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REPORT

Parameterization of the response of calcification to temperature and pCO_2 in the coral *Acropora pulchra* and the alga *Lithophyllum kotschyanum*

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Abstract The response of tropical corals and calcifying algae to ocean acidification (OA) and warming has received much attention in the past decade. However, most studies have evaluated the response of organisms to two or three temperature treatments, which does not allow the functional relationship between calcification and temperature under ambient and future pCO_2 to be determined. This study tested the hypothesis that the relationship between calcification and temperature is affected by OA in the coral *Acropora pulchra* and the calcified alga *Lithophyllum kotschyanum*. Pieces of each organism were incubated under five (24–30 °C) or six (24–31.5 °C) temperatures crossed with two pCO_2 levels (400 and 1000 µatm), and calcification was assessed in trials conducted in the spring and summer. The response of coral calcification to temperature was a positive asymmetric parabola with a maximum at ~28 °C under both pCO_2 levels and in both seasons; the effects of pCO_2 on calcification were largest at ~28 °C and lowest in both cool and warm temperatures. In contrast, calcification of the alga at both levels of pCO_2 was unaffected by temperature in spring, but declined linearly with temperature in summer. This study demonstrates that the calcification response of coral reef organisms to the crossed effect of warming and OA is complex and cannot be fully assessed without using multiple temperature treatments that are ecologically relevant.

Keywords Ocean acidification · Calcification · Climate change · pCO_2

Introduction

Anthropogenic CO_2 emissions are the cause of two major threats to coral reefs, global warming and ocean acidification (OA). Seawater warming is a major driver of mass bleaching episodes affecting tropical corals (Brown 1997), which are caused by the disruption of the symbiosis between the cnidarian host and *Symbiodinium* algae, leading to their expulsion from the host (Gates et al. 1992; Glynn 1996). In comparison with unbleached corals, bleached corals are characterized by depressed calcification, slow growth, and impaired photosynthesis, and these features can kill large numbers of corals during severe bleaching events (Glynn 1996; Hoegh-Guldberg 1999). The effects of OA on coral reef organisms have received much attention during the last decade, and most results indicate that decreasing pH negatively affects the physiology of corals and calcifying algae (Kroeker et al. 2013). The effects of OA on calcification are of particular concern, because coral calcification is expected to decline by ~10–20 % with a doubling of pre-industrial pCO_2 (Erez et al. 2011; Chan and Connolly 2013; Comeau et al. 2013, 2014b), which may lead to reduced net ecosystem accretion (Andersson and Gledhill 2013). As has been found for scleractinians (Albright 2011; Kroeker et al. 2013), OA affects tropical calcifying algae to reduce growth, recruitment, and calcification (e.g., Kuffner et al. 2008; Jokiel et al. 2008; Comeau et al. 2014b), and moreover can enhance their susceptibility to grazing (Johnson and Carpenter 2012).

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While there now are many studies on the individual effects of temperature and OA on the physiology of corals and tropical algae (e.g., Jokiel and Coles 1977; Comeau et al. 2014a), there is only a limited understanding of the combined effects of these stressors (Kroeker et al. 2013). For scleractinians, the interactive effects of temperature and OA can be either negatively synergistic for calcification, as in *Stylophora pistillata* (Reynaud et al. 2003; Anlauf et al. 2011), or show no additive effects, as in massive *Porites* spp. (Anthony et al. 2008; Edmunds et al. 2012). Similarly, for tropical coralline algae, the interaction between seawater warming and OA has negative synergistic effects on *Porolithon onkodes* (Anthony et al. 2008), but no synergistic effects in the case of *Hydrolithon reinboldii* from Moorea (Comeau et al. 2014a).

Regardless of the experimental outcome, most studies of the combined effects of OA and temperature on coral reef organisms have been performed over a limited range of temperatures, and typically with only two or three treatment levels (e.g., Reynaud et al. 2003; Comeau et al. 2014a). While contrasts of the effects of 2–3 temperatures on organism performance are informative, such studies do not allow precise parameterization of the response curve describing the relationship between temperature and performance. The general shape of these curves is well known for poikilotherms, which do not regulate their internal temperature, and they play important roles in determining organism distribution, behavior, and fitness (e.g., Huey and Berrigan 2001). Thermal response curves are characterized by an asymmetric threshold response centered on the optimum temperature, with the positive slope (to the left of the threshold) less steep than the negative slope (to the right of the threshold) (Martin and Huey 2008). For organismic traits such as respiration and photosynthesis, this shape is a summation of subcellular processes involving largely enzyme kinetics to the left of the threshold and protein damage to the right of the threshold (Hochachka and Somero 2002). As thermally mediated enzyme kinetics and protein degradation are deterministic processes that can be described mathematically, thermal response curves typically conform to a restricted family of functions (Martin and Huey 2008). These functions and their constants are valuable in characterizing and statistically comparing the response of organisms to temperature.

In the case of scleractinians and algae from tropical reefs, thermal response curves of organism performance and their interactive effects with pCO_2 are needed to understand the effects of global change and OA on performance. Calcification by scleractinians and tropical algae is particularly important in this regard, because it is responsible for the construction of coral reef ecosystems (Stoddart 1969; Allemand et al. 2011), and for some representatives of these groups, it is known in some detail. For example, calcification in tropical scleractinians is depressed at the lowest temperatures found on coral reefs (e.g., as low as ~20 °C), enhanced up to a threshold temperature of ~28 °C, and sharply declines thereafter (Pratchett et al. 2015). Although basic information is available to predict how calcification in scleractinians will respond to rising temperature (Pratchett et al. 2015), these data are distributed sparsely among taxa and temperature treatments, and typically come from experiments conducted at ambient pCO_2 and in a single season. Comparable studies of the effects of temperature on the calcification rates of tropical calcifying algae are missing, and thus, there is an assumption that they respond to temperature in broadly similar ways to scleractinians. This assumption is consistent with limited information on calcifying algae from temperate and cold-water locations (King and Schramm 1982). Together, these limitations restrict the capacity to predict how calcification in tropical scleractinians and calcifying algae will change in a warmer and more acidic future ocean.

