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Pacific-wide contrast highlights resistance of reef calcifiers to ocean acidification

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Ocean acidification (OA) and its associated decline in calcium carbonate saturation states is one of the major threats that tropical coral reefs face this century. Previous studies of the effect of OA on coral reef calcifiers have described a wide variety of outcomes for studies using comparable partial pressure of CO₂ (*p*CO₂) ranges, suggesting that key questions remain unresolved. One unresolved hypothesis posits that heterogeneity in the response of reef calcifiers to high *p*CO₂ is a result of regional-scale variation in the responses to OA. To test this hypothesis, we incubated two coral taxa (*Pocillopora damicornis* and massive *Porites*) and two calcified algae (*Porolithon onkodes* and *Halimeda macroloba*) under 400, 700 and 1000 μ atm *p*CO₂ levels in experiments in Moorea (French Polynesia), Hawaii (USA) and Okinawa (Japan), where environmental conditions differ. Both corals and *H. macroloba* were insensitive to OA at all three locations, while the effects of OA on *P. onkodes* were location-specific. In Moorea and Hawaii, calcification of *P. onkodes* was depressed by high *p*CO₂, but for specimens in Okinawa, there was no effect of OA. Using a study of large geographical scale, we show that resistance to OA of some reef species is a constitutive character expressed across the Pacific.

1. Introduction

The oceans have absorbed approximately 30% of the CO₂ released by anthropogenic activities since the eighteenth century, and therefore represent a major sink for atmospheric CO₂ [1]. In turn, the dissolution of CO₂ leads to modification of the seawater carbonate chemistry, which includes an increase in bicarbonate ions and a decrease in carbonate ion concentration, causing a decrease in pH in a process known as ocean acidification (OA) [2]. Decreasing carbonate ion concentration leads to a decrease in calcium carbonate saturation state (Ω), which is the product of the concentration of calcium and carbonate ions divided by the stoichiometric solubility product for calcium carbonate (aragonite, calcite or high-magnesium calcite, depending on which crystal form is deposited by the organism of interest). Of the three crystal forms of calcium carbonate, calcite is the least soluble, followed by aragonite, whereas the solubility of high-magnesium calcite is linked to the magnesium content (i.e. skeletal solubility increases with magnesium content). Over the last decade, numerous studies have shown that OA can have negative effects on many marine organisms, and particularly on those that calcify (reviewed in [3,4]).

Most empirical studies of the effects of OA on coral reefs have reported a positive correlation between calcification rates and aragonite saturation state (Ω_{arag}) [5], supporting pessimistic projections forecasting the disappearance of most coral reefs before the end of the current century [6–8]. However, recent studies now indicate more nuanced responses to OA for select reef calcifiers [9], with a compilation of laboratory studies of corals suggesting that coral calcification will decline approximately 10–20% (rather than ceasing) for a doubling of present-day partial pressure of CO₂ (*p*CO₂) [10]. More subtle responses to OA

Table 1. Parameters of the carbonate chemistry during incubations in Moorea, Hawaii and Okinawa, and range of carbonate chemistry conditions at the study sites. (The partial pressure of CO₂ ($p\text{CO}_2$), and the saturation states of aragonite (Ω_{arag}) and calcite (Ω_{calc}) were calculated from pH_T , total alkalinity (A_T), salinity (S) and temperature (T) using the R package seacarb. Experimental values correspond to mean \pm s.e. ($n = 56$). s.e. for the experimental pH_T in Moorea as well as temperature and salinity at the three locations were less than 0.01. Ranges of field values were estimated using MCR-LTER data [31] and personal observations in Moorea, data available in the literature [30,32] for Hawaii, and field $p\text{CO}_2$ observations using a portable $p\text{CO}_2$ system modified from [33] and environmental parameters from [34] for Okinawa.)

location	targeted $p\text{CO}_2/\text{field}$	pH_T	A_T ($\mu\text{mol kg}^{-1}$)	$p\text{CO}_2$ (μatm)	Ω_{arag}	Ω_{calc}	T ($^\circ\text{C}$)	S
Moorea	400	8.05	2323 ± 10	391 ± 3	3.73 ± 0.02	5.61 ± 0.02	27.1	36.2
	700	7.83	2320 ± 9	726 ± 8	2.50 ± 0.02	3.76 ± 0.03	27.1	36.2
	1000	7.71	2320 ± 11	993 ± 11	1.99 ± 0.02	2.99 ± 0.02	27.0	36.2
	field	8.02–8.09	2345–2385	347–437	3.5–4.1	5.2–6.2	26–30	35.8–36.4
Hawaii	400	8.01 ± 0.01	2207 ± 1	430 ± 11	3.32 ± 0.06	5.00 ± 0.09	27.0	35.4
	700	7.83 ± 0.01	2210 ± 1	696 ± 15	2.39 ± 0.04	3.59 ± 0.06	27.0	35.5
	1000	7.71 ± 0.01	2215 ± 1	959 ± 16	1.87 ± 0.03	2.81 ± 0.04	26.9	35.5
	field	7.90–8.10	2170–2260	400–620	2.7–3.3	4.0–4.9	22–28	33.8–35.2
Okinawa	400	7.99 ± 0.02	2216 ± 2	449 ± 25	3.20 ± 0.08	4.82 ± 0.12	26.9	35.5
	700	7.84 ± 0.01	2219 ± 2	679 ± 24	2.41 ± 0.06	3.63 ± 0.10	26.9	35.5
	1000	7.73 ± 0.02	2216 ± 2	921 ± 37	1.95 ± 0.08	2.94 ± 0.11	26.9	35.5
	field	7.93–8.15	2170–2240	280–500	2.4–4.3	3.7–6.5	22–28	35.2–35.5

