The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals

S. J. de Putron[1], D. C. McCorkle[2], A. L. Cohen[2], A. Dillon[3]

[1] Bermuda Institute of Ocean Sciences, 17 Biological Lane, Ferry Reach, St, Georges, GE 01, Bermuda

[2] Woods Hole Oceanographic Institution, Woods Hole, MA, 02543

[3] Ecology and Evolutionary Biology Department, Princeton University, Princeton, NJ 08544

Communicating Author:

Samantha J. de Putron

Email: Samantha.deputron@bios.edu

Tel: + 441 297 1880 ext 724

Fax: + 441 297 8143

Keywords: Coral, Calcification, Ocean Acidification, recruitment, carbonate ion

Abstract

Rising concentrations of atmospheric CO_2 are changing the carbonate chemistry of the oceans, a process known as ocean acidification (OA). Absorption of this CO_2 by the surface oceans is increasing the amount of total dissolved inorganic carbon (DIC) and bicarbonate ion (HCO_3^-) available for marine calcification, yet is simultaneously lowering the seawater pH and carbonate ion concentration ($[CO_3^{2-}]$), and thus the saturation state of seawater with respect to aragonite (Ω_{ar}). We investigated the relative importance of $[HCO_3^-]$ versus $[CO_3^{2-}]$ for early calcification by new recruits (primary polyps settled from zooxanthellate larvae) of two tropical coral species, *Favia fragum* and *Porites astreoides*. The polyps were reared over a range of Ω_{ar} values, which were manipulated by both acid-addition at constant pCO_2 (decreased total $[HCO_3^-]$ and $[CO_3^{2-}]$) and by pCO_2 elevation at constant alkalinity (increased $[HCO_3^-]$, decreased $[CO_3^{2-}]$). Calcification after two weeks was quantified by weighing the complete skeleton (corallite) accreted by each polyp over the course of the experiment. Both species exhibited the same negative response to decreasing $[CO_3^{2-}]$ whether Ω_{ar} was lowered by acid-addition or by pCO_2 elevation - calcification did not follow total DIC or $[HCO_3^-]$. Nevertheless, the calcification response to decreasing $[CO_3^{2-}]$ was non-linear. A statistically significant decrease in calcification was only detected between Ω_{ar} = < 2.5 and Ω_{ar} = 1.1 – 1.5, where calcification of new recruits was reduced by 22 – 37 % per 1.0 decrease in Ω_{ar}. Our results differ from many previous studies that report a linear coral calcification response to OA, and from those showing that calcification increases with increasing $[HCO_3^-]$. Clearly, the coral calcification response to OA is variable and complex. A deeper understanding of the biomineralization mechanisms and environmental conditions underlying these

variable responses is needed to support informed predictions about future OA impacts on corals and coral reefs.

Introduction

Rising concentrations of atmospheric carbon dioxide (CO_2) are lowering the carbonate concentration ($[CO_3^{2-}]$), pH, and aragonite saturation state (Ω_{ar}) of the surface ocean (Orr et al. 2005; Bates 2007). There is mounting concern about the potential impact of this ocean acidification on the ability of tropical reef-building corals to form their $CaCO_3$ (aragonite) skeletons (Gattuso et al. 1999; Kleypas et al. 1999). Laboratory experiments on coral colonies and mesocosm experiments on coral communities often, but not always, show a decrease in calcification in response to decreasing seawater $[CO_3^{2-}]$ and Ω_{ar} (e.g. Langdon and Atkinson 2005). However, the sensitivity and magnitude of this response is variable, and it is not yet clear whether this variability reflects inter-species differences in calcification mechanisms (e.g., control of the chemistry of the seawater-like fluid between the basal epithelial cells and the skeletal surface, hereafter called the calcifying fluid**);** interactions amongst saturation state and other variables such as nutrients; variations in experimental design (e.g., pCO_2 manipulation versus acid-addition); or in the methods used to measure calcification. Addressing the question of variability in coral responses to ocean acidification experiments is crucial if we are to understand and predict the biological consequences of anthropogenic-induced CO_2 increases over the next few decades.

Since increased atmospheric CO_2 raises both DIC and HCO_3^- concentrations in surface oceans even as $[CO_3^{2-}]$ decreases, experiments using pCO_2 enrichment are thought to mimic real world ocean acidification more accurately than acid-addition experiments, in which solution DIC

stays constant or decreases as [CO_3^{2-}] decreases. This distinction can be important if corals calcify by modifying the chemistry of the calcifying fluid to raise its saturation state; in that case, the maximum [CO_3^{2-}] that can be attained in the calcifying fluid may be limited by the DIC initially present in the solution (Cohen et al. 2009). At least one study has reported that coral calcification responds to [HCO_3^-] and not to [CO_3^{2-}] (Jury et al. 2010). In several other studies, coral calcification rates were observed to be positively correlated with increased [HCO_3^-] (Marubini and Thake 1999; Schneider and Erez 2006; Marubini et al. 2008). However, in contrast to the Jury et al. (2010) experiments, in most of these studies [CO_3^{2-}] increased at the same time as [HCO_3^-] so that the influence of bicarbonate on coral calcification cannot be separated from parallel changes in carbonate ion (see Holcomb et al. 2010 for a review). In the one case where [HCO_3^-] and [CO_3^{2-}] did not covary (the constant DIC experiment of Schneider and Erez, 2006), calcification rate followed [CO_3^{2-}] not [HCO_3^-].

Here, we compared the calcification response of two tropical coral species, *Favia fragum* and *Porites astreoides*, to a range of seawater saturation states, manipulated by both acid-addition and pCO_2 elevation, to assess the relative importance of changes in [CO_3^{2-}] and [HCO_3^-] in coral calcification. Our experiments were conducted on primary polyps (new recruits or spat) settled from non-calcifying larvae within experimentally manipulated seawater conditions. This approach ensures that all skeletal accretion (calcification) occurs under the experimental conditions. Further, by removing the polyp tissue and weighing discrete corallites of individual spat, a direct measure of calcification under ocean acidification conditions is obtained. Few previous studies have examined the effects of ocean acidification on primary polyps of corals. Albright et al. (2008) reared recruits of *P. astreoides* over a month in seawater manipulated with acid-addition and concluded that Ω_{ar} had no significant effect on settlement rates; however, they observed a linear negative correlation between declining Ω_{ar} and corallite size as measured through the live tissue.

