Coral Reefs
https://doi.org/10.1007/s00338-018-1672-3

REPORT

Effects of seawater pCO_2 and temperature on calcification and productivity in the coral genus *Porites* spp.: an exploration of potential interaction mechanisms

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Received: 19 June 2017 / Accepted: 21 February 2018

Abstract Understanding how rising seawater pCO_2 and temperatures impact coral aragonite accretion is essential for predicting the future of reef ecosystems. Here, we report 2 long-term (10–11 month) studies assessing the effects of temperature (25 and 28 °C) and both high and low seawater pCO_2 (180–750 µatm) on the calcification, photosynthesis and respiration of individual massive *Porites* spp. genotypes. Calcification rates were highly variable between genotypes, but high seawater pCO_2 reduced calcification significantly in 4 of 7 genotypes cultured at 25 °C but in only 1 of 4 genotypes cultured at 28 °C. Increasing seawater temperature enhanced calcification in almost all corals, but the magnitude of this effect was seawater pCO_2 dependent. The 3 °C temperature increase enhanced calcification rate on average by 3% at 180 µatm, by 35% at 260 µatm and by > 300% at 750 µatm. The rate increase at high seawater pCO_2 exceeds that observed in inorganic aragonites. Responses of gross/net photosynthesis and respiration to temperature and seawater pCO_2 varied between genotypes, but rates of all these processes were reduced at the higher seawater temperature. Increases in seawater temperature, below the thermal stress threshold, may mitigate against ocean acidification in this coral genus, but this moderation is not mediated by an increase in net photosynthesis. The response of coral calcification to temperature cannot be explained by symbiont productivity or by thermodynamic and kinetic influences on aragonite formation.

Keywords Ocean acidification · Seawater temperature · Coral · Calcification · *Porites* · Productivity

Introduction

Coral reefs are among the world's most biologically diverse ecosystems and are of substantial economic importance in terms of fisheries, tourism and coastal protection (Hoegh-Guldberg et al. 2007). Rising atmospheric CO_2 is causing ocean warming (IPCC 2013) and has fundamentally affected seawater carbonate chemistry, lowering seawater pH (Caldeira and Wickett 2003). Understanding the impact of these changes on the accretion of coral aragonite is essential for predicting the future of reef ecosystems. However, attempts to assess increasing pCO_2 and temperature effects, either separately or in combination, have generated contradictory reports (Gattuso and Riibesell 2011; Erez et al. 2011). Most laboratory and field studies indicate that calcification is reduced at lower seawater pH, but there is considerable disagreement on the magnitude of this effect both within and between coral species (Erez et al. 2011). The effect of increasing seawater pCO_2 and temperature on calcification also varies widely among coral taxa (e.g. Schoepf et al. 2013; Anthony et al. 2008). Unravelling the source of these discrepancies is key

Topic Editor Dr. Simon Davy

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00338-018-1672-3) contains supplementary material, which is available to authorized users.

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Published online: 10 March 2018

to predicting accurately the effects of increasing seawater pCO_2 and temperature in different coral species and reef environments.

Interpretation of seawater pCO_2 and temperature effects is complicated by dissimilarities in experimental design between studies. Coral responses can be modified by variations in other environmental parameters, e.g. light and nutrient availability (Chan and Connolly 2013; Chauvin et al. 2011), by study duration (Huang et al. 2014; Castillo et al. 2014) or by nonlinear relationships between seawater pCO_2/temperature and calcification (e.g. Anthony et al. 2008; Castillo et al. 2014). Many combined seawater pCO_2/temperature studies raise temperature above the coral thermal stress threshold (Castillo et al. 2014; Bahr et al. 2016). These studies mimic the effects of stressful, short-term increases in seawater temperature but do not simulate the impact of more subtle, long-term temperature change (Hoegh-Guldberg et al. 2007).

Relatively few studies explore the interactions of coral photosynthesis and respiration with calcification under increased seawater pCO_2/temperature, despite the potential for algal symbionts to offset ocean acidification effects. Increasing seawater pCO_2 can fertilise photosynthesis (Mackey et al. 2015) and this may ameliorate cellular acidosis (Gibbin and Davy 2014), as CO_2 consumption increases local fluid pH when HCO_3^- and H^+ react to form CO_2 and H_2O. In support of this hypothesis, the intracellular pH of symbiotic coral cells is higher in the light than in dark (Venn et al. 2009) and symbiont activities counteract the decrease in intracellular pH of host coral cells which are exposed to high pCO_2 (Gibbin et al. 2014). Photosynthesis may also provide an energy source as algal photosynthate is translocated to the coral (Muscatine and Cernichiari 1969). The responses of coral photosynthesis and respiration to elevated seawater pCO_2 are inconsistent (reviewed in Noonan and Fabricius 2016). Increasing seawater pCO_2 may stimulate (Langdon and Atkinson 2005; Crawley et al. 2010; Gibbin and Davy 2014; Noonan and Fabricius 2016) or suppress (Anthony et al. 2008; Edmunds 2012) photosynthesis. Symbiont photosynthesis may be limited by light or nutrients (rather than carbon) in the culture seawater (Chauvin et al. 2011) or may be affected by symbiont type. The growth and photosynthetic response of *Symbiodinium* to elevated pCO_2 is type specific in culture (Brading et al. 2011) and *in hospite* (Graham and Sanders 2016).

