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Coral calcification mechanisms facilitate adaptive responses to ocean acidification

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Ocean acidification (OA) is a pressing threat to reef-building corals, but it remains poorly understood how coral calcification is inhibited by OA and whether corals could acclimatize and/or adapt to OA. Using a novel geochemical approach, we reconstructed the carbonate chemistry of the calcifying fluid in two coral species using both a pH and dissolved inorganic carbon (DIC) proxy ($\delta^{11}B$ and B/Ca, respectively). To address the potential for adaptive responses, both species were collected from two sites spanning a natural gradient in seawater pH and temperature, and then subjected to three pH_T levels (8.04, 7.88, 7.71) crossed by two temperatures (control, +1.5°C) for 14 weeks. Corals from the site with naturally lower seawater pH calcified faster and maintained growth better under simulated OA than corals from the higher-pH site. This ability was consistently linked to higher pH yet lower DIC values in the calcifying fluid, suggesting that these differences are the result of long-term acclimatization and/or local adaptation to naturally lower seawater pH. Nevertheless, all corals elevated both pH and DIC significantly over seawater values, even under OA. This implies that high pH upregulation combined with moderate levels of DIC upregulation promote resistance and adaptive responses of coral calcification to OA.

1. Introduction

Rising sea surface temperatures and ocean acidification (OA) are among the most serious threats facing coral reefs today [1]. Consequently, the many ecosystem services provided by coral reefs, such as coastal protection and the income generated via tourism, fisheries and other resources [2], are also at risk. As atmospheric CO_2 concentrations continue to increase, it is therefore critical to understand if and how tropical reef corals may be able to acclimatize and/or adapt to ocean acidification and warming.

In some locations, coral reefs already naturally experience lower pH levels and/or warmer seawater temperatures that are not expected to occur elsewhere until the middle or end of this century [3–6]. Such environments represent important analogues for future ocean conditions, and are well suited to assess the capacity of reef corals to acclimatize and/or adapt to warmer, more acidic conditions in their natural environment, as well as over more realistic time scales. One such location is Hawai'i's Kāne'ohe Bay where seawater already has a lower pH throughout the year (−0.1 to −0.2 pH units), and is warmer by +1–2°C in summer, compared to present-day offshore waters, or conditions on some other nearby reefs [7–9]. Conditions similar to those in Kāne'ohe Bay are not expected to occur on many other Hawaiian reefs until at least the middle of the century under high CO_2 emissions, or the end of the century under moderate CO_2 emissions [10]. Nevertheless, abundant coral reefs not only exist within Kāne'ohe Bay, but they have recovered from catastrophic human disturbance which reduced coral cover to only a few percent by the late 1970s [9], and did so under conditions of low pH and high temperature. Thus, the environmental history of Kāne'ohe Bay provides a unique opportunity to study whether

decade-long exposure to low-pH and high-temperature conditions has influenced the ability of corals to cope with the chronic and even more extreme ocean acidification and warming that is expected by the end of this century under high CO_2 emissions.

OA is the decrease of seawater pH, carbonate ion concentration and saturation state of aragonite (Ω_{arag}) due to uptake of atmospheric CO_2 by the surface ocean. In tropical reef corals, OA often results in lower calcification rates [11–13], although this is not always the case [12,14,15]. Despite many years of research, however, it remains poorly understood why coral calcification often decreases in response to OA, and why some corals are more resistant to OA than others. Deciphering the mechanisms underlying differential OA sensitivity is challenging, in part because corals precipitate their aragonite skeleton from an extracellular, semi-isolated space between their tissue and skeleton (the calcifying fluid) that is extremely difficult to access. Using techniques such as geochemical tracers (e.g. the boron isotope pH proxy), microsensors and pH-sensitive dyes, it is now well established that corals exert significant control over the chemical composition of their calcifying fluid. By exchanging H^+ for Ca^{2+} ions (or possibly other cations), corals significantly elevate the pH of their calcifying fluid (pH_{cf}) over the external seawater pH [16–20]. This capacity to upregulate pH_{cf} is maintained even under OA conditions, although pH_{cf} typically decreases under acidification as compared to ambient seawater pH levels [16,20,21]. However, pH_{cf} not only responds to changes in seawater pH, but also to total alkalinity and dissolved inorganic carbon (DIC) [22]. These workers showed that the chemistry in the calcifying fluid is influenced by more than one component of the carbonate system, and suggested that corals may regulate the exchange of multiple chemical species in order to maintain calcification [22]. Interestingly, some coral species upregulate pH_{cf} more than others [17,23] or even maintain high pH_{cf} independent of changes in external seawater pH [24,25]. Although high pH_{cf} is not always linked to greater resistance of calcification to OA [21], strong control over pH_{cf} generally appears to be a critical mechanism involved in coral calcification, and varying capacity to upregulate pH_{cf} may underlie differential resistance of coral calcification to OA.