have also been shown in recent studies reporting signs of resistance to OA for some reef calcifiers [11–13]. Field observations at underwater CO₂ vents in Papua New Guinea and sites with high seawater $p\text{CO}_2$ in Palau have also shown that some reef calcifiers can persist in naturally acidified conditions [14,15]. For calcifying algae, the responses to increasing $p\text{CO}_2$ are diverse, with some studies reporting a decrease in calcification and an increase in bleaching in acidified conditions [16,17], and others detecting resistance to OA [12,18]. The mechanistic basis of the differing responses to OA exhibited by multiple species of corals and calcified algae remains unknown, although several causes have been proposed. These include resistance to OA mediated by strong control of pH at the site of calcification in corals [19,20], the use of bicarbonate ions in the light by both corals and algae [21,22] or the use of corallum morphology to enhance mass-transfer-mediated efflux of protons from the site of calcification to the surrounding seawater [23]. Independent of the underlying mechanism, it now seems clear that at the individual scale, the responses of reef calcifiers to OA are not described effectively by a universal functional relationship between calcification and Ω .

Tropical coral reefs have developed in regions where the mean Ω_{arag} generally is more than 3.4 [24], although there is large variation in Ω_{arag} among locations and on diel basis. At present, coral reefs are located in waters with Ω_{arag} ranging from 2.8 to 4.2, but model projections describing the chemistry of seawater in future seas suggest that the proportion of reefs located in regions with $\Omega_{\text{arag}} > 3.0$ will decline to 50% when atmospheric $p\text{CO}_2$ reaches 550 μatm , and 0% when it reaches 900 μatm [25]. While geographical disparities in the response of tropical corals to thermal stress have been demonstrated [26], there have been no broad spatial comparisons of the response of tropical corals and calcified algae to OA. The effects of OA on coral reefs have been tested in experiments at laboratories around the world, but explicit syntheses of the results are complex given the diversity of treatments employed (i.e. $p\text{CO}_2$ levels) and the differing

ancillary incubation conditions (e.g. light intensity, flow, etc.) [10,27]. These problems can be reduced by conducting identical OA experiments at multiple locations, but such work remains technically challenging, not least because the environmental conditions to which organisms have been exposed (i.e. saturation states, temperature) on the reef prior to the experiment also are likely to vary among locations, and potentially can confound the contrast of interest [28,29]. In this study, we compared the response of reef calcifiers to OA at multiple locations on a regional scale and used among-location differences in physical conditions to our advantage. Incubations were performed in Moorea (French Polynesia) where Ω_{arag} varies seasonally from approximately 3.5 to 4.0 (S. Comeau 2013, unpublished data), in Hawaii where Ω_{arag} varies from 2.7 to 3.3 [30], and in Okinawa (Japan) where Ω_{arag} varies from 2.4 to 4.3 (table 1).

To test for contrasting responses to OA over a regional scale, we measured the response to OA of four calcifiers that are common on coral reefs throughout the Pacific. Incubations were conducted in three locations using two coral taxa (massive *Porites* spp. and *Pocillopora damicornis*) and two species of calcified algae (*Porolithon onkodes* and *Halimeda macroloba*) (figure 1). We chose locations exhibiting dissimilar seawater dissolved inorganic carbon (DIC) chemistry to examine effects of geographical origin on the response to OA. Net calcification was quantified at the three locations, and close attention was paid to employing similar experimental protocols employing standardized irradiance (ca 700 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$), seawater temperature (27 $^\circ\text{C}$) and $p\text{CO}_2$ levels (400, 700 and 1000 μatm).

2. Material and methods

(a) Study organisms, organism collection and experimental design

For both coral taxa, ongoing taxonomic revisions combined with the subtle skeletal features required to distinguish taxa made it

