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Ocean acidification has no effect on thermal bleaching in the coral *Seriatopora caliendrum*

C. B. Wall · T.-Y. Fan · P. J. Edmunds

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Abstract The objective of this study was to test whether elevated pCO₂ predicted for the year 2100 (85.1 Pa) affects bleaching in the coral *Seriatopora caliendrum* (Ehrenberg 1834) either independently or interactively with high temperature (30.5 °C). Response variables detected the sequence of events associated with the onset of bleaching: reduction in the photosynthetic performance of symbionts as measured by maximum photochemical efficiency (F_v/F_m) and effective photochemical efficiency ($\Delta F/F_m'$) of PSII, declines in net photosynthesis (P^{net}) and photosynthetic efficiency (α , α), and finally, reduced chlorophyll *a* and symbiont concentrations. *S. caliendrum* was collected from Nanwan Bay, Taiwan, and subjected to combinations of temperature (27.7 vs. 30.5 °C) and pCO₂ (45.1 vs. 85.1 Pa) for 14 days. High temperature reduced values of all dependent variables (i.e., bleaching occurred), but high pCO₂ did not affect *Symbiodinium* photophysiology or productivity, and did not cause bleaching. These results suggest that short-term exposure to 81.5 Pa pCO₂,

alone and in combination with elevated temperature, does not cause or affect coral bleaching.

Keywords Ocean acidification · Temperature · Bleaching · Scleractinia · Taiwan

Introduction

Increased atmospheric CO₂ from anthropogenic activities is contributing to global climate change (GCC) and threatens coral reefs through seawater warming and ocean acidification (OA) (Hoegh-Guldberg et al. 2007). OA caused by the dissolution of CO₂ in seawater results in a decrease in ocean pH and a shift in the carbonate chemistry of seawater that is unfavorable for the deposition of calcium carbonate (Chan and Connolly 2013). During the twentieth century, rising atmospheric CO₂ has resulted in a 0.7 °C increase in mean sea surface temperatures (SST) and a decrease in sea surface pH_T of 0.1 (Caldiera et al. 2003; Raven 2005). Atmospheric CO₂ is projected to increase from current levels (~39.0 Pa) to between 49.6 and 86.1 Pa by the end of this century (van Vuuren et al. 2011) and likely will increase SST 2–3 °C and reduce sea surface pH_T by 0.3 (Meehl et al. 2007). As a result, GCC will increase the incidence of warm-water coral bleaching, and reef accretion will slow due to elevated SST and OA (Pandolfi et al. 2011).

Rising SST has contributed to the decline in abundance of corals (Hoegh-Guldberg 1999; Pandolfi et al. 2011), largely through bleaching (Loya et al. 2001). Paling of coral tissue (i.e., “bleaching”), occurs in response to multiple environmental stressors (Glynn 1996; Fitt et al. 2001), of which high light intensities and elevated temperature are most prominent in causing large-scale

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bleaching (Fitt et al. 2001). Coral bleaching progresses through a series of cellular events culminating in the reduction in the concentrations of photopigments and densities of *Symbiodinium* spp. algae within the coral, and in some instances, results in death (Glynn 1983; Fitt et al. 2001). Photoinhibition from oxidative damage to the photosynthetic apparatus of *Symbiodinium*, particularly the D1-protein, and impaired carbon fixation initiates the events leading to symbiont expulsion (Lesser 1997; Jones et al. 1998; Warner et al. 1999; Smith et al. 2005). These effects can first be detected as reductions in the quantum yield of photosystem II (PSII) (hereafter, photochemical efficiency) in response to PSII photoinactivation (Warner et al. 2010), and a reduced capacity to use light energy together with reducing agents to fix CO₂ in the Calvin cycle (hereafter, photosynthetic capacity) (Iglesias-Prieto et al. 1992; Jones et al. 1998; Warner et al. 1999). As these effects persist, impairment of the light and dark reactions of photosynthesis and damage to the photosynthetic apparatus by reactive oxygen species (Lesser 1997; Jones et al. 1998; Smith et al. 2005) leads to the expulsion of *Symbiodinium*, host cell detachment (Gates et al. 1992), or apoptosis (Dunn et al. 2007).

The effects of OA on biomineralization have received considerable attention (Chan and Connolly 2013), but the effects on photosynthesis of *Symbiodinium* within corals remain unclear. Hermatypic corals depend on *Symbiodinium* to provide photosynthetically fixed carbon to fuel metabolism (Muscatine et al. 1984). Some studies with corals suggest OA reduces net photosynthesis (P^{net}) (Reynaud et al. 2003; Anthony et al. 2008), and the maximum rate of photosynthesis (i.e., $P^{\text{net}}_{\text{max}}$) (Crawley et al. 2010), although some corals show no response of photosynthesis to pCO₂ enrichment (Leclercq et al. 2002; Godinot et al. 2011). OA has also been reported to induce bleaching in at least two corals, both alone and in concert with elevated temperature (Anthony et al. 2008). Anthony et al. (2008) hypothesized that the mechanism of OA-induced bleaching involved the disruption of carbon-concentration mechanisms used by *Symbiodinium* (Weis et al. 1989; Leggat et al. 1999), or impaired photoprotective mechanisms, such as photorespiration (Crawley et al. 2010) and nonphotochemical quenching (Hill et al. 2005). Support for these hypotheses is limited; however, as effects of OA on the photophysiology of *Symbiodinium in hospite* have not been studied in detail, and where these effects have been investigated, the outcomes are equivocal (Godinot et al. 2011; Iguchi et al. 2011; Edmunds 2012) and may be specific to *Symbiodinium* phylotypes (Brading et al. 2011).

The objective of the study was to test whether elevated pCO₂ affects bleaching in corals. An experiment was

conducted in which corals were exposed to two temperatures and two pCO₂ regimes, with the effects assessed using dependent variables that detect the early stages of bleaching (after Fitt et al. 2001): (1) the onset of reduced photochemical efficiency (2) intermediate effects manifesting in reduced photosynthetic capacity and efficiency prior to visible paling of tissue, and (3) terminal effects culminating in reduced *Symbiodinium* and photopigment densities. The experiment was conducted with the branching pocilloporid coral *Seriatopora caliendrum* (Ehrenberg 1834) that is common on the shallow reefs of southern Taiwan (Dai and Horng 2009) where this study was conducted and has previously been reported susceptible to thermal bleaching (Loya et al. 2001).