Although characterization of internal pH upregulation has significantly advanced our understanding of coral calcification, a second parameter of the carbonate system is needed to calculate the saturation state of the calcifying fluid (Ω_{cf}), which almost certainly plays a critical role in the rate of precipitation of the coral skeleton. It is often assumed that corals also elevate DIC inside the calcifying fluid (DIC_{cf}) above seawater DIC values [17,26], and when combined with pH upregulation, this results in high Ω_{arag} values (approx. 10–25) that promote the relatively rapid calcification rates typical of tropical corals. This view has been supported by studies using skeletal B/Ca as a proxy for internal carbonate ion concentration ($[carb]_{cf}$) [21,27–29], but the ubiquity of elevated DIC_{cf} and $[carb]_{cf}$ was challenged by the first direct measurements of $[carb]_{cf}$ using microsensors [18]. Cai *et al.* [18] suggested that high Ω_{cf} can also be achieved without significantly elevating DIC_{cf} above seawater values, if pH_{cf} is also upregulated to a greater degree than reported in some previous studies. Such a strategy may be advantageous to cope with OA because fewer protons need to be transported to achieve a given pH level due to the lower buffer capacity of the calcifying fluid [18]. This discrepancy highlights the need to better understand DIC dynamics inside the calcifying fluid under both ambient and low pH conditions, and to examine the potential role of DIC upregulation as a mechanism that could confer resistance to OA in tropical corals.

Some corals have considerable capacity to acclimatize and/or adapt to OA (C.P.J. & R.J.T. 2017, unpublished data). Specifically, individuals of the reef-building Hawaiian coral *Porites compressa* from Kāne'ohe Bay, where seawater pH is 0.1–0.2 units lower compared to offshore, were significantly less sensitive to acidification and calcified significantly more under simulated OA conditions than conspecifics from a nearby high-pH site where the chemistry is more representative of present-day offshore waters [30]. In contrast, *Montipora capitata* corals from the same two locations had similar pH tolerance under OA, but generally calcified faster than *P. compressa* corals [30]. Although these findings highlight the adaptive capacity of reef corals, the specific calcification mechanisms that underlie greater resistance to OA and drive such adaptive responses remain unknown. Given the clearly demonstrated role of internal pH upregulation in facilitating coral calcification, and the potential importance of DIC upregulation for calcification as well, differing capacities to upregulate pH or DIC may play critical roles in facilitating coral acclimatization and/or adaptation to OA.

To elucidate the calcification mechanisms underlying the adaptive capacity of Hawaiian corals, we analysed coral skeletons from a combined ocean acidification and warming experiment to investigate internal pH and DIC upregulation (via skeletal boron isotopes and B/Ca ratios) in *P. compressa* and *M. capitata* corals collected from the low-pH site, Kāne'ohe Bay, and the nearby high-pH site, Waimānalo Bay. This is the first time that these novel geochemical techniques [29] have been applied to understand whether coral can improve their tolerance to OA and climate change based on their environmental history. These corals were maintained at three pH levels (8.04, 7.88, 7.71 $\pm$ 0.02; reported on the total hydrogen ion scale, pH_T) in combination with two temperature levels (ambient, +1.5°C $\pm$ 0.1°C). We hypothesized that corals with higher OA tolerance (i.e. *M. capitata* from both sites and *P. compressa* from Kāne'ohe Bay) upregulate internal pH and/or DIC more than corals with lower OA tolerance (i.e. *P. compressa* from Waimānalo Bay), enabling them to calcify faster and to better resist OA than the more sensitive corals.

2. Methods

(a) Coral collection sites

Coral branches from 11 to 12 colonies of *M. capitata* and *P. compressa* were collected from each of two different sites (spanning natural gradients in seawater chemistry and temperature) on O'ahu Island, Hawai'i, in October 2011. Kāne'ohe Bay corals were collected from reefs adjacent to the Hawai'i Institute of Marine Biology (HIMB) in Kāne'ohe Bay (21°26′6″ N, 157°47′12″ W). This site represents future ocean conditions due to naturally low pH (−0.15 to −0.20 pH units below offshore; mean pH_T approx. 7.85–7.90) and elevated temperatures (+1–2°C above offshore during the May–October warm season; mean monthly maximum temperature is 28.0°C versus 26.4–27.0°C in offshore waters, or on many Hawaiian reefs) [7–9,30]. Low pH at this site is the result of net calcification and net heterotrophy within Kāne'ohe Bay, whereas elevated temperature is the result of summer heating along with shallow water depth and long residence times [7–9,30]. Waimānalo Bay corals were collected offshore Kaiona

Beach Park (21°19′36″ N, 157°40′54″ W; 18 km to the southeast of Kāne'ohe Bay). This site experiences higher pH and lower temperature than at HIMB, and these values average close to present-day offshore conditions (mean pH_T approx. 8.05; mean monthly maximum temperature approx. 27.0°C) [31]. Daily variability in seawater temperature, pH_T and DIC is similar at the two sites, with a mean temperature range of approximately 2°C, a mean pH_T range of approximately 0.2 pH units and a mean DIC range of approximately 200 µmol kg^{-1}, respectively [32]. Seawater DIC and total alkalinity, however, are both slightly lower at the Kāne'ohe site (approx. 1940 µmol kg^{-1} and approx. 2150 µeq kg^{-1}, respectively) than at the Waimānalo site (approx. 2000 µmol kg^{-1} and approx. 2300 µeq kg^{-1}, respectively) due to high rates of net calcification within Kāne'ohe Bay [7,31].

(b) Experimental protocol

After collection, corals were allowed to recover and acclimate in an indoor, flow-through aquarium system at HIMB for 2.5 months prior to the start of the experiment in December 2011. During this time, they were maintained at 26–27°C and received seawater from Kāne'ohe Bay. This acclimation period in a common garden allowed us to exclude short-term environmental history as a factor in the observed responses to experimental treatments. Corals were maintained under experimental treatment conditions representing three pH_T levels (8.04, 7.88, 7.71 ± 0.02) crossed by two temperature levels (present-day offshore, +1.5°C ± 0.1°C) for a total of 14 weeks. During the first five weeks, corals were kept at seasonal maximum temperatures (control = 26.8°C, control +1.5°C = 28.3°C), followed by nine weeks at mean annual temperatures, which were 1.5°C lower than during the first temperature phase (control = 25.3°C, control +1.5°C = 26.8°C). Further details can be found in the electronic supplementary material. Electronic supplementary material, table S1 summarizes the environmental conditions of each treatment.

