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Calcareous green alga *Halimeda* tolerates ocean acidification conditions at tropical carbon dioxide seeps

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Abstract

We investigated ecological, physiological, and skeletal characteristics of the calcifying green alga *Halimeda* grown at CO_2 seeps ($pH_{total} \sim 7.8$) and compared them to those at control reefs with ambient CO_2 conditions ($pH_{total} \sim 8.1$). Six species of *Halimeda* were recorded at both the high CO_2 and control sites. For the two most abundant species *Halimeda digitata* and *Halimeda opuntia* we determined in situ light and dark oxygen fluxes and calcification rates, carbon contents and stable isotope signatures. In both species, rates of calcification in the light increased at the high CO_2 site compared to controls (131% and 41%, respectively). In the dark, calcification was not affected by elevated CO_2 in *H. digitata*, whereas it was reduced by 167% in *H. opuntia*, suggesting nocturnal decalcification. Calculated net calcification of both species was similar between seep and control sites, i.e., the observed increased calcification in light compensated for reduced dark calcification. However, inorganic carbon content increased (22%) in *H. digitata* and decreased (−8%) in *H. opuntia* at the seep site compared to controls. Significantly, lighter carbon isotope signatures of *H. digitata* and *H. opuntia* phylloids at high CO_2 (1.01‰ [parts per thousand] and 1.94‰, respectively) indicate increased photosynthetic uptake of CO_2 over HCO_3^- potentially reducing dissolved inorganic carbon limitation at the seep site. Moreover, *H. digitata* and *H. opuntia* specimens transplanted for 14 d from the control to the seep site exhibited similar $\delta^{13}C$ signatures as specimens grown there. These results suggest that the *Halimeda* spp. investigated can acclimatize and will likely still be capable to grow and calcify in P_{CO_2} conditions exceeding most pessimistic future CO_2 projections.

Anthropogenic emissions are increasing the carbon dioxide partial pressure (P_{CO_2}) in the atmosphere (IPCC 2013). The present-day level of ~ 40 Pa (equivalent to 395 μatm) (Dlugokencky and Tans 2014) has already exceeded historic P_{CO_2} levels observed over the last two million years (Hönisch et al. 2009) and is predicted to double or triple from present-day levels within this century (Collins et al. 2013; Meinshausen et al. 2011; Moss et al. 2010). Increased P_{CO_2} consequently leads to a decrease in ocean pH and aragonite saturation state Ω_{ar}, a process called ocean acidification (OA). According to the Intergovernmental Panel on Climate Change (IPCC 2013), the surface ocean will experience a further reduction of 0.203–0.310 pH units (representative concentration pathway, RCP6.0 to RCP8.5) by the year 2100 (Ciais et al. 2013).

Potential effects of OA on life history traits, such as survival, growth, reproduction, and recruitment of marine organisms have been recently reported. It is becoming apparent that tropical coral reefs in particular are facing major ecological changes in the upcoming decades (Pandolfi et al. 2011). An emerging paradigm suggests that marine organisms will be negatively affected by OA. Indeed, some taxa may be strongly impeded and may even become extinct in future environmental conditions (Carpenter et al. 2008; Uthicke et al. 2013). However, studies also suggest species specific responses to OA and that not every organism will be affected in future OA environments and that some taxa may also be able to cope with, or even thrive, under projected CO_2 conditions (Fabricius et al. 2011; Johnson et al. 2012; Ries et al. 2009).

Most conclusions of impacts of OA on organisms and consequent extrapolations to ecosystem level are derived from laboratory experiments. Although experiments control environmental factors, allowing comparisons between studies, they mostly do not account for intraspecific and

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interspecific interactions, natural supply of nutrition, and natural fluctuation of parameters, such as light, temperature, and pH. Therefore, investigating organisms in situ in their natural environment, exposed to P_{CO_2} conditions projected for the near future could be the key in understanding acclimatization processes on organisms and changes of coral reefs at the ecosystem level.

Natural volcanic CO_2 seeps provide a unique opportunity to study the responses of organisms to increased CO_2 conditions, in their natural habitat. Benthic organisms growing close to CO_2 seeps have been exposed to these conditions throughout their life time and some may have been there for many generations. Hence, organisms living at natural volcanic seeps are acclimatized (i.e., physiologically adjusted to a changed environment) and in some cases potentially adapted (i.e., genetically changed traits over several generations) to elevated CO_2 environments. Volcanic CO_2 seeps thus provide an opportunity to identify which organisms are capable of living in CO_2 conditions projected globally in a few decades time and to investigate how these organisms are able to do so.

Volcanic CO_2 seeps in Milne Bay, Papua New Guinea (PNG), provide unique natural CO_2 conditions in shallow tropical waters (Fabricius et al. 2011), without additional freshwater or nutrient upwelling. Detailed studies thus far have also identified no other stressors such as elevated temperatures or heavy metal concentrations interfering with interpretation as OA as the only stressor. CO_2 from the ascending bubbles changes the carbonate chemistry of the seawater close to the seeps and establishes a pH gradient from ambient pH ($pH_{total} \sim 8.1$), over predicted future pH ($pH_{total} \sim 7.9$), to extremely low pH ($pH_{total} < 7$) conditions. Areas of moderate seep activity are characterized by water quality parameters which are likely to be reached worldwide in a few decades time, following RCP6.0 to RCP8.5 (Moss et al. 2010). The seep sites in PNG have been active for at least the last 80 yr, as confirmed by oral communication with traditional inhabitants, and possibly much longer (Fabricius et al. 2011). Therefore, the organisms living on the reefs impacted by those seeps are acclimatized to a high CO_2 environment for many decades.

Volcanic CO_2 seeps and areas of CO_2 upwelling have been described worldwide in temperate (Calosi et al. 2013; Cigliano et al. 2010; Hall-Spencer et al. 2008; Inoue et al. 2013; Johnson et al. 2012; Porzio et al. 2011) and tropical (Fabricius et al. 2011, 2014; Johnson et al. 2012; Noonan et al. 2013; Russell et al. 2013; Uthicke and Fabricius 2012; Uthicke et al. 2013) regions. So far, studies suggest reduced pH at CO_2 seeps in PNG lead to a decline in coral diversity with structurally complex species being particularly affected, and reduced taxonomic richness and density of coral juveniles, and low cover of crustose coralline algae (Fabricius et al. 2011, 2014). Next to direct physiological impacts on organisms, a loss of habitat complexity at CO_2 seeps indirectly leads to decreased densities of macroinvertebrate taxa (Fabricius et al. 2014). Densities and diversity of large benthic foraminifera decrease at seep sites and are absent at $pH_{total} < 7.9$, which is only a 0.2 unit reduction to present-day levels (Uthicke and Fabricius 2012; Uthicke et al. 2013). In contrast, cover of some calcareous and non-calcareous macroalgae and seagrasses increased at CO_2 seeps compared to controls (Fabricius et al. 2011; Johnson et al. 2012), indicating tolerance or acclimatization of some organisms to future P_{CO_2} conditions and possible gains in rates of photosynthesis.

Halimeda, a genus of calcareous green algae are important, fast growing primary producers associated with coral reefs. Their calcium carbonate ($CaCO_3$) skeletons contribute significantly to carbonate production and sediment formation (Freile et al. 1995; Rees et al. 2007; Wefer 1980). *Halimeda* spp. deposit aragonite, which is the more soluble form of the most common $CaCO_3$ minerals. Moreover, *Halimeda* spp. provide important habitat for invertebrate communities (Fukunaga 2008). However, impacts of low pH on *Halimeda* spp. have not been investigated at tropical CO_2 seeps. Findings from volcanic seeps in Mediterranean showed that temperate *Halimeda* spp. were absent at mean $\Omega_{ar} \leq 2.5$ (Hall-Spencer et al. 2008). Laboratory experiments revealed mixed responses of OA on *Halimeda* spp., with some species being negatively impacted, while others are not (Hofmann et al. 2014; Koch et al. 2013; Price et al. 2011; Ries et al. 2009; Sinutok et al. 2011; N. Vogel et al. pers. comm.). Moreover, the calcareous brown algae *Padina* spp. are thriving at seeps in the Mediterranean and in PNG with increased abundance at CO_2 seep sites compared to controls (Johnson et al. 2012), suggesting that some calcareous organisms can benefit from increased CO_2 availability. It is, therefore, not clear how calcifying algae, among the most important organism groups in coral reefs, respond to OA.

