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RESEARCH ARTICLE

The role of light in mediating the effects of ocean acidification on coral calcification

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SUMMARY

We tested the effect of light and P_{CO_2} on the calcification and survival of *Pocillopora damicornis* recruits settled from larvae released in southern Taiwan. In March 2011, recruits were incubated at 31, 41, 70, 122 and 226 μmol photons $m^{-2} s^{-1}$ under ambient (493 μatm) and high P_{CO_2} (878 μatm). After 5 days, calcification was measured gravimetrically and survivorship estimated as the number of living recruits. Calcification was affected by the interaction of P_{CO_2} with light, and at 493 μatm P_{CO_2} the response to light intensity resembled a positive parabola. At 878 μatm P_{CO_2}, the effect of light on calcification differed from that observed at 493 μatm P_{CO_2}, with the result that there were large differences in calcification between 493 μatm and 878 μatm P_{CO_2} at intermediate light intensities (*ca.* 70 μmol photons $m^{-2} s^{-1}$), but similar rates of calcification at the highest and lowest light intensities. Survivorship was affected by light and P_{CO_2}, and was highest at 122 μmol photons $m^{-2} s^{-1}$ in both P_{CO_2} treatments, but was unrelated to calcification. In June 2012 the experiment was repeated, and again the results suggested that exposure to high P_{CO_2} decreased calcification of *P. damicornis* recruits at intermediate light intensities, but not at lower or higher intensities. Together, our findings demonstrate that the effect of P_{CO_2} on coral recruits can be light dependent, with inhibitory effects of high P_{CO_2} on calcification at intermediate light intensities that disappear at both higher and lower light intensities.

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INTRODUCTION

The effects of light on coral calcification have been studied for decades (Yonge and Nicholls, 1931; Allemand et al., 2011), but interest in this topic waned at the end of the 20th century. Early research recognized that light is not required for coral calcification but rather it accelerates the process in symbiotic corals (Kawaguti and Sakumoto, 1948; Goreau, 1959), with faster rates in the light *versus* the dark (Allemand et al., 2011) and in shallow *versus* deep water (Dustan 1975). The relationship between calcification and light in corals was described quantitatively by Chalker (Chalker, 1981), who showed that the shape of this relationship follows a hyperbolic tangent function, as is the case for photosynthesis in many corals (Chalker and Taylor, 1978; Chalker, 1981). In the few cases where the relationship between light and calcification has been measured for tropical corals (all from shallow water <6 m deep), calcification reaches a maximum at ~400 μmol quanta $m^{-2} s^{-1}$ (Chalker, 1981; Marubini et al., 2001).

Calcification has again become a central topic in coral research as part of the effort to understand the effects of ocean acidification (OA) on coral reefs. Renewed interest has been created by rising atmospheric P_{CO_2}, which causes global temperature to rise (IPCC, 2007) and seawater to become less basic as CO_2 dissolves and liberates protons (Kleypas and Langdon, 2006). OA is expected to reduce oceanic pH by 0.3–0.5 by 2100 (IPCC, 2007), and under these conditions corals generally calcify more slowly (Erez et al., 2011). However, despite the widely publicized negative effects of OA on coral calcification (Hoegh-Guldberg et al., 2007; Erez et al., 2011), a few corals show signs of resistance to high P_{CO_2} (Edmunds, 2011; Comeau et al., 2013a), and some may acclimatize to acidic conditions (Fabricius et al., 2011; Form and Riebesell, 2012; McCulloch et al., 2012). Given the effort to understand the effect of OA on coral calcification, the complex results that are emerging (Erez et al., 2011; Tambutté et al., 2011; Edmunds et al., 2012; Comeau et al., 2012; Comeau et al., 2013a; Comeau et al., 2013b) and the incomplete understanding of the underlying biology (Allemand et al., 2011), it is not surprising that a mechanistic understanding of the effects of OA on coral calcification has proven elusive (Erez et al., 2011; Ries, 2011). However, research on this topic is advancing quickly, and the most recent work is beginning to reveal that the mechanisms involved in the depression of coral calcification by OA conditions are remarkably complex (Comeau et al., 2012; Moya et al., 2012; Kaniewska et al., 2012).

Despite the biological significance of light to symbiotic corals, in the rush to understand how coral calcification is affected by OA the role of light has been overlooked. For instance, calcification of corals under high P_{CO_2} has been measured under light intensities that are inconsistent among studies, and mostly low (e.g. Marshall and Clode, 2002; Albright et al., 2008; Edmunds et al., 2012) with limited ecological relevance to the locations from which the corals were collected. To our knowledge, only Marubini and colleagues have investigated the interactive effects of irradiance and OA on coral calcification, and they did so in the laboratory by exposing

Porites compressa to 186±94 and 641±89 μatm P_{CO_2} at 80, 150 and 700 μmol photons $m^{-2} s^{-1}$ (Marubini et al., 2001). The higher P_{CO_2} caused a greater depression of calcification at high than at low irradiances. They augmented their laboratory data with results for *P. compressa* grown at four depths (to contrast light intensities ranging from 2.7 to 29.5 μmol photons $m^{-2} s^{-1}$) in a large tank filled with seawater manipulated for dissolved inorganic carbon (DIC) chemistry (Langdon et al., 2000); they found that P_{CO_2} caused statistically similar reductions in calcification at all irradiances (Marubini et al., 2001). Combining their results, Marubini and colleagues (Marubini et al., 2001) demonstrated that the relationship between calcification and irradiance followed a hyperbolic tangent function (cf. Chalker, 1981), with increased P_{CO_2} depressing calcification more at high than at low irradiances, and reducing the sensitivity of calcification to light intensity at low irradiances.