(c) Physiological analyses

Calcification rates were determined individually for each temperature phase using the buoyant weight technique [33] and normalized to weight at the beginning of the corresponding temperature phase. These phase-specific rates guided the sampling of skeletal material for geochemical analyses, and are used here to interpret the geochemical results because only the most recently deposited skeleton was targeted for geochemical sampling (see details below). However, normalizing calcification to weight rather than surface area does not affect the interpretation of our data since the choice of normalization had little impact on the calcification rate results. Therefore, the calcification rates shown here represent growth during the second temperature phase, where corals were maintained at either 25.3 or 26.8°C for nine weeks. These calcification rates are presented here for (i) all corals used in the experiment (n = typically 11–12 colonies per treatment) and (ii) only the subset of corals that were used for geochemical analyses (n = 2–9 colonies per treatment, though typically 5–6). Please see electronic supplementary material, table S1 for details on sample size per treatment.

(d) Geochemical analyses

Corals were stained with alizarin red (15 mg l^{-1} for 7 h) before the start of the experiment but not again at the beginning of the second temperature phase. Therefore, only the most recently deposited skeleton from this second temperature phase was targeted for geochemical sampling. This phase was much longer (nine versus five weeks), providing plenty of skeletal material that could be confidently sampled (guided by known calcification rates, see the electronic supplementary material for more details). This is an established sampling approach (e.g. [23,34]) that has previously been applied successfully to the same Hawaiian coral species as in this study [34]. The uppermost layer of the bleached and dried branch tips was then gently shaved with a diamond-tipped Dremel tool [23]. Boron isotopes ($\delta^{11}B$) and B/Ca ratios were analysed following the method of McCulloch *et al*. [35]. Further details regarding the geochemical analyses can be found in the electronic supplementary material.

(e) Carbonate chemistry of the calcifying fluid

The pH of the calcifying fluid was estimated using the coral skeletal boron isotopic composition following established methods [17,36]. Biological pH regulation (ΔpH) was then calculated as the difference between pH_{cf} and seawater pH [36]. Recently, it has also been shown that the coral skeletal B/Ca concentration can be used to constrain the DIC concentration of the coral calcifying fluid (DIC_{cf}) [29,37]. We therefore estimated the carbonate ion concentration within the calcifying fluid ($[CO_3^{2-}]_{cf}$) from measurements of both pH_{cf} and coral skeletal B/Ca [29]. Biological DIC-upregulation within the calcifying fluid was calculated as the ratio of DIC_{cf} and seawater DIC (DIC_{cf}/DIC_{sw}). Further details are given in the electronic supplementary material.

(f) Statistical analyses

Generalized linear mixed model (GLMM) analysis was used to test for the effects of species, site, pH and temperature on coral $\delta^{11}B$, pH_{cf}, ΔpH, B/Ca, DIC_{cf}, DIC_{cf}/DIC_{sw}, $[CO_3^{2-}]_{cf}$, Ω_{cf} and calcification rate of the two coral species. Parent colony was a random factor nested within both species and site. Tukey adjusted p-values were used for *post hoc* tests when main effects were significant. When a significant interaction was observed, multiple pair-wise comparisons were conducted using Tukey adjusted p-values. p-values less than 0.05 were considered significant. GLMM analyses were performed using SAS software, version 9.3 of the SAS System for Windows. Linear regressions were calculated for the relationship between pH_{cf} and seawater pH as well as DIC_{cf} using SigmaPlot version 12.5.

3. Results

(a) Internal pH and DIC upregulation

Using skeletal boron isotopes ($\delta^{11}B$) as pH proxies, corals from both species and sites elevated the pH of their calcifying fluid (pH_{cf}) above seawater pH levels (by up to 0.77 pH units), even under future ocean acidification scenarios (figure 1*a–f*). We found that $\delta^{11}B$ and pH_{cf} were nevertheless highly correlated with seawater pH (electronic supplementary material, figure S1*a*), but biological pH upregulation resulted in internal pH changes that were only 20–40% as large as the changes in external seawater pH (electronic supplementary material, figure S1*b*). However, $\delta^{11}B$ and consequently also pH_{cf} and biological pH upregulation (ΔpH) differed significantly between species, sites and pH treatments (figure 1*a–f*; electronic supplementary material, table S3). Higher pH_{cf} values were generally found for *M. capitata* compared with *P. compressa*, and Kāne'ohe Bay corals of both species upregulated pH_{cf} more than Waimānalo Bay corals (figure 1*c–f*). Furthermore, pH_{cf} was lowest at the lowest seawater pH (figure 1*c*,*d*), yet corals upregulated pH_{cf} the most at these lower seawater pH levels (figure 1*e*,*f*).

