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**Resistance to ocean acidification in coral reef taxa is not gained by
acclimatization**

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Abstract

Ocean acidification (OA) is a major threat to coral reefs, which are built by calcareous species. However, long-term assessments of the impacts of OA are scarce, limiting the understanding of the capacity of corals and coralline algae to acclimatize to high pCO₂ levels. Species-specific sensitivities to OA are influenced by its impacts on chemistry within the calcifying fluid (CF). Here, we investigate the capacity of multiple coral and calcifying macroalgal species to acclimatize to elevated pCO₂ by determining their chemistry in the CF during a year-long experiment. We found no evidence of acclimatization to elevated pCO₂ across any of the tested taxa. The effects of increasing seawater pCO₂ on the CF chemistry were rapid and persisted until the end of the experiment. Our results show that acclimatization of the CF chemistry does not occur within one year, which confirms the threat of OA for future reef accretion and ecological function.

Changes in seawater carbonate chemistry caused by OA generally lead to decreasing calcification of marine organisms¹ as well as increased dissolution of existing calcium carbonate structures². The magnitude of responses to OA are species-specific and can even be intra-specific in reef taxa, although some species exhibit significant resistance to OA^{3–5}. The physiological mechanisms responsible for declines in calcification in corals and calcifying algae due to OA are, however, still unclear. Short-term experiments on small numbers of species have limited potential to assess whether calcifying organisms can acclimatize to changes in seawater carbonate chemistry, or alternatively, whether species-specific differences in calcification physiology dictates the overall magnitude of responses.

Insights into changes in calcification mechanisms can be gained by investigating the response of carbonate chemistry in the calcifying fluid (CF) from which the calcium carbonate is precipitated⁶. Corals exert a strong control on their CF chemistry, with pH in the CF (pH_{cf}) maintained above seawater $\text{pH}^{7–9}$. Similarly, the dissolved inorganic carbon (DIC) is concentrated in the CF by ~ 1.5 – 2 -fold above seawater DIC^{10–13}. As a result, the saturation state within the CF is ~ 3 – 4 times greater than the surrounding seawater, which, in association with the production of organic matrices, favours the precipitation of calcium carbonate in the CF. While less data on the calcification physiology of tropical calcifying algae are available¹⁴, the few studies on coralline algae also show that the response of their CF chemistry to seawater carbonate chemistry is similar to that of corals^{10,15,16}.

Reduced seawater pH can decrease pH_{cf} , though pH_{cf} always remains above seawater pH⁷. However, the magnitude of the effect of seawater pH on pH_{cf} is species-specific^{7,17}. In contrast, DIC_{cf} increases with temperature and declining seawater pH^{17,18}. Again, the effects on DIC_{cf} are also species-specific¹⁷. The use of laboratory studies to estimate the response of organisms to climate change *in situ* has been challenged, and this is particularly true for assessments of how climate change could modify carbonate chemistry in the CF¹¹. Massive *Porites* exhibit, for example, strong seasonal variation in CF chemistry *in situ*, where pH_{cf} varies by 0.2 units, and DIC_{cf} by 20–25% in one year^{11,19}. It was proposed that such effects of seasonal variability in CF chemistry could be masked during laboratory experiments if constant temperature and daily doses of light are employed¹¹.

Long-term OA experiments (several months to years) are rare²⁰, but they are necessary to rule out short-term responses to treatments, and to determine whether

acclimatization to such conditions is possible. Outside of laboratory experiments, field observations at naturally acidified sites such as CO₂ seeps have been used to provide valuable information on the capacity of organisms to acclimate or adapt (if locally self-recruiting) to OA^{21,22}. The survival and high abundance of massive *Porites* sp. corals near CO₂ seeps has been associated with their capacity to maintain elevated pH_{cf}²³. However, it is difficult to draw conclusions from studies made at CO₂ vents alone because those sites experience huge sporadic variations in pH (up to 1 unit) that are not representative of the future conditions expected on most reefs^{15,24}.