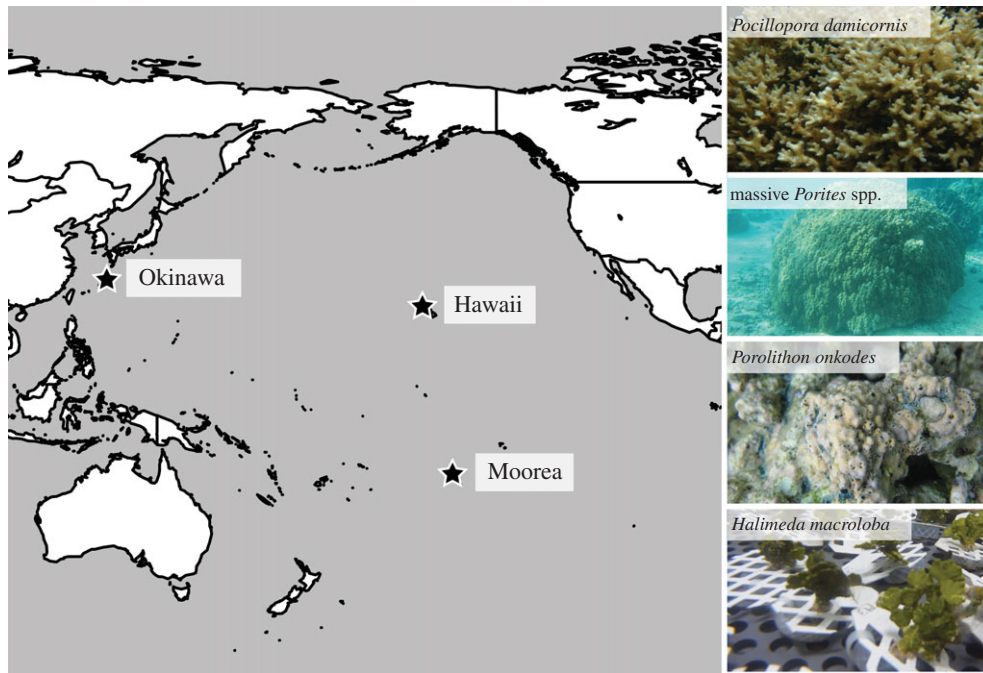


Figure 1. Map showing the study locations and photos of the four organisms investigated. Studies were performed under similar conditions of temperature (27°C), light (approx. $600\text{--}700\ \mu\text{mol quanta m}^{-2}\ \text{s}^{-1}$) and $p\text{CO}_2$ (400, 700 and $1000\ \mu\text{atm}$) in Moorea (French Polynesia), Okinawa (Japan) and Hawaii (USA), respectively. At the three locations, the response of calcification to $p\text{CO}_2$ was investigated in massive *Porites* spp., *P. onkodes* and *H. macroloba*.

unreliable to identify the study corals to species, and therefore they were considered as two functional groups. In the Pacific, massive *Porites* spp. consist of several species that are difficult to distinguish underwater [35]. Therefore, we considered these corals as members of one functional group; in Moorea, analyses of corallites (after [36]) indicated that 85% of these corals were *P. lutea* and 15% *P. lobata*. Similarly, recent genetic analyses of *P. damicornis* have detected variation throughout the Pacific that could reflect the presence of cryptic taxa [37,38].

Three out of the four study organisms precipitate calcium carbonate as aragonite (massive *Porites* spp., *P. damicornis* and *H. macroloba*) and one (*P. onkodes*) precipitates high-magnesium calcite. As calculation of the saturation state for high-magnesium calcite is challenging and depends on the proportion of magnesium in the skeleton, which was not measured in this study, we calculated saturation states only for aragonite and calcite, which is an overestimation of the actual high-magnesium calcite saturation state.

In Moorea, French Polynesia, the study was conducted in two different years at the Richard B. Gump South Pacific Research Station using organisms from approximately 1 to 2 m depth on the back reef of the north shore. Net calcification was measured in August–October 2011 for *P. damicornis*, *P. onkodes* and *H. macroloba* and in August–October 2012 for massive *Porites* spp. In Hawaii, the study was conducted in June 2012 at the Hawaii Institute of Marine Biology (HIMB) on Coconut Island. Organisms were collected from Kaneohe Bay, at 1–2 m depth for *P. damicornis* and *P. onkodes*, and 3–4 m depth for massive *Porites* spp. and *H. macroloba*. The experiment in Okinawa, Japan, was performed in May–June 2013 at the Sesoko Station, Tropical Biosphere Research Center, using specimens collected around Sesoko Island and at Bise (6 km northeast of Sesoko) at 0–3 m depth. In all experiments and for each organism, calcification was measured in duplicate trials [12] or duplicate tanks (for Hawaii and Okinawa). For each organism and at each location, 36 replicate pieces were collected, allowing for 12 replicates (six for each duplicate trial or tank) per organism for each of the three $p\text{CO}_2$ levels. While it would have been preferable to conduct the incubations during the same season using an identical protocol in all locations, this was not possible given constraints of time and logistics. In Hawaii and Okinawa, studies were performed at the end of

the spring–early summer (i.e. May/June), and at the end of the austral winter–early spring in Moorea (August/September). Similar irradiances, temperatures and $p\text{CO}_2$ levels were used at the three locations, and the organisms were allowed to recover prior to incubations to limit collection stress and reduce bias associated with recent history.

Following collection and attachment to plastic supports, samples remained in a seawater table to recover from preparation for 2 d before being transferred for acclimatization in a separate tank, or in the incubation tanks (for two weeks in Moorea, 10 days in Hawaii and 7 days in Okinawa). Acclimatization took place at 27°C and an irradiance of $600\text{--}700\ \mu\text{mol quanta m}^{-2}\ \text{s}^{-1}$, both of which are representative of the average temperature and light intensities encountered in the back reef of these locations. During acclimatization and incubation, 75 W Light Emitting Diode (LED) modules (Sol White LED Module; Aquaillumination) provided light on a 12 L : 12 D photoperiod. After acclimatization, 36 individuals of each organism were allocated randomly to the incubation tanks in which light and temperature were identical to those experienced during acclimatization (27°C and $600\text{--}700\ \mu\text{mol quanta m}^{-2}\ \text{s}^{-1}$). Positions of the organisms in all tanks were changed randomly every 2 days to eliminate position effects. Seawater, filtered through a sand filter, was replaced continuously in the acclimatization and incubation tanks at approximately $100\text{--}150\ \text{ml min}^{-1}$.