This study tested the hypothesis that the relationship between temperature and calcification in tropical scleractinians and calcified algae is affected by OA. An experimental approach was taken in which nubbins of the coral *Acropora pulchra* and pieces of the calcifying alga *Lithophyllum kotschyanum* were incubated in the laboratory under two pCO_2 levels (400 and 1000 µatm) and five (experiment 1) or six (experiment 2) temperature treatments between 24 and 30 °C (31.5 °C in experiment 2). The experiment was duplicated in September/October (experiment 1) and January/February (experiment 2) of consecutive years to test for acclimation to conditions that are unique to two times falling in the austral spring and summer.

Materials and methods

Sample collection and preparation

This study was done in Moorea, French Polynesia, at the Richard B. Gump South Pacific Research Station, first during the austral spring between 14 September and 5 October 2014, and repeated during the austral summer between 26 January and 16 February 2015. During these periods, mean seawater temperature on the outer reef was 27.1 ± 0.7 and 28.6 ± 0.5 °C, respectively (mean ± SD, $n = 728$ and $n = 722$; Leichter 2012). Branches of *A. pulchra* (~4 cm long) and fragments of *L. kotschyanum* (~4 × 4 cm) were collected from the fringing reef of the north shore at ~2 m depth and attached to plastic supports using underwater epoxy (Z-Spar, A788); 100 replicates of each taxon were prepared in 2014, and 120 replicates in 2015.

Experimental setup

Following collection and preparation, and after 48 h in flowing seawater in the laboratory, organisms were placed at random in 10 (spring) or 12 (summer) 150-L incubation tanks (10 organisms taxon^{-1} tank^{-1}). As it was not the objective of this study to statistically compare taxa, corals and algae were incubated in the same tanks. In the spring, organisms were maintained under ~400 and ~1000 µatm pCO_2 crossed with five temperatures (24.0, 25.5, 27.0, 28.5, and 30.0 °C) that covered the annual range of seawater temperatures in the back reef of Moorea (Leichter 2012). In the experiment conducted in the summer, a sixth treatment was added to test for the response to an extreme upper temperature (31.5 °C) that is likely to be more common in a warmer future ocean. For both experiments, the temperature in incubation tanks was initially set to the ambient seawater temperature when the experiments were conducted, which was ~27.0 °C for spring and ~28.0 °C in the summer incubations. Thereafter, the temperature was gradually changed at ~0.5 °C d^{-1} until the target temperatures were reached after 6 d, and then conditions were maintained for 3 weeks.

CO_2 treatments consisted of two pCO_2 levels corresponding to present-day atmospheric concentration (~400 µatm) and a value expected by the end of the twenty-first century (~1000 µatm) assuming the pessimistic RCP scenario 8.5 is maintained (IPCC 2013). For the spring experiment, CO_2 treatments were created by controlling the pH in two header tanks (each 150 L), from which seawater drained into each individual treatment tank at a constant rate of 0.3–0.4 L min^{-1}. Fresh filtered seawater was continuously delivered in the header tanks at ~2 L min^{-1}. For the summer experiment, pH was controlled directly in the incubation tanks that were supplied by fresh filtered seawater at 0.3–0.4 L min^{-1}. For both experiments, seawater pH was controlled using a digital controller attached to pH electrodes (Aquacontroller, Neptune systems, USA) and solenoid valves that were set to deliver pure CO_2 in the header tanks or the incubation tanks. Ambient air was continuously bubbled in all the incubation tanks.

For both experiments, each tank was illuminated with 75-W light-emitting diode (LED) modules (Sol White LED Module, Aquaillumination, USA) that were adjusted to provide a maximum average light intensity of ~650 µmol quanta m^{-2} s^{-1} that was similar to the average light intensity experienced in the back reef of Moorea at 2 m depth (Carpenter 2012), where the study organisms were collected. To produce a natural diel light cycle with a 12:12 light–dark photoperiod, irradiance was increased gradually from darkness to the maximum intensity over 4 h, maintained at the maximum intensity for 4 h, and then reduced gradually over 4 h. To quantify light, irradiance was measured below the seawater surface with a 4π quantum sensor (LI-193) and a LiCor LI-1400 meter in all tanks during the period of maximum light intensity. The positions of organisms in the tanks was changed randomly twice each week to minimize position effects.