This study investigates for the first time in situ ecological, physiological, and skeletal characteristics of calcareous green algae of tropical *Halimeda* after a lifetime exposure to high levels of CO_2. The distribution of six species of *Halimeda* was investigated in relation to the seawater carbonate chemistry from water samples collected at the site of occurrence. For the two most abundant *Halimeda* spp. (*Halimeda cuneata f. digitata* and *Halimeda opuntia*), in situ rates of oxygen fluxes and calcification in light and in darkness, organic-, and inorganic carbon content and carbon isotopic signatures ($\delta^{13}C$) were compared between CO_2 seep and control site.

Materials and methods

Site description

At several locations in the Milne Bay Province, PNG (Fig. 1a), volcanic CO_2 is seeping out of the seafloor (Fabricius et al. 2011). The seep sites are located at Dobu Island and Upa-Upasina (Normanby Island) close to the shore in shallow water of ~ 1 m to 15 m depth and extend over an area of ~ 20 m by 100 m with different intensities of bubble

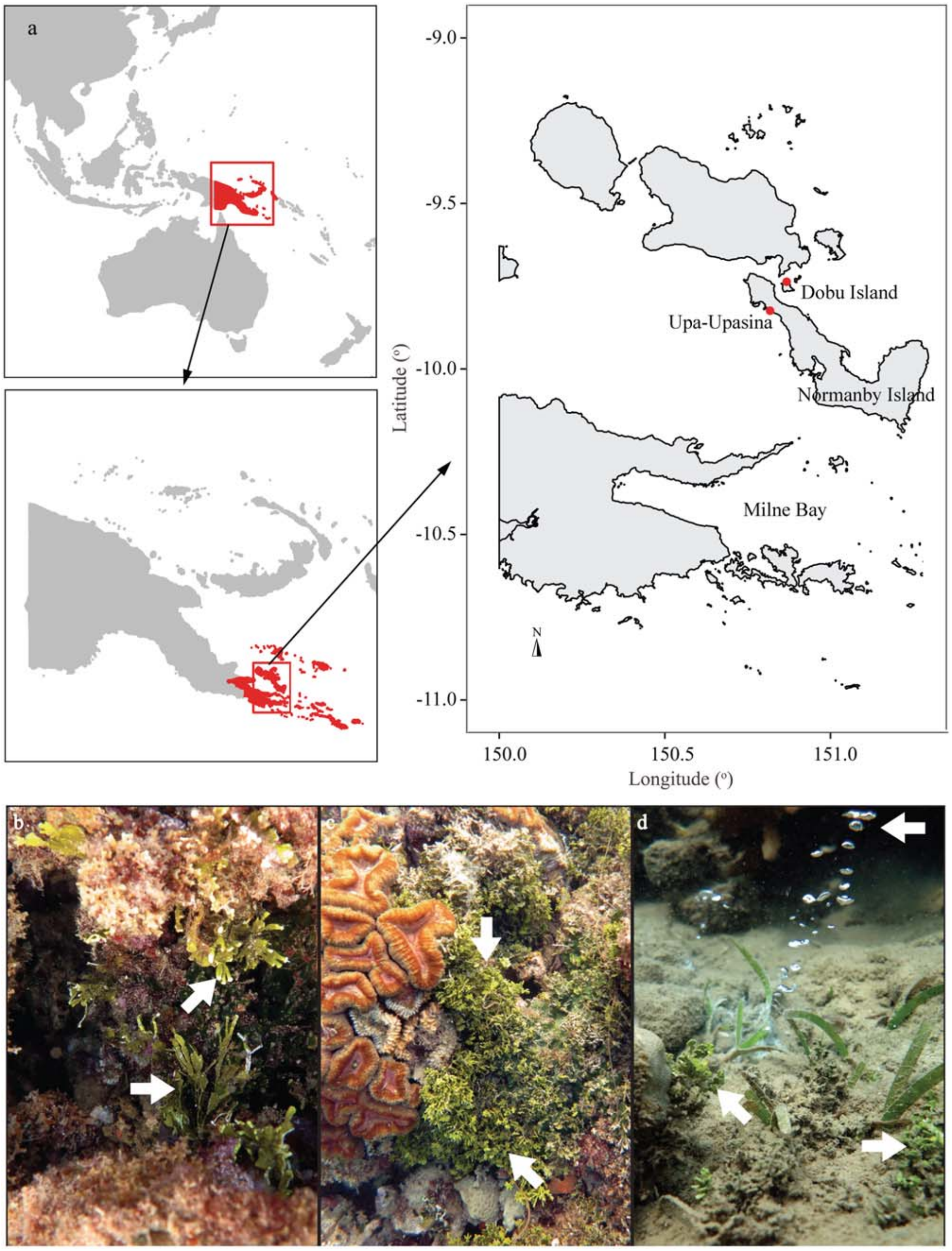

Fig. 1. (a) Map of Papua New Guinea, Milne Bay Province and Normanby Island with locations of seep sites at Dobu Island and Upa-Upasina. (b) *H. digitata* growing at CO_2 seep site (Upa-Upasina). (c) *H. opuntia* growing at CO_2 seep site (Upa-Upasina). (d) *H. opuntia* growing next to CO_2 bubbles (Dobu Island).

activity within this area. Control reefs were allocated several hundred meters away from the seep sites with no impact of the seep activity on their seawater carbonate system (Table 1). The bubbles, which consist of pure CO_2, ascend to the surface and mix with the ambient seawater, changing the carbonate chemistry. This study was confined to areas where seawater chemistry was altered to levels projected for a vast part of the globe for the end of this century (RCP6.0 to RCP8.5 scenarios) (Moss et al. 2010) (Table 1).

Sample collection

Water samples (Table 1) for occurrence/OA tolerance of *Halimeda* species were collected at Dobu Island control and seep site (S 9° 45.125′, E 150° 51.248′, and S 9° 44.199′, E 150° 52.060′, respectively) and Upa-Upasina control and seep site (S 9° 49.693′, E 150° 49.231′, and S 9° 49.446′, E 150° 49.055′, respectively) in April/May 2012 and May/June 2013. Physiological characteristics and skeletal properties of *Halimeda cuneata f. digitata* (referred as *H. digitata*) and *H. opuntia* were

Table 1. Carbonate system parameters of water samples from in situ collections (n_{total} = 86) (Dobu Island and Upa-Upasina 2012, 2013), incubations (n_{total} = 30) (Upa-Upasina 2012), and transplant experiment (n_{total} = 50) (Upa-Upasina 2012). Data are given as mean and standard deviation. kgSW, kilograms of sea water.

Treatment	pH_{NIST}	pH_{total}	Temp (°C)	TA (μmol $kgSW^{-1}$)	DIC (μmol $kgSW^{-1}$)	P_{CO_2} (Pa)	HCO_3^- (μmol $kgSW^{-1}$)	CO_3^{2-} (μmol $kgSW^{-1}$)	CO_2 (μmol $kgSW^{-1}$)	Ω_{ca}	Ω_{ar}
In situ samples/occurrence											
Control	8.23 (0.03)	8.12 (0.01)	29.8 (0.6)	2282 (32)	1907 (28)	33 (1)	1636 (24)	263 (4)	8.30 (0.20)	6.45 (0.12)	4.31 (0.09)
Impact	7.81 (0.30)	7.66 (0.30)	29.2 (0.6)	2249 (18)	2106 (129)	156 (140)	1941 (161)	125 (65)	40.02 (35.92)	3.07 (1.60)	2.04 (1.07)
Incubation experiment											
Control	8.26 (0.02)	8.17 (0.01)	29.0 (1.4)	2277 (30)	1900 (26)	31 (3)	1629 (40)	263 (22)	8.06 (0.86)	6.43 (0.56)	4.28 (0.39)
Impact	7.87 (0.10)	7.77 (0.07)	29.1 (1.5)	2330 (27)	2168 (34)	97 (13)	2014 (42)	129 (16)	25.28 (3.50)	3.16 (0.40)	2.10 (0.27)
Transplant experiment											
Control	8.25 (0.03)	8.14 (0.03)	29.2 (1.1)	2287 (27)	1915(28)	33 (3)	1646 (39)	261 (18)	8.38 (0.80)	6.38 (0.46)	4.25 (0.32)
Impact	7.90 (0.09)	7.83 (0.07)	29.1 (1.2)	2332 (24)	2142 (40)	86 (17)	1973 (58)	147 (26)	22.01 (4.72)	3.61 (0.64)	2.41 (0.43)

determined at Upa-Upasina control and seep site in April and May 2012. Specimens of *H. digitata* and *H. opuntia* (Fig. 1b,c) were sampled between 4 m and 6 m water depth at the control and CO_2 seep sites. Samples for inorganic carbon content and carbon isotopic signatures ($\delta^{13}C$) were rinsed in freshwater and dried for 48 h at 40°C for subsequent analyses.