The present study was designed to test the year-long effects of OA, in outdoor flumes²⁵, on the CF chemistry in the four corals *Pocillopora verrucosa*, *Psammocora profundacella*, *Acropora pulchra* and massive *Porites* spp. (called *Pocillopora*, *Psammocora*, *Acropora*, and *Porites* thereafter) and two calcifying algae *Lithophyllum kotschyannum* and *Halimeda minima* (*Lithophyllum* and *Halimeda* thereafter). We assessed the calcification and CF responses of these organisms exposed to four different pCO₂ conditions over one year (2 months for *Halimeda*). We were able to maintain realistic flow conditions, natural irradiance, and stable pCO₂ by using outdoor flumes²⁶. Two non-exclusive hypotheses were tested: 1) if acclimatization occurs, corals and calcifying algae would have the capacity to attain conditions of carbonate chemistry in the CF comparable to the control after one year of exposure. This would be the case if carbonate chemistry in the CF is initially affected by OA, but then returns to the level of the control during the one-year experimental period. 2) Seasonal variations in light and temperature would affect the response of the CF chemistry to OA. This would occur if carbonate chemistry in the CF varies seasonally as a function of light and temperature in a similar manner in all treatments.

Experimental conditions

Over the year-long incubation, pCO₂ treatments remained relatively stable. Overall, the average monthly mean pCO₂ values of the treatments were 356 ± 27 µatm, 561 ± 22 µatm, 714 ± 34 µatm, and 1053 ± 43 µatm for the four treatments (mean ± SE, n = 12 for all). The range of mean seawater temperatures across all four communities was 25.55– 29.37 °C, which reflected the seasonal mean at the backreef of Moorea. Mean seawater temperature integrated over the year of incubation did not

vary greatly between flumes (maximum 0.16°C between two flumes). Mean monthly daytime PAR varied seasonally from a minimum in May ($219 \pm 8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) to a maximum in November ($478 \pm 77 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Monthly A_T mean values across the flumes did not differ (from a minimum of 2286.3 ± 11.9 to a maximum of $2294.5 \pm 8.6 \mu\text{mol kg}^{-1}$ in the 561 and 714 μatm flumes, respectively) and remained similar to the total alkalinity of the back reef of Moorea.

Calcification and calcifying fluid chemistry

At the end of the 1-year incubation, calcification of *Pocillopora* and *Porites* was not affected by the treatments (Fig. 1), while conversely the calcification of *Psammocora* and *Acropora* decreased linearly with pCO_2 (slope p-value = 0.022 and 0.039, respectively, Table S1). Calcification of *Lithophyllum* also decreased linearly with pCO_2 (slope p-value = 0.032, Table S1).

pH_{cf} of the four corals exhibited contrasting responses to the treatment after 1 year. There was no significant linear relationship between pH_{cf} or DIC_{cf} and pCO_2 for *Porites*, whereas in the three other corals (*Psammocora*, *Pocillopora*, and *Acropora*) there were significant linear relationships between pH_{cf} and the pCO_2 (slope p-value < 0.01, Table S1, Fig. 2). DIC_{cf} responded in the opposite direction of pH_{cf} , with higher DIC_{cf} in the high pCO_2 treatments in these three corals (Fig. 3) (slope p-values = 0.008, 0.030, and 0.007; Table S1).

For both algae, pH_{cf} decreased linearly with increasing pCO_2 (slope p-value = 0.012 and < 0.001; for *Lithophyllum* and *Halimeda*, respectively; Table S1, Fig. 2).

There were no consistent linear relationships between the aragonite Ω_{cf} and pCO_2 in three of the four corals (*Pocillopora*, *Acropora*, and *Porites*, Fig. S1, Table S1). However, there was a decrease of Ω_{cf} with increasing pCO_2 in *Psammocora* (Table S1, Fig. S1). $\text{Ca}^{2+}_{\text{cf}}/\text{Ca}^{2+}_{\text{sw}}$ was only affected by the pCO_2 in *Pocillopora* (Fig. 4) (slope p-value = 0.009, Table S1) with maximum $\text{Ca}^{2+}_{\text{cf}}/\text{Ca}^{2+}_{\text{sw}}$ measured in the 1053 μatm treatment.