Using the aforementioned studies as a context, we tested for the effect of light on the calcification of *Pocillopora damicornis* exposed to ambient and elevated P_{CO_2}, and evaluated survivorship to gain insight into the fitness consequences of calcification. New recruits (i.e. corals ~5 days old) were selected for this analysis because they provide a tractable system in which the effects of P_{CO_2} can be studied using calcification (Albright et al., 2010; de Putron et al., 2011) and survivorship (Suwa et al., 2010; Nakamura et al., 2011) as dependent variables.

Further, recruitment is an important event in the life cycle of corals that affects their spatial distribution and population dynamics (Hughes et al., 2000; Bramanti et al., 2005), and while it plays a major role in promoting the recovery of degraded coral reefs (Babcock and Mundy, 1996), recruitment can also function as a bottleneck to population growth (Vermeij and Sandin, 2008; Arnold et al., 2010). The effects of OA on calcification of coral recruits is now being addressed in earnest (Albright, 2011); therefore, we reasoned that it is timely to ask how the results of such studies might be affected by light intensity.

MATERIALS AND METHODS

An orthogonal design was used in which irradiance and P_{CO_2} were manipulated to explore individual and combined effects on *P. damicornis* (Linneaus 1758) recruits. Two experiments were conducted, with a primary experiment in 2011, and a secondary experiment in 2012 that was designed to determine whether crucial aspects of the 2011 experiment could be repeated.

Primary experiment (March 2011)

In March 2011, eight colonies of *P. damicornis* (~20 cm diameter) were collected from 5–7 m depth on Hobihu Reef, Taiwan. Colonies were returned to the National Museum of Marine Biology and Aquarium (NMMBA, Pingtung, Taiwan) where they were placed individually into 15 l flow-through tanks and exposed to sunlight at 103±3 μmol photons $m^{-2} s^{-1}$ (mean ± s.e.m., N=85, measured with a cosine-corrected LI-192 sensor, LI-COR Biosciences, Lincoln, NB, USA). Seawater overflowing from each tank passed through mesh-lined (110 μm) cups to capture larvae, which were released during the night starting at ~22:00 h (Fan et al., 2002). Larvae released on 14 March 2011 were collected at 08:00 h and pooled among parent colonies in a 1 l beaker. These larvae were collected approximately on the inferred period of peak larval release (Fan et al., 2002).

Pooled larvae were stirred gently to mix them uniformly relative to maternal genotypes and ~2000 were placed at ~11:00 h into each of two plastic containers (24×24×12 cm) used for settlement. Containers were fitted with mesh windows (110 μm) to allow the passage of seawater, and floated in the tanks containing adult colonies. Glazed porcelain tiles (20×20×8 mm), which had accumulated natural biofilms after ~1 month incubation in aquaria receiving flowing seawater, were placed into the floating plastic container with the glazed surface upwards. Larvae were allowed to settle on these surfaces for 24 h and, thereafter, swimming larvae were discarded. Recruits on the glazed tiles were removed easily from their settlement surface, which was a crucial step necessary for weighing them individually (described below). Groups of three tiles with recruits (typically with 6–32 recruits per tile) were assigned haphazardly to each of 20 plastic containers (15×8×8 cm), which had two holes (6×4 cm) to allow for the exchange of seawater.

Each of the 20 containers was fitted with a lid of clear plastic film or neutral density (ND) filters (Lee Filters, Andover, Hants, UK) that created five light treatments. A single 0.3 ND filter blocked ~50% of the visible light, and four treatments of 50%, 25%, 12.5% and 6.25% were created by layering filters; a 5th light treatment (100% light) was created using clear plastic film (Glad Products Company, Oakland, CA, USA). ND filters block out a constant proportion of all wavelengths of photosynthetically active radiation (PAR, 400–700 nm), although ultraviolet (UV) radiation (<300 nm wavelength) is blocked completely. The clear plastic film transmitted >80% of the light between 300 and 700 nm. Transmission characteristics of the ND filter and clear plastic film were assessed using a spectrophotometer (Spectronic Genesys 5, Milton Roy, Warminster, PA, USA) (supplementary material Fig. S1).

The containers used to create the light treatments were placed into 120 liter aquaria for 5 days (16–21 March), with two aquaria at ambient P_{CO_2} and two at high P_{CO_2}. All tanks were maintained at a mean temperature of 24.0°C (s.e.m. <0.1°C, N=40) using independent heaters and chillers regulated by an aquarium controller (±0.1°C Aquacontroller Apex Neptune Systems, San Jose, CA, USA). The experimental temperature mimicked ambient seawater temperature at Hobihu Reef 1 week prior to the experiment (mean 23.6°C, s.e.m. <0.1°C, N=1008) (T.-Y.F., unpublished data), and tanks were filled with filtered seawater (1 μm), which was changed partially (10–15% volume) every day. Tanks were illuminated from 07:00 h to 19:00 h with lamps fitted with a metal halide bulb (Phillips 150 W 10,000 k) and two 39 W fluorescent bulbs (Phillips T5 460 nm) to create a mean irradiance of 265±2 μmol photons $m^{-2} s^{-1}$ (mean ± s.e.m., N=140, measured with a spherical quantum sensor LI-193, LI-COR Biosciences).

