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Species-specific consequences of ocean acidification for the calcareous tropical green algae *Halimeda*

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ABSTRACT: Ocean acidification (OA), resulting from increasing dissolved carbon dioxide (CO_2) in surface waters, is likely to affect many marine organisms, particularly those that calcify. Recent OA studies have demonstrated negative and/or differential effects of reduced pH on growth, development, calcification and physiology, but most of these have focused on taxa other than calcareous benthic macroalgae. Here we investigate the potential effects of OA on one of the most common coral reef macroalgal genera, *Halimeda*. Species of *Halimeda* produce a large proportion of the sand in the tropics and are a major contributor to framework development on reefs because of their rapid calcium carbonate production and high turnover rates. On Palmyra Atoll in the central Pacific, we conducted a manipulative bubbling experiment to investigate the potential effects of OA on growth, calcification and photophysiology of 2 species of *Halimeda*. Our results suggest that *Halimeda* is highly susceptible to reduced pH and aragonite saturation state but the magnitude of these effects is species specific. *H. opuntia* suffered net dissolution and 15% reduction in photosynthetic capacity, while *H. taenicola* did not calcify but did not alter photophysiology in experimental treatments. The disparate responses of these species to elevated CO_2 partial pressure (pCO_2) may be due to anatomical and physiological differences and could represent a shift in their relative dominance in the face of OA. The ability for a species to exert biological control over calcification and the species specific role of the carbonate skeleton may have important implications for the potential effects of OA on ecological function in the future.

KEY WORDS: Carbon dioxide · pH · Coral reef · Climate change · Benthic algae · Carbonate chemistry

INTRODUCTION

As anthropogenic carbon emissions continue to rise annually, the buffering capacity of oceanic surface seawater and its ability to take up more CO_2 decreases (IPCC 2007, Füssel 2009, Egleston et al. 2010). The resulting equilibrium of the carbonic acid system and the relative concentrations of CO_2, HCO_3^-, and CO_3^{2-} are shifting away from the pre-industrial balance, thereby decreasing pH and the amount of carbonate ions available for calcification by marine organisms (Orr et al. 2005, IPCC 2007). This process is termed ocean acidification (OA). Evidence that a depression in seawater saturation state of calcium carbonate polymorphs (aragonite and calcite) will limit the ability for calcifying marine organisms to grow, develop, and calcify is increasing (Doney et al. 2009). Concurrently, the rise in dissolved CO_2 may release photoautotrophs from carbon limitation, for algae lacking carbon concentrating mechanisms (CCMs; Hurd et al. 2009), thereby enhancing growth. Therefore, carbon emissions may

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simultaneously stimulate photosynthesis and hinder precipitation for autotrophic organisms that calcify, making the consequences of OA difficult to predict at the community level.

Coral reef ecosystems may be particularly vulnerable to the chemical ramifications of increasing partial pressure of atmospheric CO_2 (pCO_2) because most inhabitants rely upon the biogenic structure created via calcification for shelter and habitat. Thus, a large proportion of studies examining the biological consequences of OA have focused on reef-building coral and reef-cementing coralline red algal species (CRAs; Doney et al. 2009). Green calcareous algae play similarly important ecological roles but have been neglected in past OA studies. In particular, species of the genus *Halimeda* (order Bryopsidales) contribute significantly to reef calcification and productivity rates because of their fast growth and rapid turnover rates (Vroom et al. 2003, Smith et al. 2004, Nelson 2009) in comparison to corals or CRAs. In fact, *Halimeda* create bioherms, or mounds of fossilized calcareous algae, and potentially contribute more to the carbonate budget in tropical systems than corals (Rees et al. 2007). *Halimeda* often grows in mats covering extensive areas of reef habitat and is one of the most abundant algal taxa in tropical seas. *Halimeda* not only produce a large percentage of local sand and sediment in tropical ecosystems (Harney & Fletcher 2003), but they are also important sources of nutrition for many herbivorous fish. Some species of parrotfish feed preferentially on *Halimeda* (Overholtzer & Motta 1999, J. E. Smith unpubl. data), but preference is dependent upon $CaCO_3$content and algal chemical defenses (Hay et al. 1988).

Species of *Halimeda* are all unicellular (Vroom et al. 2003) and are comprised of chains of segments joined at nodes. Segments are composed of heavily branched fleshy tubes or siphons that fuse to form utricles (Hillis 1959, Verbruggen & Kooistra 2004); calcification occurs in the spaces between the siphons and utricles (i.e. the inter-utricle space). The percentage of aragonite precipitated in the segments of *Halimeda* can vary widely within and among species (see Table 1 for summary) unlike corals and CRAs, which contain approximately 95% aragonite and 95% Mg calcite, respectively. Alterations in aragonite content of the segments are achieved either by dissolution of existing skeletal structure or via

Table 1. Estimates of *Halimeda* contribution to reef calcification and mineral content of *Halimeda* from this and previous studies. Calcification rates are standing stock biomass (g $CaCO_3$ m^{-2}) × normalized growth rate (g g^{-1} $CaCO_3$ yr^{-1})

