

Effects of lowered pH and elevated nitrate on coral calcification

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ABSTRACT: Both CO₂ chemistry and nutrient concentrations of seawater affect coral calcification. The relative effects of these factors on growth of corals were studied using coral tips or 'nubbins' of the hermatypic coral *Porites compressa*. Coral nubbins were grown over 5 wk in different combinations of pCO₂ (760 and 3980 µatm), HCO₃⁻ (1670 and 1520 µM), CO₃²⁻ (110 and 20 µM), and NO₃⁻ (0.42 to 5.66 µM). The pCO₂ was increased and CO₃²⁻ decreased by adding HCl to normal seawater; NO₃⁻ was increased by adding KNO₃ to ambient seawater. Corals growing in seawater at a reduced pH of 7.2 calcified at half the rate of control corals at pH 8.0, indicating that coral growth is strongly dependent on the concentration of CO₃²⁻ ions in seawater. Reduction of calcification from lowered pH and CO₃²⁻ was greater than reduction from nitrate additions. Corals in low pH treatments recovered their initial calcification rates within 2 d of re-introduction to ambient seawater, indicating the effects of CO₂ chemistry are immediate and reversible. Changes in calcification from increases in atmospheric CO₂, and hence decreases in CO₃²⁻, may be larger than local effects from elevated nutrients.

KEY WORDS: Calcification · Scleractinian corals · Dissolved inorganic carbon · Nutrients · Eutrophication

INTRODUCTION

It is widely suggested and assumed by geochemists that community-scale biogenic calcification is positively correlated to the saturation state of the mineral in question, i.e. aragonite, calcite or Mg calcite, where saturation state is the ion product of Ca²⁺ and CO₃²⁻ (Broecker & Takahashi 1966, Smith & Pesret 1974, Mann 1986, Mackenzie & Agegian 1989, Smith & Bud-demeier 1992, Suzuki et al. 1995, Holligan & Robertson 1996). There are however other hypotheses and explanations regarding mechanisms of calcification in individual organisms (Borowitzka 1981, McConnaughey & Falk 1991, McConnaughey & Whelan 1997). Experiments have yet to elucidate the relative effects of Ca²⁺ concentration, CO₃²⁻ concentration, pH and pCO₂ or total dissolved inorganic carbon (DIC) on calcification and photosynthesis in a single calcifying organism. Mackenzie & Agegian (1989) showed a positive corre-

lation between calcification and saturation state for a marine calcareous alga, *Porolithon* sp., by controlling CO₃²⁻ concentration with pH. Recently Gattuso et al. (1998) demonstrated a decrease in calcification of *Stylophora pistillata* and *Acropora* sp. in experiments where [Ca²⁺] was decreased but [CO₃²⁻] and pH were constant.

A complication with hermatypic corals is that both nutrients and light can affect the partitioning of carbon between zooxanthellae photosynthesis and coral skeletogenesis (Dubinsky & Jokiel 1994), in principle altering the relative effects of saturation state. Increases in ambient dissolved inorganic nitrogen (DIN) increase zooxanthellae density in host tissue (Hoegh-Guldberg & Smith 1989, Muscatine et al. 1989); it is suggested that this condition promotes CO₂ limitation of net production and of calcification (Dubinsky et al. 1990, Stambler et al. 1991, Stimson 1992, Marubini & Davies 1996).

As anthropogenic inputs of nutrients and CO₂ into tropical coastal oceans increase, it is important to determine the relative effects of these compounds on calcification. Increasing CO₂ in coastal water from ris-

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Table 1. Nutrient chemistry in each treatment chamber as measured during the experimental period. pH (n = 48) is expressed on the NBS scale. Water samples (n = 24) were analysed for nutrients using Technicon Standard Methods on a Technicon Autoanalyzer II (Walsh 1989). All concentrations are μM . All data are averages \pm SE

Treatment	pH	NO_3^-	NH_4^+	PO_4^-	Si
1a	7.96 \pm 0.017	0.42 \pm 0.066	0.42 \pm 0.044	0.12 \pm 0.015	9.18 \pm 0.36
1b	7.96 \pm 0.018	0.91 \pm 0.092	0.41 \pm 0.079	0.13 \pm 0.013	9.28 \pm 0.32
1c	7.96 \pm 0.017	1.48 \pm 0.081	0.46 \pm 0.039	0.12 \pm 0.013	9.21 \pm 0.33
1d	7.96 \pm 0.016	5.66 \pm 0.037	0.42 \pm 0.122	0.11 \pm 0.025	9.20 \pm 0.35
2a	7.15 \pm 0.046	0.43 \pm 0.070	0.47 \pm 0.058	0.12 \pm 0.018	9.21 \pm 0.39
2b	7.19 \pm 0.049	0.91 \pm 0.125	0.43 \pm 0.077	0.12 \pm 0.015	9.34 \pm 0.30
2c	7.13 \pm 0.077	1.53 \pm 0.084	0.47 \pm 0.035	0.12 \pm 0.012	9.19 \pm 0.35
2d	7.21 \pm 0.037	5.51 \pm 0.350	0.45 \pm 0.063	0.11 \pm 0.023	9.28 \pm 0.41

