Mechanisms and thresholds for pH tolerance in Palau corals

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Hannah C. Barkley [a,*], Anne L. Cohen [b], Daniel C. McCorkle [b], Yimnang Golbuu [c]

[a] Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program in Oceanography and Oceanographic Engineering, Woods Hole, MA 02543, USA
[b] Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[c] Palau International Coral Reef Center, Koror 96940, Palau

A B S T R A C T

In Palau, calcification rates of two reef-building coral genera (*Porites* and *Favia*) are maintained across a strong natural gradient in aragonite saturation state (Ω_{ar}) ranging from 3.7 to 2.3. This observation contrasts the strong sensitivity to decreasing Ω_{ar} that these genera demonstrate in both laboratory CO_2 manipulation experiments and in field studies. Moreover, in contrast to other naturally more acidic coral reefs, benthic communities in Palau's low-Ω_{ar} ($\Omega_{ar} = 2.3$) Rock Island reefs display ecological indices consistent with healthy communities. A laboratory CO_2 manipulation experiment and a field-based reciprocal transplant were used to investigate whether the apparent lack of sensitivity to ocean acidification of Palau's *Porites* corals can be attributed to local adaptation to chronic acidification or to environmental factors that allow corals to thrive despite extreme pH conditions. In a two-month laboratory incubation, calcification rates of Palau *Porites* from both environments were insensitive to changes in Ω_{ar} over the range 1.5 to 3.0, suggestive of an adaptive, rather than environmental, mechanism for acidification tolerance. However, in the reciprocal transplant, corals transplanted between reefs at different ambient Ω_{ar} levels showed significant declines in calcification rates and high mortality, while corals returned back to their reef of origin were alive after 17 months in the field. Interpreted within the framework of the experimental result, the failure of pH/Ω_{ar}-tolerant corals to successfully transplant between different reef sites hints at local adaptation to other (non-pH) environmental factors such as light, temperature, and/or flow that co-vary with Ω_{ar} across Palau's natural acidification gradient.

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1. Introduction

Anthropogenic emissions of carbon dioxide (CO_2) are altering the chemistry of the world's oceans with significant implications for the structure and function of coral reef ecosystems. The absorption of excess CO_2 in surface waters drives down ocean pH, decreases carbonate ion concentration (CO_3^{2-}), and lowers the aragonite saturation state of seawater (Ω_{ar}) (Feely et al., 2004). This process, known as ocean acidification, threatens coral reef ecosystems because it decreases the availability of the carbonate ion building blocks that corals and other calcifying reef organisms need to construct the calcium carbonate ($CaCO_3$) skeletons that build reefs (Doney et al., 2009). A majority of laboratory CO_2 manipulation experiments have demonstrated that coral calcification rates are highly sensitive to decreasing pH and Ω_{ar} (Kroeker et al., 2010; Kroeker et al., 2013), although a few studies have also shown a non-significant response to acidification (e.g. Comeau et al., 2014a; Edmunds, 2012). Furthermore, several studies of naturally low-Ω_{ar} reefs reveal that in situ coral calcification rates decline under chronic low-pH conditions (Crook et al., 2011; Enochs et al.,

2015; Manzello et al., 2014), with the exception of the Papua New Guinea CO_2 vents (Fabricius et al., 2011). Together, experimental and field-based observations imply significantly reduced coral calcification rates and eventual shifts from net accretion to net erosion for coral reefs globally.

In light of predictions for coral reef responses to ocean acidification, finding coral reef communities that may be resilient to ocean acidification and elucidating mechanisms for acidification tolerance are increasingly important conservation priorities. However, identification of pH-tolerant reefs and reef communities is challenging because, unlike thermal stress events that cause visible bleaching, pH tolerance and impacts must be identified experimentally. To date, only the coral reefs that fringe Palau's karstic Rock Islands stand in stark contrast to the depauperate coral communities observed at most low-Ω_{ar} reefs. Even though they currently exist at predicted end-of-century Ω_{ar} levels ($\Omega_{ar} = 2.3$), these coral reef communities remain diverse and coral-dominated (Barkley et al., 2015; Shamberger et al., 2014). Moreover, calcification rates of two reef-building coral genera (*Porites* and *Favia*) do not change across Palau's steep Ω_{ar} gradient ($\Omega_{ar} = 3.7$ to 2.3). This observation contradicts other field-based and experimental results for *Porites* and *Favia*, where significant calcification declines were observed for these genera with declining Ω_{ar} (Albright et al., 2008; Anthony et al., 2008;

Crook et al., 2013, 2011; DePutron et al., 2010; Drenkard et al., 2013; Enochs et al., 2015; Iguchi et al., 2012; Manzello et al., 2014; Muehllehner and Edmunds, 2008). This apparent tolerance to acidification raises two key questions: 1) by what mechanisms are Palau's corals able to maintain calcification rates in chronically low-pH conditions, and 2) are Palau's corals currently at their thresholds for pH tolerance?

There are two likely explanations for coral reef community acidification tolerance. First, the particular combination of environmental conditions in Palau's more acidic bays could alleviate impacts of low Ω_{ar} on calcification. Laboratory experiments suggest high light, strong flow, and elevated nutrients or food can offset acidification impacts on coral calcification rates (Cohen and Holcomb, 2009; Comeau et al., 2014b; Drenkard et al., 2013; Suggett et al., 2012; Tanaka et al., 2014). In addition, field studies of acidified reef sites have revealed that environmental variables other than Ω_{ar} (e.g. temperature) may have a stronger influence on coral calcification rates than shifts in carbonate chemistry (Fabricius et al., 2011). Many of these environmental factors co-vary with Ω_{ar} across Palau's acidification gradient, although not necessarily in the direction required for modulation of pH effects (Barkley et al., 2015). Alternatively, Palau's thriving Rock Island coral reef communities may have acclimatized or adapted to naturally low-Ω_{ar} conditions. Today, the low-pH Rock Island reefs are relatively isolated within bays and inlets and were even more isolated prior to the mid-Holocene high stand when sea level was significantly lower than it is today. It is possible that strong selective pressure over hundreds to thousands of years has driven community-wide acclimatization or adaptation to chronically low-Ω_{ar} levels. Distinguishing between these mechanisms can help inform best practices for conservation by protecting environmental features that promote tolerance (e.g. maintaining trees that provide shade, protecting coral food populations, or preserving water quality) or by prioritizing connectivity between pH-adapted and pH-sensitive communities, and can aid in the identification of any additional acidification-tolerant reefs.