Light inside each container was measured twice daily using a small (1 mm diameter) cosine-corrected PAR sensor attached to a pulse amplitude-modulated fluorometer (Diving-PAM, Heinz Walz GmbH, Effeltrich, Germany). The small size of the sensor allowed light to be measured ~1 cm above the coral recruits while they were inside the plastic container and beneath the treatment lids. This sensor was calibrated using a separate light meter (LI-1400 Datalogger fitted with a LI-192 sensor, LI-COR Biosciences). To place the experimental light conditions in an ecological context, irradiance at 5 m depth on Hobihu Reef was measured between 5 and 10 March using a 4π light sensor (MDS-MkV/L, Alec Electronics, Rockland Oceanographic, Vancouver, Canada) that recorded PAR at 0.002 Hz. The mean (±s.e.m.) light intensity recorded by this sensor from 06:00 h to 18:00 h was 392±33 μmol photons $m^{-2} s^{-1}$ (N=72) and the maximum daily intensity was 1151±205 μmol photons $m^{-2} s^{-1}$ (N=6 days).

The DIC content of seawater was manipulated by bubbling with a premixed gas of a known P_{CO_2}, or by bubbling with unmodified air. To mix the gas for the high P_{CO_2} treatment, a system employing a variable timed solenoid valve was used, which controlled the flow of air and CO_2 into a mixing chamber to reach the target P_{CO_2} of

900 µatm. The target value was selected to provide a treatment P_{CO_2} expected within ~100 years under RCP8.5 (van Vurren et al., 2011). The solenoid valve was connected to an infrared gas analyzer (S151, Qubit Systems, Kingston, ON, Canada), which monitored the output gas and provided dynamic control of the duty cycle of the solenoid.

Throughout the experiment, total alkalinity (TA) and pH were measured following standard operating procedure (SOP) 3b and 6b (respectively) (see Dickson et al., 2007). TA was measured using an open cell automatic titrator (Model DS50, Mettler-Toledo, Columbus, OH, USA) filled with certified HCl titrant supplied by Dr A. Dickson (Scripps Institute of Oceanography), and TA was calculated using an Excel spreadsheet (Fangue et al., 2010). The accuracy and precision of the TA measurements were tested against certified reference material (CRM, from Dr A. Dickson) and maintained within 0.7% of certified values. pH was measured on the total scale (±0.001 pH) using *m*-Cresol Purple dye in a spectrophotometric assay (SOP 6b). Salinity was measured with a conductivity meter (±0.1 accuracy, model 340i, WTW GmbH, Weilheim, Germany), and temperature with a certified digital thermometer (±0.05°C accuracy, Fisher Scientific Traceable Digital Thermometer, Pittsburgh, PA, USA). TA and pH were used to calculate P_{CO_2}, $[HCO_3^-]$, $[CO_3^{2-}]$ and aragonite saturation state (Ω_A) using CO2SYS (Pierrot et al., 2006).

Upon completion of the experiment, recruits were sampled for calcification, protein and survivorship. First, three recruits per tile were harvested for protein analysis and frozen. Frozen recruits were solubilized in 0.1 mol l^{-1} NaOH with sonication (15 s at 10% amplitude) and heating (5 h at 50°C), then neutralized with 1 mol l^{-1} HCl (to pH 7.0–7.5) and were processed in triplicate. Protein concentration was determined using the BioRad protein assay (BioRad Laboratories, Hercules, CA, USA) scaled to microtiter plates with absorbance at 595 nm read on a 96-well plate spectrophotometer (Synergy H4 Hybrid Reader, Biotek, Winooski, VT, USA; the assay was calibrated using BSA). To measure calcification, recruits were cleaned of tissue in bleach (6% NaOCl for 8 h), removed from the tiles with a razor blade, and weighed individually (±100 ng, UMT2 balance, Mettler-Toledo) after drying (72 h at 27°C); calcification was normalized to protein and time (mg $CaCO_3$ mg^{-1} protein day^{-1}). Survival was assessed daily throughout the experiment by photographing the tiles with a digital camera (10 megapixel, Canon 40d) fitted with a macro lens. Live coral tissue was readily seen in the images, and presence of the tissue was used to categorize corals as alive or dead.

Secondary experiment (June 2012)

The secondary experiment in June 2012 was conducted in an identical way to the primary experiment, using the same equipment in a slightly different configuration. Notably, logistical problems prevented duplicate tanks being used in each treatment condition, and therefore the effect of P_{CO_2} was tested without tank replication. As the lamps were less bright in 2012 than in 2011 because the bulbs were older, and temperature and P_{CO_2} were regulated precisely but slightly inaccurately, it was not possible to create identical physical and chemical conditions in the two experiments. Further, it was not possible to conduct both experiments in the same season, and the second experiment was conducted in the summer when ambient seawater temperature was ~28.0°C.

For the second experiment, eight colonies of *P. damicornis* were collected from 5–7 m depth on Hobihu Reef in early June, which was unusually wet and stormy. Larvae were released from these colonies at NMMBA on 24 June, and at 08:00 h on the day of release were pooled among parent colonies and settled on tiles as described above. These larvae were released approximately on the inferred peak larval release for June, which typically follows more quickly after the new moon in June than in March (T.-Y.F., unpublished data). Once settled, the containers holding the recruits in the five light treatments were allocated to the P_{CO_2} treatments on 26 June, and the recruits were left to grow for 5 days. Experimental tanks were maintained at ~27.5°C, illuminated at a mean (±s.e.m.) irradiance of 235±1 µmol photons m^{-2} s^{-1} (N=40), and exposed to target P_{CO_2} values of ambient conditions in the laboratory and ~940 µatm.