CO_2 treatments consisted of three $p\text{CO}_2$ levels corresponding to present-day conditions (approx. $400\ \mu\text{atm}$), an average value expected by the end of the century (approx. $700\ \mu\text{atm}$, representative concentration pathway (RCP) scenario 6.0, [39]) and a pessimistic value expected by the end of the twenty-first century (approx. $1000\ \mu\text{atm}$, RCP scenario 8.5) (table 1). Treatments were created by bubbling the tanks with CO_2 -manipulated air that was created with solenoid-controlled gas regulation systems (Model A352, Qubit Systems) in Moorea, mass-flow controllers (Model C100L, Sierra Instruments Inc.) in Hawaii and a direct $p\text{CO}_2$ control system in Okinawa (developed with the cooperation of Kimoto Electric Co., Ltd., Osaka, Japan). The flow of CO_2 -manipulated air and seawater was adjusted in each tank to correct for deviations from target $p\text{CO}_2$ detected by pH measurements made twice daily.

(b) Carbonate chemistry

At the three locations, pH was measured twice daily using a portable pH meter (Orion 3-stars) fitted with a DG 115-SC pH probe (Mettler) calibrated every other day with Tris/HCl buffers (A. Dickson, San Diego, CA, USA). In Moorea and in Hawaii, pH also was measured spectrophotometrically once a week using m-cresol dye [40]. The two methods provided similar results with a mean difference less than or equal to 0.009 pH. Analysis of total alkalinity (A_T) was conducted by open-cell potentiometric titrations using 50 ml samples of seawater collected every 2–3 days on the day of seawater sampling [40]. A_T was calculated using a modified Gran function applied to pH values ranging from 3.5 to 3.0 [40]. Parameters of the carbonate system in seawater were calculated from salinity, temperature, A_T and pH_T using the R package seacarb [41].

(c) Net calcification

To quantify net calcification, the difference between the initial and final buoyant weight [42] after 14 days of incubation was converted to dry weight using an aragonite density of 2.93 g cm^{-3} for corals and *H. macroloba*, and a calcite density of 2.71 g cm^{-3} for *P. onkodes*. Net calcification was normalized to surface area, obtained using the foil technique for corals, and image analysis (IMAGEJ, US NIH) of aerial photographs for *P. onkodes*. Net calcification was normalized to ash-free dry mass for *H. macroloba*, because determination of surface area was not possible for this species. For corals, biomass (tissue dry mass) was also used to normalize calcification to take into account the effects of $p\text{CO}_2$ on tissue growth. Biomass was not measured in *P. onkodes*, but previous observations have shown no effects of $p\text{CO}_2$ on tissue growth of this species (S. Comeau 2012, personal observation). To quantify biomass, corals were fixed in approximately 5% formalin in seawater, their skeletons dissolved in 5% HCl and the remaining formalin-fixed tissue dried for 24–48 h at 60°C prior to weighing ($\pm 1 \text{ mg}$).

(d) Statistical analysis

The assumptions of normality and equality of variance were evaluated through graphical analyses of residuals. All analyses were performed using R software (R Foundation for Statistical Computing). Differences in seawater carbonate chemistry within treatments between locations were compared with a two-way ANOVA, with treatment and sites as fixed effects. Area-normalized and biomass-normalized calcification rates were analysed using a three-way ANOVA, where treatments ($p\text{CO}_2$ levels) and locations were fixed effects, and duplicate tanks were a nested effect. When there was no statistical effect of the duplicate tanks ($p > 0.25$), the tank effect was dropped from the analysis. Data were deposited in the PANGAEA database (<http://doi.pangaea.de/10.1594/PANGAEA.832834>).

3. Results

(a) Treatment conditions

At the different locations, the chemical conditions within the tanks were regulated precisely (table 1). The $p\text{CO}_2$ levels did not differ among locations ($F_{2, 1494} = 2.67$, $p = 0.069$), but the Ω_{arag} did ($F_{2, 1494} = 68.17$, $p < 0.001$). Exploratory Tukey HSD post hoc analyses revealed that the aragonite saturation state was significantly higher in Moorea than in Hawaii ($p < 0.001$) and Okinawa ($p < 0.001$), but there was no difference between Hawaii and Okinawa ($p = 0.983$). Temperature was significantly different among locations ($p < 0.001$), although the magnitude of the effect was probably biologically trivial (means differing between locations by less than 0.2°C).

(b) Effects of ocean acidification on corals

For massive *Porites* spp., the maximum area-normalized net calcification was measured in Okinawa in the control treatment ($1.89 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ cm}^{-2}$) and the minimum in Moorea in the $1000 \mu\text{atm}$ treatment ($1.02 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ cm}^{-2}$) (figure 2a). For *P. damicornis*, maximum area-normalized net calcification rate was found in Okinawa in the control and at $1000 \mu\text{atm}$ ($1.18 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ cm}^{-2}$), and the minimum in Hawaii at $700 \mu\text{atm}$ ($0.53 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ cm}^{-2}$) (figure 2c).