Primary polyps of *F. fragum* reared for 8 days in seawater manipulated with acid-addition also showed a reduction in the size and weight of the primary corallite with decreasing Ω_{ar} (Cohen et al. 2009).

Methods

Larval collection and settlement

Mature colonies of the brooding corals *F. fragum* and *P. astreoides* were collected from inshore patch reefs in Bermuda just prior to their predicted time of larval release in July 2007 (*F. fragum*), August 2007 (*P. astreoides*), and July 2008 (both species). Colonies were maintained at the Bermuda Institute of Ocean Sciences (BIOS) in outdoor flow-through seawater aquaria under near-ambient temperature and light conditions, and were held in either jars or mesh bags of aerated seawater during the nights of release to isolate the larvae. Zooxanthellate larvae were collected daily as they were released by the adults and settled on preconditioned tiles in small (0.5 L) plastic containers of seawater at the saturation state of each experimental aquarium (see below). Tiles were preconditioned by leaving racks of tiles on nearby reefs for 4-6 weeks, allowing them to obtain the biofilms and algae needed to facilitate larval settlement. After a settlement period of 24-48 h, the tiles containing metamorphosed primary polyps were transferred to the experimental aquaria. The polyps were grown for two weeks, after which the polyp tissue was removed by bleaching to reveal the underlying corallite. The skeleton of each polyp was removed from the tile and individually weighed using a micro balance (Cohen et al. 2009). Since all skeletal carbonate retrieved from the experiments was formed under the experimental conditions, total corallite

weight provides a direct measure of the amount of calcification ($CaCO_3$ production) achieved by each polyp under the different experimental conditions. For statistical analysis, corallite weight data were square root transformed to meet assumptions and were analyzed using One-Way ANOVA followed by Multiple Comparison of Means TK, GT2, T' tests (BIOMstat33).

Experimental conditions

Glass-lidded aquaria (30 L) containing reef seawater (static, not flow-through) were pre-adjusted to a range of seawater saturation states (Table 1). In 2007, the aquarium seawater alkalinity was decreased by addition of 1.0 N HCl (0, 17, 38, and 64 mL per aquarium for the four treatments) two days prior to the start of the experiment, and each aquarium was bubbled with lab air for the duration of the experiment. The average seawater pCO_2 during the 2007 experiment, calculated from alkalinity and DIC, was approximately 450 ppmv, reflecting the elevated CO_2 of air inside the lab. In 2008, the aquarium seawater pCO_2 and DIC levels were set by continuous and direct bubbling via a micropore bubble 'wand' into each aquarium with air from a compressor room separate from the lab, and with air+CO_2 mixtures produced with pairs of mass flow controllers. The composition of the bubbling gas mixtures in 2008 was monitored daily using a Qubit infra-red CO_2 analyzer and mean ppmv ± SD were: 394 ± 9 (ambient air; control), 753 ± 12 (mid CO_2), and 2327 ± 23 (high CO_2). The bubbling rates were set to insure that the water in each tank stayed well mixed and flowed actively over the corals. The seawater temperature in all aquaria in each experiment was monitored every half an hour using Hobo temperature loggers (Onset Corp.) Average seawater temperatures for the two week period were: 25 °C ± 0.5 (mean ± SD) for 2007 *F. fragum*; 28.5 ± 0.2 for 2007 *P. astreoides*; and 29.4 ± 1.3 for both species in 2008. The polyps were not fed during the two week experiments (apart from particulate matter initially

present in the aquaria), and were kept on a 12/12 hr light-dark cycle with the maximum light levels achievable with the aquarium lights: mean (± SD) of 61 ± 6 μmol m^{-2} s^{-1}.

The chemical conditions for all treatments in each experiment are summarized in Table 1. Salinity was determined with an Autosal salinometer. Discrete water samples for analysis of salinity, alkalinity (Alk), and dissolved inorganic carbon (DIC) were collected weekly (at the beginning, mid-point, and end of each experiment); the Alk/DIC samples were poisoned with mercuric chloride immediately after collection. Alk and DIC were measured using a closed cell titration with non-linear curve fitting on ~100 mL samples, standardized using certified reference materials obtained from Dr. A. Dickson (SIO). In between these discrete sampling points, the pH(NBS) of each tank was monitored every one to three days using an Orion pH meter and temperature-compensated electrode, and a single high-resolution pH monitoring test (every 6 hours for 1.5 days) was carried out to assess the possibility of short-term variations in carbonate chemistry. The carbonate chemistry of each tank was stable - variations in pH(NBS) within treatments (on both sub-weekly and sub-daily time scales) were always small (± a few hundredths of a pH unit) relative to the pH differences between treatments (tenths of a pH unit). The discrete sample seawater temperature, salinity, Alk, and DIC data were used to calculate other carbonate system parameters ($[HCO_3^-]$, $[CO_3^{2-}]$ and Ω), using a spreadsheet version of the CO2SYS program of Lewis and Wallace (1998), with the dissociation constants of Roy et al. (1993) and the aragonite solubility of Mucci (1983). The precision of the titrations was ± 0.2 % for both alkalinity and DIC in ambient seawater, but only ± 0.6 % and ± 1.7 %, respectively, in the most strongly acidified treatment. This resulted in an analytical uncertainty in calculated saturation state of roughly ± 0.5 % at ambient conditions and ± 16 % in the lowest Ω treatment.