Materials and methods

Experimental design

Four treatments contrasted ambient temperature–ambient pCO₂ (AT–ACO₂), ambient temperature–high pCO₂ (AT–HCO₂), high temperature–ambient pCO₂ (HT–ACO₂), and high temperature–high pCO₂ (HT–HCO₂). Ambient temperature referred to the seawater on shallow reefs in Nanwan Bay when the experiment was conducted in July and August 2011 (28.0 ± 0.2 °C at 3 m depth [mean $\pm$ SE, $n = 21$ days]) and therefore was set at 27.5 °C. Ambient pCO₂ reflected ambient CO₂ conditions, although this routinely was above the global mean atmospheric value of ~ 39.0 Pa. High temperature was relative to the maximum seawater temperature recorded at 3 m depth on the study reef in summer (31.0 °C) (T-Y Fan pers comm) and was 30.5 °C. High pCO₂ represented conditions projected to occur by the year 2100 (~ 86 Pa) under the high CO₂ emission scenario RCP 6.0 (van Vuuren et al. 2011).

We hypothesized that OA would cause bleaching as defined by decreased photochemical efficiency, reduced photosynthetic capacity and efficiency, depressed chlorophyll *a* content, and lowered *Symbiodinium* densities and that these effects would be exacerbated with high temperature. To test this hypothesis, corals were exposed to treatments in 8 tanks ($n = 2$ tanks treatment⁻¹) filled with 130 L of filtered (1.0 μm) seawater. Treatments were maintained at a salinity of 33.4 (measured with a YSI 3100 Conductivity Meter, YSI Inc., USA) with 20 % water changes each evening. Temperatures were maintained independently by microsensor-based regulators (Aqua-Controller, Neptune Systems, USA) connected to a 300-W heater (Taikong Corp.), and chiller (Aquatek, Aquasystems, Taiwan), and the seawater was mixed with a pump (1,451 L h⁻¹). Light was provided to each tank by two

18-W fluorescent bulbs (TL-D Blue, Phillips, USA) and two 150-W metal-halide bulbs on a 12 h light: 12 h dark cycle that created mean irradiances ranging from 251 to 279 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ as measured beneath the seawater using a spherical light sensor (LI-193, Li-Cor, USA).

Treatments of pCO_2 were maintained by bubbling ambient air (A- CO_2) or CO_2 -enriched air into the tanks (H- CO_2). To prepare high pCO_2 treatments, CO_2 was mixed with ambient air by solenoid-controlled gas mixing technology (Model A352, Qubit Systems, Canada). CO_2 and ambient air were mixed in a chamber, and the pCO_2 measured using an infrared (IR) gas analyzer (S151, Qubit Systems) calibrated against certified reference gas (1,793 ppm CO_2 , San Ying Gas Co., Taiwan). The pCO_2 treatments were maintained dynamically by the IR gas analyzer, which regulated a solenoid valve controlling the flow of CO_2 gas. The final pCO_2 was logged in ppm on a PC using LabPro software (Vernier Software and Technology, USA), and a pump delivered the gas mixture to the high- pCO_2 tanks at $\sim 15 \text{ L min}^{-1}$. Ambient pCO_2 tanks received ambient, non- CO_2 -enriched air at a similar flow rate.

Treatments were monitored daily at 0900, 1200, and 1700 hrs for temperature and salinity; irradiance was measured at 1200 hrs; pH and carbonate chemistry of the treatments were determined daily on seawater samples ($\sim 250 \text{ mL}$) taken from all tanks at 0900 hrs. Temperature was measured using a certified digital thermometer (Fisher Scientific 15-077-8, $\pm 0.05^\circ\text{C}$), and seawater was assessed for total alkalinity (TA, $\mu\text{mol kg}^{-1}$) and pCO_2 by potentiometric titrations following standard operating procedures (SOP) 3 (Dickson et al. 2007); pH_T was determined spectrophotometrically using *m*-cresol purple (SOP 6B, Dickson et al. 2007). Seawater samples were titrated using an open-cell autotitrator (Model DL50, Mettler-Toledo, USA) filled with certified acid titrant (from A. Dickson, Scripps Institution of Oceanography) and equipped with a DG115-SC pH probe (Mettler-Toledo). TA was evaluated for precision and accuracy using certified reference materials (CRM) of known TA (from A. Dickson, Scripps Institution of Oceanography) with our analyses differing $<0.9\%$ from certified values. pH_T , salinity, temperature, and TA were used in CO2SYS software in Microsoft Excel (Fangue et al. 2010) to calculate the components of the dissolved inorganic carbon (DIC) system in seawater.

Coral collection

Sixty juvenile *S. caliendrum* ($<4 \text{ cm diam.}$) were collected on July 22, 2011, from Hobihu Reef ($21^\circ 56.799'\text{N}$, $120^\circ 44.968'\text{E}$), Nanwan Bay. Colonies were collected from 3 to 4 m depth and transported to a flow-through aquarium

at the National Museum of Marine Biology and Aquarium (NMMBA) where they were allowed to recover from collection for 24 h. The recovery tank was filled with flowing, filtered seawater ($50 \mu\text{m}$) and mixed with a pump ($1,451 \text{ L h}^{-1}$). Temperature was maintained at ambient conditions ($28.07 \pm 0.10^\circ\text{C}$, $\pm \text{SE}$, $n = 24$) and light was supplied at $164 \pm 4 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ on a 12 h light: 12 h dark cycle.