Palau's Rock Island coral communities exist today at pH/Ω_{ar} levels predicted for many coral reefs by 2100. However, it is unknown how Ω_{ar} will change within these bays over the next few decades as ocean acidification progresses in the open ocean and whether these coral communities are approaching or already at their acidification thresholds. While trends in Ω_{ar} on inshore reefs might proceed independently of changes in open ocean Ω_{ar}, acidification in the open ocean may also lead to concurrent decreases in Ω_{ar} in already low-Ω_{ar} areas. If this is the case, corals currently living under low-Ω_{ar} conditions might reach their acidification thresholds much sooner than those on high-Ω_{ar} reefs. On the other hand, equivalent incremental decreases in Ω_{ar} across all reefs may have less of an impact on the coral communities chronically exposed and possibly adapted to and/or environmentally buffered against low Ω_{ar} levels. Thus, understanding both the mechanism of coral tolerance to acidification and the Ω_{ar} thresholds of coral calcification are critically important in predicting how apparently acidification-tolerant communities will cope with changes in Ω_{ar} in the future.

To address these questions, combined onsite laboratory CO_2 manipulation and reciprocal transplant experiments were conducted to 1) determine sensitivities of corals from a high-Ω_{ar} reef and a low-Ω_{ar} reef to a range of Ω_{ar} conditions and 2) establish whether the calcification insensitivity to Ω_{ar} observed in the field may be best explained by environmental mitigation of acidification stress or by coral adaptation to low-Ω_{ar} conditions. Specifically, if environmental mitigation explains acidification-tolerance in low-pH reefs, corals should be dependent on the presence of a specific suite of environmental variables in order to survive transplantation into highly acidified conditions. If adaptation is responsible for acidification tolerance, Palau's corals should be able to calcify in a different Ω_{ar} environment without the presence of particular environmental conditions. Thus, laboratory CO_2 perturbation experiments test coral responses solely to Ω_{ar} with all other environmental variables held constant, while reciprocal transplants examine coral responses to Ω_{ar} in the context of all the other environmental variability

present between high-Ω_{ar} barrier reefs and low-Ω_{ar} Rock Island reefs. In addition, observing coral responses to a range of CO_2 levels can help to evaluate Ω_{ar} thresholds and improve predictions of how coral communities currently living under low-Ω_{ar} conditions will respond to future changes in seawater chemistry.

2. Methods

2.1. Coral collection

Coral plugs were collected in December 2012 from massive *Porites* colonies at a naturally low-Ω_{ar} reef site (7.324°N, 134.493°E; mean Ω_{ar} = 2.3; n = 78) and a naturally high-Ω_{ar} reef site (7.268°N, 134.522°E; mean Ω_{ar} = 3.7; n = 75; Fig. S2). At each reef site, small skeletal cores (diameter = 3.5 cm) were removed from massive colonies (one core per colony) at 2–3 m depth using underwater pneumatic drills, and cores were cut with a lapidary table saw to approximately 1 cm below the tissue layer. The plugs were affixed to nylon square base screws with marine epoxy, secured to egg crate racks, and returned to their original reefs to allow the corals to recover from the coring procedure. All corals survived two months of recovery on the reef, and on all corals living tissue had fully overgrown the sides of the plugs so that no underlying skeleton was exposed. Corals were recovered in February 2013.

2.2. CO_2 manipulation experiment

Corals from two reefs were cultured at three CO_2 levels for eight weeks in March to May 2013 (n = 10 corals per treatment, n = 60 corals total). The corals were individually incubated in independently-manipulated plastic cups (volume = 750 ml) to increase statistical power. Cups were placed within a large, temperature-controlled water bath. The corals were maintained at mean ($\pm$SD) temperatures of 29.4 °C $\pm$ 0.1 °C. Light was provided by LED aquarium lights (Coralife) at average levels of 334 $\pm$ 48 µmol photons m^{-2} s^{-1} (measured by an underwater quantum sensor, LI-COR) on a 12 h:12 h light:dark schedule. Corals were fed live *Artemia* brine shrimp larvae every other evening by pipetting 1 ml of concentrated brine shrimp in filtered seawater into each cup. Coral cups were cleaned weekly to prevent algae overgrowth.

Mean pH (total scale)/Ω_{ar} levels for the three treatment conditions were 7.98/3.0, 7.83/2.3, and 7.60/1.5 (Table 1). In each coral cup, carbon system chemistry was regulated using a combination of flow-through, pre-equilibrated water and bubbling of mixed air/CO_2 gas. Incoming seawater (filtered to 0.35 µm) from the reef was aerated and split into three header tanks. In the low-CO_2 header tank, water was bubbled with air. In the mid-CO_2 and high-CO_2 header tanks, CO_2 levels were regulated by a pH controller (Drs. Foster and Smith) connected to a solenoid valve that introduced CO_2 gas into the header tank through a column diffuser. Water was siphoned from the three header tanks into each coral cup at a rate of approximately 375 ml h.$^{-1}$. Each coral cup was also bubbled with either compressed air (low CO_2 treatment) or mixed compressed air and CO_2 gas (mid and high CO_2 treatment) controlled by pairs of mass flow controllers (Aalborg Instruments) at approximately 200 ml min^{-1}. Low alkalinity levels in the source water to the Palau International Coral Reef Center (drawn from within the lower-alkalinity Rock Islands) prevented Ω_{ar} in the low-CO_2 condition (Ω_{ar} = 3.0) from reaching values that were as high as those measured on the barrier reef site (Ω_{ar} = 3.7).