ing atmospheric CO_2 lowers seawater pH, lowering the CO_3^{2-} concentration in seawater, and potentially having an even more deleterious effect on reef growth than nutrient input. In this paper we report on an experiment to determine whether a decrease in the saturation state of aragonite decreases calcification to a greater extent in the coral *Porites compressa* than an increase in nitrate.

METHODS

Branches of the Pacific hermatypic coral *Porites compressa* were collected from a single colony at 1 m depth at Point Reef, Coconut Island, Kanehoe Bay, Hawaii, during June 1995. Similar-sized tips (nubbins) were cut, ground flat, glued on acrylic tiles with SuperGlue and placed in a holding tank with continuous water flow for a week prior to the experiment.

To determine the combined effect of pH and nitrate on calcification a 2-factor experiment was designed. The factor 'pH' had 2 levels: ambient and reduced by the addition of HCl. The factor 'nitrate' had 4 levels: ambient ($0.4 \mu\text{M NO}_3^-$) and increased by additions of 0.5, 1 and $5 \mu\text{M KNO}_3$. Water quality for each of the 8 treatments is shown in Table 1.

Corals were maintained in eight 9 l glass aquaria. The aquaria were covered with neutral-density shade cloth so to reduce full solar radiation by approximately 20%. Seawater from Kanehoe Bay flowed from a 50 l header tank through each aquarium at an inflow of 2 l min^{-1} (4 to 5 min water residence time). A peristaltic pump continuously dripped solutions of HCl and KNO_3 next to the seawater inflow in each experimental tank, thus maximising mixing within 10 s.

While the KNO_3 addition was maintained constant throughout the experiment, the HCl was added only during the initial 38 d (experimental period) followed by a period of no HCl addition of 11 d (recovery

period). Total carbon in seawater is partitioned among CO_2 aqueous, HCO_3^- , and CO_3^{2-} . When acid is added both HCO_3^- and CO_3^{2-} protonate, increasing CO_2 and HCO_3^- and decreasing CO_3^{2-} . Thus as CO_3^{2-} decreases so does the saturation state ($\text{Ca}^{2+} \times \text{CO}_3^{2-}$). From measurements of pH and total alkalinity in the chambers, values for pCO_2 , aragonite saturation (Ω), total DIC and the proportion of the available CO_2 species were calculated as in Table 2.

In total, 80 nubbins were weighed in seawater (buoyant weighed) with a Mettler balance accurate to 0.5 mg.

Data were converted to skeletal dry weight using the methods described by Davies (1989). Following initial weighing, ten nubbins were randomly assigned to each of the 8 treatment groups. Buoyant weight was measured every 5 to 7 d for 7 wk. The weight of nubbins is directly related to surface area within the size range used in this experiment (Marubini 1996).

The effect of pH and nitrate on coral growth rate was tested using a factorial ANOVA in which the slope of the regression of coral weight over time was the dependent variable. The assumptions of normality and homogeneity of variance were met. All statistical analyses were performed using the software package SPSS (version 6.0).

RESULTS

There was no significant difference between initial weights of the nubbins in each treatment group (ANOVA: $F_{7,75} = 0.72$, $p > 0.6$); nor was there a correlation between growth rate and initial weight (Fig. 1).

Table 2. CO_2 parameters. Data are averages \pm SE for chambers in pH Treatment 1 (ambient seawater) and pH Treatment 2 (with HCl addition). CO_2 speciation was computed from pH and total alkalinity using the constants of Mehrbach et al. (1973). The aragonite saturation states Ω were calculated according to Mucci (1983)

	Treatment 1	Treatment 2
pH (NBS)	7.962 \pm 0.008	7.173 \pm 0.026
TA ($\mu\text{eq kg}^{-1}$)	1956 \pm 10.69	1565 \pm 32.5
TCO_2 ($\mu\text{mol kg}^{-1}$)	1840 \pm 9.5	1641 \pm 18.2
pCO_2 (μatm)	757 \pm 37.7	3982 \pm 202
$\text{CO}_2 \times$ ($\mu\text{mol kg}^{-1}$)	20 \pm 0.98	103 \pm 7
HCO_3^- ($\mu\text{mol kg}^{-1}$)	1672 \pm 11.9	1518 \pm 23
CO_3^{2-} ($\mu\text{mol kg}^{-1}$)	112 \pm 3.97	18 \pm 0.91
$\Omega_{\text{aragonite}}$	1.81 \pm 0.065	0.28 \pm 0.014