For both massive *Porites* spp. and *P. damicornis*, there was no significant effect of $p\text{CO}_2$ on area-normalized net calcification ($p = 0.561$ and $p = 0.149$, respectively; table 2), but net calcification differed among locations ($p < 0.001$ for both species; table 2) with highest area-normalized net calcification among $p\text{CO}_2$ treatments in Okinawa compared with Moorea and Hawaii. Area-normalized calcification pooled among $p\text{CO}_2$ treatments was approximately 37% lower in Hawaii and Moorea compared with Okinawa for massive *Porites* spp., and approximately 45% and approximately 47% lower in Hawaii and Moorea compared with Okinawa for *P. damicornis*. Also there was no significant interaction between $p\text{CO}_2$ treatment and location on area-normalized calcification of both massive *Porites* spp. and *P. damicornis* ($p < 0.798$ and $p < 0.690$; table 2).

Biomass-normalized net calcification of both massive *Porites* spp. and *P. damicornis* was not significantly affected by $p\text{CO}_2$ ($p = 0.910$ and $p = 0.586$, respectively; table 2) or the interaction between $p\text{CO}_2$ and location ($p < 0.987$ and $p = 0.405$, respectively; table 2). However, biomass-normalized net calcification of massive *Porites* spp. differed among locations ($p < 0.001$; table 2), because of higher biomass-normalized calcification among $p\text{CO}_2$ treatments in Okinawa compared with Moorea and Hawaii (figure 2b,d). Biomass-normalized net calcification of massive *Porites* spp. was approximately 59% lower in Hawaii and Moorea compared with Okinawa and approximately 50% and approximately 57% lower in Hawaii and Moorea compared with Okinawa for *P. damicornis*.

(c) Effects of ocean acidification on algae

The algal species exhibited contrasting results compared with corals for the effects of OA among locations. For *P. onkodes*, area-normalized net calcification was highest in Hawaii in the control ($1.92 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ cm}^{-2}$), and lowest in Moorea at $700 \mu\text{atm}$ ($0.69 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ cm}^{-2}$). Area-normalized net calcification was affected by $p\text{CO}_2$ ($p = 0.024$; table 3) and location ($p < 0.001$; table 3 and figure 3a), but the interaction between these effects was not significant ($p = 0.148$; table 3 and figure 3a). The effect of $p\text{CO}_2$ was striking in Hawaii, where *P. onkodes* calcified faster than in the other locations under all three $p\text{CO}_2$ treatments.

For *H. macroloba*, biomass-normalized net calcification was highest in Okinawa at $700 \mu\text{atm}$ ($12.76 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ g}^{-1}$), and lowest in Moorea in the control ($9.36 \text{ mg CaCO}_3 \text{ d}^{-1} \text{ g}^{-1}$) (figure 3b). Biomass-normalized net calcification was unaffected by $p\text{CO}_2$ ($p = 0.900$), location ($p = 0.737$) or the interaction between the two ($p = 0.704$; table 3).

4. Discussion

This study tested the response to OA for four reef calcifiers from three locations characterized by dissimilar seawater DIC