Occurrence/OA tolerance

To determine OA tolerance, water samples (n_{total} = 86) were collected 5–10 cm above *Halimeda* spp. thalli, growing at the control and seep sites of Dobu and Upa-Upasina by snorkeling and scuba diving. Water samples were analyzed for pH_{NIST}, temperature and voltage in millivolts (mV) with a temperature corrected bench top pH meter (OAKTON, USA) and a refillable pH probe (Eutech), calibrated on NIST (National Institute of Standards and Technology) scale. Additional pH readings were performed with Tris-buffer in artificial seawater supplied by A. Dickson (Scripps Institute for Oceanography) to determine the accuracy of pH measurements in 2012 and 2013 (n = 19, pH = 8.15 ± 0.05 SD, temperature = 29.3 ± 1.2°C). Millivolt and temperature measurements were used to convert pH values to total scale (pH_{total}). In some instances, conversion to total scale, lowered the variance of pH readings, as indicated in Table 1. Water collections were repeated on several days in 2012 and 2013 in the mornings and evenings, to incorporate diurnal pH fluctuations, over a total of six sampling events.

Seawater carbonate system parameters

Subsamples (50 mL) of seawater were directly titrated for total alkalinity (TA) on a Metrohm 855 robotic titrosampler (Metrohm, Switzerland) by gran titration, using 0.5 mol L^{-1} HCl as described in (Uthicke and Fabricius 2012). TA was calculated by nonlinear regression fitting of hydrogen ion concentration and the volume of titrant between pH 3.5 and pH 3.0, following the Standard Operating Procedure SOP3b outlined in the “Guide to Best Practices for Ocean CO_2 Measurements” (Dickson et al. 2007). Acid concentration was corrected by titrating Certified Reference Material (CRM Batch 106, A. Dickson, Scripps Oceanographic Institute). The accuracy of TA measurements was determined by CRM titrations in 2012 and 2013 (n = 38, TA = 2218 ± 11 SD). Carbonate system parameters (Table 1) of incubations and field samples were calculated utilizing pH_{total} and TA measurements by CO2calc software (Robbins et al. 2010) using CO_2 constants from Lueker et al. (2000).

Calcification and photosynthesis

Calcification rates in the light and dark, as well as net photosynthesis and respiration rates, were measured in situ at control (pH_{total} = 8.17) and seep sites (pH_{total} = 7.77, *see* Table 1 for carbonate chemistry). Branches 5–8 cm in height and with ~ 20 phylloids of *H. digitata* and *H. opuntia* were collected and retained at the site of collection until incubations commenced. Light incubations were conducted in situ

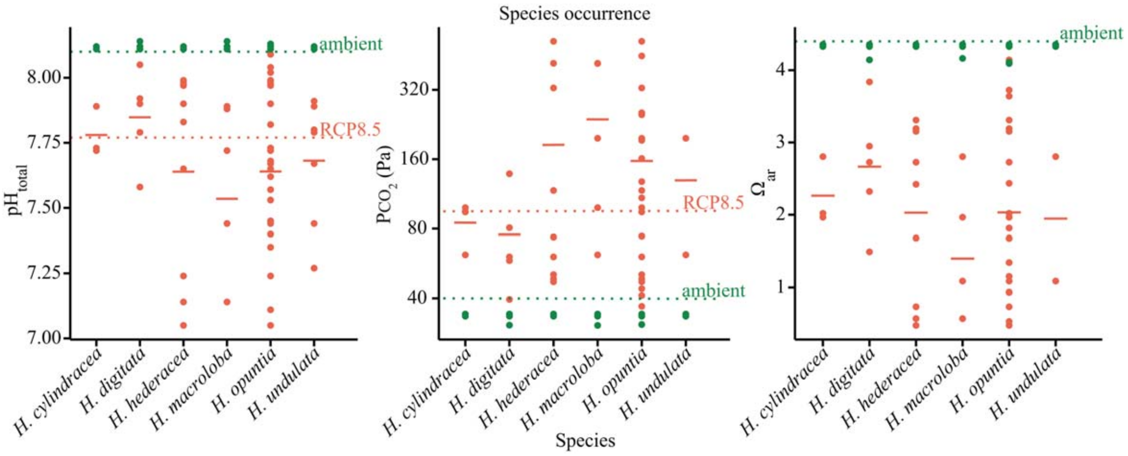

Fig. 2. Carbonate system parameters of water samples collected above *Halimeda* species growing at Dobu Island and Upa-Upasina control and seep site. Each dot represents a water sample collected above the corresponding species (green = control site, red = seep site). Dotted lines indicate ambient (green) levels and predicted future (red) levels following the most pessimistic 'representative concentration pathway' RCP8.5. Solid lines (red) represent mean values of water samples for each species, collected at the seep site.

at 5 m water depth at midday. Specimens were placed into 0.5-L clear Perspex chambers, simultaneously at control and seep sites, by two separate SCUBA diving teams. After ~ 3 h incubation under ambient light, incubation chambers were retrieved and a water subsample was directly analyzed for TA (as described above). Oxygen concentration was determined in each incubation chamber including two blank incubations per treatment (to correct for seawater production/respiration) with a hand-held dissolved oxygen meter (HQ30d, Hach) as described elsewhere (Uthicke and Fabricius 2012; Witt et al. 2012). Light intensities of incubation conditions were recorded by two light loggers (Odyssey, New Zealand) each at control and seep site. Photosynthetically available radiation (PAR) was dependent on weather conditions and averaged 34 and 39 μmol photons m^{-2} s^{-1} at the control and seep site, respectively, for *H. digitata* and 259 and 281 μmol photons m^{-2} s^{-1} for *H. opuntia* incubations. Dark incubations were conducted on board the research vessel for ~ 3 h in the evening. The incubation chambers were filled with water from the site of origin of the plants (control vs. seep site). Chambers were placed in black plastic bins (45L) with lids for darkening and flow-through seawater for temperature control. Rates of calcification were determined with the alkalinity anomaly technique (Chisholm and Gattuso 1991). Calcification rates (in μmol L^{-1} C h^{-1} gFW^{-1}) and oxygen fluxes (in μg O_2 h^{-1} gFW^{-1}) were calculated in relation to blank incubations and standardized to the fresh weight (FW) of the plants. Daily net calcification rates were calculated by 12 h of daylight and 12 h of darkness.

C and N contents and stable isotope signatures

Apical phylloids of dried *Halimeda spp*. were crushed with mortar and pestle and the homogenate was analyzed for total carbon (C_{tot}) and total nitrogen (N) on a Flash EA 1112 elemental analyzer (Thermo Fisher Scientific). In addition, organic carbon (C_{org}) contents were measured after acidifying the sample with 150 μL concentrated HCl to drive out C_{inorg}. Inorganic carbon content was calculated by subtracting C_{org} from C_{tot}. Stable isotope signatures were measured in a subset of these samples using a Delta S mass spectrometer (Thermo Fisher Scientific) coupled with the elemental analyzer.

Transplant experiment

A transplant experiment was carried out at Upa-Upasina in 2012 over a period of 14 d. Branches (~ 20 to 30 phylloids) of *H. digitata* and *H. opuntia* were collected at the control and seep sites and attached onto plastic trays, assuring specimens were physically separated. Three replicate trays were deployed at each site in 5 m of water. Six individuals of each species were transplanted from control to control (CC) and control to impact site. Two light loggers (Odyssey, New Zealand) were deployed next to each experimental site, to record PAR throughout the course of the experiment. Recordings of daily light sums averaged 5.31 and 4.34 mol photons m^{-2} d^{-1} for control and seep site, respectively, with light maxima of 667 μmol and 707 μmol photons m^{-2} s^{-1}. One layer of thin wire mesh (~ 3 cm mesh size) was wrapped around each tray to assure protection from large herbivore fish. After two weeks, specimens were sampled, rinsed in fresh water, and dried for 48 h at 40°C for subsequent carbon, nitrogen, and stable isotope signature analyses as described above.