One day following collection, the colonies of *S. caliendrum* were suspended in the recovery tank using nylon line and left to recover for 5 days. On July 28, 2011, they were placed randomly into the treatment tanks ($n = 7 \text{ tank}^{-1}$) for incubations lasting 14 days. On August 11, 2011, corals were processed over 3 days for the dependent variables described below and retained in experimental conditions during this time. To maintain comparable exposure periods among like-temperature treatments, corals from the HT treatments were processed first, followed by corals in the AT treatments, with corals randomly selected for processing within each temperature treatments.

Photochemical efficiency

The effects of temperature and pCO_2 on photochemical efficiency were tested by measuring the maximum photochemical efficiency of open RCII in the dark (F_v/F_m) and the effective photochemical efficiency of RCII in the light ($\Delta F/F_m'$) using pulse amplitude modulation (PAM) fluorometry. PAM fluorometry is an effective tool to assess noninvasively the photophysiology of *Symbiodinium in hospite* (Warner et al. 1996, 2010) and provides an indication of PSII photochemical activity and the transport of electrons through PSII (Cosgrove and Borowitzka 2010). F_v/F_m provides a measure of photochemical quenching (qP) reflecting the rate of charge separation across PSII in the open (i.e., dark-adapted) state, while $\Delta F/F_m'$ accounts for photochemical and nonphotochemical quenching (NPQ), including mechanisms for the dissipation of excess absorbed light energy as heat through the PSII antennae complex (Hill et al. 2005). NPQ is of particular biological importance as a mechanism of photoprotection and avoidance of photoinhibition under peak daily irradiance and under conditions causing bleaching (Warner et al. 1996; Jones et al. 1998; Hoegh-Guldberg and Jones 1999).

Photochemical efficiency was assessed using a Diving-PAM (Waltz, GmbH, Effeltrich, Germany) operated at a gain of 6, intensity of 9, and a slit width of 0.8. Prior to the start of the experiment, PAM settings were adjusted to obtain a range of minimum fluorescence yield (F_o) between 200 and 400 (arbitrary units) and stable maximum fluorescence yield (F_m). $\Delta F/F_m'$ was measured to quantify changes in quantum yield relative to the dark-adapted state

due to excess thermal energy dissipation and NPQ, and F_v/F_m was measured to quantify the maximum efficiency of open RCII in the dark-adapted state. Photochemical efficiency was measured using a 5-mm-diameter fiberoptic probe held ~ 5 mm above the tissue and ~ 1 cm behind branch tips. $\Delta F/F_m'$ was measured under actinic irradiance ($\sim 265 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and F_v/F_m under weak indirect red lighting ($\leq 2.0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). $\Delta F/F_m'$ was measured every second day of the incubation at 1230 hrs, and F_v/F_m was measured every second day at 1730 hrs. A pilot study was used to determine the duration of dark adaptation necessary to stabilize values of F_v/F_m , to identify effects of prolonged darkness on F_v/F_m (i.e., dark-induced reduction in the PQ pool; Hill and Ralph 2008), and to test whether weak indirect red light (as produced from small lamps used during nocturnal PAM measurements) affected F_v/F_m . Results indicated F_o stabilized after <0.5 h of darkness, and F_v/F_m was statistically indistinguishable when measured following dark adaptation lasting 0.5, 1.0, or 2.0 h ($F_{2,27} = 0.137$, $P = 0.872$), or measured with and without weak red light ($F_{1,18} = 0.352$, $P = 0.561$).

Photosynthesis–irradiance (P/I) curves

To test for the effects of $p\text{CO}_2$ and temperature on the ability for *Symbiodinium* to utilize light and perform photosynthesis, net photosynthesis (P^{net}), determined from changes in O_2 concentrations in seawater, was measured under different irradiances using three corals selected randomly from each treatment tank ($n = 6$ treatment $^{-1}$). Two respirometers were used to measure P^{net} , and each housed a single coral in trials lasting ~ 1.5 – 2.0 h. Measurements of P^{net} began on the 14th day of incubations, and 3 days were required to process all corals in the experiment. Temperatures were maintained by placing the respirometers in a water bath. Water motion in each chamber was provided by a stir-bar, and the flow rate quantified by photographing hydrated *Artemia* spp. eggs (Sebens and Johnson 1991), revealing the mean flow rate near the center of the respirometer to be $5.43 \pm 0.32 \text{ cm s}^{-1}$ ($\pm \text{SE}$, $n = 20$). Prior to each trial, corals were maintained in darkness for 1 h to allow the stimulatory effect of light on respiration to abate (Edmunds and Davies 1988). O_2 flux then was measured at ten irradiances supplied in an ascending sequence between 0 and $747 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Light intensities were created by adjusting the height of a 400-W metal-halide lamp (Osram Sylvania, USA) above the respirometer, and measuring the irradiance using a cosine-corrected light sensor recording photosynthetically active radiation (PAR). The light sensor was 1.0 mm diameter and attached to a Diving-PAM (Waltz, GmbH, Effeltrich, Germany) and was calibrated using a

Li-Cor LI-192 quantum sensor. O_2 fluxes were adjusted for changes in O_2 concentrations in control chambers filled with seawater alone, and controls were run at each combination of temperature and $p\text{CO}_2$ for each irradiance, and during darkness ($n = 3$ treatment $^{-1}$).

The O_2 saturation of seawater was measured using an optrode (FOXY-R, 1.58 mm diameter, Ocean Optics, USA) connected to a spectrophotometer (USB2000, Ocean Optics), which logged O_2 concentrations on a PC running Ocean Optics software (OOISensors, version 1.00.08, Ocean Optics). The optrode was calibrated using water-saturated air at the measurement temperature and a zero solution of sodium sulfite (Na_2SO_3) and 0.01 mol L^{-1} sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$). O_2 saturation during the trials was maintained between 80 and 100 % by replenishing chambers with filtered ($1.0 \mu\text{m}$) seawater from respective temperature and $p\text{CO}_2$ treatments. Changes in O_2 saturation were converted to O_2 concentrations ($\mu\text{mol L}^{-1}$) using tabulated gas solubility at a known temperature and salinity [N. Ramsing and J. Gundersen at www.unisense.com, based on Garcia and Gordon 1992]. Rates of change in O_2 concentrations were determined by regressing O_2 concentration against time, and standardizing to the surface area of the coral tissue (cm^2) as determined by wax dipping (Stimson and Kinzie 1991). The relationship between P^{net} and irradiance was described with a hyperbolic tangent function (Jassby and Platt 1976) that included an exponent for photoinhibition (e.g., β) at high irradiances (Platt et al. 1980) to account for photoinhibitory effects of high-light or high-temperature exposure known to occur in phytoplankton (Platt et al. 1980) and *Symbiodinium* (Smith et al. 2005):