To characterize the carbonate chemistry in each cup, total alkalinity (TA), pH, temperature, and salinity were measured weekly. Spectrophotometric pH measurements were made with 2 mM *m*-Cresol purple indicator dye using a spectrometer with a 100 mm flow cell (Ocean Optics, mean precision = 0.005) following procedures in Clayton and Byrne (1993) and Dickson et al. (2007) and using the equation of Liu et al. (2011). Samples for TA were collected in 20 ml glass vials and

Table 1
Mean (1 SD) seawater carbonate system conditions for laboratory CO_2 manipulation experiment and field reciprocal transplant. Reciprocal transplant site chemistry data were previously reported in Barkley et al. (2015).

Experiment	CO_2 treatment	Salinity (psu)	T (°C)	pH (total scale)	TA (µmol kg^{-1})	DIC (µmol kg^{-1})	pCO_2 (µatm)	$[CO_3^{2-}]$ (µmol kg^{-1})	Ω_{ar}
CO_2 manipulation	Low CO_2	33.7 (0.1)	29.4 (0.1)	7.98 (0.02)	2029 (36)	1762 (37)	429 (24)	184 (7)	3.0 (0.1)
	Mid CO_2	33.7 (0.1)	29.4 (0.1)	7.83 (0.02)	2061 (27)	1866 (33)	652 (46)	142 (6)	2.3 (0.1)
	High CO_2	33.7 (0.1)	29.4 (0.1)	7.60 (0.03)	2063 (25)	1960 (28)	1198 (83)	90 (5)	1.5 (0.1)
Reciprocal transplant	Low CO_2	33.6 (0.3)	29.9 (0.8)	8.04 (0.02)	2179 (30)	1863 (36)	384 (26)	222 (10)	3.7 (0.2)
	Mid CO_2	32.4 (0.6)	30.3 (1.0)	7.84 (0.03)	1977 (27)	1783 (25)	604 (48)	140 (10)	2.3 (0.2)

poisoned with saturated mercuric chloride. Automated gran titrations for TA were run on duplicate 1 ml samples using a Metrohm Titrando 808 and 730 Sample Changer (mean precision = 4 µmol kg^{-1}), and TA values were standardized to certified reference materials obtained from Andrew Dickson [Scripps Institution of Oceanography (Dickson, 2001)]. Salinity was measured in each cup using an YSI salinity probe, and temperatures were measured using an Omega thermocouple (accuracy = 0.1 °C). Full CO_2 system parameters were calculated from temperature, salinity, TA, and pH using CO2SYS (Lewis and Wallace, 1998) with the constants of Mehrbach et al. (1973) as refit by Dickson and Millero (1987).

2.3. Coral calcification analysis

Calcification rates were measured using both buoyant weight (Davies, 1989) and alkalinity anomaly (Chisholm and Gattuso, 1991) techniques. Buoyant weights for each coral were collected at the beginning of the experiment, after three weeks in experimental CO_2 conditions, and then weekly during weeks four to eight. Corals were weighed using a balance with a weigh-below hook (Sartorius GC803S), which allows for beneath-balance weighing of coral plugs that remain entirely submerged in experimental cups maintained at treatment Ω_{ar} levels. Wet weight data were converted to dry weights using an aragonite density of 2.93 g cm^{-3} and the density of seawater determined using a standard of known weight and density. Repeated buoyant weight measurements on the same coral yielded mean precision estimates of ±0.03 g.

Day/night alkalinity depletion experiments were conducted at the end of the eight-week experiment. Water flow to each coral cup was stopped during this time, but gas bubbling was continued in order to maintain pH levels. Samples for TA were collected for each coral cup at the beginning and end of two four-hour periods (one four-hour period during the day and one at night). Alkalinity depletion incubations were simultaneously run in control cups containing only filtered seawater (n = 3 per experiment). Because the net change in TA values in control cups was within analytical precision (mean = 3 µmol kg^{-1}), coral calcification was assumed to be the only process impacting the alkalinity in the cups, where 2 mol of alkalinity were consumed for every 1 mol of calcium carbonate produced. TA pre and post incubation was determined following the titration procedure described in Section 2.2 with samples run in triplicate.

Calcification rates for both buoyant weight and alkalinity anomaly measurements were normalized to coral tissue surface areas. Surface areas were measured following the general procedure for aluminum foil wrapping, in which the weight of aluminum foil needed to cover the entire surface of the coral skeleton is converted to area using a calibration curve (Marsh, 1970). However, skeletons were wrapped with electrical tape instead of aluminum foil because the use of electric tape provided tighter control and minimization of tape overlap, which can significantly overestimate surface area. The area of each coral skeleton occupied by living tissue was wrapped in electrical tape that was

carefully trimmed to eliminate any overlay. The weight of tape used to cover the coral tissue for each skeleton was converted to surface area using a weight-to-area calibration, where ten pieces of electrical tape of known area were weighed to build a weight-per-unit area curve. Replicated electrical tape surface area estimates on ten coral skeletons produced a mean precision of 0.43 cm^2, or ~1% of calculated surface areas.