Statistical analyses

To determine significant differences of responses between controls and seep sites, statistical analyses were conducted with the software R (R Development Core Team 2014). For each response variable measured, we performed Linear

Models with location (control and seep site) as fixed factor. To test data for equal variance and homogeneity, we performed Levene's tests on each response variable. In case the null hypothesis was rejected (i.e., the variance between groups was unequal), we transformed ($\log_{10}$ or arcsine dependent on variable) the data prior to subsequent analyses.

Results

In situ samples

At control and seep site a total of six different *Halimeda* species were identified with either lightly calcified (LC), calcified (C), and heavily calcified (HC) phylloids. Species included *H. cylindracea* (C), *Halimeda cuneata f. digitata* (LC), *Halimeda cuneata f. undulata* (LC), *Halimeda hederacea* (HC), *Halimeda macroloba* (LC), and *H. opuntia* (HC) (Littler and Littler 2003). Water samples above the thalli ranged from $pH_{total} = 8.14$ at the control sites to $pH_{total} = 7.05$ at the seep sites (Fig. 2, Table 1). Water samples collected above *Halimeda* spp. at the control sites yielded mean pH_{total} of 8.12 and samples from the seep sites yielded mean pH_{total} of 7.66 (Table 1). Calculated mean P_{CO_2} and Ω_{ar} from collected water samples were 33 (± 1 SD) Pa and 4.31 (± 0.09 SD) at the control site and 156 (± 140 SD) Pa and 2.04 (± 1.07 SD) at the seep site, respectively. Based on personal observations during ~ 47 dive hours the most abundant species at both control and seep sites appeared to be *H. digitata* and *H. opuntia*.

Mean rates of light calcification of both *H. digitata* and *H. opuntia*, were significantly increased at the seep site (131% and 41%, respectively) compared to the control site (Fig. 3, Table 2, Linear Models $p = 0.020$ and $p = 0.049$, respectively). Rates of calcification in the dark of *H. digitata* was not affected by CO_2, while rates of *H. opuntia* were significantly decreased (−167%) at the seep site resulting in $CaCO_3$ dissolution in the dark (Fig. 3, Table 2, $p = 0.013$). Calculated net calcification rates of both *H. digitata* and *H. opuntia* were not significantly affected by CO_2 (Fig. 3, Table 2).

Net photosynthesis of both species did not differ between seep and control site under experimental light conditions (Fig. 4, Table 2), but mean respiration rate of *H. digitata* was significantly increased (96%) at the seep site compared to the control site (Table 2, $p = 0.029$). Gross photosynthesis of both *H. digitata* and *H. opuntia* was not affected by elevated CO_2 (Table 2).

Total carbon content of *H. digitata* tissue was significantly higher (15%) at the seep, compared to the control site (Fig. 5, Table 2, $p = 0.010$), while organic carbon content was significantly lower (−29%) at the seep compared to the control site (Fig. 5, Table 2, $p = 0.0022$). *H. digitata* grown at the seep site showed significantly higher inorganic carbon content (22%), compared to control site (Fig. 5, Table 2, $p = 0.003$). Total and organic carbon contents of *H. opuntia* tissue did not differ between sites. However, inorganic carbon content of *H. opuntia* was significantly lower (−8%) at the seep compared to the control site (Fig. 5, Table 2, $p = 0.037$). Moreover, $C_{org} : C_{inorg}$ ratio of *H. digitata* was significantly lower at the seep compared to control site (Fig. 5, Table 2, $p = 0.0015$), while $C_{org} : C_{inorg}$ ratio of *H. opuntia* did not significantly differ between sites.

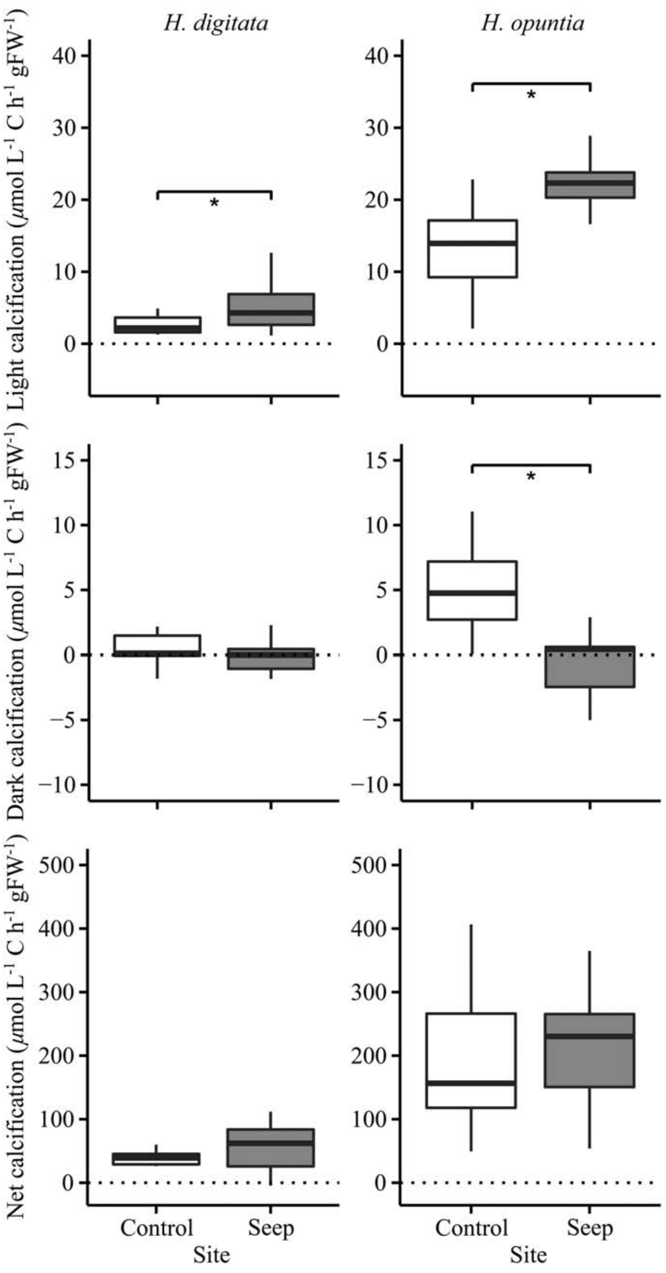

Fig. 3. In situ light-, dark- and net-calcification rates of *H. digitata* and *H. opuntia* grown at control and CO_2 seep site. Brackets indicate significant differences in ANOVA's, with significance levels * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

Stable carbon isotope signatures of both *H. digitata* and *H. opuntia* specimens were significantly lower (5% and 8%, respectively) at the seep compared to the control site (Fig. 6,

Table 2. Linear Model Analysis of Variance results for physiological and skeletal parameters of *H. digitata* and *H. opuntia* with control and seep site as source of variation. Asterisk's indicate significant differences of response variables between control and seep site.

Response variable	Species	Source of variation	df	*F*-value	*p*-value
Light calcification	*H. digitata*	Site	1	6.34	0.0200*
		Residuals	21		
	H. opuntia	Site	1	4.32	0.0495*
		Residuals	22		
Dark calcification	*H. digitata*	Site	1	0.59	0.4497
		Residuals	21		
	H. opuntia	Site	1	7.33	0.0129*
		Residuals	22		
Net calcification	*H. digitata*	Site	1	0.46	0.5060
		Residuals	21		
	H. opuntia	Site	1	0.14	0.7126
		Residuals	22		
Net photosynthesis	*H. digitata*	Site	1	3.19	0.0886
		Residuals	21		
	H. opuntia	Site	1	1.09	0.3084
		Residuals	22		
Respiration	*H. digitata*	Site	1	6.16	0.0216*
		Residuals	21		
	H. opuntia	Site	1	0.23	0.6362
		Residuals	22		
Gross photosynthesis	*H. digitata*	Site	1	0.25	0.6197
		Residuals	21		
	H. opuntia	Site	1	1.23	0.2786
		Residuals	22		
C_{tot}	*H. digitata*	Site	1	8.15	0.0098*
		Residuals	20		
	H. opuntia	Site	1	2.22	0.1520
		Residuals	20		
C_{org}	*H. digitata*	Site	1	12.36	0.0022*
		Residuals	20		
	H. opuntia	Site	1	3.11	0.0930
		Residuals	20		
C_{inorg}	*H. digitata*	Site	1	11.83	0.0026*
		Residuals	20		
	H. opuntia	Site	1	5.00	0.0369*
		Residuals	20		
$C_{org} : C_{inorg}$	*H. digitata*	Site	1	13.59	0.0015*
		Residuals	20		
	H. opuntia	Site	1	3.56	0.0737
		Residuals	20		
$\delta^{13}C$	*H. digitata*	Site	1	9.50	0.0056*
		Residuals	21		
	H. opuntia	Site	1	19.07	0.0003*
		Residuals	20		
$\delta^{13}C$ (transplant)	*H. digitata*	Site	1	9.07	0.0093*
		Residuals	14		
	H. opuntia	Site	1	8.16	0.0114*
		Residuals	16		