$$P^{\beta} = P_s^{\beta} (1 - e^{-a}) e^{-b} \quad (1)$$

where $a = \alpha I/P_s^{\beta}$, $b = \beta I/P_s^{\beta}$, P^{β} is the rate of net primary productivity (P^{net}), P_s^{β} is the maximum rate of net photosynthesis accounting for photoinhibition, α is the initial slope of the light-limited portion of the curve, and I is irradiance in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Hyperbolic tangent functions were fit to the productivity data by nonlinear regression using Systat 11 software (Systat, Inc., USA) and used to characterize the photosynthetic efficiency (α) and P^{net} under high-light conditions. The aforementioned curves describe the full biological relationship between P^{net} and I for a large range of irradiances supplied in the laboratory, with these intensities exceeding maximum intensities found on the study reef. Mean PAR at 3 m depth on Hobihi reef (measured between March 6 and 10, 2011, using a 4π spherical quantum sensor (MkV-L, JFE Advantech Co., Kobe, Japan) between 0900–1500 hrs was $660 \pm 30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($\pm \text{SE}$, $n = 148$). To compare the photosynthetic performance of corals under ecologically relevant conditions and following exposure to

treatments, best-fit curves were used to calculate P^{net} at 660 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (hereafter P_{660}^{net}), and this metric, along with α , and dark respiration, was compared among treatments. While inclusion of an exponent for photoinhibition (β) improved the fit of the response curves to the empirical data, in situ irradiances were not sufficiently high to elicit photoinhibition on the reef, and therefore, β was not employed as a dependent variable in the analysis.

Chlorophyll *a* concentration and *Symbiodinium* density

Chlorophyll *a* concentration and *Symbiodinium* density were quantified by removing coral tissue from the skeleton using an airbrush filled with filtered seawater (1.0 μm). Colonies were airbrushed into a plastic bag, producing 8–40 mL of slurry that was homogenized (Polytron PT2100, Kinematica, USA) prior to separating the *Symbiodinium* by centrifugation (1,500 $\times g$). The *Symbiodinium* pellet was resuspended by vortexing in filtered seawater and used to measure chlorophyll *a* concentration and symbiont density.

Symbiodinium used for chlorophyll determinations were frozen (-4°C for 24 h) and subsequently thawed (4°C for 24 h) and filtered onto a cellulose acetate membrane filter (3.0 μm pore size, Critical Process Filtration, USA) to which 3 mL of 90 % acetone was added. Samples were refrigerated (4°C) in darkness for 36 h, centrifuged (1,500 $\times g$ for 3 min), and absorbances at 630 and 636 nm measured and used to calculate chlorophyll *a* concentration using the trichromatic equations of Jeffrey and Humphrey (1975) for dinoflagellates. Chlorophyll *a* concentration was standardized by algal cell (pg cell^{-1}) and surface area ($\mu\text{g cm}^{-2}$) of the coral. *Symbiodinium* density (cells cm^{-2}) was determined by counting *Symbiodinium* in the homogenized slurry stripped from the coral colonies, with the

counts completed using a hemocytometer ($n = 4$ counts). Preliminary data showed that the mean and standard deviation of replicate determinations of *Symbiodinium* density stabilized after four counts.

Statistical analysis

Response variables were compared among treatments using three-way nested ANOVA in which pCO_2 and temperature were fixed effects, and tank was a random factor nested within treatment. The physical and chemical conditions in the treatments also were analyzed with this statistical model. Tank was removed from the model when not significant at $P \geq 0.25$ (Quinn and Keough 2002). Significant interactive effects were analyzed using a post hoc Tukey test. To test the statistical assumption of ANOVA, graphical analyses of residuals were employed. Analyses were performed using Systat 11 in a Windows operating system.

Results

Tank parameters

Physical and chemical conditions in the treatment tanks were maintained precisely (Table 1). Mean pCO_2 treatments were 45.1 ± 0.2 and 85.1 ± 0.5 Pa ($\pm\text{SE}$, $n = 55\text{--}56$) with mean pCO_2 across treatment tanks ranging from 43.6 to 47.0 Pa (A- CO_2) and 83.2 to 87.2 Pa (H- CO_2). pCO_2 differed between replicate tanks ($F_{4,103} = 3.673$, $P = 0.008$) and treatments ($F_{1,4} = 1798.18$, $P = < 0.001$), and pH_T differed between replicate tanks ($F_{4,103} = 5.956$, $P < 0.001$) and between pCO_2 treatments ($F_{1,4} = 1120.272$, $P = < 0.001$). The tank effects for pCO_2 and pH reflected differences of < 0.04 pH_T and ≤ 4.0 Pa pCO_2 . For seawater temperatures, tank effects were not detected ($F_{4,176} = 1.261$,

Table 1 Summary of physical and chemical conditions in the 8 treatment tanks between July 28 and August 11, 2011