Here, we present a long-term (17–22 week acclimation) study designed to test the effect of increasing seawater pCO_2 (from 180 to 750 µatm) and temperature (25 and 28 °C) on massive *Porites* spp. corals. Massive poritids are major components of coral reefs in the Indo-Pacific, are found in almost all reef habitats and can be the most important reef framework builders (Pichon 2011). Responses of coral colonies to temperature and seawater pCO_2 are highly variable for a range of coral species (Kavousi et al. 2015; Shaw et al. 2016), and we measured the individual responses of colonies of a range of genotypes. We utilised seawater pCO_2 which were both lower and higher than the present day, and we measured the effect of environmental change on colony calcification, photosynthesis and respiration to explore how these physiological processes interact.

Methods

We tested the impact of variations in seawater pCO_2 and temperature on calcification, respiration and photosynthesis of massive *Porites* spp. corals in 2 experiments. In each experiment, corals were acclimated to altered seawater pCO_2 for at least 17 weeks before study. We cultured 3 different genotypes in experiment 1 and 4 different genotypes in experiment 2. All corals were harvested in Fiji by a collector and imported into the UK. We defined different genotypes as coral heads collected from large, spatially separate (non-adjoining) colonies. We cut multiple colonies (each $\sim$ 12 cm in diameter) from each head to enable the study of large individuals of the same genotype in each treatment. Small experimental colonies do not provide accurate estimates of the physiological performance of larger colonies, even when area-normalised (Edmunds and Burgess 2016). In both experiments, corals were cultured at seawater pCO_2 of $\sim$ 180 µatm (the CO_2 atmosphere during the last glacial maximum, Petit et al. 1999), $\sim$ 400 µatm (the present day) and $\sim$ 750 µatm (projected to occur by the end of the present century, IPCC 2013). In experiment 2, corals were also cultured at $\sim$ 260 µatm (the CO_2 atmosphere during the pre-industrial, Gattuso and Lavigne 2009). In experiment 1, corals were maintained at 25 °C and this demonstrated that photosynthesis and respiration rates could vary significantly between replicate colonies of the same genotype. In experiment 2, the same coral colonies were cultured at both 25 and 28 °C for separate periods to enable us to measure the performance of each individual as a function of temperature. Our upper experimental temperature is close to the maximum seasonal temperature at the coral source site (Fiji, where seasonal seawater temperatures typically range from 25.5 to 29 °C) and is likely to be below the thermal stress threshold for these specimens. Culture system parameters are summarised in Table 1.

Coral culturing

Corals were maintained in a purpose-built large-volume aquarium system constructed of low CO_2 permeability

Table 1 Summary of the physical and chemical composition of seawater in each culture treatment, measured over each 5-week experimental period

Treatment	Temperature (°C)	Salinity	DIC (μmol kg^{-1})	Gas stream pCO_2 μatm	TA (μeq kg^{-1})	Estimated pH_T	Estimated $\Omega_{aragonite}$
Experiment 1—25 °C							
180 μatm	25.3 ± 0.1	35.1 ± 0.04	1831 ± 11	180 ± 5	2289 ± 14	8.313	5.34
400 μatm	25.2 ± 0.2	35.2 ± 0.08	1999 ± 8	403 ± 8	2290 ± 8	8.039	3.38
750 μatm	25.1 ± 0.2	35.2 ± 0.05	2113 ± 11	754 ± 6	2293 ± 5	7.809	2.18
Experiment 2—25 °C							
180 μatm	25.0 ± 0.10	35.1 ± 0.1	1862 ± 7	178 ± 2	2194 ± 48	8.322	5.46
260 μatm	25.0 ± 0.16	35.2 ± 0.0	1947 ± 17	260 ± 3	2228 ± 45	8.197	4.49
400 μatm	25.2 ± 0.25	35.1 ± 0.1	2025 ± 13	405 ± 6	2255 ± 15	8.041	3.42
750 μatm	25.0 ± 0.21	35.1 ± 0.0	2129 ± 20	751 ± 9	2235 ± 29	7.812	2.20
Experiment 2—28 °C							
180 μatm	28.0 ± 0.09	35.1 ± 0.05	1757 ± 39	178 ± 2	2189 ± 38	8.300	5.47
260 μatm	28.0 ± 0.18	35.1 ± 0.07	1910 ± 11	259 ± 3	2282 ± 18	8.192	4.84
400 μatm	28.0 ± 0.22	35.1 ± 0.09	1999 ± 14	402 ± 3	2281 ± 16	8.041	3.74
750 μatm	28.0 ± 0.09	35.1 ± 0.09	2113 ± 23	752 ± 6	2281 ± 17	7.813	2.45