Results

We observed a significant negative response of early calcification (measured as corallite weight – the mass of skeleton accreted per polyp in 14 days) to decreased saturation state (Ω_{ar}), for new recruits of both *F. fragum* (Fig. 1a) and *P. astreoides* (Fig. 1b, ANOVA's, $P<0.001$). The sensitivity of skeletal growth to changes in Ω_{ar} was the same whether Ω_{ar} was manipulated by open system acid-addition (dashed lines in Fig. 1a, b) or pCO_2 elevation (solid lines in Fig. 1a, b) over the comparable range of Ω_{ar}. However, calcification did not decrease linearly with declining saturation state (Fig. 1). Multiple comparison of means analysis after the significant ANOVA results showed that corallite weights of polyps reared at ambient saturation state (Ω_{ar} = ~3.8 - 4.2) were not significantly different from those reared at the next treatment level (Ω_{ar} = 2.5 for acid-addition experiments and Ω_{ar} = 2.8 for pCO_2 elevation experiments, $P>0.05$). Rather, a significant effect of changing Ω_{ar} on corallite weight was observed only between Ω_{ar} = 2.5/2.8 and the next treatment level (Ω_{ar} = <1.5).In the acid-addition experiments (dashed lines, Figure 1), the polyps reared at $\Omega_{ar} \leq 1.1$ and below for *F. fragum* (Fig. 1a), and at $\Omega_{ar} \leq 1.2$ and below for *P. astreoides* (Fig. 1b) weighed significantly less than those reared at Ω_{ar} = 2.5 and above ($P<0.05$). *F. fragum* declined 51 % and *P. astreoides* declined 29 % between these treatments, which equates to a decline of 37 % and 22 % respectively per 1.0 decrease in Ω_{ar}. A similar result was observed for both species when saturation state was lowered by pCO_2 elevation (solid line, Fig. 1): polyps reared at the lowest saturation state (Ω_{ar} = 1.4 for *F. fragum*, and Ω_{ar} = 1.5 for *P. astreoides*) weighed significantly less than polyps reared at Ω_{ar} = 2.8 and above ($P<0.05$). There was a 37 %

(*F. fragum*) and 36 % (*P. astreoides*) decline in corallite weight between these treatments, which equates to 26 % decline in corallite weight per 1.0 decrease in Ω_{ar} for both species.

The actual skeletal masses for *F. fragum* were similar in both the acid-addition and pCO_2 elevation experiments (Fig. 1a). In contrast, the 2008 *P. astreoides* weights (pCO_2 experiment) were approximately 20 % lower than the 2007 weights (acid-addition experiment) at the same omega value (note different y axes, Fig. 1b). Despite the difference in mean weight between the populations, the sensitivity of calcification to changing Ω_{ar} was practically identical.

In both the acid-addition (at constant pCO_2) and pCO_2 elevation experiments, $[CO_3^{2-}]$ is linearly correlated with Ω_{ar} (Fig. 2a). In contrast, $[HCO_3^-]$ decreased in response to acid-addition but increased in response to pCO_2 elevation (Fig. 2b). Like $[HCO_3^-]$, DIC decreased as Ω_{ar} was lowered by acid-addition, and increased as Ω_{ar} was lowered by pCO_2 elevation (Table 1). We observed that calcification (corallite weight) in both *F. fragum* and *P. astreoides* was positively correlated with $[HCO_3^-]$ (and DIC) only in the acid-addition experiments, where $[HCO_3^-]$ and $[CO_3^{2-}]$ co-vary; conversely, calcification was negatively correlated with $[HCO_3^-]$ (and DIC) in the pCO_2 elevation experiments, where $[HCO_3^-]$ and $[CO_3^{2-}]$ are anticorrelated (*F. fragum,* Fig. 3a; *P. astreoides*, Fig. 3b).

Discussion

Calcification by new recruits of two tropical coral species, *F. fragum* and *P. astreoides*, showed the same negative response to decreasing $[CO_3^{2-}]$ whether Ω_{ar} was lowered by acid-addition (which also lowered both DIC and $[HCO_3^-]$) or by pCO_2 elevation (which raised both DIC

and [HCO_3^-]). The experiments were conducted over two summers with different parent colonies providing the larvae. Thus, natural variability in the larvae of this species may explain the different starting weights of the *P. astreoides* spat in the the acid-addition and pCO_2 elevation experiments. The mean seawater temperature in the experimental aquaria also varied between the two years – by ~1 °C for *P. astreoides* (28.5°C for acid-addition, 29.4°C for pCO_2 elevation) and by ~4 °C for *F. fragum* (25 °C for acid-addition and 29.4 °C for the pCO_2 elevation experiment). Prior studies have shown that simultaneous elevation of temperature and pCO_2 may exacerbate (e.g., Reynaud et al. 2003) or reduce (e.g., Anthony et al. 2008) the impact of pCO_2 alone on calcification over a certain temperature and pCO_2 range. Conversely, Rodolfo-Metalpa et al. (2010) showed that elevation of temperature had no effect on the calcification response of the temperate coral *Cladocora caespitosa* to elevated pCO_2. We find no evidence that temperature influenced the response of *F. fragum* to decreased Ω_{ar} in the acid-addition versus pCO_2 elevation experiments. Absolute calcification rates at a given Ω_{ar} for this species were similar in the acid-addition (25 °C) and pCO_2 elevation (29.4 °C) experiments. Conversely, the temperature differences in the experiments with *P. astreoides* was only ~1 °C yet these populations did exhibit a difference in mean calcification rates at a given Ω_{ar}. Despite the difference in *P. astreoides* polyp size between the cohorts, and the temperature difference between the two *F. fragum* experiments, the response of each species to changing Ω_{ar} was the same each year and for both coral species- calcification (corallite weight) followed Ω_{ar} (i.e., [CO_3^{2-}]), and was not positively influenced by elevated [HCO_3^-].