2.4. Reciprocal transplant experiment

The reciprocal transplant experiment was conducted concurrently with the CO_2 manipulation experiment. Initial buoyant weight measurements were obtained for all corals prior to transplantation. Of the corals collected from the low-Ω_{ar} reef and from the high-Ω_{ar} reef (n = 44 for each reef), approximately half of the corals in each group were returned to their reef of origin (low-Ω_{ar} to low-Ω_{ar}: n = 23, high-Ω_{ar} to high-Ω_{ar}: n = 21), while the remaining corals were transplanted to the opposite reef (low-Ω_{ar} to high-Ω_{ar}: n = 21, high-Ω_{ar} to low-Ω_{ar}: n = 23). All corals were transplanted to the same depth (5 m). In May 2013, after eight weeks in the field (and at the end of the CO_2 manipulation experiment), approximately half of the corals from each reef (n = 10–12 per transplant group) were recovered and weighed. The remaining corals were left out on the two reefs for 17 months (n = 10–11 per group) and were recovered in August 2014. Corals were evaluated for partial or total mortality and were judged to be alive (no visible tissue death observed), partially dead (visible tissue death and/or tissue recession but some living tissue remaining), or dead (no living tissue remaining). Buoyant weights were collected for all corals to determine overall calcification rates during the reciprocal transplant period. Because coral tissue is assumed to be neutrally buoyant (i.e. it does not contribute to buoyant weight measurements) and the timing of tissue death during the 17-month transplant was unknown, the overall change in skeletal mass was calculated and reported for all corals regardless of mortality status.

2.5. Statistical analyses

All statistical analyses were conducted in R (version 3.0.1). For the CO_2 manipulation experiment, calcification rates derived from overall buoyant weight changes (final–initial) and alkalinity depletion measurements were fit to generalized linear models to explore the relationship between Ω_{ar} and coral calcification rates. Calcification rates calculated from weekly changes in buoyant weight were fit to linear mixed effects models using the *nlme* package (Pinherio et al., 2012). The Akaike Information Criterion (AIC) best-fit model included Ω_{ar}, reef of origin, and week as fixed effects and considered individual coral colonies as random effects to account for repeated weight measurements on the same coral. All reciprocal transplant calcification data met assumptions for normality (Shapiro-Wilk test) and heteroscedasticity (Levene's Test) and were thus analyzed with two-way ANOVAs with post-hoc Tukey tests. Mortality count data for the

reciprocal transplant corals (classified as live, partially dead, or dead) were analyzed using Fisher's Exact Tests.

3. Results

3.1. CO_2 manipulation experiment

Regardless of reef of origin, corals in the CO_2 manipulation experiment showed no calcification sensitivity to Ω_{ar} after eight weeks in experimental conditions. Comparisons between alkalinity anomaly calcification measurements and buoyant weight estimates collected during the last week of the experiment showed that calcification rates produced by both methods were significantly correlated for individual corals (Fig. 1; $r = 0.67$, $p < 0.001$). Buoyant weight measurements collected at the beginning and end of the eight-week incubation indicated no significant effect of reef of origin (generalized linear model, $p = 0.85$), Ω_{ar} level ($p = 0.18$), or their interaction ($p = 0.72$) on coral calcification rates ($R^2 = 0.06$; Fig. 2A; Table S1). Similarly, day/night alkalinity depletion experiments conducted after eight weeks in experimental treatments indicated no difference in calcification rates across all corals regardless of reef of origin ($p = 0.35$), Ω_{ar} level ($p = 0.15$), or their interaction ($p = 0.62$, $R^2 = 0.10$; Fig. 2B; Table S1). Linear mixed effects models showed that calcification rates changed significantly over time. Calcification rates were lower at the beginning of the incubation than in later weeks (estimate = 1.55 mg cm^{-2} week^{-1}, $p < 0.001$; Fig. 3, Table S2), and 53% of corals lost weight during the first three weeks of the experiment. However, weekly calcification rates were not significantly impacted by Ω_{ar}, reef of origin, or their interaction ($p > 0.05$), although during the final month of the experiment the low CO_2 treatments from each reef consistently showed the highest calcification rates.

3.2. Reciprocal transplant experiment

After two months in the field, there were significant differences in calcification rates (two-way ANOVA, $F_{1,40} = 12.87$, $p < 0.001$ for the interaction of original reef and transplant reef) and mortality (Fisher's Exact Test,
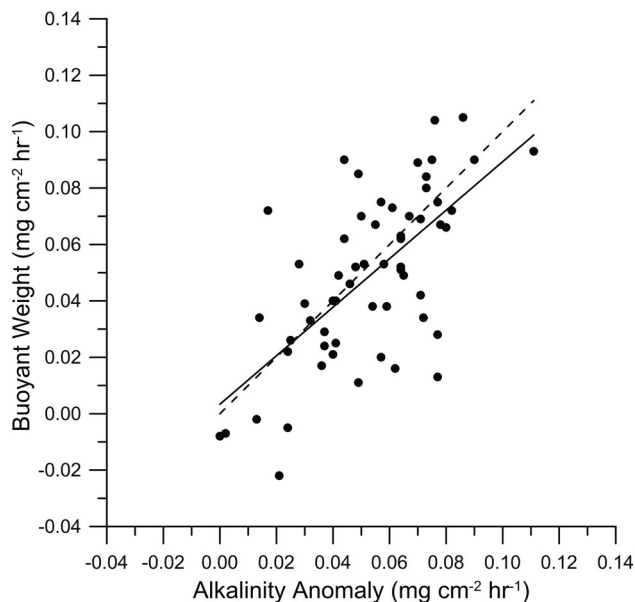
Fig. 2. Calcification rates of *Porites* corals collected from a naturally high-Ω_{ar} reef (gray) and a naturally low-Ω_{ar} reef (black) incubated at three Ω_{ar} conditions. (A) Overall coral calcification rates calculated as the surface area normalized difference between weight at the end of the 8-week experiment and initial weight (measured by buoyant weighing). (B) Calcification rates derived from paired 4-hour day/night alkalinity depletion measurements conducted at the end of the 8-week experiment. Coral colony calcification rates are plotted against the average Ω_{ar} measured in each experimental cup (n = 10 corals per treatment group). Neither set of rate measurements showed significant differences as a function of either Ω_{ar} or reef of origin.

