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REPORT

Ocean acidification and calcifying reef organisms: a mesocosm investigation

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Abstract A long-term (10 months) controlled experiment was conducted to test the impact of increased partial pressure of carbon dioxide (pCO_2) on common calcifying coral reef organisms. The experiment was conducted in replicate continuous flow coral reef mesocosms flushed with unfiltered sea water from Kaneohe Bay, Oahu, Hawaii. Mesocosms were located in full sunlight and experienced diurnal and seasonal fluctuations in temperature and sea water chemistry characteristic of the adjacent reef flat. Treatment mesocosms were manipulated to simulate an increase in pCO_2 to levels expected in this century [midday pCO_2 levels exceeding control mesocosms by 365 ± 130 µatm (mean ± sd)]. Acidification had a profound impact on the development and growth of crustose coralline algae (CCA) populations. During the experiment, CCA developed 25% cover in the control mesocosms and only 4% in the acidified mesocosms, representing an 86% relative reduction. Free-living associations of CCA known as rhodoliths living in the control mesocosms grew at a rate of 0.6 g buoyant weight year^{-1} while those in the acidified experimental treatment decreased in weight at a rate of 0.9 g buoyant weight year^{-1}, representing a 250% difference. CCA play an important role in the growth and stabilization of carbonate reefs, so future changes of this magnitude could greatly impact coral reefs throughout the world. Coral calcification decreased between 15% and 20% under acidified conditions. Linear extension decreased by 14% under acidified conditions in one experiment. Larvae of the coral *Pocillopora damicornis* were able to recruit under the acidified conditions. In addition, there was no significant difference in production of gametes by the coral *Montipora capitata* after 6 months of exposure to the treatments.

Keywords Ocean acidification · Climate change · Coral · Calcification · Coralline algae · Aragonite saturation

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Introduction

Results of extensive research over the past decade has lead to increasing concern that rising atmospheric carbon dioxide (CO_2) concentrations will cause changes in the ocean's carbonate chemistry system with a resulting impact on calcifying marine organisms (e.g., Kleypas et al. 1999; Langdon et al. 2003; Feely et al. 2004; Orr et al. 2005; Hoegh-Guldberg et al. 2007). The atmospheric partial pressure of CO_2 (pCO_2) will continue to increase from the present value of ~382 µatm in 2007 to greater than 700 µatm in the year 2100, if a business-as-usual CO_2 emission scenario is followed (IPCC 2001, 2007). The oceans absorb CO_2 (Mackenzie et al. 2001; Sabine et al. 2004), resulting in surface water increases in dissolved inorganic carbon and decreases in pH and carbonate saturation state (Feely et al.

2004). The lowering of ocean pH due to increasing atmospheric CO_2 has been termed "ocean acidification" (Bacastow and Keeling 1973; Caldeira and Wickett 2003). Marine calcifying organisms may experience increasing difficulty in secreting their skeletons as ocean pH decreases and the consequences may be severe for many marine ecosystems including coral reefs (Gattuso et al. 1999; Kleypas et al. 1999; Leclercq et al. 2000; Orr et al. 2005).

Until recently, the major focus of studies concerning effects of global climate change on coral reefs has been directed at the impact of higher global sea surface temperature on coral "bleaching" and mortality (e.g., Hoegh-Guldberg 1999). At present, there is a growing interest in the effects of ocean acidification (Marubini and Atkinson 1999; Marubini et al. 2001, 2003; Reynaud et al. 2003; Renegar and Riegl 2005; Schneider and Erez 2006), with predicted decreases in coral calcification rates of the order of 17–35% relative to pre-industrial rates. However, the biological conclusions drawn from the models are based on results of relatively a few experimental studies on living organisms. Previous experimental work has been carried out on corals (Gattuso et al. 1998; Marubini et al. 2001; Reynaud et al. 2003; Ohde and Hossain 2004; Renegar and Riegl 2005), coralline algae (Agegian 1985; Mackenzie and Agegian 1989), and coral reef mesocosm communities (Leclercq et al. 2000, 2002; Langdon et al. 2000, 2003; Langdon and Atkinson 2005). These earlier studies each have one or more experimental limitations such as use of closed systems, small water volumes, artificial or low irradiance, lack of treatment replication, and/or very short incubation times. Thus, to further this area of investigation a long-term, replicated experiment under full solar irradiance was conducted in a series of outdoor flow-through mesocosms. This system was located at the Hawaii Institute of Marine Biology and was designed to simulate the biological and physical coral reef environment on the adjacent reef. Conditions in these mesocosms follow the natural diurnal cycles in solar radiation, temperature, and seawater chemistry typically occurring on the adjacent tropical reef flat. Algal spores and larvae of various organisms settled in the mesocosms and developed communities over the course of the experiment. Recruitment, growth, and mortality of typical coral reef calcifiers could be measured directly. Response of numerous taxa could thereby be evaluated simultaneously in a system that mimiced the physical, chemical, and biological conditions on a reef under various conditions of ocean acidification.

