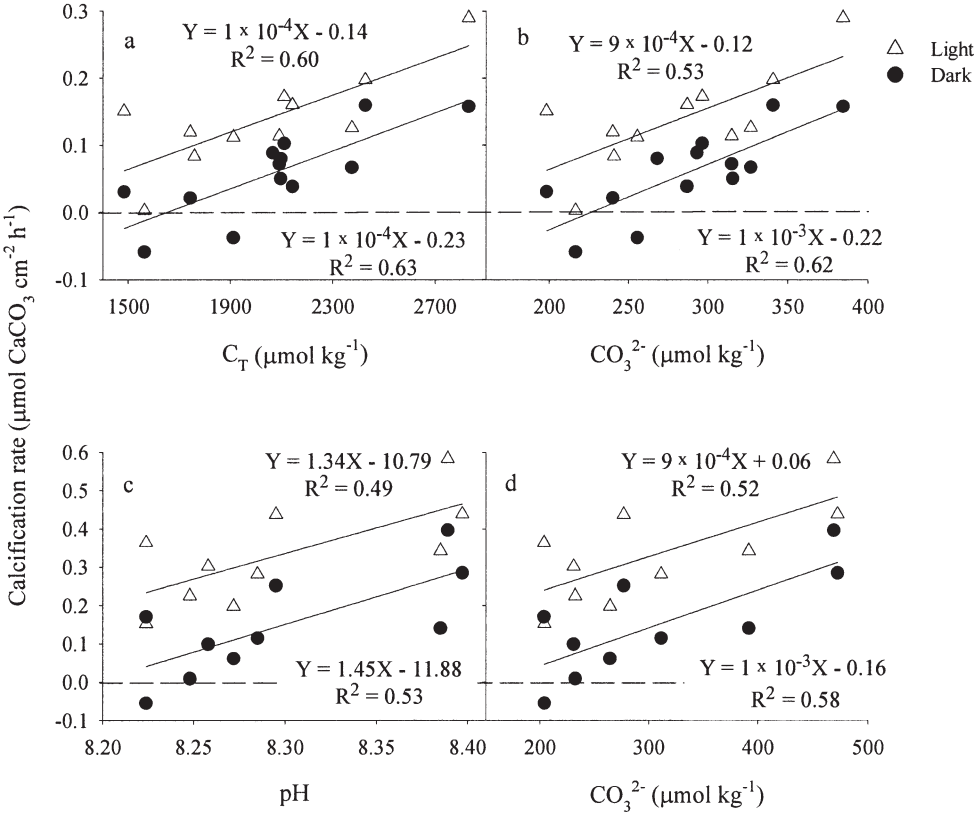

Fig. 3. Light and dark calcification in constant pH as a function of (a) C_T and (b) CO_3^{-2}, and in constant CO_2(aq) as a function of (c) pH and (d) CO_3^{-2}.

Constant pH experiments—In this experiment the relative proportions between the different carbonate species [CO_2(aq), HCO_3^{-1}, and CO_3^{2-}] remained constant but their concentrations varied with C_T changes. Calcification showed a significant positive and linear correlation with C_T (C_T = 1.2–3 mmol kg^{-1}; $p < 0.006$ and $p = 0.002$ for light and dark, respectively) and with CO_3^{2-}(CO_3^{2-} = 145–437 μmol kg^{-1}; $p < 0.011$ and $p = 0.002$ for light and dark, respectively) in both light and dark experiments (Fig. 3a,b, respectively). The slopes in both cases were similar, in agreement with the results of the constant C_T experiment (Fig. 1). This suggests that the CO_3^{2-} concentration (and/or Ω_{arag}) and not the pH is the main factor that controls calcification. The constant offset between the light and dark calcification is about 0.1 μmol $CaCO_3$ cm^{-2} h^{-1} (Fig. 3a).

Photosynthesis did not show any correlation with the C_T or CO_2(aq) (which were changed between 1,000 and 2,800 μmol kg^{-1} and between 5 and 14 μmol kg^{-1}, respectively; Fig. 2c). This is consistent with the results of the previous constant C_T experiment. P_N, P_G, and R did not change significantly with changes in inorganic carbon speciation (Fig. 2a).