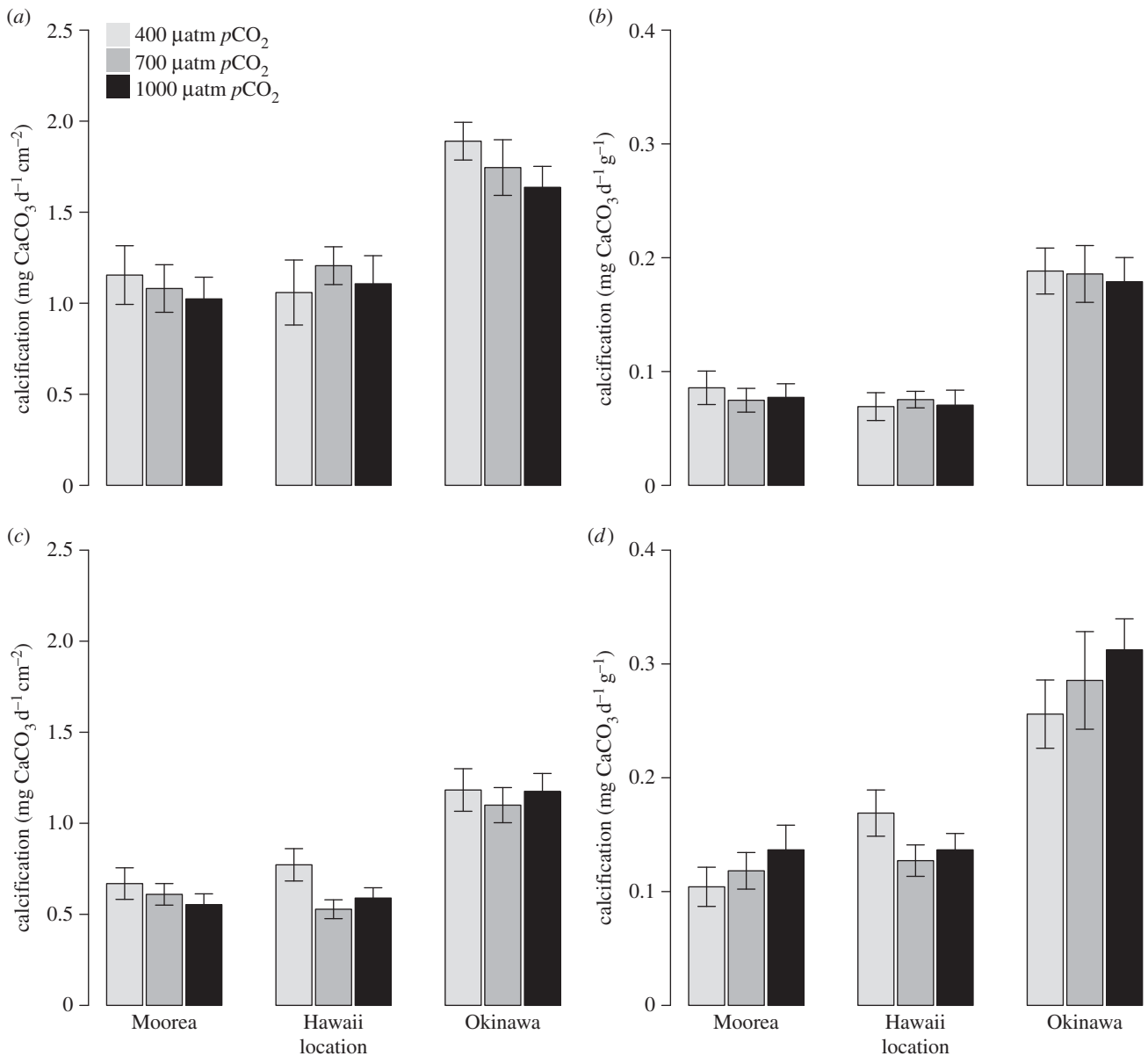


Figure 2. Calcification of the corals massive *Porites* spp. and *P. damicornis* maintained in three pCO₂ levels (400, 700 and 1000 μatm) in Moorea, Okinawa and Hawaii, respectively. The first row represents: (a) the area-normalized calcification and (b) the biomass-normalized calcification of massive *Porites* spp. The second row shows: (c) the area-normalized calcification and (d) the biomass-normalized calcification of *P. damicornis*. The bars correspond to the mean calcification and the vertical error bars show the s.e. in the measurement of calcification ($n = 12$).

chemistry. In contrast to our hypothesis—that geographical origin, or local acclimatization, may drive dissimilar responses to OA—location was not related to the response to OA in either of the corals and in only one of the algae.

Our results confirm the more nuanced effects of OA that have been recorded for several tropical coral and algal taxa exposed in laboratory experiments to elevated pCO₂ [12,27], as well as for temperate corals and molluscs transplanted into naturally acidified waters [43]. Moreover, they suggest that a linear relationship between calcification and Ω_{arag} for corals [5] is unlikely to be universal for the diversity of organisms responsible for reef construction, although this does not exclude the possibility that it applies at a whole reef scale [24]. Organisms from Moorea, where Ω_{arag} remains high year-round (approx. 4.0), were no more affected by OA than organisms from regions where Ω_{arag} is seasonally low (as in Kaneohe Bay, Hawaii [30] and Okinawa (table 1)). In Hawaii, Ω_{arag} is depressed to approximately 2.7 in winter because of cold seawater (22–23°C) and high pCO₂ caused

by calcification and long residence times of seawater over the reef (at least in Kaneohe Bay [30]). Similarly, low winter temperature in Okinawa [44] leads to low saturation states (i.e. Ω_{arag} 2.4; table 1). Our results suggest that responses to winter conditions, perhaps through seasonal acclimatization (*sensu* [45]) or perhaps by local adaptation to low Ω_{arag} [15], does not appear to drive the response of these reef calcifiers to OA. However, this outcome does not exclude the possibility that reef calcifiers are acclimatized or adapted to other physical conditions unique to each location (e.g. differing nutrient concentrations, temperature, etc.). Although corals are well known to alter their biomass and allocation of resources to reproduction throughout the year [46], these effects are unlikely to have biased our analyses as experiments were carried out in spring (Hawaii and Okinawa) or at the end of the austral winter (Moorea), when corals typically have large food reserves [46]. Potentially, the presence of high biomass in our study corals may have enhanced resistance to OA treatments in all three locations [46]. In terms of allocation of

Table 2. Comparison of area-normalized and biomass-normalized calcification for the corals massive *Porites* spp. and *P. damicornis*. (Analyses were performed using a two-way ANOVA in which treatment and location were fixed effects. MS, mean square.)

organism	dependent variable	effect	d.f.	MS	F	p-value
massive <i>Porites</i> spp.	area-normalized calcification (mg CaCO ₃ d ⁻¹ cm ⁻²)	treatment	2	0.32	0.58	0.561
		location	2	5.08	22.43	<0.001
		treatment × location	4	0.09	0.41	0.798
		residuals	99	0.27		
	biomass-normalized calcification (mg CaCO ₃ d ⁻¹ g ⁻¹)	treatment	2	2.9 × 10 ⁻⁴	0.10	0.910
		location	2	0.14	45.72	<0.001
		treatment × location	4	2.6 × 10 ⁻⁴	0.08	0.987
		residuals	99	3.1 × 10 ⁻³		
<i>P. damicornis</i>	area-normalized calcification (mg CaCO ₃ d ⁻¹ cm ⁻²)	treatment	2	0.16	1.94	0.149
		location	2	3.40	41.84	<0.001
		treatment × location	4	0.05	0.56	0.690
		residuals	99	0.08		
	biomass-normalized calcification (mg CaCO ₃ d ⁻¹ g ⁻¹)	treatment	2	3.8 × 10 ⁻³	0.54	0.586
		location	2	0.28	39.90	<0.001
		treatment × location	4	7.2 × 10 ⁻³	1.01	0.405
		residuals	99	7.1 × 10 ⁻³		