At the end of the second experiment, recruits were sampled for calcification as described above with rates normalized to protein and time (mg $CaCO_3$ mg^{-1} protein day^{-1}). Logistical constraints prevented protein assays in 2012, and therefore the protein content of each recruit was assumed to be the same as that measured in 2011. Survivorship was measured at the end of the experiment (not daily as in 2011) using a dissecting microscope (×40 magnification) to detect the presence of live tissue.

Statistical analysis

Calcification was analyzed using two-way nested ANOVA with tank as a random nested factor in light and P_{CO_2} treatments; the nested factor was dropped from the analysis when it was not significant (at P>0.25) (Underwood, 1997). This analysis was modified in 2012 because replicate tanks were not employed, and in this case a two-way ANOVA was employed with corals treated as statistical replicates. To test the statistical assumptions of ANOVA, normality was assessed using a Shapiro–Wilks test, and homogeneity of variances was assessed graphically using residuals. To analyze survivorship, a Kaplan–Meier (K–M) product-limit analysis was used (Machin et al., 2006), in which the probability of individual recruits surviving was assumed to be independent among recruits. As K–M analyses cannot accommodate nested experimental designs, replicate corallites were pooled within treatments and log-rank tests (Machin et al., 2006) were used to test for differential survival among light and P_{CO_2} treatments. All statistical analyses were conducted using JMP software (version 9.0.2, SAS Institute Inc.).

RESULTS

Primary experiment (March 2011)

The clear film and ND filters created light treatments characterized by mean (±s.e.m.) irradiances of 31±2, 41±2, 70±3, 122±4 and 226±9 µmol photons m^{-2} s^{-1} (N=10). P_{CO_2} treatments were maintained at mean (±s.e.m.) values of 493±27 µatm (ambient P_{CO_2}) and 878±26 µatm (high P_{CO_2}) (N=5) (Table 1).

Pocillopora damicornis larvae settled onto tiles at a density of 19±2 corals per tile (mean ± s.e.m., N=61) and grew rapidly, depositing 104–558 µg $CaCO_3$ per recruit over 5 days. Protein content of the recruits was unaffected by light or P_{CO_2} (ANOVA light: $F_{4,25}$=1.512, P=0.249; P_{CO_2}: $F_{1,25}$=0.134, P=0.720) and averaged 0.057±0.001 mg per recruit across treatments (mean ± s.e.m., N=35). Calcification standardized to overall mean protein content ranged from 1.12 to 1.57 mg $CaCO_3$ mg^{-1} protein day^{-1}, and was significantly affected by the interactive effect of light and P_{CO_2} ($F_{4,130}$=3.57, P=0.009, Fig. 1A) and the main effect of P_{CO_2} ($F_{1,130}$=9.01, P=0.003) but not light ($F_{4,130}$=1.221, P=0.306).

Calcification as a function of irradiance at ambient P_{CO_2} resembled a positive parabola, with calcification maximized at 70 µmol photons m^{-2} s^{-1} and decreasing slightly thereafter (Fig. 1A). In contrast, calcification as a function of irradiance at high P_{CO_2} resembled a negative parabola. Relative to ambient P_{CO_2}, high P_{CO_2}

Table 1. Physical and chemical features of seawater in the treatment tanks over the primary (March 2011) and secondary (June 2012) experiments

Experiment	Treatment	Temperature (°C)	Salinity	TA (μmol kg^{-1} SW)	pH$_{Total}$	P_{CO_2} (μatm)	HCO_3^- (μmol kg^{-1} SW)	CO_3^{2-} (mmol kg^{-1} SW)
Primary	Ambient P_{CO_2}	24.01±0.14	33.9±0.1	2276±13	7.97±0.02	493±27	1846±30	175±3
	High P_{CO_2}	23.98±0.10	33.9±0.1	2274±10	7.75±0.01	878±26	1992±20	115±2
Secondary	Ambient P_{CO_2}	27.56±0.02	30.4±0.1	2236±2	7.98±0.01	483±9	1793±6	182±3
	High P_{CO_2}	27.49±0.02	30.4±0.1	2222±8	7.73±0.01	938±11	1954±5	111±2

Experiments lasted 5 days. Values are means ± s.e.m. Temperature and salinity were measured twice daily (*N*=10) with the remaining parameters measured daily (*N*=5).
TA, total alkalinity.

had the most conspicuous effects on calcification at intermediate light intensities (41, 70 and 122 μmol photons m^{-2} s^{-1}), with virtually no effect at the lowest (31 μmol photons m^{-2} s^{-1}) and highest irradiance (226 μmol photons m^{-2} s^{-1}). The greatest difference in calcification between ambient and high P_{CO_2} occurred at 70 μmol photons m^{-2} s^{-1}, where calcification was depressed 29% by high P_{CO_2}.