Materials and methods

Six $1 \times 1 \times 0.5$ m fiberglass mesocosm tanks (Fig. 1) were supplied with seawater pumped directly from a depth of 2 m offshore of the adjacent Coconut Island Reef in Kāne'ohe Bay, Hawai'i. An adjustable head box-standpipe arrangement provided each mesocosm with an inflow of sea water at ~ 8 l min^{-1} resulting in a complete turn over rate of ~ 1 h. The natural diurnal and seasonal fluctuations that occur on inshore reef and coastal areas (Kayanne et al. 1995; Bates et al. 2001; Bates 2002) were thereby retained in the mesocosms (Fig. 2). Three mesocosms were randomly chosen to be held under ambient (control) conditions. These were controls in the sense that the seawater chemistry was not artificially manipulated. However, the water chemistry of the controls as well as the acidified mesocosms was influenced by metabolic activity of the contained communities in a manner analogous to what occurs on the adjacent reef flat. Three other mesocosms were maintained at a daytime pCO_2 level exceeding the pCO_2 of the control mesocosms by 365 ± 130 µatm (mean ± sd) (Fig. 2), which approximates increasing pCO_2 levels predicted by the BAU IS92a CO_2 scenario within this century (IPCC 2001, 2007). Carbonate chemistry in the treatment mesocosms was altered by adding a 10% solution of hydrochloric acid (HCl) diluted with tap water at a controlled rate (1.3 ml min^{-1}) into the inflow pipes of each treatment mesocosm using a 205CA Watson Marlow multi-channel peristaltic pump. Control mesocosms received plain tap water at the same rate. Treatments were initiated on October 31, 2005 and ended on August 8, 2006.

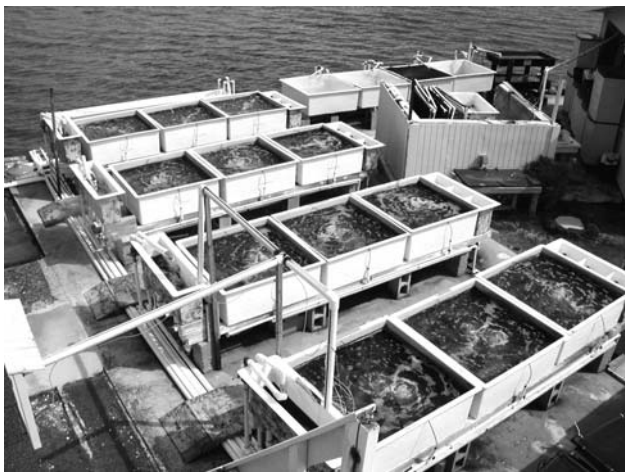

Fig. 1 Continuous-flow mesocosm facility located at the Hawaii Institute of Marine Biology, Coconut Island

Temperature, salinity, dissolved oxygen, and pH were measured in all six mesocosms at least once per week at midday. Water samples for dissolved inorganic carbon (DIC) and total alkalinity (TA) were collected at this time. All seawater chemistry measurements and analyses were completed following procedures outlined in Kuffner et al. (2008). Diurnal sampling was conducted on several