Values are means ± 1 standard deviation. pH_T (total scale) and $\Omega_{aragonite}$ were estimated from DIC and pCO_2 using CO_2 sys (Pierrot et al. 2006) using the equilibrium constants for carbonic acid from Mehrbach et al. (1973), refit by Dickson and Millero (1987) and for $KHSO_4$ from Dickson (1990). Total seawater boron was set to 416 μmol kg^{-1} (Uppstrom 1974)

materials designed to control temperature, salinity and dissolved inorganic carbon (DIC) system parameters within narrow limits (see Cole et al. 2016). Corals were housed in 21-l cast acrylic tanks, recirculated with seawater from high-density polyethylene reservoirs containing ~ 900 l of seawater. At the start of each experiment, the reservoir seawater was ~ 80–85% fresh artificial seawater (Red Sea Salt, Red Sea Aquatics, UK) diluted with either artificial seawater from a mixed coral/fish aquarium (Experiment 1) or with local seawater (Experiment 2). 10–15 l of seawater was usually removed from each reservoir each week (during removal of microalgae from the tank surfaces) and was replaced with fresh artificial seawater. No seawater replacement occurred during the experimental periods. The reservoirs were bubbled (at ~ 10 l min^{-1}) with gas mixes set to reach the target seawater pCO_2 compositions. The 400-μatm treatment was aerated with untreated ambient air drawn from outside the building approximately 150 m from the open coast. The high- and low-CO_2 treatments were aerated with ambient or low-CO_2 air, respectively, combined with high-purity CO_2 (Foodfresh, BOC, UK) to achieve the required gas composition. Low-CO_2 air was produced by bubbling ambient air through a caustic solution (0.9 M NaOH and 0.1 M $Ca(OH)_2$) and rinsing it by bubbling through deionised water. The [CO_2] of this low-CO_2 air was monitored every 2 h by automated non-dispersive infrared CO_2 analysers (WMA04, PP systems, USA) and ranged from ~ 20 to 100 μatm depending on the age of the caustic solution. Flow rates of air (ambient or low-CO_2) and CO_2 were regulated by high-precision mass flow controllers (SmartTrak 50 Series, Sierra USA) controlled by a purpose-written MATLAB® program. The [CO_2] of the gas streams (after any addition of CO_2) were monitored automatically 3–4 times per day and were held within narrow limits (Table 1). Corals were maintained under LED lighting (Maxspect R420R 160w-10000k) on a 12-h light/12-h dark cycle, with wavelength settings of 100% A and 20% B such that photosynthetically active radiation (PAR) intensity at coral depth was ~ 300 μmol photons m^{-2} s^{-1}. Corals were fed weekly with rotifers.

Acclimation and study timing

In experiment 1, imported corals were maintained at ambient seawater pCO_2 conditions for 2 months, adjusted to pCO_2 treatment conditions over another 2 months and then acclimated at the final treatment pCO_2 for 5 months before study, all at 25 °C. This was followed by a 5-week experimental period in which calcification, respiration and net and gross photosynthesis were measured in each coral colony on usually 3 or 4 occasions. In experiment 2, imported corals were maintained at ambient seawater pCO_2 conditions for 2 months, adjusted to pCO_2 treatment conditions over 1 month and then acclimated at the final treatment pCO_2 for 4 months at 28 °C. This was followed by a 5-week experimental period in which physiological rates were measured as before. At the end of this time, a

small slice of each colony (including tissue and skeleton) was removed by rock saw and preserved for future analysis. This reduced the surface areas of each colony by ~ 25%. Seawater temperatures were then reduced to 25 °C over a period of 4 weeks and then acclimated at this temperature for another month during which time the sawn edges of the coral colonies were overgrown by coral tissue. Finally, physiological rates were measured in each coral over a 5-week period as before. In experiment 2, the coral polyps did not extend in the colonies cultured at 400 µatm and at 28 °C and there was little microalgae growth on the tank surfaces in this treatment. The seawater in this reservoir was removed and replaced with seawater from the remaining 3 reservoirs (bubbled to bring it to the correct seawater pCO_2 before use). Physiological measurements were only made in these colonies at 25 °C.

Coral isolations

To measure physiological rates, individual coral colonies were isolated in 21-l culture tanks for 5 h (in the light) or 7 h (in the dark). Net photosynthesis and respiration rates were estimated from measurements of dissolved oxygen (DO_2, Thermo Orion 5 star meter with RDO sensor) at the start and end of each incubation (Schneider and Erez 2006). Net photosynthesis was defined as the production of oxygen in the light and respiration as the consumption of oxygen in the dark. Gross photosynthesis was calculated as net photosynthesis minus respiration. The precision of repeat DO_2 measurements was always better than 0.3% (1σ). [DO_2] typically increased by up to ~ 80 µmol l^{-1} (from ~ 200 µmol l^{-1}) in the light and decreased by up to ~ 40 µmol l^{-1} in the dark. Calcification rates were estimated from the change in seawater total alkalinity (typically ~ 50 µeq kg^{-1}) over the course of the isolation (Schneider and Erez 2006). Light and dark calcification rates were combined to yield a total rate per day. Isolations were repeated 3–4 times (with the exception of 2 coral colonies which were only isolated twice) at approximately weekly intervals over the 4-week study period. All metabolic rates were normalised to coral surface area. Coral tissue surface areas were estimated by multiplying the tissue area measured on scaled photographs using image processing software (ImageJ, National Institute of Health, USA) by a hummockiness factor to correct for colony topography. The hummockiness factor was calculated for each colony by dividing the actual length of the colony surface (measured with a piece of thread) by the length of the colony surface estimated from ImageJ.

