Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment

C. Langdon
Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida, USA

M. J. Atkinson
Hawaii Institute of Marine Biology, Kaneohe, Hawaii, USA

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[1] An investigation was conducted to determine the effects of elevated pCO_2 on the net production and calcification of an assemblage of corals maintained under near-natural conditions of temperature, light, nutrient, and flow. Experiments were performed in summer and winter to explore possible interactions between seasonal change in temperature and irradiance and the effect of elevated pCO_2. Particular attention was paid to interactions between net production and calcification because these two processes are thought to compete for the same internal supply of dissolved inorganic carbon (DIC). A nutrient enrichment experiment was performed because it has been shown to induce a competitive interaction between photosynthesis and calcification that may serve as an analog to the effect of elevated pCO_2. Net carbon production, NP_C, increased with increased pCO_2 at the rate of 3 ± 2% (μmol CO_2aq $kg^{-1})^{-1}$. Seasonal change of the slope NP_C-[CO_2aq] relationship was not significant. Calcification (G) was strongly related to the aragonite saturation state Ω_a. Seasonal change of the G-Ω_a relationship was not significant. The first-order saturation state model gave a good fit to the pooled summer and winter data: $G = (8 \pm 1$ mmol $CaCO_3$ m^{-2} $h^{-1})(\Omega_a - 1)$, $r^2 = 0.87$, $P = 0.0001$. Both nutrient and CO_2 enrichment resulted in an increase in NP_C and a decrease in G, giving support to the hypothesis that the cellular mechanism underlying the decrease in calcification in response to increased pCO_2 could be competition between photosynthesis and calcification for a limited supply of DIC.

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1. Introduction

[2] The rate of skeletal growth of corals is a major determinant of their fitness and ecological success. Skeletal growth determines a coral colony's ability to compete for space and light, and its ability to repair structural damage caused by humans, storms, grazers or bioeroders. Large coral colonies have a greater reproductive output and a competitive advantage over smaller colonies [*Koop et al.*, 2001]. The rate of growth also governs how long new coral recruits and coral fragments take to reach the critical colony size for sexual maturity and start reproducing [*Harrison and Wallace*, 1990; *Lirman*, 2000; *Sakai*, 1998; *Smith and Hughes*, 1999; *Soong*, 1993; *Szmant*, 1986; *Wood*, 1999]. Coral growth or skeletogenesis is driven by calcification, the process whereby Ca^{2+} and CO_3^{2-} ions obtained from seawater precipitate in the calcioblastic epithelium of the coral polyp to form crystals of the calcium carbonate mineral aragonite. The presence of zooxanthellae is critical to the ability of corals to calcify at the high rate necessary to build reefs. Azooxanthellate corals do not build reefs. Corals that bleach largely lose the ability to calcify. Recently it was found that the strain of zooxanthallae can affect the rate of growth of juvenile colonies when *Little et al.* [2004] found that corals containing the thermally resistant clade D strain grew 2–3 times slower than corals containing clade C1 zooxanthallae.

1.1. Effect of Light, Temperature, and Nutrients on Calcification

[3] Environmental conditions are known to exert a strong control on the rate of coral calcification. Calcification increases with increasing light up to a limit and then saturates [*Barnes*, 1982; *Chalker and Taylor*, 1975, 1978; *Marubini et al.*, 2001]. Calcification increases with increasing temperature up to a thermal optimum at or 1°–2°C below the normal peak summer temperature and then declines steeply with further increase in temperature [*Coles and Jokiel*, 1978; *Houck et al.*, 1977; *Marshall and Clode*,

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2004]. In laboratory studies, nutrient concentration of the experimental incubation water during growth also affects coral calcification. Enrichment of nitrate to levels of 5–20 μM result in increased zooxanthallae density and photosynthesis, and decreased calcification [*Marubini and Davies*, 1996; *Marubini and Thake*, 1999]. High ammonium enrichment produces similar results [*Ferrier-Pages et al.*, 2000; *Hoegh-Guldberg and Smith*, 1989; *Stambler et al.*, 1991]. The increase in zooxanthallae density and photosynthesis and decrease in calcification has led to the working hypothesis that nitrogen enrichment enhances the net growth of the zooxanthallae, which, in turn, limits the supply of dissolved inorganic carbon (DIC) available to the animal host for calcification [*Stambler et al.*, 1991]. The exception to this explanation is the study by *Ferrier-Pages et al.* [2001] who observed a 23% decrease in calcification in response to nitrate enrichment (2 μM) but no change in zooxanthallae density or photosynthesis.

[4] In the field with daily exposure to high nutrients, *Koop et al.* [2001] found that few growth responses were detected in any of the nutrient treatments during the low-loading phase of ENCORE (+N, +P, +NP;11 μM NH_4, 2.3 μM PO_4). Some seasonal differences in calcification were measured in *Acropora longicyathus* between nutrient treatments, however, there were no significant differences when calcification was integrated over a full year. During the high-loading phase of ENCORE (36 μM NH_4, 5.1 μM PO_4) calcification decreased in the presence of +N, +P and +NP in small colonies of *P. damicornis* but not in *A. longicyathus*. Ammonium enrichment led to a decrease in the rate of calcification of *A. palifera* but had no effect on *A. aspera* or *S. pistillata*. It should be noted here that there was no control of carbonate chemistry in these experiments, and it is now well known that carbonate ion can affect calcification more than nitrogen [*Marubini and Atkinson*, 1999]. The effect of ammonium enrichment on larger (>20 cm) colonies of *A. longicyathus* was seasonally variable but integrated over the full year resulted in an overall increase. The calcification rate of both *A. longicyathus* and *A. palifera* increased in +P treatments but skeletal density was reduced. +P treatments had no effect on *A. aspera* and tended to decrease calcification in *S. pistillata* [*Takabayashi*, 1996] and *P. damicornis*. Significant effects on coral reproductive capacity were observed in this study. Corals exposed to ammonium enrichment produced significantly fewer and smaller eggs than unexposed corals, and gametes exposed to +N and +NP had very low fertilization rates.

[5] The different responses of coral to nutrients are probably related to the very different experimental conditions and lack of control of actual nutrient uptake. Experiments on coral physiology and nutrients are conducted by either growing corals in very high nutrient water (often unrealistically high) or exposing corals to high concentration for brief periods of time. Few experiments report nutrient uptake or nutrient loading. The ENCORE experiment reported actual nutrient loading [*Steven and Atkinson*, 2003] (not in *Koop et al.* [2001]). The low-loading period corresponded to 0.66 mmol P m^{-2} per low tide and 3.5 mmol N m^{-2} per low tide, both about the same as typical daily loading rates of coral reefs [*Atkinson and Falter*, 2003]. Thus it is no surprise that there were no effects from the low-loading nutrient treatment. The high-loading treatment corresponded to 3.9 mmol P m^{-2} per low tide and 18 mmol N m^{-2} per low tide, a factor of 2–4 higher than typical loading rates. We might expect only weak responses by the biota with only a 2–4 factor increase in nutrient loading.

1.2. Effect of Calcium Carbonate Saturation State on Calcification

[6] Recently there has been a great deal of interest in the aragonite saturation state as an environmental variable that can influence the rate of calcification of marine organisms. The aragonite saturation state (Ω_a) is the ratio of the ion concentration product ($[Ca^{2+}] \times [CO_3^{2-}]$) to the solubility product (K^*_{sp}) for the mineral aragonite at the in situ conditions of temperature, salinity and pressure. Studies have determined that chemical precipitation is proportional to Ω: the greater the ion concentration product the greater the rate of formation of the mineral [*Burton and Walter*, 1987; *Inskeep and Bloom*, 1985; *Zhong and Mucci*, 1989; *Zuddas and Mucci*, 1998]. The relationship is described by a rate law of the form

$$R = k(\Omega a - 1)^n, \qquad (1)$$

where k is the rate constant and n is the order of the reaction. There is some controversy as to whether in the case of aragonite precipitation in seawater n = 1 [*Inskeep and Bloom*, 1985] or n = 1.8–2.4 [*Zhong and Mucci*, 1989]. Studies on hermatypic corals [*Gattuso et al.*, 1998; *Marubini et al.*, 2001, 2002; *Reynaud et al.*, 2003; *Marshall and Clode*, 2002; *Ohde and Hossain*, 2004], coralline algae [*Agegian*, 1985; *Borowitzka*, 1981; *Gao et al.*, 1993a], coccolithophorids [*Riebesell et al.*, 2000; *Sciandra et al.*, 2003; *Zondervan et al.*, 2001], foraminifera [*Bijma et al.*, 1999], echinoderms [*Shirayama*, 2005], mesocosm coral reef communities [*Langdon et al.*, 2000, 2003; *Leclercq et al.*, 2000, 2002] and natural coral reef ecosystems [*Broecker et al.*, 2001; *Ohde and van Woesik*, 1999; *Suzuki et al.*, 1995] have shown that the calcification of a diverse selection of organisms and natural systems is also strongly dependent on Ω.

1.3. Connection Between Fossil Fuel CO_2 and Saturation State

[7] The saturation state of the surface ocean is to a large extent controlled by the concentration of CO_2 in the overlying atmosphere. The response time the oceanic mixed layer to equilibration with the atmosphere lies in the range of several months to several years [*Broecker and Peng*, 1974]. This means that as the CO_2 of the atmosphere builds up the ocean will follow the forcing by the atmosphere with a time lag of several months to several years. Currently the oceans are taking up 2 Pg C yr^{-1} and the cumulative uptake between preindustrial times and 1990 is estimated to have been 118 Pg [*Sarmiento and Gruber*, 2002]. When CO_2 dissolves in seawater, less than 1% remains as CO_2aq, the balance forms carbonic acid which disassociates to form H^+ and HCO_3^-. The decrease in pH causes some of the CO_3^{2-} to combine with H^+ to form HCO_3^-. The end result is an increase in $[CO_2\ aq]$ and $[HCO_3^-]$ and a decrease in $[CO_3^{2-}]$. Since $[CO_3^{2-}]$ decreases the saturation state also decreases. *Kleypas et al.* [1999] calculated that the average Ω_a in the

tropics was 4.6 in 1880. It has dropped to a present day value of 4.0, and it could drop to 3.1 by 2065 and to 2.8 by 2100 if CO_2 emissions continue as projected by the *Intergovernmental Panel on Climate Change* [1995] IS92a "Business as Usual" scenario.