Species	Lineage	Location	Calcification (g $CaCO_3$ m^{-2} yr^{-1})	% $CaCO_3$	Source
H. discoidea	*Halimeda*	Jamaica		48.6	Böhm (1973)
		Puerto Rico		47.8	Stark et al. (1969)
		Tahiti	14.23		Payri (1988)
		New Caledonia	13.11	65.0	Garrigue (1991)
H. incrassata	*Rhipsalis*	Jamaica		76.4	Böhm (1973)
		West Antigua	60.74–114.31		Multer (1988)
		Florida	0.1–62.3	25.6	Bach (1979)
		New Caledonia	31.9	72	Garrigue (1991)
		Tahiti	74.5	86.5	Payri (1988)
		Bermuda	50		Wefer (1980)
		Yucatan Peninsula		72.3–86.4	Van Tussenbroek & Van Dijk (2007)
		Tahiti	48.91–170.82		Payri (1988)
H. monile	*Rhipsalis*	Jamaica		72.8	Böhm (1973)
		Florida	0.1–6.9	16.0	Bach (1979)
H. opuntia	*Opuntia*	Jamaica	59.6–113.7	83.8–89.7	Böhm (1973)
		Puerto Rico		84.7	Stark et al. (1969)
		Tahiti	95.63–2922.0		Payri (1988)
		Palmyra Atoll	9.8–126.6	93.7–96.2	Present study
H. taenicola	*Halimeda*	Palmyra Atoll	1.5–9.6	81.1–86.5	Present study
H. tuna	*Halimeda*	Yugoslavia		62.8	Prat & Hamackova (1946)
		Florida Keys	24.76–100.86	75.0–82.9	Vroom et al. (2003)
		South Africa		32.5–34.6	Böhm (1973)
		Mediterranean	314	45.7	Ballesteros (1991)

physiological changes in the relative deposition rate of $CaCO_3$ versus fleshy tissue. This plasticity in the allocation of carbon to different tissue types may allow certain species of *Halimeda* to adapt to fluctuating environmental conditions.

Most organisms that precipitate calcium carbonate do so intracellularly, where the saturation state is highly regulated via ion pumping such as in articulated CRAs (Borowitzka 1979, Lee & Carpenter 2001), corals (Cohen & Holcomb 2009), sea urchins (Wilt 2002), and bivalves (Marin et al. 1996). However, *Halimeda* regulate carbonate chemistry in the semi-isolated inter-utricle space by passive diffusion of ions across the siphons (De Beer & Larkum 2001, Lee & Carpenter 2001). During the day, biomineralization occurs in *Halimeda* when the cell wall extracts CO_2 (or bicarbonate) for photosynthesis (Borowitzka & Larkum 1977), thereby raising pH and aragonite saturation state (Ω-aragonite) (Borowitzka & Larkum 1976a,b). Once the crystallization nuclei are formed, abiogenic precipitation of aragonite fills the space unoccupied by fleshy tissue (Borowitzka & Larkum 1977, Macintyre & Reid 1995). Calcification rates across *Halimeda* species are typically faster in the light than in the dark (Borowitzka & Larkum 1976a) and are often tightly linked with photosynthesis (Jensen et al. 1985, Semesi et al. 2009a). Because the calcifying space in *Halimeda* is only semi-isolated, the total dissolved inorganic carbon in the boundary layer surrounding the algae may directly influence calcification rates (Lee & Carpenter 2001). Thus, photophysiology and changing ambient seawater chemistry may have stronger influence over calcification and dissolution rates for *Halimeda* than for other coral reef calcifiers.

To date little evidence exists regarding the potential biological consequences of OA for different species of *Halimeda*. Because *Halimeda* are passive calcifiers, they are presumably at risk to changing seawater chemistry (Ries 2009). The few studies that have used projected oceanographic conditions as an experimental treatment have ignored the relative importance of photophysiology to the calcification process (but see Anthony et al. 2008) and have only been conducted using one species of *Halimeda* (e.g. Ries et al. 2009, Robbins et al. 2009). Here, we used a controlled mesocosm study to (1) examine whether experimental CO_2 enrichment affects calcification, growth and photophysiology of 2 common species of *Halimeda*, (2) determine if the magnitude of any effects are species specific, and (3) examine the species traits that likely enhance or reduce susceptibility to OA.

MATERIALS AND METHODS

Site description

All research was conducted at Palmyra Atoll located in the Northern Line Island chain in the Central Pacific. Palmyra represents a unique ecosystem because of its isolation and lack of direct local human impacts. The coral reefs of Palmyra are considered to be relatively healthy with reef building corals and CRAs making up greater than 50% of the benthic cover on the forereef slope (10 m) and shallow reef terrace (5 m depth) (54 and 78% cover, respectively; Sandin et al. 2008). The articulated calcareous green algae *Halimeda* are by far the most common macroalgae on Palmyra's reefs and account for on average 22 and 10% total benthic cover on the forereef and reef terrace habitats, respectively (J. E. Smith unpubl. data). We worked with 2 species common in both habitats on Palmyra that differed greatly morphometrically and genetically (Verbruggen et al. 2005). *Halimeda opuntia* (*Opuntia* lineage) is a heavily calcified and dense, matt-forming species that attaches to the substratum in numerous locations using small rhizoidal attachments. *H. taenicola* (*Halimeda* lineage) has large segments but is less calcified and attaches to the benthos via one large, robust holdfast (Fig. 1).

SCUBA divers collected samples of *Halimeda opuntia* and *H. taenicola* at 5 m depth on the shallow western terrace of Palmyra Atoll (5° 53.1696′ N, 162° 7.5756′ W) for a CO_2 bubbling experiment. All species of *Halimeda* were also collected within 0.7 × 0.9 m plots from 6 other sites to estimate standing stock biomass per species per unit area and to examine the natural variability in the proportion of calcified to fleshy tissue *in situ* (see 'Calcification and growth'). In all instances, algae used in the experiment (1.5 ± 0.1 g; mean ± SE) were removed at the holdfast or rhizoids to minimize damage to the siphons; samples were free of epiphytes and displayed actively growing tips.