Carbonate chemistry measurements

pH was monitored daily at 0900 hrs in all tanks using a portable pH meter (Orion 3-stars, Thermo Scientific, USA) mounted with a combination pH probe (Orion Ross Ultra, Thermo Scientific, USA) that was calibrated every other day with Tris/HCl buffer (after Dickson et al. 2007). pH also was measured spectrophotometrically in all tanks once per experiment using m-cresol dye (Dickson et al. 2007); these determinations yielded values similar to those of the pH meter (within 1 %). To detect potential dial variations in pH in the tanks due to biological activity (i.e., photosynthesis and respiration), discrete measurements of pH were taken at 0600 and 1800 hrs, and these analyses showed that pH varied by ~0.05 units between these times. Total alkalinity (A_T) was measured every 3 d in each tank and remained stable ($\leq$15 µmol kg^{-1} variation) because of the fast renewal rate of seawater within tanks (water was replaced at 0.3–0.4 L min^{-1}). A_T titrations were performed within 1 d of seawater collection using open-cell potentiometric titration (Dickson et al. 2007) with an automatic titrator (T50, Mettler-Toledo, Switzerland). Titrations of certified reference material (batch 140) provided by A. Dickson were performed prior to each set of titrations and yielded an average value ±2.9 µmol kg^{-1} of the nominal value (SE = 2.8 µmol kg^{-1}, n = 12). Salinity was measured every week with a conductivity meter (YSI 3100, YSI, USA), and temperature was measured daily with a certified thermometer (Fisher-Scientific, USA). Parameters of the carbonate system were calculated from salinity, temperature, A_T, and pH_T using the R package seacarb (Gattuso et al. 2015).

Net calcification

Buoyant weights (Spencer-Davies 1989) of the corals and algae were recorded at the beginning of the incubation and after 3 weeks in the treatments. The difference between the initial and final buoyant weight was converted to dry weight of skeleton using the aragonite density of 2.93 g cm^{-3} for *A. pulchra* and calcite density of 2.71 g cm^{-3} for *L. kotschyanum*, in accordance with the mineral form of $CaCO_3$ deposited by each taxon. Rates of net calcification were normalized to the area of organisms estimated using wax dipping (Stimson and Kinzie 1991).

Statistical analyses

To test for differences in treatment conditions in the tanks, temperature and pCO_2 in the tanks were analyzed with a two-factor partially nested ANOVA in which treatment (pCO_2 or temperature) was a fixed effect and tank a random nested factor.

Since the objective of our study was to parameterize the relationship of calcification with temperature and pCO_2, we favored the use of multiple treatments (i.e., five or six temperatures crossed with two pCO_2) over the replication of few treatments. While our measurements from individual organisms in each tank are pseudoreplicated, the potential implications of pseudoreplication were reduced by the large volume of the tanks (150 L) and the open flow of seawater. These design features reduced the potential for the corals and algae within a single tank to affect one another (e.g., through microbial infections or the release of dissolved organic material). Based on this rationale, individual organisms were treated as statistical replicates. Calcification of corals and algae was analyzed with a three-way ANOVA in which temperature, pCO_2 treatment, and time (spring vs. summer) were fixed effects. Statistical analyses were conducted using R software, and the statistical assumptions were tested through graphical analyses of residuals.

To investigate the shape of the relationships between calcification and temperature, nonlinear (for corals) and/or linear (for algae) curve fits were tested. For *A. pulchra*, three mathematical functions that have previously been used to fit the response of a physiological parameter to temperature—sinusoidal, third-degree polynomial, and Gaussian–Gompertz (G–G) (after Martin and Huey 2008 as described below)—were tested for goodness of fit by least squares of the response of calcification to temperature under the two pCO_2 levels tested. The Akaike information criterion (AIC) was used to determine which function best described this relationship. The AIC weight was used to determine which functions could not be rejected from the study, and confirmed that the G–G model was the best fit to the data for three of four cases (two pCO_2 × two times). Due to its biological significance in capturing the effects of Arrhenius kinetics and thermal disruption of proteins (Martin and Huey 2008), the G–G function was used to describe the results for *A. pulchra*:

$$G = Me^{(-e^{(\beta(T-T_m)} - \alpha(T-T_m)^2)}$$

where G is the calcification (units of mg $CaCO_3$ cm^{-2} d^{-1}), M is the maximum calcification (units of mg $CaCO_3$ cm^{-2} d^{-1}), β is the descending slope (units of mg $CaCO_3$ cm^{-2} d^{-1} $°C^{-1}$) to the right of the thermal optimum, T is the seawater temperature (°C), T_m is the temperature at which calcification is maximal, and α is the ascending slope to the left of the thermal optimum. The best fit of the G–G function (least squares) was determined using the function *nls* in R; *t* tests were used to compare the constants between pCO_2 treatments and times.

For *L. kotschyanum*, the AIC also was used to determine the best fit between the G–G function, a linear model, and nonlinear logarithmic fits. When a linear fit was chosen, ANCOVAs were used to test for an effect of pCO_2 and time on the calcification–temperature relationships.

Results

Treatment conditions

For the experiments completed in spring 2014 and summer 2015, mean pCO_2 differed between pCO_2 treatments ($p < 0.001$), and there was no difference between duplicate tanks within each pCO_2 treatment ($p = 0.131$ and $p < 0.312$, respectively; Tables 1, 2). Mean temperature differed between temperature treatments ($p < 0.001$ in both cases), and there was no difference between duplicate tanks within each temperature treatment ($p = 0.266$ and $p = 0.734$).

Acropora pulchra

Calcification of *A. pulchra* was affected by temperature ($p < 0.001$; Table 3), pCO_2 treatment ($p = 0.001$; Table 3), time ($p < 0.001$; Table 3), and by the temperature × time interaction ($p < 0.001$).