Table 3. Comparison of area-normalized and biomass-normalized calcification for the algae *P. onkodes* and *H. macroloba*, respectively. (Analyses were performed using a two-way ANOVA in which treatment and location were fixed effects. MS, mean square.)

species	dependent variable	effect	d.f.	MS	F	p-value
<i>P. onkodes</i>	area-normalized calcification (mg CaCO ₃ d ⁻¹ cm ⁻²)	treatment	2	0.95	3.88	0.024
		location	2	5.80	23.72	<0.001
		treatment × location	4	0.42	1.73	0.148
		residuals	99	0.24		
<i>H. macroloba</i>	biomass-normalized calcification (mg CaCO ₃ d ⁻¹ g ⁻¹)	treatment	2	5.62	0.11	0.900
		location	2	16.33	0.31	0.737
		treatment × location	4	29.02	0.54	0.704
		residuals	99	53.33		

resources to reproduction, *P. damicornis* releases larvae year round [47,48] and massive *Porites* spp. were likely to be at a similar stage of their reproductive cycle [47,48]. Therefore, reproduction is unlikely to have an interactive effect with the timing of our experiments.

Resistance of massive *Porites* spp. to short-term OA conditions has been demonstrated in Moorea where corals showed no response of area-normalized net calcification to OA [13,49]. The resistance of this taxon to OA has also been reported in one location in Papua New Guinea where naturally acidified waters occur at a shallow volcanic CO₂ seep where massive *Porites* ecologically is dominant in the reef community [14]. Similar to massive *Porites* spp., previously we have shown that calcification of *P. damicornis* in Moorea is resistant to pCO₂, at least over 15 days of incubations [12]. It has also been reported from mesocosm experiments that OA does not affect settlement of *P. damicornis* larvae released from adult colonies maintained in acidified conditions [50].

Previous studies of *P. onkodes* have reported deleterious effects of OA of differing magnitudes that consist of decreased net calcification [12], higher sensitivity to grazing [51], depressed net productivity and increased net dissolution [52] and bleaching [16]. Different responses of *P. onkodes* to high pCO₂ in different locations in this study (as well as among previous studies [12,52]) suggest that in contrast to *H. macroloba*, massive *Porites* and *P. damicornis*, the response of *P. onkodes* to OA may be influenced by the environmental history prior to collection. At local scale, coralline algae experiencing naturally diel pH variations are acclimatized to variable pH [29], and some temperate species found in habitats exposed to large pH changes (i.e. tidal pools) are more resistant to OA than species living in habitats characterized by more stable pH [28]. However, more pronounced negative effects of OA were found on the temperate coralline alga *Arthrocardia corymbosa* exposed to fluctuating pH [53], which suggests a limit to the capacity of coralline algae to

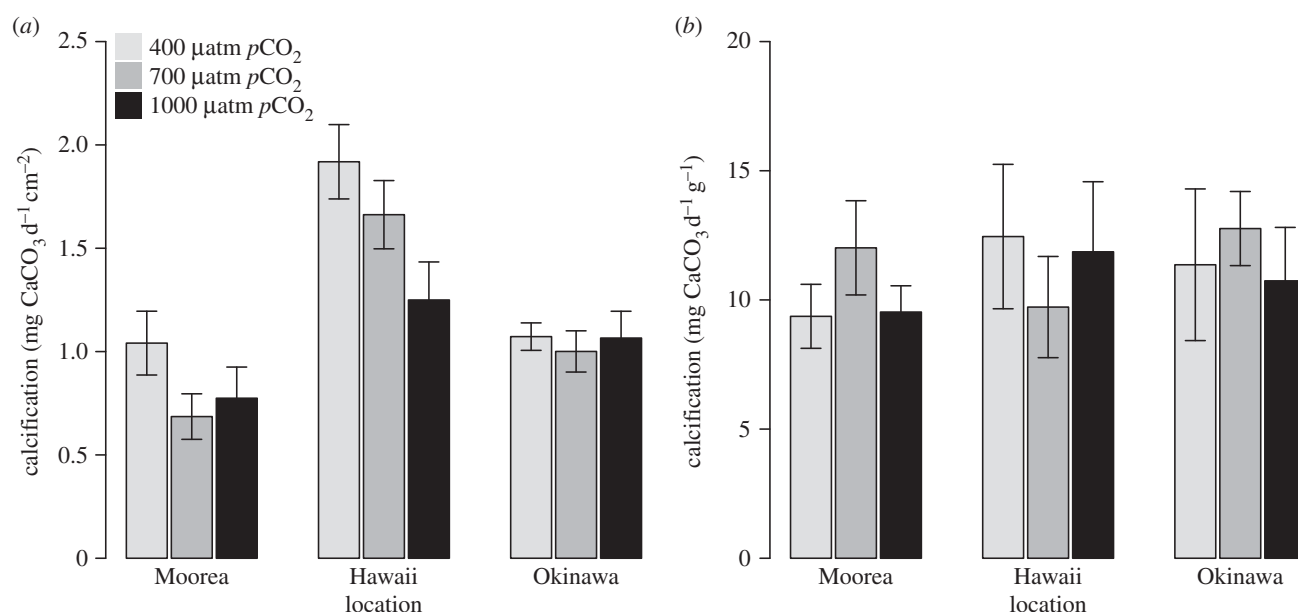


Figure 3. Calcification of the algae *P. onkodes* and *H. macroloba* maintained in three $p\text{CO}_2$ levels (400, 700 and 1000 μatm) in Moorea, Okinawa and Hawaii. (a) Area-normalized calcification of *P. onkodes*. (b) Biomass-normalized calcification of *H. macroloba*. The bars correspond to the mean calcification and the vertical error bars show the s.e. in the measurement of calcification ($n = 12$).