To our knowledge, only two prior studies have examined the relative influence of [HCO_3^-] and [CO_3^{2-}] on coral calcification in experiments where these two carbonate parameters were not themselves positively correlated. In a set of constant-DIC experiments, Schneider and Erez (2006) observed that calcification by *Acropora eurystoma* was positively correlated with [CO_3^{2-}], and

inversely correlated with [HCO_3^-]. In contrast, pCO_2 enrichment experiments by Jury et al. (2010) showed that calcification by *Madracis auretenra* was more closely linked to [HCO_3^-] than to [CO_3^{2-}]. In the present study, calcification by new recruits of the corals *F. fragum* and *P. astreoides* was negatively correlated with [HCO_3^-] (and DIC) in the pCO_2 elevation experiments, where [HCO_3^-] and DIC increased and [CO_3^{2-}] decreased. Our results are in agreement with those of Schneider and Erez (2006), where Ω_{ar} or [CO_3^{2-}] exerted the strongest influence on calcification. Reasons for the discrepancy between our results and those of Scneider and Erez (2006) compared to Jury et al. (2010) remain unclear. Both Jury et al. (2010) and Schneider and Erez (2006) conducted short-duration alkalinity anomaly measurements that gave an estimate of calcification rate during the 1-2 hour measurement period. Conversely, our approach of measuring total skeletal weight provides an integrated estimate of calcification over the two-week experiment. Light levels in both Jury et al. (2010) and Schneider and Erez (2006) were relatively high (200 µmol m^{-2} s^{-1} and 350 µmol m^{-2} s^{-1} respectively) whereas those in our study were significantly lower; 61 µmol m^{-2} s^{-1}. However, at least one difference is that in studies where coral calcification was shown to increase with increasing [HCO_3^-] (Marubini and Thake 1999; Marubini et al., 2008, Jury et al, 2010), levels of total DIC were often significantly higher (~3500 to 3900 µmol/kg seawater) than those used here, and in the study of Schneider and Erez (2006). Perhaps corals reared in high-DIC, low-Ω_{ar} seawater are able to utilize the additional HCO_3^- ions for calcification, and thus compensate for reduced [CO_3^{2-}].

Coral nutritional status may also be an important factor in the coral response to elevated DIC. For example, corals in the Jury et al. (2010) study were fed twice-weekly and corals in the Scneider and Erez (2006) study were kept in situ in between incubations receiving natural food levels, while the new recruits in the present study were not fed and may have been energetically depleted for at least part of the two-week experiment. These nutrition-related differences may be

significant since increased nutrient availability or higher energy reserves in fed corals may enable them to utilize bicarbonate ions more efficiently than can unfed corals. For instance, Langdon and Atkinson (2005) found that corals reared under nutrient replete conditions were significantly less sensitive to decreased Ω_{ar} than corals reared under ambient nutrient conditions, and Holcomb et al. (2010) found that calcification by nutrient-replete corals reared under 780 ppm CO_2 was not statistically different from calcification under ambient CO_2.

The response of calcification to $[CO_3^{2-}]$ rather than to $[HCO_3^-]$ in this study raises an important question: if, in order to nucleate and grow aragonite crystals, corals elevate the Ω_{ar} (i.e., $[CO_3^{2-}]$) of the calcifying fluid at the site of calcification by converting aqueous CO_2 and bicarbonate to carbonate ions (e.g., Cohen and McConnaughey 2003; Allemand et al. 2004), then why is the initial $[CO_3^{2-}]$ of the external seawater so important in influencing the calcification outcome? The answer may lie in the energetic cost of converting bicarbonate and aqueous CO_2 to CO_3^{2-} (for example, by removing protons from the calcifying fluid). If the seawater in the calcifying space starts with elevated $[HCO_3^-]$ and lowered $[CO_3^{2-}]$, the coral must expend more energy to reach a given Ω_{ar} (Cohen and Holcomb 2009). Well-nourished corals may be able to invest this energy, and convert the elevated DIC and $[HCO_3^-]$ in CO_2-enriched water to $[CO_3^{2-}]$ for calcification; corals without adequate energetic reserves may be more sensitive to the $[CO_3^{2-}]$ of the ambient water. The influence of nutritional status may vary among coral species, and in different environmental settings.

Calcification by new recruits of *F. fragum* and *P. astreoides* reared under the experimental conditions of this particular study clearly responded to Ω_{ar} and not to $[HCO_3^-]$ or total DIC. However, the calcification response to Ω_{ar} was non-linear. A significant decrease in the amount of aragonite accreted after 14 days was detected only in the treatments with Ω_{ar} lower than 2.5 in the acid-addition experiment, and with Ω_{ar} lower than 2.8 in the pCO_2 elevation experiment. Although

the exact Ω_{ar} value at which calcification declined cannot be determined from our data, somewhere between Ω_{ar}= 2.8/2.5 and Ω_{ar} =1.4/1.1 a threshold Ω_{ar} exists, above which there was no significant change in calcification and below which, calcification declined sharply. Many previous studies that have reported a response in coral calcification to lowered saturation state documented a linear decline (see Langdon and Atkinson 2005 for review). Similarly, Albright et al. (2008) observed a linear decrease in the growth of new recruits of *P. astreoides* during the first month post-settlement. In their study, skeletal growth was estimated not by weight measurements of the primary corallite, but via surface area measurements of the skeleton as seen through the tissue of live spat, an approach that does not include the influences of vertical extension or of changes in skeleton density. However, many other experimental studies have reported a non-linear calcification response to changing Ω_{ar} or no response at all between ambient and Ω_{ar} ~1.5 – 2 (e.g., Gattuso et al. 1998; Ries et al. 2009, 2010; Reynaud et al. 2003 (only the 25 °C experiment); Holcomb et al. 2010; Houlbreque et al. 2010; Rodolfo-Metalpa et al. 2010). Field observations on coral growth rates across natural Ω_{ar} gradients also document a range of calcification responses to Ω_{ar}. For instance, Bates et al (2010) observed a strong correlation of *in situ* rates of calcification and Ω_{ar} in Bermuda. Manzello (2010) reported a species specific response in extension rates of corals growing in the Eastern Pacific along a natural Ω_{ar} gradient, with some species showing a decrease in growth with decreasing Ω_{ar}, others showing no response, and still other showing higher growth rates under lowered Ω_{ar}. The coral calcification response to omega may be intrinsically variable, or it may be that many factors influence calcification, and their relative importance varies depending on specific conditions in the field, or in laboratory experiments.