Experimental design

To test the hypothesis that pCO_2 enrichment affects calcification, growth and photophysiology of 2 common species of *Halimeda*, we used a bubbling experiment with premixed gas enriched in CO_2. Individual algal samples were placed in small glass aquaria holding 700 ml of seawater that had been collected offshore and subjected to treatment conditions to

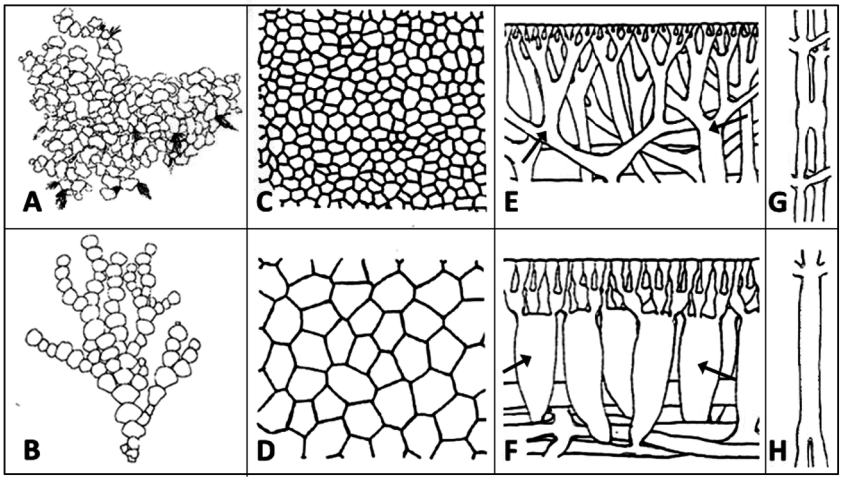

Fig. 1. *Halimeda opuntia* and *H. taenicola*. Schematics of (A,C,E,G) *H. opuntia* (lineage *Opuntia*) and (B,D,F,H) *H. taenicola* (lineage *Halimeda*) showing (A,B) thallus morphology that includes a thallus and holdfast or rhizoid structure (reprinted from Verbruggen & Kooistra 2004) and (C–H) segment and node anatomy as determined by light microscopy at 100× magnification, except for (E) that is at 50× (reprinted from Hillis 1959). (C,D) Surface views of the peripheral utricles. (E,F) Sagittal sections showing the structure the utricles of the cortex (arrow pointing at utricles). (G,H) Organization and fusion of the medullary siphons at the node

achieve equilibrium before the start of the experiment (~24 to 48 h) (as per Hurd et al. 2009). Experimental conditions were created by bubbling premixed gas enriched with excess pCO_2 (AirGas Pro) to 900 ± 90 µatm. The treatment level was chosen based on 'extreme' projections for 2100 under 'business as usual' (Scenario IV; IPCC 2007), which have since been adjusted to 'likely' conditions given 'business as usual' carbon emissions (Füssel 2009). Ambient conditions were created using an air pump (Coralife Super Luft Air Pump) delivering ambient air to the control aquaria. Each aquaria (n = 4 per treatment and per species) was covered with Parafilm to reduce evaporation of seawater due to constant bubbling. Reserve tanks of experimentally treated and ambient water were used for 100% water changes every 48 h. Temperature was regulated by immersing all aquaria in a water bath maintained at 28°C (the seasonal average for Palmyra) using a heater and chiller (1/4 HP model). Treatment conditions were monitored daily in each aquarium with a YSI Quarto and maintained to the following error level: salinity ± 0.1, pH of seawater (pH_{SW}) ± 0.01 pH units; temperature ± 0.1°C, dissolved oxygen ± 0.2 mg l^{-1}; Table 2). During one day, measurements were also taken morning, noon, evening, and midnight to quantify diurnal fluctuations in the aquaria. To control for the relative contribution of algal metabolism to experimental conditions, 'empty' aquaria (n = 2) were employed and subjected to the same 2 bubbling treatments described above. Water samples for total alkalinity (A_T) and total dissolved inorganic carbon (DIC_T) were collected at Days 2, 8, and 14 of the experiment in 500 ml Corning-brand Pyrex sample bottles and fixed with 200 µl saturated $HgCl_2$ solution (1% headspace).

All of the experimental aquaria were placed under a shade cloth to mimic the natural light environment at 5 m depth on the forereef and thus none of the algae received direct sunlight. We measured photosynthetically active radiation (PAR) above the experimental aquaria using a Pulse Amplitude Modulated (PAM) fluorometer (WALZ) with a light cosine microsensor (WALZ) (180° collection, calibrated against a Li-Cor LI 192 4π quantum sensor), which averaged 150 ± 30 µM photons m^{-2} s^{-1} at mid-day for all treatments. Growth, calcification, photophysiology, calcium carbonate tissue content, and shedding rate of senescent segments were quantified to determine the relative responses of *Halimeda opuntia* and *H. taenicola* to experimentally induced OA at the end of the 14 d experiment.