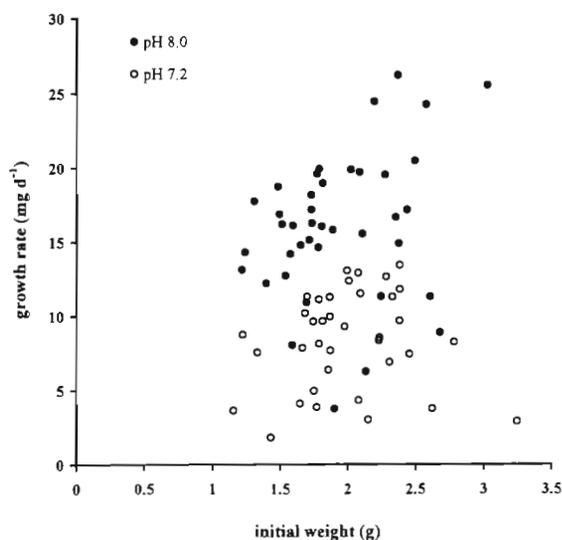


Fig. 1. *Porites compressa*. Growth rates of nubbins during the experimental period as a function of initial skeletal weight. All nutrient treatments are grouped by pH treatment

Initial weights ranged from 1 to 3 g. The mean daily calcification rates of nubbins ranged 2.7-fold, from 7.1 to 18.9 mg CaCO₃ d⁻¹ (Table 3). Growth rates of nubbins were remarkably constant throughout the whole experimental period within each treatment, and there was no interaction between pH and NO₃⁻ (ANOVA: $F_{3,68} = 13.01$, $p > 0.4$). Corals grown in ambient pH calcified significantly faster (Fig. 2a) than corals in low pH treatments (Fig. 2b) (ANOVA: $F_{1,68} = 80.56$, $p < 0.0001$). NO₃⁻ had only a marginal effect on calcification (ANOVA: $F_{3,68} = 1.97$, $p = 0.126$). Thus changes in weight of corals in the ambient and low pH treatments were averaged across NO₃⁻ treatments for both the experimental and recovery periods. Corals growing at pH 8.0 deposited an average of 16.4 mg CaCO₃ d⁻¹, while corals at pH 7.2 deposited only half as much, or 8.4 mg CaCO₃ d⁻¹ (Fig. 3). When addition of HCl stopped (recovery period), the growth rate of nubbins in the low pH treatments immediately increased to the higher rates of ambient pH; the pH effect was no longer significant (ANOVA: $F_{1,68} = 0.52$, $p > 0.4$).

DISCUSSION

Calcification was not significantly affected by increases in nitrate. Marubini & Davies (1996) and Marubini & Thake (1999) showed that 5 to 20 μM NO₃⁻ increased zooxanthellae density

and decreased calcification. The increased zooxanthellae resulted in much darker corals than those grown in open ocean water. The high-nutrient corals in the present study showed no change in colour, even after 7 wk; we suggest that there was sufficient NH₄⁺ in the water (0.44 μM) with sufficient flushing of the tanks (giving 1.27 mmol NH₄⁺ chamber⁻¹ d⁻¹) to inhibit the activity of nitrate reductase (Syrett 1981; see Thomas & Atkinson 1997 for detailed study of water velocity on ammonia uptake by corals).

Growth rate of *Porites compressa* incubated at pH 7.2 was almost half that of nubbins grown at pH 8.0. This reduction in calcification was constant during the experimental period, and occurred readily. In fact, within the first 5 d of the experiment, corals incubated at pH 8.0 grew by 15.6 mg CaCO₃ d⁻¹, while those at pH 7.2 grew by 10.1 mg CaCO₃ d⁻¹ (ANOVA: $F_{1,76} = 24.12$, $p < 0.0001$). Similarly, calcification was restored as soon as the addition of HCl was stopped (Fig. 3). This rapid change in calcification rate indicates the effect of pH did not have long-term deleterious effects on coral physiology or coral tissue, and is suggestive of a chemical kinetic response without an adaptive response to environmental conditions.