Although the calcification response to Ω_{ar} in this study was non-linear, a very strong negative response was observed below the threshold Ω_{ar}. Below this threshold, there was a 22 – 37 % decrease in the amount of aragonite accreted over 14 days per 1.0 decrease in Ω_{ar}. This is

substantially stronger than the average response of corals in the experiments summarized by Langdon and Atkinson (2005); and may reflect particular conditions in our experiments (e.g., lack of feeding), or may indicate a general tendency for early coral calcification to be more sensitive to decreases in Ω_{ar} once Ω_{ar} has dropped below some threshold. The observed range of coral responses to ocean acidification amongst published studies may reflect differences in other stressors or in growth conditions (e.g., light, nutrition) among both field and laboratory studies. Whatever its cause, this variation suggests that accurate predictions of how coral calcification will respond to ocean acidification will require a better understanding of the mechanisms and conditions that underlay these variable responses.

Acknowledgements

This study was supported by NSF award 0648157 (Cohen and McCorkle), NSF 1041106 (Cohen, McCorkle), NSF 1041052 (de Putron), the VITA foundation (de Putron), WHOI Ocean Life Institute (Cohen), PEI and EEB Departments at Princeton University, Bill and Anne Charrier, and the Anthony B. Evnin, Dean's Roundtable, and Edmund Hayes Sr. senior thesis funds (Dillon). We thank Kathryn Rose, Becky Belastock (WHOI), and Kascia White, Dustin Long, Katherine Yates and Julia Lawson (BIOS interns) for assistance with field and lab work. Helpful comments on the manuscript were provided by Michael Holcomb and two anonymous reviewers. This is BIOS contribution number 2009.

Reference List

Albright R, Mason B, Langdon C (2008) Effect of aragonite saturation state on settlement and post-settlement growth of *Porites astreoides* larvae. Coral Reefs 27:485-490

Allemand D, Ferrier-Pagès C, Furla P, Houlbrèque F, Puverel S, Reynaud S, Tambutté E, Tambutté S, Zoccola D (2004) Biomineralization in reef-building corals: from molecular mechanisms to environmental control. C R Palevol 3:453-467

Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc of the Nat Acad of Sci of the USA 105**:** 17442-17446

Bates NR (2007) Interannual variability of the oceanic CO_2 sink in the subtropical gyre of the North Atlantic Ocean over the last 2 decades. J Geophys Res 112, C09013 [doi:09010.01029/02006JC003759]

Bates NR, Amat A, Andersson, AJ (2010) Feedbacks and responses of coral calcification on the Bermuda reef system to seasonal changes in biological processes and ocean acidification. Biogeosciences 7: 1-22

Cohen AL, McConnaughey TA (2003) Geochemical perspectives on coral mineralization In: Dove PM, Weiner S, deYoreo JJ (eds) Biomineralization, Reviews in mineral geochemistry, vol 54. The Mineralogical Society of America, Washington, DC pp 151-187

Cohen AL, McCorkle DC, de Putron SJ, Gaetani GA, Rose KA (2009) Morphological and compositional changes in the skeletons of new coral recruits reared in acidified seawater: Insights into the biomineralization response to ocean acidification. Geochem Geophys Geosyst 10, Q07005 [doi:10.1029/2009GC002411]

Cohen AL, Holcomb M (2009) Why corals care about ocean acidification: uncovering the mechanism. Oceanography 22 (4): 118-127

Gattuso J-P, Frankignoulle M, Bourge I, Romaine S, Buddemeier RW (1998) Effect of calcium carbonate saturation of seawater on coral calcification. Global Planet Change 18:37-46

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

Herfort L, Thake B, Taubner I (2008) Bicarbonate stimulation of calcification and photosynthesis in two hermatypic corals. J Phycol 44:91-98

Holcomb M, McCorkle C, Cohen AL (2010) Long-term effects of nutrient and CO2 enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786). J Exp Mar Biol Ecol 386:27–33

Houlbreque F, Rodolfo-Metalpa R, Ferrier-Pages C, Boisson F, Al-Trabeen K, Oberhaensli F, Jeffree R (2010) Effects of increased pCO_2 on zinc bioaccumulation and calcification in the tropical coral *Stylophora pistillata*. Eos Trans. AGU, 91(26), Ocean Sci. Meet. Suppl., Abstract BO53A-04

Jury CP, Whitehead RF, Szmant AM (2010) Effects of variations in carbonate chemistry on the calcification rates of *Madracis auretenra* (= *Madracis mirabilis sensu* Wells, 1973): Bicarbonate concentrations best predict calcification rates. Global Change Biol [doi: 10.1111/j.1365-2486.2009.02057.x]

Kleypas JA, Buddemeier RW, Archer D, Gattuso JP, Langdon C, Opdyke BN (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science 284:118-120

Langdon C, Atkinson MJ (2005) Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J Geophy Res 110, C09S07 [doi:10.1029/2004JC002576]

Langdon C, Takahashi T, Sweeney C, Chipman D, Goddard J, Marubini F, Aceves H, Barnett H, Atkinson MJ (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. Global Biogeochem Cycles 14:639-654

Leclercq N, Gattuso JP, Jaubert J (2000) CO_2 partial pressure controls the calcification rate of a coral community. Global Change Biol 6:329-334

Lewis E, Wallace DWR (1998) Program developed for CO_2 system calculations. ORNL/CDIAC-105, Carbon Dioxide Inf Anal Cent Oak Ridge Natl Lab, US Dept of Energy, Oak Ridge, TN