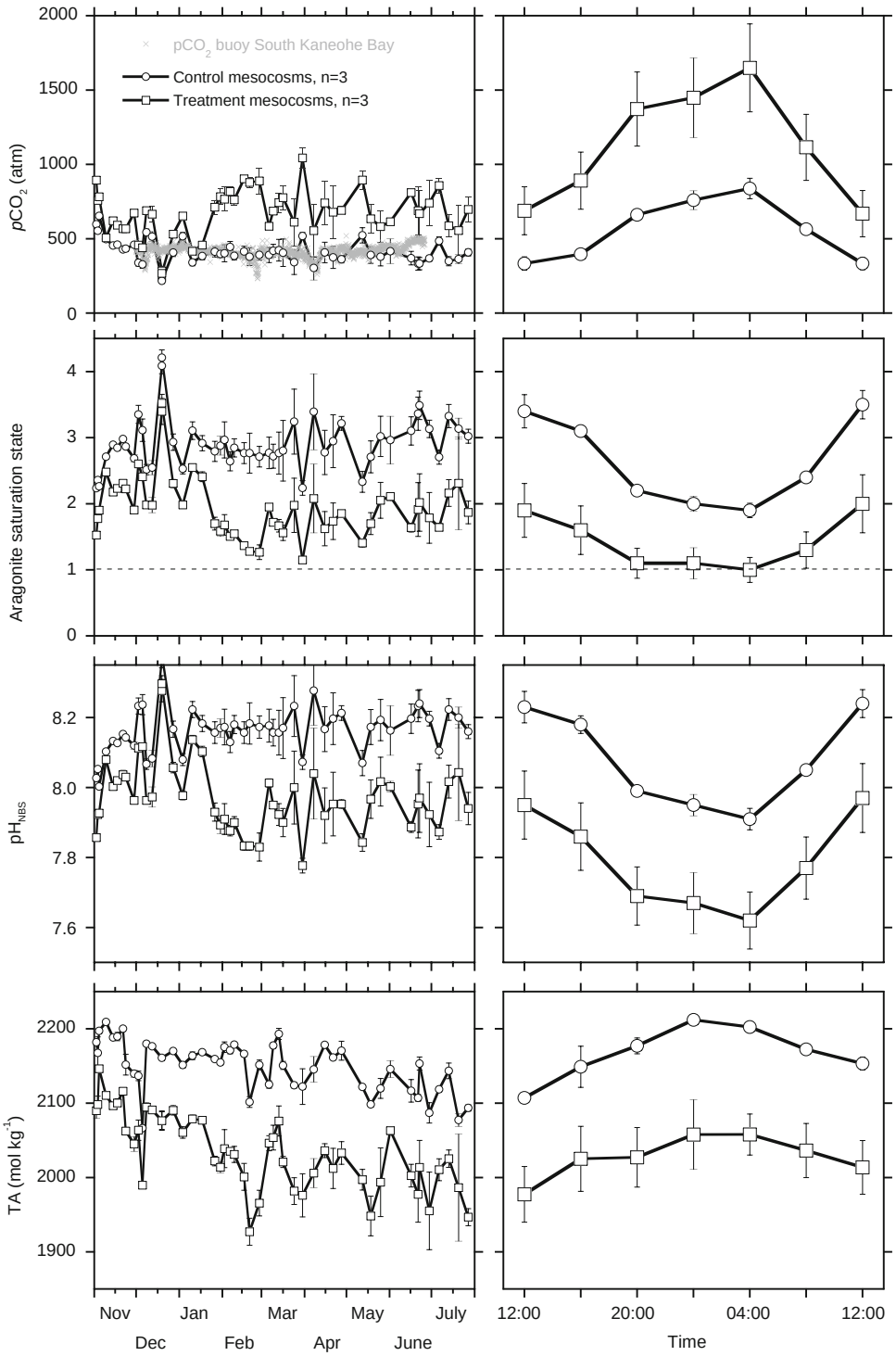

Fig. 2 Seawater chemistry. Left column shows average seawater carbonate chemistry observed at midday during the experiment (November 2005 to July 2006) with *squares* for acidification treatment and *circles* for control treatment. Right column shows a representative full diurnal cycle (June 21–22, 2006; 12:00–12:00 h) for the control treatment (*circles*) and acidifications treatment (*squares*). The pairs of graphs, from top to bottom, show partial pressure of CO_2 (pCO_2), saturation state with respect to aragonite (the *dashed line* illustrates equilibrium, $\Omega = 1$), pH measured on the NBS scale, and total alkalinity (TA) in the mesocosms. Error bars $\pm$ SD, $n = 3$ mecocosms

occasions to quantify the daily natural variability (e.g., Fig. 2). During the course of this period, a number of subexperiments were run within the mesocosms to study various aspects of the calcification question. These included the impact of ocean acidification on recruitment and growth of crustose coralline algae (CCA) (Kuffner et al. 2008).

Coral growth

The coral growth experiments used the reef coral *Montipora capitata*, which is a major reef forming species in Hawaii. This species is abundant in Kaneohe Bay and thrives in the mesocosms. Conditions in the three replicate experimental and three replicate control tanks were

allowed to stabilize prior to the introduction of the first set of coral colonies. Three coral growth trials were conducted over the course of the experiment. In each case, the corals skeletons were stained with alizarin red-*S* (Lamberts 1974) to later measure skeletal linear extension. Coral incubation in staining solution was limited to 8 h because stress bands have been reported in skeletons of corals left more than 12 h in the dye (Hudson 1981; Dodge et al. 1984). Corals were also weighed periodically using the buoyant weight technique (Jokiel et al. 1978).

Trial 1 used small nubbins secured with underwater epoxy (Z-spar Splash Zone compound) to 8 × 8 cm ceramic tiles ($n = 5$ per mesocosm) and ran from November 18, 2005 to August 7, 2006. The average initial dry weight of the nubbins calculated from buoyant weight was 3.2 ± 0.8 g (mean ± sd). Buoyant weights were measured six times over the time period of trial 1. Analysis of data after the first weighing period suggested that use of nubbins and a sample size of $n = 5$ per mesocosm might not be sufficient for statistical confidence in the result, hence, a second trial was initiated.

Trial 2 used large [101 ± 43 g dry weight (mean ± sd)] colonies of the coral *M. capitata* rather than nubbins, a larger sample size ($n = 20$ colonies per mesocosm), and ran from January 4 to August 7, 2006. The measured daytime pCO_2 of the treatment mesocosms was elevated by an average of 310 μatm relative to the control mesocosms during this time period (Fig. 2). Unattached colonies of comparable size and morphology (~10 cm diameter) were collected from reef flats in Kaneohe Bay at a depth of 1 m. Colonies were labeled for identification, buoyant weights measured, and then stained with alizarin red-S. After staining and weighing, the 20 colonies were randomly assigned to each of three replicate experimental and three replicate control treatments in the mesocosms. At the end of the experiment, linear extension was measured with calipers as the distance between the original stain line and the growing branch tip. Ten replicate vertical growth measurements were taken from each colony and the average was calculated. Buoyant weights of all colonies were measured at various intervals ranging from 18 to 80 days over the experimental period.

