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Decreased light availability can amplify negative impacts of ocean acidification on calcifying coral reef organisms

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ABSTRACT: Coral reef organisms are increasingly and simultaneously affected by global and local stressors such as ocean acidification (OA) and reduced light availability. However, knowledge of the interplay between OA and light availability is scarce. We exposed 2 calcifying coral reef species (the scleractinian coral *Acropora millepora* and the green alga *Halimeda opuntia*) to combinations of ambient and increased pCO_2 (427 and 1073 µatm, respectively), and 2 light intensities (35 and 150 µmol photons m^{-2} s^{-1}) for 16 d. We evaluated the individual and combined effects of these 2 stressors on weight increase, calcification rates, O_2 fluxes and chlorophyll *a* content for the species investigated. Weight increase of *A. millepora* was significantly reduced by OA (48%) and low light intensity (96%) compared to controls. While OA did not affect coral calcification in the light, it decreased calcification in the dark by 155%, leading to dissolution of the skeleton. *H. opuntia* weight increase was not affected by OA, but decreased (40%) at low light. OA did not affect algae calcification in the light, but decreased calcification in the dark by 164%, leading to dissolution. Low light significantly reduced gross photosynthesis (56 and 57%), net photosynthesis (62 and 60%) and respiration (43 and 48%) of *A. millepora* and *H. opuntia*, respectively. In contrast to *A. millepora*, *H. opuntia* significantly increased chlorophyll content by 15% over the course of the experiment. No interactive effects of OA and low light intensity were found on any response variable for either organism. However, *A. millepora* exhibited additive effects of OA and low light, while *H. opuntia* was only affected by low light. Thus, this study suggests that negative effects of low light and OA are additive on corals, which may have implications for management of river discharge into coastal coral reefs.

KEY WORDS: pH · Turbidity · Calcification · Dissolution · Photosynthesis · Corals · Algae · *Acropora millepora* · *Halimeda opuntia*

INTRODUCTION

Anthropogenically increased carbon dioxide (CO_2) introduced into the atmosphere is changing the earth's climate. In addition to aggravating the greenhouse effect and thus driving global warming, approximately one-third of the atmospheric CO_2 is taken up by the oceans (Bindoff et al. 2007). CO_2 which is added to the oceanic carbonate system increases hydrogen ion concentrations and thus leads to a reduction of seawater pH (ocean acidification, OA) (Golubik et al. 1979, Kleypas & Langdon 2006). Depending on the Representative Concentration Pathways (RCP) followed, atmospheric CO_2 is predicted to rise from ~395 µatm at present (Dlugokencky & Tans 2014) to between 850 and 1370 µatm by the year 2100 (RCP6.0 and RCP8.5, respectively), which is correlated with a further decrease in ocean pH unless drastic reductions in output and/or an increase in carbon capture are achieved (RCP 2.6

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and 4.5) (Moss et al. 2010). In turn, a reduction of pH leads to a shift in the oceanic carbonate system, which results in a decreased calcium carbonate ($CaCO_3$) saturation state (Ω) of seawater. Recent studies revealed negative effects of decreased Ω on growth/calcification of a vast range of coral reef organisms, leading to predictions of shifts in community structures, loss of framework builders and loss of coral reef biodiversity under future conditions (Gattuso et al. 1998, Langdon et al. 2000, Orr et al. 2005, Ries et al. 2009, Fabricius et al. 2011, 2014, Uthicke & Fabricius 2012).

In addition to increasing sea surface temperature (SST) and OA, often local disturbances (such as elevated organic and inorganic nutrients, increased turbidity or decreased salinity) present additional pressures on coral reef organisms at inshore reefs exposed to land-runoff (Bell 1992, Fabricius 2005, 2011, Wooldridge et al. 2006, Uthicke et al. 2011). Water quality is known to affect inshore reef communities, leading to declines in hard coral diversity and increased macroalgae richness (Fabricius et al. 2005, Schaffelke et al. 2005, De'ath & Fabricius 2010). Decrease in water quality on the Great Barrier Reef (GBR) has been linked to anthropogenic activities associated with land-use, and has been in decline since European settlement (McCulloch et al. 2003, Roff et al. 2013). Predominantly during summer months, an increase in precipitation and hence riverine runoff results in more severe consequences to near-shore reef communities. Combinations of global and local stressors may have additive or synergistic effects and may push organisms closer to tolerance thresholds. For instance, interactive effects of OA and irradiance, OA and eutrophication, ocean warming (OW) and herbicides, or OW and eutrophication have all been shown to have impacts on several coral reef organisms (Langdon & Atkinson 2005, Chauvin et al. 2011, Negri et al. 2011, Uthicke et al. 2011, Comeau et al. 2014).

For many calcareous reef organisms, photosynthesis is essential for energy supply, calcification and/or survival, either because they are autotrophic primary producers or exhibit mixotrophic carbon acquisition. Scleractinian corals host photosynthetically active dinoflagellates as endosymbionts, which provide important energy to the host (Goreau 1959, Wainwright 1963). Moreover, by fixing CO_2 from the environment in the light, they increase cellular, surface and boundary layer pH levels and therefore facilitate the precipitation of $CaCO_3$ by elevating the aragonite saturation state (Ω_{ar}) (Goreau 1959, Al-Horani et al. 2003). For the calcifying green alga genus *Halimeda*, photosynthesis is important for calcification, since some species do not possess active calcification mechanisms. In fact, in some *Halimeda* species, calcification is a byproduct of increased intracellular pH from photosynthesis, which results in abiotic precipitation of aragonite needles (de Beer & Larkum 2001). By increasing the pH levels in the environment, photosynthesis may even protect organisms against OA, as long as sufficient light is available (de Beer et al. 2000, Al-Horani et al. 2003).