Manzello DP (2010) Coral growth with thermal stress and ocean acidification: lessons from the eastern tropical Pacific. Coral Reefs 29: 749-758

Marubini F, Thake B (1999) Bicarbonate addition promotes coral growth. Limnol Oceanogr 44:716-720

Marubini F, Ferrier-Pages C, Furla P, Allemand D (2008) Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism. Coral Reefs 27:491-499

Mucci A (1983) The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. Am J Sci 283(7): 780-799

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A,Joos F,. Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G-K, Rodgers KB,. Sabine CL, Sarmiento JL, Schlitzer R,. Slater RD, Totterdel l IJ, Weirig M-F, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681-686

Reynaud S, Leclercq N, Romaine-Lioud S, Ferrier-Pagès C, Jaubert J, Gattuso JP (2003) Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. Global Change Biol 9:1660-1668

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology 37:1131-1134

Ries JB, Cohen AL, McCorkle DC (2010) The temperate coral *Oculina arbuscula* exhibits a non-linear, threshold calcification response to *p*CO2-induced ocean acidification. Coral Reefs Doi: 10.1007/s00338-010-0632-3

Rodolfo-Metalpa R, Martin S, Ferrier-Pagès C, Gattuso J-P (2010) Response of the temperate coral *Cladocora caespitosa* to mid- and long-term exposure to pCO_2 and temperature levels projected for the year 2100 AD. Biogeosciences 7:289-300

Roy RN, Roy LN, Vogel KM, Portermoore C, Pearson T, Good CE, Millero FJ, Campbell DM (1993) The dissociation-constants of carbonic-acid in seawater at salinities 5 to 45 and temperatures 0 °C to 45 °C. Mar Chem 44:249-267

Schneider K, Erez J (2006) The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. Limnol Oceanogr 51:1284-1293

Figure Legends

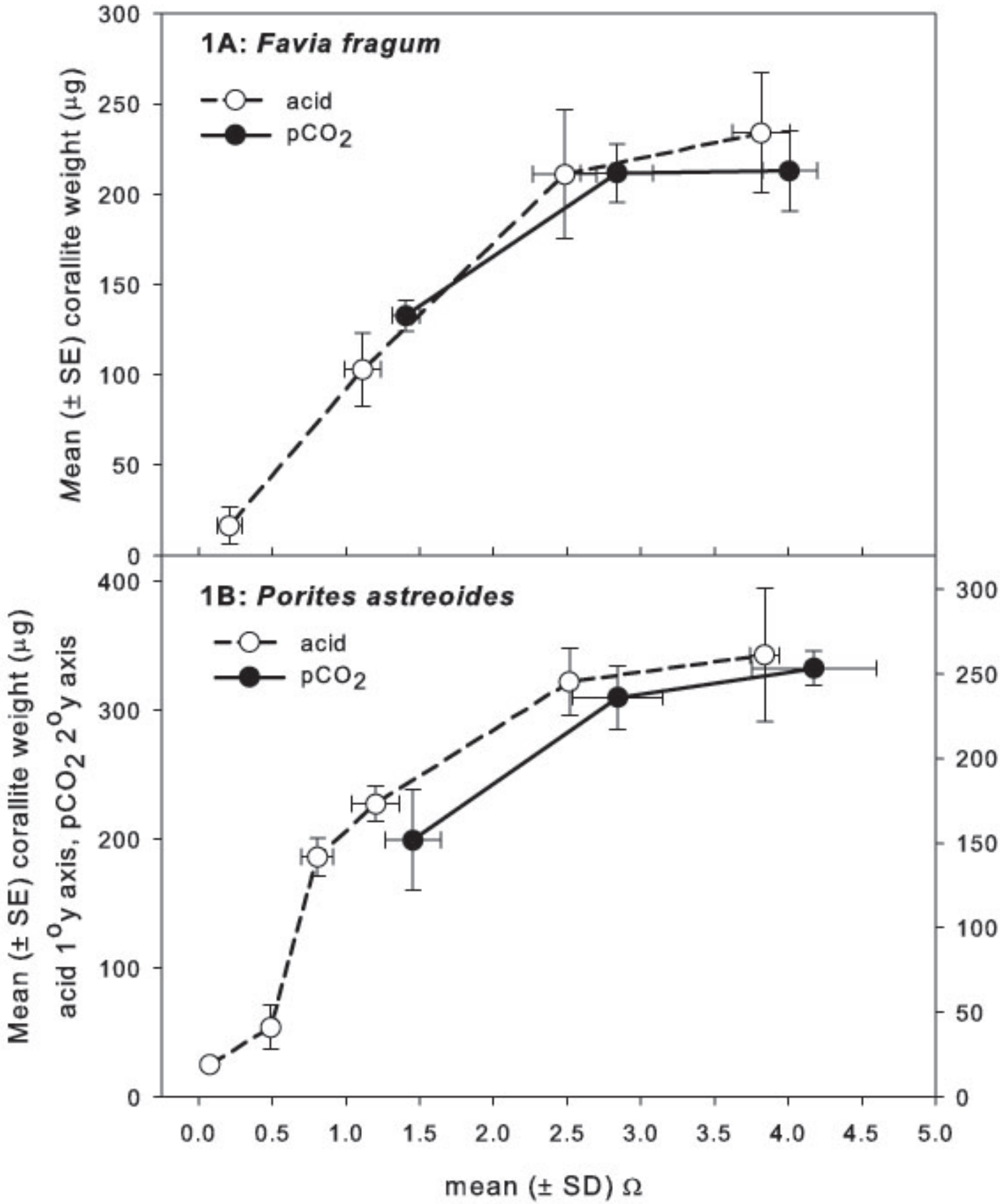
Table 1: Mean seawater chemistry conditions for each treatment in the acid-addition and pCO_2 elevation experiment. See text for the analytical procedures.