1.4. Source of Inorganic Carbon for Photosynthesis and Calcification

[8] There are very limited data on the source and transport mechanisms of the inorganic carbon used for coral calcification. Radioisotopic tracer experiments have demonstrated that DIC from seawater is incorporated into the skeleton [*Goreau*, 1961, 1963; *Taylor*, 1983]. *Erez* [1978] showed using a double labeling experiments (^{14}C and ^{45}Ca) that a large fraction of the CO_3^{2-} incorporated into coral skeletons could come from metabolic CO_2. Considerably more is known about the source and transport mechanisms of inorganic carbon used for photosynthesis by the zooxanthallae. This may be applicable to calcification if the two processes share the same internal pool of DIC. *Allemand et al.* [1998] have reviewed what is known about the acquisition of carbon for endosymbiont photosynthesis in corals. The immediate source of the carbon used by the zooxanthallae is that available in the cytoplasm of the host anthozoan cell. This in turn is derived to a significant extent from the external seawater. *Al-Moghrabi et al.* [1996] and *Goiran et al.* [1996] demonstrated that HCO_3^- is actively transported across the epithelia of the host cell by an anion carrier that is sensitive to DIDS. Once in the cytoplasm of the host cell the HCO_3^- is converted to CO_2 and CO_3^{2-} by carbonic anhydrase. This carbon concentrating mechanism (CCM) produces the high concentration of CO_2 that is required by the Form II Rubisco enzyme of the zooxanthallae to efficiently fix carbon. The photosynthetic rate of corals does not seem to be limited by the DIC supply in seawater [*Burris et al.*, 1983; *Goiran et al.*, 1996]. However, *Weis* [1993] showed that photosynthesis of the sea anemone *Aiptasia pulchella* did not saturate until a DIC concentration of 5 mM. Also CO_2 enrichment has been found to increase the photosynthesis of microalgae [*Riebesell et al.*, 1993], macroalgae [*Borowitzka and Larkum*, 1976; *Gao et al.*, 1993b] and seaweeds [*Zimmerman et al.*, 1997]. It is possible that under conditions of CO_2 enrichment the energy-costly CCM mechanism in corals may be deactivated resulting in usage of external CO_2 as it becomes more available relative to HCO_3^- [*Beardall et al.*, 1998]. Finally, active transport of CO_3^{2-} is known in animal systems [*Boron*, 2001] but has not been demonstrated to occur in corals [*Goiran et al.*, 1996].

1.5. Interactions Between Photosynthesis and Calcification

[9] The interactions between photosynthesis and calcification in corals are complex. There is a very strong correlation between photosynthesis and calcification at both the organism and community level [*Gattuso et al.*, 1999]. However, as noted above, nutrient enrichment can result in an uncoupling resulting in an increase in photosynthesis and a decrease in calcification [*Ferrier-Pages et al.*, 2000; *Hoegh-Guldberg and Smith*, 1989; *Marubini and Davies*, 1996; *Marubini and Thake*, 1999; *Stambler et al.*, 1991]. This observation has given rise to the hypothesis that the photosynthesis and calcification may compete for the same supply of DIC. Environmental conditions that stimulate the photosynthetic activity of the zooxanthallae such as enrichment of nutrients or CO_2 may draw down the internal DIC pool in the host cell and result in less CO_3^{2-} available for calcification. In this situation the increase in $[CO_3^{2-}]$ due the pH shift caused by photosynthesis is out weighed by the decrease in $[CO_3^{2-}]$ due to the overall reduction of DIC due to demand outstripping supply by the active uptake of HCO_3^-. For the scheme to work the zooxanthallae must have a greater call on the available DIC than the calcioblastic epithelial cells. This may be explained by the carbon concentrating mechanism.

1.6. Cellular Mechanism of Saturation State Control of Calcification

[10] Calcification is performed by the cells of calicoblastic epithelium. These cells are separated from the calcium carbonate skeleton by a thin layer of fluid known as the extracellular calcifying fluid or ECF. It is here that the Ca^{2+} and HCO_3^- or Ca^{2+} and CO_3^{2-} combine to form the calcium carbonate mineral aragonite. The cells of the calicoblastic epithelium are separated from the seawater within the oral cavity or coelenteron by a single-cell-thick aboral ectoderm. The advective exchange of water within the coelenteron may be too slow to supply the calcium and carbon required for photosynthesis and calcification [*Wright and Marshall*, 1991]. The calcium and DIC pools in the coelenteron can also be resupplied by passive or active transport of Ca^{2+} and HCO_3^- across the two-cell-thick oral epithelial layer that separates the coelenteron from the ambient seawater. The reader should see *Gattuso et al.* [1999] for an excellent review on the pathways whereby Ca^{2+} ions and DIC can reach the ECF. Ca^{2+} can reach the ECF by transcellular transport (energy-dependent), by paracellular diffusion or by advection (both energy-independent), or by a combination of all three processes. The active pathway involves an enzyme-mediated step that is saturated at the ambient concentration of Ca^{2+} in seawater in some species [*Chalker*, 1976; *Tambutte et al.*, 1996] and not in others [*Chalker*, 1976; *Krishnaveni et al.*, 1989].

[11] Calcification and photosynthesis are thought to share a common DIC pool which is the cytosol of the host cell. Ultimately the DIC could come from seawater via active or passive pathways or internally via metabolism. Irrespective of its source, the calicoblastic cells obtain DIC for calcification by transporting HCO_3^- from this pool via some form of anion carrier mechanism [*Tambutte et al.*, 1996].

[12] The cellular mechanism involved in the response of calcification to increased pCO_2 observed widely at the organismal and community level is not known. Unfortunately there are no data available on the response of the chemistry of the ECF to changes in the pCO_2 or saturation state of the ambient seawater. Therefore this discussion of the mechanism of calcification must be based on conjecture. The simplest mechanism to explain how changes in the $[Ca^{2+}]$ and $[CO_3^{2-}]$ in the seawater can influence the ECF is to assume that there is a paracellular pathway whereby the ions diffuse through the junctions between cells without crossing cell membranes directly to the site of calcification. Most published models of coral calcification include this pathway although active transport is thought to more

important [*Furla et al.*, 2000; *Gattuso et al.*, 1999; *Tambutte et al.*, 1996]. This mechanism has the attraction of explaining why Sr, Cd, Pb, Mn, Ba, U are incorporated into the skeleton in the same ratios they are found in seawater [*Dunbar and Cole*, 1993].

[13] Active transport of calcium could be consistent with the saturation state hypothesis if the rate of calcium transport saturates at a Ca^{2+} concentration above the ambient concentration of seawater as has been reported for some coral species [*Chalker*, 1976; *Krishnaveni et al.*, 1989]. Elevated pCO_2 results in an increase in the external HCO_3^- concentration so a limitation of the supply of DIC cannot be invoked to explain the decrease in calcification. Increased pCO_2 could result in decreased calcification if photosynthesis and calcification compete for the same internal supply of DIC in the host cell (see section 1.5). Finally, as *Gattuso et al.* [1999] noted, the pH decrease associated with the pCO_2 increase could cause changes membrane permeability and conductance or in the activity of an enzyme involved in some critical pathway.

[14] An understanding of how coral calcification responds to increased pCO_2 and how that response may vary depending on changes in other environmental factors is critical to predicting how coral reefs may change in the next 50–100 years in response to global environmental change. In this study we report the effects of a doubling in pCO_2 on an assemblage of Hawaiian corals (*Porites compressa* and *Montipora capitata*) under summer and winter conditions. Both net photosynthesis and calcification were measured because they both draw from the same internal pool of DIC and hence might be expected to interact. We also investigated the effect of a 10 × nutrient loading because previous work has shown that its effect on calcification may provide an analog for how elevated pCO_2 affects calcification. Owing to logistical considerations we had to limit the elevated pCO_2 treatments to 1.5 hours. We also did not have the ability to heat the water in the flume to simulate the effects of greenhouse warming. In recognition of these limitations caution should be exercised in extending the results of this study to predicting the response to global environmental change.

2. Methods

2.1. Experimental Setup

[15] Specimens of *Porites compressa* and *Montipora verucosa* (*capitata*) were collected from the Coconut Island reef flat, Hawaii Institute of Marine Biology, Kaneohe, Hawaii, USA, brought to the island, and then placed in an experimental flume, 24 m long, 0.40 m wide and 0.4 m deep (see *Atkinson and Bilger* [1992] for a discussion of the design of the flume). The experimental community covered a 2.2 m^2 (5.5 m by 0.4 m) area of the flume. Water was recirculated through the flume throughout the experiments. Water velocities past the experimental community were controlled at 20 or 40 cm s^{-1}. At night and between the experiments fresh seawater from 100 m offshore was pumped thru the flume at a rate of 20 L min^{-1} resulting in a water residence time of 2 hours. The ratio of water volume to planar surface of the coral community was 1.1 m. Collections of coral were performed before the summer and winter experiments. Care was taken to prepare the coral assemblage in the flume the same each time. The flume received full natural sunlight. Photosynthetically available irradiance incident on the flume was measured with a LiCor 192 cosine collector sensor located on the top of a nearby building. The temperature and O_2 concentration of the water in the flume was monitored continuously with an Endeco 1127 pulsed O_2 sensor. The accuracy of the O_2 sensor was checked frequently against Winkler determined oxygen concentrations. Water samples were drawn from the flume for analysis of total alkalinity (TA) and total dissolved inorganic carbon (DIC). The CO_2 parameters (pH, [CO_2], [HCO_3^-], [CO_3^{2-}] and Ω_a) were computed from TA, DIC, temperature and salinity using the program CO2SYS [*Lewis and Wallace*, 1998]. Nutrient concentrations and nutrient uptake were measured only during the January 2000 experiments.

2.2. Overview of Experiments

[16] Prior to beginning the CO_2 experiments, net production and calcification of the coral assemblage were measured throughout the light period over three days (21–24 August 1999) in a series of five to six 1 hour incubations. These data allowed us to determine that net production and calcification were light saturated between 0900 and 1730 LT during the summer experiment and between 1030 and 1500 LT during the winter experiment. All future experiments were run between these hours to minimize variability due to fluctuations in irradiance. A preliminary experiment was also conducted to investigate the effect of flow velocity. Ten incubations were performed at a flow of 40 cm s^{-1} and eight at a flow of 20 cm s^{-1}. It was found that the rates at the two velocities were not significantly different. All subsequent incubations were performed at 20 cm s^{-1} because this minimized gas exchange.