Calcification and growth

Organismal calcification rates for both species of *Halimeda* were estimated using the buoyant weight method (Davies 1989) and standardized by initial weight. Before subjecting the *Halimeda* samples to treatment conditions, each alga was placed in a weigh-below basket on a balance (Denver SI-403) to obtain its initial weight to the nearest mg. Changes in buoyant weight over the course of the experiment (including the shed segments), normalized to initial thallus mass, were used to estimate mean calcification rates for each species in the control and experimental treatments (n = 4). To estimate the current contribution of $CaCO_3$ from *Halimeda* to the reef, *in situ* estimates of calcification were calculated as the product of the biomass per species per unit area of reef (g wet wt m^{-2}) and the expected net change in

Table 2. *Halimeda opuntia* and *H. taenicola.* Mean (±SE) measurements for aquaria treatment conditions in CO_2 enrichment experiment. Measurements were taken at the same time each day, averaged across each species of *Halimeda* or control (n = 4 aquaria each) and then averaged for the duration of the experiment (n = 14 d). All treatments were also averaged at a given time point during one day to examine diurnal variability (n = 16 aquaria at each time point). Parameters were measured using the YSI Quatro handheld water quality meter. pCO_2: partial pressure CO_2; DO: dissolved oxygen; pH_{SW}: pH of seawater

Time period	Treatment	Species	Salinity	Temperature (°C)	DO (mg l^{-1})	pH_{SW}
Full duration (14 d)	Ambient air	Empty aquaria	34.95 ± 0.04	29.31 ± 0.07	4.95 ± 0.13	8.08 ± 0.02
		H. opuntia	34.78 ± 0.07	29.26 ± 0.08	4.57 ± 0.16	8.03 ± 0.04
		H. taenicola	34.73 ± 0.08	29.29 ± 0.08	4.73 ± 0.12	7.99 ± 0.03
	High pCO_2	Empty aquaria	34.98 ± 0.05	29.33 ± 0.06	4.66 ± 0.26	7.68 ± 0.04
		H. opuntia	34.85 ± 0.07	29.23 ± 0.08	4.16 ± 0.21	7.63 ± 0.02
		H. taenicola	34.80 ± 0.08	29.25 ± 0.07	4.38 ± 0.19	7.62 ± 0.02
Morning	Ambient air		34.83 ± 0.04	28.98 ± 0.11	4.98 ± 0.16	8.04 ± 0.05
	High pCO_2		34.92 ± 0.04	29.01 ± 0.10	4.76 ± 0.10	7.60 ± 0.05
Noon	Ambient air		34.94 ± 0.10	29.45 ± 0.03	4.79 ± 0.16	7.99 ± 0.06
	High pCO_2		35.08 ± 0.05	29.48 ± 0.01	4.90 ± 0.23	7.60 ± 0.02
Evening	Ambient air		34.65 ± 0.02	29.41 ± 0.03	5.43 ± 0.19	8.07 ± 0.01
	High pCO_2		35.71 ± 0.03	29.43 ± 0.03	5.13 ± 0.11	7.73 ± 0.02
Midnight	Ambient air		35.13 ± 0.00	29.36 ± 0.01	4.56 ± 0.10	7.99 ± 0.01
	High pCO_2		35.18 ± 0.01	29.38 ± 0.02	4.68 ± 0.08	7.70 ± 0.03

thallus mass standardized by initial thallus size (mg dry wt mg^{-1} d^{-1}) as estimated from the control replicates in the bubbling experiment.

Photophysiology

To estimate the photosynthetic efficiency for both species at the beginning and end of the experiment, we used rapid light curves (RLC) generated by exposing samples for 10 s to 9 incremental steps of irradiance using a red PAM fluorometer (Walz, GmbH) (Saroussi & Beer 2007). The fiber-optic sensor of the diving PAM was clipped to an unepiphytized but mature segment midway up one of the branch axes of each alga to control for bias due to developmental stage. For each algal sample, 2 curves were generated on 2 separate segments from independent branches to increase precision within a replicate. The average electron transport rates by actinic irradiance intensity (ETRs) for each algal sample were then used to fit a 3 parameter nonlinear model for each of 4 replicates per treatment as described by Frenette et al. (1993). Estimates of I_k (compensation irradiance), α (initial slope of the RLC), $rETR_{max}$ (maximum electron transport at I_k), and β (decline in slope of RLC after $rETR_{max}$ or photoinhibition past I_k) were determined and compared among treatments and species. We used the relative measure of rETR because the exact absorbance of the thallus for either species of *Halimeda* is unknown. Curves were fitted with a Gauss-Newton nonlinear regression algorithm in R (version 2.10, R Development Core Team 2009), and the estimates of $rETR_{max}$, α and β were used to describe photosynthetic efficiency.

Tissue content and senescence

To quantify morphological changes in response to experimental conditions, we also measured the percentage of $CaCO_3$ in the thallus and the shedding rate of segments for each algal sample. At the end of the experiment, each alga was briefly rinsed in fresh water, dried for 48 h at 60°C (until a constant weight was achieved), weighed, treated with several changes of a 5% HCl solution until all carbonate was dissolved, rinsed with fresh water and then dried again as above and reweighed. The ratio of the dry calcified weight (total dry minus fleshy dry) weight to the total dry weight was used to calculate the percent $CaCO_3$ for each sample. Shedding rate was calculated as the mean number of segments lost from each alga divided by the number of branch axes on each alga. To quantify the natural variability of $CaCO_3$ content in *Halimeda* from multiple sites around Palmyra we used the same drying and weighing methods described above for each species from 7 sites; see Table 1).

Water chemistry analysis

Carbonate chemistry and salinity analyses were conducted by the Dickson lab at the Scripps Institution of Oceanography. Total dissolved inorganic carbon (DIC_T) was determined using a Single Operator Multi-parameter Metabolic Analyzer (SOMMA) and a UIC Model 5011 CO_2 coulometer. Total alkalinity (A_T) was determined by open cell acid titration using a Metrohm Dosimat Model 665 and Thermo Scientific Ross potentiometric pH probe and meter. Salinity was determined using a Metler Toledo Model DE45 density meter. Seawater dissolved inorganic carbon parameters (HCO_3^-, CO_3^{2-}, CO_2, pCO_2), pH_{SW}, and saturation state of carbonate minerals (Ω-calcite and Ω-aragonite) were calculated based on measured DIC_T and A_T using the computer program CO2SYS (version 14; Pierrot et al. 2006) and stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson & Millero (1987); data are presented in Table 3.