Treatment	Tank	Temperature ($^\circ\text{C}$)	$\text{pH}_{\text{total}}^\dagger$	TA ($\mu\text{mol kg}^{-1}$)	pCO_2 (Pa)	HCO_3^- ($\mu\text{mol kg}^{-1}$)	CO_3^{2-} ($\mu\text{mol kg}^{-1}$)
AT- ACO_2	2	27.6 ± 0.02 (23)	7.99 (14)	2196 ± 8 (14)	45.4 ± 0.3 (14)	1724 ± 5.1 (14)	192 ± 1.7 (14)
	4	27.7 ± 0.13 (23)	7.99 (14)	2184 ± 7 (14)	44.6 ± 0.3 (14)	1712 ± 4.5 (14)	192 ± 1.4 (14)
HT- ACO_2	3	30.4 ± 0.14 (23)	8.01 (14)	2217 ± 8 (14)	43.6 ± 0.5 (14)	1687 ± 6.3 (14)	216 ± 1.8 (14)
	8	30.6 ± 0.02 (23)	7.98 (14)	2219 ± 7 (14)	46.7 ± 0.4 (14)	1711 ± 5.1 (14)	207 ± 1.7 (14)
AT- HCO_2	1	27.7 ± 0.03 (23)	7.76 (14)	2208 ± 7 (14)	86.1 ± 0.6 (14)	1904 ± 4.6 (14)	124 ± 1.2 (14)
	7	27.7 ± 0.10 (23)	7.75 (13)	2200 ± 8 (13)	87.1 ± 0.9 (13)	1903 ± 6.3 (13)	121 ± 1.4 (13)
HT- HCO_2	5	30.6 ± 0.13 (23)	7.77 (14)	2227 ± 6 (14)	83.2 ± 1.2 (14)	1884 ± 3.4 (14)	141 ± 2.1 (14)
	6	30.5 ± 0.05 (23)	7.77 (14)	2222 ± 7 (14)	84.2 ± 0.8 (14)	1883 ± 4.7 (14)	139 ± 1.5 (14)

Seawater chemistry was assessed daily and temperature three times daily (0900, 1200, 1700 h) in all tanks. Values displayed are mean $\pm$ SE (n) TA total alkalinity, AT- ACO_2 ambient temperature–ambient pCO_2 , HT- ACO_2 high temperature–ambient pCO_2 , AT- HCO_2 ambient temperature–high pCO_2 , HT- HCO_2 high temperature–high pCO_2

† SE < 0.1

$P = 0.287$), and mean temperatures ($\pm$ SE, $n = 92$) were 27.65 ± 0.04 °C (AT) and 30.53 ± 0.05 °C (HT).

Photochemical efficiency

Corals were acclimated to laboratory conditions prior to being placed into treatments. The progress of laboratory acclimation was monitored through daily measures of F_v/F_m using 10 corals selected randomly each day. We inferred that acclimation was complete when F_v/F_m did not vary among days. After 5 days (when F_v/F_m stabilized), corals were placed in treatments and $\Delta F/F_m'$ and F_v/F_m measured every second day (data not shown) with this regime revealing declines at HT (but not other treatments) after 7 days. After 13 days, corals at HT experienced a 15 % (HT-HCO₂) and 16 % (HT-ACO₂) reduction in $\Delta F/F_m'$ ($P < 0.05$) compared to corals in the AT-ACO₂ treatment (Fig. 1). Mean F_v/F_m was depressed 13 and 9 % in HT-ACO₂ and HT-HCO₂ treatments, respectively, versus AT-ACO₂ ($P < 0.05$) (Fig. 1). No significant effect ($P > 0.25$) of tank was detected for F_v/F_m , and tank was dropped from this analysis. While tank also was not significant for $\Delta F/F_m'$ ($P = 0.193$), it was retained in the analysis. $\Delta F/F_m'$ was affected by temperature ($F_{1,4} = 96.726$, $P = 0.001$) and was reduced at HT relative to AT. However, $\Delta F/F_m'$ was not affected by pCO₂ ($F_{1,4} = 0.775$, $P = 0.428$), and there was no temperature $\times$ pCO₂ interaction ($F_{1,4} = 0.0001$, $P = 0.993$). Similarly, corals at HT exhibited reduced F_v/F_m and were affected by temperature ($F_{1,48} = 63.711$, $P < 0.001$) but not pCO₂ ($F_{1,48} = 2.595$, $P = 0.114$), and there was no temperature $\times$ pCO₂ interaction ($F_{1,48} = 0.307$, $P = 0.582$).

Fig. 1 Effective photochemical efficiency of RCII in actinic light ($\Delta F/F_m'$) (~ 266 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and maximum photochemical efficiency of open RCII (F_v/F_m) for juvenile *Seriatopora caliendrum* exposed for 14 days to combinations of temperature and pCO₂ (Table 1). Values displayed are mean $\pm$ SE ($n = 13$ –14 treatment⁻¹)

P/I curves

Net photosynthesis standardized to area (cm^2) increased with irradiance and developed asymptotes at >400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in 7 cases, with slight declines in P^{net} at high irradiances for 2 corals in the HT-ACO₂ treatment. Overall, however, mean ($\pm$ SE, $n = 5$ –6) values for β ranged from 0.030 ± 0.020 (HT-ACO₂) to 0.006 ± 0.002 $\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$ ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)⁻¹ (HT-HCO₂), and these declines did not begin until ecologically relevant irradiances from the collection depth had been exceeded. The hyperbolic tangent functions with photoinhibition fit the photosynthesis data well (mean $r^2 = 0.95$) and were used to calculate values for α and P_{660}^{net} for corals in each treatment ($n = 5$ –6 treatment⁻¹). Dark respiration was calculated directly from raw data (Table 2). Tank effects were not significant for respiration or P_{660}^{net} ($P > 0.25$), and therefore, tank was dropped from these analyses. Tank was retained in the analysis of α (Tank $P = 0.216$).

Area-normalized respiration ranged from 0.96 ± 0.06 to 0.73 ± 0.07 $\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$ (mean $\pm$ SE, $n = 5$ –6, Table 2) and was not affected by temperature, pCO₂, or the interaction between the two (Table 3). Temperature significantly affected P_{660}^{net} standardized to area ($P < 0.001$); no effect of pCO₂ or the temperature $\times$ pCO₂ interaction was detected (Table 3). Area-normalized P_{660}^{net} decreased 95 % at HT-ACO₂ compared to AT-ACO₂ and was affected similarly at HCO₂, being reduced 89 % at HT compared to AT (Fig. 2). Alpha was affected by temperature ($P < 0.001$) but not pCO₂, or the interaction between the two.