[17] The interactive effects of CO_2 and seasonal change in temperature and irradiance on net production and calcification were investigated in a series of incubations performed during summer (26 August to 1 September 1999) and winter (7–18 January 2000) conditions. During the summer experiment eighteen incubations were done at ambient pCO_2 (460 μatm) and nine at 1.7 × ambient (789 μatm). During the winter experiment a nutrient enrichment was added as a factor. Six incubations were run under ambient nutrients; three at ambient pCO_2 (391 μatm), two at 1.3 × (526 μatm) and one at 2.0 × pCO_2 (781 μatm). These experiments were followed by a four day period during which the flume was spiked each day with 0.03 mol of PO_4 and 0.3 mol of NH_4. This raised [PO_4] to 13 μM and [NH_4] to 109 μM. The corals were exposed to the nutrient enriched water for 4 h. Nutrient concentrations were measured at the beginning and end to determine the uptake rate. Following four hours of exposure to the elevated nutrients the flume was drained, refilled, and continuously flushed with low-nutrient ambient seawater until the next incubation. The intention of the enrichment treatment was not to simulate a particular eutrophication scenario but to fully saturate the internal nutrient pools of the corals and thereby gain a perspective of the maximum impact that nutrients might have on the response of net production and calcification to elevated pCO_2 in the short term. Following this conditioning, with the corals again exposed to ambient nutrient concentrations but their internal pools saturated with nutrients, we ran

incubations measuring net photosynthesis, calcification and nutrient uptake at four different pCO_2 levels; six at ambient (380 μatm), two at 1.4 × (527 μatm), six at 1.9 × (733 μatm) and one at 0.6 × (219 μatm).

2.3. Experimental Protocol

[18] Every morning, before the experiments began, the sidewalls and bottom of the flume were brushed to remove filamentous algae. The seawater in the flume was drained and the flume was filled with fresh seawater. The seawater inlet was closed. The water was recirculated in the flume at a velocity of 20 or 40 cm s^{-1}. One ambient and one elevated pCO_2 incubation was run each day. If the incubation was an elevated pCO_2 run 20–40 mL of concentrated HCl was added. In the case of the subambient incubation 40 mL of concentrated NaOH was added. The acid or base was prediluted in a 10 gal bucket and slowly added to the flowing water. This prevented a large spike in pH and ensured rapid mixing. After 30 min of mixing, water sampling began. Initially water samples were taken every 30 min but it was determined that this did not add much to the accuracy of the rates or to the determination of the average chemical conditions during the 1.5 hour incubations. Therefore during the incubations in January 2000 only initial and final water samples were taken. Following the incubation the flume was immediately drained and refilled with ambient seawater. During the draining the corals were exposed to the air for no more than 2–3 min. There was no evidence that the aerial exposure or the elevated pCO_2 treatments were detrimental to the corals as indicated by the fact that net production at ambient pCO_2 remained constant from the beginning to the end of the experiment.

2.4. Control of Carbonate Chemistry

[19] In these experiments pCO_2 was adjusted by manipulating TA by addition of acid or base while holding DIC constant. In the ocean the opposite occurs. The DIC concentration of the surface ocean increases due to invasion of CO_2 from the atmosphere while TA remains constant. There are some subtle differences in the carbonate chemistry of seawater between when the pCO_2 is doubled by increasing DIC and when it is doubled by decreasing the TA. To illustrate, assume that we start with seawater at a temperature of 25°C and 35 psu and an initial TA of 2300 μEq kg^{-1} and DIC of 1973 μmol kg^{-1}. This water will have a pCO_2 of 350 μatm and a pH of 8.08 on the seawater scale. If the DIC is increased to 2109 μmol kg^{-1}, the pCO_2 will become 700 μatm, the pH will drop to 7.83 and the $[HCO_3^-]$ and $[CO_3^{2-}]$ will become 1944 and 145 μmol kg^{-1}, respectively. Now if instead we lower the TA of the same initial seawater to 2144 μEq kg^{-1} without changing the DIC the resulting seawater has a pCO_2 of 700 μatm, a pH of 7.80, $[HCO_3^-]$ of 1825 μmol kg^{-1} and a $[CO_3^{2-}]$ of 128 μmol kg^{-1}. The pH of the resulting seawater is virtually identical and in both cases the HCO_3^- increases and the CO_3^{2-} decreases. The differences are that in the decreased TA case the $[HCO_3^-]$ increase is smaller (5% versus 12%) and the $[CO_3^{2-}]$ decrease is larger (45% versus 37%) compared to the natural setting. The ratio of $[HCO_3^-]/[CO_3^{2-}]$ following a doubling in pCO_2 is 13.4 in the natural case and 14.3 in the reduced TA case, a difference of 6%. These differences should be borne in mind but it would seem unlikely that experiments based on the manipulation of TA will yield results fundamentally different from the natural case (DIC increase at constant TA).

[20] Addition of acid or base was made 30 min before the start of a run in order to allow time for the system to become well mixed. The chemical addition raised or lowered the pCO_2 putting the system out of equilibrium with the atmosphere. As a result there was a flux of CO_2 into or out of the water due to gas exchange. Our calculations of net production and calcification take gas exchange into account. However, gas exchange will cause the carbonate chemistry to change over the course of the measurement period. It is straight forward to calculate this change. During the 2 × pCO_2 runs the air-water pCO_2 differential is 370 μatm. The flux of CO_2 to the atmosphere is (5 m d^{-1})(1.5 hours/24 hours)(0.027 mmol m^{-3} μatm^{-1})(370 μatm)(8 m^2 flume SA)/(2.4 m^3 flume volume) or 10 mmol m^{-3} (1.5 hours)$^{-1}$. This loss of CO_2 would cause a 7% decrease in pCO_2, 0.03 unit pH increase, and a 5% increase in $[CO_3^{2-}]$ and Ω_a. It is apparent that the effect of gas exchange in negligible. More important is the effect that photosynthesis and calcification will have on the carbonate chemistry. The dimensions of the flume and the duration of the experiments were carefully chosen to ensure that the changes in TA, DIC and O_2 would be big enough to quantify the rates precisely but not so big that the carbonate chemistry would change significantly between the beginning and end of a run. In practice, the decrease in pCO_2 from start to end of an incubation averaged 12% at ambient pCO_2 and 23% at 2 × pCO_2. The variability in terms of standard deviation in pCO_2, pH and Ω_a over a 1.5 hour incubation was ±60 μatm, ±0.03 units, and ±0.1, respectively.

2.5. Analytical Methods

[21] Water samples for total dissolved inorganic carbon (DIC), total alkalinity (TA) and dissolved oxygen were collected in glass bottles at the beginning and end of each 1.5 hour measurement period. DIC was determined coulometrically [*Chipman et al.*, 1993]. Analyses were run in triplicate, and the precision (1σ) and accuracy were estimated to be ±1 μmol kg^{-1}. The TA was determined in triplicate using an automated Gran titration. The precision was typically ±2–3 μEq kg^{-1}. Accuracy of both the DIC and TA analyses were checked against certified seawater reference material prepared by Andrew Dickson (Scripps Institute of Oceanography). The concentrations of HCO_3^-, CO_3^{2-}, pH, pCO_2 and Ω_a were calculated using the CO2SYS program written by Ernie Lewis (Brookhaven National Laboratory) using the dissociation constants of *Mehrbach et al.* [1973] as refit by *Dickson and Millero* [1987]. Dissolved oxygen concentration was measured by Winkler titration using an automated titrator employing amperometric endpoint detection. Precision is estimated to be ±0.2 μmol L^{-1}. Analyses for nutrients (phosphate, nitrate, nitrite, ammonium and silicate) were performed using a Technicon AAII system, with standard procedures modified for high-precision analyses (Technicon Industrial Systems; Industrial methods for water, seawater, and wastewater analysis). Nutrient analyses were performed by Ted Walsh, SOEST Nutrient Analytical Lab.

2.6. Measurements of Net Production and Calcification

[22] The calcification rate was determined by the alkalinity anomaly method. This method assumes that precipitation of one mole of $CaCO_3$ reduces the total alkalinity by two equivalents. Deviations from this simple stoichiometry can occur in response to production and degradation of organic carbon through assimilation and remineralization of nitrate [*Brewer and Goldman*, 1976]. However, it is unlikely that such processes would be occurring in the flume. Changes in the concentration of NO_3 and NH_4 during an incubation could contribute no more than ±0.3 μEq kg^{-1} to the change in TA, much less than the precision of the analysis. The net calcification rate of the corals in the flume was computed according to

$$G = -0.5\rho\left(\frac{1000\ \mathrm{L}}{1\ \mathrm{m}^3}\right)\left(\frac{1\ \mathrm{mmol}}{1000\ \mu\mathrm{mol}}\right)\left(\frac{V_F}{A_C}\right)\Delta\mathrm{TA}/\Delta t, \quad (2)$$

where G is the net calcification rate in mmol $CaCO_3$ m^{-2} h^{-1}, −0.5 is the stoichiometric conversion from μEq of TA removed to μmoles of $CaCO_3$ produced, ρ is the density of seawater (1.025 kg L^{-1}), V_F is the volume of seawater in the flume (2.4 m^3), A_C is the planar surface of the assemblage of coral (2.2 m^2), ΔTA is the change in total alkalinity in μEq kg^{-1} and Δt the elapsed time between TA measurements in hours. The net production of organic carbon was computed according to

$$\mathrm{NP_C} = -\left(\frac{1}{A_C}\right)\left[V_F\rho\left(\frac{\Delta\mathrm{DIC}}{\Delta t} - 0.5\frac{\Delta\mathrm{TA}}{\Delta t}\right) + A_F S k_{\mathrm{CO_2}}\left(\mathrm{pCO}_{2,W} - 370\right)\right], \quad (3)$$

where NP_C is the net carbon production in mmol C m^{-2} h^{-1}, ΔDIC is the change in total dissolved inorganic carbon, A_F is the surface area of the flume (8 m^2), S is the solubility of CO_2 in seawater at ambient temperature and salinity (0.027mmol CO_2 m^{-3} μatm^{-1}), k_{CO_2} is the gas exchange coefficient for CO_2 in m h^{-1}, $pCO_{2,W}$ is the partial pressure of CO_2 in the water, and 370 is the partial pressure of CO_2 in the overlying atmosphere in μatm. The net oxygen production was computed according to

$$\mathrm{NP_O} = \left(\frac{1}{A_C}\right)\left[V_F\frac{\Delta[\mathrm{O_2}]}{\Delta t} + A_F k_{\mathrm{O_2}}\left([\mathrm{O_2}] - C^*\right)\right], \quad (4)$$

where $\Delta[O_2]$ is the change in dissolved oxygen concentration in mmol m^{-3}, k_{O_2}] is the gas exchange coefficient for O_2 in m h^{-1} and C^* is the oxygen concentration in equilibrium with the atmosphere at the prevailing temperature and salinity of the seawater (205.1 mmol m^{-3} in August and 217.6 mmol m^{-3} in January).