Survivorship of *P. damicornis* recruits varied significantly across light and P_{CO_2} treatments (Fig. 1A, Fig. 2, Table 2). Survivorship remained >90% in all treatments until day 4, but thereafter treatment effects became apparent. Despite similar calcification at ambient and high P_{CO_2} at both the lowest and highest irradiance, survivorship was 34% higher (P=0.012, Table 2) in ambient than in high P_{CO_2} at 31 μmol photons m^{-2} s^{-1}, while at 222 μmol photons m^{-2} s^{-1} survivorship was 38% greater under high than under low P_{CO_2} (P<0.001). In ambient P_{CO_2}, only 34% of recruits survived at 70 μmol photons m^{-2} s^{-1}, the lowest of any irradiance, but in high P_{CO_2}, survivorship was lowest (19%) at 41 μmol photons m^{-2} s^{-1} and highest at 70 μmol photons m^{-2} s^{-1} (71%). Under both P_{CO_2} regimes, survivorship was maximized at 122 μmol photons m^{-2} s^{-1}. Survivorship was not correlated with calcification at ambient P_{CO_2} (r=0.173, d.f.=3, P=0.477) or high P_{CO_2} (r=0.424, d.f.=3, P=0.771), or with light intensity at ambient P_{CO_2} (r=0.154, d.f.=3, P=0.805) or high P_{CO_2} (r=0.733, d.f.=3, P=0.159).

Secondary experiment (June 2012)

The physical and chemical conditions in the treatments for the secondary experiment were similar to those created in the primary experiment (Table 1). However, the reef environment from which the parent colonies were collected in June 2012 was different from that encountered in March 2011, notably with higher seawater temperatures and elevated turbidity. A wet and stormy period during June 2012 resulted in heavy rainwater run off, thick clouds and reduced sunlight. The clear film and ND filters created light treatments characterized by mean (±s.e.m.) irradiances of 14±0.6, 27±2, 56±3, 96±3 and 191±4 μmol photons m^{-2} s^{-1} (N=10). P_{CO_2} treatments were maintained at mean (±s.e.m.) values of 483±9 μatm (ambient P_{CO_2}) and 938±11 μatm (high P_{CO_2}) (N=5; Table 1).

The initial *P. damicornis* larvae released under the adverse conditions of June 2012 were unusual in shape and color, but larvae released subsequently appeared normal in color and size, and were similar to those obtained in March 2011. Only the later released larvae were used in the present analysis. The June larvae settled at a density of 17±1 corals per tile (mean ± s.e.m., N=100) and deposited 17.8–441.3 μg $CaCO_3$ per recruit in 5 days. Calcification was standardized to the protein concentration of recruits measured in the primary experiment (0.057±0.001 mg per recruit), and this generated calcification rates ranging from 0.06 to 1.55 mg $CaCO_3$ mg^{-1} protein day^{-1}. Although calcification was reduced in June 2012 compared with that in March 2011, it again was affected significantly by the main effect of P_{CO_2} ($F_{1,240}$=16.16, P<0.001), but was not affected by light ($F_{4,240}$=0.99, P=0.413) or the interaction

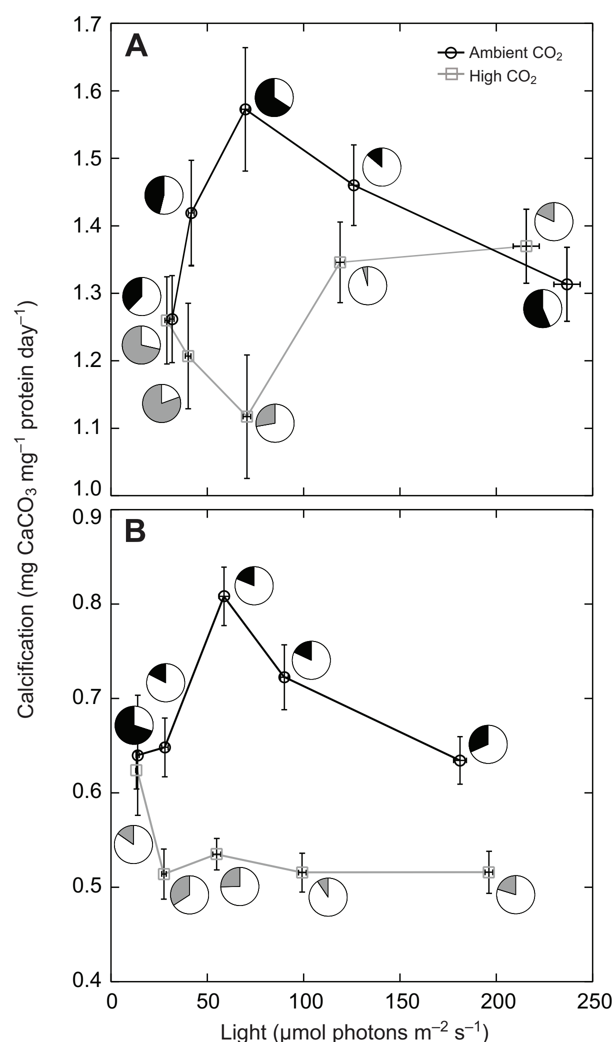

Fig. 1. Calcification of *Pocillopora damicornis* recruits standardized by protein (0.057 mg per recruit) in five light treatments in the primary experiment in March 2011 (A) and the secondary experiment in June 2012 (B). Corals were incubated for 5 days in ambient or high P_{CO_2}. Values are means ± s.e.m. (calcification N≈6–20 in March 2011, N≈7–39 in June 2012; photosynthetically active radiation N=18–20 in March 2011, N=10–20 in June 2012). Pie charts in each plot show the percentage of recruits alive (white) and dead (black, ambient P_{CO_2}; gray, high P_{CO_2}) as calculated by the Kaplin–Meier analysis at the end of the experiment (N≈92–209 in March 2011, N≈66–196 in June 2012).