Chlorophyll a and Symbiodinium density

After 2 weeks in the treatments, corals in AT treatments appeared a normal color while corals in the HT treatment showed signs of bleaching, although no corals died. When normalized to area, mean concentration of chlorophyll *a* ranged from 13.35 ± 0.43 $\mu\text{g cm}^{-2}$ in AT-ACO₂ to 3.73 ± 0.43 $\mu\text{g cm}^{-2}$ in HT-ACO₂ ($\pm$ SE, $n = 12$ –14) (Fig. 3a). The interaction of temperature and pCO₂ was significant ($F_{1,48} = 5.074$, $P = 0.029$) due to a 13 % reduction in chlorophyll *a* cm^{-2} between AT-ACO₂ and AT-HCO₂, and a 25 % increase in HT-HCO₂ compared to HT-ACO₂ (Fig. 3a). Chlorophyll *a* concentration was affected by temperature ($F_{1,48} = 193.646$, $P < 0.001$), but not pCO₂ ($F_{1,48} = 0.457$, $P = 0.502$), and post hoc analyses revealed A-CO₂ and H-CO₂ conditions within temperature treatments (i.e., 27.7 and 30.5 °C) were not significantly different from each other ($P > 0.05$) (Fig. 3a). When normalized to *Symbiodinium* cells, chlorophyll *a* concentration was unaffected by temperature ($F_{1,28} = 0.143$, $P = 0.709$),

Table 2 Dark respiration and parameters describing the relationship between net photosynthesis ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) and irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) (P/I) standardized by area for juvenile *Seriatopora caliendrum*

Parameter	Treatments			
	AT-ACO ₂	AT-HCO ₂	HT-ACO ₂	HT-HCO ₂
R ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$)	0.96 ± 0.06 (6)	0.75 ± 0.04 (5)	0.73 ± 0.07 (5)	0.75 ± 0.08 (6)
P_{660}^{net} ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$)	1.80 ± 0.44 (6)	1.96 ± 0.29 (6)	0.08 ± 0.09 (5)	0.22 ± 0.09 (6)
α ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) ⁻¹	0.013 ± 0.0007 (6)	0.011 ± 0.0004 (6)	0.004 ± 0.0005 (5)	0.004 ± 0.0003 (6)

Corals were incubated for 14 days in combinations of temperature ($^{\circ}\text{C}$) and pCO_2 (Pa) (Table 1). Parameters were obtained from the best-fit hyperbolic tangent with an exponent for photoinhibition (Platt et al. 1980). Values displayed are mean $\pm$ SE (n = number of corals)

R respiration; $\text{Chl } a$ chlorophyll a , P_{660}^{net} rate of net photosynthesis as measured at $660 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, α the initial slope of the light-limited portion of the curve

Table 3 Statistical analysis of photosynthesis versus irradiance (P/I) curve parameters standardized by surface area for juvenile *Seriatopora caliendrum* exposed to different treatments

Dependent variable	Effect	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Respiration ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$)	pCO_2	1	0.044	1.823	0.194
	Temp	1	0.070	2.930	0.104
	$\text{pCO}_2 \times \text{Temp}$	1	0.070	2.912	0.105
	Error	18	0.024		
P_{660}^{net} ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$)	pCO_2	1	0.132	0.291	0.596
	Temp	1	17.078	32.728	<0.001
	$\text{pCO}_2 \times \text{Temp}$	1	0.001	0.001	0.974
	Error	19	0.453		
α ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) ⁻¹	pCO_2	1	4.00×10^{-6}	2.162	0.215
	Temp	1	3.43×10^{-4}	171.417	<0.001
	$\text{pCO}_2 \times \text{Temp}$	1	2.00×10^{-6}	1.103	0.353
	Tank ($\text{pCO}_2 \times \text{Temp}$)	4	2.00×10^{-6}	1.639	0.216
	Error	15	1.00×10^{-6}		

Analyses were performed using a partly nested ANOVA with two fixed factors (pCO_2 and Temperature) and one nested factor (Tank). Tank was dropped from the analysis when $P > 0.25$; significant values ($P < 0.05$) are in bold

$\text{Chl } a$ chlorophyll a , *df* degrees of freedom, *MS* mean sum of squares, parameter definitions in Table 2

pCO_2 ($F_{1,28} = 0.181$, $P = 0.673$), or the interaction between the two ($F_{1,28} = 0.001$, $P = 0.975$) (Fig. 3a-inset).

Symbiodinium densities standardized to area were affected by temperature ($F_{1,28} = 104.676$, $P < 0.001$) but not pCO_2 ($F_{1,28} = 0.315$, $P = 0.579$), or the interaction between the two ($F_{1,28} = 0.343$, $P = 0.563$). Mean *Symbiodinium* densities decreased 69 % in HT-ACO₂ compared to AT-ACO₂, and 65 % in HT-HCO₂ compared to AT-HCO₂ (Fig. 3b).

Discussion

OA and coral bleaching

Large-scale thermal bleaching is a major cause of declining coral cover (Wilkinson 2008), and therefore, reports that

elevated pCO_2 can induce bleaching at a magnitude equivalent to thermally induced bleaching (Anthony et al. 2008) have received much attention. Despite this attention, there has not been a rigorous test of the hypothesis that OA affects the same physiological processes that underpin thermal bleaching (Warner et al. 1996; Fitt et al. 2001; but see Brading et al. 2011). In the present study, bleaching of *S. caliendrum* at elevated temperature and high pCO_2 was evaluated at three functional levels corresponding to the sequence of events taking place during the onset of bleaching (Fitt et al. 2001). High pCO_2 (85.1 Pa) did not cause bleaching, either individually, or interactively with high temperature.