At reefs susceptible to land-runoff, increased turbidity leads to reduced light availability and therefore decreased photosynthetically available radiation (PAR) for photosynthesizing organisms. While sediment from rivers and dredging activities directly increase turbidity, elevated nutrient levels from agricultural land-runoff increase turbidity indirectly. Inshore eutrophication can enhance the abundance of chlorophyll, phytoplankton and microalgae blooms in the water column (Bell 1992, Devlin & Schaffelke 2009), which in turn leads to a reduction of PAR. Consequently, reduced PAR due to increased turbidity with increasing OA may have additional negative effects on growth, calcification and other responses of coral reef organisms. As shown in previous studies, calcification and photosynthesis in corals decrease with increasing turbidity (Kendall et al. 1983, 1985) and decreasing light intensity (Marubini et al. 2001, Mass et al. 2007). However, knowledge of the interaction between OA and low light conditions is scarce, even though the interplay between these stressors may be crucial. Local stressors that affect light availability are generally easier to manage than global stressors; therefore, it is important to understand these interactions, as findings from manipulative experiments can be used to take action via environmental management plans aiming to reduce stressors on coral reef organisms. Given that photosynthesis plays a crucial role in calcification, and OA has impacts on calcification of many organisms, it is surprising that the present study is one of the first to investigate this interaction.

The aim of the present study was to investigate the individual and interactive effects of OA and decreased PAR on 2 different coral reef taxa, the scleractinian coral *Acropora millepora* and the calcifying green alga *Halimeda opuntia*. *A. millepora* is common and widespread over tropical coral reefs and contributes to primary productivity, carbonate production and reef development. *H. opuntia* is an important major, fast-growing primary producer, commonly found on tropical coral reefs. *Halimeda* spp. contribute considerably to carbonate produc-

tion, sediment formation and play an important role in the benthic community by providing habitat for many invertebrate species (Wefer 1980, Freile et al. 1995, Rees et al. 2007, Fukunaga 2008). Thus, we conducted a laboratory experiment using controlled conditions and determined the response parameters, growth rates (measured by buoyant weight), calcification rates in light and in dark (measured by alkalinity anomaly), O_2 fluxes (productivity and respiration) and chlorophyll *a* (chl *a*) content.

MATERIALS AND METHODS

Specimen collection and preparation

Colonies of the coral *Acropora millepora* were collected from an inshore fringing reef next to Pelorus Island (central section of the GBR; 18° 33.001′ S, 146° 29.304′ E) between 2 and 4 m below lowest astronomical tide (LAT). After colonies were fragmented, individual coral nubbins were glued onto stubs and kept at the Australian Institute of Marine Science (AIMS, Townsville) in flow-through (recirculating flow ~1200 l h^{-1}) aquaria facilities under plasma light (150 µmol photons m^{-2} s^{-1}) for >3 mo. Nubbins were transferred into experimental tanks 2 wk prior to the start of experiment to acclimate to experimental control conditions. Specimens of the calcifying green alga *Halimeda opuntia* were collected from a fringing reef next to Orpheus Island, also an inshore reef in the central section of the GBR (18° 36.737′ S, 146° 29.110′ E) between 0.5 and 1.0 m below LAT. Alga fragments (each with 50 to 100 phylloids) were acclimated in experimental aquaria under control conditions for 2 wk until the start of the experiment.

Light regimes at the collection site of *Acropora millepora* were similar to control light conditions in the experiment. *Halimeda opuntia* was subjected to lower light conditions in the experiment than occurred in the field, due to collection from shallower depths. However, both organisms were acclimated to the same 'control light' conditions for 2 wk before the start of the experiment. Light levels chosen as the control and low light conditions were well within average ranges found between 3 and 6 m below LAT at mid-shelf and inshore reefs on the GBR, respectively (Uthicke & Altenrath 2010). Moreover, light data were collected with light loggers (Odyssey) simultaneously at a mid-shelf location (Rib Reef; 18° 28.785′ S, 146° 52.256′ E) and an inshore location (Orpheus Island; 18° 38.949′ S, 146° 29.183′ E) at 5 m below LAT over a period of 18 d in February 2013. At Rib Reef, daily light sums averaged 10.45 mol photons m^{-2} d^{-1}, ranging from 4.58 to 13.35 mol photons m^{-2} d^{-1}. Daily light sums at Orpheus Island averaged 1.95 mol photons m^{-2} d^{-1}, ranging from 0.22 to 4.66 mol photons m^{-2} d^{-1}. Hence, experimental conditions (daily light sums of 6.48 mol photons m^{-2} d^{-1} for controls and 1.51 mol photons m^{-2} d^{-1} for low light regimes) were well within naturally occurring light intensities at ~5 m below LAT at mid-shelf and inshore locations of the GBR.

Experimental setup

The manipulative aquaria experiment was carried out in flow-through conditions over a period of 16 d between July and August 2012 at AIMS. After a 2 wk acclimation period, 4 nubbins of *Acropora millepora* and 2 fragments of *Halimeda opuntia* were allocated to each of the 12 experimental aquaria. Four treatments with 3 replicate tanks (working volume 17.5 l) were placed in alternating order. Treatments consisted of combinations of ambient pCO_2 (427 µatm), high pCO_2 (1073 µatm), low light (35 µmol photons m^{-2} s^{-1}) and control light (150 µmol photons m^{-2} s^{-1}). High pCO_2 conditions corresponded to projections between the RCP 6.0 and RCP 8.5 scenario for the year 2100 (Moss et al. 2010). Light intensities were chosen from average PAR readings from an inshore and mid-shelf reef at ~5 m below LAT, present during the summer months. Water flow was provided with fresh filtered (0.5 µm) seawater at 25°C, with a salinity of 34.5, at a flow rate of 150 ml min^{-1}. Irradiance was delivered by white light LED (6000 K, Aqua Illumination), covering the full color spectrum. Light levels were set to a 12 h:12 h light:dark cycle. Additional aquarium pumps (AquarWorld, 250 l h^{-1}) were fitted into each tank to ensure water movement. Target pH levels were achieved by an automatic CO_2 injection system (Aqua Medic) controlled by potentiometric pH sensors, as described in Vogel & Uthicke (2012).