In spring, maximum calcification was 1.29 ± 0.08 mg $CaCO_3$ cm^{-2} d^{-1} (mean ± SE, $n = 10$), and this was measured under ambient pCO_2 at 28.5 °C. Under high pCO_2, maximum calcification was lower than at ambient pCO_2 (1.08 ± 0.05 mg $CaCO_3$ cm^{-2} d^{-1}) and also occurred at 28.5 °C (Fig. 1a). There were no differences in α and β (Table 4) between pCO_2 treatments (*t* test, $p = 0.509$ and $p = 0.902$, respectively). T_m (the temperature at which calcification is maximal) was not affected by pCO_2 ($p = 0.595$) and was 27.42 ± 0.44 °C in the ambient treatment and 27.97 ± 0.94 °C in the high pCO_2 treatment. At these T_m values, M (the maximum calcification rate in the G–G function) was affected by pCO_2 ($p < 0.001$) and was 1.33 ± 0.06 mg $CaCO_3$ cm^{-2} d^{-1} at ambient pCO_2, and 1.08 ± 0.05 mg $CaCO_3$ cm^{-2} d^{-1} at high pCO_2.

During summer, maximum calcification was 1.11 ± 0.09 mg $CaCO_3$ cm^{-2} d^{-1} (mean ± SE, $n = 10$) in ambient pCO_2 at 28.5 °C, and 0.97 ± 0.08 mg $CaCO_3$ cm^{-2} d^{-1} at high pCO_2 at 28.5 °C (Fig. 1b). There were no differences in α and β (Table 4) between pCO_2 treatments (*t* test, $p = 0.950$ and $p = 0.733$). Mean T_m was 28.41 ± 0.80 °C in ambient

Table 1 Mean (±SE, $N = 21$) temperature and carbonate chemistry in ten incubation tanks during a three-week incubation in austral spring (September–October 2014)

Tank	Treatment	pCO_2 (µatm)	A_T	pH_T	Ω_{arag}	Temperature (°C)
1	24.0-Amb	330 ± 8	2329 ± 1	8.11 ± 0.01	3.79 ± 0.06	24.08 ± 0.01
2	24.0-OA	929 ± 39	2328 ± 1	7.74 ± 0.02	1.89 ± 0.07	24.01 ± 0.04
3	25.5-Amb	344.4 ± 8	2324 ± 2	8.10 ± 0.01	3.83 ± 0.05	25.42 ± 0.03
4	25.5-OA	939 ± 28	2326 ± 2	7.73 ± 0.01	1.95 ± 0.04	25.52 ± 0.03
5	27.0-Amb	359 ± 9	2325 ± 2	8.08 ± 0.01	3.93 ± 0.06	27.13 ± 0.05
6	27.0-OA	992 ± 31	2327 ± 1	7.71 ± 0.01	1.99 ± 0.05	27.03 ± 0.07
7	28.5-Amb	386 ± 7	2324 ± 2	8.05 ± 0.01	3.90 ± 0.05	28.37 ± 0.06
8	28.5-OA	1006 ± 32	2326 ± 2	7.71 ± 0.02	2.07 ± 0.05	28.49 ± 0.05
9	30.0-Amb	396 ± 8	2322 ± 2	8.04 ± 0.01	4.02 ± 0.05	29.89 ± 0.06
10	30.0-OA	991 ± 40	2326 ± 4	7.71 ± 0.02	2.21 ± 0.07	29.85 ± 0.07

Organisms were incubated under six (24–31.5 °C) temperatures crossed with ambient (Amb = 400 µatm) and high (OA = 1000 µatm) pCO_2 levels. Measured pH_T, total alkalinity (A_T), temperature, and salinity ($S = 36.3$ during the whole incubation) were used to calculate pCO_2, and the saturation state of aragonite (Ω_{arag}) and calcite (Ω_{calc}) using the R package Seacarb (Gattuso et al. 2015)

Table 2 Temperature and carbonate chemistry treatments in 12 incubation tanks during austral summer (January–February 2015)

Tank	Treatment	pCO_2 (µatm)	A_T	pH_T	Ω_{arag}	Temperature (°C)
1	24.0-Amb	326 ± 7	2281 ± 3	8.11 ± 0.01	3.67 ± 0.04	23.99 ± 0.06
2	24.0-OA	990 ± 19	2284 ± 3	7.70 ± 0.01	1.70 ± 0.02	24.00 ± 0.08
3	25.5-Amb	348 ± 7	2283 ± 2	7.72 ± 0.01	3.69 ± 0.04	25.41 ± 0.04
4	25.5-OA	974 ± 18	2287 ± 2	7.71 ± 0.01	1.82 ± 0.03	25.46 ± 0.07
5	27.0-Amb	374 ± 8	2282 ± 2	8.06 ± 0.01	3.71 ± 0.04	26.97 ± 0.06
6	27.0-OA	941 ± 12	2281 ± 2	7.72 ± 0.01	1.97 ± 0.02	27.01 ± 0.06
7	28.5-Amb	388 ± 6	2284 ± 2	8.05 ± 0.01	3.79 ± 0.03	28.42 ± 0.07
8	28.5-OA	963 ± 12	2281 ± 3	7.71 ± 0.00	2.05 ± 0.02	28.55 ± 0.06
9	30.0-Amb	420 ± 7	2280 ± 3	8.02 ± 0.01	3.77 ± 0.04	29.87 ± 0.09
10	30.0-OA	960 ± 8	2283 ± 4	7.72 ± 0.00	2.16 ± 0.01	29.88 ± 0.04
11	31.5-Amb	466 ± 10	2284 ± 3	7.98 ± 0.01	3.72 ± 0.05	31.33 ± 0.06
12	31.5-OA	984 ± 13	2289 ± 2	7.71 ± 0.01	2.24 ± 0.02	31.25 ± 0.05

Organisms were incubated under six (24–31.5 °C) temperatures crossed with ambient (Amb = 400 µatm) and high (OA = 1000 µatm) pCO_2 levels. Measured pH_T, total alkalinity (A_T), temperature, and salinity ($S = 35.7$ during the whole incubation) were used to calculate pCO_2, and the saturation state of aragonite (Ω_{arag}) and calcite (Ω_{calc}) using the R package Seacarb (Gattuso et al. 2015). All the values shown are mean ± SE ($N = 21$)

pCO_2, and 27.99 ± 0.69 °C in the high pCO_2 (Table 4), but was not affected by pCO_2 ($p = 0.691$). M was 1.01 ± 0.05 mg $CaCO_3$ cm^{-2} d^{-1} at ambient pCO_2, and 0.88 ± 0.06 mg $CaCO_3$ cm^{-2} d^{-1} at high pCO_2, but this difference was not significant ($p = 0.121$).