Statistical analysis

Two-way analyses of variance (ANOVAs) were used to test whether there were physiological or morphological responses to OA and if the responses were species-specific (JMP 8 version 8; SAS). Factor I (fixed) was the CO_2 treatment (ambient or elevated) and factor II (fixed) was the species of *Halimeda*; each factor had 2 levels. When appropriate, *t*-tests were also used to distinguish if net loss or accumulation of segments and calcium carbonate were occurring. Normality and homoscedasticity were verified for each data set using the Kolmogorov-Smirnov normality test.

RESULTS

Calcification and growth

Exposure to elevated concentrations of CO_2 over a 2 wk period negatively affected the ability of both species of *Halimeda* to calcify. Both species showed significant declines in net calcification with increased pCO_2 (Fig. 2), but the magnitude of the effect was species specific (Table 4). In the CO_2 enrichment treatment conditions, *H. opuntia* exhibited net dissolution ($t = 2.78$, df = 3, $p = 0.007$) while the net calcification rate of *H. taenicola* was not significantly different from zero ($t = 2.57$, df = 3, $p = 0.70$) (Fig. 2). In contrast, both *H. opuntia* and *H. taenicola* showed positive growth under control conditions (Fig. 2), as evidenced by the presence of new seg-

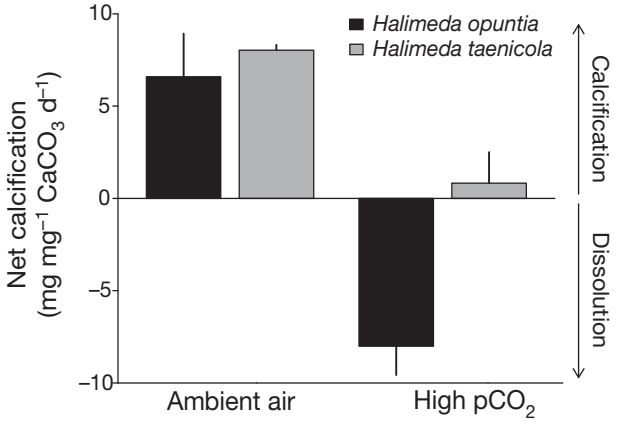

Fig. 2. *Halimeda opuntia* and *H. taenicola*. Mean (+SE) calcification rate for *H. opuntia* and *H. taenicola* in treatment and control aquaria (n = 4) determined with the buoyant weight technique. Change in weight was measured as mg $CaCO_3$ d^{-1} for each thallus. See Table 3 for descriptions of experimental conditions

Table 3. Mean (SE) seawater chemistry at 20°C for experimental aquaria (n = 4 per treatment) for control (400 ppm) and experimental treatments (900 ppm). Seawater pH (pH_{SW}), dissolved inorganic carbon parameters (pCO_2, CO_2, HCO_3^-, CO_3^{2-}), and saturation state of carbonate minerals (Ω-calcite , Ω-aragonite) were calculated from measured values of total dissolved inorganic carbon (DIC_T) and total alkalinity (A_T) with the computer program CO2SYS (version 14; Pierrot et al. 2006). Seawater values are considered to be 'natural' as they were measured from discrete samples at the benthos on the forereef taken during the day at varying tides

Treatment	Salinity	A_T (µmol kg^{-1})	DIC_T (µmol kg^{-1})	pH_{SW}	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	CO_2 (µmol kg^{-1})	pCO_2 (ppm)	Ω-calcite	Ω-aragonite
Seawater	34.2–34.9	2178–2290	1876–1973	7.93–8.10	1658–1741	209–222	9.1–9.6	281–296	5.0–5.3	3.3–3.5
Control	34.8 (0.1)	2221 (10)	1994 (4)	8.0 (0.02)	1817 (4)	163 (5)	14.2 (0.5)	440 (17)	3.9 (0.1)	2.5 (0.1)
High pCO_2	34.8 (0.1)	2236 (5)	2148 (2)	7.7 (0.03)	2019 (11)	88 (5)	32.8 (2.4)	946 (41)	2.1 (0.1)	1.4 (0.1)

Table 4. *Halimeda opuntia* and *H. taenicola.* Two-way ANOVA results for various response variables to the effects of CO_2 (control and enrichment) crossed with species of *Halimeda* (n = 4 per treatment). Response variables are categorized by physiology (net calcification and photophysiology) and thallus characteristics (shedding rate and tissue content). Photosynthetic efficiency is characterized by parameter values from the fitted model (Platt et al. 1980). Significant differences are shown in **bold**. $rETR_{max}$: relative maximum electron transport rate; α: initial slope of rapid light curve; β: photoinhibition

Metric	Effect of CO_2		Effect of species		Effect of CO_2 × Species	
	F	p	*F*	p	*F*	p
Physiology						
Net calcification	**6.63**	**0.0002**	**3.12**	**0.01**	**2.25**	**0.05**
$rETR_{max}$ (μmol electrons m^{-2} s^{-1})	2.15	0.06	**3.96**	**0.003**	**2.18**	**0.05**
α (μmol electrons μmol $quanta^{-1}$)	0.68	0.51	1.11	0.29	0.47	0.65
β (μmol electrons μmol $quanta^{-1}$)	0.74	0.48	**2.76**	**0.02**	0.05	0.96
Morphology						
Segments shed	**2.88**	**0.02**	**3.57**	**0.004**	**2.47**	**0.03**
% $CaCO_3$ and $MgCO_3$	**3.63**	**0.005**	**10.23**	**<0.0001**	**3.77**	**0.004**

ments on all control samples and by an average 5.5 and 5.0% increase in buoyant weight, respectively. Calcification rates of samples in control conditions were significantly greater than zero for both species ($t \geq 2.78$, df = 3, $p \leq 0.047$).