Carbonate system parameters

Total alkalinity (TA) was determined by gran titration with a Metrohm 855 robotic titrosampler (Metrohm) using 0.5 M HCl (see also Uthicke & Fabricius 2012). Total alkalinity was calculated by non-linear regression fitting between pH 3.5 and 3.0 and was corrected to certified reference material (CRM Batch 106, A. Dickson, Scripps Oceanographic Institute). Seawater pH, temperature and millivolts

(mV) were measured daily (including early morning and evening measurements to incorporate diurnal fluctuations) with a temperature corrected, handheld pH meter (WTW, Germany), calibrated on the NIST (National Institute of Standards and Technology, USA) scale. Millivolt and temperature readings were utilized to calculate pH on a total (pH_{total}) scale. Carbonate system parameters (Table 1) were calculated with CO2calc software (Robbins et al. 2010) utilizing TA and pH_{total} values and CO_2 constants from Lueker et al. (2000). Carbonate system parameters were calculated from measurements in each aquarium and 3 sampling events over the course of the experiment. Calculated pCO_2 levels yielded averages of 427 µatm for controls and 1073 µatm for future scenario conditions (Table 1). Ω_{ar} yielded averages of 3.3 and 1.7 for controls and high pCO_2 treatments, respectively.

Growth rates

Growth of organisms was determined by the buoyant weight technique. Individual specimens were single-weighted (accuracy: 0.1 mg, Mettler Toledo) in a custom-built buoyant weight set-up with water jacket and seawater of constant temperature (25°C) and salinity (34.5 ppt) at the start and at the end of the experiment. Growth of organisms was expressed as daily percentage of change.

Calcification in light and dark, net photosynthesis and respiration

After 16 d in experimental conditions, 2 individuals of each species and replicate tank were incubated for 1 h in the light and thereafter 1 h in the dark to determine calcification and photosynthetic rates. Light intensity and seawater pH of incubations corresponded to treatment condition of each organism. One experimental run consisted of 12 parallel incubations in 200 ml incubation chambers, including 2 blanks per treatment. To assure constant water temperature during incubation, chambers were placed into a flow-through water bath at 25°C. Additionally, magnetic stirrer bars ensured water movement within the incubation chambers.

Calcification rates in light and dark were determined by the alkalinity anomaly technique (Chisholm & Gattuso 1991). A subsample of 50 ml was pipetted from the incubation seawater and directly titrated for TA on a Metrohm 855 (as described above). $CaCO_3$ precipitation or dissolution in µM C h^{-1} was calculated following Gao & Zheng (2010) and standardized to organism surface area (*Acropora millepora*) or buoyant weight (*Halimeda opuntia*). Daily net calcification was calculated by 12 h of daylight and 12 h of darkness. We determined surface areas of coral nubbins using the wax-weight method (Veal et al. 2010) and chose buoyant weight as standardization for the algae due to their highly 3-dimensional structures and lowest variability in data.

Net photosynthesis in the light or dark respiration were monitored consecutively during the incubations by 3 Firesting 4-channel oxygen meters (Pyroscience), which were connected to each chamber with fiber optic cables. Gross photosynthesis, net photosynthesis and respiration rates were expressed as µM O_2 h^{-1} and standardized to organism surface area (*A. millepora*) or buoyant weight (*H. opuntia*).

Pigment content

Chl *a* content of algae tissue was determined spectrophotometrically. Organisms were frozen to –80°C after incubations. Similar to the chl *a* extraction

Table 1. Carbonate system parameters (mean with SD in parentheses) of experimental conditions. TA = total alkalinity, DIC = dissolved inorganic carbon, pCO_2 = carbon dioxide partial pressure, HCO_3^- = bicarbonate, CO_3^{2-} = carbonate, Ω_{ar} = aragonite saturation state, SW = seawater

Treatment	pH_{total}	Temp (°C)	TA (µmol kg SW^{-1})	DIC (µmol kg SW^{-1})	pCO_2 (µatm)	HCO_3^- (µmol kg SW^{-1})	CO_3^{2-} (µmol kg SW^{-1})	CO_2 (µmol kg SW^{-1})	Ω_{ar}
Control pCO_2 + control light	8.038 (0.031)	25.4 (0.2)	2276 (13)	1990 (17)	421 (19)	1774 (21)	203 (5)	12 (1)	3.2 (0.1)
High pCO_2 + control light	7.707 (0.038)	25.3 (0.1)	2281 (7)	2160 (5)	1069 (71)	2026 (8)	104 (6)	30 (2)	1.7 (0.1)
Control pCO_2 + low light	8.008 (0.015)	25.7 (0.8)	2278 (7)	1983 (11)	433 (15)	1762 (17)	209 (7)	12 (1)	3.4 (0.1)
High pCO_2 + low light	7.693 (0.016)	25.5 (0.3)	2288 (5)	2164 (8)	1076 (18)	2029 (9)	106 (2)	30 (1)	1.7 (0.0)

described in Schmidt et al. (2011) and Vogel & Uthicke (2012), apical segments of algae were placed in 15 ml Falcon tubes on ice and 4 ml of cold ethanol (95% EtOH) was added. After crushing the segments with a homogenizer, extracts were heat-shocked in a water bath (78°C for 5 min), and left in a fridge for 24 h extraction. Absorbencies on 750 and 664 nm were read on a Powerwave microplate reader (BioTek). Chl *a* content was calculated with equations by Nusch (1980) and standardized to segment fresh weight.

Chl *a* content of coral *Acropora millepora* was determined after coral tissue was stripped from the skeleton with an air gun utilizing fresh, ultra-filtered (0.2 µm) seawater. Zooxanthellae were isolated from the host tissue and re-suspended in 2 ml of ethanol (EtOH 95%), heat-shocked and extracted for 24 h in the cold. Absorbencies were read (as described above) and chl *a* contents were calculated standardized to nubbin surface area.