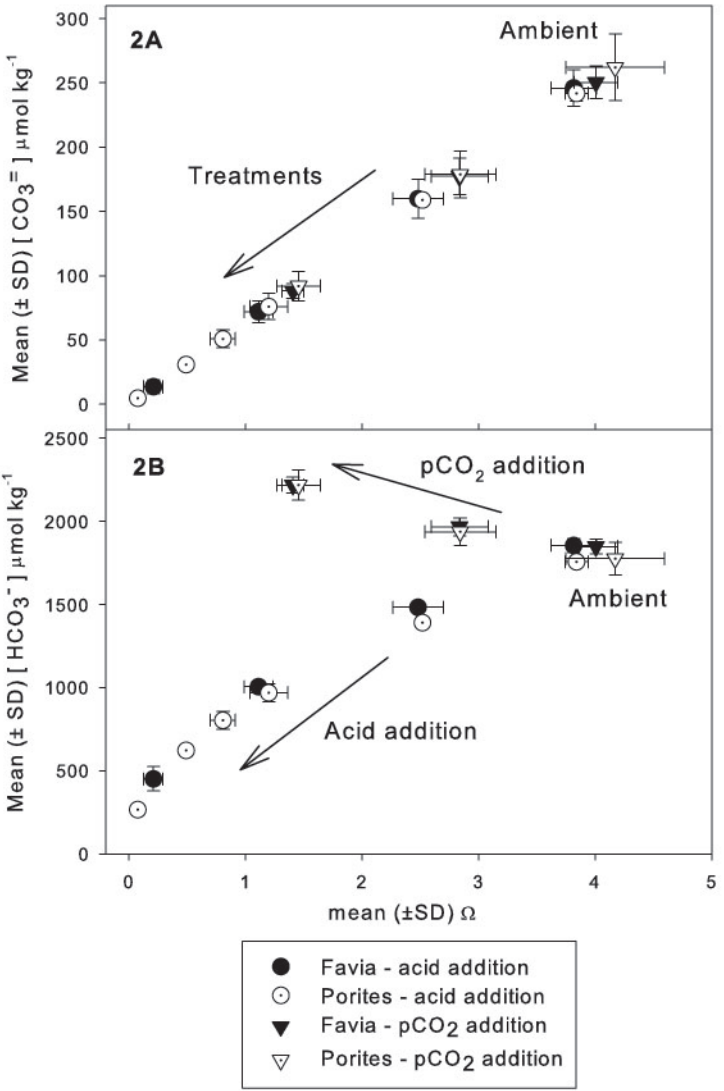
Figure 1: Mean (±SE) corallite weight of 2-week old *Favia fragum* (1A) and *Porites astreoides* (1B) plotted as a function of mean (±SD) aragonite saturation state (Ω), for the 2007 acid-addition experiments (open circles, dashed line) and the 2008 pCO_2 elevation experiments (closed circles, solid line). Calcification declines with decreasing Ω.

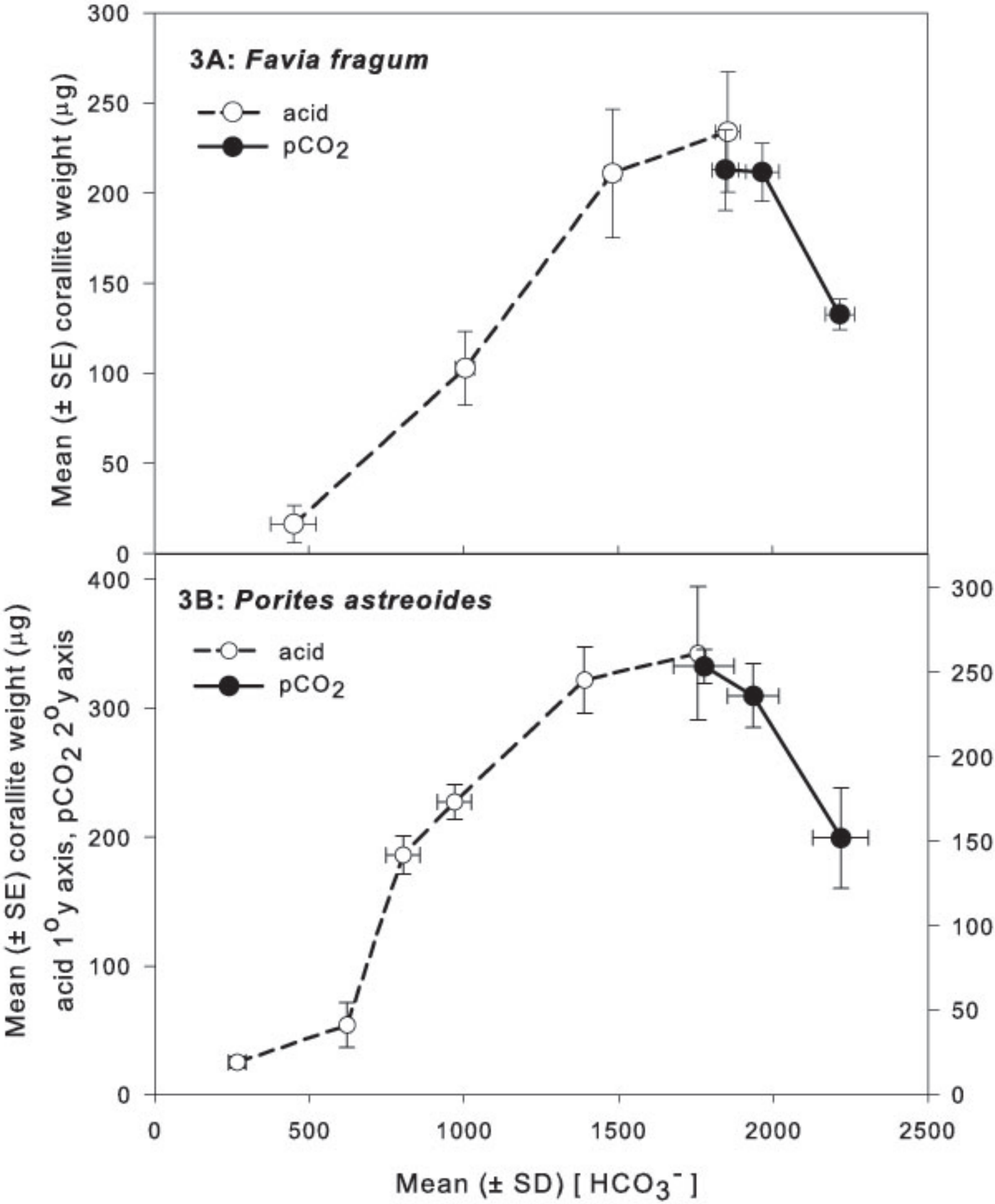
Figure 2: 2A, Mean (±SD) carbonate ion concentration ($[CO_3^{2-}]$) plotted against mean (±SD) aragonite saturation state (Ω) shows a linear correlation in both acid-addition and pCO_2 elevation experiments. 2B, Mean (±SD) bicarbonate ion concentration ($[HCO_3^-]$) plotted against Ω showing a decrease in $[HCO_3^-]$when Ω is lowered by acid-addition, and an increase when Ω is lowered by pCO_2 addition.

