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Original Article

Ocean acidification affects productivity but not the severity of thermal bleaching in some tropical corals

Sam H. C. Noonan* and Katharina E. Fabricius

Australian Institute of Marine Science, PMB 3, Townsville MC, QLD 4810, Australia

**Corresponding author: tel: + 61 7 4753 4131; fax: + 61 7 4772 5852; e-mail: s.noonan@aims.gov.au*

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Increasing carbon dioxide (CO_2) emissions are raising sea surface temperature (SST) and causing ocean acidification (OA). While higher SST increases the frequency of mass coral bleaching events, it is unclear how OA will interact to affect this process. In this study, we combine *in situ* bleaching surveys around three tropical CO_2 seeps with a 2-month two-factor (CO_2 and temperature) tank experiment to investigate how OA and SST in combination will affect the bleaching susceptibility of tropical reef corals. Surveys at CO_2 seep and control sites during a minor regional bleaching event gave little indication that elevated pCO_2 influenced the bleaching susceptibility of the wider coral community, the four most common coral families (Acroporidae, Faviidae, Pocilloporidae, or Poritidae), or the thermally sensitive coral species *Seriatopora hystrix*. In the tank experiment, sublethal bleaching was observed at 31°C after 5 d in *S. hystrix* and 12 d in *Acropora millepora*, whereas controls (28°C) did not bleach. None of the measured proxies for coral bleaching was negatively affected by elevated pCO_2 at pH_T 7.79 (vs. 7.95 pH_T in controls), equivalent to ~780 µatm pCO_2 and an aragonite saturation state of 2.5. On the contrary, high pCO_2 benefitted some photophysiological measures (although temperature effects were much stronger than CO_2 effects): maximum photosystem II quantum yields and light-limited electron transport rates increased in both species at high pCO_2, whereas gross photosynthesis and pigment concentrations increased in *S. hystrix* at high pCO_2. The field and laboratory data in combination suggest that OA levels up to a pH_T of 7.8 will have little effect on the sensitivity of tropical corals to thermal bleaching. Indeed, some species appear to be able to utilize the more abundant dissolved inorganic carbon to increase productivity; however, these gains offset only a small proportion of the massive bleaching-related energy losses during thermal stress.

Keywords: carbon dioxide, carbon limitation, coral reef, global climate change, interactive effects, photophysiology.

Introduction

The continual anthropogenic pollution of Earth's atmosphere with carbon dioxide (CO_2) is causing planetary warming and ocean acidification (OA). These global changes are ongoing and projected to be exacerbated into the future as atmospheric CO_2 levels continue to rise (IPCC, 2013). In the marine environment, climate change is raising sea surface temperature (SST), while the oceanic uptake of CO_2 is causing a suite of chemical changes, increasing dissolved inorganic carbon (C_T), and reducing carbonate saturation states and pH (Langdon and Atkinson, 2005). The warming and OA stressors are occurring simultaneously and are considered among the greatest threats to marine biodiversity (Kleypas, 1999; Hoegh-Guldberg *et al.*, 2007). It is thus necessary to consider the effects of these global stressors in conjunction with each other as their effects may differ in combination and isolation (Harvey *et al.*, 2013).

Coral reefs are among the most vulnerable ecosystems to the changes associated with CO_2 emissions (Hoegh-Guldberg, 1999; Anthony *et al.*, 2011). The primary concern under increasing SST is the breakdown of the symbiosis between scleractinian corals and dinoflagellate algae of the genus *Symbiodinium* (zooxanthellae) in a process known as bleaching (e.g. Brown, 1997; Lesser, 2011). During thermal bleaching, damage to the photosynthetic machinery in *Symbiodinium*, including photosystem II (PSII), reduces their photosynthetic capacity and may eventually lead to their expulsion from the coral host (Lesser, 2011). Loss of autotrophy can starve the coral, and large-scale coral mortality has been observed when conditions that induce bleaching persist for some time (e.g. Brown, 1997; Berkelmans *et al.*, 2004). Periodic heat stress events, where temperatures exceed the long-term summer maximum for weeks at a time, are superimposed upon the gradual warming trend and

often prompt bleaching (Brown, 1997; Berkelmans *et al.*, 2004). The frequency of extreme weather conditions and subsequent heat stress events are projected to increase with rising atmospheric CO_2 (IPCC, 2013). However, it remains unclear how the thermal bleaching susceptibility of corals will be influenced by OA.

The issue of OA has received less attention than global warming; however, recent years have seen a surge in research effort. To date, coral reef research has primarily focused on the effects of OA on rates of calcification (e.g. Anthony *et al.*, 2008; Uthicke and Fabricius, 2012) and the metabolic demands that potentially increase to maintain high calcification rates (Cohen and Holcomb, 2009; Cyronak *et al.*, 2016). However, other effects of increasing pCO_2 remain less clear, especially on the corals' *Symbiodinium* partners, and many results are contradictory. Some authors report that pCO_2 increases can enhance primary production in *Symbiodinium* in a range of host taxa and suggest that carbon is the limiting substrate for photosynthesis (Crawley *et al.*, 2010; Brading *et al.*, 2011; Uthicke and Fabricius, 2012), while other studies have seen negligible (Wall *et al.*, 2013) and even negative effects of increased pCO_2 on photophysiology (Anthony *et al.*, 2008).

Studies examining the interactive effects of elevated SST and dissolved inorganic carbon on the bleaching susceptibility and photobiology of corals are few, and often have contradictory results. Some investigators have found increases in pigment content and the number of *Symbiodinium* per coral cell under elevated temperatures and CO_2 (Reynaud *et al.*, 2003), whereas others have found null (Schoepf *et al.*, 2013; Wall *et al.*, 2013) or opposite results. Anthony *et al.* (2008) observed declining pigmentation and oxygen production in two species of coral exposed to OA and temperature, suggesting that elevated CO_2 may increase thermal bleaching severity in corals. On the other hand, pCO_2 increases may boost primary production and could reduce the severity of thermally induced bleaching (Hoogenboom *et al.*, 2012).

An important factor determining responses to rising SST and OA is acclimatization through prolonged or repeated prior exposure to the stressor. While short-term experimental studies are certainly informative, in isolation they are unable to account for potential acclimatization. Longer term tank experiments provide more robust results as study organisms become acclimatized with the experimental environment (Krief *et al.*, 2010). Some evidence is emerging which suggests corals from reefs with a history of heat stress tend to bleach less severely during subsequent heat stress events (Berkelmans *et al.*, 2004; Maynard *et al.*, 2008). Tropical CO_2 seeps also allow studies to be conducted on organisms that have been acclimatized to higher levels of pCO_2 throughout their lifetime (Fabricius *et al.*, 2011). More longer term experiments and *in situ* studies around CO_2 seeps are needed to more confidently predict how chronic OA will interact with warming SST to shape coral reefs into the future.

This two-part study combines field observations and an experiment to investigate the effects of increased pCO_2 on coral thermal bleaching susceptibility. Part 1 reports on field data collected during a mild bleaching event in Milne Bay Province, Papua New Guinea in April 2011, including at coral reefs surrounding three CO_2 seeps. To determine if zooxanthellate corals are more or less susceptible to thermal bleaching if they had been exposed to elevated levels of pCO_2 since settlement, surveys were used to quantify coral pigmentation near and away from the seeps. This represents the first set of direct measurements of *in situ* coral thermal bleaching susceptibility in the face of OA [but see Manzello (2010)]. The field data were complemented by a 2-month crossed two-factor laboratory experiment to investigate the interactive effects of elevated pCO_2 and temperature on the bleaching susceptibility, photobiology, photosynthetic production, and *Symbiodinium* pigment dynamics in two common coral species, namely *Seriatopora hystrix and Acropora millepora*.