Statistical analysis

We statistically tested growth rates, net-, light- and dark-calcification rates, gross photosynthesis, net photosynthesis, respiration, and chl *a* content for significant differences between experimental treatment conditions. Levene's tests for equal variances were performed on datasets in the software program R (R Development Core Team 2014). If necessary, response variables were $\log_{10}$ transformed prior to analyses to fulfill assumptions of equal of variances. Mixed effect ANOVAs were conducted on datasets with NCSS software (Hintze 2007) with pH and light treatment as fixed factors. Replicate tanks were considered as nested (random) factor. To distinguish significantly differing groups we conducted Tukey-Kramer multiple comparison tests.

RESULTS

The interaction between pCO_2 and light intensity was not significant for any treatment parameter (Table 2). However, the coral *Acropora millepora* exhibited additive negative effects of high pCO_2 and low light conditions on growth rates and calcification rates in the dark (Table 3).

Mean growth rates (Fig. 1) of *Acropora millepora* were significantly ($p = 0.032$) reduced in high pCO_2 (Tables 2 & 3) by 48% compared to controls, while the growth rate of *Halimeda opuntia* was not impacted by high pCO_2. Low light significantly ($p < 0.0001$) reduced growth rates of *A. millepora* by 96% compared to controls, while growth of *H. opuntia* was not significantly reduced (Table 2, $p = 0.069$).

Net calcification rates (Fig. 1) of *Acropora millepora* and *Halimeda opuntia* (measured by alkalinity anomaly) followed similar trends as growth rates measured by buoyant weight (Tables 2 & 3). The buoyant weight method (integrated over longer

Table 2. Mixed effect ANOVA results for the effect of different pCO_2 and light conditions on growth rates; net, light, and dark calcification; gross and net photosynthesis; respiration; and chlorophyll *a* content in the coral *Acropora millepora* and the alga *Halimeda opuntia*. Values in **bold** are significant at $p < 0.05$

Source of variation	*A. millepora* df	*F*	p	*H. opuntia* df	*F*	p
Growth rate						
pCO_2	1,29	6.78	**0.032**	1,8	0.60	0.460
Light	1,29	64.98	**<0.001**	1,8	4.41	0.069
pCO_2:light	1,29	0.17	0.692	1,8	0.05	0.833
Tank	8,29	2.44	**0.037**	8,8	1.71	0.231
Net calcification						
pCO_2	1,11	4.78	0.060	1,12	3.59	0.095
Light	1,11	25.95	**0.001**	1,12	1.09	0.328
pCO_2:light	1,11	1.02	0.342	1,12	0.31	0.594
Tank	8,11	1.09	0.434	8,12	2.26	0.098
Light calcification						
pCO_2	1,11	0.14	0.722	1,12	0.01	0.910
Light	1,11	13.72	**0.006**	1,12	0.48	0.509
pCO_2:light	1,11	0.18	0.684	1,12	0.45	0.523
Tank	8,11	1.61	0.227	8,12	2.14	0.113
Dark calcification						
pCO_2	1,11	21.45	**0.002**	1,12	57.53	**<0.001**
Light	1,11	21.36	**0.002**	1,12	2.81	0.132
pCO_2:light	1,11	2.54	0.150	1,12	0.09	0.772
Tank	8,11	0.41	0.892	8,12	4.15	**0.014**
Gross photosynthesis						
pCO_2	1,12	0.00	1.000	1,11	3.51	0.098
Light	1,12	208.60	**<0.001**	1,11	38.76	**0.003**
pCO_2:light	1,12	0.00	1.000	1,11	0.41	0.542
Tank	8,12	1.07	0.441	8,11	1.53	0.252
Net photosynthesis						
pCO_2	1,12	0.00	1.000	1,11	3.08	0.117
Light	1,12	267.99	**<0.001**	1,11	35.85	**<0.001**
pCO_2:light	1,12	0.21	0.661	1,11	0.47	0.512
Tank	8,12	0.77	0.636	8,11	1.40	0.297
Respiration						
pCO_2	1,12	0.0	0.947	1,11	2.85	0.130
Light	1,12	31.31	**0.001**	1,11	25.38	**0.001**
pCO_2:light	1,12	0.23	0.646	1,11	0.06	0.817
Tank	8,12	4.13	**0.014**	8,11	3.89	**0.020**
Chlorophyll *a*						
pCO_2	1,12	0.34	0.577	1,12	1.09	0.326
Light	1,12	2.23	0.174	1,12	6.69	**0.032**
pCO_2:light	1,12	0.02	0.895	1,12	1.10	0.324
Tank	8,12	3.17	**0.035**	8,12	1.16	0.398

Table 3. Summary of effects (given as % change) of treatment variables on response parameters for the coral *Acropora millepora* and the alga *Halimeda opuntia*. Decreases >100% indicate decalcification. ns: no significant treatment effect ($p \geq 0.05$); measured additive effects: differences of means between the control pCO_2/high light and high pCO_2/low light treatment

Response parameter	Species	pCO_2	Light	Additive effect: Predicted	Additive effect: Measured
Growth rate	*A. millepora*	−48	−96	−144	−114
	H. opuntia	ns	ns	ns	ns
Net calcification	*A. millepora*	−57	−99	−156	−127
	H. opuntia	ns	ns	ns	ns
Light calcification	*A. millepora*	ns	−83	ns	ns
	H. opuntia	ns	ns	ns	ns
Dark calcification	*A. millepora*	−155	−155	−310	−204
	H. opuntia	−164	ns	ns	ns
Gross photosynthesis	*A. millepora*	ns	−56	ns	ns
	H. opuntia	ns	−57	ns	ns
Net photosynthesis	*A. millepora*	ns	−62	ns	ns
	H. opuntia	ns	−60	ns	ns
Respiration	*A. millepora*	ns	−43	ns	ns
	H. opuntia	ns	−48	ns	ns
Chlorophyll *a* content	*A. millepora*	ns	ns	ns	ns
	H. opuntia	ns	−15	ns	ns

term) and alkalinity anomaly method (determined in the short term) showed compatible results, and a similar pattern for treatment effects.

Elevated pCO_2 had no effect on organisms' calcification in the light (Fig. 1). While low light significantly (Table 2, $p = 0.006$) reduced light calcification of *Acropora millepora* by 83%, *Halimeda opuntia* light calcification was not reduced in low light conditions.