Monitoring seawater composition

Seawater temperatures were measured hourly (TinyTag Aquatic, Gemini Data Loggers, UK) in experiment 1. Temperatures in experiment 2 and salinities in both experiments were measured twice daily on 4 days of each week in the experimental periods (Thermo Orion 5 star meter, calibrated to NIST conductivity standards). Total alkalinity was measured by automated Gran titration (Metrohm, 888 Titrando) twice daily on 4 days of each week in the experimental periods. Precision of duplicate ~ 30 ml analyses was typically ± 2 µeq kg^{-1}. The precision of multiple measurements of synthetic Na_2CO_3 standards was consistently ± 3 µeq kg^{-1} (1σ, between days). The total alkalinity, [Ca] and [Sr] of the culture seawater were maintained by additions of 0.6 M Na_2CO_3 and a mixture of 0.58 M $CaCl_2$ + 0.02 M $SrCl_2$ by 200 µl volume solenoid diaphragm pumps, evenly spaced over a 24-h period, controlled by a custom-written MATLAB® dosing control program. DIC was measured weekly in each reservoir using a CO_2 differential, non-dispersive, infrared gas analyser (Apollo SciTech; AS-C3). Samples were calibrated against a natural seawater certified reference material (CRM; A. Dickson, Scripps Institution of Oceanography). Internal reproducibility was calculated from the standard deviation of 8 replicate measurements of a single sample ($\sigma/\sqrt{n}$) and was always ≤ 0.1%. Multiple measurements of the CRM analysed as an unknown were in excellent agreement with the certified value (within 5 µmol kg^{-1}, Cole et al. 2016).

Results

Calcification, gross and net photosynthesis and respiration rates for all corals are illustrated in Figs. 1 and 2. Two replicate colonies of genotype 3 were cultured and measured in 400 and 750 µatm seawater pCO_2 (Fig. 1). Calcification rates, normalised to coral surface area, did not vary significantly between replicates, but both gross photosynthesis and respiration rates did vary significantly ($p \leq 0.05$ two-tailed t test). Light variations over the tank footprint were small (< 50 µmol photons m^{-2} s^{-1}), but photosynthesis variations between replicates may reflect individual topography or modulation of light scattering (Marcelino et al. 2013). We maintained each colony in the same tank position for the duration of each experiment ensuring that measurements of the same colony in different temperatures are directly comparable.

Physiological rates, particularly calcification, were highly variable between genotypes. For example, genotype 7 maintained calcification rates that were ≥ ×2 higher than genotypes 5 and 6 regardless of seawater pCO_2 or

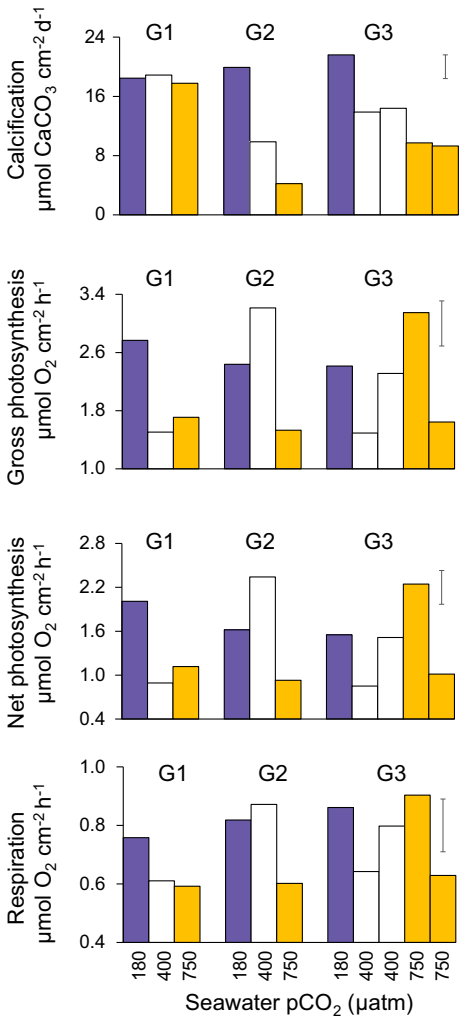

Fig. 1 Calcification, gross and net photosynthesis and respiration rates for each coral genotype (G1, G2 and G3) at 25 °C and over a range of seawater pCO_2. Two replicate colonies of G3 were cultured at 400 and 750 μatm. Bars represent means of 3–4 incubations over a 5-week period. The error indicates the typical standard deviation of these multiple incubations

temperature (Fig. 2). To ease comparison of the effect of seawater pCO_2 on physiological processes, we calculated the relative calcification, respiration, gross and net photosynthesis rates of each coral genotype as a proportion of the rate observed at the lowest seawater pCO_2 (180 μatm, Fig. 3). We compared physiological rates between individuals of the same genotype in different seawater pCO_2 treatments (one-way ANOVA, Tables 2, 3). We combined data from the 2 replicate genotype 3 colonies in experiment 1 for these analyses.