Seasonal variation in calcifying fluid composition

Seasonal variations in calcifying fluid composition were determined on the two corals *Acropora* and *Porites*. These corals were selected because they exhibit

contrasting morphologies and different potential sensitivities to ocean acidification. In *Acropora*, pH_{cf} was consistently higher in the 356 μ atm treatment (Fig. 5), with a significant effect of the treatment (ANOVA, $p < 0.001$). There was also a significant effect of treatment (ANOVA, $p = 0.045$) and time ($p = 0.023$) on Ω_{cf} that was driven by slightly higher Ω_{cf} in the lowest pCO₂ treatment, particularly at the end of the incubation (Fig. S2). In contrast, DIC_{cf} (ANOVA, $p = 0.096$) and Ca²⁺_{cf} / Ca²⁺_{sw} ($p = 0.107$) were not affected by the treatment, but were affected by time (both $p < 0.001$) (Fig. 5 and S2). Seawater temperature and the mean daily light did not affect pH_{cf} and DIC_{cf}, while the mean daily light had a positive effect on Ca²⁺_{cf} / Ca²⁺_{sw} and Ω_{cf} (Table S2).

For *Porites*, there was no significant effect of the treatment on pH_{cf} (ANOVA, $p = 0.773$), DIC_{cf} ($p = 0.835$), Ω_{cf} ($p = 0.711$) and Ca²⁺_{cf}/Ca²⁺_{sw} (Fig. 5 and S2). There was a seasonal trend with the lowest pH_{cf}, and the highest DIC_{cf}, values recorded during the austral winter (Fig 5). This was driven by a negative effect of temperature and light on pH_{cf} and positive effect of these parameters on DIC_{cf} (Table S3).

Discussion

During our year-long experiment we found no evidence of acclimatization to elevated pCO₂ across four coral and two calcifying algal species. If acclimatization occurred, we would have expected carbonate chemistry in the calcifying fluid to be initially affected by the treatments, then return to levels comparable to that of the controls after one year. Rather, we found that the responses to pCO₂ were species-specific, occurred rapidly, and persisted over one year. Three of the four coral species and the two macroalgal species exhibited a decrease in pH_{cf}, and an increase in DIC_{cf} for the corals, when seawater pCO₂ was increased. This is similar to what has been found previously during shorter term experiments^{17,18}. In contrast, the chemistry in the calcifying fluid of *Porites* did not respond to pCO₂. The two species whose calcification was unaffected by OA were from different genera (*Pocillopora* and *Porites*), and exhibited different morphologies (branching vs massive). These results confirm the difficulty in predicting species-specific responses to ocean acidification based on functional traits^{3,27}. Additionally, the calcifying fluid chemistry of both species exhibited different responses to increased seawater pCO₂, with *Porites* maintaining homeostatic conditions with changing pCO₂ and *Pocillopora*

compensating a decrease in pH_{cf} by increasing $[\text{Ca}^{2+}]_{\text{cf}}$. It is these different mechanisms of maintaining favourable CF chemistry that enable constant calcification, observed here and in our previous work¹⁰ (Fig. 6).

Two lines of evidence demonstrate the lack of acclimatization of the chemistry at the site of calcification to external changes in seawater. First, pH_{cf} measured in the corals at the end of the experiment exhibited the commonly described decreases in pH_{cf} with seawater pH in three out of four taxa^{7,28}. The similar relationship between pH_{cf} and seawater pH found in the macroalgae also matched previous results from shorter-term laboratory studies^{10,15,16}. Second, the effects of seawater pCO_2 on the CF chemistry were consistent across time and varied in a similar manner in all treatments for *Acropora* and massive *Porites* sampled at regular intervals (every 3 and 4 months, respectively). Our results therefore suggest that seawater carbonate chemistry acts relatively quickly to alter CF chemistry and that this effect is maintained after one year. Hence, one year of exposure to the different treatments did not improve or worsen the capacity of five of the six corals and macroalgae investigated to up-regulate their proton export²⁹ to maintain pH_{cf} at ambient levels.