Comparison of the temperature–calcification response between the two times shows that under ambient pCO_2, maximal calcification differed between times and was higher during spring than in summer (t test, $p < 0.001$), but α, β, and T_m did not differ between times ($p = 0.349$, $p = 0.778$, and $p = 0.307$, respectively). Under high pCO_2, maximum calcification differed between times ($p = 0.014$), but α, β, and T_m did not ($p = 0.639$, $p = 0.666$, and $p = 0.986$, respectively).

Lithophyllum kotschyanum

For the alga, a three-way ANOVA showed that pCO_2 and time affected calcification ($p < 0.001$ in both cases; Table 3). The interaction of temperature and time was not significant ($p = 0.093$), despite a trend toward a positive effect of temperature during spring and a negative effect during summer (Fig. 2a, b).

During the spring, maximum calcification occurred at 30.0 °C under both pCO_2 conditions, while the minimum calcification rates were measured at 24.0 °C under ambient pCO_2, and at 25.5 °C under elevated pCO_2 (Fig. 2a). The AIC test showed that a linear fit best represented the

Table 3 Results of a three-way ANOVA used to test the effects of temperature, pCO_2, and time (spring vs. summer) on calcification of the coral *Acropora pulchra* and the alga *Lithophyllum kotschyanum*

Taxon	Effect	*df*	SS	*F* values	*p* values
A. pulchra	Temperature	4	4.787	17.584	<0.001
	pCO_2	1	0.706	10.374	0.001
	Time	1	0.880	12.930	<0.001
	Temperature × pCO_2	4	0.357	1.312	0.267
	Temperature × time	4	1.407	5.169	<0.001
	pCO_2 × time	1	0.005	0.078	0.780
	Temperature × time × pCO_2	4	0.420	1.541	0.192
	Residuals	196	13.204		
L. kotschyanum	Temperature	4	0.087	0.542	0.705
	pCO_2	1	0.487	12.094	<0.001
	Time	1	0.720	17.884	<0.001
	Temperature × pCO_2	4	0.006	0.034	0.997
	Temperature × time	4	0.325	2.017	0.093
	pCO_2 × time	1	0.000	0.000	0.985
	Temperature × time × pCO_2	4	0.033	0.205	0.935
	Residuals	196	7.890		

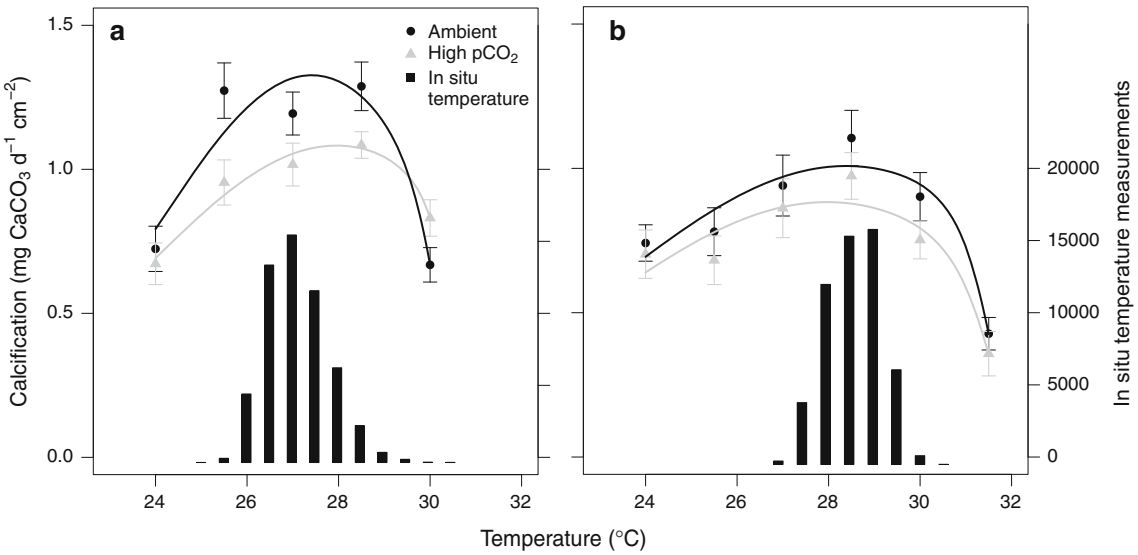

Fig. 1 Calcification rates of the coral *Acropora pulchra* measured at two pCO_2 levels and five or six temperatures during **a** austral spring (September–October 2014) and **b** austral summer (January–February 2015). *Black dots* represent calcification rates (mean ± SE, $n = 10$) under ambient pCO_2 level (~400 µatm), and *gray triangles* are calcification rates measured under elevated pCO_2 level (~1000 µatm). Gaussian–Gompertz relationships were used to fit the nonlinear response of calcification to temperature. The *black columns* show the mean distribution of temperatures recorded close to the collection site over the last 8 yr during August, September, and October for the spring, and January, February, and March for the summer. Temperature was recorded in situ at 0.833 mHz

relationship between calcification and temperature, but calcification did not vary significantly with temperature under ambient (slope = 0.013 mg $CaCO_3$ cm^{-2} d^{-1} $°C^{-1}$; $p = 0.196$) or elevated pCO_2 (slope = 0.007 mg $CaCO_3$ cm^{-2} d^{-1} $°C^{-1}$; $p = 0.499$).