acclimatize to OA. Interestingly, *P. onkodes* was the most sensitive to OA in Hawaii (this study), where calcification was the highest, which is consistent with the hypothesis that organisms characterized by rapid calcification are more affected by OA than those that calcify slowly [13].

In addition, recent observations in the North Pacific have shown that coralline algae forming a thick thallus have been more affected during the past 30 years by changes in seawater carbonate chemistry than species with thinner thalli [54]. While thickness of the thallus was not measured in our study, it is possible that *P. onkodes* was thicker in Hawaii where calcification rates were the highest, which would be consistent with the higher sensitivity to OA of coralline algae with thick thalli and rapid calcification. Aside from growth rates as a means to explain varied responses to OA, differential sensitivities to OA among sites in the calcified algae might reflect different mineral compositions of the skeletons. For example, a varying proportion of magnesium, or the presence of dolomite and magnesite that are less sensitive to dissolution, could underlie differential responses of algae to OA [55].

Using comparable manipulative experiments conducted at three locations spanning an unprecedented large spatial scale, our study demonstrates resistance to OA for two corals and one alga, with a second alga (*P. onkodes*) resistant to OA in one of the three locations. This alone is an important discovery as it demonstrates that local examples of resistance of corals to aspects of OA [11,12] may have general application over a scale of thousands of kilometres. While it was beyond the scope of our study to identify the mechanistic basis of the resistance of these two corals to OA, there are three hypotheses with potential to explain this pattern. First, the tissue of corals could function as a protective barrier isolating the site of calcification from the external seawater [23,43], thereby protecting it from decreasing pH attributed to OA [19,20]. Of the two corals studied, massive *Porites* is well known for an unusually thick tissue layer that permeates deeply into the skeleton [49]. In this study, a thick tissue layer

could have played an important role in protecting massive *Porites* from OA at the three locations. Second, the ability of corals to use bicarbonate ions in the light, which increases under OA conditions [21,22], could mitigate negative responses driven by Ω_{arag} alone. Such capacity has been demonstrated in *Madracis auretenra* and *Porites rus* [21,22], and this could be a common feature of tropical scleractinians. Third, calcification in corals is driven by their ability to maintain high pH at the site of CaCO₃ deposition by the export of protons from the site of calcification, but proton export might become more energetically costly under OA conditions [19,20,23]. This hypothesis could explain the resistance of *P. damicornis* to OA, because it exhibits a highly branched corallum [56] that favours efflux of protons from the site of calcification to the surrounding seawater [23].

As described above for two corals, for *H. macroloba* there was no effect of $p\text{CO}_2$ on net calcification. Previous studies on the response of net calcification to $p\text{CO}_2$ in *Halimeda* report variable results of exposure to high $p\text{CO}_2$, including decreased calcification [57], a threshold relationship [18], no effect (in *H. macroloba*) and a linear decrease in *H. minima* [12]. The variation in responses reported in previous studies is probably caused both by species-specific responses to OA as well as methodological artefacts. These might include experiments conducted at different irradiances or varying water motion that could affect rates of photosynthesis and calcification in *Halimeda*.

The mechanisms underlying the resistance of *H. macroloba* to OA are unlikely to be the same as those for corals (described above). It has been demonstrated using microsensors that calcification in *H. discoidea* is not controlled actively by the alga, but rather is linked directly to the pH in intercellular spaces that is controlled by the ratio of photosynthesis to respiration [58]. It is possible that in our experiment, increases in $p\text{CO}_2$ and HCO_3^- in seawater favoured photosynthesis during daylight [59] leading to an increase in pH in intercellular spaces that increased calcification. By contrast, at night, respiration and the absence of photosynthesis leads to a

decrease in pH in intercellular spaces, which could result in night-time dissolution of CaCO₃. As a result, net calcification was not affected by OA.

The resistance of three of four corals and calcified algae to short-term exposure to OA conditions, with a consistent effect across a very large spatial scale, suggests that this ability may represent a constitutive and geographically conserved capacity to resist some of the effects of OA. However, while our study suggests some reef calcifiers will persist in a more acidic future, it remains to be determined whether they can endure the challenges of rising temperature that are likely to occur at a more rapid pace than declining seawater pH, and moreover, whether they can survive if the carbonate framework beneath them begins to dissolve [60]. Our results, as well as past studies demonstrating species-specific responses to pCO₂ in reef

calcifiers [12,13], suggest that OA may function as a taxonomic filter, favouring the persistence of species that are resilient to OA. As a result, OA could drive the formation of less diverse coral reefs populated across the Pacific with resistant species exemplified by massive *Porites*, *P. damicornis* and *H. macroloba*.

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