As pH was reduced from 8.0 to 7.2, HCO₃⁻ decreased by 10% (from 1672 to 1518 μM) while CO₃²⁻ decreased by a factor of 6 (from 112 to 18 μM). It is likely that calcification was responding to the 6-fold decrease in CO₃²⁻, not the 10% decrease in HCO₃⁻. Experiments by Marubini & Thake (1999) are also consistent with the hypothesis that [CO₃²⁻] rather than [HCO₃⁻] governs coral calcification. In their experiments the growth rate of *Porites porites* nubbins was doubled by an addition of 2 mM NaHCO₃. This addition doubled HCO₃⁻ but also resulted in a 3-fold increase in CO₃²⁻. While these data support the hypothesis that saturation state [Ca²⁺] × [CO₃²⁻] may have a large control on calci-

Table 3. *Porites compressa*. Mean initial weight and daily calcification rate (mg CaCO₃ d⁻¹) of nubbins exposed to 4 nitrate treatments (a, no addition; b, +0.5 μM NO₃⁻; c, +1 μM NO₃⁻; d, +5 μM NO₃⁻) in seawater of pH 8.0 (1) and with the addition of HCl to pH 7.2 (2). Rates are the average slope (*b*) of the linear regression model fitted to the weights of each nubbin over time, during the experimental and recovery periods (38 and 11 d respectively)

Treatment	pH	NO ₃ ⁻ (μM)	Initial wt. (g)	Experiment <i>b</i> mg d ⁻¹	SE	n	Recovery <i>b</i> mg d ⁻¹	SE	n
1a	8.0	-	2.09	18.9	1.81	10	13.7	2.66	10
1b	8.0	+0.5	1.86	14.9	1.16	10	13.9	1.48	10
1c	8.0	+1.0	1.85	16.0	4.50	9	14.3	2.34	9
1d	8.0	+5.0	1.89	15.8	1.05	10	14.3	1.18	10
2a	7.2	-	2.08	9.5	1.12	10	17.7	1.45	10
2b	7.2	+0.5	1.79	9.2	1.19	8	16.0	2.01	8
2c	7.2	+1.0	2.00	7.1	1.20	9	12.4	1.86	9
2d	7.2	+5.0	2.09	7.8	0.81	10	14.0	1.75	10

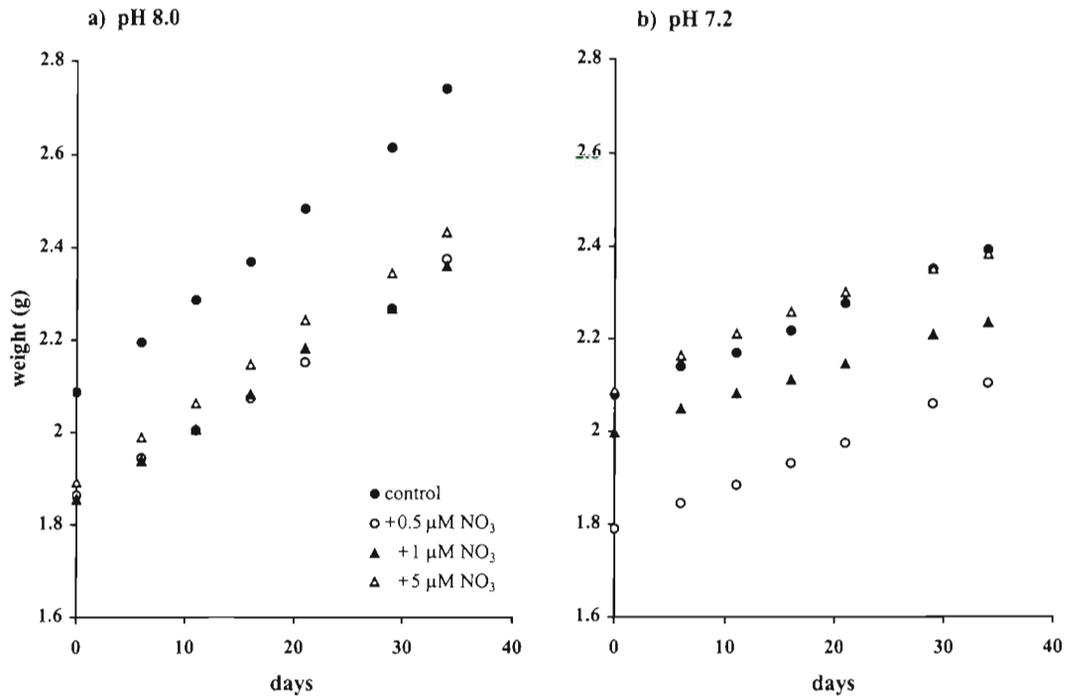


Fig. 2. *Porites compressa*. Mean weight of nubbins during the experimental period. Corals were incubated with different nitrate concentrations in (a) seawater (pH 8.0) and (b) with the addition of HCl (pH 7.2). Error bars are omitted for clarity

fication, they do not necessarily exclude other mechanisms involving increases in just CO_3^{2-} or a combination of HCO_3^- and CO_3^{2-} .