2.7. Estimation of Gas Exchange Rate

[23] The gas exchange rate of oxygen was determined by bubbling the water in the flume with pure O_2 gas to elevate the concentration and then observe the rate with which it decreased over time. Dead corals were placed in the flume to simulate the bottom roughness of the live corals. Current speed was varied between 14 and 34 cm s^{-1}. A regression of k_{O_2} versus current speed yielded the relationship

$$k_{\mathrm{O_2}} = 0.167U - 1.38, \quad (5)$$

where U is the current speed in cm s^{-1}. The value of k_{CO_2} was calculated from k_{O_2} according to the Schmidt number relationship

$$k_{\mathrm{CO_2}} = k_{\mathrm{O_2}}\left(\frac{487}{432}\right)^{-0.5}, \quad (6)$$

where 487 and 432 are the Schmidt numbers of CO_2 and O_2, respectively.

2.8. Statistical Analysis

[24] The significance of CO_2 and nutrient treatment effect on photosynthesis and calcificaton was tested by Student's *t*-test. Least squares linear regression was used to determine the significance of relationships between photosynthesis and $[CO_2aq]$ and between calcification and Ω_a. Significance reported below indicates that the probability of falsely rejecting the null hypothesis is <0.05. All uncertainties in the text, tables and figures are 95% confidence intervals unless $n = 2$ or 3, in which case the standard error (SE) is given.

3. Results

3.1. Physical and Chemical Setting

[25] The seasonal variability in water temperature and photosynthetically available radiation (PAR) over the period 1 January 1999 to 31 December 2000 are shown in Figures 1a and 1b. Winter temperatures can be quite variable ranging from 21.0°C to 25.0°C. Summer temperatures are less variable ranging from 25.2°C to 27.6°C. PAR irradiance goes through a minimum of 17–21 mol quanta m^{-2} d^{-1} in December and January and peaks around 40 mol quanta m^{-2} d^{-1} in June and July. It can be seen that the conditions during the experimental periods of this study, signified by the bars, fall close to the annual minimum and maximum in temperature and light. The mean and 95% confidence intervals of the ambient physical and chemical conditions during the experiments are given in Table 1. The pCO_2 of the water at the intake to the flume from 23 August to 2 September 1999 was 513 ± 22 μatm and from 7 to 17 January 2000 was 408 ± 9 μatm. The pCO_2 of nearby offshore waters at the Hawaiian Ocean Time Series station was 370 μatm in August 1999 and 341–355 μatm during the period December 1999 to February 2000 (http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html). The oxygen concentration of the water over the same periods was 209 ± 7 μmol kg^{-1} (2.6% supersaturated) in August 1999 and 230 ± 7 μmol kg^{-1} (5.6% supersaturated) in January 2000. The positive pCO_2 differential (ΔpCO_2) of 38 to 143 μatm between the water at the study site and the atmosphere indicates that the southern portion of Kaneohe Bay is a source of CO_2 to the atmosphere. There must be significant rates of net calcification and/or respiration in the bay to support that flux given that the ΔpCO_2 of the oceanic water entering the bay is 0 to −22 μatm. Given that the bay water is supersaturated with respect to oxygen indicates that

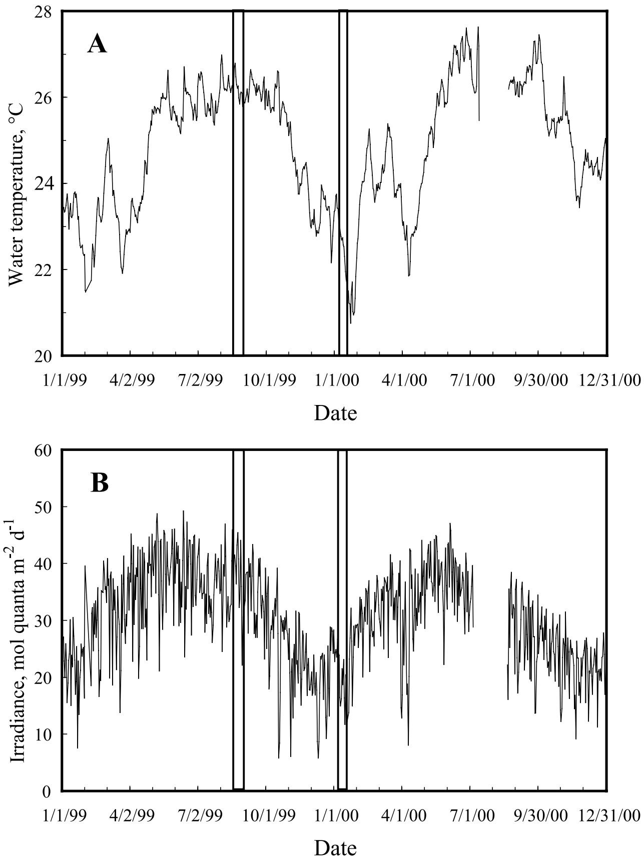

Figure 1. (a) Water temperature and (b) surface water photosynthetically available irradiance recorded at the Coconut Point weather station at the Hawaiian Institute of Marine Biology. The bars indicate the periods of this study.

community carbon production exceeds respiration and is a sink for CO_2. The fact that the bay is a net source of CO_2 indicates that calcification is producing more CO_2 than is consumed by net production. The elevated pCO_2 in the bay water causes a depression of the CO_3^{2-} concentration (173–185 μmol kg^{-1}) and Ω_a (2.8–2.9) relative to that of nearby offshore waters at the Hawaiian Ocean Time Series Station, 229 ± 6 μmol kg^{-1} and 3.6 ± 0.1, respectively. Nutrient concentrations were only measured in January. They were as follows: PO_4 0.17 ± 0.02 μM, $NO_3 + NO_2$ 0.3 ± 0.1 μM, NH_4 0.28 ± 0.06 μM and SiO_3 6.0 ± 0.3 μM. These are typical levels for coral reefs [*Atkinson and Falter*, 2003].

3.2. Effects of Irradiance on Net Production and Calcification

[26] The photosynthesis-irradiance and calcification-irradiance relationship for the coral assemblage was determined in August by measuring the rate of production and calcification throughout the course of the day for several days. The rate of NP_C was found to be well described by a hyperbolic tangent function (Figure 2a), $r^2 = 0.75$ ($n = 34$). The best fit parameters defining the curve were: NP_{max} = 50 ± 3 mmol C m^{-2} h^{-1}, α = 0.13 ± 0.03 mmol C m^{-2} h^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$, R = 10 ± 6 mmol C m^{-2} h^{-1}. The light saturation parameter, I_k, was 586 ± 108 μmol quanta m^{-2} s^{-1} and the compensation intensity, I_c, was 80 ± 33 μmol quanta m^{-2} s^{-1}. The calcification data had too much scatter to justify the fitting of a hyperbolic tangent curve, however, rates did exhibit a general trend with irradiance, increasing from a dark rate of 3 ± 3 mmol $CaCO_3$ m^{-2} h^{-1} to a rate of 20–27 mmol $CaCO_3$ m^{-2} h^{-1} at 1300–1700 μmol quanta m^{-2} s^{-1} (Figure 2b).

[27] From these curves we learned that the net production of the coral assemblage saturated at an irradiance of 586 μmol photons m^{-2} s^{-1}. Examination of the light data revealed that the irradiance exceeded this level between 0900 and 1730 LT in August and between 1030 and 1500 LT in January. The calcification data did not yield a calcification-irradiance relationship with a well defined saturation response. However, *Marubini et al.* [2001] found that the calcification rate of *P. compressa* saturated at an irradiance of 250 μmol photons m^{-2} s^{-1}. On the basis of these results we decided that it would be possible to run two incubation experiments each day; one from 1000 to 1130, and a second from 1230 to 1400. By restricting the incubations to the hours 1000–1400 and centering them around noon we ensured that light levels would generally be saturating and the corals would receive approximately the same amount of light.

[28] Taking the measurements of incident irradiance made at the Coconut Point Reef weather station for the period 21 August to 1 September 1999 and applying a diffuse attenuation coefficient (k_{PAR}) of 0.18 m^{-1}, based on measurements in Kaneohe Bay, we computed the average hourly irradiance at 3 m depth, the mean depth of the reef flats in Kaneohe Bay. Using the photosynthesis- and calcification-irradiance models given in Figures 2a and 2b we computed how much organic and inorganic carbon a patch of reef with the physiological characteristics of the corals in this study would produce over a typical August day, P_G 452 mmol C m^{-2} d^{-1}, NP_C 248 mmol C m^{-2} d^{-1} and G 238 mmol $CaCO_3$ m^{-2} d^{-1}. The ratio of midday calcification to gross production (G:P_G) was 0.33. The ratio of daily calcification to gross production was 0.53. The ratio of midday calcification to night time calcification was 5.3. In the calculation of P_G we assume that the respiration rate in the light is the same as the dark. This assumption has been widely made in the coral reef literature. However, two recent studies have found that the rate of respiration in the light can be 2–

Table 1. Prevailing Ambient Physical and Chemical Conditions During the Flume Experiment[a]

Dates	Temperature, °C	Light, mol quanta m^{-2} d^{-1}	TA, μEq kg^{-1}	TCO_2, μmol kg^{-1}	HCO_3^-, μmol kg^{-1}	CO_3^{2-}, μmol kg^{-1}	CO_2 aq, μmol kg^{-1}	pCO_2, μatm	pHsw	Ω_{arag}	O_2, μmol L^{-1}
21 Aug–1 Sep 1999	27.3 ± 0.3	37 ± 6	2171 ± 10	1929 ± 12	1742 ± 15	173 ± 6	14 ± 1	513 ± 22	7.92 ± 0.02	2.8 ± 0.1	209 ± 7
7–18 Jan 2000	23.4 ± 0.3	19 ± 4	2197 ± 7	1935 ± 8	1737 ± 10	185 ± 2	11.8 ± 0.3	408 ± 9	8.01 ± 0.01	2.91 ± 0.04	230 ± 4

[a]Mean ±95% CI.