$p < 0.001$) related to transplant treatments (Fig. 4; Fig. 5; Table S3). Corals originally collected from the high-Ω_{ar} site had similar growth rates and >80% survival at both high and low Ω_{ar} (Tukey HSD, $p = 0.99$) and grew as fast in low-Ω_{ar} conditions as corals originally from the low-Ω_{ar}

Fig. 3. Weekly coral calcification rates in the CO_2 manipulation experiment. Mean (± 1 SE) calcification rates of *Porites* corals (n = 10 per treatment group) collected from a naturally high-Ω_{ar} reef (solid lines and points) and a naturally low-Ω_{ar} reef (open lines and points) were incubated for 8 weeks at three CO_2/Ω_{ar} conditions: $\Omega_{ar} = 1.5$ (black), 2.3 (dark gray), and 3.0 (light gray). Buoyant weight data were collected at the beginning of the experiment and then weekly from weeks 3–8. Weekly calcification estimates were calculated as the mean change in area normalized weight per week, with calcification estimates for week 3 calculated as the change in weight between initial and week 3 weight divided by 3.

Fig. 1. Comparison of calcification estimates derived from buoyant weight and alkalinity anomaly techniques. Day/night average alkalinity anomaly estimates for calcification rates of each coral colony at the end of the 8-week experiment plotted against buoyant weight calcification rates collected during the last week of the experiment (n = 60). The solid line shows the line of best fit ($r = 0.67$, $p < 0.001$), and the dotted line represents a theoretical 1:1 relationship between calcification estimates derived by the two techniques.
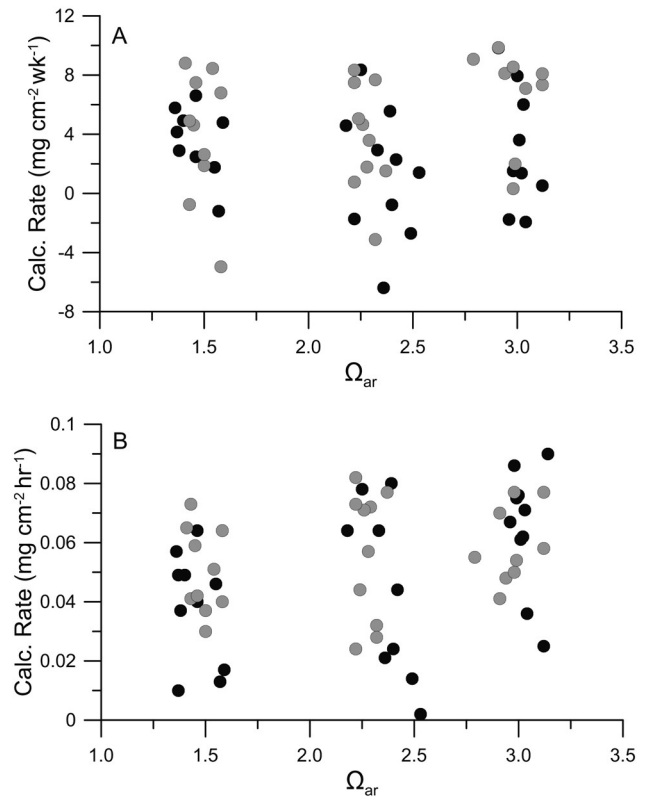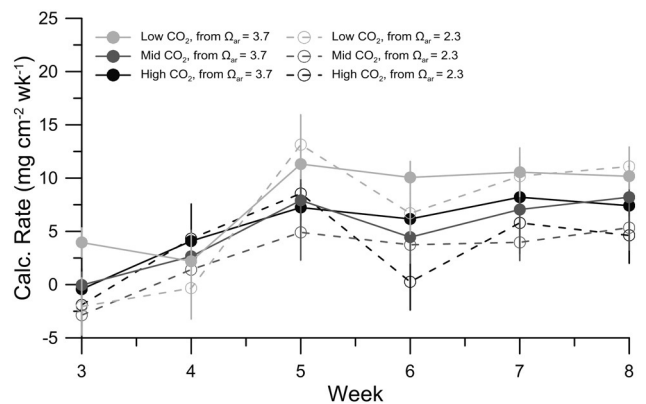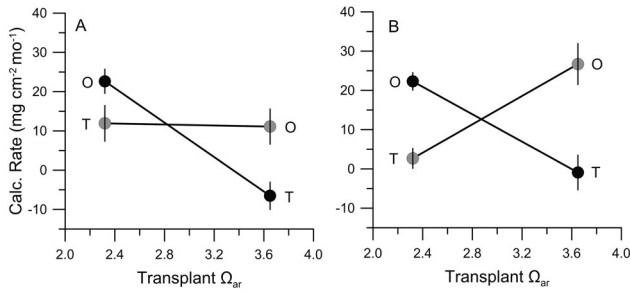
Fig. 4. Coral calcification rates in the reciprocal transplant experiment. Mean calcification rates of *Porites* corals collected from a naturally high-Ω_{ar} reef (gray) and a naturally low-Ω_{ar} reef (black) and either returned to their original reef ("O") or transplanted to the opposite reef ("T"). Transplant experiments were conducted for (A) 2 months and (B) 17 months (n = 10–12 per treatment group). Mean calcification rates per group (± 1 SE), determined by buoyant weighing before and after the specified time period, are colored by reef of origin and plotted by the Ω_{ar} of the reef to which corals were transplanted. Groups of corals originally collected from the same reef are connected by solid lines.

site (p = 0.20). However, the corals transplanted from the low-Ω_{ar} reef to the high-Ω_{ar} reef had significantly lower calcification rates relative to the other three transplant groups (p < 0.01 for all pairs), with 64% of individuals showing partial or total mortality after two months.