Material and methods

Bleaching surveys of reefs with elevated and ambient pCO_2

Bleaching surveys were conducted at three locations with volcanic CO_2 seeps and adjacent control sites (namely Upa Upasina, Esa'Ala, and Dobu, in Milne Bay Province, Papua New Guinea) over a 1-week period in April 2011. Seep and control sites are described in detail by Fabricius *et al.* (2011). The emerging gas is >98% CO_2, and the seep and control sites are very similar in their geomorphology, flow, light, wave exposure, and nutrients (Tables S1 and S2 of Fabricius *et al.*, 2011). Three years of continuous benthic temperature logging since April 2011 (Reef net, Census Ultra, Canada) has shown that temperatures are similar between seep and control sites (Supplementary Figure S2 and Table S1). Spatial maps of mean seawater carbonate chemistry (Fabricius *et al.*, 2011) were used to confine surveys to seep areas with mean seawater pH_T ~7.8 ± 0.2 to 7.9 ± 0.2 *SD*. We observed mild bleaching (i.e. a proportion of colonies were pale and a very small number was almost white) at all visited reefs (Supplementary Figure S1).

The first set of surveys was a series of 20 × 0.5 m belt transects at the six seep and control sites, at 3–4 m depth ($n = 4$ each at the seep and control sites of Upa Upasina and Dobu, and $n = 2$ per site at the smaller Esa'Ala reef). A single observer recorded the taxonomic identity (mainly genus but family for less common taxa) and pigmentation of any zooxanthellate hard coral and octocoral colonies within the belts ($n = 874$ colonies). Pigmentation was estimated on the upper surface of the colony to the nearest 0.25 colour chart units of the Coral Watch Coral Health Chart (http://www.coralwatch.org) on a scale of 1 (severely bleached) to 6 (dark pigmentation), which correlates well with symbiont density and chlorophyll *a* content (Siebeck *et al.*, 2006) (Supplementary Figure S1d). Of the 874 colonies surveyed, 780 came from the families Acroporidae, Faviidae, Pocilloporidae, or Poritidae.

During the surveys, the coral *S. hystrix* was noted to be particularly bleached. As the belt transects failed to capture enough of these colonies for statistical analyses, a second set of surveys was conducted at the Upa Upasina between 2 and 6 m depth, recording the depth and pigmentation on the upper surface of the first 14–15 colonies of *S. hystrix* encountered each at the control and seep site.

Laboratory experiment

A 2-month, two-factor aquarium experiment (two temperatures: 28 and 31°C, and two pH_T levels: 7.8 and 8.0, Table 1) was conducted at the Australian Institute of Marine Science in September to December 2011 to investigate coral bleaching susceptibility to pCO_2 and temperature exposures at levels projected to occur before the end of the century (atmospheric CO_2 ~750 ppm in the representative concentration pathway 8.5; Moss *et al.*, 2010; IPCC, 2013). The elevated temperature treatment of 31°C is considered the 10-d summer bleaching threshold for corals from the study area (long-term summer mean ~28°C; Berkelmans *et al.*, 2004). The two common and widely distributed coral species used, *S. hystrix* and *A. millepora*, are both highly susceptible to thermal bleaching.

Eighteen partial colonies of *S. hystrix* and *A. millepora* were collected from two reefs in the central Great Barrier Reef (Orpheus

Table 1. Treatment conditions in the 54-d flow-through experiment: pH_T and temperature (measured daily), and seawater carbonate parameters (measured weekly).

	Measured parameters				Calculated parameters		
Treatment	**pH_T**	**Temperature (°C)**	**A_T (μmol kg^{-1} SW)**	**C_T (μmol kg^{-1} SW)**	**pCO_2 (μatm)**	**HCO_3^- (μmol kg^{-1} SW)**	**Ω_{ar}**
TL.CL	7.97 (0.05)	28.07 (0.17)	2318 (22)	2026 (13)	479 (38)	1819 (45)	3.27 (0.31)
TL.CH	7.79 (0.05)	27.85 (0.25)	2325 (24)	2116 (35)	738 (65)	1950 (47)	2.44 (0.21)
TH.CL	7.99 (0.05)	30.81 (0.33)	2328 (23)	2019 (9)	500 (32)	1789 (15)	3.57 (0.30)
TH.CH	7.79 (0.03)	30.83 (0.36)	2326 (23)	2119 (35)	835 (85)	1940 (41)	2.56 (0.26)

The four experimental treatments are a combination of low and high temperature (TL and TH) and low and high pCO_2 (CL and CH). Measured values of temperature, salinity (35 ppt), total alkalinity (A_T) and dissolved inorganic carbon (C_T) were used to calculate the seawater carbonate parameters ($n = 9$ per treatment) including the saturation state of aragonite (Ω_{ar}). Standard deviations are shown in brackets.

Island and Davies Reef), Australia, between 3 and 5 m of depth. Each colony was divided into four ~5 cm nubbins ($n = 72$ nubbins per species) and one nubbin per colony was assigned to one of the four experimental treatments to remove any effects of parental colony identity. Initial measurements of net oxygen production and PAM fluorometer parameters, taken after the acclimation period (outlined below), did not differ between corals collected from the two sites (ANOVA, all $p > 0.05$).

Nubbins were allowed 7 d of acclimation in partially shaded (maximum irradiance ~500 μmol photons m^{-2} s^{-1}), outdoor holding tanks with flow through seawater (~28°C) before being distributed across 12 glass aquaria (17 l volume) in a temperature-controlled room (25 ± 1°C), supplied (400 ml min^{-1}) with filtered (5 μm) seawater at 28°C and ambient atmospheric pCO_2 for a further 17-d acclimation. There were three aquaria per treatment, each containing six nubbins per species. Illumination was delivered in 12 h cycles by white fluorescent light (10 000 K), and was consistent between aquaria (180 μmol photons m^{-2} s^{-1}, 15.6 mol photons d^{-1}, meter LI205A, sensor LI201SA, LICOR, USA). Each aquarium had an individual power head for circulation.

After acclimation, the elevated pCO_2 treatment was applied directly for 21 d before the onset of heat stress by controlling pH in header tanks through a diffused feedback control CO_2 gas injection system (Aquamedic, Germany). Daily pH_{NBS} measurements were conducted in each experimental aquarium (meter: Oakton pH 1100, USA; electrode: Eutech, USA), with measurements being compared with the Dickson seawater TRIS pH standard (Table 1) and converted to pH_T (Dickson, 2007).

A heat stress event was then simulated by ramping the temperature in heated treatments at 0.5°C per 12 h with a separate feedback control system (Neptune Apex aqua controller, USA), then maintained in the header tanks with a computer-controlled data logger (CR 1000, Campbell Scientific, Australia). Treatments were alternated to eliminate any potential environmental effects within the room. Aquarium water temperatures were monitored daily and remained constant (Table 1). Water samples were taken weekly throughout the experiment for C_T and A_T analyses (Marianda VINDTA 3C, Germany), which were used to calculate carbonate system parameters with the program CO2SYS (Lewis and Wallace, 1998; Table 1).

Coral nubbins were inspected for survivorship and visual signs of bleaching nearly daily. To ensure there would be living samples for final analyses, the experiment was terminated once ~60% of nubbins per species within the heated treatments showed visual signs of bleaching. Nubbins were immediately snap-frozen in liquid nitrogen and stored at −80°C for later analyses. We used photosynthetically active radiance (PAR) absorptivity and F_v/F_m measurements (outlined below) as bleaching indices throughout the experiment and further examined the content of different pigments at the experiment's end.

Pulse amplitude-modulated fluorometry

Pulse amplitude-modulated (PAM) fluorometry measurements of dark adapted (>30 min) maximum quantum yields (F_v/F_m) and PAR absorptivity were taken with an Imaging PAM fluorometer (IPAM, Walz, Germany; Unit IMAG-CM fitted with a Maxi head). Measurements of all 144 nubbins were recorded weekly from the start of the acclimation period, then every 4 d once the heat treatment began. F_v/F_m provides a measure of the maximum proportion of available light that can be photochemically quenched through PSII. PAR absorptivity is the fraction of incident red light that is absorbed by photosynthetically active pigments (Ralph *et al.*, 2005).