At 25 °C, calcification was significantly lower at 750 μatm pCO_2 compared to 180 μatm pCO_2 in 4 of the 7 *Porites* spp. genotypes and significantly lower at 750 μatm pCO_2 compared to 400 μatm pCO_2 in 1 genotype (Tables 2, 3). At 28 °C, calcification was significantly lower in only one of the 4 genotypes cultured at 750 μatm pCO_2 compared to 180 μatm pCO_2 (and was significantly higher in 1 genotype). Gross and net photosynthesis and respiration rates were significantly lower at 750 μatm pCO_2 than at 180 μatm pCO_2 in 2 of the 7 genotypes cultured at 25 °C. At 28 °C, the pattern was more complicated with some genotypes exhibiting significantly higher photosynthesis or respiration rates at high or low seawater pCO_2 (Table 3).

To assess the effect of temperature changes, we compared physiological rates at 25 and 28 °C for each coral colony (two-tailed *t* test, Table 4). To avoid inter-colony differences (such as those observed in experiment 1) affecting the results, we measured the responses of the same individual colonies to seawater pCO_2 first at 28 °C and then 3 months later at 25 °C. We cannot discount the possibility that physiological rates were affected by the time that individual corals spent in the culture system, but we believe that any effect is likely to be small. Individual *Porites* spp. colonies which we have cultured in our aquaria for > 4 years at constant temperature exhibit similar calcification rates (unpublished data) to those observed for massive *Porites* spp. in the field (Allison et al. 1996), indicating that corals maintain approximately constant calcification rates in the aquaria. Increasing seawater temperature usually increased calcification but decreased respiration and gross and net photosynthesis rates (Fig. 2). Increasing temperature significantly increased calcification in three of the 4 genotypes at 750 μatm pCO_2 but did not significantly affect calcification at 180 or 260 μatm (Table 4). Increasing temperature significantly decreased gross and net photosynthesis in only one genotype at 750 μatm pCO_2 but in all genotypes at 180 μatm.

Discussion

Increasing seawater pCO_2 decreased calcification significantly in 4 of 7 massive *Porites* spp. genotypes at 25 °C but in only 1 of 4 genotypes at 28 °C. Our results are broadly comparable to 2 previous shorter (11 days and 8 weeks) studies of massive *Porites* spp. in which calcification rates were reduced by high seawater pCO_2 at 25–26 °C (Anthony et al. 2008) but were not significantly affected at 28–29 °C (Anthony et al. 2008; Edmunds 2012). While the majority of studies indicate that coral calcification is reduced by ocean acidification (Erez et al. 2011), massive *Porites* spp. appear to be relatively insensitive to increasing seawater pCO_2 (Crook et al. 2012) and have higher rates of calcification at some natural high seawater pCO_2 sites compared to their counterparts at adjacent low-pCO_2 sites (Fabricius et al. 2011).