The most distinct effect of elevated pCO_2, however, was observed in dark incubations (Fig. 1). Elevated pCO_2 significantly reduced calcification of *Acropora millepora* and *Halimeda opuntia* (Table 2, $p = 0.002$ and < 0.001, respectively) by 155 and 164%, respectively, with decalcification of their skeletons occurring in high pCO_2 conditions. Reduction of calcification by more than 100% indicates decalcification. Moreover, low light conditions significantly (Table 2, $p = 0.002$) reduced dark calcification of *A. millepora* by 155% compared to controls.

High pCO_2 did not show any effect on gross photosynthesis, net photosynthesis or respiration of *Acropora millepora* or *Halimeda opuntia* (Fig. 2). However, low light levels significantly reduced gross photosynthesis by 56 and 57% (both $p < 0.001$), net photosynthesis by 62 and 60% (both $p < 0.001$), and respiration by 43 and 48% (both $p = 0.001$) for *A. millepora* and *H. opuntia*, respectively (Table 2).

Chl *a* content (Fig. 2) of *Halimeda opuntia* was significantly (Table 2, $p = 0.032$) increased by 15% in low light conditions compared to controls. Chl *a* within the coral *Acropora millepora* was not significantly different among treatments (Table 2).

DISCUSSION

Negative effects of OA on a range of marine calcifying organisms have been well documented (e.g. Orr et al. 2005, Guinotte & Fabry 2008, Kleypas & Yates 2009, Hendriks et al. 2010, Pandolfi et al. 2011, Fabricius et al. 2014). The present study demonstrated that *Acropora millepora* in particular was negatively affected by elevated pCO_2, and that decreased light availability can have an additional impact on both organisms. Although the factors were not synergistic (i.e. higher than the effect of individual stressors added together, see Table 3), additive effects on some response parameters clearly suggest that some corals may better cope with global OA if PAR is not reduced at the same time. With increasing OA, many corals will experience lower growth rates in future. If PAR is reduced at the same time, the inhibitors (elevated pCO_2 and reduced PAR) are additive (Dunne 2010) and growth rates of corals impacted by reduced PAR at inshore reefs will be more compromised than those of corals on mid-shelf reefs. Therefore, by improving water quality the additional stressor of low light availability for inshore corals can be reduced.

In the present study we observed a significant reduction of growth rates for *Acropora millepora* in high pCO_2 conditions after 16 d in experimental conditions. To date, experiments have revealed mixed responses of coral reef calcifiers towards altered pCO_2 conditions, showing decreased growth/calcification in elevated pCO_2, or no effect at all (e.g. Ries et al. 2009, Comeau et al. 2013b). Yet, the present study revealed that *A. millepora* belongs to the group which is likely to experience negative impacts under future environmental conditions. Presumably, reduced Ω_{ar} is the driving factor of decreased calcification of corals in a high pCO_2 environment (Schneider & Erez 2006, Marubini et al. 2008). Due to a decrease of Ω_{ar} in OA conditions, many organisms become im-