After 17 months in the field, only the corals that had been returned to their original reef (i.e. low-Ω_{ar} to low-Ω_{ar} and high-Ω_{ar} to high-Ω_{ar}) had high growth and survival rates. There was no significant difference in calcification between the two groups of corals returned to their reef of origin (p = 0.84), and calcification rates were significantly higher than those of transplanted corals from both reefs (p < 0.01 for all pairs). Corals transplanted from one reef to the other showed very low, mostly net negative growth. Mortality varied significantly between treatment groups (Fisher's Exact Test, p < 0.001) and was highest in the transplanted populations (73–90% partial or total mortality). However, a small number of corals transplanted to the opposite reef were able to survive transplantation: 27% (3 individuals) of the corals transplanted from high-Ω_{ar} to low-Ω_{ar} and 10% (1 individual) of the corals transplanted from low-Ω_{ar} to high-Ω_{ar} were alive with no visible tissue death after 17 months in the field.

4. Discussion

The coral reef communities in Palau's low-Ω_{ar} Rock Island bays provide a unique opportunity to evaluate pH tolerance in corals chronically exposed to naturally more acidic conditions. As strategies for coral reef conservation focus increasingly on supporting sites with high resilience potential, a robust understanding of both the mechanisms that increase tolerance and the pH thresholds will be important to incorporate into conservation planning and management decisions. Where tolerance is afforded by a specific combination of environmental conditions (e.g. high light, strong flow, or elevated nutrients/food), controlling coastal anthropogenic activities that negatively affect those features will become of paramount importance. Where tolerance is identified as adaptive, ensuring the protection of those communities and their connectivity with neighboring reefs should be a high priority.

Palau *Porites* corals incubated at three pCO_2 levels in a laboratory manipulation experiment demonstrated no overall calcification response to acidification and were able to maintain calcification rates over a Ω_{ar} range from 3.0 to 1.5. This result is consistent with the absence of a calcification response in *Porites* corals at the same reef sites in situ over a narrower range in Ω_{ar} (Barkley et al., 2015). The Ω_{ar} tolerance observed in the CO_2 manipulation experiments is suggestive of adaptation, as *Porites* coral populations were able to maintain their calcification rates despite extreme declines in Ω_{ar} and in the absence of variability in other environmental factors. In addition, these corals were incubated under experimental pH and Ω_{ar} levels much lower than those they experience in situ. Thus, the maintenance of calcification rates at pH = 7.6 and Ω_{ar} = 1.5 indicates that corals in Palau are not living close to their Ω_{ar} threshold and are tolerant to acidification levels far below those to which they are currently exposed.

There was no significant trend in coral calcification with decreasing Ω_{ar}, but individual corals exhibited a large range in calcification rates within each treatment group. The range in calcification rates observed in the experiment is comparable to that observed in the field at both reef sites (Fig. 6C), suggesting that this observed variance is a natural feature of Palau's coral populations rather than differing responses to treatment conditions. To ensure statistical power, this experiment examined the Ω_{ar} sensitivities of distinct populations of corals within each treatment group rather than the response of the same individual colonies to multiple Ω_{ar} levels. Thus, it is not possible to distinguish whether the observed range in calcification rates relates solely to the variability in growth rates inherent in these coral populations or, instead, to differences in the Ω_{ar} tolerance of individual corals. If the latter is the case, it is likely that, even within a population with no overall sensitivity to acidification, not all colonies will be able to tolerate declining Ω_{ar} conditions. At the same time, those corals able to maintain high rates of growth despite decreasing Ω_{ar} might be those best able to survive and repopulate any communities that succumb to future ocean acidification.
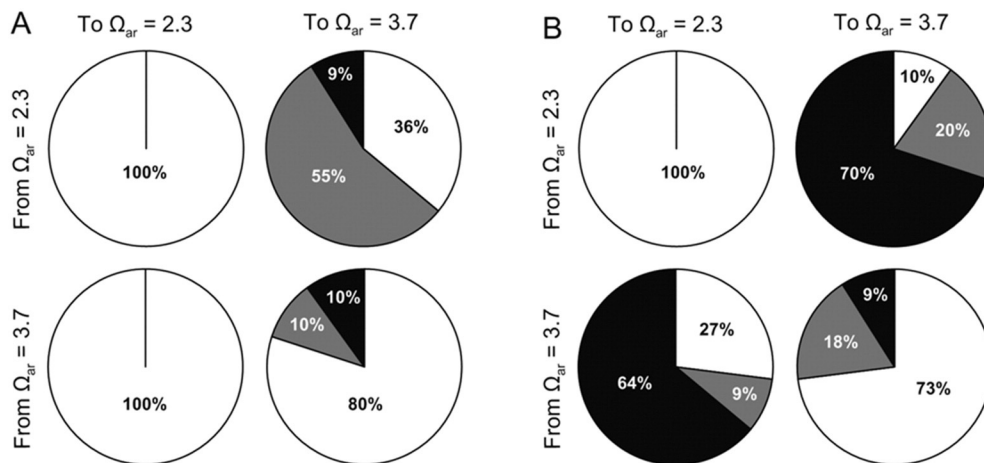