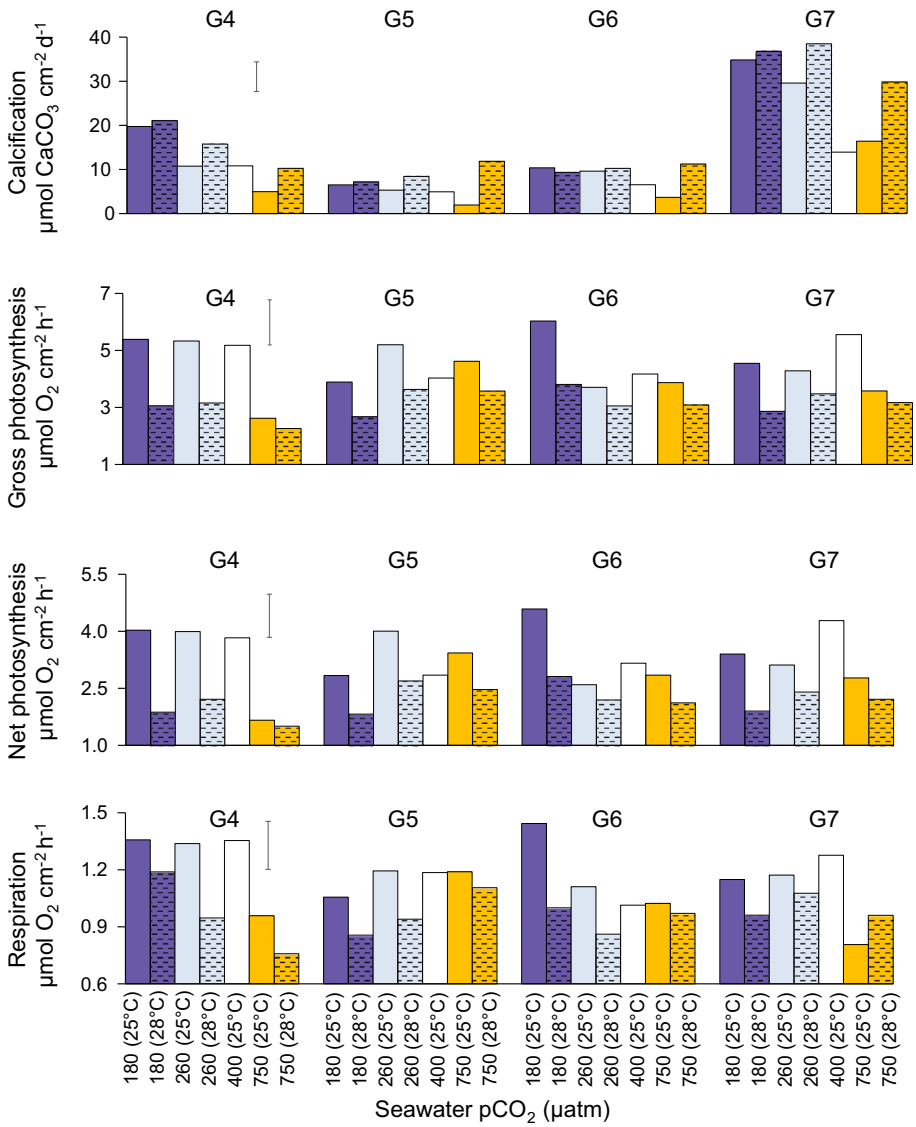

Fig. 2 Calcification, gross and net photosynthesis and respiration rates for each coral genotype (G4, G5, G6 and G7) over a range of seawater pCO_2 and at 2 seawater temperatures. Bars represent means of 3–4 incubations over a 5-week period. The error indicates the typical standard deviation of these multiple incubations. Data for 25 and 28 °C are presented by clear and hatched bars, respectively. Rates were measured in corals at 400 µatm at 25 °C only

Increasing seawater temperature enhanced calcification in almost all corals, and the magnitude of this effect was seawater pCO_2 dependent. The 3 °C temperature increase enhanced calcification on average by 3% at 180 µatm, by 35% at 260 µatm and by > 300% at 750 µatm. At high seawater pCO_2, this increase substantially mitigated the effects of ocean acidification on calcification: at 25 °C the mean *Porites* spp. calcification rate at seawater pCO_2 750 µatm is ∼ 40% of the rate observed at 180 µatm. In contrast, at 28 °C this figure is ∼ 100%. The temperature driven increases in calcification were not accompanied by increases in symbiont gross or net productivity (Fig. 2). Rather, net production was reduced at the higher temperature in all colonies regardless of seawater pCO_2 (Fig. 2). Our results concur with a previous massive *Porites* spp. study in which net productivity was reduced at high seawater temperature and seawater pCO_2, but calcification was not affected (Anthony et al. 2008). Our study indicates that increasing seawater temperature can offset the suppression of calcification by ocean acidification in this coral species. However, this offset is not associated with an increase in net algal productivity.

To understand how increasing temperature may offset ocean acidification effects requires some understanding of the coral calcification process. The aragonitic coral skeleton precipitates from a calcifying fluid, derived from seawater (Tambutte et al. 2012) and enclosed in a semi-isolated extracellular space between the coral tissue and underlying skeleton (Clode and Marshall 2002). Corals increase the pH of the calcification fluid above that of seawater (Al-Horani et al. 2003; Venn et al. 2011) shifting the fluid DIC equilibrium in favour of CO_3^{2-} at the expense of CO_2. The CO_2 concentration gradient thus formed facilitates the diffusion of CO_2 from the overlying