Rapid light curve (RLC) measurements were taken with the IPAM (Ralph *et al.*, 2005) on the day before each species was removed from the aquaria ($n \geq 15$ per species per treatment), applying 10 s exposures to increasing irradiances (38, 88, 160, 264, 309, 369, 504, 658, 861 and 995 μmol photons m^{-2} s^{-1}). Measured RLCs were fitted with an exponential function to derive light-limited electron transport rate (α) and the maximum electron transport rate (ETR_{max}) following standard procedures (Ralph and Gademann, 2005).

Oxygen flux

Gross photosynthesis and respiration were measured after the acclimation period, after 21 d of CO_2 treatment, and in the closing days of the experiment ($n > 6$ per species per treatment per time point). Real time changes in oxygen concentration were measured in stirred and temperature-controlled 210 ml clear Perspex incubation chambers fitted with oxygen sensor spots ("optodes", Ø 0.5 cm, Presens, Germany), and an Oxy-4 fibre-optic oxygen meter [Presens; for details see Uthicke *et al.* (2012)]. The same lights and intensities used in the aquaria were used in the gross photosynthesis runs. Treatment water was obtained from the header tanks of the experiment and further filtered to 0.5 μm. Measurements lasted ~30 min and each run included a blank chamber. Respiration and gross photosynthesis rates were normalized to nubbin surface area. Net production was calculated by subtracting hourly respiration from hourly gross photosynthesis.

Protein and pigment content

Three nubbins per species per tank were water-picked to remove coral tissue in 10 ml of ultra-filtered seawater (0.05 μm). This slurry was homogenized and a supernatant of coral tissue was prepared for spectrophotometric protein quantification following Dove *et al.* (2006) using the DC protein assay kit (Bio-Rad Laboratories, Australia). Protein content was standardized to

nubbin surface area, determined using the single wax dipping technique (Veal *et al.*, 2010).

Pigments from the *Symbiodinium* pellet obtained after centrifuging the water-picked coral nubbins were sonicated and extracted on ice in the dark in two 1-h extractions in 1 ml of chilled (4°C) buffered methanol (98% MeOH/2% 0.5 M tetrabutylammonium acetate [TBAA] pH 6.5). Extracts were prepared for analysis with an ultra-performance liquid chromatography (UPLC) system (Waters Acquity UPLC) following Uthicke *et al.* (2012) and were standardized to nubbin surface area for analysis.

Statistical analyses

In the wider bleaching surveys, two-factor analysis of variance (ANOVA) was used to compare mean pigmentation between locations and CO_2 exposures for all taxa combined, and separately for the four common families. Tukey's HSD was used for *post hoc* examinations. ANOVA was used in the *S. hystrix* bleaching surveys to compare the mean pigmentation of colonies between the seep and control site, with colony depth as a covariate.

In the experiment, data were averaged across nubbins for each species within aquaria. Unless otherwise stated, all reported statistics satisfied the assumptions of homoscedasticity, Gaussian distributions, and independence. Generalized additive mixed models (GAMMs) were fitted to assess trends in PAR absorptivity and quantum yields across treatments over time. One-way and two-factor ANOVA were used to compare quantum yields and PAR absorptivity between the treatments at specific dates, as well as RLC parameters, protein and pigment contents, pigment ratios, and oxygen flux at three time points (after the acclimation period, after 21 d CO_2 treatment, and in the last days of the experiment). The statistical program R (version 3.0.2) was used including the packages mgcv and nlme (R Development Core Team, 2014).

Results

Bleaching surveys

Mild bleaching was observed at all six sites. Coral pigmentation data from all 874 colonies combined displayed a significant interaction between location and CO_2 exposure (two-factor ANOVA, Location × CO_2: $F_{(2,868)} = 5.616$, $p = 0.004$), as the mean pigmentation at the Dobu control site was ~0.2 colour chart units darker than that of the Upa-Upasina control and Esa'Ala and Dobu seep sites (Figure 1, Tukey's HSD: $p < 0.05$, Supplementary Table S2). No differences were detected between any of the other CO_2 and site combinations for mean pigmentation across all coral taxa (Figure 1).

The pigmentation values for the Acroporidae and Faviidae differed between some specific sites (Location × CO_2 interaction), but no differences were attributable to CO_2 exposure as a main effect (Figure 1 and Supplementary Table S2). Pigmentation values in the Poritidae ($n = 466$ colonies) displayed a significant main effect of CO_2 exposure, with colonies at the seep sites being ~4% paler (3.59 ± 0.55 *SD* colour chart units) than the control sites (3.76 ± 0.74). No differences in pigmentation were detected in the Pocilloporidae.

In *S. hystrix*, most colonies were obviously pale (mean 1.81 ± 0.47), indicating moderate bleaching in this species, and 57 and 33% of colonies had a pigmentation value of ≤1.5 at the high CO_2 and control site, respectively (i.e. were almost completely

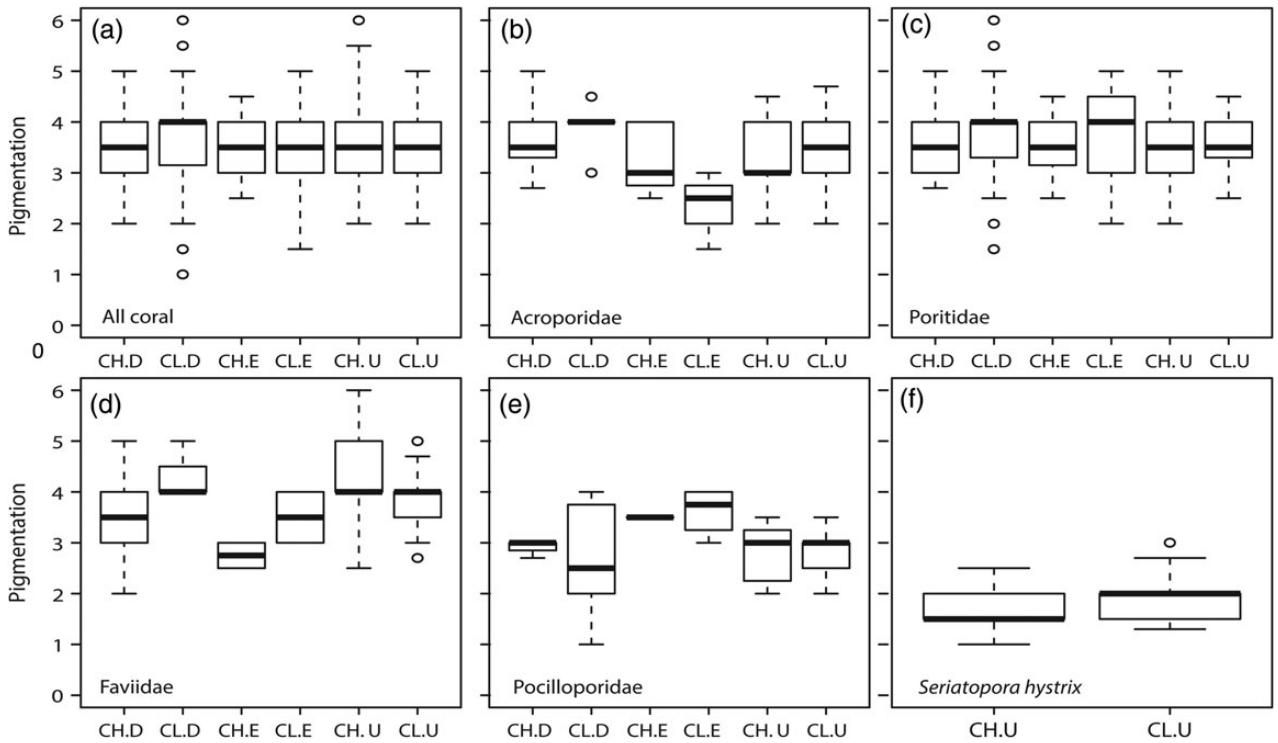

Figure 1. Field surveys of coral pigmentation during a mild bleaching event. Coral colour chart scores (6 = darkest and 1 = lightest) for all coral taxa combined (a: $n = 874$), the Acroporidae (b: $n = 128$), Poritidae (c: $n = 466$), Faviidae (d: $n = 145$), Pocilloporidae (e: $n = 41$), and *S. hystrix* (f: $n = 29$) at seep and control sites (pCO_2 high and low: CH and CL) at the three reefs Dobu (D), Esa'Ala (E), and Upa Upasina (U). Plots are standard boxplots, with the top and bottom of the box enclosing the first to third quartiles (horizontal bar: median; the whiskers: 1.5 interquartile ranges; and the circles: outliers).