Coral skeletal B/Ca ratios also differed significantly between species and sites, and among pH treatments (figure 2*a*,*b*; electronic supplementary material, table S4). Again, *M. capitata* had generally higher B/Ca than *P. compressa*, and Kāne'ohe Bay corals from both species had higher B/Ca

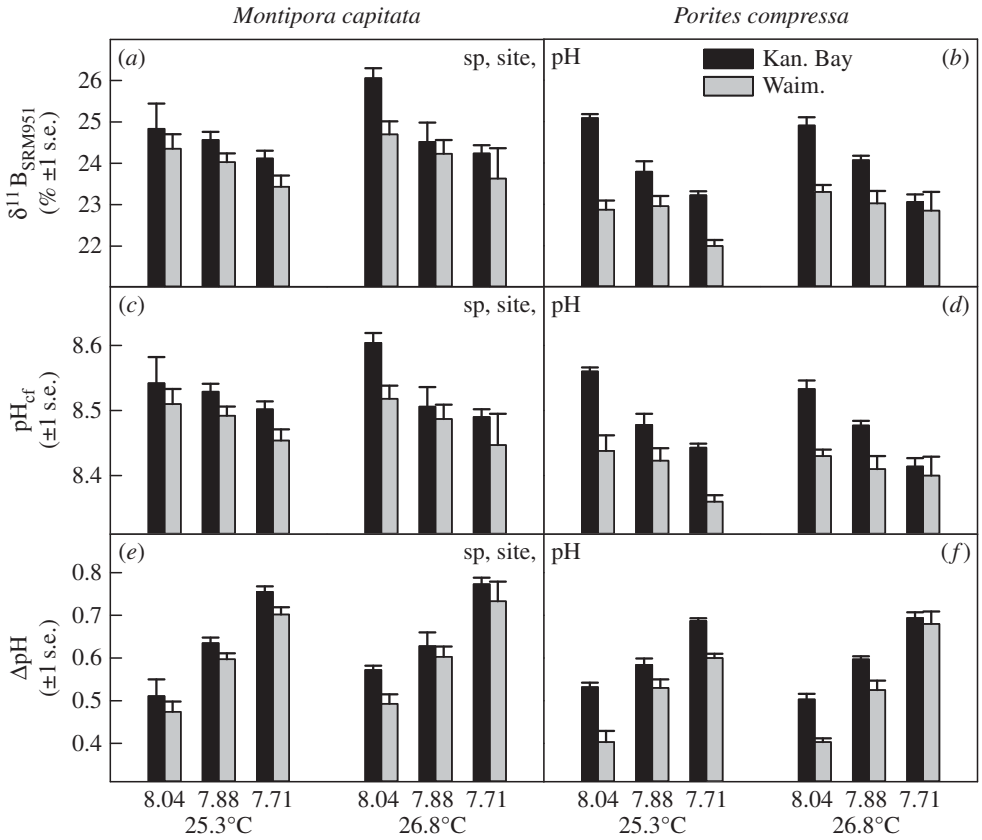

Figure 1. Coral skeletal boron isotopes ($\delta^{11}B$) (*a*,*b*), calcifying fluid pH (pH_{cf}) (*c*,*d*) and biological pH upregulation (ΔpH) (*e*,*f*) in *M. capitata* and *P. compressa* from Kāne'ohe Bay and Waimānalo Bay, Hawai'i. Mean ± 1 s.e. are shown. Significant species (sp), site, pH and temperature (temp) effects are indicated when present (electronic supplementary material, table S3).

than Waimānalo Bay corals. The lowest B/Ca values were observed at medium and low pH treatments. When calcifying fluid DIC concentrations (DIC_{cf}) and internal DIC upregulation (DIC_{cf}/DIC_{sw}) were estimated from B/Ca and pH_{cf}, all corals were found to significantly upregulate DIC_{cf} above seawater DIC values (by a factor of 1.6–2.1×) (figure 2*c*–*f*).

However, for both DIC_{cf} and DIC_{cf}/DIC_{sw}, significant interactive effects between species and site as well as species and pH were observed (figure 2*c*–*f*; electronic supplementary material, table S4). *Porites compressa* from Waimānalo Bay had higher DIC_{cf} and thus higher DIC_{cf}/DIC_{sw} than conspecifics from Kāne'ohe Bay, which in turn had higher DIC_{cf} and DIC_{cf}/DIC_{sw} than *M. capitata* from Waimānalo Bay. The lowest DIC_{cf} and DIC_{cf}/DIC_{sw} values were observed in *M. capitata* from Kāne'ohe Bay. Regarding interactive effects between species and pH, *P. compressa* at low and medium seawater pH had the highest DIC_{cf} and DIC_{cf}/DIC_{sw} values, respectively, whereas *M. capitata* at high and low seawater pH had the lowest such values. Additionally, DIC_{cf} and DIC_{cf}/DIC_{sw} were also significantly affected by temperature, with generally greater values observed at 25.3 than at 26.8°C (electronic supplementary material, table S4). The pH_{cf} values were inversely correlated with DIC_{cf} values for all species and sites, although *P. compressa* had much higher R^2 values than *M. capitata* (electronic supplementary material, figure S2).

(b) Calcifying fluid saturation state and calcification rates

Since all corals elevated DIC_{cf} significantly above seawater values, the carbonate ion concentration ($[CO_3^{2-}]_{cf}$) and aragonite saturation state of the calcifying fluid (Ω_{cf}) were also elevated significantly above seawater values (figure 3*a*–*d*), with Ω_{cf} reaching values between 13.3 and 17.0. For both $[CO_3^{2-}]_{cf}$ and Ω_{cf}, a significant interaction between species, site and pH was observed (electronic supplementary material, table S5): *P. compressa* corals from Kāne'ohe Bay at high seawater pH had the highest values, whereas *M. capitata* from Waimānalo Bay at low seawater pH had the lowest values (figure 3*a*–*d*).