During the summer, calcification was greatest at 25.5 °C under both pCO_2 regimes, and lowest at 31.5 °C under both pCO_2 regimes. In both pCO_2 treatments, calcification decreased with temperature at both ambient (slope = −0.039 mg $CaCO_3$ cm^{-2} d^{-1} $°C^{-1}$, $p < 0.001$) and elevated pCO_2 (slope = −0.023 mg $CaCO_3$ cm^{-2} d^{-1} $°C^{-1}$, $p = 0.006$). Overall, neither the slopes nor the elevations of the regression lines differed between pCO_2 treatments (ANCOVA, slopes: $p = 0.710$, and elevations: $p = 0.081$).

Table 4 Parameters of the Gaussian–Gompertz curves that fit the relationship between calcification and temperature in *Acropora pulchra*

Time	Parameter	Ambient values	OA values	*p* values
Spring	M	1.33 ± 0.06	1.08 ± 0.05	0.003
Spring	α	0.04 ± 0.02	0.03 ± 0.02	0.509
Spring	β	2.74 ± 0.66	2.99 ± 2.01	0.902
Spring	T_m	27.42 ± 0.44	27.97 ± 0.94	0.595
Summer	M	1.01 ± 0.05	0.88 ± 0.06	0.121
Summer	α	0.02 ± 0.01	0.02 ± 0.01	0.950
Summer	β	2.46 ± 0.72	2.16 ± 0.52	0.733
Summer	T_m	28.41 ± 0.80	27.99 ± 0.69	0.691

Parameters (mean ± SD) are given for the spring (October 2014) and summer (February 2015) experiments. M is the maximum calcification (mg $CaCO_3$ cm^{-2} d^{-1}), α is the ascending slope to the left of the thermal optimum and β is the descending slope (mg $CaCO_3$ cm^{-2} d^{-1} $°C^{-1}$), and T_m is the temperature at which calcification is maximal (°C). p values show the results of the t tests applied on each parameter of the curves between ambient and OA

Discussion

Acropora pulchra

Calcification in this coral followed a Gaussian–Gompertz (G–G) relationship as a function of temperature for both times and under both pCO_2 conditions. During spring, only the maximum calcification was affected by pCO_2, while during summer none of the parameters of the G–G curves were affected by pCO_2. These results indicate that OA does not affect the manner in which coral calcification responds to temperature in either spring or summer at this location. Interestingly, pCO_2 did not affect the optimal temperature for calcification or the slope of either the upward or the inhibitory portions of the relationship with temperature, which suggests that there is no interactive effect of elevated temperature and OA on calcification. The absence of an interactive effect of temperature and pCO_2 on calcification in this coral is similar to the results of studies in which corals have been exposed to a limited number of temperature treatments. For example, no interactive effect was reported for massive *Porites* and *P. rus* exposed to two pCO_2 levels (~416 and 815 µatm) at 25.6 and 29.3 °C (Edmunds et al. 2012), or *P. lobata* and *A. intermedia* incubated under three pCO_2 levels (~300, 600, and 1200 µatm) and two temperature treatments (~25.5 and 28.5 °C; Anthony et al. 2008). In contrast, when incubated at 25 °C, OA did not affect *Stylophora pistillata*, but in a warm treatment in which temperature was increased to 28 °C, temperature and OA acted in synergy to reduce calcification by 50 % (Reynaud et al. 2003).

Variable responses to OA and temperature have also been reported in four other corals (*A. millepora*, *Pocillopora damicornis*, *Montipora monasteriata*, and *Turbinaria reniformis*), with positive, negative, or neutral crossed effects of temperature and OA (Schoepf et al. 2013). While these studies seem to produce results that differ from those