Constant CO_2(aq) experiments—In this experiment, the HCO_3^- and CO_3^{2-} concentrations varied, whereas the CO_2(aq) remained constant (8.29 ± 0.53 μmol kg^{-1}), and pH varied between 8.22 and 8.39. Light and dark calcification showed a significant positive and linear correlation with pH (Fig. 3c; $p < 0.025$ and $p < 0.017$, respectively) and with CO_3^{2-} (Fig. 3d; $p < 0.019$ and $p < 0.011$, respectively). In agreement with both previous experiments, the slopes of light and dark calcification were parallel, and the offset was about 0.2 μmol $CaCO_3$ cm^{-2} h^{-1} (Fig. 3c). P_N, P_G, and R did not change significantly with changes in inorganic carbon speciation (Fig. 2d).

Discussion

Calcification—Calcification rates increased linearly with CO_3^{2-} concentration under conditions of constant C_T, pH, and CO_2(aq) (Figs. 1 and 3). This is in agreement with results of previous studies on individual corals (Marubini et al. 2001, 2003; Ohde and Hossain 2004), and on the artificial coral communities of Biosphere 2 and mesocosms (Langdon et al. 2000; Leclercq et al. 2002). This general trend was suggested in natural reef communities based on a comprehensive field study in the Red Sea (Silverman et al., pers. comm.). In a number of previous studies, coral calcification displayed a Michaelis-Menten kinetics as a function of Ca^{2+} concentrations (Chalker 1976; Gattuso et al. 1998*a*). According to these studies, at seawater concentrations ($Ca^{2+} \sim 10.6$ mmol L^{-1}) calcification was saturated for the corals *Stylophora pistillata*, *A. cervicornis*, and *A. formosa*. However, recently it was shown that addition of 2.5 and 5 mmol L^{-1} of Ca^{+2} to natural seawater (as opposed to the artificial seawater that was

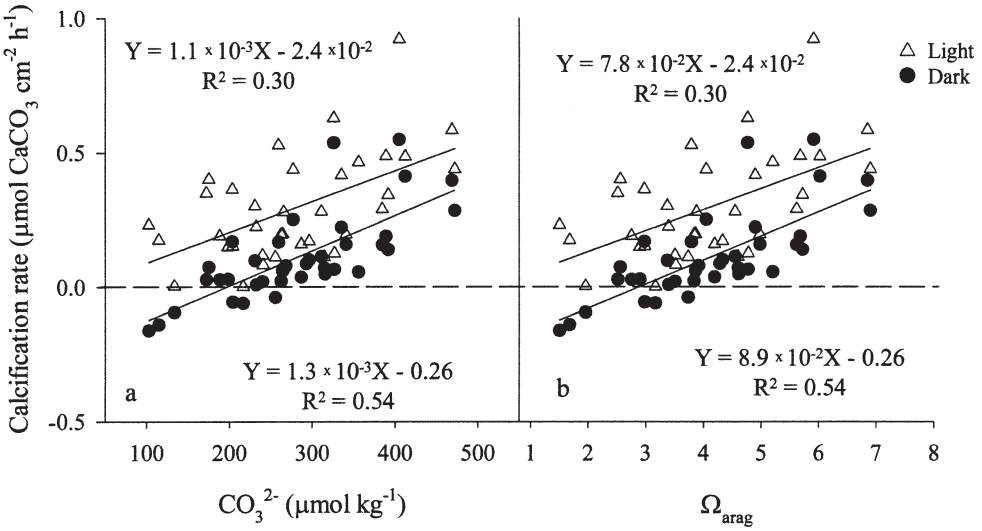

Fig. 4. Light and dark calcification of all three experiments versus (a) CO_3^{-2} and (b) Ω_{arag}.

used in the previous studies) caused a calcification increase by 30–61% and 54–84%, respectively, in *Galaxea fascicularis* (Marshall and Clode 2002). Therefore it is possible that Ω_{arag}, which depends on the product of Ca^{2+} and CO_3^{2-} (Eq. 1), controls coral calcification. Because the concentration of Ca^{2+} in the ocean is relatively constant compared with variability in CO_3^{2-}, Ω_{arag} is predominantly controlled by changes in CO_3^{2-} concentration (changes in $K'sp_{arag}$ are relatively small in the range of temperatures, salinities, and depth typical for coral reef abundance in the ocean).