Calcification rates obtained using the buoyant weight method differed significantly between species and sites, but not among pH or temperature treatments (figure 3*e*,*f*; electronic supplementary material, table S5) for the subset of corals examined for geochemistry. *Montipora capitata* generally calcified more than *P. compressa*, and Kāne'ohe Bay corals calcified more than corals from Waimānalo Bay. When calcification rates were analysed for all corals in the experiment, rather than just the subset analysed for geochemistry, a significant interactive effect between pH and temperature was also observed (in addition to the same species and site effects as above): corals calcified fastest at high seawater pH and 25.3°C, and slowest at the same temperature combined with low seawater pH (figure 3*g*,*h*; electronic supplementary material, table S5). Notably, only the 25.3°C/pH = 7.71 treatment resulted in negative calcification rates, or net dissolution, in *P. compressa* corals from Waimānalo Bay (figure 3*f*,*h*).

4. Discussion

(a) Drivers of long-term acclimatization and/or adaptation to ocean acidification

Corals that originated from Kāne'ohe Bay (where seawater is 1–2°C warmer in summer and pH is 0.1–0.2 units lower

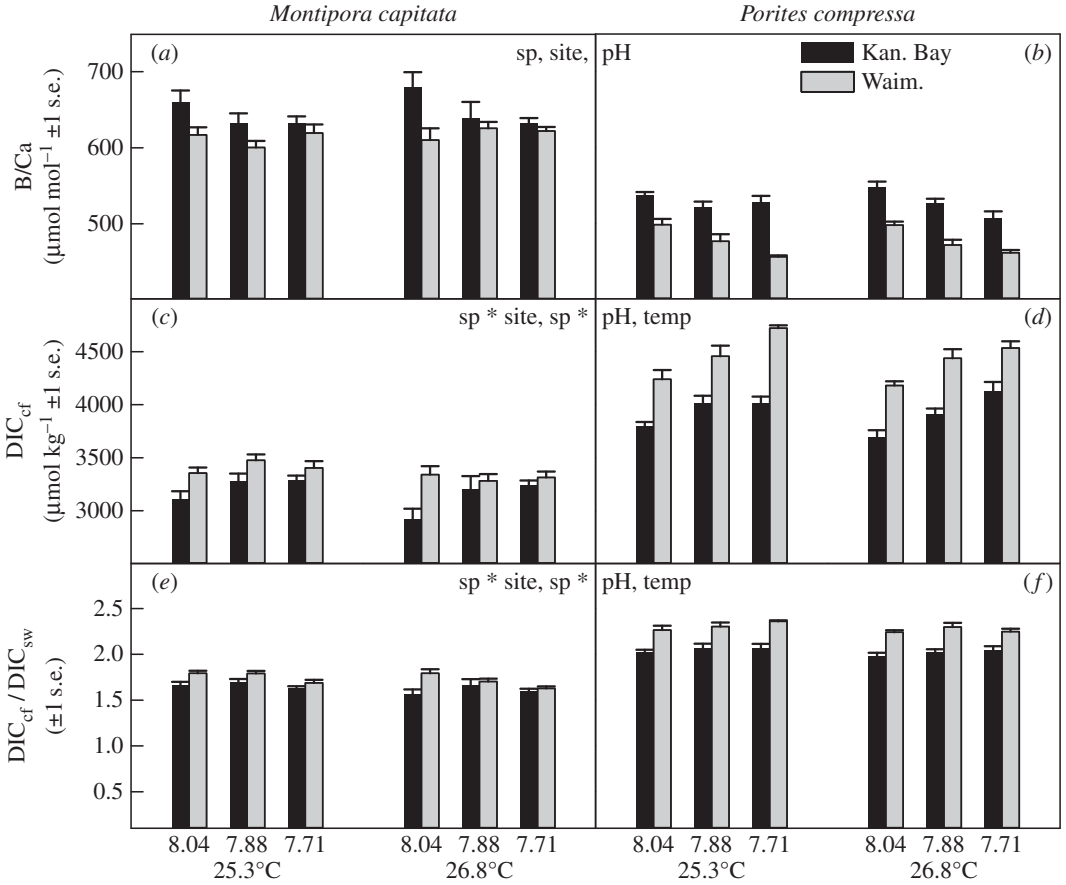

Figure 2. Coral skeletal B/Ca ratios (*a*,*b*), calcifying fluid DIC concentration (DIC_{cf}) (*c*,*d*) and biological DIC upregulation (DIC_{cf}/DIC_{sw}) (*e*,*f*) in *M. capitata* and *P. compressa* from Kāne'ohe Bay and Waimānalo Bay, Hawai'i. Mean $\pm$ 1 s.e. are shown. Significant species (sp), site, pH and temperature (temp) effects are indicated when present (electronic supplementary material, table S4).

compared with offshore) calcified faster and significantly more under simulated OA conditions than conspecifics from Waimānalo Bay, a nearby high-pH site where water chemistry and temperature are more similar to present-day offshore waters (figure 3). Interestingly, this resistance to acidification was consistently linked to a combination of higher pH_{cf} and lower DIC_{cf} in Kāne'ohe Bay corals compared to Waimānalo Bay corals (figures 1 and 2). This pattern was particularly evident regarding differences in DIC_{cf}, as demonstrated by the significant interactive effects between species and pH, and species and site (electronic supplementary material, table S4). Although similar interactive effects were not observed for calcification rates, they nevertheless mirrored trends in DIC_{cf}, with highest rates being correlated with lowest DIC_{cf} (figures 2*c*,*d* and 3*e*–*h*).