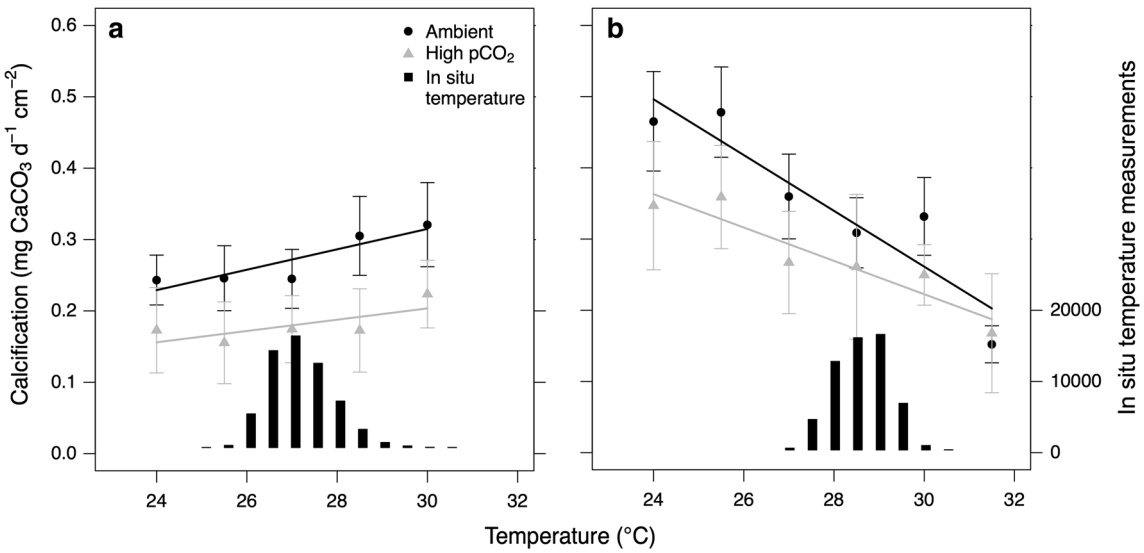

Fig. 2 Calcification rates of the alga *Lithophyllum kotschyanum* measured at two pCO_2 levels and five or six temperatures during **a** austral spring (September–October 2014) and **b** austral summer (January–February 2015). *Black dots* represent calcification rates (mean ± SE, $n = 10$) under ambient pCO_2 level (~400 µatm), and *gray triangles* are calcification rates measured under elevated pCO_2 level (~1000 µatm). Linear relationships were used to fit the response of calcification to temperature. The *black columns* show the mean distribution of temperatures recorded close to the collection site over the last 8 yr during August, September, and October for the austral spring, and January, February, and March for the austral summer. Temperature was recorded in situ at 0.833 mHz

presented here, our experiments suggest a way to reconcile these discrepancies. Notably, it is likely that both the narrow range of temperatures and the number of temperature treatments (usually two) in previous studies did not capture the complexity of the response of calcification to temperature as revealed by the best fit of a G–G curve in the present study. For example, the comparison of calcification rates of *A. pulchra* at 24 and 30 °C in this study would have led to the conclusion that OA and temperature do not affect calcification, while the comparison of rates at 24 and 28 °C would have led to contradictory results (i.e., stronger effect of OA at elevated temperature).

The effects of time (spring vs. summer) on the shape of calcification–temperature relationships were weak, but time had significant effects on maximum calcification under both ambient and elevated pCO_2; maximum calcification was higher in the spring than in the summer. Higher calcification rates in the spring could reflect seasonal variation in biomass, tissue energy reserves, and *Symbiodinium* density (Fagoonee et al. 1999; Fitt et al. 2000; Thornhill et al. 2011), which could have translated to variation in the allocation of metabolic resources to calcification. Seasonal differences in the calcification rates of corals have previously been demonstrated in Moorea for *A. pulchra*, which exhibited higher rates of calcification during spring (1.41 ± 0.08 mg $CaCO_3$ d^{-1} cm^{-2} at 400 µatm pCO_2 and 27 °C; Comeau et al. 2013) versus summer (1.02 ± 0.05 mg $CaCO_3$ d^{-1} cm^{-2} at 400 µatm pCO_2 and 27 °C; Comeau et al. 2014a). Seasonal differences in area-normalized calcification were accompanied by variation in biomass, which was 33 % lower in the summer compared to spring (Comeau et al. 2013, 2014a). Biomass was not measured in the present study, but the decrease in area-normalized calcification in summer could have resulted from declining biomass and constant biomass-normalized rates of calcification.

Despite the variation in the maximum rate of calcification between spring and summer, there was no shift in the temperature at which the maximum rate was attained at either pCO_2. This result differs from previous studies with corals that have shown an increase in the thermal optimum for calcification during summer (Jokiel and Coles 1977), or an increase in the thermal optimum for corals inhabiting regions exposed to higher temperature (Coles et al. 1976; Coles and Jokiel 1977). The discrepancy between our results (which show no shift in optimum temperature between spring and summer) and previous studies may be explained by two nonexclusive hypotheses.

First, it is possible that the corals in Moorea did not adjust their thermal optimum for calcification because seawater temperatures in this habitat do not vary greatly between spring and summer, which were the periods to which logistical constraints limited our study. For example, over the last 8 yr, the mean daily temperature of seawater in the fringing reef was 27.1 ± 0.7 °C (SD, $n = 728$) during austral spring, and it was only 1.5 °C warmer in the austral summer (28.6 ± 0.5 °C, $n = 722$; Leichter 2012). In the present study, the temperatures at which calcification was maximized in *A. pulchra* were 27.7 and 28.2 °C (for spring and summer, respectively, pooled between pCO_2 treatments), which fall within the range of mean seawater temperatures typically occurring in spring and summer on the fringing reefs of Moorea. Second, specimens of *A. pulchra* were collected from shallow fringing reefs (≤2-m depth) where they are exposed on a daily basis to a relatively wide range of seawater temperatures. Mean diel variation in seawater temperature in this habitat is $\sim 0.7 \pm 0.3$ °C (SD, $n = 728$) during the spring, whereas daily variation in the temperature of offshore seawater is $\sim 0.4 \pm 0.2$ °C (SD, $n = 728$; Leichter 2012). Therefore, it is likely that corals on this fringing reef, where daily variation in seawater temperature can exceed the variation in mean seawater temperature between spring and summer, exhibit smaller changes in their thermal optima between these periods than might occur in corals inhabiting more thermally stable environments such as the outer reef habitat of Moorea.