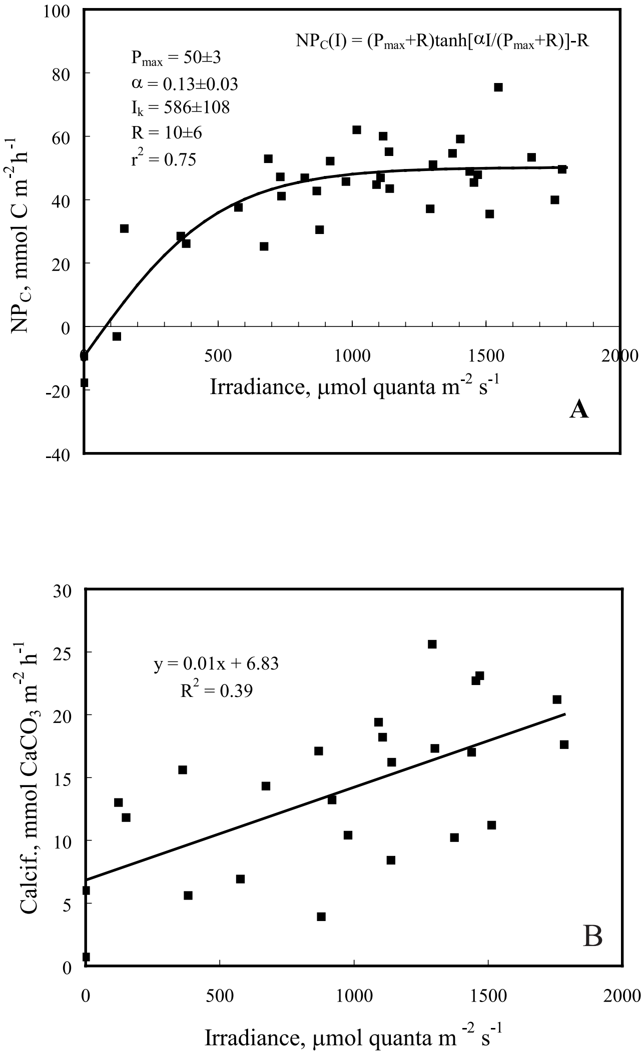

Figure 2. (a) Net photosynthesis-irradiance relationship of the coral assemblage in the flume in August 1999 showing the measurements of net carbon production (NP_C) and the hyperbolic tangent curve fit to the data. (b) Calcification-irradiance relationship of the same coral assemblage and the best fit straight line to the data.

12 times higher than in the dark [*Al-Horani et al.*, 2003; *Langdon et al.*, 2003]. In the case of the *Langdon et al.* [2003] study the assumption that light equaled dark respiration resulted in a 40% underestimation of the true rate of gross production.

3.3. Effect of Flow Velocity on Net Photosynthesis and Calcification

[29] Experiments were conducted in August to see whether variations of flow velocity would affect net photosynthesis or calcification rates. Runs were made under ambient pCO_2 at a velocities of 20 cm s^{-1} (n = 8) and 40 cm s^{-1} (n = 10). The effect of elevated pCO_2 on flow dependence was not tested. There was a slight increase in NP_C (43 ± 6 versus 46 ± 3 mmol C m^{-2} h^{-1}) and NP_O (36 ± 5 versus 42 ± 4 mmol O_2 m^{-2} h^{-1}) and no change in calcification (16 ± 4 versus 16 ± 2 mmol $CaCO_3$ m^{-2} h^{-1}) but the changes were not significant (Student's *t*-test, $P > 0.05$). There was no significant difference in the temperature or irradiance between the 20 and 40 cm s^{-1} runs. There was a small but significant increase in [CO_2aq] from 11.6 ± 0.6 to 13.1 ± 0.6 μmol kg^{-1} and decrease in Ω_a from 3.1 ± 0.1 to 2.8 ± 0.1. Within the limits of our data we see no evidence that an increase in current speed from 20 to 40 cm s^{-1} causes a significant increase in net production or calcification. We conclude that the rates of net production and calcification that we obtained in the flume are close to optimal and probably representative of those in the natural setting. On the basis of these results all experiments in January were performed at a current speed of 20 cm s^{-1} because this minimized gas exchange and the change in carbonate chemistry over the course of an experimental run.

3.4. Seasonal Changes in Net Production and Calcification at Ambient pCO_2 and Nutrient Conditions

[30] Midday rates of net production showed strong seasonality (Figure 3). NP_C declined from 45 ± 3 mmol C m^{-2} h^{-1} (n = 18) in August to 23 ± 8 (n = 3) in January, a significant 49% decrease ($P < 0.05$). NP_O also decreased significantly from 37 to 23 mmol O_2 m^{-2} h^{-1} ($P < 0.05$). Calcification did not change significantly, 16 ± 2 mmol $CaCO_3$ m^{-2} h^{-1} in August versus 15.4 ± 0.8 in January.

3.5. Nutrient Enrichment

[31] The flume was spiked with 0.03 mol PO_4 and 0.3 mol NH_4 on four successive afternoons (10–13 January 2000). This raised [PO_4] to 13 μM and [NH_4] to 109 μM. The corals took up 5.8 ± 0.3 mmol PO_4 m^{-2} (n = 4) and 39 ± 5 mmol NH_4 m^{-2} during the 4 hour exposure periods (Table 2). Subsequent to the 4 day nutrient enrichment period, the uptake rates of PO_4, NH_4 and NO_3 were measured under ambient conditions. Ambient concentrations of PO_4, NH_4 and NO_3 were 0.16 ± 0.03, 0.24 ± 0.03 and 0.26 ± 0.05 μmol L^{-1}, respectively. The measured rates of uptake were 0.3 ± 0.2 mmol PO_4 m^{-2} d^{-1} (n = 10), 1.4 ± 0.6 mmol NH_4 m^{-2} d^{-1} and 2.7 ± 0.8 mmol NO_3 m^{-2} d^{-1} (n = 10). The N:P uptake ratio under ambient conditions was 13.7. The enrichment constituted a loading of 14 times the daily P uptake and 10 times the daily N uptake under ambient nutrient conditions. The effect of

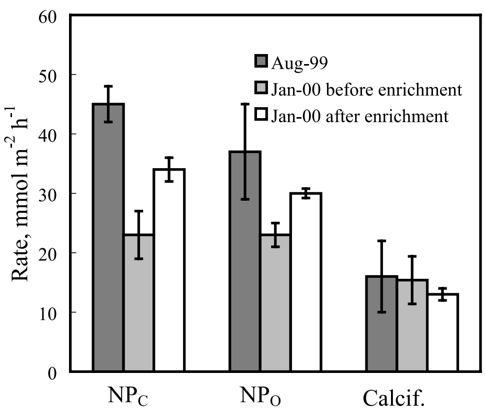

Figure 3. Rates of net production and calcification of the coral assemblage under ambient pCO_2 and nutrient concentration in August and under ambient pCO_2 before and after a nutrient enrichment in January.

Table 2. Nutrient Uptake Rates During and After Nutrient Enrichment

Date and Time, LT	$[PO_4]$, mmol m^{-3}	$[NH_4]$, mmol m^{-3}	Uptake: PO_4, mmol m^{-2} 4-h^{-1}	Uptake: NH_4, mmol m^{-2} 4-h^{-1}
10 Jan 2000, 1430	13.1	104		
10 Jan 2000, 1845	8.1	77	5.5	29.6
11 Jan 2000, 1442	12.8	109		
11 Jan 2000, 1856	7.4	73	5.9	39.8
12 Jan 2000, 1510	12.8	115		
12 Jan 2000, 1918	7.8	75	5.5	43.9
13 Jan 2000, 1452	13.1	109		
13 Jan 2000, 1950	7.4	70	6.2	42.6
Average			**5.8**	**39.0**
SD			**0.3**	**5.6**
95% CI			**0.3**	**5**

Date and Time, LT	$[PO_4]$, mmol m^{-3}	$[NH_4]$, mmol m^{-3}	$[NO_3]$, mmol m^{-3}	Uptake: PO_4, mmol m^{-2} d^{-1}	Uptake: NH_4, mmol m^{-2} d^{-1}	Uptake: NO_3, mmol m^{-2} d^{-1}
11 Jan 2000, 1010	0.21	0.27	0.44			
11 Jan 2000, 1143	0.20	0.21	0.31	0.2	1.0	2.2
12 Jan 2000, 1007	0.20	0.29	0.43			
12 Jan 2000, 1137	0.15	0.20	0.11	0.9	1.6	5.6
13 Jan 2000, 1007	0.20	0.21	0.32			
13 Jan 2000, 1142	0.16	0.16	0.17	0.7	0.8	2.5
13 Jan 2000, 1237	0.16	0.25	0.13			
13 Jan 2000, 1407	0.16	0.15	0.09	0.0	1.7	0.7
14 Jan 2000, 1013	0.17	0.32	0.42			
14 Jan 2000, 1148	0.16	0.16	0.27	0.2	2.6	2.5
14 Jan 2000, 1223	0.14	0.26	0.16			
14 Jan 2000, 1413	0.12	0.18	0.09	0.3	1.1	1.0
15 Jan 2000, 1007	0.15	0.17	0.32			
15 Jan 2000, 1126	0.13	0.16	0.14	0.4	0.2	3.6
15 Jan 2000, 1237	0.15	0.28	0.37			
15 Jan 2000, 1353	0.15	0.22	0.19	0.0	1.2	3.7
16 Jan 2000, 1007	0.16	0.30	0.35			
16 Jan 2000, 1140	0.14	0.22	0.25	0.3	1.4	1.7
16 Jan 2000, 1237	0.14	0.28	0.35			
16 Jan 2000, 1345	0.14	0.29	0.23	0.0	−0.2	2.8
17 Jan 2000, 1007	0.16	0.42	0.41			
17 Jan 2000, 1137	0.22	0.20	0.22		3.8	3.3
Average	**0.16**	**0.24**	**0.26**	**0.3**	**1.4**	**2.7**
SD	**0.03**	**0.07**	**0.11**	**0.3**	**1.1**	**1.3**
95% CI	**0.01**	**0.03**	**0.05**	**0.2**	**0.6**	**0.8**

the nutrient enrichment at ambient pCO_2 was to cause NP_C to increase from 23 ± 8 mmol $CaCO_3$ m^{-2} h^{-1} to 34 ± 6 ($P > 0.05$) and NP_O to increase from 23 ± 2 mmol O_2 m^{-2} h^{-1} to 30 ± 4 ($P < 0.05$) (Figure 3). We can infer from this response to nutrient enrichment that the coral assemblage in January was nutrient limited. Calcification decreased from 15.4 ± 0.8 mmol $CaCO_3$ m^{-2} h^{-1} to 13.4 ± 1 ($P > 0.05$). More on the interactions between the nutrient and CO_2 treatments is given in the following section.

3.6. Effect of Elevated pCO_2 on Photosynthesis

[32] The chemical conditions imposed on the corals and the corresponding rates of net production are shown in Table 3. Elevated pCO_2 had the effect of enhancing the rate of NP_C (Figure 4). In August, NP_C increased significantly from 45 ± 3 ($n = 18$) to 55 ± 6 ($n = 9$) mmol C m^{-2} h^{-1} (two-tailed Student's t-test, $P < 0.02$) in response to a 1.7-fold increase in pCO_2 and [CO_2 aq]. The slope of the relationship was 1.2 ± 0.4 mmol C m^{-2} h^{-1} (μmol kg^{-1})$^{-1}$. The slope of the relationship was similar in January, 1.0 ± 0.2 mmol C m^{-2} h^{-1} (μmol kg^{-1})$^{-1}$. The effect of nutrient enrichment was to cause an increase in NP_C at all CO_2 levels. The slope of the relationship was still significant but less than that immediately before the enrichment, i.e., 0.5 ± 0.4 mmol C m^{-2} h^{-1} (μmol kg^{-1}).