Figure 3: Mean (±SE) corallite weight of 2-week old *Favia fragum* (3A) and *Porites astreoides* (3B) (same data as Figure 1) plotted as a function of mean (±SD) bicarbonate ion concentration ($[HCO_3^-]$). Calcification rate is not controlled by $[HCO_3^-]$.

Experiment	Species	Date	Treatment	Salinity (psu ± SD)	Alkalinity (ueq/kg ± SD)	DIC (µmol/kg ± SD)	pH (NBS ± SD)	HCO3- (µmol/kg ± SD)	CO32- (µmol/kg ± SD)	omega (± SD)
Acid-addition	*F. fragum*	Jul-07	Control (1)	37.9 ± 1.0	2451 ± 73	2113 ± 54	8.16 ± 0.01	1855 ± 41	246 ± 14	3.82 ± 0.2
			2	37.9 ± 1.0	1890 ± 64	1655 ± 41	8.07 ± 0.02	1483 ± 27	160 ± 15	2.48 ± 0.2
			3	38.1 ± 1.2	1212 ± 53	1091 ± 39	7.88 ± 0.03	1006 ± 31	72 ± 9	1.11 ± 0.1
			4	38.0 ± 1.1	506 ± 91	479 ± 78	7.48 ± 0.1	452 ± 74	14 ± 5	0.21 ± 0.1
	P. astreoides	Aug-07	Control (1)	36.9 ± 0.1	2344 ± 2	2010 ± 9	8.14 ± 0.01	1757 ± 14	242± 6	3.84 ± 0.1
			2	37.3 ± 0.2	1797 ± 8	1560 ± 9	8.05 ± 0.0	1389 ± 8	159 ± 0	2.52 ± 0.01
			3	37.5 ± 0.3	1185 ± 79	1056 ± 64	7.88 ± 0.04	969 ± 55	76 ± 10	1.20 ± 0.2
			4	37.2 ± 0.1	958 ± 71	866 ± 62	7.79 ± 0.04	803 ± 55	51 ± 7	0.81 ± 0.1
			5	37.3 ± 0.2	726 ± 15	664 ± 13	7.69 ± 0.02	622 ± 12	31 ± 1	0.49 ± 0.02
			6	36.3 ± 0.2	291 ± 31	284 ± 29	7.24 ± 0.05	266 ± 28	5 ± 1	0.07 ± 0.02
pCO_2 addition	*F. fragum*	Jul-08	Control	37.0 ± 1.0	2449 ± 54	2110 ± 47	8.11 ± 0.03	1847 ± 44	250 ± 13	4.01 ± 0.19
			Mid CO_2	37.0 ± 0.5	2393 ± 40	2165 ± 47	7.93 ± 0.03	1966 ± 53	177 ± 14	2.84 ± 0.25
			High CO_2	37.3 ± 0.8	2429 ± 54	2359 ± 52	7.58 ± 0.02	2218 ± 47	88 ± 6	1.41 ± 0.09
	P.astreoides	Jul-08	Control	37.5 ± 0.6	2411 ± 119	2050 ± 106	8.14 ± 0.04	1776 ± 98	262 ± 26	4.17 ± 0.42
			Mid CO_2	37.7 ± 0.6	2369 ± 61	2135 ± 74	7.94 ± 0.04	1936 ± 83	179 ± 18	2.84 ± 0.3
			High CO_2	38.0 ± 0.8	2439 ± 90	2362 ± 93	7.58 ± 0.05	2218 ± 89	92 ± 11	1.46 ± 0.19


1A: Favia fragum
acid
pCO2
Mean (± SE) corallite weight (µg)
300
250
200
150
100
50
0
1B: Porites astreoides
acid
pCO2
Mean (± SE) corallite weight (µg)
acid 1°y axis, pCO2 2°y axis
400
300
200
100
0
300
250
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
mean (± SD) Ω


2A
Ambient
Treatments
Mean (± SD) [$CO_3^{=}$] µmol kg^{-1}
2B
pCO_2 addition
Ambient
Acid addition
Mean (± SD) [HCO_3^{-}] µmol kg^{-1}
mean (±SD) Ω
Favia - acid addition
Porites - acid addition
Favia - pCO_2 addition
Porites - pCO_2 addition


3A: Favia fragum
acid
pCO2
Mean (± SE) corallite weight (µg)
300
250
200
150
100
50
0
3B: Porites astreoides
acid
pCO2
Mean (± SE) corallite weight (µg)
acid 1° y axis, pCO2 2° y axis
400
300
200
100
0
300
250
200
150
100
50
0
0
500
1000
1500
2000
2500
Mean (± SD) [HCO3-]