Trial 3 (June 9 to August 7, 2006) was run in the same manner as trial 2 but the *M. capitata* colonies were smaller [$n = 20$, 60 ± 24 g dry weight (mean ± sd)] and were weighed more frequently (every 2 weeks) in an attempt to detect possible changes in calcification rate due to acclimation during the early weeks of exposure to acidified treatment.

Coral calcification data for all three growth trials of *M. capitata* were analyzed using one-way analysis of covariance (ANCOVA) by mesocosm (covariate was initial dry weight of the colony) considering growth over the entire time period that each group was in the tanks, with an *a priori* contrast to test for differences between control and treatment mesocosms. In trial 3, the data were square-root transformed to meet the assumptions of the ANCOVA. Survivorship was high (>95%) in all treatments, with no differences in mortality detected between the control and treatment mesocosms (data not shown). Corals that died or showed partial mortality during the study were not included in the analyses. Statistical analyses were conducted using Statistix version 8.0. Assumptions of normally distributed residuals and equal variance among ANOVA cells were tested and met in all cases.

Skeletal linear extension was measured in trials 2 and 3, but nubbins in trial 1 were not large enough to allow accurate linear extension measurements. For trial 2, the data did not meet the assumptions for a parametric one-way ANOVA, so a Kruskal–Wallis test was performed instead. The Kruskal–Wallis test produces *F*-statistic produced by performing a parametric ANOVA on rank-transformed data. For colonies grown in trial 3, a one-way ANOVA was applied (the assumptions were met), and the treatment effect was tested using an *a priori* contrast of control versus treatment tanks. In trials 2 and 3 both mean linear extension rate of the branches and mean calcification were measured. Therefore, calcification divided by extension for each colony was calculated, providing possible insight into density of new skeletal material deposited on branches during the growth period. For trial 2, the data did not meet the assumptions of a one-way ANOVA (even after transformation), so a Kruskal–Wallis test was performed. Data from trial 3 were arcsine transformed ($y' = 2 \arcsin\sqrt{y}$) before analysis using a one-way ANOVA.

Rhodolith growth

Rhodoliths form as unattached accretions of various CCA. Rhodoliths of ~10 g weight were collected from reefs in Kaneohe Bay and randomly assigned to the mesocosms ($n = 4$ per mesocosm). The rhodoliths used in the experiment consisted of a mixed CCA community including *Lithophyllum* cf. *pallescens*, *Hydrolithon* sp. and *Porolithon* sp. Rhodolith buoyant weight was measured on November 14, 2005 and final weight on August 8, 2006. Growth rate was expressed as change in grams buoyant weight per year. Results were analyzed using a one-way ANOVA by mesocosm on rank transformed data, with an *a priori* contrast to test for a difference between control and treatment mesocosms.

Coral spawning

The *M. capitata* colonies in trial 2 of the growth study were used to estimate the impact of acidification on gamete

production. The corals had been living under the treatment and control conditions starting in January 2006 and the first spawning occurred ~6 months later on June 25, 2006. Sexual reproduction in *M. capitata* occurs following the summer new moons, with gamete release over a period of several consecutive days between 20:45 and 22:30 h (Kolinski and Cox 2003). *M. capitata* is a simultaneous hermaphrodite, broadcasting egg–sperm bundles with a variable number of eggs.

Prior to spawning, 10 colonies from each mesocosm were randomly selected for collection of egg–sperm bundles. These colonies were placed in 4 l plastic flower pots 3 h prior to each predicted spawning event. Openings in the bottom of the containers allowed free exchange of water with the coral. The tops of the containers were supported above the water level using floatation collars to contain the positively buoyant bundles produced by each coral. Nylon netting was positioned over the drainage openings to prevent intrusion of bundles from corals spawning below the floating containers.

During spawning periods in June, July, and August, all bundles were collected from each colony. When only a few bundles were present, all were removed by pipette and counted. When larger numbers of bundles were released, the volume of the bundles was measured and transformed to counts using a curve calculated from counts of known volumes. All bundles released over the entire reproductive season were combined to estimate total reproductive output of a particular colony. Reproductive output was normalized to colony buoyant weight. Subsamples of bundles were placed individually in Petri dishes to determine the number of eggs per bundle. Approximately 1 h after release, bundles break apart and the individual eggs can be counted.

Several colonies from the treatment and control mesocosms that had suffered partial mortality or fragmentation were not included in the analysis. Total number of gamete bundles g^{-1} was not normally distributed, even after transformation. Therefore, data were analyzed using a nonparametric Kruskal–Wallis one-way analysis of variance. The number of eggs $bundle^{-1}$ was normally distributed and therefore, was analyzed using ANOVA.