[33] Unlike NP_C, NP_O did not change consistently in response to elevated pCO_2. In August a 1.7-fold increase in pCO_2 resulted in no significant change, i.e., 37 ± 4 (n = 18) versus 36 ± 5 (n = 9) mmol O_2 m^{-2} h^{-1}. In January, NP_O increased significantly in response to a 1.4-fold increase in pCO_2 (23 ± 2 versus 31 ± 0 mmol C m^{-2} h^{-1}) but then declined to 21 mmol C m^{-2} h^{-1} when pCO_2 was doubled.

[34] The rates of NP_C and NP_O were normalized to the rate measured at ambient [CO_2 aq] and then regressed against [CO_2 aq] as a means of pooling the data and extracting the underlying dependence on [CO_2 aq] (Figure 5). The regression of normalized NP_C versus [CO_2 aq] was highly significant ($r^2 = 0.60$, $P = 0.015$) with a slope of 3 ± 2% (mmol kg^{-1})$^{-1}$. In comparison, the regression of normalized NP_O versus [CO_2 aq] was not significant ($r^2 = 0.006$, $P = 0.84$).

3.7. Effect of Elevated pCO_2 on Calcification

[35] Calcification exhibited great sensitivity to altered seawater chemistry decreasing significantly in August from 16 ± 2 mmol $CaCO_3$ m^{-2} h^{-1} (n = 18) under ambient

Table 3. Metabolic Rates and the Physical Conditions and Seawater Carbonate Chemistry During the Experiments Performed on the Coral Assemblage in the Flume[a]

Date	Run	n	Temperature, °C	Irradiance, μmol quanta m^{-2} s^{-1}	HCO_3^-, μmol kg^{-1}	CO_3^{2-}, μmol kg^{-1}	CO_2 aq, μmol kg^{-1}	pCO_2, μatm	pH (sws)	Ω_a	Calcif, mmol $CaCO_3$ m^{-2} h^{-1}	NP_C, mmol C m^{-2} h^{-1}	NP_O, mmol O_2 m^{-2} h^{-1}
Aug 1999	1.0×	18	27.3 ± 0.3	1216 ± 151	1691 ± 14	185 ± 6	12.3 ± 0.6	460 ± 20	7.96 ± 0.02	3.0 ± 0.1	16 (2)	45 (3)	37 (4)
Aug 1999	1.7×	9	27.3 ± 0.4	1138 ± 233	1720 ± 26	113 ± 6	21 ± 2	789 ± 52	7.74 ± 0.02	1.82 ± 0.09	9 (4)	55 (6)	36 (5)
							Before Nutrient Enrichment						
Jan 2000	1.0×	3	23.8 ± 0.7	712 ± 328	1709 ± 21	191 ± 5	11.3 ± 0.3	391 ± 10	8.02 ± 0.01	3.01 ± 0.09	15.4 (0.8)	23 (8)	23 (2)
Jan 2000	1.3×	2	23.8 ± 0.2	831 ± 283	1717 ± 24	144 ± 4	15.4 ± 0.8	526 ± 34	7.90 ± 0.02	2.27 ± 0.07	12 (1)	32 (1)	31 (0)
Jan 2000	2.0×	1	24.4	851	1740	104	22.9	781	7.74	1.65	3	35	21
							After Nutrient Enrichment						
Jan 2000	1.0×	6	23.2 ± 0.5	758 ± 141	1701 ± 18	191 ± 5	11.2 ± 0.6	380 ± 22	8.04 ± 0.02	3.00 ± 0.08	13 (1)	34 (6)	30 (4)
Jan 2000	1.4×	2	23.8 ± 0.7	625 ± 164	1723 ± 26	145 ± 11	15 ± 1	527 ± 41	7.90 ± 0.03	2.3 ± 0.2	13 (1)	35 (6)	33 (2)
Jan 2000	1.9×	6	22.8 ± 0.4	711 ± 91	1734 ± 19	103 ± 4	22 ± 1	733 ± 36	7.76 ± 0.02	1.62 ± 0.06	13 (2)	43 (4)	34 (3)
Jan 2000	0.6×	1	23.7	605	1599	298	5.5	219	8.25	4.7	16	34	29

[a]Number of runs, n. Mean ±95% CI. Variability (1σ) in chemical conditions during individual incubations were typically pCO_2 ± 60 μatm, pH ± 0.03, Ω_a ± 0.1.

conditions to 9 ± 4 (n = 9) under the 1.7 × conditions (P < 0.05). In January, calcification decreased from 15.4 ± 0.8 mmol $CaCO_3$ m^{-2} h^{-1} (n = 3) under ambient conditions to 11.7 ± 1 (n = 2) at 1.4 × and 3 (n = 1) at 2.0 ×. The decrease from ambient to the 1.4 × level was significant (P < 0.05).

[36] Nutrient enrichment had the effect of depressing calcification slightly in the ambient chemistry runs, i.e., 15.4 ± 0.8 (n = 3) versus 13 ± 1 (n = 6) mmol $CaCO_3$ m^{-2} h^{-1}, however, the decrease was not significant (P > 0.05). The most notable effect of the nutrient enrichment was the increased rate of calcification at 1.4 × and 2.0 ×. As a result, following the nutrient enrichment elevated pCO_2 did not cause a depression in calcification.

[37] Calcification was strongly related to Ω_a (Figure 6). In August the slope of the relationship was 6.4 ± 1.9 (SE) mmol $CaCO_3$ m^{-2} h^{-1} per unit change in Ω_a and in January it was 9.0 ± 2.6 (SE). The difference in slopes was not significant (P > 0.05). We found that first-order saturation state model, equation (1), gave a good fit to the pooled summer and preenrichment winter data (r^2 = 0.87, P = 0.0001). The fit of the second-order saturation state model was not as good (r^2 = 0.78, P = 0.0036).

[38] While seasonal change in temperature and light did not have a significant effect the calcification-Ω relationship the nutrient enrichment did. The loading of N and P reduced the slope of the calcification-Ω_a relationship from 9 ± 2.6 (SE) to 1.0 ± 0.2 (SE) mmol $CaCO_3$ m^{-2} h^{-1} per unit change in Ω_a (Figure 7). The apparent uncoupling between calcification and Ω_a was observed to persist for 5 days after the nutrient enrichment was discontinued.

[39] The possibility of a competitive interaction between calcification and photosynthesis was investigated by plotting the rate of calcification against the rate of NP_C for the data from the CO_2 and nutrient enrichment experiments (Figure 8). The plot indicates that there was a negative interaction. In August and in January before the nutrient enrichment, CO_2 enrichment resulted in a decrease in calcification and increase in NP_C at a ratio of −0.7 to −0.8 moles of $CaCO_3$ m^{-2} h^{-1} to 1.0 moles of organic C m^{-2} h^{-1}. Following the nutrient enrichment this relationship broke down and NP_C increased with little or no change in calcification.

4. Discussion

4.1. Rates of Photosynthesis and Calcification Under Ambient Seawater Conditions

[40] The rates of net production (23–45 mmol C m^{-2} h^{-1}) and calcification (15–16 mmol $CaCO_3$ m^{-2} h^{-1}) of the corals in the flume in this study compare favorably with measurements made in the field under natural conditions. *Gattuso et al.* [1993] reported that midday rates of NP_C and G were 40–80 mmol C m^{-2} h^{-1} and 9–25 mmol $CaCO_3$ m^{-2} h^{-1}, respectively, on a reef flat in Moorea, French Polynesia. On Yonge Reef, in the northern end of the Great Barrier Reef, *Gattuso et al.* [1996] reported midday rates of NP_C and G of 47–111 mmol C m^{-2} h^{-1} and 8–27 mmol

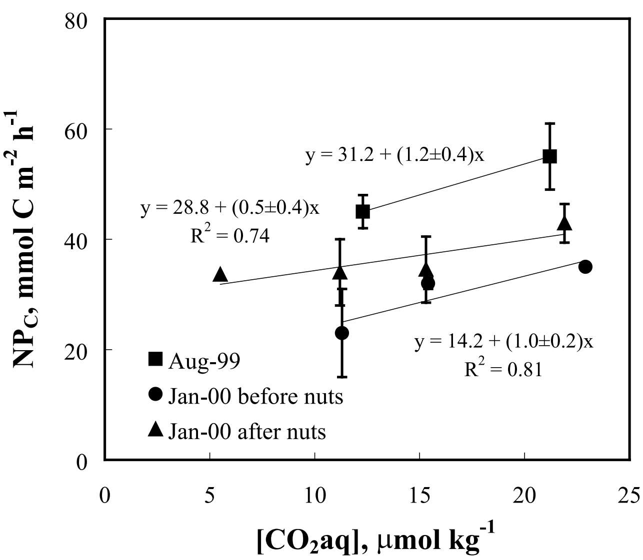

Figure 4. Effect of [CO_2aq] on NP_C in August and in January before and after nutrient enrichment. Uncertainty is SE.

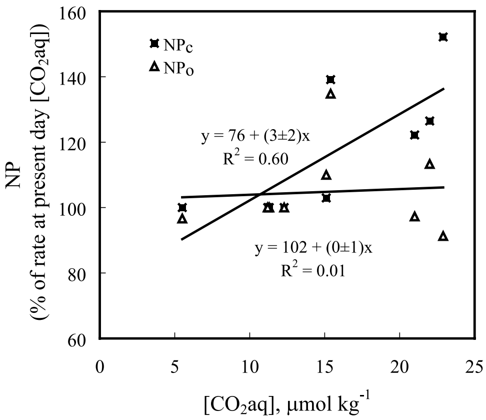

Figure 5. Effect of $[CO_2aq]$ on the rate of net production expressed as a percentage of the rate at present day $[CO_2aq]$. Uncertainty is SE.

$CaCO_3\ m^{-2}\ h^{-1}$, respectively. *Bates et al.* [2001] and *Ohde and van Woesik* [1999] have reported average daytime rates for reef flats in Bermuda and Okinawa of 12–17 mmol C $m^{-2}\ h^{-1}$ and 9–12 mmol $CaCO_3\ m^{-2}\ h^{-1}$ for NP_C and calcification, respectively. Perhaps most relevant to this study, *Atkinson and Grigg* [1984] observed similar summer and winter rates of NP_C at French Frigate Shoals located in the same chain of islands as the present study and containing a fairly similar community. Midday rates of NP_C averaged 51 mmol C $m^{-2}\ h^{-1}$ during the summer and 25 mmol C $m^{-2}\ h^{-1}$ during the winter, remarkably similar to the 45 and 23 mmol C $m^{-2}\ d^{-1}$ obtained in this study. We take this as evidence that the seasonal differences in net production that we observed in this study reflect real differences in the metabolism of the corals on the reef and were not an artifact of fact that we used two different assemblages of coral in our experiments. Midday calcification rates at French Frigate Shoals were similar in magnitude to the rates measured in this study but exhibited a larger decline in the winter, i.e., 23 mmol $CaCO_3\ m^{-2}\ h^{-1}$ during the summer and 6 mmol $CaCO_3\ m^{-2}\ h^{-1}$ during the winter compared to the results in this study of 16 mmol $CaCO_3\ m^{-2}\ h^{-1}$ in the summer and 15 mmol $CaCO_3\ m^{-2}\ h^{-1}$ during the winter.