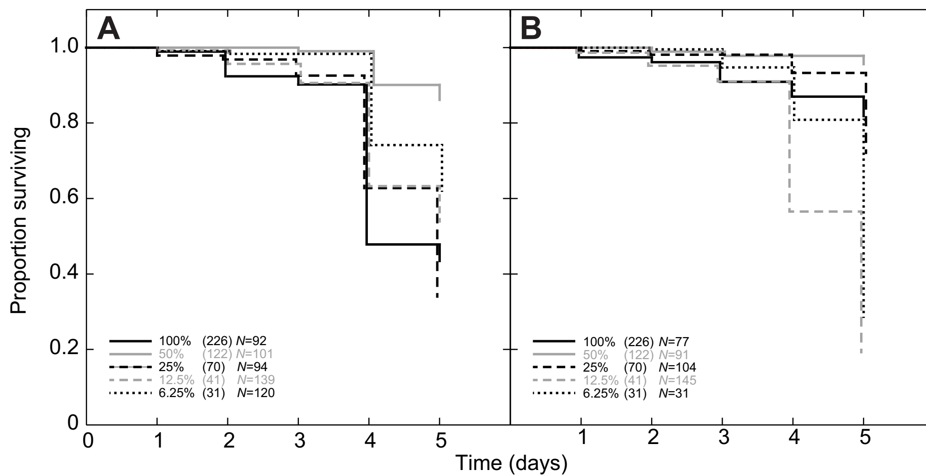

Fig. 2. Survivorship of *P. damicornis* recruits in March 2011 calculated from the Kaplan–Meier analysis in ambient (A) and high (B) P_{CO_2} in each of five light treatments.

of light and P_{CO_2} ($F_{4,240}$=1.14, P=0.339, Fig. 1B). Exploratory t-tests comparing calcification between P_{CO_2} treatments revealed no effect of P_{CO_2} at 14 μmol photons m^{-2} s^{-1} (t=0.137, d.f.=60, P=0.892) or 191 μmol photons m^{-2} s^{-1} (t=1.765, d.f.=68, P=0.082), but a significant difference at the intermediate irradiance of 56 μmol photons m^{-2} s^{-1} (t=3.011, d.f.=67, P=0.004).

As observed in 2011, calcification in recruits as a function of irradiance resembled a positive parabola at ambient P_{CO_2} (Fig. 1B), while at high P_{CO_2} the relationship between calcification and light differed slightly from that recorded in 2011. In 2012 and at elevated P_{CO_2}, calcification was highest at 14 μmol photons m^{-2} s^{-1} but thereafter declined with increasing irradiance and remained similar between 27 and 191 μmol photons m^{-2} s^{-1} (Fig. 1B). Nevertheless, the shape of the calcification–light relationships at ambient and elevated P_{CO_2} demonstrated that the relative effect of P_{CO_2} differed as a function of light intensity, with no effect at the lowest light intensity, the strongest effect (a 34% reduction) at 56 μmol photons m^{-2} s^{-1} and an intermediate effect at the highest light intensity.

Survivorship of *P. damicornis* recruits from 2012 varied across light and P_{CO_2} treatments (Fig. 1B). Despite similar calcification at ambient and elevated P_{CO_2} at the lowest irradiance, survivorship was 55% higher in ambient than in elevated P_{CO_2}. The lowest survivorship in ambient P_{CO_2} was recorded at 14 μmol photons m^{-2} s^{-1} (30%), but at elevated P_{CO_2} the lowest survivorship was recorded at 27 μmol photons m^{-2} s^{-1} (66%); the highest survivorship in ambient P_{CO_2} occurred at 27 μmol photons m^{-2} s^{-1} (83%), and in elevated P_{CO_2} it occurred at 96 μmol photons m^{-2} s^{-1} (91%). As was observed in 2011, survivorship was not correlated with either calcification or light intensity at either P_{CO_2} (r≤0.486, d.f.=3, P≥407).

DISCUSSION

Similar results from two experiments repeated in different years strengthen the conclusion that the effects of P_{CO_2} on calcification of *P. damicornis* recruits are influenced by light intensity. In the only previous study that has addressed the interactive effect of P_{CO_2} (186 *versus* 641 μatm P_{CO_2} achieved through HCl addition) and light on coral calcification, a calcification–irradiance response describing a hyperbolic tangent was reported, with calcification depressed by irradiances >81 μmol photons m^{-2} s^{-1} (Marubini et al., 2001). In contrast, our experiments conducted in 2011 demonstrated that the effect of light on calcification resembled a positive parabola at ambient P_{CO_2}, but at high P_{CO_2} the shape of the relationship changed to resemble an inverse parabola. Although the effects of light were less clear in the replicate experiment in 2012, nevertheless the qualitative shape of the calcification–light response differed between P_{CO_2} treatments, and P_{CO_2} effects were most strongly developed at intermediate light intensities. In both experiments, therefore, the relative effect of high P_{CO_2} on calcification was absent at the lowest light intensity, absent-to-weak at the highest light intensities, and strongly developed at intermediate light intensities (25–75 μmol photons m^{-2} s^{-1}).