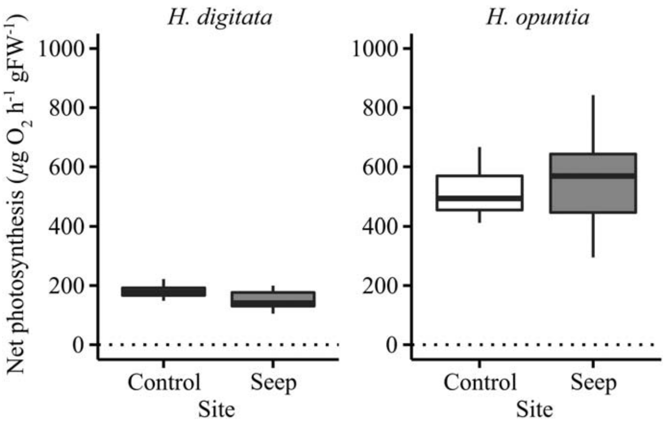

Fig. 4. In situ rates of net photosynthesis of *H. digitata* and *H. opuntia* grown at control and CO_2 seep site.

Table 2, $p = 0.006$ and $p < 0.001$, respectively). Thus, both the species showed proportionally increased fixation of lighter ^{12}C at the seep compared to the control site.

Transplant experiment

After 14 d, stable carbon isotope signatures of newly grown phylloids of both, *H. digitata* and *H. opuntia* were significantly lower (9% and 15%, respectively) in thalli that were transplanted from the control to the seep site (Fig. 6, Table 2, $p = 0.010$ and $p = 0.011$, respectively). $\delta^{13}C$ values became more negative and matched with carbon isotope signatures from *Halimeda* spp. that originally grew at the seep site. As negative control, thalli transplanted from the control to the control site matched with the carbon isotope signatures of *Halimeda* spp. that originally grew at the control site.

Discussion

We investigated ecological, physiological, and skeletal characteristics of *Halimeda* spp. acclimatized to elevated CO_2 environments at volcanic seep sites and compared these to control reefs. Notably, we recorded six different *Halimeda* species growing within areas close to CO_2 seeps and at control sites at Dobu Island and Upa-Upasina, with all species observed down to a pH_{total} level of at least ~ 7.7 (P_{CO_2} ~ 100 Pa). At several locations, we observed *Halimeda* spp. growing directly next to ascending CO_2 bubble streams (Fig. 1d). Water parameters showed some *Halimeda* spp. were still capable to grow in occasional extreme pH conditions ($pH_{total} < 7$) and Ω_{ar} under-saturation ($\Omega_{ar} < 1$) (Fig. 2). Thus, *Halimeda* spp. at seep sites were growing in P_{CO_2} conditions that exceed the most negative "representative concentration pathway" RCP8.5 (IPCC 2013; Moss et al. 2010). This observation stands in contrast to observations at CO_2 seeps in the Mediterranean, where temperate *Halimeda* spp. were absent at the site impacted by CO_2 seeps (Hall-Spencer et al. 2008).

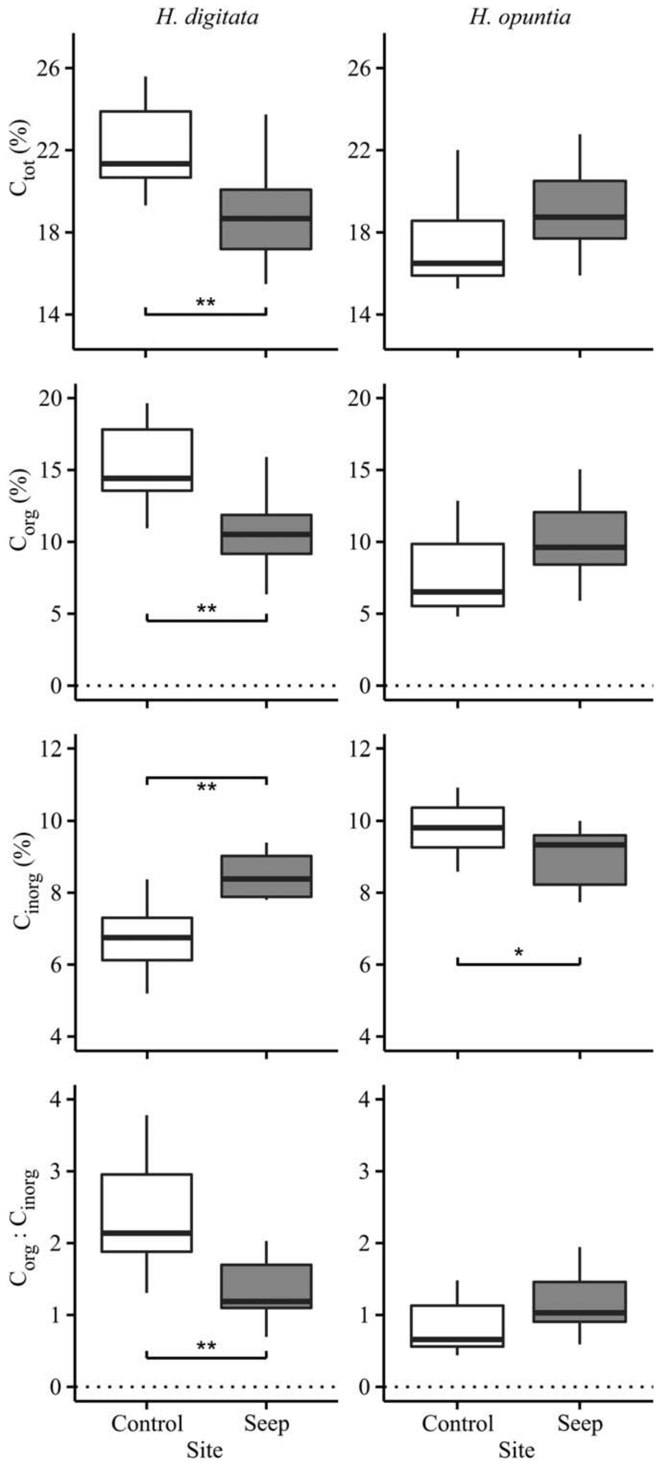

Fig. 5. Total-, organic- and inorganic-carbon content and $C_{org} : C_{inorg}$ ratio of *H. digitata* and *H. opuntia* grown at control and CO_2 seep site. Brackets indicate significant differences in ANOVA's, with significance levels * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

Why temperate *Halimeda* spp. are absent in elevated CO_2 conditions, while tropical *Halimeda* spp. are not, is unclear. Potentially, different oceanographic conditions between sites