Coral recruitment

Corals recruit into the mesocosms from the continuous flow of seawater at a rate of ~1 colony $year^{-1}$ (Jokiel and Guinther 1978). However, past experience has shown that a high rate of coral settlement can be obtained by including adult colonies of the reef coral *Pocillopora damicornis* within the mesocosm tanks. This common species broods and releases planula larvae on a monthly cycle throughout the year which readily settle and grow in the mesocosms system (Jokiel and Guinther 1978). Five small colonies of this species were added to each mesocosm at the beginning of the experiment. At the end of the experiment both size and number of polyps of newly settled colonies were enumerated on tank walls. All of the settlements on the mesocosm walls were *P. damicornis* assumed to be from planula larvae produced by the adult colonies placed in the mesocosms for this purpose. Results were analyzed using a two-tailed, two-sample *t*-test comparing the total number of recruits for each replicate ($n = 3$) of treatment versus control mesocosms.

Recruitment of other calcifying organisms

Various organisms such as algae, barnacles, oysters, and vermetid mollusks recruit into the mesocosms in high numbers as they do under suitable field conditions. At the end of the experiment, the mesocosms were drained. Nonoverlapping digital images were taken of the tank side wall communities. Percent coverage by the oyster, *Dendrostrea sandwichensis*, turf algae, and calcareous algae was quantified using "PhotoGrid" software that generates random points and facilitates digital recording of coverage data by taxonomic group (Bird 2002). Fifty randomly selected points were projected onto each of the 48 nonoverlapping digital images that covered the entire area of the four tank walls. Images of the tank bottom were not included because fine sediment had excluded settlement of encrusting organisms. As in the case of the coral settlements, individual settlements of the vermetid mollusk *Serpulorbis* sp. and the barnacle *Balanus* sp. were measured directly using a magnifier and calipers. Results were analyzed using two-sample *t*-tests.

Results

The mesocosms followed diurnal cycles in solar radiation, temperature, and seawater chemistry (Fig. 2) similar to those observed on other tropical reef flats (e.g., Δ0.6 pH units, Kayanne et al. 1995; Ohde and van Woesik 1999). Various organisms recruited into the mesocosms as larvae during the experiment, leading to the development of a complex mesocosm community.

Coral growth

In trial 1 using the coral nubbins there were no significant differences in calcification rate between the treatment and control mesocosms, probably because of the small sample size ($n = 5$ nubbins per mesocosm). In trials 2 and 3 the sample size was therefore raised to $n = 20$ to increase the statistical power.

In trial 2 the coral colonies in the acidification treatment showed a 15% reduction in calcification rate compared to the control conditions (one-way ANCOVA, mesocosm effect: $F_{(5,\ 89)} = 2.9$, $P = 0.018$, covariate effect: $F_{(1,\ 89)} = 64.2$, $P < 0.0001$, *a priori* treatment contrast $P = 0.013$; Table 1 and Fig. 3). Linear extension rates were not significantly different among mesocosms.

In trial 3, colonies in the acidification treatment showed a 20% reduction in calcification rate compared to the control conditions (one-way ANCOVA on square-root transformed data, mesocosm effect: $F_{(5,\ 86)} = 2.5$, $P = 0.038$, covariate effect: $F_{(1,86)} = 22.6$, $P < 0.0001$, *a priori* treatment contrast $P = 0.0193$; Table 1 and Fig. 4). Linear extension was significantly reduced due to acidification by 14%, (one-way ANOVA, $F_{(df=5,\ 114)} = 4.68$, $P = 0.0006$, *a priori* treatment contrast $P = 0.005$).

In both trials 2 and 3 there was no difference in calcification divided by extension (Table 1) between treatments.

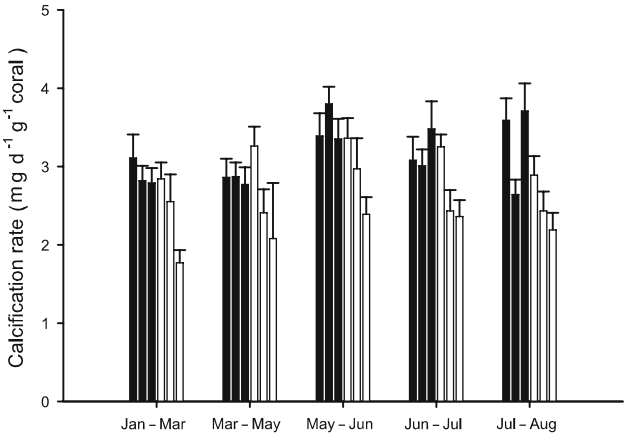

Fig. 3 *Montipora capitata*. Calcification rate (mg d^{-1} g^{-1} coral) of whole colonies (trial 2) over the 215 days experimental period. The time periods between sampling days were not equal (62, 64, 40, 21 and 28 days). Black bars are control tanks and white bars are treatment tanks. Error bars ± SE, $n = 20$

Rhodolith growth

Rhodoliths in the control mesocosms grew at a rate of 0.6 ± 0.3 g $year^{-1}$ (mean ± SE) while those in the acidification treatment decreased in weight at a rate of −0.9 ± 0.3 g $year^{-1}$ (Table 2) representing a 250% difference from positive to negative growth (rank transformed data, one-way ANOVA $F_{(5,\ 17)} = 11.5$, $P = 0.0001$, *a priori* contrast $P < 0.0001$).