DIC_{cf} also displayed a lack of acclimatization. The inverse relationship between DIC_{cf} and seawater pH (and therefore pH_{cf}) found at the end of the experiment has been previously shown both during shorter term experiments lasting only a few weeks or months¹⁰ and during *in situ* seasonal observations^{11,30,31}. Similar trends were also found in *Goniopora* sp. exposed for 6 months to variable and constant pH¹⁴. Together, this identifies seawater carbonate chemistry as the main driver of this inverse relationship in numerous corals. It also indicates that the ability to increase DIC_{cf} as seawater pH decreases under ocean acidification could be a common feature in many coral genera to limit its negative effects on the precipitation of calcium carbonate.

Chemistry in the CF of *Porites* did not respond to seawater pCO_2 from the start to the end of the experiment. This result suggests that the capacity of some corals to control pH_{cf} and DIC_{cf} independently of external seawater conditions (i.e massive *Porites*) is an inherent characteristic. Observations on *Porites cylindrica* also showed that this species was able to maintain a constant pH_{cf} when exposed to low pH during a 6-month *in situ* free ocean CO_2 enrichment experiment¹⁹. The ability of massive *Porites* to survive under the low pH conditions at CO_2 vents has also been linked with its capacity to maintain elevated pH_{cf} ²³. Taken together, the calcification rates and

pH_{cf} of *Porites* spp. appear to be relatively insensitive to the impacts of OA. However, the response of the *Porites* genus to OA may be atypical amongst corals. Furthermore, this tolerance may be limited only to particular *Porites* species, since a pronounced decrease in pH_{cf} with seawater pH was found in *P. compressa* from Hawaii¹⁷ and in some massive *Porites* sp. genotypes³².

pH_{cf} homeostasis²⁴ is not the only mechanism of resistance to OA. Here, calcification of *Pocillopora* was unaffected by the treatments despite a decrease in pH_{cf} with seawater pH. Similarly, pH_{cf} of *Pocillopora damicornis* decreased with seawater pH during a two-month laboratory study but calcification remained constant¹⁸. The lack of an effect of seawater pH on the calcification of *Pocillopora damicornis* was explained by the increase in calcium concentration in the calcifying fluid that enabled the corals to maintain constant precipitation of calcium carbonate despite decreasing pH_{cf}³³. Here, we observed a similar increase in the calcium concentration of *Pocillopora verrucosa* when seawater pH decreased, which favoured the maintenance and even the slight (but not statistically significant) increase of Ω_{arag} cf with pCO₂. This result suggests that increasing [Ca²⁺] in response to decreasing pH is a common feature for corals of the genus *Pocillopora* and could explain the resistance of some *Pocilloporidae* to OA reported previously^{34–36}. The mechanism responsible for increases in [Ca²⁺] still need to be confirmed. However, previous evidence points toward an active transport of calcium by various intracellular pathways to the site of calcification³⁷.

It has been suggested that short-term laboratory experimental results can be misleading because they do not consider seasonal variations in biotic and abiotic factors. For example, seasonal cycles of pH_{cf} and DIC_{cf} have been documented on massive *Porites* sp. from the Great Barrier Reef and Ningaloo Reef, Australia¹¹, and *Acropora youngae* and *Pocillopora damicornis* from Rottnest Island, Western Australia³⁰. Here, the seasonal cycles in *Porites* pH_{cf} and DIC_{cf} was conserved across pH treatments, suggesting that such cycles are not driven by seawater pH (which were constant across seasons) but rather by other abiotic (light, temperature, nutrient) or biotic parameters (photosynthetic rates, tissue thickness, reproduction cycles, growth rates). Ross et al.³⁰ attributed the seasonal variations in pH_{cf} and DIC_{cf} to temperature. We found similar effects of temperature on pH_{cf} and DIC_{cf} of *Porites*. Contrary to conclusions of previous studies¹¹, the presence (*Porites*) and absence (*Acropora*) of these seasonal trends in pH_{cf} and DIC_{cf} are related to species-specific effects, rather