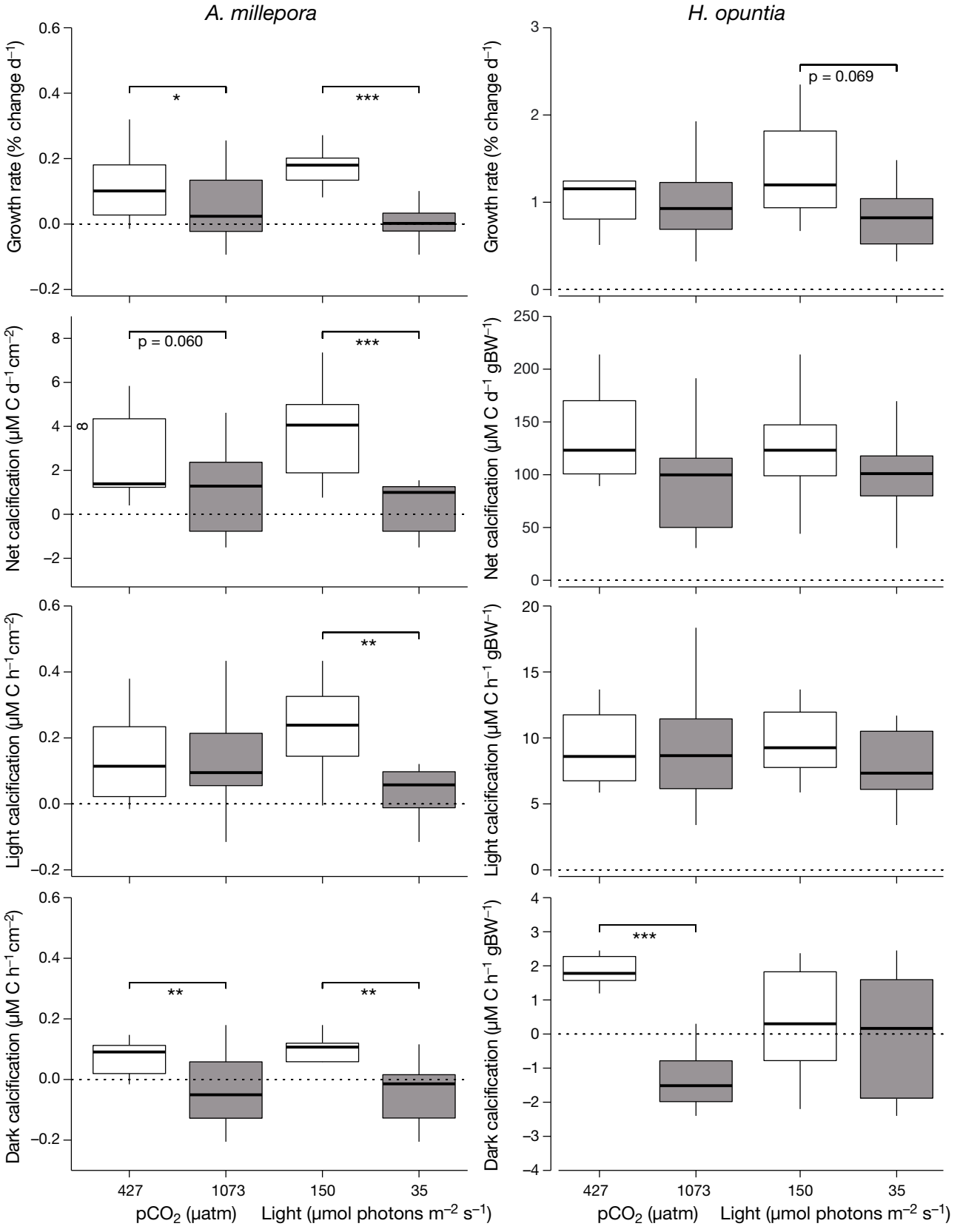

Fig. 1. Growth rates and net, light and dark calcification rates of the coral *Acropora millepora* and the alga *Halimeda opuntia* after 16 d exposure to experimental conditions. Data were pooled across pCO_2 and light treatment because there was no significant interaction. Whiskers represent lower and upper extremes. White boxes: control treatments; grey boxes: changed treatments. Dashed lines: zero lines. BW: buoyant weight. Brackets indicate significant differences (or nearly significant; exact p-value given) in ANOVAs. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$

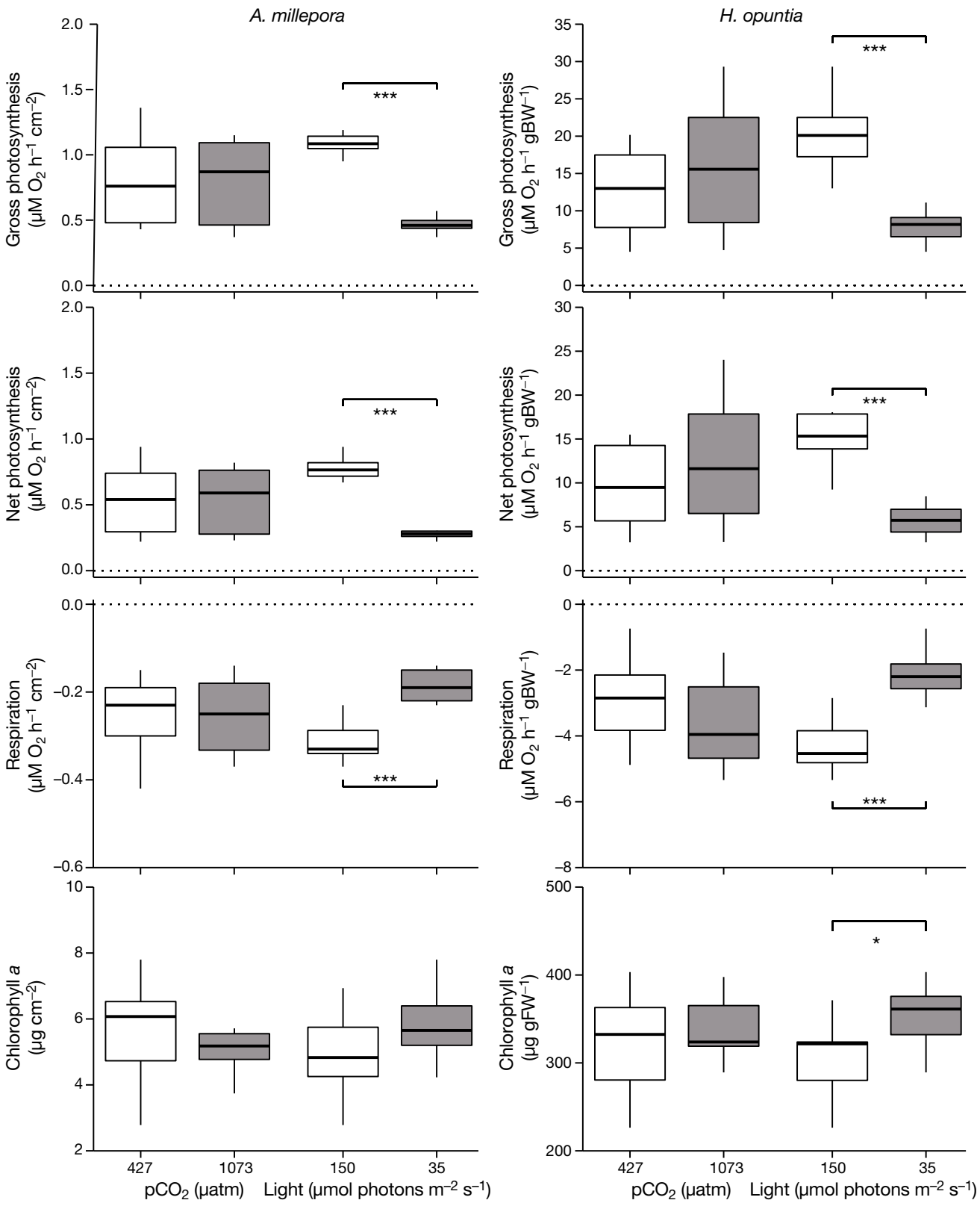

Fig. 2. Gross- and net photosynthesis, and respiration and chlorophyll *a* content of the coral *Acropora millepora* and the alga *Halimeda opuntia* after 16 d exposure to experimental conditions. Data was pooled across pCO_2 and light treatment because there was no significant interaction. Whiskers represent lower and upper extremes. White boxes: control treatments; grey boxes: changed treatments. Dashed lines: zero lines. BW: buoyant weight; FW: fresh weight. Brackets indicate significant differences in ANOVAs. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$