These observations suggest that combining high pH_{cf} and moderately elevated DIC_{cf} is a critical calcification mechanism underlying the potential for corals to mount adaptive responses under OA and warming. By modulating the interactive dynamics of pH and DIC upregulation within their calcifying fluid, these corals have a significant ability to acclimatize and/or adapt to OA by modifying the physiological processes involved in calcification. Since all corals were given 2.5 months to acclimate in a common garden prior to the start of the experiment, any fast-acting mechanisms of pH acclimatization should have been exhausted before the start of the experiment, leaving long-term acclimatization (i.e. physiological changes which operate over a time scale of greater than 2.5 months) or local adaptation (i.e. fixed, heritable differences among coral genotypes) as possible explanations for the distinct responses to acidification we observed. Although rapid mechanisms involved in coral temperature acclimation can occur within a few weeks [38,39], previous studies have failed to find any evidence that corals acclimatize to acidification even after 1 year of exposure [13,20,40], suggesting that local adaptation may be the primary driver of the differences in OA tolerance shown here. It is also important to consider that low pH may or may not be the ultimate driving force behind the differential calcification rates and OA resistance we observed. Instead, it is possible that separate environmental factors, such as elevated temperature, have selected for fast-growing, pH-tolerant corals in Kāne'ohe Bay, and that their enhanced OA resistance is a by-product of selection due to other factors. Whether or not low pH is the primary agent leading to increased OA resistance among these corals, this study is the first to detail mechanisms which allow corals to mount adaptive responses to acidification.

Despite clear site-specific differences in pH and DIC upregulation, both coral species significantly elevated their internal pH_{cf} and DIC_{cf} above seawater values (figures 1 and 2; electronic supplementary material, figure S1). pH_{cf} values were elevated above seawater pH by up to 0.77 pH units, supporting the idea that biological pH upregulation is a universal mechanism employed for calcification in scleractinian corals [16–20,24,25,36]. Similarly, our study confirms that internal pH_{cf} typically decreases linearly under OA, albeit with a much lower sensitivity (20–40%) because pH upregulation increases under such conditions (figure 1*e*,*f*; electronic supplementary material, figure S1) [16,17,20]. Remarkably, this relationship holds true for most coral species studied to date,