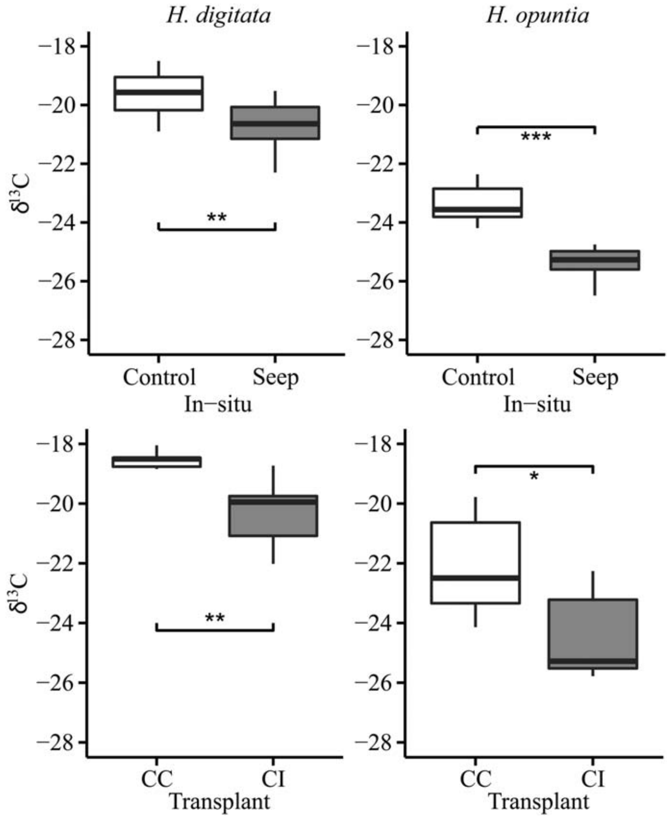

Fig. 6. $\delta^{13}C$ and $\delta^{15}N$ signatures of *H. digitata* and *H. opuntia* grown at control and CO_2 seep site and transplanted from CC and control to seep site. Brackets indicate significant differences in ANOVA's, with significance levels * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

contributed to observed differences. For instance, water temperature affects the solubility of $CaCO_3$ with favorable conditions for organisms in tropical regions. However, the saturation state of aragonite in the present study was lower ($\Omega_{ar} \sim 2$) compared to the seep site in the Mediterranean where *Halimeda* spp. were absent ($\Omega_{ar} \leq 2.5$). Potentially more stable conditions throughout the year in PNG compared to the Mediterranean led to observed differences. Similarly, hard corals were absent under high CO_2 conditions at temperate seeps in Japan (Inoue et al. 2013), while coral cover was not impacted at seeps in PNG but the diversity of species changed with increasing CO_2 (Fabricius et al. 2011). Laboratory experiments investigating the impacts of OA on *Halimeda* spp. also arrived at varying conclusions, with some suggesting that growth and calcification of several *Halimeda* spp. may be impacted under future CO_2 conditions (Price et al. 2011; Ries et al. 2009; Sinutok et al. 2011), while others suggest that several others are unlikely to be impacted by OA alone (Comeau et al. 2013; Hofmann et al. 2014; N. Vogel et al. pers. comm.). Morphological distinctions, such as surface area to volume ratio of phylloids may contribute to different responses of different *Halimeda* species to OA where thicker phylloids may reduce OA impacts. In addition, different morphologies affect diffusion of inorganic carbon to sites of calcification and photosynthesis. Moreover, different organisms possess different mechanisms of calcification. While aragonite deposition in *Halimeda* takes place in the interutricular spaces (Borowitzka 1989), *Padina* calcification is initiated intracellular (Okazaki et al. 1986) and corals deposit $CaCO_3$ at their calicoblastic epithelium (Allemand et al. 2004). However, in this study, *Halimeda* growing at the seep sites did not show any pattern related to their morphology and included lightly and heavily calcifying species, as well as rock-anchoring and sand-dwelling species. Our measured seawater carbonate system parameters provide evidence for the existence of *Halimeda* in high CO_2 environments, suggesting several tropical *Halimeda* spp. can acclimatize to future OA conditions. This observation is in agreement with a previous study on the slightly calcareous brown algae *Padina* sp., which occurs at volcanic CO_2 seep sites in PNG and the at the Mediterranean (Johnson et al. 2012). However, seep sites investigate the effects of OA in isolation and it is possible that other co-occurring factors predicted for the future (e.g., warming or increase of terrestrial runoff) may interact to affect *Halimeda* spp.

We investigated *H. digita* and *H. opuntia* physiology in detail as they were most abundant at both control and seep site. By selecting the most abundant species, the potential of a bias toward more resilient species cannot be excluded. Nevertheless, occurrence of *H. cylindracea*, *H. hederacea*, *H. macroloba*, and *H. undulata* at the seep sites suggests that several other species can tolerate this particular environment. Net and gross photosyntheses of both, *H. digita* and *H. opuntia*, did not differ between control and seep site. Increased dissolved inorganic carbon (DIC) availability did not positively affect the photosynthesis of *Halimeda* spp. grown at volcanic seep sites incubated in otherwise present environmental conditions (i.e., present light conditions). In contrast, previous studies observed increased productivity of benthic foraminifera at the Upa-Upasina seep site, suggesting endosymbiotic algae hosted by foraminifera may be carbon limited and thus benefit from increased DIC availability (Uthicke and Fabricius 2012). Similar results were observed in an experiment with coral *Acropora eurystoma*, which showed increased photosynthesis in elevated DIC concentrations, presuming carbon limitation of zooxanthellae in ambient water conditions (Chauvin et al. 2011). As shown by Borowitzka and Larkum (1976b) *Halimeda tuna* photosynthesis saturates at DIC < 3 mmol L^{-1} (DIC ~ 1900 μmol $kgSW^{-1}$ in present study, $kgSW^{-1}$ = per kg seawater). *Halimeda* photosynthesis utilizes dissolved CO_2 as the primary carbon source however HCO_3^- can also be used but at a reduced rate (Borowitzka and Larkum 1976b). Moreover, in experiments *H. tuna* photosynthesis saturated at 27 μmol L^{-1} CO_2 and 2274 μmol L^{-1} HCO_3^- (Borowitzka and Larkum 1976b), both indicating photosynthesis should be DIC limited under present environmental conditions at control sites (CO_2 = 7.78 μmol kgSW^{-1}, Table 1). Potentially, ambient PAR level of experimental incubations for *H. digitata* and *H. opuntia* (39 and 281 μmol photons m^{-2} s^{-1}, respectively) were below light saturation and organisms were subjected to light limitation before DIC limitation could be observed.

In situ calcification rates showed that both *H. digitata* and *H. opuntia* had increased calcification rates in the light at the seep compared to the control site. This is an indication that calcification of some *Halimeda* spp. may benefit from increased DIC availability. Increased bicarbonate concentrations at the seep site may thus have relieved the organisms of limiting conditions for calcification. Borowitzka and Larkum (1976b) showed that *H. tuna* calcification is saturated at about 5 mmol L^{-1} ΣCO_2, indicating carbon limitation at control conditions of the present study (DIC = 1.892 mmol kgSW^{-1}, Table 1). Calcification in *Halimeda* spp. is dependent on diffusion of CO_3^{2-} and Ca^{2+} into the intercellular space, suggesting the supply of DIC can become limiting (Borowitzka and Larkum 1976a, b, 1977). Thus, elevated DIC concentrations at seep sites (DIC = 2163 μmol kgSW^{-1}, Table 1) may explain increased calcification rates of *H. digitata* and *H. opuntia*, compared to control sites. However, low water motion in chambers may also have increased the thickness of boundary layers on the organisms' surface and thus exacerbated the positive effect of elevated DIC on calcification as discussed by Langdon and Atkinson (2005) and seen for coral photosynthesis (Chauvin et al. 2011). Therefore, potentially a combination of DIC undersaturation at ambient seawater conditions (1.892 mmol kgSW^{-1}) and increased boundary layers in incubation chambers may have resulted in increased calcification rates at the seep site, as presumed by Chauvin et al. (2011).

In contrast incubations in darkness showed calcification rates of *H. opuntia* were strongly and negatively impacted by decreased pH leading to decreased calcification and dissolution at the seep compared to the control site. Positive calcification rates were still observed at the control site in darkness, despite respiratory CO_2 release. While Borowitzka (1986) showed some decalcification in ambient seawater conditions due to respiratory CO_2 during the night, he also showed much of the DIC, which is released into the intracellular space, can be refixed in the morning. A potential reason why *H. opuntia* showed significant impacts of elevated P_{CO_2} during darkness, but *H. digitata* did not may emerge from the different morphology of both species. *H. opuntia* phylloids have a larger surface area to volume ratio and thus calcified areas are more exposed to their physical environment. This may have an advantage during the day, when a proportionally larger surface area facilitates diffusion processes and thus increases productivity and calcification. However, at night, this property may be a disadvantage, where a higher exposure to elevated CO_2 conditions, may increase negative impacts, as seen in the present study. The observed $CaCO_3$ dissolution of *H. opuntia* in the present study is in agreement with a laboratory experiment, which showed no negative effect of OA on two photosynthesizing and calcifying organisms (*Acropora millepora* and *Halimeda opuntia*) in the light but during the dark (N. Vogel et al. pers. comm.). The latter study also observed this phenomenon in incubation conditions with water movement, suggesting low water motion did not exacerbate dissolution in darkness in the present study. Moreover, this observation agrees with results from Borowitzka & Larkum (1976b), which showed that respiration can inhibit calcification of *Halimeda* by decreasing pH and [CO_3^{2-}] and presumed that respiratory CO_2 production could lead to $CaCO_3$ dissolution. In contrast, during light no negative impacts of OA on calcification could be observed. Photosynthesis may thus offset impacts of OA by buffering pH during light, increase Ω_{ar} and, therefore, facilitate deposition of $CaCO_3$ (Al-Horani et al. 2003; Borowitzka and Larkum 1976b; Goreau 1959; N. Vogel et al. pers. comm.).