white, Figure 1f). There was no difference in the mean pigmentation of the upper surfaces of *S. hystrix* between the seep and control site ($F_{(1,26)} = 2.073$, $p = 0.16$), nor was depth a significant covariate ($F_{(1,26)} = 0.753$, $p = 0.39$).

Laboratory experiment

Mortality and visual signs of bleaching

In the laboratory experiment, none of the nubbins displayed visual loss in pigmentation at control temperatures (28°C). In *S. hystrix*, the first visual signs of bleaching were recorded at 31°C five days after the temperature ramp finished, 60% of *S. hystrix* appeared visually bleached 4 d later, and the final measurements were taken before removing the surviving nubbins from the experiment. All bleached and dead nubbins ($n = 3$ total) were confined to, and evenly distributed across, the two heated treatments. In *A. millepora*, visual signs of bleaching were first observed 12 d after the temperature ramp finished, and ~60% of nubbins in the heated treatments appeared visually bleached and the experiment ended on the 16th d after the temperature ramp. No mortality was recorded in *A. millepora*.

The PAR absorptivity initially increased in both species during the acclimation period, and all but plateaued by the time the pCO_2 treatments began and remained relatively constant throughout the pCO_2 exposure period (Supplementary Figures S3 and S4). Absorptivity values did not differ in either species at the end of the acclimation period between tanks that would later become treatments, or after 3 weeks of CO_2 treatment (ANOVA: all $p > 0.05$). However, absorptivity values declined once temperatures were ramped (Supplementary Figures S3 and S4), and final values were significantly lower in the heated treatments compared with the control temperature (Table 2). Absorptivity values from both species changed significantly over the course of the experiment (GAMM smooth term: *S. hystrix*: $F_{(5.984)} = 14.05$, $p < 0.001$; *A. millepora*: $F_{(5,27)} = 8.75$, $p < 0.001$); however, pCO_2 did not affect absorptivity ($p > 0.1$), while the heated treatments had significant reductions in both species (GAMM: *S. hystrix*: $T = 14.05$, $p < 0.05$; *A. millepora*: $T = 2.97$, $p < 0.01$). The models explained 42 and 35% of the variation in PAR absorptivity for *S. hystrix* and *A. millepora*, respectively (Supplementary Figures S3 and S4).

Quantum yields and rapid light curves

After acclimation, the F_v/F_m for both species did not differ between tanks that would later become treatments (two-factor ANOVA, all $p > 0.05$). F_v/F_m was 0.65 ± 0.03 (*SD*) and 0.67 ± 0.03 for *S. hystrix* and *A. millepora*, respectively. After 3 weeks of pCO_2 exposure, F_v/F_m in *S. hystrix* was significantly higher at elevated compared with ambient pCO_2 (0.53 ± 0.03 vs. 0.63 ± 0.04, one-way ANOVA: $F_{(1,10)} = 22.43$, $p < 0.001$). In contrast, F_v/F_m in *A. millepora* remained similar between pCO_2 treatments (0.61 ± 0.05 vs. 0. 64 ± 0.04, one-way ANOVA, $p > 0.05$). While declines in F_v/F_m were observed in all treatments, values in the final days after heat stress, for both species, were influenced significantly by both pCO_2 and temperature in an additive fashion (Figure 2c and d, and Table 2). In both species, the highest mean F_v/F_m values were recorded in the high pCO_2 + low temperature treatment, whereas the lowest values were observed in the low pCO_2 + high temperature treatment. This decline was ~20% in both species (*S. hystrix*: 0.64 ± 0.03 vs. 0.50 ± 0.03; *A. millepora* 0.62 ± 0.02 vs. 0.53 ± 0.09). The changes over time in F_v/F_m were significant in both species (GAMM smooth term: *S. hystrix*: $F_{(6.18)} = 14.43$, $p < 0.001$; *A. millepora*: $F_{(4.58)} = 16.94$, $p < 0.001$), with CO_2 addition significantly increasing F_v/F_m in *S. hystrix* (GAMM: $T = 2.66$, $p < 0.01$) and elevated temperature significantly reducing it in *A. millepora* (GAMM: $T = 2.67$, $p < 0.01$). The models explained 62 and 52% of the variation in F_v/F_m for *S. hystrix* and *A. millepora*, respectively (Supplementary Figures S5 and S6).

At the end of the experiment, RLCs in *S. hystrix* indicated that light-limited electron transport rates (α) were influenced by both pCO_2 and temperature, and that their effects were additive (Figure 2e and f, and Table 2). Elevated pCO_2 increased α, while increasing temperature lowered them. Mean values of α were lowest in the low pCO_2 + high temperature treatment (0.07 ± 0.01), being 43% of those observed in the high pCO_2 + low temperature treatment (0.16 ± 0.01, Table 2). Maximum electron transport rate (ETR_{max}) in *S. hystrix* was influenced by temperature alone

Table 2. ANOVA results comparing photophysiological parameters for *S. hystrix* and *A. millepora* between the experimental treatments of CO_2 (C), temperature (T), and their interaction (C : T): mean absorptivity and maximum quantum yield (F_v/F_m, both averaged over the final two measurements); alpha and ETR_{max} values derived from rapid light curves, and final net oxygen flux and chlorophyll *a* concentration at the end of the 54-d experiment.

	S. hystrix			*A. millepora*	
	d.f.	***F***	***p***	***F***	***p***
Absorptivity					
C	1	0.34	0.54	0.93	0.35
T	1	15.17	<0.01	11.18	<0.01
C : T	1	0.50	0.49	0.92	0.35
Res	20				
F_v/F_m					
C	1	64.14	<0.01	5.78	0.03
T	1	11.68	<0.01	11.27	<0.01
C : T	1	0.72	0.41	1.19	0.29
Res	20				
Alpha (α)					
C	1	17.28	<0.01	17.44	<0.01
T	1	32.98	<0.01	11.72	<0.01
C : T	1	0.13	0.73	0.10	0.76
Res	8				
ETR_{max}					
C	1	2.92	0.13	2.31	0.17
T	1	24.72	<0.01	0.81	0.39
C : T	1	0.36	0.57	0.80	0.40
Res	8				
Respiration					
C	1	1.00	0.33	0.60	0.45
T	1	1.55	0.23	0.22	0.64
C : T	1	0.56	0.46	0.97	0.34
Res	20				
Production					
C	1	4.99	0.04	0.01	0.93
T	1	31.36	<0.01	38.40	<0.01
C : T	1	0.7	0.41	0.81	0.38
Res	20				
Net O_2 production					
C	1	6.52	0.02	0.01	0.93
T	1	28.51	<0.01	38.40	<0.01
C : T	1	1.13	0.30	0.81	0.38
Res	20				
Chlorophyll *a*					
C	1	12.62	<0.01	0.34	0.58
T	1	51.80	<0.01	67.28	<0.01
C : T	1	1.72	0.23	1.57	0.24
Res	8				