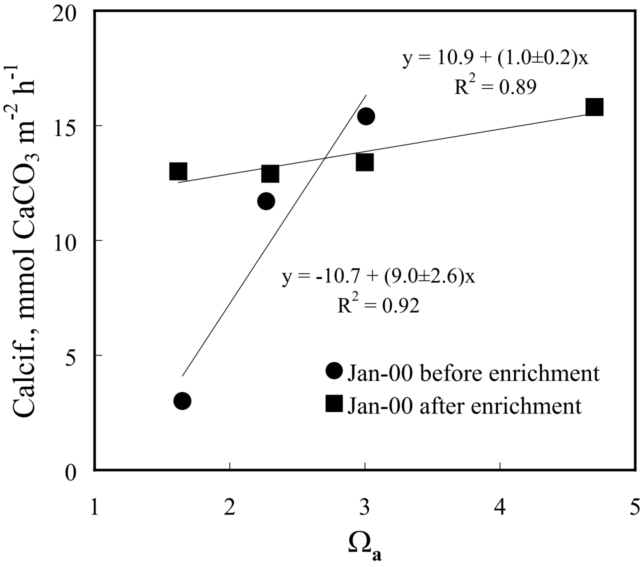

Figure 7. Impact of nutrient enrichment on the calcification-Ω_a relationship in January. Uncertainty is SE.

4.2. Effect of Current Speed on Photosynthesis and Calcification

[41] It is important to understand how current speed affects rates of coral photosynthesis and calcification both

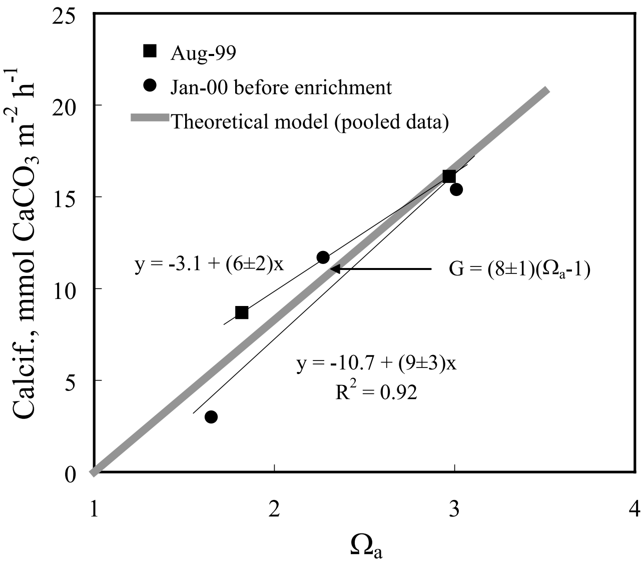

Figure 6. Effect of reduced aragonite saturation state (Ω_a) on the calcification rate of the coral assemblage in August 1999 and in January 2000 before the nutrient enrichment. The bold shaded line represents the fit of the theoretical rate law relationship (equation (1)) to the pooled data. Uncertainty is SE.

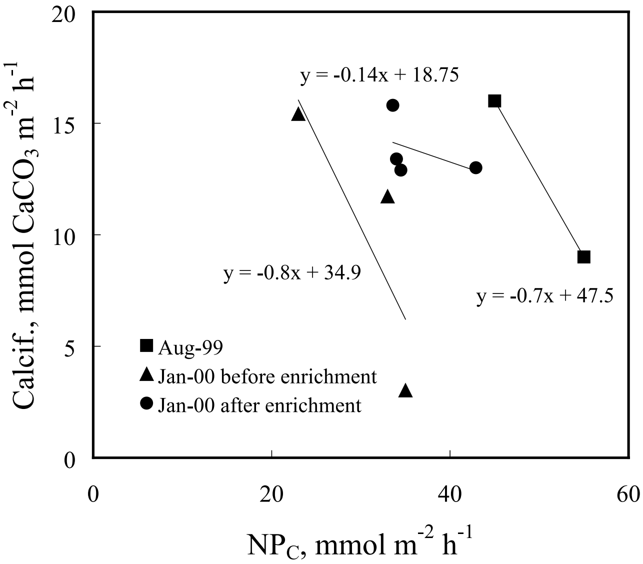

Figure 8. Calcification-NP_C property-property plot showing the negative interaction between net production and calcification when the corals were subjected to elevated pCO_2.

from the standpoint of designing reproducible experiments and for understanding its possible role in affecting carbon limitation in the field. Current speed affects the thickness of the diffusive boundary layer (DBL) at the interface between the coral and the ambient seawater. The thickness of the DBL is inversely related to flux of gaseous molecules and ions between the coral and the environment. If the current speed is slow enough the DBL can become thick enough to cause mass transport limitation of the supply of a critical building block or disposal of a toxic waste product. In this study we varied the current speed between 20 and 40 cm s^{-1} and observed that there was no significant effect on net production or calcification. Previously, *Atkinson et al.* [1994] varied current speed between 5.6 and 56.9 cm s^{-1} in the same flume and found no significant change in respiration or calcification of *P. compressa*. They did not look at net production. There is experimental evidence that current speed can limit net production of corals at very low current speeds such as may be encountered in sheltered environments. *Lesser et al.* [1994] placed colonies of *Pocillopora damicornis* in respirometry chambers and found that increasing current speed from 1 to 11 cm s^{-1} had a significant impact on NP_O causing it to increase from 38 to 56 mmol O_2 m^{-2} h^{-1}. Here we showed that current speeds in the range of 20–40 cm s^{-1} were sufficient to ensure that net production was not mass transport limited.

4.3. Response of Net Production to pCO_2, Temperature, Irradiance, and Nutrients

[42] In this study we report for the first time evidence that a manipulation of the carbonate chemistry of seawater designed to simulate the change that may happen in the next 50–100 years had a significant effect on the net carbon production of a coral assemblage. We found that NP_C increased at the rate of 3 ± 2% per 1 μmol kg^{-1} increase in [CO_2aq] (Figure 5). Previous studies by *Burris et al.* [1983] and *Goiran et al.* [1996] found that net production of the corals *Seriatopora hystrix*, *Stylophora pistilla* and *Galaxea fascicularis* did not increase with increased DIC. *Weis* [1993] showed that net production of the sea anemone *Aiptasia pulchella* increased up to a DIC of 5 mM. These three studies manipulated the carbonate chemistry in a very unrealistic way. They added $NaHCO_3$ and increased the DIC 2–5 times the present day level. In the natural setting we expect DIC to increase by only 7–10%. More recent studies have modified the carbonate chemistry more realistically and they found that increasing pCO_2 up to 658 or 918 μatm caused no significant increase in net production [*Leclercq et al.*, 2002; *Reynaud et al.*, 2003]. These studies only looked at NP_O. In this study we found that NP_O did not respond to elevated [CO_2aq] but that NP_C did. This result suggests that the photosynthetic quotient (PQ) defined as mol of O_2 evolved: mol of CO_2 fixed must decrease as [CO_2aq] is increased. The production of more carbohydrates at the expense of proteins and lipids could result in a decline in PQ. If the CO_2 enrichment stimulated photosynthesis but there was not a sufficient supply of nutrients you might expect such a shift.

[43] The increased rates of NP_C observed in this study could theoretically be due to increased availability of CO_2 or HCO_3^- both of which increased in these experiments. However, the increase in [HCO_3^-] is tiny, varying from 1691 to 1740 μmol kg^{-1} in the 1× through 1.8× runs. It seems unlikely that a 3% increase in [HCO_3^-] could be responsible for the 22% increase in NP_C observed in August or the 52% in January. It is more likely that the increase in NP_C was due to the twofold increase in [CO_2aq]. Perhaps under conditions of CO_2 enrichment the energy-costly CCM mechanism is deactivated resulting in usage of external CO_2 as it becomes more available relative to HCO_3^- [*Beardall et al.*, 1998]. The increased net production would result from the energy savings in not having to produce the enzymes required for the CCM mechanism.

4.4. Response of Calcification to pCO_2, Temperature, Irradiance, and Nutrients

[44] We found that calcification of the coral assemblage was responsive to a short-term change in the carbonate chemistry of the seawater (ΔpCO_2 increase of 329–386 μatm, $\Delta[CO_3^{2-}]$ decrease of 72–87 μmol kg^{-1} and ΔpH drop of 0.22–0.28 units) designed to mimic the change that may be experienced in the next 50–100 years if atmospheric pCO_2 doubles. This sensitivity was evident under summer (−44%) and winter conditions (−80%). Calcification was much more sensitive to change in pCO_2 than to seasonal change in temperature and irradiance (+4% increase from winter to summer at ambient pCO_2) and to a 10× loading of N and P (−16% at ambient pCO_2).

[45] The lack of change in calcification of the *P. compressa*/*M. capitata* assemblage despite a 3.8°C change in temperature at first seems surprising. It is known that the calcification rate of many coral species increases with increasing temperature up to a thermal optimum and then declines [*Coles and Jokiel*, 1978; *Jokiel and Coles*, 1977; *Kajiwara et al.*, 1995]. The results of *Coles and Jokiel* [1978] are particularly relevant because they studied *M. verrucosa* (*capitata*) and found that there was an interaction between light and the calcification-temperature relationship. At 40% of surface irradiance, calcification increased smoothly up to 28°C. However, at 70 and 100% of surface irradiance calcification peaked at 26°C. Looking at their curve of calcification versus temperature it can be seen that the rates at 23.4°C and 27.3°C are almost the same. If we had done experiments during the spring or fall we might have observed an effect of temperature on calcification. Another example of an interaction between temperature and CO_2 effects is the study of *Reynaud et al.* [2003] who found that elevated pCO_2 had no effect on calcification of *S. pistillata* at 25.3°C but did cause a strong decrease at 28.3°C. These results suggest that elevated temperature aggravates the sensitivity to CO_2 while in this study we found that sensitivity to CO_2 was similar across the normal seasonal range in temperature. Only more work will reveal which response is more typical of corals in general.