Our results draw attention to the inconsistencies in the reported effects of high pCO_2 in directly (or indirectly) causing coral bleaching. For instance, our findings are contrary to reports that pCO_2 as high as 152.0 Pa causes

Fig. 2 Net photosynthesis (P_{net}) versus irradiance (P/I) curves for juvenile *Seriatopora caliendrum* exposed for 14 days to combinations of temperature and $p\text{CO}_2$ as described in Table 1. Symbols correspond to treatments AT- ACO_2 (dark circles), HT- ACO_2 (open circles), AT- HCO_2 (dark triangles), and HT- HCO_2 (open triangles). At each irradiance, values are mean $P_{\text{net}} \pm \text{SE}$ ($n = 5-6$); best-fit lines are fit to mean P_{net} values

bleaching in *Acropora intermedia* and *Porites lobata* (Anthony et al. 2008) and leads to declines in net productivity of *Stylophora pistillata* (Reynaud et al. 2003), *A. intermedia* and *P. lobata* (Anthony et al. 2008; Crawley et al. 2010). Additionally, while elevated $p\text{CO}_2$ reduced photochemical efficiency in *Porites australiensis* (F_v/F_m ; Iguchi et al. 2011) and massive *Porites* spp. (F_v/F_m and $\Delta F/F_m'$; Edmunds 2012), and increased concentration of chlorophyll *a* per algal cell in *S. pistillata* (e.g., photoacclimation; Crawley et al. 2010), we found no effect of $p\text{CO}_2$ on photochemical efficiency and the content of chlorophyll *a* cm^{-2} or chlorophyll *a* cell^{-1} . However, our results are consistent with several other studies showing that $p\text{CO}_2$ as high as 227 Pa has no effect on photosynthesis in *Acropora eurytoma* (Schneider and Erez 2006) or *S. pistillata* (Godinot et al. 2011). Further, high $p\text{CO}_2$ has no effect on F_v/F_m in *S. pistillata* (Godinot et al. 2011), or chlorophyll content ($a + c_2$) and *Symbiodinium* density in *P. australiensis* (Iguchi et al. 2011). While 14-day exposure to elevated $p\text{CO}_2$ in the present study did not result in bleaching, elevated temperature reduced both photosynthetic performance and symbiont and photopigment density, as has previously been reported (Hoegh-Guldberg and Smith 1989; Warner et al. 1999; Anthony et al. 2008). This result reaffirms the importance of rising SST in negatively affecting corals (Hoegh-Guldberg et al. 2007) and underscores the possibility that corals could succumb to the effects of high temperature before they are impacted seriously by high $p\text{CO}_2$ (Hoegh-Guldberg et al. 2007).

Fig. 3 Chlorophyll *a* normalized to area and *Symbiodinium* cells, and the density of *Symbiodinium* spp. in juvenile *Seriatopora caliendrum* exposed for 14 days to combinations of temperature and $p\text{CO}_2$ (Table 1). **a** Chlorophyll *a* ($\mu\text{g cm}^{-2}$) ($n = 12-14$ treatment $^{-1}$) with inset showing chlorophyll *a* content per *Symbiodinium* cell at 27.7 °C (white columns), 30.5 °C (gray columns), and 45.1 and 85.1 Pa CO_2 ; **b** *Symbiodinium* spp. density per surface area of coral tissue (cm^{-2}) ($n = 8$ treatment $^{-1}$). Values displayed are mean $\pm$ SE; letters indicate post hoc multiple comparisons with dissimilar letters marking treatments that differed ($P < 0.05$)

Onset of bleaching

Previous evidence that OA directly affects one of the proximal processes (i.e., photochemical efficiency) driving coral bleaching is equivocal, but the present results clearly support a null effect for high $p\text{CO}_2$. It is unclear what factors are responsible for conflicting $p\text{CO}_2$ effects on *Symbiodinium* photochemical efficiency among studies; however, OA effects on coral calcification are modulated by light intensity (Dufault et al. 2013; Suggett et al. 2013) and light intensity may also provide insight into the disparate effects of OA on photochemical efficiency. Numerous OA studies have been performed under subsaturating low light intensities ($<10-150 \mu\text{mol photons}$

$\text{m}^{-2} \text{s}^{-1}$) (e.g., Crawley et al. 2010; Iguchi et al. 2011) that may not be ecologically relevant to corals in situ. We acknowledge light intensities employed here ($265 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were below field irradiances; however, light intensity exceeded the saturation irradiance ($I_k = P_{\text{max}}/\alpha$) for photosynthesis in these corals ($I_k = 144 \pm 3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ [$\pm\text{SE}$, $n = 3$], C.B. Wall pers comm). Under subsaturating irradiances, energy dissipation pathways (e.g., NPQ) are less active than under saturating irradiances. However, OA appears to prematurely activate energy dissipation pathways (Crawley et al. 2010), which may result in reduced photochemical efficiency and productivity. At low light intensities, the effects of OA on photochemical efficiency may be masked due to various factors, including the coral's previous light history, low levels of NPQ (i.e., reducing $\Delta F/F_m'$) and PSII photoinactivation (i.e., reducing F_v/F_m), and efficient turnover of the D1 protein of PSII. Therefore, CO_2 effects under low light intensities may only manifest under prolonged incubations, or under short incubation at high irradiances (Edmunds 2012). It is clear that further research on the interaction of light and OA in affecting photochemical efficiency will be required to resolve these issues.

Intermediate events associated with bleaching

The pCO_2 -enrichment alone, or in combination with high temperature (HT), did not affect the dark respiration of *S. caliendrum*, or their photosynthetic efficiency (α) and photosynthetic capacity (P_{660}^{net}). However, in HT treatments, concurrent with decreased F_v/F_m and $\Delta F/F_m'$, large reduction in P_{660}^{net} and α was observed, while dark respiration remained unchanged. Under prolonged exposure to high light intensities or elevated temperatures, photoprotective mechanisms that normally shift energy away from PSII begin to breakdown, resulting in photo-oxidative damage to PSII, reduced photosynthetic productivity, and ultimately, bleaching (Lesser 1997; Warner et al. 1999; Smith et al. 2005). In the present study, a reduction in P_{660}^{net} associated with elevated temperature may be attributed to several processes including reduced densities of *Symbiodinium* or the number of photosynthetic units (PSU), and a slower turnover rate of PSII and the D1 reaction center protein (Falkowski and Raven 1997; Warner et al. 1999). Photodamage and decreased turnover rates of the D1 protein are associated with the onset of thermal bleaching in corals (Warner et al. 1999), as well as in heat-stressed *Symbiodinium* in vitro (Iglesias-Prieto et al. 1992) and more generally, in marine diatoms and natural phytoplankton assemblages undergoing photoinhibition in situ (Behrenfeld et al. 1998). Decreases in α of colonies of *S. caliendrum* exposed to HT treatments indicates a

diminished integrity of RCII, potentially arising from photo-oxidative damage to the D1 protein of RCII (Lesser 1997; Warner et al. 1999), and fewer photons absorbed by the antennae complex being transferred to the acceptor side of PSII (Falkowski and Raven 1997).