Available data on growth rates of *Porites* spp. by buoyant weighing, knowing HCO_3^- , CO_3^{2-} , and NO_3^- or NH_4^+ (Marubini & Davies 1996, Marubini & Thake 1999, present study) are plotted in Fig. 4. Growth rates are expressed as percentage of the calcification rate in ambient seawater (low nutrient, no chemical additions) for each given experiment. Increases in CO_3^{2-} concentration increase growth rate over a larger range (40 to 160% of control) than DIN (50 to 100% of control). We conclude that DIC can have a paramount effect on coral calcification. These results are consistent with the hypothesis that reef growth decreases as CO_2 rises in the atmosphere, lowering CO_3^{2-} in surface seawater.

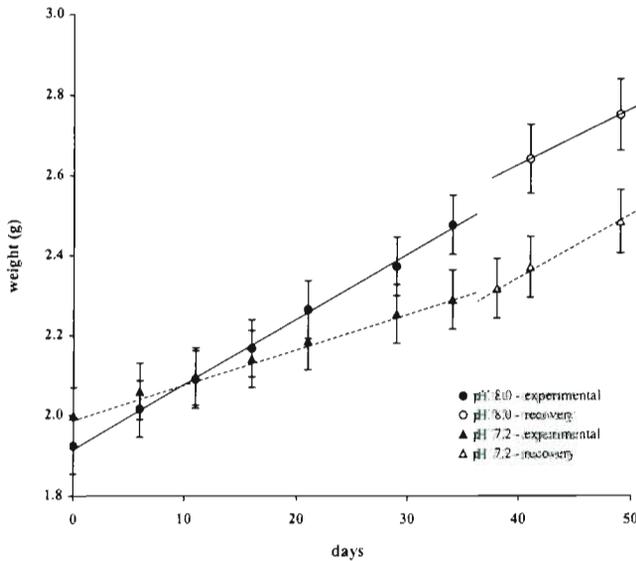


Fig. 3. *Porites compressa*. Mean weight of nubbins in seawater and with the addition of HCl (all nitrate treatments grouped together) during the experimental and recover periods. Bars are \pm SE

At the organismal scale, further studies on the interaction of pH and DIC will significantly improve the current understanding of the mechanisms underlying coral calcification. The next issue is to determine the variability of calcification in relation to pH and DIC, both within a coral species and among species. Then, extrapolation of the results to the scale of a reef will be possible. In addition, we propose that experiments on calcification maintain accurate control of DIC parameters. Conflicting results in the literature regarding effects of nutrients on calcification might be explained by differences in seawater carbonate chemistry between experiments.

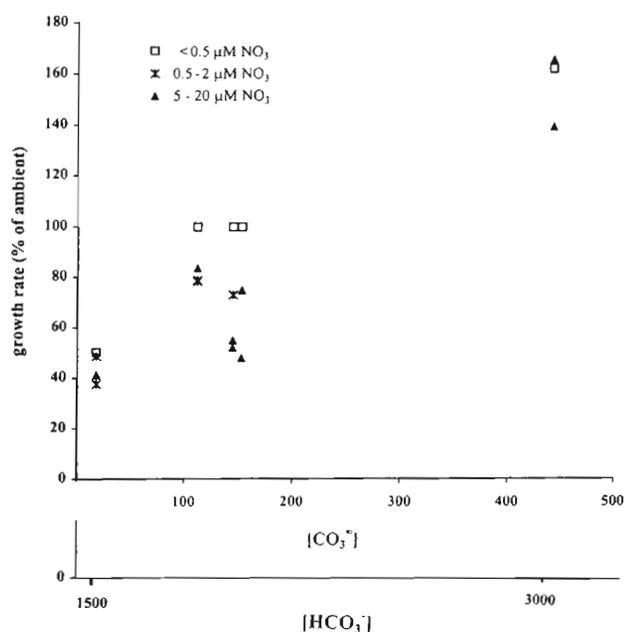


Fig. 4. Relationship between skeletal growth rate and carbonate or bicarbonate ion concentrations for nubbins of *Porites* spp. exposed to different NO₃⁻ concentrations. Rates are expressed as percentage of the calcification rate in ambient seawater (control treatment) for each given experiment. Data were compiled from Marubini & Davies (1996), Marubini & Thake (1999), and this paper

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