[46] The nutrient effect observed in this study (−16%) falls at the lower end of the range reported in the literature of −16% to −62% [*Ferrier-Pages et al.*, 2000; *Marubini and Atkinson*, 1999; *Marubini and Davies*, 1996; *Marubini and Thake*, 1999; *Stambler et al.*, 1991]. This may reflect the way the nutrients were administered. In the previous studies nutrient stock solutions were constantly pumped into running seawater aquaria to achieve a steady state concentration of 1–20 μM NO_3 or NH_4 and 2–3 μM PO_4. The treatments were typically maintained for 3–4 weeks. These

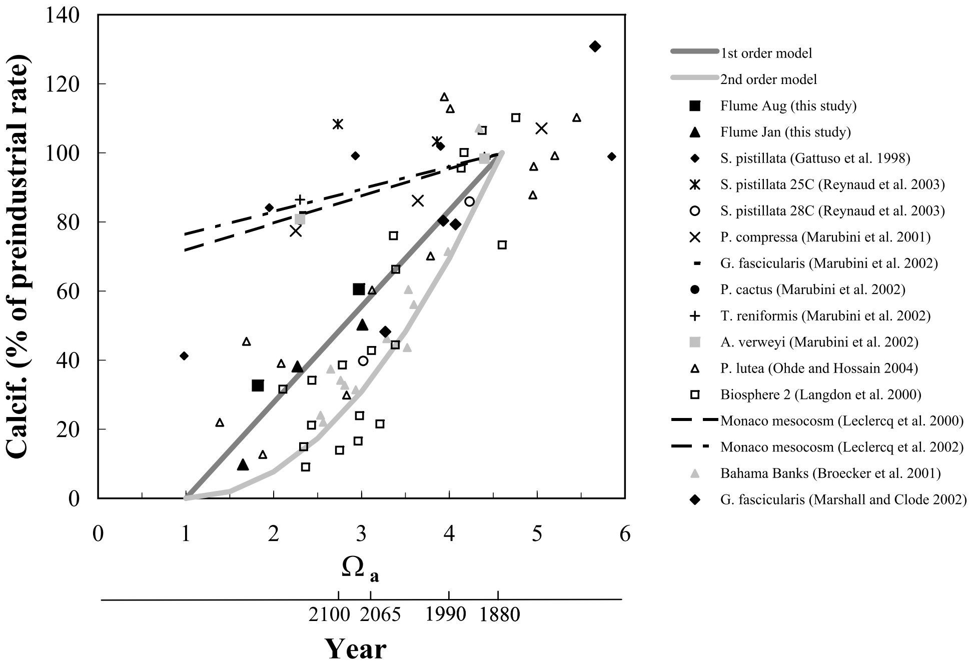

Figure 9. Effect of Ω_a on calcification rate expressed as a percentage of the preindustrial rate (Ω_a = 4.6). Data are from the present study and published studies on corals or coral reef communities. Bold shaded lines indicate the relationship predicted by the saturation state model (equation (1)) assuming the reaction is first or second order.

experiments characterize the response to chronic, high-level eutrophication, while, the present study reflects the response to episodic nutrient pulses such as might be experienced following a heavy rain and runoff event. We also found that nutrient enrichment caused an increase in the intercept and decrease in the slope of the calcification-Ω relationship (Figure 7), making the corals less sensitive to a change in Ω. This is contrary to the findings of *Marubini and Thake* [1999] who reported that nutrient enrichment aggravated the sensitivity of *P. porites* to change in Ω. It remains to be seen which response will be found to be more representative of corals in general.

[47] In this study we found that the saturation state model (equation (1)) did an excellent job explaining the response of calcification to change in seawater carbonate chemistry (Figure 6). Not only did a straight line through the data explain 87% of the variability but the *x* intercept (0.9 ± 0.3 SE) was not significantly different from 1. However, until a study is performed with corals where both [Ca^{2+}] and [CO_3^{2-}] are varied and the data are found to fit equation (1) we cannot consider that the saturation state hypothesis has been proven conclusively. At this point alternatives such as the competition hypothesis must also be considered. Photosynthesis and calcification both draw carbon from the same internal pool within the cytoplasm of the animal host cells. It has been suggested that the two processes may actually compete for carbon when demand exceeds supply [*Dubinsky et al.*, 1990; *Stambler et al.*, 1991]. We have looked for evidence of carbon competition between photosynthesis and calcification by plotting one against the other (Figure 8). We found that the ratio of the decrease in calcification to increase in NP_C was −0.7:1 in August and −0.8:1 in January. This is close to the −1:1 that would be expected if the two processes were competing for the same supply of carbon. The interpretation of the postnutrient enrichment results is that supplying nutrients somehow prevents the competition.

4.5. Projected Declines in Coral Calcification in the 21st Century

[48] It is of interest to use the data from this study and other published studies to make a tentative projection of how coral reef calcification may change as a consequence of the rise in atmospheric CO_2. It is important to recognize the limitations of these data at the outset. None of the studies take into account the possible synergistic effects of superoptimal temperature and elevated CO_2. Nor do they take into account the effect of eutrophication because we do not have a good model of how nutrient fluxes to reefs may change in the future. Finally, the timescale of these studies ranges from one hour to one month. It is possible that corals that may possess the capability to adapt to elevated CO_2 if given more time.

[49] In order to facilitate comparison, the rates of calcification were expressed as a percentage of the extrapolated rate at a Ω_a of 4.6, the estimated saturation state of the tropical ocean in 1880 when atmospheric pCO_2 was 280 ppm [*Kleypas et al.*, 1999]. The normalized rates were then plotted as a function of Ω_a (Figure 9). The predicted relationship between calcification and saturation state based on the rate law used to explain the kinetics of chemical precipitation (equation (1)) is indicated on the figure for the cases where the reaction is first or second order. It can be seen that the data from the present study fall along the line predicted by the saturation state model assuming a first-order relationship (n = 1). Data from the studies of *Langdon et al.* [2000], *Broecker et al.* [2001] and *Reynaud et al.* [2003] cluster within the space defined by the two curves

describing the predicted first- and second-order relationships. These data predict a decline in coral and coral reef calcification of 60% (range 40–83%) by the year 2065. There is a second cluster of data from the studies of *Gattuso et al.* [1998], *Leclercq et al.* [2000], *Marubini et al.* [2001, 2002] and *Reynaud et al.* [2003] that fall along a line that describes a much smaller sensitivity to change in Ω_a. These data predict a decline of only 1–18% by 2065.

[50] The reason for the large difference between the low- and high-sensitivity data sets is not known. The species in the upper cluster may have evolved a mechanism that makes them less sensitive to changes in saturation state or to low pH and $[CO_3^{2-}]$. However, we also need to consider the possibility that the differences between the two clusters of data are a result of how the experiments were performed. The study of CO_2 effects on coral calcification is a new field. Methods have varied from study to study and intercomparisons of methodologies have not yet been performed. Three examples illustrate the problem. Data for *P. compressa* fall in the low-sensitivity group in one study [*Marubini et al.*, 2001] and in the high-sensitivity group in another (this study). Differences include the method of measuring calcification (buoyant weight versus TA change) and the duration of the treatments (4 weeks versus 1.5 hours). Treatment duration may be an issue, however, the data from the present study based on 1.5 hour exposure to reduced saturation state cluster with the data from two studies where the exposure to reduced saturation state was held for months [*Broecker et al.*, 2001; *Langdon et al.*, 2000]. The second example is from the study of *Reynaud et al.* [2003] who found that data for *S. pistillata* fell in the low-sensitivity cluster at 25°C but in the high-sensitivity cluster at 28°C indicating that temperature effects should not be ignored. The third example is the study by *Gattuso et al.* [1998] that varied Ω_a by manipulating $[Ca^{2+}]$ and not the carbonate chemistry. Their data for *S. pistillata* fall in the low-sensitivity cluster. Was this because the study was performed at 27°C or because *S. pistillata* or corals in general are not sensitive to changes in Ω_a per se but to some aspect of the change in carbonate chemistry associated with the rise in pCO_2? To resolve these questions we need studies that compare the buoyant weight and TA change methods of measuring calcification, studies that look the interaction of temperature and CO_2 sensitivity, studies that test the saturation state hypothesis by manipulating both $[Ca^{2+}]$ and $[CO_3^{2-}]$, and finally studies that look at how the sensitivity to elevated pCO_2 changes with time of exposure.

5. Conclusion

[51] A short-term, 1.7- to 2.0-fold increase in pCO_2 caused a 22–52% increase in NP_C and a 44–80% decrease in calcification of a *P. compressa*/*M. capitata* assemblage in an outdoor flume. A short-term 10× nutrient loading of N and P had a comparable impact on NP_C (+48%) but a lesser impact on calcification (−16%). The winter to summer change in temperature (3.8°C) and irradiance (19–37 mol quanta m^{-2} d^{-1}) had a greater impact on NP_C (+96%) and a slight positive impact on calcification (+4%). We conclude that the impact of a doubling of pCO_2 is quite significant compared to the other environmental factors that affect coral calcification. There was minimal interaction between CO_2 effects and normal seasonal change in temperature and irradiance. The intercept of the NP_C-CO_2aq relationship changed from summer to winter but the slope of the relationship hardly changed. Neither the intercept nor slope of the calcification-Ω_a relationship changed significantly from summer to winter. The interaction between CO_2 and nutrient enrichment was quite different. Nutrient enrichment had the effect of making NP_C and calcification less sensitive to change in $[CO_2aq]$ and Ω_a, respectively, and somehow resulted in substantially higher rates of NP_C and calcification at CO_2aq and Ω_a levels that were formerly limiting. The mechanism is not clear but it would appear that nutrient enrichment increases the supply of DIC to both photosynthesis and calcification.

[52] We demonstrated that the first-order saturation state model did an excellent job of explaining the effect of the elevated pCO_2 treatments on the calcification. However, we also found evidence to support the hypothesis that elevated pCO_2 stimulates photosynthesis resulting in a reduced supply of DIC to calcification. A challenge for the future will be to combine what we know about the active and passive pathways of Ca^{2+} and HCO_3^-, the competing demands of photosynthesis and calcification for the internal pool of DIC and the evidence that calcification is controlled by the external concentrations of Ca^{2+} and CO_3^{2-} into a single unified theory of coral calcification. The future of coral reefs will also be shaped by thermal effects on calcification [*Coles And Jokiel*, 1978; *Houck et al.*, 1977; *Marshall and Clode*, 2004] and photosynthesis (see *Hoegh-Guldberg* [2000] for a review of thermally induced bleaching). Given that a future high-CO_2 world will bring a warmer and more acidic ocean it is clear that we need to know how the thermal and CO_2 effects will interact.

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M. J. Atkinson, Hawaii Institute of Marine Biology, 46-007 Lilipuna Road, Kaneohe, HI 96744, USA. (mja@hawaii.edu)

C. Langdon, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149, USA. (clangdon@rsmas.miami.edu)