In previous studies changes in the carbonate system were caused by manipulating pH or pCO_2 of the air bubbled in the experiment, which resulted in changes of the CO_3^{2-} concentration. Manipulating pCO_2 is the best way to mimic the changes in the carbonate chemistry expected from global CO_2 increase. In the present study changes in the carbonate chemistry were obtained keeping C_T, $CO_2(aq)$, or pH constant. This was necessary in order to identify which parameter of the carbonate system control the rate of calcification. In all these experiments calcification increased with CO_3^{2-} concentration (Figs. 1 and 3). This suggests that pH, pCO_2, C_T, and A_T does not control the calcification rate, but the CO_3^{2-} concentration is the dominating factor. The speciation of C_T controls how much CO_3^{2-} is available for calcification, and CO_3^{2-} concentration can be used to describe the response of coral calcification to changes in ocean carbonate chemistry. Even when the results of all of the experiments are pooled together we can still observe the general relations between CO_3^{2-} and the rates of calcification in light and dark conditions (Fig. 4a). Obviously the linear correlations are not so good as in the individual experiments, but the main features (i.e., linearity and constant offset between light and dark calcification) are maintained (Fig. 4a). It should be noted, however, that during geological history the concentration of Ca^{2+} in the ocean has changed significantly (e.g., Horita et al. 2002), and hence for long-term estimates of coral reef $CaCO_3$ precipitation, Ω_{arag} should be used. The data presented here suggest that present day calcification of *A. eurystoma* is ~20% lower than that expected for preindustrial conditions. Calcification of *A. eurystoma* is expected to decrease by an additional ~35%, for a total of 55%, when atmospheric CO_2 will double relative to the preindustrial level, which is expected by the year 2050–2100 (Houghton et al. 2001). This estimate is higher than previous ones for other corals species and artificial coral reef communities, which are in the range of a 10–50% decrease (Table 2). The differences in these estimates may be related to the different species used (e.g., branched vs. massive), the length of the experiments, and the method used to estimate the coral calcification. Our experiments were short (~2 h), whereas those mentioned above were on a longer scale of days to years, which may cause slight acclimation (Langdon et al. 2000). Alternatively, the difference may be related to the fact that the corals in our experiment were well fed (*see Discussion*).

In all of the experiments presented in this study, light calcification was higher than dark calcification, a phenomenon known as light-enhanced calcification (LEC), which has been documented in many previous studies (e.g., Barnes and Chalker 1990; Gattuso et al. 1999). However, in most of our experiments the regression lines of light and dark calcification as a function of CO_3^{2-} were parallel, indicating an average constant offset between light and dark conditions. (Figs. 1b, 3b,d, and 4). Similar results were obtained for *Porites lutea* in a similar constant C_T experimental setup (Ohde and Hossain 2004). In previous studies the LEC was presented as a ratio between light and dark calcification calculated for colonies subject to normal environmental conditions (e.g., Gattuso et al. 1999), which ranged between <1 and >5 (with a median of 3). In our experiments the ratio can vary from roughly 2 in high pH to higher then 26 at lower pH values and up to infinity when dark calcification becomes zero or negative (Fig. 5). Although this may be a phenomenon that is observed mainly for experiments with the carbonate system, it may be better to discuss LEC in terms of the difference between

Table 2. Reduction in calcification rates of corals expected in response to doubling of atmospheric CO_2 concentrations relative to preindustrial levels. Data are from different published sources.

Coral system	Calcification reduction at doubling atmospheric CO_2 (%)	Reference
Biosphere 2	40	Langdon et al. 2000
Open top mesocosm	22	Leclerq et al. 2000
Open top mesocosm	13	Leclerq et al. 2002
Porites compressa	10	Marubini et al. 2001
Galaxea fascicularis	16	Marubini et al. 2003
Pavona cactus	18	Marubini et al. 2003
Turbinaria reniformis	13	Marubini et al. 2003
Acropora verweyi	18	Marubini et al. 2003
Stylophora pistillata	50 (28°C) −5 (25°C)	Reynaud et al. 2003
Acropora eurystoma	55	Present study

light and dark calcification and not as a ratio between the two processes. A possible explanation for this constant shift between light and dark calcification will be given later in the text.