A comparison of calcification rates of coral recruits in the present analysis with those reported elsewhere (reviewed in Albright, 2011) reveals a high degree of variability in the response to P_{CO_2}. Based on the interactive effects of light and P_{CO_2} reported here, we hypothesize that this variability can be attributed to different experimental light intensities. For instance, Albright and colleagues (Albright et al., 2008) measured the planar area of *Porites astreoides* recruits exposed to acidified seawater (pH 7.8, P_{CO_2} 720 ppm and 25.4–26.6°C) under artificial lights delivering on average <10 μmol photons m^{-2} s^{-1}, and reported 72–84% reductions by high P_{CO_2}. Subsequently, Albright and Langdon tested the effects of high P_{CO_2} (obtained with gas bubbling) on *P. astreoides* recruits under natural light (intensities not provided), and found that growth (by area) was

Table 2. Results from log rank tests of the survivorship of *Pocillopora damicornis* recruits calculated by Kaplan–Meier analysis in the primary and the secondary experiment

Experiment	Comparisons	Level	χ^2	d.f.	P-value
Primary	P_{CO_2} treatment	Ambient	60.5	4	<0.001
		High	209.9	4	<0.001
	Light treatment	226	22.8	1	<0.001
		122	4.9	1	0.027
		70	34.0	1	<0.001
		41	22.5	1	<0.001
		31	6.3	1	0.012
Secondary	P_{CO_2} treatment	Ambient	67.9	4	<0.001
		High	36.1	4	<0.001
	Light treatment	191	1.95	1	0.162
		96	3.5	1	0.062
		56	1.2	1	0.270
		27	7.6	1	0.006
		14	73.2	1	<0.001

Comparisons between all light treatments (in μmol photons m^{-2} s^{-1}) in ambient and high P_{CO_2} treatments as well as individual tests of light treatments between P_{CO_2} treatments are shown.

reduced by 35% at 775 μatm *versus* 330 μatm (Albright and Langdon, 2011). de Putron and colleagues grew recruits of *P. astreoides* and *Favia fragum* under 61 μmol photons $m^{-2} s^{-1}$, and found that calcification (by mass) at 29.4°C was reduced by 41% and 38% (respectively) at 1441 μatm P_{CO_2} delivered with gas bubbling (de Putron et al., 2011); Cohen and colleagues grew recruits of *F. fragum* at 61 μmol photons $m^{-2} s^{-1}$ and 25°C and found that calcification (by mass) was reduced by 26% at saturation state of aragonite Ω_{arag}=2.40 *versus* Ω_{arag}=3.7 (Cohen et al., 2009); and Anlauf and colleagues grew recruits of *P. panamensis* at 134 μmol photons $m^{-2} s^{-1}$ and reported that calcification (by mass) was reduced by 40% at 950 μatm P_{CO_2} (at 29.4°C and created by gas bubbling) compared with controls at 486 μatm P_{CO_2} and 28.4°C (Anlauf et al., 2011). Recently, we grew recruits of *Seriatopora caliendrum* at 305 μmol photons $m^{-2} s^{-1}$ and 25.1–25.4°C, and found that calcification (by mass) was unaffected by 623 *versus* 456 μatm P_{CO_2}, but was elevated 50% by 830 *versus* 464 μatm P_{CO_2}, in both cases with P_{CO_2} manipulated by gas bubbling (Dufault et al., 2012). Together, the aforementioned studies support a trend for the negative effect of elevated P_{CO_2} on calcification of coral recruits to be related inversely to light intensity, at least above a threshold value (here ~50 μmol photons $m^{-2} s^{-1}$). Indeed, if the results of Albright and Langdon (Albright and Langdon, 2011) are excluded from our data compilation (as they did not record light intensity), the inverse association between the effect size of P_{CO_2} on calcification and light is significant (r=–0.882, d.f.=6, P<0.05). Studies of the effects of high P_{CO_2} on the calcification of coral recruits appear more likely to reveal inhibitory effects at intermediate light intensities than at high light intensities.

Light intensities used in the present experiments (~14–226 μmol photons $m^{-2} s^{-1}$) were lower than those recorded at the depth from which the parent colonies were collected (5–7 m), but nonetheless are ecologically relevant for *P. damicornis* at Hobihu Reef where this species occurs from at least 2 to 10 m depth. The mean light intensity on exposed surfaces at 5 m depth at Hobihu Reef just prior to the March 2011 experiment was 392 μmol photons $m^{-2} s^{-1}$, but intensities lower than this would be found in the sheltered microenvironments favored for coral settlement (Nozawa, 2008). Interestingly, *P. damicornis* larvae from Hobihu Reef tended not to settle on exposed surfaces at high light intensities (>120 μmol photons $m^{-2} s^{-1}$) in the laboratory, and instead favored cracks and crevices; at lower light intensities (~50–75 μmol photons $m^{-2} s^{-1}$) they settled on exposed surfaces (A.M.D., unpublished data). These observations of the settlement behavior of *P. damicornis* larvae contextualize the present analysis, and suggest that coral recruits may normally settle *in situ* at light intensities under which they are most susceptible to high P_{CO_2}. Conversely, coral larvae that settle in the 'wrong' conditions under ambient P_{CO_2} (i.e. in high light intensities) might be better able to resist the effects of elevated P_{CO_2}. The possibility that high P_{CO_2} could act as a selective force on coral larvae to affect the beneficial consequences of settlement location deserves further attention.