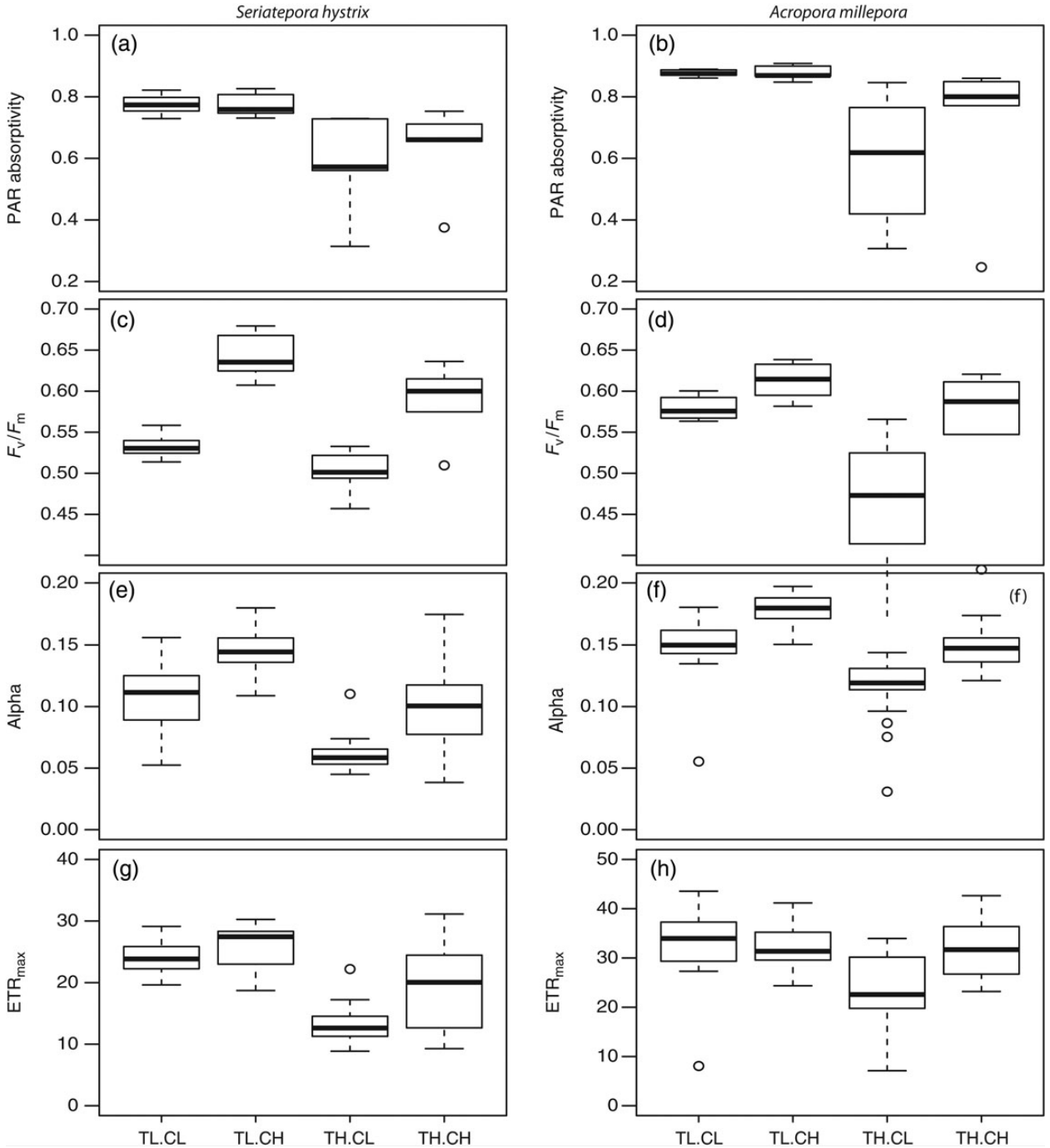

Figure 2. Photophysiological responses in corals in the laboratory to the treatments of low and high temperature (TL and TH) and low and high pCO_2 (CL and CH). Shown are PAR absorptivity (a and b) and maximum PSII quantum yield (F_v/F_m, c and d) in the last 5 d of heat stress, and light-limited electron transport rate (photosynthetic efficiency, α; e and f), and the maximum electron transport rate (ETR_{max}; g and h) at the end of the experiment. $n = 3$ tanks per treatment (averaging 6 colonies per species) for F_v/F_m and absorptivity; $n \geq 15$ per treatment for α and ETR_{max}. See Figure 1 legend for boxplot description.

(Figure 2f and h, and Table 2). ETR_{max} values in the higher temperature treatment (0.09 ± 0.02) were 63% of those observed in the lower temperature treatment (0.14 ± 0.02, Figure 3). The α values in *A. millepora* were also significantly affected by both CO_2 and temperature in an additive fashion (Table 2). As per *S. hystrix*, they were lowest in the low CO_2 + high temperature treatment (0.11 ± 0.03), being 64% of the values observed in the high CO_2 + low temperature treatment (0.18 ± 0.01). No significant differences in ETR_{max} values were detected between the experimental treatments in *A. millepora* (Table 2).