paired in building $CaCO_3$ skeletons (Raven et al. 2005, Kleypas & Langdon 2006, Hoegh-Guldberg et al. 2007). In contrast, *Halimeda opuntia* showed no significant trend on growth rates in relation to Ω_{ar}/pCO_2. Some previous studies suggest that *Halimeda* spp. may be impacted in future OA conditions, showing reduced growth in elevated pCO_2 (Ries et al. 2009, Price et al. 2011, Sinutok et al. 2011). However, similar to corals, *Halimeda* spp. exhibit different growth forms with associated morphological distinctions. *Halimeda* spp. occur as heavily calcified and less calcified species, sand-dwellers and rock-anchored species as well as species with different sizes and shapes of phylloids. *Halimeda* spp. with smaller phylloids have a higher surface to volume ratio than with larger phylloids and hence have a higher exposure to their physical environment. As shown by Comeau et al. (2013b), *H. macroloba* showed no impact of increased pCO_2 on calcification, but *H. minima* showed reduced calcification in elevated pCO_2. However, different outcomes may also arise from different methodologies implemented, such as flow conditions, nutrient availability, size of organisms, level of pCO_2 condition implemented, or combinations of different stressors (e.g. OA and OW). The impact of elevated pCO_2 on growth of *H. opuntia* in Price et al. (2011) compared to the lack of response to elevated pCO_2 in the present study is unclear. We propose that different results mainly arose due to different methodologies being implemented. The present study used flow-through conditions with a constant supply of fresh filtered seawater and associated nutrients, while in Price et al. (2011), 0.7 l tanks were utilized with water exchange every 48 h, not accounting for nutrient depletion. Moreover, daily light sums of control light in the present study were considerably higher and closer to natural light conditions than reduced natural light regimes in Price et al. (2011), where light maxima at midday averaged 150 µmol photons m^{-2} s^{-1}. In the present study we chose a pCO_2 level which is likely to be reached by the year 2100 under projections between the RCP 6.0 and RCP 8.5 scenario (Moss et al. 2010), the experimental set-up provided flow-through conditions with continuous supply of nutrients and light regimes naturally found on mid-shelf and inshore locations of the GBR at 5 m below LAT. One explanation for why *H. opuntia* is capable of growth in a high pCO_2 environment while *A. millepora* is not may be that calcification rates in *Halimeda* spp. are generally higher than in corals (when both standardized to either surface area or buoyant weight). For *H. opuntia*, daytime calcification rates were approximately one order of magnitude higher than dissolution rates in the dark under elevated pCO_2 conditions. Thus, even when some dissolution is taking place in the dark, higher light calcification rates sum up to positive net calcification rates. In contrast, for *A. millepora*, dark dissolution and light calcification rate were in a similar range, indicating net dissolution if impacts of future OA and low light conditions become additive (Table 3).

Notably, light calcification rates of both organisms, determined using the alkalinity anomaly technique, were unaffected by increased pCO_2. To our knowledge, this is the first study to show that calcification of *Acropora millepora* and *Halimeda opuntia* in elevated pCO_2 is not impacted in the light and supports the assumption that in the light, photosynthetic activity can counteract negative impacts of OA by increasing intracellular, surface and boundary-layer pH. By utilizing CO_2, photosynthesis increases pH and Ω_{ar} (de Beer et al. 2000, de Beer & Larkum 2001, Glas et al. 2012). As shown by Al-Horani et al. (2003), pH increases under the calcioblastic layer of corals in light, which elevated the super saturation of Ω_{ar} from 3.2 up to 25, facilitating deposition of $CaCO_3$ (Goreau 1959, Al-Horani et al. 2003).

However, the present study also suggested that for dark calcification rates, the opposite effect is the case. During respiration in the dark, additional CO_2 further reduces pH and Ω_{ar} (already lowered by OA) and impedes deposition of $CaCO_3$. Hence, in the absence of light, both organisms were strongly negatively impacted by high pCO_2 conditions, leading to dissolution of their skeleton. In contrast, under present-day conditions, both organisms can calcify in the dark (i.e. in the absence of photosynthesis). This observation is in agreement with a previous study, showing decalcification of *Acropora eurystoma* in high pCO_2 and darkness, while $CaCO_3$ was still deposited under control conditions or in high pCO_2 in the light (Schneider & Erez 2006). Previous studies have also shown that reef communities can change the diurnal local seawater carbonate chemistry by photosynthesis, respiration, calcification and dissolution, and that $CaCO_3$ dissolution is primarily taking place in the dark (Chisholm 2000, Langdon & Atkinson 2005, Kleypas et al. 2011, Anthony et al. 2013). While respiration is taking place in the dark, additional CO_2 is added to the carbonate system and already lowered pH levels from OA are further reduced, leading to an additional reduction of Ω_{ar} as already provoked by OA. Consequently, *A. millepora* and *Halimeda opuntia* were incapable of depositing $CaCO_3$ in the dark and even experienced dissolution of their skeletons under these conditions.

Considering the negative impacts of OA in darkness, we demonstrated that low light conditions may likewise result in additional negative implications on organisms, once PAR is reduced below a level at which photosynthesis cannot buffer reduced pH by OA. Presumably, this threshold level is below 35 µmol photons m^{-2} s^{-1} as tested in the present study. As shown in the present study by measurements in the light, OA showed no impact on calcification rates of either organism. Thus, the availability of sufficient light, associated with photosynthetic activity and apparent buffer capacity, mitigated negative effects of OA in light incubations. However, turbidity decreases PAR and therefore reduces the capability of the organisms' photosynthesis to buffer the negative impacts of OA, even during the day. As shown by our O_2 flux measurements, low light regimes significantly decreased gross and net photosynthesis in both organisms. This enhances negative impacts of OA during the day, especially at inshore reefs, where riverine runoff leads to reduced PAR (Devlin & Schaffelke 2009). Light data from mid-shelf and inshore GBR reefs at 5 m below LAT show that light availability can be extremely reduced at inshore reefs, considerably impacting organisms' photosynthetic capacities. Moreover, reduced photosynthetic activity of organisms experiencing reduced light availability may also change DIC/carbonate chemistry on inshore reefs compared to mid-shelf locations. Under low light conditions, mean growth rates of both the coral and algae were reduced compared to higher light. Light-enhanced photosynthesis and calcification of coral and algae is a well-documented phenomenon (Goreau 1959, Chalker & Taylor 1975, Chalker 1981, de Beer et al. 2000, de Beer & Larkum 2001). With increasing OA and the additive negative effects of low light on coral growth, as demonstrated in the present study, the mechanism of light-enhanced calcification may gain in importance. Moreover, under lower light conditions, when photosynthetic activity is reduced, organisms obtain less energy supply, thus reducing the scope for growth.