For spring and summer, differences between calcification rates in ambient versus elevated pCO_2 were maximal when temperatures were close to optimal temperatures for this physiological process. In contrast, at low (~24 °C) or elevated (~30 °C) temperatures, calcification was similar in both pCO_2 treatments. These results may have ecological importance in suggesting that under cool conditions, when calcification is reduced, the negative effects of pCO_2 (at least at ~1000 µatm) are reduced. While it has been suggested that species-specific sensitivity to OA in corals can be determined by the rate of calcification (Comeau et al. 2014b), the present results indicate that the reduced calcification rates occurring as a result of low temperature can also mask the effects of OA. Increased sensitivity to OA in corals with high rates of calcification is potentially due to the necessity of exporting large quantities of protons [H^+] from the site of calcification. This requirement arises from the need to maintain a chemical microenvironment conducive to mineralization through high pH (>8.6; Venn et al. 2013) and elevated $CaCO_3$ saturation state (Ω; >15; Cohen et al. 2009; McCulloch et al. 2012). With OA, the concentration of protons in seawater increases, which steepens the concentration gradient of this cation between the site of calcification within the coral and the surrounding seawater. As a result, the energy required to transport protons up this gradient would increase the metabolic costs of calcification (Ries 2011). Corals that calcify more slowly, either because of an intrinsic feature of the taxon (Comeau et al. 2014b), or because of thermally mediated

effects (this study), have a reduced rate of proton production in conjunction with their reduced $CaCO_3$ deposition, and therefore are hypothesized to be less sensitive to OA.

Lithophyllum kotschyanum

Calcification in the alga *L. kotschyanum* decreased with temperature during the summer, while there was no effect of temperature in spring despite a trend toward an increase with temperature. The linearity of the response to temperature, or the absence of an effect of temperature, appears inconsistent with the well-known and fundamental effects of temperature on poikilotherms (Martin and Huey 2008). However, the linear calcification–temperature response of *L. kotschyanum* may have reflected the physiological consequences of the thermal regime from which they were collected, as well as the range of temperature treatments employed in the study. For example, exposure to the seasonal and diel fluctuations in seawater temperature characteristic of the fringing reef of Moorea (Leichter 2012) could have favored the development of a strong capacity for thermal acclimatization in *L. kotschyanum*, which would dampen the apparent effects of temperature on calcification. Interestingly, however, if such a mechanism was important for *L. kotschyanum*, similar effects appeared not to be well developed in *A. pulchra* growing in the same habitat. Perhaps, exposure to a wider range of experimental temperatures would have revealed inflection points in the calcification–temperature response of *L. kotschyanum*, as observed for *A. pulchra*.

Previous studies on the physiological responses of macroalgae to temperature have revealed a broad range of optimal temperatures. For example, *Asparagopsis taxiformis* from California exhibited constant rates of net photosynthesis at temperatures between 14 and 21 °C, which corresponded to the annual temperature range at the collection site (Padilla-Gamiño and Carpenter 2007a). Over a larger range of temperatures, photosynthetic efficiency of *A. taxiformis* varied <25 % between 10 and 35 °C. In Hawaii, where the same alga inhabits an environment where annual temperatures vary from 24 to 27 °C, net photosynthesis was constant between 20 and 30 °C (Padilla-Gamiño and Carpenter 2007a). In the case of *A. taxiformis* from Hawaii, detection of the inflection point in the relationship of net photosynthesis with temperature was possible only over a large range of temperatures (10–35 °C) that exceeded the natural range of seawater temperatures experienced in situ. A similar trend was found for the algae *Laurencia pacifica* and *L. nidifica* (from California and Hawaii, respectively) that maintained constant rates of net photosynthesis and respiration over a range of temperatures that exceeded the annual in situ seawater temperatures (Padilla-Gamiño and Carpenter 2007b). The effects of temperature on calcification of coralline algae are not well known, but they have been reported for the cold-water species *Phymatolithon calcareum* (King and Schramm 1982); in this alga, calcification increased linearly from 10 to 24 μg $CaCO_3$ g^{-1} h^{-1} between 0 and 20 °C. While our study reveals a linear relationship for the effect of calcification on temperature for *Lithophyllum kotschyanum*, the reduction in calcification at 31.5 °C suggests this temperature may be close to the thermal maximum for calcification in this species.

Temperature and OA did not have interactive effects on the calcification of *L. kotschyanum* during spring or summer. Results from previous studies suggest the response of coralline algae to the crossed effects of temperature and pCO_2 is species-specific. For instance, Anthony et al. (2008) described synergistic effects of pCO_2 and warming on calcification, net productivity, and bleaching of *Porolithon onkodes*. Likewise, the temperate coralline alga *L. cabiochae* was unaffected by elevated temperature (ambient + 3 °C) and pCO_2 (700 μatm) in isolation, but showed a 50 % reduction in net calcification when exposed to a combination of these two parameters, but only during summer (Martin and Gattuso 2009). In contrast, the diel calcification rates of the temperate coralline alga *Lithothamnion corallioides* were negatively affected by pCO_2 (ranging from 380 to 1000 μatm), but were unaffected by the interaction of pCO_2 with temperature (10–19 °C; Noisette et al. 2013). We have also shown that calcification of the tropical coralline alga *Hydrolithon reinboldii* was unaffected by the crossed effects of pCO_2 with temperature and light (Comeau et al. 2014a). In contrast to our results for corals, the present results for *Lithophyllum kotschyanum* do not offer a way to reconcile these disparate results, which likely indicates that the response of coralline algae to temperature is species-specific.

This study demonstrates the complexity of the relationships between calcification in reef organisms and two environmental factors, pCO_2 and temperature. An experimental approach using a large range of temperatures allowed us to clearly define the crossed effects of temperature and OA on *A. pulchra*. The response of *L. kotschyanum* to temperature was not curvilinear despite being based on a range of temperatures representing in situ seawater temperature in Moorea. Overall, our study suggests that the interaction between pCO_2 and temperature is neither additive nor synergistic for the two taxa tested, and that the effects of OA on *A. pulchra* are strongest under optimal thermal conditions.

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