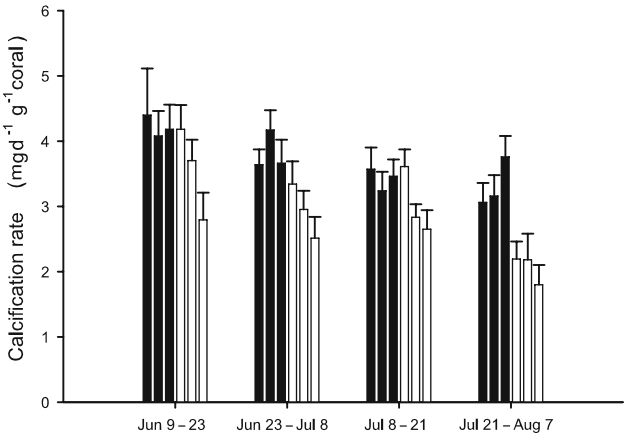

Fig. 4 *Montipora capitata*. Calcification rate (mg d^{-1} g^{-1} coral) of whole colonies (trial 3) over the 59 days experimental period. Sampling frequency was approximately every 2 weeks. *Black bars* are control tanks and *white bars* are treatment tanks. Error bars ± SE, $n = 20$

Coral spawning

There were no significant differences between the total number of bundles g^{-1} colony buoyant weight produced by the corals in the acidified treatment and those in the control mecocosms, nor in the mean number of eggs $bundle^{-1}$, nor in total egg production (Table 1).

Table 1 *Montipora capitata*. Growth rate and spawning of corals in the control ($n = 3$) and acidification treatment ($n = 3$) mesocosms

Measured variable	Trial	Control treatment	Acidification treatment	Percent difference	Statistical significance
Calcification rate (mg d^{-1} g^{-1} coral)	2	4.1 ± 0.91	3.5 ± 0.9	**−15**	$\mathbf{P = 0.013}$[a]
	3	4.1 ± 0.7	3.3 ± 0.7	**−20**	$\mathbf{P = 0.0193}$[a]
Linear extension (μm d^{-1})	2	88 ± 11	82 ± 13		$P = 0.065$[b]
	3	97 ± 17	83 ± 14	**−14**	$\mathbf{P = 0.005}$[c]
Calcification/extension (mg g^{-1} μm^{-1})	2	0.048 ± 0.012	0.043 ± 0.010		$P = 0.29$[b]
	3	0.047 ± 0.014	0.042 ± 0.012		$P = 0.17$[c]
Total no. bundles g^{-1} colony buoyant wt	2	5.2 ± 3.1	7.2 ± 5.8		$P = 0.47$[b]
Mean no. eggs $bundle^{-1}$	2	15.2 ± 1.7	13.3 ± 2.1		$P = 0.060$[c]

Means ± SE. Bold type signifies $P < 0.05$

[a] *P*-value for *a priori* contrast between treatment and control mesocosms, one-way ANCOVA by tank (data square-root transformed in trial 3)

[b] *P*-value for nonparametric one-way Kruskal–Wallis ANOVA by tank

[c] *P*-value for *a priori* contrast between treatment and control mesocosms, one-way ANOVA by tank

Table 2 Recruitment and growth of various calcifying organisms in control ($n = 3$) and acidification treatment ($n = 3$) mesocosms

Recruitment	Control treatment	Acidification treatment	Percent difference	Two-sample *t*-test
Coral				
Settlements (no. m^{-2})	55 ± 14	49 ± 18		$P = 0.81$
Spat diameter (mm)	2.5 ± 0.2	2.8 ± 0.1		$P = 0.44$
No. of polyps of newly settled colonies	4.4 ± 0.9	5.3 ± 0.6		$P = 0.47$
Other organisms				
Rhodolith accretion (g buoyant wt. yr^{-1})	0.6 ± 0.3	−0.9 ± 0.3	**−250**	**$P < 0.0001$**[a]
Crustose coralline algae (% cover)	25 ± 4	3.6 ± 0.9	**−86**	**$P = 0.03$**[b]
Turf algae (% cover)	16.6 ± 4.0	14.5 ± 2.6		$P = 0.69$
Vermetids (no. m^{-2})	78.4 ± 35.1	7.7 ± 3.1		$P = 0.18$[b]
Oysters (% cover)	5.7 ± 1.9	4.4 ± 0.8		$P = 0.56$
Barnacles (no. m^{-2})	8.3 ± 3.5	4.5 ± 1.6		$P = 0.37$
Barnacle size (mm)	5.1 ± 0.6	6.0 ± 0.2		$P = 0.25$
Bare substratum (% cover)	53.2 ± 2.1	77.5 ± 1.1	**+46**	**$P = 0.0006$**

Means ± SE. Bold type signifies $P < 0.05$

[a] *P*-value for *a priori* contrast between control versus treatment mesocosms, one-way ANOVA by tank

[b] Two-sample *t*-test assuming unequal variances

Coral recruitment

Larvae, presumably produced by the reef coral *P. damicornis* held in the mesocosms, successfully settled on tank walls. Due to adult colony mortalities over the 10-month period, it was not possible to confirm that the number of larvae released was equal among tanks. However, the condition of the colonies was quantitatively assessed on January 20, 2006, and the mean percentage of live tissue per colony did not vary among tanks (Kruskal–Wallis test), there was no treatment effect, and no differences between the size of settlements either as diameter or number of polyps (Table 2).