Fig. 5. Coral morality in the reciprocal transplant experiment. Observed mortality in *Porites* corals collected from a naturally high-Ω_{ar} reef and a naturally low-Ω_{ar} reef and either returned to their original reef or transplanted to the opposite reef (n = 10–12 per treatment group). Coral mortality was evaluated in groups of corals collected either after (A) 2 months or (B) 17 months in the field. Corals were judged to be alive (white), partially dead (gray), or dead (black). After 17 months, corals that had been transplanted showed significantly higher mortality than corals returned to their original reef.
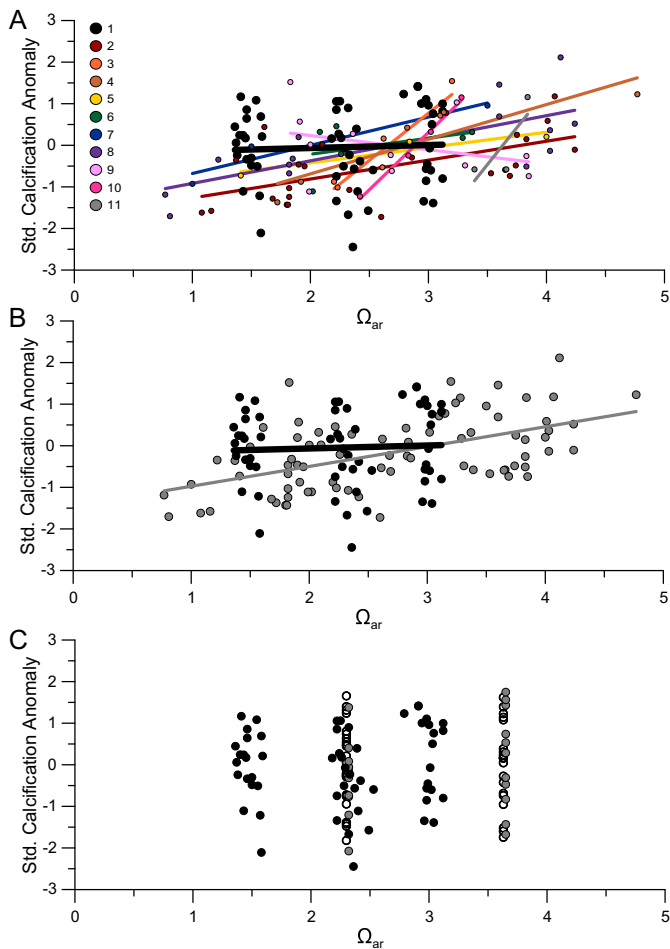
Fig. 6. *Porites* calcification sensitivity to acidification across field observations and laboratory experiments. (A) *Porites* coral calcification responses to declining Ω_{ar} in a CO_2 manipulation experiment in Palau versus the calcification responses observed in ten other studies of massive *Porites* corals. Legend refers to 1: this study, 2: Ohde and Hossain, 2004, 3: Albright et al., 2008, 4: Anthony et al., 2008, 5: dePutron et al., 2011, 6: Edmunds, 2012, 7: Iguchi et al., 2012, 8: Crook et al., 2013, 9: Comeau et al., 2014, 10: Manzello et al., 2014, and 11: Enochs et al., 2015. (B) Palau *Porites* calcification responses to Ω_{ar} (black) versus the overall calcification sensitivity observed across ten other studies of the same genus (gray). (C) Palau *Porites* calcification responses to Ω_{ar} in the CO_2 manipulation experiment (black) and reciprocal transplant (in corals returned to their original reef; white), plotted with calcification rates measured in *Porites* coral skeletal cores (gray) from the two reefs included in this study (Barkley et al., 2015). All data are plotted as standardized calcification anomalies, which were calculated for each study by subtracting the measured calcification rate of each coral from the overall calcification mean and dividing by the standard deviation. Lines represent the linear line of best fit for each data set. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Somewhat surprisingly, calcification responses to Ω_{ar} were no different between corals collected from naturally high-Ω_{ar} and naturally low-Ω_{ar} reefs. Corals collected from the chronically low-Ω_{ar} reef ($\Omega_{ar} = 2.3$) calcified as fast in experimental conditions and were equally insensitive to declining Ω_{ar} as corals collected from the high-Ω_{ar} reef ($\Omega_{ar} = 3.7$). This suggests that at least two *Porites* coral populations, separated by a distance of about 10 km, are able to acclimatize or adapt to Ω_{ar} conditions lower than those to which they are presently exposed. Hydrodynamic models of the Palau archipelago suggest that the isolated coral communities of the Rock Island bays are largely self-seeded and have high rates of larval retention but that some larval exchange may occur between lagoon and barrier reefs (Golbuu et al., 2012). If there is even a small degree of larval connectivity between these populations, any evolved trait that allows Rock Island corals to survive under low-Ω_{ar} conditions could be shared by barrier reef communities and may

explain the pH tolerance of barrier reef corals that do not currently experience low-Ω_{ar} conditions.