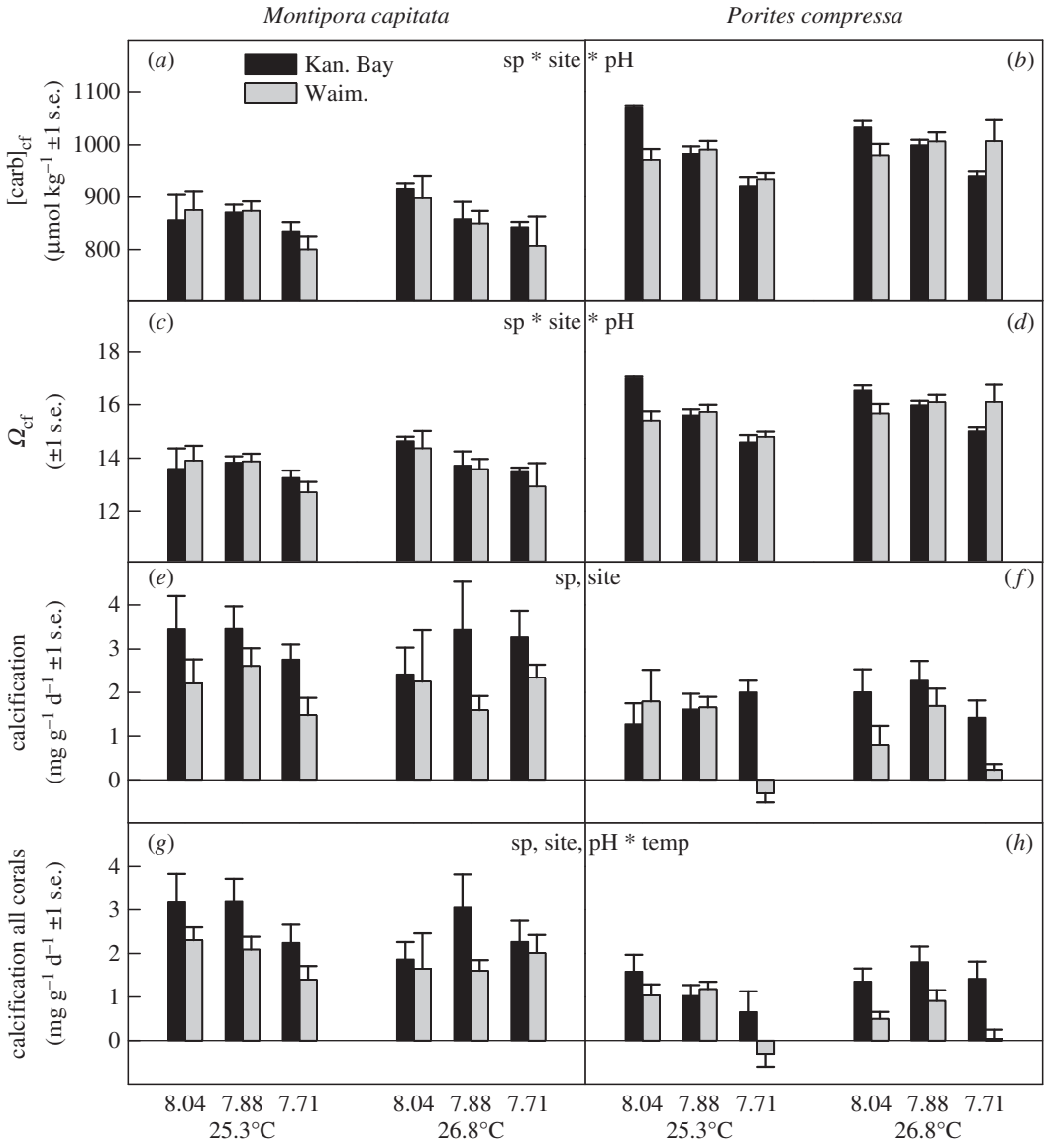

Figure 3. Calcifying fluid carbonate ion concentration ($[carb]_{cf}$) (*a*,*b*), calcifying fluid aragonite saturation state (Ω_{cf}) (*c*,*d*), calcification rates for corals used for geochemical analyses (*e*,*f*) and calcification rates for all corals in the experiment (*g*,*h*) in *M. capitata* and *P. compressa* from Kāne'ohe Bay and Waimānalo Bay, Hawai'i. Mean ± 1 s.e. are shown. Significant species (sp), site, pH and temperature (temp) effects are indicated when present (electronic supplementary material, table S5).

except for *Porites* corals from a highly variable reef flat and natural CO_2 vents [24,25].

Our study is among the first to infer internal DIC_{cf} concentrations for tropical reef corals, and the first to assess how long-term acclimatization and/or adaptation to naturally lower seawater pH and higher summer temperatures modulate the response of DIC_{cf} to OA. In *M. capitata* and *P. compressa*, DIC_{cf} concentrations ranged from approximately 3300–4200 μmol kg^{-1} and were thus approximately 1.6–2.2 times higher than seawater DIC, respectively (figure 2*e*,*f*). These results are generally consistent with other boron-based studies showing that corals significantly elevate DIC_{cf} above seawater concentrations [21,28,29]. However, our findings contradict recent microsensor measurements of the calcifying fluid showing carbonate ion concentrations of 600–1500 μmol kg^{-1} and DIC_{cf} values close to seawater [18]; in addition, our estimates give much narrower species-specific values that co-vary (figures 2 and 3). Given the spatially dependent sensitivity of microsensor studies and the inability to conduct pH and carbonate ion microsensor profiles simultaneously within the same pocket of calcifying fluid [18], direct comparisons between microsensor and stable isotope studies are challenging, at best. Importantly, our findings are in strong contrast to the study of Allison *et al*. [27], which estimated that DIC_{cf} would be less than half that of seawater DIC if borate is assumed to substitute for carbonate ions during mineral precipitation. Instead, the authors argued that in order to make sense of their geochemical data, borate must substitute for bicarbonate ions or a combination of bicarbonate and carbonate ions in the crystal lattice, which is then followed by recrystallization of the calcium bicarbonate into aragonite, but without boron exchange occurring during this process. A more parsimonious explanation is that borate substitutes for carbonate ions alone during mineral precipitation, as we assume here, and that the discrepancy between these two studies can now be explained by the order of magnitude difference between the experimentally established partitioning coefficient used in our study and the one used by Allison *et al*. [27].

The observed correlation between high calcification rates, high pH_{cf} and relatively low DIC_{cf} in Kāne'ohe Bay corals provides novel insights into the calcification mechanisms underlying coral resistance to acidification and informs our

understanding of the adaptive capacities of corals under OA and climate change. Clearly, maintaining high pH_{cf} is favourable for calcification as it promotes the conversion of bicarbonate ions and carbon dioxide to carbonate ions, resulting in a high saturation state within the calcifying fluid. The advantages of maintaining only moderately elevated DIC_{cf} under acidification, however, are less clear because higher DIC_{cf} levels would result in a proportional increase in saturation state at a given pH. However, if moderately elevated DIC_{cf} is combined with higher pH_{cf}, as observed in *M. capitata*, this also results in high saturation states. Such a strategy can be advantageous because compared with a high DIC_{cf} /low pH_{cf} scenario, it is less energetically expensive to maintain a given pH_{cf} at lower DIC_{cf} because the lower buffer capacity of the fluid requires the removal of fewer protons to achieve that pH. Consequently, a low DIC_{cf}/high pH_{cf} scenario may have allowed Kāne'ohe corals to grow faster than Waimānalo Bay corals, especially if the energy savings were invested in other key processes, such as the production of organic matrix or proton excretion from the coelenteron. Cai *et al*. [18] already pointed out that a lower DIC_{cf} scenario may be particularly advantageous under OA if proton removal from the coelenteron becomes increasingly difficult due to rising seawater proton concentration [41]. Interestingly, lower sensitivity of calcification to OA was also linked to lower DIC_{cf} values in *Pocillopora damicornis* from subtropical Western Australia, though this species also maintained lower pH_{cf} than OA-sensitive *Acropora yongei* from the same location [21]. The low DIC_{cf}/high pH_{cf} scenario could also have drawbacks because the lower buffering capacity in this scenario also means that pH upregulation is more vulnerable to changes in the physiological and biochemical conditions that control proton pumping [18]. Alternatively, it is also possible that DIC transport is rate limited, such that lower DIC_{cf} may be a by-product of higher calcification rates, rather than a driver of them. Consequently, fast calcification rates would result in low DIC_{cf} due to the more rapid depletion of DIC within the calcifying fluid. While our findings show that the low DIC_{cf}/high pH_{cf} scenario is clearly linked to adaptive responses and resistance to OA, the underlying reasons remain currently poorly understood. Further research into the sources, pathways and energetic requirements of DIC_{cf} transport is required to resolve this.