Dissolution—In many of the dark incubations at the lower end of CO_3^{2-} concentrations, the rates of calcification were negative, suggesting that the corals were dissolving (Fig. 4). This is surprising because Ω_{arag} values of the water were always >1 (ranging between 1.5 and 7). In dark incubations, Ohde and Hossain (2004) showed that dissolution in the coral *Porites lutea* occurred at $\Omega_{arag} < 4$. The most probable cause for this dissolution is the net respiration rates, which may lower the internal Ω_{arag} conditions. If we lower the external pH by 0.3 pH units as needed to match the light and dark calcification (as done below), Ω_{arag} may approach values that are <1.

Photosynthesis—Photosynthesis had no significant response to changes in the carbonate system (Fig. 2). This finding is in agreement with a previous study (Burris et al. 1983; Reynaud et al. 2003). However, it contradicts other observations that suggest the photosynthesis of algal symbionts is carbon-limited (Dennison and Barnes 1988; Lesser et al. 1994; Kühl et al. 1995). This contradiction can be partly settled because the C_T range in our experiments is lower than in the experiment of Goiran et al. (1996). P_N in the coral *G. fascicularis* increased with C_T when it was changed between 0 and ~1,650 μmol kg^{-1}, and then remained constant to ~2,500 μmol kg^{-1}. In our experiment C_T varied between 1,640 and 2,700 μmol kg^{-1} (except at two points in the constant pH experiment), which is well within the saturation concentrations of Goiran et al. (1996; Fig. 2; Table 1). C_T in the surface tropical and equatorial ocean is around 2,000 μmol kg^{-1}, and hence C_T values below 1,650 μmol kg^{-1} do not represent a realistic scenario for coral existence. Using a different approach, it was shown that the increase in water flow around coral colonies showed an increase in photosynthesis and calcification (Dennison and Barnes 1988; Lesser et al. 1994). This was explained by an increase in C_T availability but may also be explained by an increase in aeration, disposal of metabolic wastes, and other physiological effects that are associated with reduction of the thickness of the stagnant boundary layer around the coral. In our experiments the seawater medium was intensively stirred to obtain conditions that are similar to the natural environment of coral reefs where water is usually in continuous motion. Microsensor measurements of CO_2(aq) in light incubations near the surface of the coral *Favia* sp. were very low and probably not in thermodynamic equilibrium with the rest of the carbonate system (de Beer et al. 2000). However, these experiments were carried out at a pH range between 7.0 and 8.0 (dark and light, respectively), roughly 1 pH unit lower than natural seawater. Using inhibitors and competitor for anion transport (Al-Moghrabi et al. 1996; Furla et al. 2000), it was shown that photosynthesis was reduced by 50–100%; hence it was suggested that an active mechanism of HCO_3^- uptake exists for the photosynthesis in the corals *G. fascicularis* and *S. pistillata*. It may, therefore, be concluded that both CO_2(aq) and HCO_3^- are carbon sources for photosynthesis; however, because the Rubisco can utilize only CO_2(aq), the HCO_3^- need to be converted into CO_2(aq) within the organism (see below).

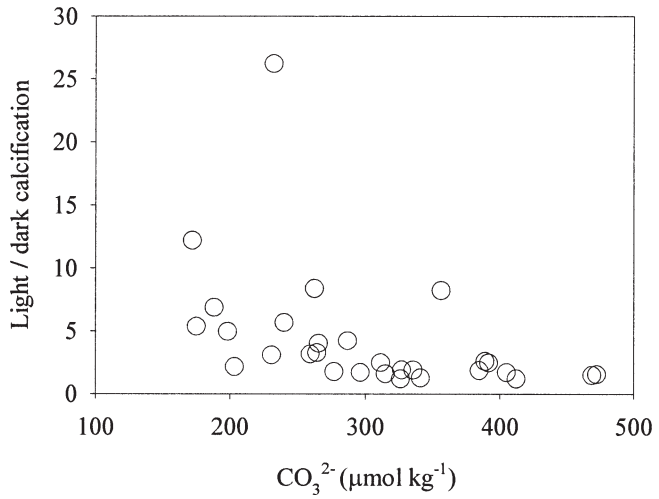

Fig. 5. Light to dark calcification ratio of all experiments versus CO_3^{-2}.