A review of all laboratory-based and in situ studies of *Porites* sensitivity to ocean acidification indicates this genus is, in general, not particularly tolerant to declining Ω_{ar}. Indeed, most laboratory experiments demonstrate significant declines in *Porites* calcification across ranges in Ω_{ar} comparable to those tested in this study (Albright et al., 2008; Anthony et al., 2008; DePutron et al., 2010; Iguchi et al., 2012; Ohde and Hossain, 2004). Field observations of calcification rates across natural acidification gradients in the Yucatan *ojos*, Eastern Tropical Pacific low-Ω_{ar} upwelling regions, and Maug CO_2 vent sites show similar responses to acidification (Crook et al., 2013; Enochs et al., 2015; Manzello et al., 2014) (Fig. 6). Of the ten other laboratory and field studies that explored massive *Porites* responses to Ω_{ar} across three or more treatments or acidification levels, eight found that *Porites* calcification was highly sensitive to changes in Ω_{ar} (Albright et al., 2008; Anthony et al., 2008; Crook et al., 2013; DePutron et al., 2010; Enochs et al., 2015; Iguchi et al., 2012; Manzello et al., 2014; Ohde and Hossain, 2004), while two found no sensitivity (Comeau et al., 2014a; Edmunds, 2012) (Fig. 6A). To quantitatively compare *Porites* acidification responses across all studies, standardized calcification anomalies were calculated for each experiment by subtracting the overall population calcification mean from each data point and dividing by the standard deviation. The overall calcification response curve constructed with standardized calcification anomaly data from all ten studies shows significant declines in *Porites* calcification with decreasing Ω_{ar} (generalized linear model, estimate = 0.48, p < 0.001). This general *Porites* sensitivity to Ω_{ar} was significantly different from the response observed in Palau *Porites* corals (generalized linear model, estimate = 0.41, p = 0.04), where standardized calcification anomalies showed no response to changes in Ω_{ar} (generalized linear model, estimate = 0.07, p = 0.72) (Fig. 6B).

There are several possible mechanisms by which corals can maintain calcification rates under low-pH conditions. Some studies have suggested that acidification-tolerant corals are able to use both bicarbonate and carbonate ions for calcification (Jury et al., 2010; Comeau et al., 2013). However, if this were the case, then calcification would increase under ocean acidification as bicarbonate ion concentrations rise. Elevated nutrient concentrations and heterotrophic feeding have also been shown to increase calcification rates of corals in low-pH conditions relative to low-nutrient/unfed corals (Holcomb et al., 2010; Edmunds, 2011; Drenkard et al., 2013; Towle et al., 2015). There is currently no compelling evidence that Palau corals have adopted this strategy, as nutrient levels do not vary across Palau's reef environments (Barkley et al., 2015). However, food availability and heterotrophic feeding levels are not known. Another hypothesized mechanism is that corals maintain the pH and Ω_{ar} of the extra-cellular calcifying medium despite low seawater pH (Ries, 2011; McCulloch et al., 2012; Venn et al., 2013; Vidal-Dupiol et al., 2013). This may occur through increased activity of Ca^{2+} ATPase, an enzyme concentrated in the calicoblastic epithelial cells that removes protons and elevates pH/Ω_{ar} of the coral's calcifying medium (Cohen and McConnaughey, 2003; Zoccola et al., 2004). Boron isotope analyses of two *Porites* corals each from the low-Ω_{ar} and high-Ω_{ar} reefs considered in this study suggest that this may be the case. On average, the two low-pH corals elevated their calcifying fluid pH by 0.51 units above that of the external seawater (pH = 7.84) to achieve an average calcifying pH of 8.35. Conversely, the two high-pH corals elevated their calcifying fluid pH by only 0.38 units above that of the external seawater (pH = 8.04) to achieve a calcifying pH of 8.42 (DeCarlo et al., 2016). Thus, the elevation of calcifying medium pH may be a likely mechanism by which corals in Palau's low-pH reefs are able maintain calcification rates equivalent to those living in high-pH reef environments.

Corals in the reciprocal transplant experiment showed extreme sensitivity to differences in the suite of environmental variables that change across reef types in Palau. Corals collected from a low-Ω_{ar} Rock Island reef and returned to the low-Ω_{ar} reef and corals collected from and

returned to a high-Ω_{ar} barrier reef had equivalent calcification rates and high survival rates after 17 months in the field. However, corals collected from one reef and then transplanted to the other had very low growth rates and very high mortality over the same time period, with only a small number of individuals alive at the end of the experiment. The high survival of control populations and the fact that most transplanted individuals were still alive after two months rule out the transplantation procedure itself as the primary cause of mortality. The poor growth and survival of both groups of transplanted corals are striking, as corals collected from the same initial reef populations demonstrated very high Ω_{ar}-tolerance in the laboratory CO_2 manipulation experiment. This implies that environmental variables other than Ω_{ar} (e.g. temperature, light, water flow, etc.), or alternatively, ecological factors (e.g. predation, competition), were responsible for the very low survival of transplanted corals. In addition, variables other than Ω_{ar} appear to have a stronger impact on coral health and growth. The reciprocal transplant, in which each population showed the highest fitness in its native environment, displays archetypical local adaptation (Reed and Martiny, 2007; Savolainen et al., 2013). Combined with the laboratory Ω_{ar} manipulation results, the reciprocal transplant thus supports an adaptive mechanism for acidification tolerance in Palau.

Results from the CO_2 manipulation experiment and reciprocal transplant suggest both that adaptive mechanisms are at play and that Palau *Porites* corals could maintain calcification rates with progressive ocean acidification. Calcification rates of *Favia* corals were similarly maintained across the natural acidification gradient in Palau, indicating that more than one genus may have evolved the capability to deal with pH stress (Barkley et al., 2015). However, while coral calcification rates may be insensitive to acidification in Palau, rates of coral skeletal bioerosion become significantly elevated as pH falls in Palau and will likely increase under future levels of acidification (Barkley et al., 2015; DeCarlo et al., 2015). In order for Palau's pH-tolerant coral reefs to survive ocean acidification over the next several decades, rates of calcification must continue to outpace those of bioerosion. For this to be the case, local management efforts must regulate any coastal threats – such as nutrient eutrophication – that can accelerate rates of bioerosion and alter the balance between reef accretion and dissolution (DeCarlo et al., 2015). If properly managed, Palau's unique acidification-tolerant coral reefs may be among the coral reef communities best able to persist under predicted changes in ocean chemistry over the course of the 21st century.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2017.01.003.

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