(b) Drivers of species-specific calcification resistance to ocean acidification

A growing body of literature shows that some coral species are naturally more resistant to OA than others [12,15]. The mechanisms underlying resistance to OA in these species, however, are poorly understood, although factors such as calcification rate or growth form may play a role [42]. In our study, branching *M. capitata* generally calcified faster and also maintained growth better under OA conditions than branching *P. compressa* (figure 3). As already observed for Kāne'ohe Bay corals, the high calcification rates of *M. capitata* were linked to high pH_{cf} and moderately elevated DIC_{cf}, whereas the lower calcification rates of *P. compressa* were linked to lower pH_{cf} but higher DIC_{cf} compared with *M. capitata* (figures 1–3). This suggests again that high pH in combination with moderate DIC upregulation promotes faster growth and resistance to OA.

It should be noted that Ω_{cf} was somewhat higher in *P. compressa* (approx. 15–17) than in *M. capitata* (approx. 13–15), yet the latter species calcified faster. The higher calcification rates observed under lower Ω_{cf} are counterintuitive, given that the rate of abiotic mineral precipitation is proportional to the saturation state. Even more counterintuitive is the finding that *P. compressa* corals from Waimānalo had negative calcification rates at 25.3°C and a pH of 7.71 (figure 3*f*,*h*), yet nevertheless maintained high Ω_{cf} values of approximately 15 (figure 3*d*). Similar maintenance of high Ω_{cf} despite impaired growth rates has also been observed in severely heat-stressed corals [28]. The apparent decoupling between calcification rates and Ω_{cf} in more than just this treatment points to the importance of additional, biological factors for mediating coral calcification. This includes the production and delivery of organic matrix molecules [43], which can induce aragonite precipitation *in vitro* at Ω_{arag} ~1.3 [44]. Particularly in OA-sensitive species such as *P. compressa*, it may be that low pH impairs calcification primarily via disruption of processes such as organic matrix production, while maintenance of high Ω_{cf} appears unaffected. Alternatively, OA may reduce net coral calcification indirectly by increasing rates of carbonate dissolution in previously deposited skeleton. Indeed, many *P. compressa* from Waimānalo showed axial growth well beyond the alizarin stain line under the lowest pH, allowing us to sample these corals for geochemical analyses, and yet they also experienced a net decrease in skeletal mass over time.

Our study confirms previous findings that corals with high resistance to OA also have high pH_{cf} levels, and that pH_{cf} is only moderately responsive to external seawater pH [24,25]. For example, massive *Porites* corals exposed to a natural gradient of seawater pH at the CO_2 seeps in Papua New Guinea maintained high internal pH_{cf} down to seawater pH levels expected by the end of this century, and pH_{cf} decreased slightly only when seawater pH declined further [25]. Calcification estimates derived from coring these *Porites*, as well as a calcification model, suggest that their growth will not be affected by OA until at least end-of-century conditions [3,25]. Similarly, *Porites cylindrica* from the highly dynamic Heron Island reef flat maintained high pH_{cf} and skeletal growth independent of large fluctuations in external seawater pH or superimposed OA treatments [24]. Although these studies highlight the importance of biological pH upregulation, they were not able to fully constrain the carbonate chemistry of the calcifying fluid using a second parameter, as we do here with DIC_{cf}. It is unknown if these other, acidification-resistant coral species also maintain moderately elevated DIC_{cf} concentrations. Our study is therefore among the first to demonstrate that coral resistance to OA is a complex interplay between pH and DIC upregulation.

It is important to note here that our findings are potentially biased towards more stress-resistant genotypes due to the strict criteria that we imposed while selecting corals for geochemical analyses (see electronic supplementary material, methods). These criteria were imposed as quality control during the sampling process but may also have resulted in selecting more stress-resistant genotypes. However, the same criteria were applied to all corals so that the observed differences between species, sites and treatments are nevertheless valid. Moreover, our robust samples sizes in most treatments (electronic supplementary material, table S1) ensured that intraspecific diversity was still present, with both more and less stress-resistant genotypes present in each treatment.

(c) Implications for the future of coral reefs

Our findings demonstrate that as oceans continue to warm and acidify, coral pH and DIC upregulation will largely be unaffected by small increases in temperature (within the seasonal range). However, both *a priori* resistance and long-term acclimatization and/or adaptation of coral calcification to OA seem to be facilitated by achieving high pH_{cf} yet only moderately elevated DIC_{cf}. We demonstrate here that reef corals have a significant capacity to adjust their calcification strategies in response to long-term exposure to naturally lower seawater pH and warmer summer temperatures, as occurs naturally on the reefs of Kāne'ohe Bay. Therefore, shifts to higher pH_{cf} yet only moderately elevated DIC_{cf} are probably also possible in response to OA and warming occurring over the coming decades, potentially allowing at least some corals to develop resistance to OA and maintain calcification rates in the future.

Data accessibility. All data from this manuscript will be submitted to the Biological and Chemical Oceanography Data Management Office (BCO-DMO) of the U.S. National Science Foundation after publication.

Authors' contributions. C.P.J. and R.J.T. designed the experiment. C.P.J. conducted the experiment and physiological analyses. V.S. conducted the geochemical analyses and analysed the data. All authors were involved in the preparation of the paper.

Competing interests. We have no competing interests.

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