Photophysiology

Elevated pCO_2 affected the photophysiology of *Halimeda opuntia*, but not *H. taenicola*. Overall, *H. opuntia* had higher photosynthetic potential than *H. taenicola*, regardless of treatment conditions, at the beginning (data not shown) and end of the experiment (Fig. 3). Otherwise, initial RLCs were similar across treatments within each species. By the end of the experiment, the mean $rETR_{max}$ was consistently higher for *H. opuntia* in both treatments, despite evidence for relatively stronger inhibition at high irradiances (higher β) in this species; the initial slope of the RLC (α) remained similar across species and treatments (Tables 4 & 5). However, *H. opuntia* exhibited a significant negative photophysiological response to enrichment with CO_2 ($rETR_{max}$: $t = 2.65$, df = 6, p = 0.038), while *H. taenicola* did not respond differently to treatment conditions. *H. opuntia* samples exposed to pCO_2 enrichment showed a 16% decrease in $rETR_{max}$ relative to ambient conditions by the end of the experiment (Fig. 3). The percent dissolved oxygen in the mesocosms was equivalent across both species and treatments (Table 2).

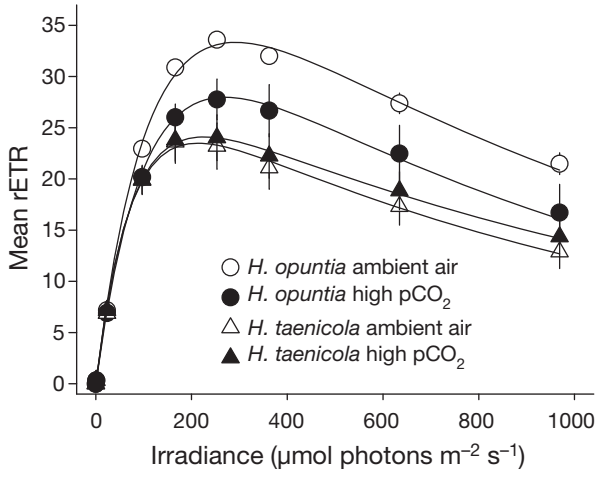

Fig. 3. *Halimeda opuntia* and *H. taenicola.* Mean (±SE) relative electron transport rates (rETR) measured with a diving Pulse Amplitude Modulated (PAM) fluorometer for *H. opuntia* and *H. taenicola* exposed to ambient air or elevated pCO_2 experimental conditions, which demonstrates the photophysiological effects of ocean acidification. Curves overlaying the data points were fit based on a 3 parameter non-linear model (Platt et al. 1980) using a Gauss-Newton nonlinear regression algorithm (n = 4)

Table 5. *Halimeda opuntia* and *H. taenicola.* Mean (±SE) effects of ocean acidification on photosynthetic efficiency for 2 species of *Halimeda* (n = 4 per treatment) estimated from rapid light curve fits using Platt et al. (1980). $rETR_{max}$: relative maximum electron transport rate (μmol electrons m^{-2} s^{-1}); α: initial slope of rapid light curve (μmol electrons μmol $quanta^{-1}$); β: photoinhibition (μmol electrons μmol $quanta^{-1}$)

Treatment	$rETR_{max}$	α	β
H. opuntia			
Ambient air	33.40 ± 0.54	0.380 ± 0.008	0.039 ± 0.003
High pCO_2	28.00 ± 1.97	0.343 ± 0.012	0.038 ± 0.005
H. taenicola			
Ambient air	23.48 ± 2.13	0.389 ± 0.016	0.028 ± 0.002
High pCO_2	24.14 ± 2.12	0.383 ± 0.020	0.024 ± 0.004

Tissue content and senescence

The morphology of each species of *Halimeda* was also affected by CO_2 enrichment but by different mechanisms. In the presence of enriched pCO_2, *H. opuntia* shed thallus segments at 12 times the rate of *H. taenicola*; however this process was also more common for *H. opuntia* under ambient conditions (Fig. 4A). The addition of CO_2 significantly increased the shedding rate for *H. opuntia* (t = 2.71, df = 3, p = 0.03) by 82% while shedding of *H. taenicola* segments remained unaffected (t = 1.26, df = 3, p = 0.25), resulting in a significant interaction term (Table 4). Conversely, the proportion of calcified tissue in *H. opuntia* was unaffected by CO_2 enrichment, while calcified tissue content decreased by 6% in *H. taenicola* (Table 4, Fig. 4B). Finally, *H. taenicola* had a lower $CaCO_3$ content than *H. opuntia* both under control conditions (Fig. 4B) and in field collections (Table 1).