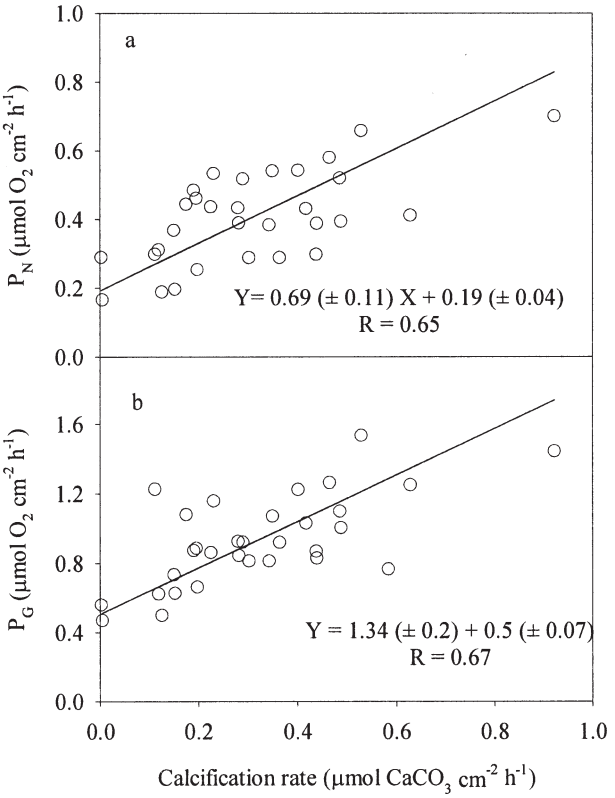

Fig. 6. (a) P_N and (b) P_G versus calcification in the light for all experiments. Because calcification, P_N, and P_G are subjected to measurement errors and natural variability, we describe their relations using a geometrical mean regression (Ricker 1973).

Relation between calcification and photosynthesis—In the present study, in all of the experiments regardless of the one kept constant [C_T, pH, or CO_2(aq)], calcification increased with CO_3^{2-}, whereas P_N and P_G remained constant. This suggests a certain decoupling between symbionts photosynthesis and coral calcification under these experimental conditions (Figs. 1–3). However, when data of all the experiments are grouped together, there is a significant positive correlation between photosynthesis and calcification (Fig. 6). The common explanation for such a correlation is that photosynthesis enhances calcification (e.g., Barnes and Chalker 1990), but as mentioned above this was not observed within the individual experiments of the present study. Another possible explanation for these observations is that calcification enhances photosynthesis through indirect carbon supply. It was suggested previously that protons are transported from the calcification site by a $Ca^{2+}/2H^+$-ATPase pump located at the basal membrane of the calicoblastic epithelium (McConnaughey and Whelan 1997). The main purpose of this pump is to elevate the pH at the calcification site and obtain there a higher supersaturation for aragonite precipitation. The protons that arrive to the coelenteron cavity can then combine with HCO_3^- to form CO_2(aq), which can support the high photosynthetic demand of the symbiotic algae. Indeed, plasma-membrane Ca^{2+}-ATPase, most probably the Ca^{2+}/H^+ exchanger, was cloned and localized within the calicoblastic cells of the coral *S. pistillata* (Zoccola et al. 2004). Such a Ca^{2+}/H^+ exchanger can explain the high Ca^{2+} and pH levels of ~10.7 μmol L^{-1} and 9.3, respectively, observed using Ca^{2+} and pH microsensors under the calicoblastic layer of the coral *G. fascicularis* (Al-Horani et al. 2003). Furthermore, Al-Horani et al. (2003) showed that Ca^{2+} and pH dynamics below the calicoblastic epithelium are light-dependent, and these activities can be inhibited with Ruthenium Red, which is a known Ca^{2+}-ATPase inhibitor. Symbionts P_N in *G. fascicularis* were inhibited by about 35% when treated with 100 μmol L^{-1} of the Ca^{2+}-channel inhibitor verapamil (Al-Moghrabi et al. 1996), which also completely inhibited calcification in *S. pistillata* (Tambutté et al. 1996). In addition, incubations with Ca^{2+}-free artificial seawater caused a decrease in P_N of *G. fascicularis* by 55% (Al-Moghrabi et al. 1996). All of these observations support the idea that coral calcification may stimulate symbiont photosynthesis. On the other hand, experiments preformed on *S. pistillata* with low Ca^{2+} (2.85 mmol kg^{-1}) or with HEBP (1-hydroxyethylidene-1,1-bisphosphonic acid, an inhibitor of $CaCO_3$ precipitation) showed that P_N was not inhibited or even slightly increased (Yamashiro 1995; Gattuso et al. 2000). Some of these experiments may not represent healthy functional conditions for corals as suggested by Marshall and Clode (2004) who described various abnormal phenomena associated with low Ca^{2+} conditions. They suggested that this can be caused by the inhibition of regulatory function of Ca^{2+} in the coral cells. Similarly, experiments with HEBP showed a reduction in P_N and R, which was followed by bleaching (Marshall and Clode 2002). It therefore seems that most of the experimental evidence supports the idea that calcification may enhance photosynthesis.