The analysis of the survivorship of *P. damicornis* recruits reveals two interesting trends. First, given the relationship between rapid growth and survival in small corals (Jackson, 1977), it is surprising that survival and calcification were unrelated. As calcification creates the skeleton upon which coral tissue is located, and is a primary means by which size increases through asexual division and coloniality, we expected that survival would increase with calcification-driven increases in size. This was not the case, however, and at ambient P_{CO_2} in 2011 the lowest survival (34%) coincided approximately with the highest calcification at 70 μmol photons $m^{-2} s^{-1}$. At ambient P_{CO_2} in 2012, the highest survival remained >80% at intermediate light treatments (27–96 μmol photons $m^{-2} s^{-1}$), while calcification varied by up to 25%. These results suggest that the survival of small corals is determined by more than rapid growth (and increased size), and perhaps in an aquarium (as in the present study) that is safe from the risks of extrinsic mortality, mortality due to intrinsic effects (*sensu* Kirkwood and Austad, 2000) is elevated.

Second, even though calcification in coral recruits generally was depressed at low light intensities (especially in the 2011 experiment), and elevated P_{CO_2} has negative fitness consequences for at least one invertebrate (Wood et al., 2008), our results reveal that survivorship of *P. damicornis* recruits was elevated at high P_{CO_2} (878–938 μatm) compared with that at ambient P_{CO_2} (483–493 μatm) in three out of five irradiances tested in both 2011 and 2012. Although these results must be interpreted with caution as they were obtained in an aquarium, where sources of extrinsic mortality like competition and predation were absent, nevertheless it is intriguing to speculate they reflect the role of tissue quality in determining resistance to intrinsic sources of mortality. If this speculation is correct, then it is reasonable to hypothesize that tissue quality is modulated indirectly by elevated P_{CO_2} accelerating photosynthesis by *Symbiodinium* (Anthony et al., 2008; Crawley et al., 2010), particularly at high light intensities. *Symbiodinium* utilize HCO_3^- as a carbon source in photosynthesis, converting it to CO_2 with a carbon-concentrating mechanism prior to fixation in the Calvin cycle (Furla et al., 2000). As rising P_{CO_2} increases seawater [HCO_3^-], high P_{CO_2} has the potential to accelerate photosynthesis (Erez et al., 2011), although experimental evidence in support of this trend is equivocal (e.g. Schneider and Erez, 2006; Crawley et al., 2010; Brading et al., 2011). Nevertheless, it might be valuable to further explore the role of high P_{CO_2} (and hence elevated HCO_3^-) in accelerating photosynthesis in *Symbiodinium*, hence affecting the translocation of photosynthetically fixed carbon to the animal host (Muscatine, 1990), and its role in augmenting holobiont biomass (Rodrigues and Grottoli, 2007) and (as in the present case) perhaps modulating survival.

Finally, the most intriguing aspect of the current analysis is the contrasting shape of the calcification–irradiance responses at ambient and high P_{CO_2}. Following our 2011 research, it was clear that the P_{CO_2}–light interaction relied heavily on the two calcification rates recorded at 70 μmol photons $m^{-2} s^{-1}$ (Fig. 1), and therefore the experiment was repeated in order to evaluate the possibility that the results could be attributed to chance alone. Although calcification rates were lower in June 2012 than in March 2011 – most likely reflecting seasonality in coral performance (Fitt et al., 2000; Thornhill et al., 2011) – the results of the two experiments were similar for ambient P_{CO_2} and deviated under high P_{CO_2} when light was >56 μmol quanta $m^{-2} s^{-1}$. The altered response of corals at high irradiances under high P_{CO_2} in the second experiment may be the result of seasonal changes to holobiont physiology caused by increased temperature during the second experiment (27.5 *versus* 24.0°C), or by changes to seawater quality caused by heavy rainfall just prior to the start of the second experiment.

While it was beyond the scope of the present study to experimentally test for a mechanistic basis to the common response recorded in 2 years, it is intriguing to speculate that our results reflect light-dependent use of HCO_3^- for coral calcification (Comeau et al., 2013b). According to Comeau and colleagues (Comeau et al., 2013b), tropical corals calcify by utilizing CO_3^{2-} in the day and night – and therefore calcification is depressed at high P_{CO_2} (which reduces [CO_3^{2-}]) (Erez et al., 2011) – but additionally corals use

[HCO_3^-] in a light-dependent manner during the day. The research by Comeau and colleagues (Comeau et al., 2013b) can be used to reconcile aspects of the variation in response of coral recruits to high P_{CO_2} that have been reported previously (cited above) and in the present study. In the case of the present study, it is possible, therefore, that high P_{CO_2} depresses calcification at intermediate light intensities because of lowered CO_3^{2-}, but as light intensities increase, light-dependent use of HCO_3^- causes calcification to again increase. At ambient P_{CO_2}, HCO_3^- limitation may then cause calcification to decline at high light intensities (see Comeau et al., 2013b).

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AUTHOR CONTRIBUTIONS

All authors contributed to lab work and field collections. A.M.D., V.R.C. and P.J.E. designed the study. Data analysis was carried out by A.M.D., A.N., L.B., V.R.C. and P.J.E. The manuscript was written by A.M.D., A.N., L.B., V.R.C. and P.J.E. All authors commented on the manuscript and contributed to revisions.

COMPETING INTERESTS

No competing interests declared.

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