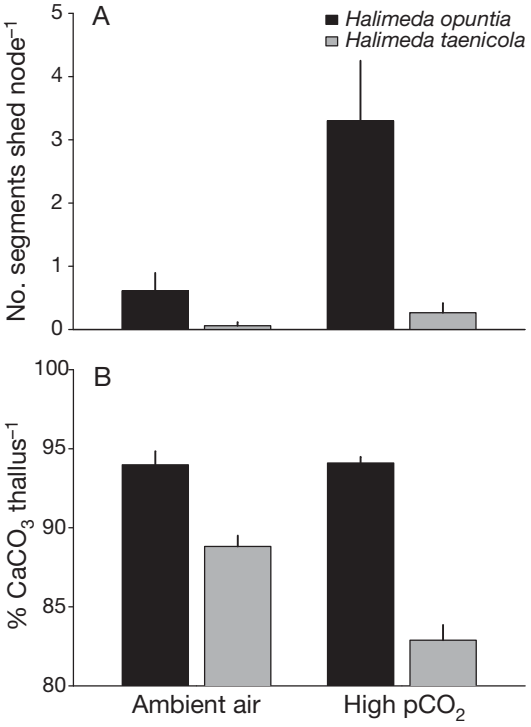

Fig. 4. *Halimeda opuntia* and *H. taenicola*. Mean (+SE) (A) loss of segments per node and (B) percent $CaCO_3$ per thallus for each *H. opuntia* and *H. taenicola* sample (n = 4) in treatment and control aquaria. See Table 2 for description of conditions

DISCUSSION

While experimental studies on organismal responses to the threat of OA are becoming more common, most have examined the consequences for reef building corals (Doney et al. 2009), coccolithophores (Iglesias-Rodriguez et al. 2008), commercially important invertebrates (O'Donnell et al. 2009, Talmage & Gobler 2009, Todgham & Hofmann 2009) and fish species (Munday et al. 2009). In contrast, few studies have addressed the potentially conflicting responses to OA for calcareous macroalgae that simultaneously photosynthesize and calcify (but see studies on CRAs: Anthony et al. 2008, Jokiel et al. 2008, Kuffner et al. 2008, Semesi et al. 2009b). Tropical green calcareous algae, such as *Halimeda*, use photophysiology to precipitate calcium carbonate and thus complicate our ability to predict their response to OA. Our study is the first to examine both of these physiological processes under projected seawater chemistry conditions given current anthropogenic carbon emission rates. Both species of *Halimeda* examined here exhibited consistent negative responses to simulated acidification despite the relatively low replication used in the study. Further, we identified that the magnitude of the response to OA was species specific. *H. opuntia* experienced net dissolution, increased segment shedding, and reduction in photosynthetic capacity after 2 wk. In contrast, *H. taenicola* showed potential for acclimatization to OA by adjusting the percent $CaCO_3$ required to maintain structural integrity, although growth was significantly curtailed in low pH conditions. The results of this short-term study suggest that projected levels of OA will likely have dramatic effects on growth, calcification and photophysiology of *Halimeda.* As discussed below, OA may alter *Halimeda* species abundances, palatability, sand production, primary productivity and carbonate production on coral reefs if these algae are unable to acclimatize or adapt over the next century.

Three recent studies looking at the effects of OA on calcification of *Halimeda* species have reported conflicting results. Ries et al. (2009) did not detect an effect of CO_2 addition on the recruitment of an unidentified species of *Halimeda*, but they did find a non-linear response in the calcification rate of *Halimeda* recruits to simulated OA. In both cases, their results may have been confounded by the unappreciated effects of nutrient addition that commonly occur when heating temperate seawater (from coastal Massachusetts, USA) for the maintenance of tropical species. Release from phosphorus or nitrogen limitation enhances productivity of some *Halimed*a species (Smith et al. 2004) and may have enabled these algae to photosynthesize in experimental aquaria at rates

greater than those possible in nature on oligotrophic reefs. Thus, rearing *Halimeda* in heated temperate seawater, as done in previous studies, dampens the ability to detect potential effects of OA, unless pH is depressed to prohibitively low levels. In contrast, Stanley et al. (2010) reported decreased calcification and linear extension for *H. incrassata* grown under 'calcite sea' conditions (artificially altered Mg/Ca ratios) because calcite was precipitated more readily than aragonite. Unfortunately, none of these studies reported the light levels used in the experiments or measured changes in photosynthetic potential, which makes interpretation of their results challenging. However, the limited data available to date do corroborate our findings and suggest that the tight link between calcification and photosynthesis for *Halimeda* spp. may render these algae extremely susceptible to OA under changing chemical oceanographic conditions.

For every response variable measured, there was a species-specific response whereby *Halimeda taenicola* demonstrated the greatest ability to acclimatize to treatment conditions while *H. opuntia* suffered reduced calcification and productivity. A combination of unique anatomical characteristics and differing physiological processes could explain the relative differences in physiological responses to OA between these algae, which belong to genetically and morphologically distinct lineages (Verbruggen & Kooistra 2004, Verbruggen et al. 2005). *H. opuntia* has more heavily branched, widely spaced siphons that may explain its high $CaCO_3$ content in comparison to *H. taenicola*, which has less branched but much thicker and more tightly packed siphons (Hillis 1959, Verbruggen et al. 2005) (Fig. 1C,D), creating a smaller inter-utricular calcifying space that is more isolated from ambient seawater. Thus, the higher surface to volume ratio between siphons of *H. opuntia* may allow tighter regulation of chemistry within this calcifying space via photophysiology. Furthermore, at the nodes between the segments, *H. opuntia* has short fusions of the siphons (Bandeira-Pedrosa et al. 2003) (Fig. 1E), which can weaken as dissolution occurs and contribute to the likelihood of segment shedding. In contrast, *H. taenicola* has extended fusions (Fig. 1F), which may strengthen the junction at the node, even in the absence of $CaCO_3$ skeletal support (Hillis 1959, Verbruggen et al. 2005). Thus, the dependence of an organism on calcification for structural integrity may predict its relative vulnerability to the consequences of OA.