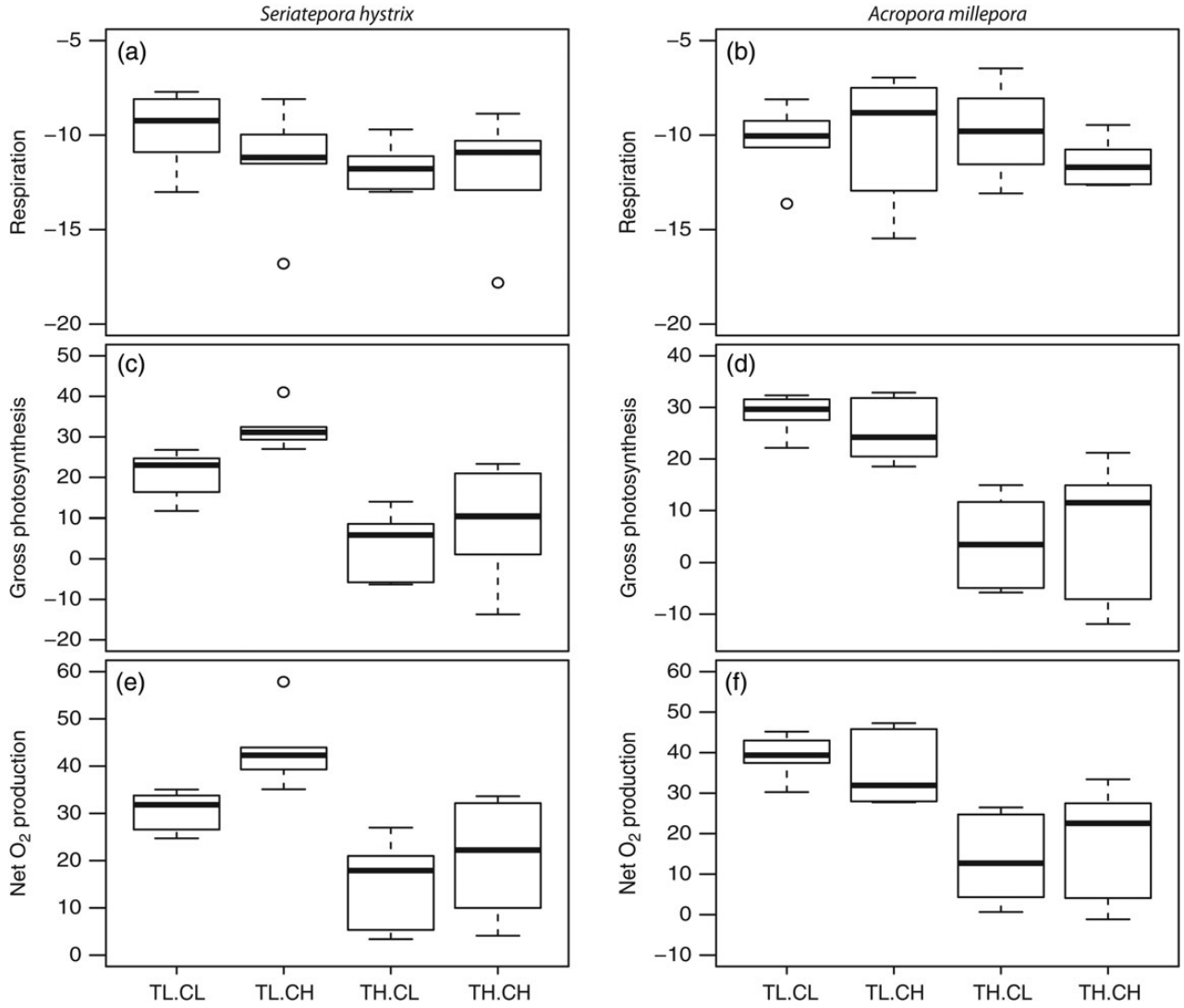

Figure 3. Respiration (a and b), gross photosynthesis (c and d), and average hourly net production (e and f) (all in μg O_2 cm^{-2} h^{-1}) in *S. hystrix* and *A. millepora* at the end of the experiment. Values are standardized per unit surface area of the coral nubbins ($n = 6$ per treatment). Legends as in Figure 2.

Oxygen flux

Rates of respiration and photosynthesis did not differ after 3 weeks of differential pCO_2 exposure (before heat treatment) in either species (one-way ANOVA: $p > 0.05$), and final rates in the control treatments closely matched initial rates. Respiration rates after 3 weeks of pCO_2 treatment were -9.65 ± 0.90 and -9.90 ± 2.61 μg O_2 cm^{-2} h^{-1} for *S. hystrix* and *A. millepora*, respectively, whereas gross photosynthesis rates were 29.64 ± 5.35 and 36.80 ± 10.79 μg O_2 cm^{-2} h^{-1}. In *S. hystrix*, the final net oxygen production rates were influenced by both pCO_2 and temperature in an additive fashion (Table 2), with CO_2 addition increasing and increased temperature reducing net production (Figure 3e). This was driven by changes in gross photosynthesis, as respiration remained unchanged between treatments (Figure 3a and c). Net production was highest in the high pCO_2+ low temperature treatment (43.47 ± 7.75 μg O_2 cm^{-2} h^{-1}) and lowest in the low pCO_2 + high temperature treatment (15.42 ± 9.29), i.e. a 2.5-fold difference (Figure 3e). In *A. millepora*, net O_2 production measurements at the end of the experiment were influenced by temperature alone (Table 2). Corals in the two heated treatments produced ~2.5 times less O_2 than in low temperature treatments (15.88 ± 12.10 vs. 37.30 ± 7.19 μg O_2 cm^{-2} h^{-1}; Figure 3f). This difference was driven by changes in gross photosynthesis, as respiration rates remained unaffected (Table 2). Analyses of O_2 flux and pigment contents (see below), standardized by units of protein, gave no further insights compared with the values standardized by surface area.

Coral protein content

Protein content per unit surface area at the end of the experiment showed no difference between treatments in either species (two-factor ANOVA: all $p > 0.1$). *Seriatopora hystrix* nubbins had 3.43 ± 0.65 mg cm^{-2} protein (mean of all treatments), whereas in *A. millepora* this value was 5.10 ± 0.92 mg cm^{-2} and was significantly higher than in *S. hystrix* (one-way ANOVA: $F_{(1,22)} = 26.36$, $p < 0.001$).

Symbiodinium *pigment content*

At the end of the experiment, many of the *Symbiodinium* pigment concentrations in *S. hystrix* were influenced by both pCO_2 exposure and temperature, without major interactions between these treatments (Supplementary Table S3). Temperature and pCO_2 changes had an additive effect on concentrations of chlorophyll *a* and *c*2 and peridinin, which increased with elevated pCO_2 and declined at high temperature (Figure 4a–d and f). Pigment concentrations in the high pCO_2 + low temperature treatment were ~5-fold higher than at low pCO_2 + high temperature. β-Carotene and the combination of diadinoxanthin (Ddx) and dinoxanthin (Dnx) were significantly reduced at high temperatures (Supplementary Table S3). Conversely, the concentration of diatoxanthin (Dtx), the relative proportion of Dtx to the total xanthophyll pool, and

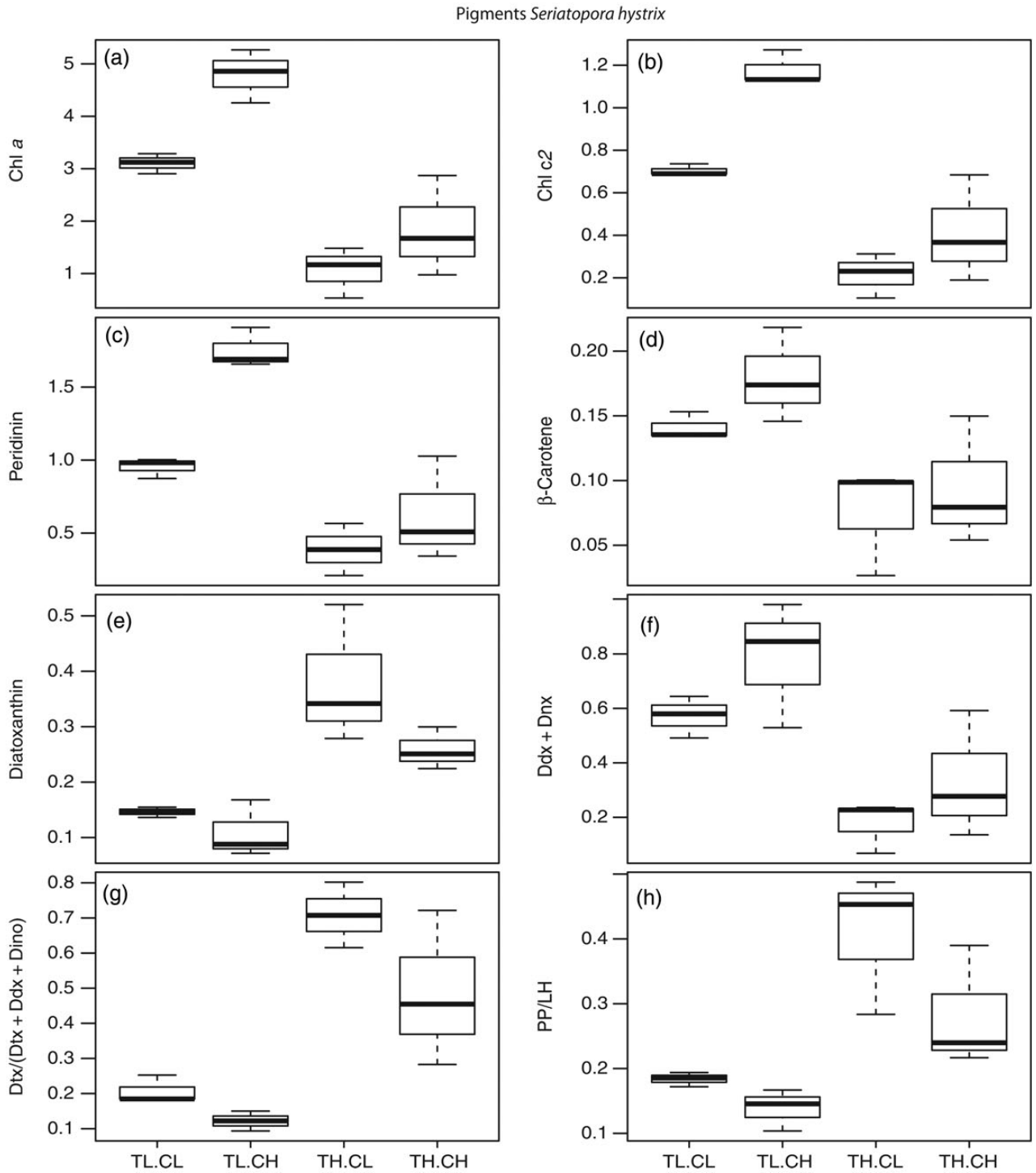

Figure 4. Molar concentrations of pigments (nmol cm^{-2} coral surface area) and pigment ratios from the *Symbiodinium* of *S. hystrix* at the end of the experiment ($n = 9$ per treatment). Legend as in Figure 2.

the ratio of photoprotective (PP: Ddx, Dnx, Dtx, and β-carotene) to light harvesting (LH: chlorophyll *a*, chlorophyll *c*2, and peridinin) pigments showed the opposite patterns, increasing with temperature and declining with elevated pCO_2.