Recruitment of other calcifying organisms

Besides an 86% reduction in mean coral cover of CCA in the acidified treatment (two-sample *t*-test, $P = 0.03$), there were no significant differences in the other calcifying organisms monitored in the study (Table 2). For the vermetids (*Serpulorbis* sp.) and the barnacles (*Balanus* sp.) there were particularly large variances in the data which together with the low sample size may have masked any differences. Overall, there was significantly more (46%) bare substratum exposed on the tank walls in the acidified treatment compared to the controls (two tailed two-sample *t*-test, $P = 0.0006$; Table 2). Space made available from the reduction in CCA was not colonized by other organisms.

Discussion

The calcification rate decreased between 15% and 20% (Table 1) in the coral *M. capitata* under experimental conditions that increased daytime seawater pCO_2 by ~300 µatm, a level which is expected to occur within this century. Skeletal extension in *M. capitata* was also reduced by 14% in trial 3, but did not decrease in trial 2. These findings are generally in agreement with other reports (e.g., Marubini and Atkinson 1999; Marubini et al. 2001, 2003; Reynaud et al. 2003; Renegar and Riegl 2005; Schneider and Erez 2006). Some investigations show a greater response in coral calcification than were observed in these experiments. For example, coral skeletal growth declined 80% in branch tips of the coral *Acropora cervicornis* grown at 700–800 µatm in closed systems with pCO_2 adjusted by bubbling with CO_2 gas (Renegar and Riegl 2005).

The most striking observation in this experiment was the impact of ocean acidification on CCA. CCA growth that developed on the mesocosm walls during the experiment covered 25% of the surfaces in the control treatment and only 3.6% of the surfaces in the acidified treatments, an 86% relative reduction. Conditions of acidification clearly interfered at the recruitment stage as well as during early growth, and corroborate results documented earlier in this experiment by Kuffner et al. (2008). The rhodoliths in the mesocosms were CCA that had accreted to form unattached nodules. Rhodolith facies are a common feature of modern and ancient carbonate shelves worldwide and rhodolith communities contribute significantly to the global coastal ocean calcium carbonate budget (Bosence 1983; Foster et al. 1997; Frantz et al. 2000; Hinojosa-Arango and Riosmena-Rodríguez 2004). Rhodoliths that were held in the acidified mesocosms showed a 250% decrease compared to the controls, changing from positive to negative accretion. CCA are primary calcifiers on reefs and form

wave-resistant structures such as the massive buttresses of the spur and groove formations, and are instrumental in the process of cementing carbonate fragments into a solid structure and acting as reef framework organisms (Adey and Macintyre 1973; Adey 1998). Furthermore, CCA provide preferred substrata and positive settlement cues for reef-building coral larvae (Morse et al. 1988; Heyward and Negri 1999). In addition, CCA have a skeletal mineralogy of high magnesium calcite, a phase more soluble than aragonite or calcite, and hence they may be the first responders to ocean acidification by dissolution (Morse et al. 2006; Andersson et al. 2007).

At the conclusion of the experiment, there was no significant difference in the cover of noncalcifying algae on the mesocosm walls. There was significantly more uncolonized substratum, with 77% cover in the acidified treatment compared to 53% in the control, a relative increase of 46% (Table 2). Initial results from this experiment (Kuffner et al 2008) using plastic cylindrical fouling surfaces showed a significant increase in noncalcifying algae that accompanied a significant decrease in CCA. By the end of the experiment, however, a larger biomass of herbivores consisting of gastropod mollusks, opisthobranch mollusks, crabs, amphipods, polychaetes, and other organisms had developed. This is a normal pattern of succession in the mesocosms. These organisms graze algae on the walls and may have been responsible for cropping much of the area down to bare substratum. It is possible that any advantage of noncalcifying algae on reefs under future levels of acidification could be offset by increased herbivory in areas that are ecologically and trophically intact. In any event, the mesocosm approach can provide us with such insights and provides a means of testing such hypotheses.

Acclimation to ocean acidification

Repetitive measurement of growth in coral over the course of many months failed to produce a trend that would suggest a strong acclimation response of corals to ocean acidification (Figs. 3 and 4). Likewise, Langdon et al. (2000) compared the sensitivity of calcification to short-term (days) and long-term (months to years) changes in saturation state and could not detect any evidence of acclimation.