Calculated net calcification rates did not differ between control and seep site for neither *H. digitata* nor *H. opuntia*. Increased light calcification and decreased dark calcification rates at the seep site cancelled out each other to no difference of net calcification rates between sites. This observation is in agreement with results derived from laboratory experiments on *H. opuntia* (N. Vogel et al. pers. comm.), and reemphasizes our in situ observations that show *Halimeda* spp. are capable to grow and calcify at high CO_2.

Elevated CO_2 showed opposite effects on inorganic carbon content of the two species with increased C_{inorg} in *H. digitata* but decreased values in *H. opuntia* at the seep site, compared to controls. $CaCO_3$ dissolution during the dark may lead to a marginally lowered C_{inorg} content of *H. opuntia*. Similarly, increased C_{inorg} content of *H. digitata* at the seep site may be explained by elevated calcification rates during the light at the seep site. Decreased C_{inorg} content (despite unaffected net calcification rates) of *H. opuntia* was previously observed by Hofmann et al. (2014). Moreover, a previous study on *Padina* showed lower $CaCO_3$ content at PNG seep sites compared to controls (Johnson et al. 2012). Increased C_{inorg} content of *H. digitata* is in contrast to previously discussed observations but is in agreement with the calcification rates measured, showing a trend (nonsignificant) toward slightly increased net calcification rates at the seep compared to control site. Decreased C_{org} and increased C_{inorg} of *H. digitata* also reflected in a decreased $C_{org}:C_{inorg}$ ratio at the seep site compared to controls. Notably, despite changes in C_{inorg} of *H. digitata* and *H. opuntia*, both were still capable to grow and to deposit $CaCO_3$ even in conditions temporary corrosive to aragonite (Ω_{ar} under saturation).

Both *H. digitata* and *H. opuntia* tissues showed increased negative $\delta^{13}C$ signatures (i.e., increased fractionation of carbon isotopes) at the seep compared to the control site, indicating either ^{13}C depletion or proportionally higher ^{12}C in tissues. In addition, tissues of both species showed depletion in ^{13}C after 14 d transplantation to the seep site while thalli that remained at the control site showed the same isotopic signatures as originally determined. Thus, we showed that the environment at the seep site led to a depletion of ^{13}C and an increased fractionation of carbon isotopes in *Halimeda* spp. tissue compared to controls and that these changes are detectable after as little as 14 d. This was most likely due to increased CO_2 availability at the seep site. As CO_2 is isotopically light compared to HCO_3^- (~ 10‰ [parts per thousand]) (Laws et al. 2002) an increased fractionation of carbon isotopes indicates an increased utilization of CO_2 over HCO_3^- at the seep site. This observation is an indication that *Halimeda* spp. may benefit from increased CO_2 availability at the seep site for photosynthetic carbon acquisition and organic carbon assimilation in their tissue. In *Halimeda* spp., photosynthesis utilizes CO_2 as primary source of inorganic carbon. Therefore, elevated CO_2 availability at the seep sites may facilitate the diffusion process and thus the uptake of CO_2 compared to the control sites. This observation has also been demonstrated for noncalcifying algae (Carvalho et al. 2010). Theoretically, calcification may alter fractionation of δ in organic tissue due to supply of CO_2 for photosynthesis derived from heavier HCO_3^- during calcification ($Ca^{2+} + 2\ HCO_3^- \rightarrow CaCO_3^- + CO_2 + H_2O$) (Laws et al. 2002). However, Laws et al. (2002) also provide evidence that calcification does not supply heavier CO_2 from calcification for photosynthesis (Buitenhuis et al. 1999; Riebesell and Wolf-Gladrow 1995). Unaltered rates of net photosynthesis suggested that both species did not benefit from elevated CO_2 at the seep site and thus were not DIC (i.e., CO_2) limited under the experimental conditions. However, as discussed above, it is possible that the light conditions during incubations were below saturation explaining why DIC limitation in net photosynthesis was not detected. Nonetheless, carbon isotope signatures from transplants indicate *Halimeda* spp. may benefit from increased CO_2 at the seep site, when integrated over several days.

With this study we provide evidence that several *Halimeda* spp. are tolerant of increasing P_{CO_2}. Some species (e.g. *H. opuntia*) that are found at the seep site are reported to be sensitive to OA. However, this conclusion is derived from laboratory experiments in artificial conditions, while the results from the present study are based on long-term exposure in a natural environment with natural light, nutrient, and flow regimes. Therefore, we suggest re-evaluating the impact of OA as single stressor on *Halimeda* spp. However, in future environmental conditions, organisms will not only have to deal with OA but also with other environmental stressors, such as ocean warming and land runoff, which may have additive or synergistic effects. Additional investigations are necessary to evaluate impacts of several stressors combined.

References

Al-Horani, F. A., S. M. Al-Moghrabi, and D. De Beer. 2003. The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. Mar. Biol. **142**: 419–426. doi:10.1016/S0022-0981(02)00578-6

Allemand, D., and others 2004. Biomineralisation in reef-building corals: From molecular mechanisms to environmental control. C. R. Palevol **3**: 453–467. doi:10.1016/j.crpv.2004.07.011

Borowitzka, M. 1986. Physiology and biochemistry of calcification in the Chlorophyceae. Biomineralization in the lower plants and animals, p. 107–124. Oxford University Press.

Borowitzka, M. 1989. Carbonate calcification in algae-initiation and control. Biomineralization: Chemical and biochemical perspectives, p. 63–94. VCH.

Borowitzka, M. A., and A. W. D. Larkum. 1977. Calcification in the green alga *Halimeda* I. An ultrastructure study of thallus development. J. Phycol. **13**: 6–16. doi:10.1111/j.1529-8817.1977.tb02879.x

Borowitzka, M. A., and A. W. D. Larkum. 1976a. Calcification in the green alga *Halimeda* II. The exchange of Ca^{2+} and the occurrence of age gradients in calcification and photosynthesis. J. Exp. Bot. **27**: 864–878. doi:10.1093/jxb/27.5.864

Borowitzka, M. A., and A. W. D. Larkum. 1976b. Calcification in the green alga *Halimeda* III. The sources of inorganic carbon for photosynthesis and calcification and a model of the mechanism of calcification. J. Exp. Bot. **27**: 879–893. doi:10.1093/jxb/27.5.879

Buitenhuis, E. T., H. J. De Baar, and M. J. Veldhuis. 1999. Photosynthesis and calcification by *Emiliania huxleyi* (Prymnesiophyceae) as a function of inorganic carbon species. J. Phycol. **35**: 949–959. doi:10.1046/j.1529-8817.1999.3550949.x

Calosi, P., and others 2013. Adaptation and acclimatization to ocean acidification in marine ectotherms: An in situ transplant experiment with polychaetes at a shallow CO_2 vent system. Philos. Trans. R. Soc. B: Biol. Sci. **368**: 20120444. doi:10.1098/rstb.2012.0444

Carpenter, K. E., and others. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science **321**: 560–563. doi:10.1126/science.1159196

Carvalho, M. C. D., K.-I. Hayashizaki, and H. Ogawa. 2010. Effect of pH on the carbon stable isotope fractionation in photosynthesis by the kelp *Undaria pinnatifida*. Coastal Mar. Sci. **34**: 135–139. doi: http://hdl.handle.net/2261/51663.