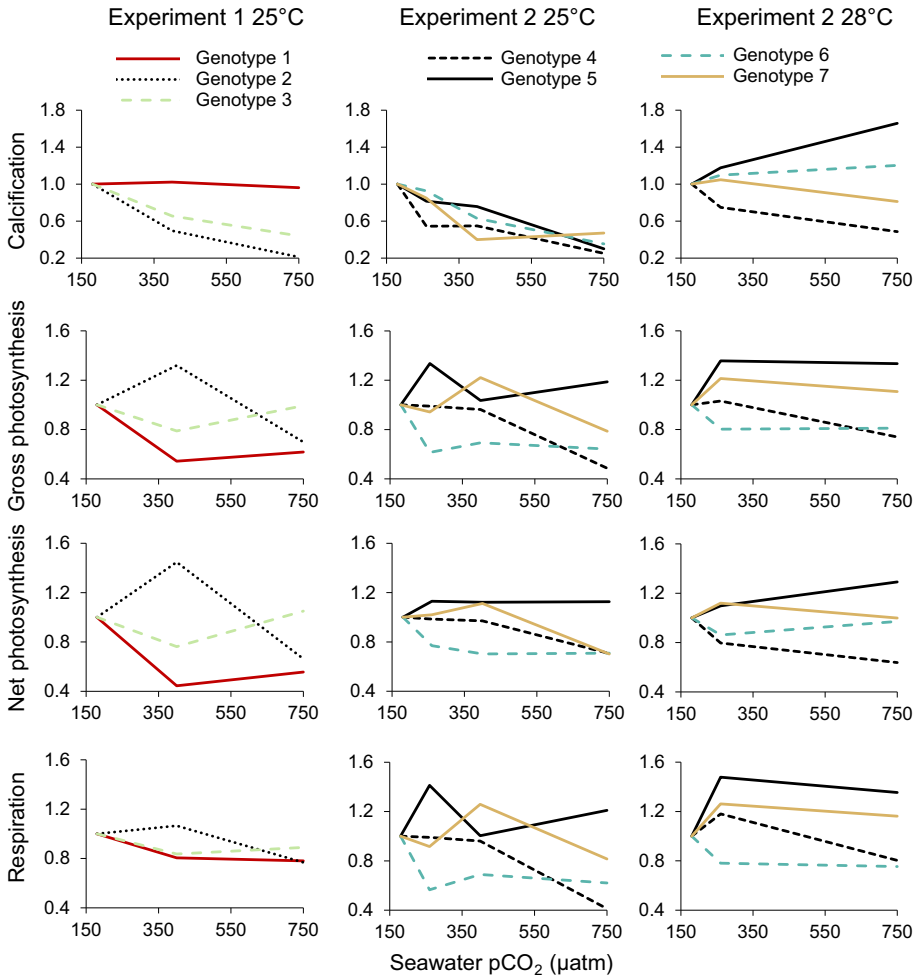

Fig. 3 Relationships between seawater pCO_2 and calcification, gross and net photosynthesis and respiration rates in each *Porites* spp. genotype. Rates were calculated as a proportion of the rate observed at 180 µatm for each genotype to eliminate variations in physiological rates between genotypes

Table 2 Summary of significant differences ($p \leq 0.05$ one-way ANOVA followed by Tukey's pairwise comparisons) in physiological rates between individuals of the same coral genotype in different seawater pCO_2 treatments (180, 400 or 750 µatm) in experiment 1

	Calcification	Gross/net photosynthesis	Respiration
Genotype 1	ns	180 > 400	180 > 400
P. lutea		180 > 750	180 > 750
Genotype 2	180 > 400	400 > 750	ns
P. lutea	180 > 750		
Genotype 3	180 > 400	ns	ns
P. murrayensis	180 > 750		
	400 > 750		

The same results were observed for gross and net photosynthesis in all colonies

ns no significant differences between seawater pCO_2 treatments

coral tissue into the calcification fluid (Erez 1978) where it reacts to form HCO_3^- and CO_3^{2-}. It is unclear which DIC species is/are involved in aragonite precipitation. CO_3^{2-} is usually preserved in the aragonite lattice, but HCO_3^- could also be involved in precipitation. Wolthers et al. (2012) successfully modelled observed growth rates of the $CaCO_3$ polymorph calcite by assuming both aqueous HCO_3^- and CO_3^{2-} attach to the growing crystal. HCO_3^- subsequently deprotonates, leaving CO_3^{2-} preserved in the carbonate lattice. Von Euw et al. (2017) observed HCO_3^- in both coral and synthetic aragonite which may be a remnant of attached un-deprotonated HCO_3^-. Aragonite precipitation rates correlate positively with fluid aragonite saturation state (Burton and Walter 1987), a function of $[Ca^{2+}]$ and $[CO_3^{2-}]$, suggesting that CO_3^{2-} is critical in precipitation. However, similar positive relationships are also observed

Table 3 Summary of significant differences ($p \leq 0.05$, one-way ANOVA followed by Tukey's pairwise comparisons) in physiological rates between individuals of the same coral genotype in different seawater pCO_2 treatments (180, 260, 400 or 750 µatm) in experiment 2

	Calcification		Gross/net photosynthesis		Respiration	
	25 °C	28 °C	25 °C	28 °C	25 °C	28 °C
Genotype 4 *P. murrayensis*	180 > 750	180 > 750	ns	ns	180 > 750 260 > 750 400 > 750	
Genotype 5 *P. murrayensis*	ns	750 > 180	ns	260 > 180[a] 750 > 180[b]	ns	750 > 180 750 > 260
Genotype 6 *P. murrayensis*	ns	ns	180 > 260 180 > 400 180 > 750	180 > 750 180 > 260[b]	180 > 400 180 > 750	180 > 260
Genotype 7 *P. lutea*	180 > 400 180 > 750 260 > 400 260 > 750	ns	400 > 260[a] 400 > 750	ns	180 > 750 260 > 750 400 > 750	ns

The same results were observed for gross and net photosynthesis unless noted

ns no significant differences between seawater pCO_2 treatments. Rates were measured in corals at 400 µatm at 25 °C only

[a]This result was significant for net photosynthesis but not gross photosynthesis

[b]This result was significant for gross photosynthesis but not net photosynthesis

Table 4 Summary of *p* values (two-tailed *t* test) comparing physiological rates at 25 and 28 °C in each coral colony

pCO_2 µatm	Calcification			Gross photosynthesis			Net photosynthesis			Respiration		
	180	260	750	180	260	750	180	260	750	180	260	750
Genotype 4	0.64	0.29	0.18	**0.013**	**0.0092**	0.67	**0.012**	**0.014**	0.84	0.41	**0.0011**	**0.053**
Genotype 5	0.60	0.11	**0.0016**	**0.019**	**0.041**	0.15	**0.023**	0.056	0.096	**0.027**	**0.0050**	0.65
Genotype 6	0.72	0.82	**0.0037**	**0.0036**	0.11	**0.00043**	**0.011**	0.32	**0.0012**	**0.00094**	**0.013**	0.36
Genotype 7	0.62	0.052	**0.017**	**0.024**	**0.020**	0.40	**0.022**	**0.028**	0.20	0.070	0.12	0.17

p values ≤ 0.05 are highlighted in bold

between aragonite precipitation rate and fluid $[HCO_3^-]$ or $[HCO_3^- + CO_3^{2-}]$ (see supplementary information), so this dataset does not demonstrate sole involvement of CO_3^{2-} in precipitation. Regardless of the DIC species involved in aragonite formation, the rise in calcification fluid pH and subsequent CO_2 invasion increases the availability of both HCO_3^- and/or CO_3^{2-} at the calcification site.