Coral reproduction and recruitment

The *M. capitata* corals grown under acidified conditions in this experiment had reduced calcification rates, but did not show a decrease in gamete production. This observation is consistent with the findings of Fine and Tchernov (2007) who reported normal gametogenesis in *Oculina patagonica* and *Madracis pharencis* when exposed to acidified conditions. In their experiments, tissue biomass was maintained even though calcification ceased and the existing skeletal material began to dissolve.

There are a number of possible explanations for the lack of reduction in gamete production in the coral *M. capitata* under the acidified conditions. Perhaps resources were channeled into reproduction at the expense of other metabolic activities. Gametogenesis has not been studied in *M. capitata*, but in related species gametogenesis extends over 9–11 months (Willis et al. 1985; Babcock et al. 1986; Vargas-Ángel et al. 2006). In this study, the exposure to acidification began in January 2006, so corals in the experimental tanks presumably had begun the process of gametogenesis prior to exposure to acidified seawater. Perhaps once the process had begun, the allocation of resources continued for the rest of the cycle. *M. capitata* increases heterotrophy following the stress of bleaching (Grottolli et al. 2006), and can maintain lipid stores following loss of zooxanthellae. Such increased lipid can allow this species to continue sexual reproduction following a bleaching event (Cox 2007). If *M. capitata* responds to acidification with a similar increase in heterotrophy, the supplemental resources may permit this species to complete its reproductive cycle under acidified conditions.

Larvae produced by the reef coral *P. damicornis* held in the mesocosms successfully settled and grew on tank walls and produced a mean of 49 new colonies m^{-2} during the experiment (Table 2). Previous work in the mesocosms using the same methodology has shown that reproductive success of this species in the mesocosms is readily influenced by a variety of factors. Recruitment success of *P. damicornis* changes by several orders of magnitude with a shift of only one degree away from the optimum reproductive temperature of 27°C (Jokiel and Guinther 1978). Settlement success in the mesocosms is also very sensitive to minor variations in water motion and light and is influenced by herbivore grazing (Brock 1979). In this experiment, however, recruitment was not responsive to acidification.

Experimental design and replication

The mesocosm approach described here has many advantages, but also has limitations. The primary advantage is that continuous flow mesocosms are located under full solar radiation and provided with a continual supply of unfiltered sea water pumped directly from Kaneohe Bay, Oahu. Thus, conditions within the mesocosms are realistic and follow natural cycles observed on the reef. The response of many different organisms and processes can be studied in the same experiment. Many species recruit naturally into the mesocosms, allowing an opportunity to

study the response of recruitment and early life stages to a given set of conditions. The calcifying organisms in this experiment were grown side-by-side under the same conditions, so inter-comparisons were very powerful. Further, this approach allows for the measurement of community metabolism and water chemistry. Thus, the mesocosms provide a more realistic ecological setting that enhances the predictive value of the result, and represent a valuable technique in the study of ocean acidification impacts on ecosystems over a wide scale from individual organism response to integrated whole community metabolic response and change in community composition. There are also a number of limitations. Although mesocosms of this type are simplified "ecosystems," there is still a considerable amount of variation within and among mesocosms caused by factors such as complex interactions between organisms (e.g., predation, competition) and differences in rates of succession in the different mesocosms. As biomass within the communities develops, community metabolism changes the chemical environment within the mesocosm. Community photosynthetic rate, respiration rate, and calcification rate increase with time. Detritus begins to accumulate in the mesocosms. Such changes involve biological variation at many levels and result in increasing variability between replicates over time.

The results of this investigation show that the mesocosm approach was very effective at detecting the relative importance of various calcifying organisms in accounting for declines in reef community calcification under acidified conditions. This study was able to identify those groups of organisms that show a profound response to conditions of ocean acidification. The experiments suggest that certain sensitive processes such as coral reproduction and recruitment might not show a dramatic response to future ocean acidification.

The small number of replicates ($n = 3$) and the high level of variation in these more complex communities appear to have limited the ability to detect statistical differences for some of the more subtle changes that might be occurring. Mesocosms are large units and therefore, it is costly and difficult to increase replication. Previous investigators have utilized metabolic community measurements over time while changing pCO_2 in a mesocosm community design with an $n = 1$. For example, the classic "Biosphere 2" experiments (Langdon et al. 2000, 2003) were conducted on the same community with one set of measurements held at present levels of pCO_2 and the experimental treatment run a year later using doubling of the pCO_2 concentration. Another approach is to monitor the response of a single community to a spike in pCO_2 (Langdon and Atkinson 2005). Leclercq et al. (2000, 2002) exposed a single mesocosm to various levels of pCO_2 and monitored the physiological response over time. There is a need for more detailed information on the impact of anticipated increases in global temperature and pCO_2 on long-term processes such as fecundity, recruitment, grazing, and competition (Hoegh-Guldberg et al. 2007). Thus, it appears that future studies of biological response of coral reef communities to anticipated global changes must include long-term experiments of the type described here with sufficient treatment replication to establish statistical confidence in the results.

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