Chauvin, A., V. Denis, and P. Cuet. 2011. Is the response of coral calcification to seawater acidification related to nutrient loading? Coral Reefs **30**: 911–923. doi:10.1007/s00338-011-0786-7

Chisholm, J. R. M., and J. P. Gattuso. 1991. Validation of the alkalinity anomaly technique for investigating calcification and photosynthesis in coral-reef communities. Limnol. Oceanogr. **36**: 1232–1239. doi:10.4319/lo.1991.36.6.1232

Ciais, P., and others. 2013. Carbon and Other Biogeochemical Cycles. In T. F. Stocker et al. [eds.], Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Cigliano, M., M. Gambi, R. Rodolfo-Metalpa, F. Patti, and J. Hall-Spencer. 2010. Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. Mar. Biol. **157**: 2489–2502. doi:10.1007/s00227-010-1513-6

Collins, M. and others 2013. Long-term climate change: Projections, commitments and irreversibility. In T. F. Stocker et al. [eds.], Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Comeau, S., P. J. Edmunds, N. B. Spindel, and R. C. Carpenter. 2013. The responses of eight coral reef calcifiers to increasing partial pressure of CO_2 do not exhibit a tipping point. Limnol. Oceanogr. **58**: 388–398. doi:10.4319/lo.2013.58.1.0388

Dickson, A. G., C. L. Sabine, and J. R. Christian. 2007. Guide to best practices for ocean CO_2 measurements. PICES special publication **3**: p. 191. doi:http://aquaticcommons.org/id/eprint/144

Dlugokencky, E., and P. Tans. 2014. Trends in atmospheric carbon dioxide: Recent global CO_2. NOAA/ESRL.

Fabricius, K. E., G. De'ath, S. Noonan, and S. Uthicke. 2014. Ecological effects of ocean acidification and habitat complexity on reef-associated macroinvertebrate communities. Proc. R. Soc. B: Biol. Sci. **281**: 20132479. doi:10.1098/rspb.2013.2479

Fabricius, K. E., and others. 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat. Clim. Change **1**: 165–169. doi:10.1038/nclimate1122

Freile, D., J. Milliman, and L. Hillis. 1995. Leeward bank margin *Halimeda* meadows and draperies and their sedimentary importance on the western Great Bahama Bank slope. Coral Reefs **14**: 27–33. doi:10.1007/BF00304068

Fukunaga, A. 2008. Invertebrate community associated with the macroalga *Halimeda kanaloana* meadow in Maui, Hawaii. Int. Rev. Hydrobiol. **93**: 328–341. doi:10.1002/iroh.200711063

Goreau, T. F. 1959. The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biol. Bull. **116**: 59–75. doi:10.2307/1539156

Hall-Spencer, J. M., and others. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature **454**: 96–99. doi:10.1038/nature07051

Hofmann, L. C., J. Heiden, K. Bischof, and M. Teichberg. 2014. Nutrient availability affects the response of the calcifying chlorophyte *Halimeda opuntia* (L.) JV Lamouroux to low pH. Planta **239**: 231–242. doi:10.1007/s00425-013-1982-1

Hönisch, B., N. G. Hemming, D. Archer, M. Siddall, and J. F. Mcmanus. 2009. Atmospheric carbon dioxide concentration across the mid-Pleistocene transition. Science **324**: 1551–1554. doi:10.1126/science.1171477[19541994

Inoue, S., H. Kayanne, S. Yamamoto, and H. Kurihara. 2013. Spatial community shift from hard to soft corals in acidified water. Nat. Clim. Change **3**: 683–687. doi:10.1038/nclimate1855

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, p. 1535. In T. F. Stocker et al. [eds.]. IPCC.

Johnson, V. R., B. D. Russell, K. E. Fabricius, C. Brownlee, and J. M. Hall-Spencer. 2012. Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO_2 gradients. Global Change Biol. **18**: 2792–2803.

Koch, M., G. Bowes, C. Ross, and X. H. Zhang. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Global Change Biol. **19**: 103–132. doi:10.1111/j.1365-2486.2012.02791.x

Langdon, C., and M. J. Atkinson. 2005. Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J. Geophys. Res. **110**: C09S07. doi:10.1029/2004JC002576

Laws, E. A., B. N. Popp, N. Cassar, and J. Tanimoto. 2002. ^{13}C discrimination patterns in oceanic phytoplankton: Likely influence of CO_2 concentrating mechanisms, and implications for palaeoreconstructions. Funct. Plant Biol. **29**: 323–333. doi:10.1071/PP01183

Littler, D. S., and M. M. Littler. 2003. South Pacific reef plants: A divers' guide to the plant life of South Pacific coral reefs. Offshore Graphics Inc.

Lueker, T. J., A. G. Dickson, and C. D. Keeling. 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: Validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. Mar. Chem. **70**: 105–119. doi:10.1016/S0304-4203(00)00022-0

Meinshausen, M., and others. 2011. The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. Clim. Change **109**: 213–241. doi:10.1007/s10584-011-0156-z

Moss, R. H., and others. 2010. The next generation of scenarios for climate change research and assessment. Nature **463**: 747–756. doi:10.1038/nature08823

Noonan, S. H., K. E. Fabricius, and C. Humphrey. 2013. *Symbiodinium* community composition in scleractinian corals is not affected by life-long exposure to elevated carbon dioxide. PloS One **8**: e63985. doi:10.1371/journal.pone.0063985

Okazaki, M., A. Pentecost, Y. Tanaka, and M. Miyata. 1986. A study of calcium carbonate deposition in the genus *Padina* (Phaeophyceae, Dictyotales). Br. Phycol. J. **21**: 217–224. doi:10.1080/00071618600650251

Pandolfi, J. M., S. R. Connolly, D. J. Marshall, and A. L. Cohen. 2011. Projecting coral reef futures under global warming and ocean acidification. Science **333**: 418–422. doi:10.1126/science.1204794

Porzio, L., M. C. Buia, and J. M. Hall-Spencer. 2011. Effects of ocean acidification on macroalgal communities. J. Exp. Mar. Biol. Ecol. **400**: 278–287. doi:10.1016/j.jembe.2011.02.011

Price, N. N., S. L. Hamilton, J. S. Tootell, and J. E. Smith. 2011. Species-specific consequences of ocean acidification for the calcareous tropical green algae *Halimeda*. Mar Ecol Prog Ser **440**: 67–78. doi:10.3354/meps09309

R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. doi:10.1007/s10985-007-9065-x[18000755]

Rees, S. A., B. N. Opdyke, P. A. Wilson, and T. J. Henstock. 2007. Significance of *Halimeda* bioherms to the global carbonate budget based on a geological sediment budget for the Northern Great Barrier Reef, Australia. Coral Reefs **26**: 177–188. doi:10.1007/s00338-006-0166-x

Riebesell, U., and D. Wolf-Gladrow. 1995. Growth limits on phytoplankton. Nature **373**: 28. doi:10.1038/373028b0

Ries, J. B., A. L. Cohen, and D. C. Mccorkle. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology **37**: 1131–1134. doi:10.1130/G30210A.1

Robbins, L. L., M. E. Hansen, J. A. Kleypas, and S. C. Meylan. 2010. CO2calc—A user-friendly seawater carbon calculator for Windows, Max OS X, and iOS (iPhone). U.S. Geological Survey Open-File Report 2010-1280, p. 17.

Russell, B. D., S. D. Connell, S. Uthicke, N. Muehllehner, K. E. Fabricius, and J. M. Hall-Spencer. 2013. Future seagrass beds: Can increased productivity lead to increased carbon storage? Mar. Pollut. Bull. **73**: 463–469. doi:10.1016/j.marpolbul.2013.01.031

Sinutok, S., R. Hill, M. A. Doblin, R. Wuhrer, and P. J. Ralph. 2011. Warmer more acidic conditions cause decreased productivity and calcification in subtropical coral reef sediment-dwelling calcifiers. Limnol. Oceanogr. **56**: 1200–1212. doi:10.4319/lo.2011.56.4.1200

Uthicke, S., and K. E. Fabricius. 2012. Productivity gains do not compensate for reduced calcification under near-future ocean acidification in the photosynthetic benthic foraminifer species *Marginopora vertebralis*. Global Change Biol. **18**: 2781–2791. doi:10.1111/j.1365-2486.2012.02715.x

Uthicke, S., P. Momigliano, and K. Fabricius. 2013. High risk of extinction of benthic foraminifera in this century due to ocean acidification. Sci. Rep. **3**. Article number 1769, 1–5. doi:10.1038/srep01769.

Wefer, G. 1980. Carbonate production by algae *Halimeda*, *Penicillus* and *Padina*. Nature **285**: 323–324. doi:10.1038/285323a0

Witt, V., C. Wild, and S. Uthicke. 2012. Interactive climate change and runoff effects alter O_2 fluxes and bacterial community composition of coastal biofilms from the Great Barrier Reef. Aquat. Microb. Ecol. **66**: 117. doi:10.3354/ame01562

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