The precipitation rates of synthetic aragonites are positively correlated with temperature and fluid saturation state (Fig. 4, replotted from Burton and Walter 1987). We overlay our observed increases in coral calcification rate as a function of temperature onto Fig. 4 assuming that the calcification fluid aragonite saturation states (Ω) of all corals growing at 25 °C are $\sim$ 8. Coral calcification fluid $[Ca^{2+}]$ is similar to seawater (Al-Horani et al. 2003), suggesting that [precipitating DIC] drives calcification rate. The mean calcification site pH_{total} of modern massive

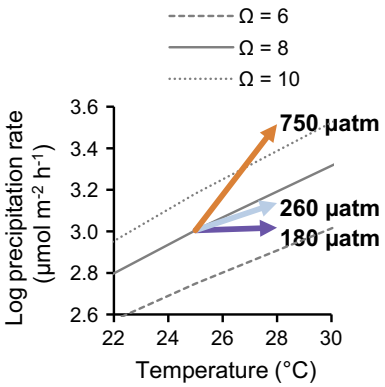

Fig. 4 The temperature dependence of coral calcification rate at 180, 260 and 750 µatm seawater pCO_2. The effect of temperature and fluid saturation state (Ω) on inorganic aragonite precipitation rate is replotted from Burton and Walter (1987)

Porites spp. field corals growing at ambient pCO_2 and a mean annual seawater temperature of 25 °C is ~ 8.5 (Allison et al. 2014). In paired laboratory measurements of both calcification fluid pH and $[CO_3^{2-}]$ in a range of coral species, a coral calcification fluid of pH_{total} 8.5 has an aragonite saturation state of ~ 8 (Cai et al. 2016). Corals cultured at high seawater pCO_2 are unable to attain the same high calcification fluid pH observed in their low seawater pCO_2 counterparts (Venn et al. 2012), perhaps because low seawater pH may inhibit the dissipation of H^+ from the coral to local seawater (Jokiel 2011). It is therefore unlikely that they concentrate DIC at the calcification site to the same extent or reach the same high fluid saturation states. Decreasing $\Omega_{aragonite}$ to 6 reduces aragonite precipitation rate by about half, while raising it to 10 (as we hypothesise may occur at low seawater pCO_2) increases precipitation by ~ 50%. While our selection of calcification fluid saturation states at high and low seawater pCO_2 is arbitrary, it serves to illustrate a key point. Inorganic aragonite precipitation rates are temperature dependent, increasing × 1.6 from 25 to 28 °C at $\Omega_{aragonite} = 8$, but saturation state has little effect on this temperature dependence (i.e. this figure changes from 1.5 to 1.7 at $\Omega_{aragonite} = 6$ and 10, respectively, Fig. 4). In our study, temperature had little effect on coral calcification at low seawater pCO_2 and any observed increase in aragonite precipitation is much lower than that observed in inorganically precipitated aragonites. In contrast at high seawater pCO_2, the temperature-mediated increase in calcification is much larger (× 3) than observed in inorganic aragonites. Clearly, the response of coral calcification to temperature is not driven solely by thermodynamic and kinetic influences on aragonite precipitation. Rather other factors act to negate and augment the effect of temperature on aragonite precipitation at low and high seawater pCO_2, respectively. While it is not immediately clear what these are, calcification responses of the magnitude observed here can potentially be explained by changes in the saturation state of the calcification fluid (Fig. 4). The fluid saturation state is influenced by enzyme activities. For example, Ca-ATPase extrudes protons from the calcification fluid (Al-Horani et al. 2003), while carbonic anhydrases catalyse the interconversion of CO_2 and HCO_3^- (Zoccola et al. 2016), and these activities may be both temperature and seawater pCO_2 dependent. For example, ocean acidification suppresses the gene expression and enzyme activities of carbonic anhydrases localised to the coral calcifying cells but increasing temperatures enhance these enzyme activities and may counteract the effect of seawater pCO_2 (Zoccola et al. 2016).

Our data indicate that temperature increases can mitigate against the effect of ocean acidification on calcification in massive *Porites* spp. It is possible that future seawater temperature increases may offset the effects of ocean acidification in this coral genus at reef locations where corals live below their upper thermal stress thresholds. However, in practice, seawater temperature increases are unlikely to enhance calcification at most tropical reef locations where corals already exist close to their upper thermal tolerance limits. Future minor increases in seawater temperature at these sites are likely to induce widespread coral bleaching and thereby suppress coral calcification (Frieler et al. 2013; Hooidonk et al. 2013). Recent decreases in growth rates of massive *Porites* spp. at some Indo-Pacific reef locations are likely driven by local increases in local seawater temperatures (Cantin and Lough 2014), indicating that this process is already in progress.

Acknowledgements This work was supported by the UK Natural Environment Research Council (Award NE/I022973/1) to AAF and NA. We thank Dave Steven, Mark Robertson, Casey Perry, Mike Scaboo and Andy Mackie for their assistance with the culture system build.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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