Photosynthesis of algae and coral can be limited by dissolved inorganic carbon (DIC) availability (Borowitzka & Larkum 1976, de Beer & Larkum 2001, Marubini et al. 2008, Crawley et al. 2010, Chauvin et al. 2011). Carbonic anhydrase can utilize elevated bicarbonate availability to increase the CO_2 pool available for photosynthetic activity. Thus we assumed that photosynthesis could be enhanced under higher pCO_2. However, photosynthesis of the organisms investigated here could not benefit from increased DIC concentrations. This may have 2 different reasons: (1) the organisms were not DIC-limited in experimental control conditions; (2) under present light conditions, photosynthesis/calcification of organisms was not saturated and hence there was no detectable benefit from increased DIC availability. Studies conducted by Marubini et al. (2008) and Crawley et al. (2010) indicating DIC limitation both utilized higher light intensities than the present study (~300 and ~1000 µmol photons m^{-2} s^{-1}, respectively). This suggests that under present experimental conditions (i.e. present light intensities) calcification and photosynthesis were not DIC-limited.

Moreover, we showed that in decreased PAR, *Halimeda opuntia* increased its tissue chl *a* content in order to compensate for less light availability, while the coral was not able to do so over the period of the experiment. By adjusting its chl *a* content, the alga might acclimate to reduced light availability in the short term, and increase its photosynthetic capacity in low light. Increased productivity changes the carbonate chemistry to the advantage of the algae by facilitating deposition of $CaCO_3$. In contrast, *Acropora millepora* did not have this advantage because it could not increase chl *a* over the 16 d experimental period and thus may not be able to acclimate in the short term to decreased light availability. As shown by previous studies, corals alter their chl *a* content by having either a higher number of zooxanthellae per unit area, or by an increase of chl *a* content in the zooxanthellae (Coles & Jokiel 1978, Chauvin et al. 2011). Field data suggest that *A. millepora* show increased pigmentation with decreasing water clarity from mid-shelf to inshore (Fabricius 2006). However, this might also be a response towards a more chronic exposure to low light conditions and other water quality parameters (i.e. increased nutrient availability at inshore reefs). Therefore, the algae may show more immediate responses towards changing light regimes and thus have an advantage in acclimation compared to the coral.

Acropora millepora and *Halimeda opuntia* did not exhibit significant interactive effects on response parameters measured, which is an indication that effects were not synergistic (Dunne 2010). Similarly, Comeau et al. (2013a, 2014) found no interactive effects of OA and irradiance on calcification rates of *Porites rus* and *Acropora pulchra*, respectively, after 3 wk exposure to experimental conditions. In contrast, a study on *Pocillopora damicornis* recruits presented interactive effects of OA and light after 5 d of experimentation (Dufault et al. 2013). However, the responses were non-linear and impacts of OA on calcification rates were only found at intermediate light

intensities (70 µmol photons m^{-2} s^{-1}) and not at lower or higher light levels (31, 41, 122 and 226 µmol photons m^{-2} s^{-1}). Moreover, a study on *Acropora horrida* and *Porites cylindrica* showed impacts of OA on calcification that were greatest in light calcification of corals grown in lower light conditions (100 µmol photons m^{-2} s^{-1}) compared to corals grown in higher light (400 µmol photons m^{-2} s^{-1}) after 5 wk (Suggett et al. 2013). This is unexpected and in contrast to the present study, where the impact of OA on calcification was not significant in the light, but was strong in the dark. In the present study *A. millepora* did not show interactive effects of OA and light; however, there were additive effects of both stressors. We detected reduced growth rates (–48%) when exposed to high pCO_2 conditions and also reduced growth rates when exposed to low light regimes (–96%), which resulted in a predicted additive growth rate of –144% (which is similar to the measured –114% growth rate) (Table 3). This may have an ecological implication for corals inhabiting inshore reefs susceptible to land-runoff and thus decreased light availability. Turbidity changes the attenuation of light penetrating the water column, decreasing PAR with increasing depth more rapidly than in clear water conditions. This, in turn, may lead to a stronger depth limitation for corals and thus to potential habitat decline in future OA conditions, because they gain less light with lower water depth compared to clear water habitats.

CONCLUSIONS

In the present experiment, we confirmed that the marine calcifiers investigated are negatively impacted by ocean acidification, with *Acropora millepora* showing more negative impacts than *Halimeda opuntia*. As long as sufficient light is available during the day, photosynthesis aids organisms to counteract negative impacts of OA. However, if there is not sufficient light available (e.g. due to high turbidity), there may be impacts of OA on calcification also during the day. Thus, low light conditions inshore remove this advantage from photosynthesizing organisms. As suggested by the dark incubations, respiration potentially aggravates the impacts of OA on the organisms, leading to dissolution of their skeleton. This highlights the importance of considering light-dependent impacts of OA on photosynthesizing calcifiers. Moreover, we showed that decreased light availability is an additive stressor with OA, particularly for the coral *A. millepora*, because the coral exhibits reduced calcification in OA conditions as well as in low light conditions. *H. opuntia*, on the other hand, grows marginally less in low light, but was not negatively impacted by OA in its overall growth. Consequently, the combination of OA and low light conditions may contribute to a changing coral reef ecosystem with even less hard corals as framework builders and more macroalgae on inshore reefs of the future. Potential acclimatization to environmental stressors in the long term could lead to different responses of organisms. Therefore, further investigations are needed to test the effects of OA in combination with light availability on coral reef organisms. Management of coastal runoff could also play an important role, as by improving water clarity on inshore reefs, the additional stressor of low light availability for corals would be reduced.

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