The null effect of pCO_2 treatments on coral respiration agrees with studies, showing no effect of pCO_2 (up to 217 Pa) on the dark respiration of *S. pistillata* (Reynaud et al. 2003; Crawley et al. 2010; Godinot et al. 2011), *Acropora intermedia* or *Porites lobata* (120.1 Pa) (Anthony et al. 2008), *A. eurystroma* (56.0 Pa) (Schneider and Erez 2006), and *Porites* spp. at 76.6 Pa (Edmunds 2012) and 99 Pa (Wall and Edmunds 2013), but conflict with studies reporting OA reduces coral respiration (Edmunds 2012). In the present study, high flow rates may have stimulated coral respiration across treatments by reducing the diffusion boundary layer (DBL) across the corals tissue (Lesser et al. 1994), thereby reducing the ability to detect a treatment effect on respiration. Alternatively, the disparity in OA effects on coral respiration may reflect differential responses to elevated external CO_2 or reduced extracellular pH, or the magnitude of the pCO_2 treatments (Edmunds 2012).

The decrease in α and P_{660}^{net} of *S. caliendrum* kept at 30.5°C is consistent with studies showing a decrease in the photosynthetic performance of *Symbiodinium in hospite* (Coles and Jokiel 1977) and in vitro (Iglesias-Prieto et al. 1992) for corals exposed to elevated temperatures ($>28^\circ\text{C}$). However, 85.1 Pa pCO_2 did not affect the light-limited efficiency of photosynthesis (α) or the rate of photosynthesis of *S. caliendrum* at mean in situ irradiances (i.e., P_{660}^{net}). These findings are in agreement with studies with corals showing no effect of pCO_2 (up to 152 Pa) on the capacity for photosynthesis (Langdon and Atkinson 2005; Schneider and Erez 2006) and photosynthetic efficiency (Crawley et al. 2010) of *Symbiodinium*. While coral photosynthesis appears insensitive to changes in pH (Goiran et al. 1996; Schneider and Erez 2006), it has been suggested that rates of *Symbiodinium* photosynthesis are carbon limited under ambient [DIC] of $\sim 2,000 \mu\text{mol kg}^{-1}$ (Herfort et al. 2008), and therefore, increase dissolved CO_2 or elevated [DIC] ($\sim 200 \mu\text{mol kg}^{-1}$ primarily in the form of HCO_3^-) from OA could stimulate photosynthesis, although the general consensus is that *Symbiodinium* productivity is not stimulated by 10 % increase in [DIC].

Variability in abiotic factors, such as PAR or seawater temperature, may influence the impacts of OA on corals and the performance of their *Symbiodinium*, particularly in short- versus long- term experiments. However, disparities in effects of OA on photosynthesis of corals may also reflect genetic variability in the response of *Symbiodinium* to environmental disturbance (Ragni et al. 2010; Putnam

et al. 2012), or differential reliance of the algae on CCMs versus $\text{CO}_{2(\text{aq})}$ to supply carbon for photosynthesis (sensu Brading et al. 2011). For instance, the response of four cultured *Symbiodinium* phylotypes (Brading et al. 2011) to high pCO_2 is phylotype-specific. However, the role(s) played by *Symbiodinium* phylotypes in the response of corals to OA is uncertain, first because it is unknown what phylotypes associate with *S. caliendrum* in southern Taiwan (although, *S. hystrix* on the Great Barrier Reef associates with 5 phylotypes of clade C *Symbiodinium*, Bongaerts et al. 2010), and further, the microenvironment surrounding *Symbiodinium in hospite* differs from the external environment (Venn et al. 2009). The coral host is thought to exert strong control over the photosynthetic performance of *Symbiodinium* (Gates et al. 1999), and therefore, the effects of OA on *Symbiodinium in hospite* are likely to be modulated by the host.

Terminal stages of bleaching

The terminal stages of coral bleaching involve a reduction in photopigment content of *Symbiodinium* and the expulsion of symbionts (Fitt et al. 2001). In the present study, 14 days at 30.5 °C led to declines in the density of *Symbiodinium* and reductions in their chlorophyll *a* content, but neither trait was affected by high pCO_2 . Crawley et al. (2010) reported no change in *Symbiodinium* densities, but an increase in chlorophyll *a* content cell^{-1} in response to high pCO_2 (38.5 vs. 70.9 and 111.5 Pa), and suggested that OA elicits photoacclimation by *Symbiodinium*. While chlorophyll concentration in *S. caliendrum* was not affected by pCO_2 alone, the $\text{pCO}_2 \times$ temperature interaction led to reduction in chlorophyll *a* at 27.7 °C and increases at 30.5 °C relative to 45.1 Pa. Similarly, 77.0 Pa pCO_2 decreased chlorophyll *a* (mg protein^{-1}) in *S. pistillata* at 25.2 °C and increased chlorophyll *a* at 28.3 °C, relative to 46.6 Pa (Reynaud et al. 2003). However, in the present study, chlorophyll *a* (pg cell^{-1}) and *Symbiodinium* densities did not change in response to pCO_2 ; therefore, the change in chlorophyll *a* content is likely to be caused by symbiont expulsion and not photoacclimation. Using *S. caliendrum*, our results suggest OA interacts with temperature to affect the chlorophyll content of *Symbiodinium*, but this modulation of pigment content does not result in increased photosynthetic performance or ameliorated effects of thermal bleaching on the holobiont.

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