In *A. millepora*, the *Symbiodinium* pigment concentrations and ratios were influenced by temperature only (Figure 5 and Supplementary Table S3). Concentrations of chlorophyll *a* and *c*2, peridinin, β-carotene, and the combination of Ddx and Dnx all declined in the heated treatments (Figure 5a–d and f), whereas Dtx, the relative proportion of Dtx to the total xanthophyll pool, and the ratio of PP to LH pigments increased (Figure 5e, g, and h). Chlorophyll *a* and *c*2, peridinin, and β-carotene showed a fivefold reduction in heated compared with control temperatures, whereas Ddx + Dnx was reduced approximately threefold. Concentrations

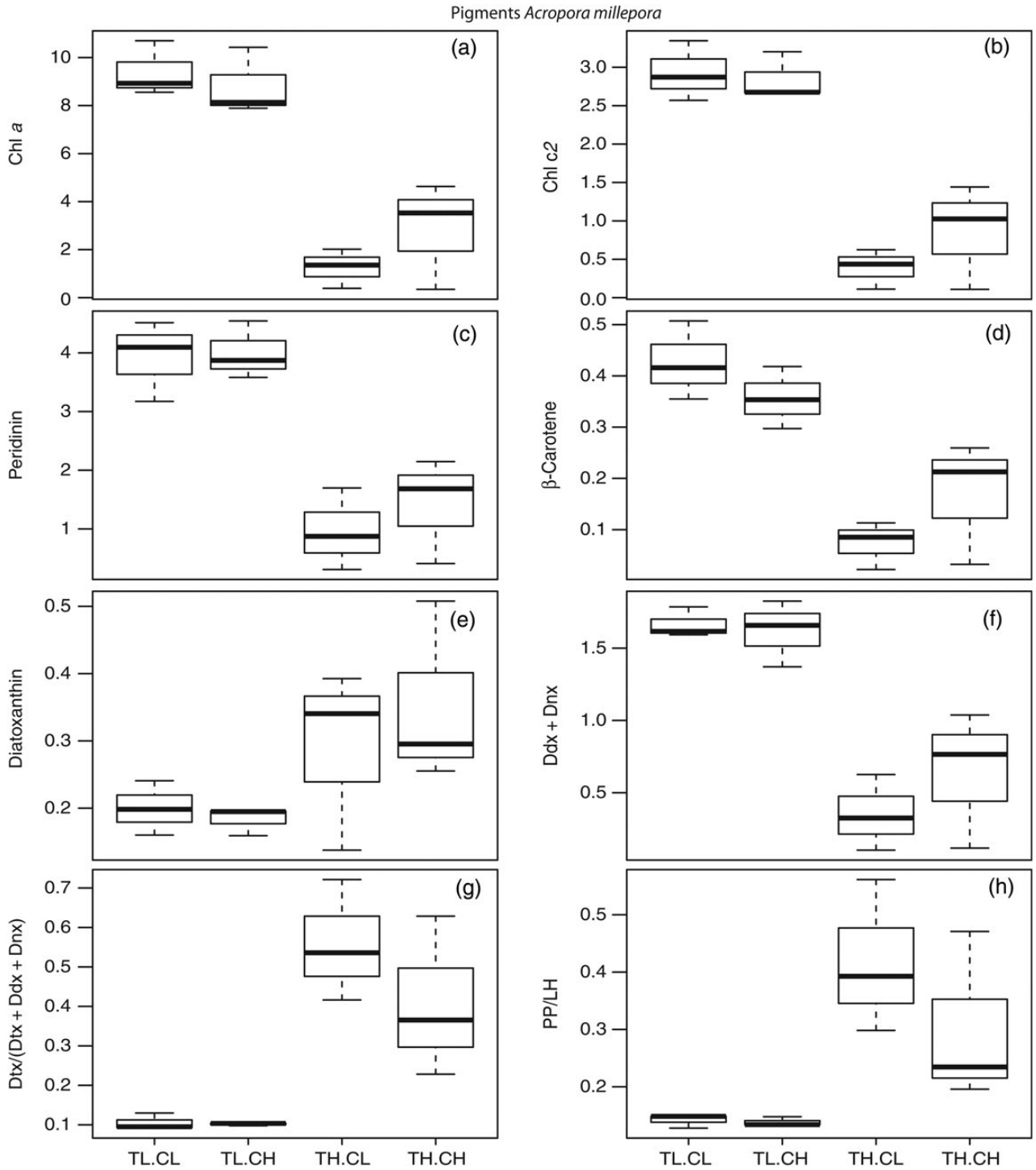

Figure 5. Molar concentrations of pigments (nmol cm^{-2} coral surface area) and pigment ratios from the *Symbiodinium* of *A. millepora* at the end of the experiment ($n = 9$ per treatment). Legend as in Figure 2.

of Dtx were approximately twice as high in the heated compared with the control treatments, while xanthophyll cycling and PP : LH increased five- to sixfold (Figure 5).

Discussion

Despite rapidly progressing global climate change and OA, which is irreversible on a time-scale of thousands of years, science is still largely uncertain about the interactive effects of these changes on marine organisms. The present study investigated whether the thermal bleaching tolerance of tropical reef corals would be influenced by elevated pCO_2 at levels predicted for later this century under OA. Contrary to some earlier observations and predictions (e.g. Anthony *et al.*, 2008), our field and experimental data showed minimal negative effects of CO_2 exposure on the bleaching susceptibility of symbiotic corals. Furthermore, we found that pCO_2 increases can provide some benefits to the symbionts' photosystem, enhancing maximum PSII quantum yields and light-limited electron transport rates in both species, and additionally promoting photosynthetic productivity and pigment concentrations in *S. hystrix*. This study shows that although OA may provide an avenue to improve photosynthetic carbon gains for some corals, even during heat stress, the detrimental effects of warming temperatures remained disproportionately stronger, and did not fully offset bleaching-related losses in productivity.

The bleaching surveys at the three CO_2 seep and control sites represent the first *in situ* data that specifically investigate coral thermal bleaching susceptibility under elevated pCO_2. They gave little indication that the thermal bleaching susceptibility of the major components of the coral community was influenced by elevated pCO_2. Slight CO_2 effects were detected in the Poritidae at all sites, and in the Faviidae at Dobu; however, differences were very minor (~4% change in pigmentation in the Poritidae). Pervious work in the same study area also detected reduced colour chart scores in massive *Porites* at the seep compared with the control sites during winter when bleaching was not observed (Fabricius *et al.*, 2011). We therefore conclude that the observed minor reductions in *Porites* pigmentation at the seeps cannot be unequivocally attributed to a significant increase in bleaching sensitivity.