In all the experiments presented here (Fig. 2), P_N was practically constant regardless of the pH, C_T, or CO_2(aq) in the surrounding water. At the same time, calcification increased by a factor of 3–4 with increasing CO_3^{2-} concentration (Figs. 1b and 3b,d). If we accept McConnaughey's model (McConnaughey and Whelan 1997), which is now well supported by the observations of Al-Horani et al. (2003) and Zoccola et al. (2004), it is possible that under high CO_3^{2-} conditions enhanced calcification is causing an increase in proton translocation from the calcification site. These protons may shift the carbonate system in the coelenteron toward lower pH and increased CO_2(aq) concentration. This may enhance the diffusion flux of CO_2(aq) toward the symbiotic algae within the oral endodermis. Indeed the pH in the coelenteron of the coral *G. fascicularis* was relatively low (8.19), similar to that of ambient seawater (Al-Horani et al. 2003) despite the high photosynthetic activity that should have raised the pH there. This is indeed observed in the symbiotic noncalcifying sea anemone *Anemonia viridis* where the pH in the coelenteron was ~0.8 pH units higher than seawater (Furla et al. 1998). In our low pH or high CO_2(aq) incubations, the CO_2(aq) levels were probably sufficient to maintain a normal photosynthetic rate for the symbiotic algae. In the high pH or low CO_2(aq) conditions, however, the protons released during enhanced calcification could elevate the

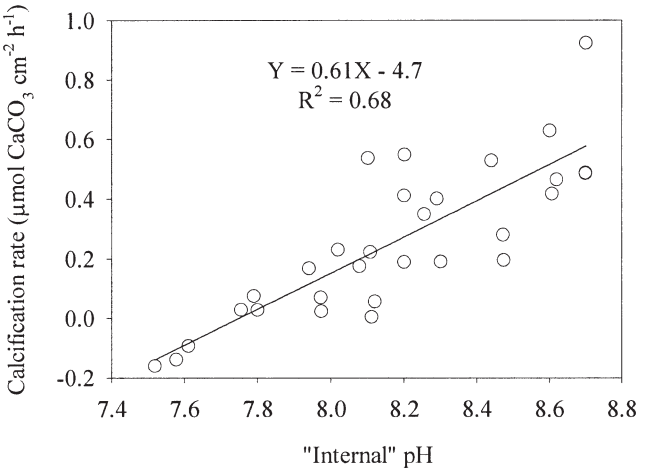

Fig. 7. Constant C_T experiment calcification versus "internal" pH. The "internal" pH was calculated by adding 0.05 pH units to the initial pH of light incubation and subtracting 0.3 pH units in dark incubations.

CO_2(aq) levels in the coelenteron and prevent CO_2 limitation of the symbiotic algae. In order for this mechanism to function, the rates of photosynthesis should be similar to those of calcification. Indeed as can be observed in Fig. 6a, this is the case in many of our experiments.

It is also possible that the constant difference between light and dark calcification observed in all of our experiments (Figs. 1b, 3b,d, and 4) is caused by a shift in the pH (and the entire carbonate chemistry) associated with the light to dark transition in the coelenteron. Indeed light and dark pH shifts of roughly 0.3–0.6 were reported in the coelenteron of *G. fascicularis* (Al-Horani et al. 2003). In this context it is interesting that the difference between the light and dark linear correlation lines in Figure 1a, for example, is about 0.35 pH units. This suggests that the LEC values can be obtained from the dark values by increasing the pH by ~0.35 units. By adding 0.05 pH units to the external pH in the light and subtracting 0.3 pH units in the dark, we can fit the data of the constant C_T experiment to a single linear regression with relatively high R^2 of 0.7 (Fig. 7). This may suggest that the coelenteron is an important mediator for carbon supply to both photosynthesis and calcification.

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