While dissolved CO_2 (and lowered carbonate saturation state) slows calcification, it also provides a necessary substrate for photosynthesis. Photosynthetic capacity, as measured by relative chlorophyll fluorescence, did not respond positively to pCO_2 enrichment for either *Halimeda* species, suggesting that these photoautotrophs were not carbon limited in our short experiment, although irradiance was fairly low. Instead, *H. opuntia* experienced a significant reduction in photosynthetic potential in response to enriched pCO_2 in treatment mesocosms, while *H. taenicola* remained unaffected (Fig. 3). Similar depressions of productivity have been documented for some species of corals and for crustose coralline algae in response to elevated pCO_2 (Anthony et al. 2008), but other studies have found enhancement of photosynthesis for red calcified algae (Semesi et al. 2009b) and coccolithophores (Iglesias-Rodriguez et al. 2008). This discrepancy could arise because some species of *Halimeda* (not used in this study) possess CCMs and use bicarbonate as an alternate source of inorganic carbon during photosynthesis (Borowitzka & Larkum 1976b, Matsuda et al. 1998, De Beer & Larkum 2001, Giordano et al. 2005). While CCMs can enhance photosynthetic efficiency, they are used at an energetic cost to the organism due to the production of carbonic anhydrase to catalyze the hydration of CO_2 and active ion pumping across membranes. Seawater more replete with dissolved CO_2 can degrade CCM induction, causing the alga to rely on passive diffusion of CO_2 alone, thus leading to reduced potential efficiency of carbon assimilation (Collins et al. 2006). Our limited study shows that excess dissolved CO_2 not only depresses Ω-aragonite for calcification but also compromises photophysiology for *H. opuntia*, suggesting that OA may cause negative synergistic challenges for this species.

A key function of the carbonate skeleton in calcified algae, other than to secure the alga to the substrate and support the thallus in the water column, is to inhibit grazing. The degree to which an alga is calcified can deter herbivory (Hay et al. 1988, Paul & van Alstyne 1988, Pennings & Paul 1992). In fact, plant toughness can be a stronger deterrent than chemical defenses against herbivory (Pennings & Paul 1992). For *Halimeda*, herbivory largely occurs at the tips of branches where new growth is initiated and young segments have not yet fully calcified; mature, heavily calcified segments do not hold the same relative nutritional value (Hay et al. 1988). While *H. taenicola* did not experience any change in photophysiology or segment shedding rate in treatment conditions, this species did suffer reduced calcification and $CaCO_3$ content in the thallus. If the

decreased $CaCO_3$ content in the thallus renders those segments more palatable, then increasing pCO_2 may further indirectly affect the abundance of this alga on tropical reefs. Changes in grazing intensity and preference due to algal acclimatization to OA may lead to shifts in benthic community structure not predicted by the physiological response of calcification alone.

Our results suggest that general predictions about the biological consequences of OA for benthic calcified macroalgae are difficult to make as individual species, even within a genus, can vary widely in their responses to increased pCO_2. However, responses to CO_2 enrichment may be related to morphological characteristics, such as the degree to which an alga relies on its $CaCO_3$ skeleton for structural integrity and the relative isolation of the calcifying space, and organismal physiology, such as the biological mechanism of calcification and the relative dependence of photophysiology on CCMs. Increased pCO_2 resulted in reduced growth rates, exacerbated segment shedding, dissolution of carbonate, and reduced photosynthetic efficiency for *Halimeda opuntia,* a highly calcified species that disintegrates without the structural support of a skeleton. This species showed less plasticity to reduced pCO_2 than *H. taenicola*, which only experienced reduced calcification yet maintained structural integrity, in part because it has more robust and densely packed siphons that give it a thicker fleshy thallus than other species. The responses to OA may be yet predicted by taxonomy; the heavily calcified and more predominant species (e.g. species from the *Opuntioid* lineage) appear to be more susceptible to OA than less calcified and less abundant but more resilient *Halimeda* (e.g. species from the *Halimeda* or *Rhipsalis* lineages; see Table 1).

The results of this study are novel and intriguing but are constrained by the nature of short-term mesocosm 'shock' experiments. The algae were exposed to rapidly changing chemical conditions and did not have the chance to acclimatize or adapt to slowly decreasing pH, as is anticipated with OA. There is an urgent need to conduct field experiments that harness the naturally varying carbonate chemistry (driven by upwelling, pollution or reef metabolism) across coral reefs to study the long-term response of coral reef organisms to changes in dissolved inorganic carbon. Additionally, the interaction of the potential global threat of increased carbon emissions (including rising sea surface temperature) with more localized anthropogenic stressors such as overfishing and/or pollution may exacerbate or negate biological consequences predicted from OA alone. While we are beginning to gain an understanding of some of the species specific responses to increased pCO_2, few data exist on the potential synergies of multiple stressors on species interactions and benthic reef community structure. Our results represent some of the first evidence of the potential effects of OA on 2 common and important calcifying species of the green algae *Halimeda* in the Pacific, and we detected large species specific responses that could potentially alter ecosystem structure and functioning on tropical reefs. The growing body of research investigating the effects of increased pCO_2 on marine organisms, including the results reported here, continues to highlight the variability in species responses and thus identifies the complexity in predicting the community and ecosystem wide consequences of OA in marine environments.

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