Thermal bleaching in corals can be co-determined by natural dynamics in food supply, water flow, and light regimes (Fabricius, 2006; Anthony *et al.*, 2009; Hoogenboom *et al.*, 2012), the combined effects of which cannot be effectively replicated in the laboratory. Furthermore, experiments are relatively short term by nature and hence unable to fully account for potential acclimatization. Seep sites are not perfect representations of future reefs, as temperatures remain at ambient levels and CO_2 regimes are more temporarily variable. However, organism acclimatization and ecological interactions between taxa can be examined. The use of seep sites as natural laboratories, in conjunction with controlled experiments, provide the best available information to predict how OA will impact marine ecosystems.

In the laboratory experiment, heat stress induced a bleaching response in both coral species. *Seriatopora hystrix* was more sensitive to thermal stress than *A. millepora*, but heat stress was not exacerbated by increased CO_2 in either species as shown in the field. While CO_2 addition reduced the severity of temperature effects on some photophysiological parameters, thermal bleaching (as quantified by commonly used photophysiological measures) was still observed in both coral species in both pCO_2 treatments, and the temperature effects were much stronger than the pCO_2 effects. It remains to be seen whether this finding will hold for other species and other experimental conditions. For example, corals in the present study were relative sensitive species, were not fed, and were kept under moderate light levels, and previous studies have shown that all these factors co-determine thermal tolerance (Marshall and Baird, 2000; Anthony *et al.*, 2009; Hoogenboom *et al.*, 2012). The fact that our field study resulted in similar findings for both highly sensitive and more thermally tolerant taxa (e.g. *S. hystrix* vs. *Faviidae*), under natural levels of light, flow, and food supply, confirms that the findings from the laboratory study are likely to apply to other species and other study conditions.

The extent of coral thermal bleaching is influenced by factors within the host coral and their *Symbiodinium*. While different *Symbiodinium* types have been shown to vary in their temperature tolerance (Berkelmans and van Oppen, 2006), previous work at the same CO_2 seep locations did not detect any difference in *Symbiodinium* types due to CO_2 exposure in six common corals (Noonan *et al.*, 2013). In our laboratory experiment, the parental corals were divided evenly across all experimental treatments to prevent differences in genotypes or symbiont identity from confounding our results.

The results of previous works that have examined the effects of elevated pCO_2 or the interactive effect of elevated pCO_2 and increased temperature on coral bleaching and photobiology have been highly inconsistent. Anlauf *et al.* (2011) showed that *Porites panamensis* bleached at increased temperature under ambient pH, but not under reduced pH (the number of *Symbiodinium* per coral polyp remained unaffected). Schoepf *et al.* (2013) documented a range of responses to OA and increased temperature across four species of corals, with no clear pattern emerging. Reynaud *et al.* (2003) showed an increase in chlorophyll *a* and the number of *Symbiodinium* per coral cell in *Stylophora pistillata* with increased temperature and CO_2, respectively, whereas Anthony *et al.* (2008) found pigmentation (measured by luminance) decreased in two species of coral under similar treatments. Moreover, Wall *et al.* (2013) concluded that changes in CO_2 had no influence on the bleaching susceptibility of *Seriatopora caliendrum*. Anthony *et al.* (2008) attributed the differences between their results and that of Reynaud *et al.* (2003) to the higher light levels used in their study; however, the null effects observed by Wall *et al.* (2013) were in corals exposed to saturating light levels. The use of different species, methodologies, and metrics of bleaching may be contributing to the disparities seen between works to date.

The present study confirmed that increases in pCO_2 can stimulate photosynthesis in corals, suggesting that carbon supply may limit their photosynthesis. A greater proportion of quanta were funnelled through PSII for use in photosynthesis under higher pCO_2, and light-limited electron transport rate and maximum quantum yields in both *S. hystrix* and *A. millepora* increased. In *S. hystrix*, this further manifested in greater oxygen production. Carbon limitation has been reported in *Symbiodinium* in many taxa including corals and in culture (Brading *et al.*, 2011; Uthicke and Fabricius, 2012), with photosynthesis being stimulated by the addition of CO_2 (Crawley *et al.*, 2010) or bicarbonate (Herfort *et al.*, 2008). These results are not universal however, with some authors reporting either null or negative effects of elevated pCO_2 on photosynthesis (Langdon and Atkinson, 2005; Anthony *et al.*, 2008; Edmunds, 2012; Wall *et al.*, 2013). Carbon may only become limiting in high light, as well as in relatively nutrient-rich waters (Chauvin *et al.*, 2011) such as those of the inshore GBR lagoon used in the present experiment and that conducted by Crawley *et al.* (2010). In contrast, experiments conducted using relatively

oligotrophic waters (Langdon and Atkinson, 2005; Anthony *et al.*, 2008; Edmunds, 2012) may experience limitation in other substrates required for photosynthesis before carbon supply becomes limiting. Moreover, other carbonate system changes associated with OA, such as pH declines, may contribute to increased productivity and warrant further investigation.

In the present study, the effects of increased pCO_2 were more evident in *S. hystrix* than in *A. millepora*. In *S. hystrix*, pigment dynamics, including the xanthophyll cycle which non-photochemically quenches excess light energy, net photosynthetic oxygen production, maximum PSII quantum yields, and ETRs, were all up-regulated at high pCO_2 and reduced with temperature stress. In contrast, pigments and net photosynthetic oxygen production responded only to temperature stress in *A. millepora*. Such species-specific responses may help explain the disparities seen between different experimental works conducted to date and may be due to differences in the efficiency of their carbon concentrating mechanism (Comeau *et al.*, 2012). Furthermore, many of the negative effects of elevated pCO_2 on coral photophysiology and photosynthetic production documented in previous studies have occurred in treatments where CO_2 values were experimentally increased to very high levels (Krief *et al.*, 2010). For example, Anthony *et al.* (2008) found that net productivity in *Acropora intermedia* and *Porites lobata* did not change with moderately increased pCO_2 (similar to those in the present study); however, productivity dramatically declined once pCO_2 was further increased. Similarly, Crawley *et al.* (2010) documented a 38% increase in photosynthetic capacity in *Acropora formosa* under conservative but not under high emission scenarios. Such non-linear (Gil, 2013; Schoepf *et al.*, 2013), species-specific (Marshall and Baird, 2000; Schoepf *et al.*, 2013) responses to environmental pressures are not uncommon. It may be that groups of closely related species have separate non-linear responses to CO_2 where minor increases have negligible effects or are beneficial for net photosynthesis, while additional increases in CO_2 may result in negative effects.

Maintaining rates of calcification in corals potentially becomes increasingly energy demanding with increasing seawater pCO_2 (Cohen and Holcomb, 2009; Comeau *et al.*, 2013; Cyronak *et al.*, 2016; Jokiel, 2016). During times of thermal stress, energetic demands are also placed on corals to maintain the symbiosis with their *Symbiodinium* partners (Lesser, 2011). With OA progressing and SST anomaly frequencies increasing, the opportunity cost for corals to maintain the *status quo* may include declines in calcification, bleaching resistance, fecundity, or other energetically demanding processes. As carbon emissions continue to increase, we are likely to see the gradual deterioration of coral species that are more susceptible to OA effects on calcification (Comeau *et al.*, 2013) and temperature stress (Marshall and Baird, 2000) and decline in those species that are unable to utilize the more abundant CO_2 for photosynthesis in eutrophic waters (Crawley *et al.*, 2010; Brading *et al.*, 2011). Unfortunately for the most reef associated taxa, it appears that the most competitive species of coral, in the face of OA and increasing SST, are massive varieties that support a low diversity of associates (Marshall and Baird, 2000; Fabricius *et al.*, 2011, 2014). While it is difficult to be sanguine in the face of projected trajectories for coral reefs, the only feasible option to prevent the exacerbation of these effects is to reduce anthropogenic CO_2 emissions.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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