236 than being artefacts arising from a lack of exposure to natural fluctuations of light and
237 temperature that could ultimately alter interpretations of how seawater pH controls
238 pH_{cf} . This is supported by the fact that decreases in pH_{cf} in response to seawater pH
239 have also occurred in the presence of natural daily pH and oxygen variability in recent
240 prior work¹⁵.

241 In conclusion, our study demonstrates that despite the strong capacity of corals
242 and coralline algae to modulate the chemistry at their site of calcification^{10,15,17}, the
243 effects of ocean acidification were manifested after one year under realistic
244 experimental conditions in three corals and in the algae. The magnitude of these
245 effects is species-specific, and species' resistance to OA over one year can be linked
246 to two main mechanisms studied here. Corals whose calcification was unaffected by
247 OA either (1) maintained pH_{cf} homeostasis (*Porites*), or (2) increased $\text{Ca}^{2+}_{\text{cf}}$
248 (*Pocillopora*) (Fig. 6). Increasing DIC_{cf} under OA is also a compensatory mechanism
249 for decreasing pH_{cf} , but it was not sufficient here to provide resistance to OA. Our
250 results thus suggest that these mechanisms are likely inherent characteristics, and that
251 some corals and calcifying algae do not have the capacity to acclimate to OA, even
252 over 1-year of experimental treatment. While acclimatization over one year did not
253 occur in our study, further work assessing effects over multiple generations will be
254 necessary to investigate the capacity to acclimate across generations. Finally, the
255 results described here confirm that existing species-specific differences in sensitivities
256 to OA will likely shape the composition of future reefs.

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Author contributions

SC wrote the paper, prepared the experiment, run the geochemical analyses and analysed the data. CC wrote the paper and conducted geochemical analyses. TMD performed geochemical analyses. SD performed the flume experiment. RC and MTM provided vital laboratory facilities. All authors edited the manuscript, contributed intellectual expertise and approved of its submission.

Data availability

All data are available on the public data repositories BCO-DMO (<https://www.bco-dmo.org/dataset/756211>).

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Methods

Sample collection and preparation

This study was carried out in Moorea, French Polynesia, at the Richard B. Gump Research station between November 2015 and November 2016. For this experiment four taxa of coral were used, *Psammocora profundacella* (*Psammocora*), *Acropora pulchra* (*Acropora*), *Pocillopora verrucosa* (*Pocillopora*), and massive *Porites* spp. (*Porites*), and two taxa of calcifying macroalgae, the coralline alga *Lithophyllum kotschyianum* (*Lithophyllum*) and the green *Halimeda minima* (*Halimeda*). *Porites*, *Lithophyllum* and *Halimeda* were part of the back reef communities recreated in the flumes²⁵. To increase the number of taxa tested, *Psammocora*, *Acropora*, and *Pocillopora* were maintained in the upstream side of the flumes on separated racks next to the tested communities. These taxa were selected because these taxa are dominant members of the benthic community in the back reef of Moorea³⁸. Twenty-four *Acropora* and *Pocillopora* were sampled from a common garden at 3 m depth on the North Shore of Moorea (See ³⁹ for details). The 5-cm long branches were selected from 6 different colonies for each species. One coral of each colony was used for each pCO₂ condition. Twenty-four ~ 5-cm in diameter *Psammocora* were from the back reef where they commonly are found growing on calcareous rubble. *Halimeda* were hand-picked from the back reef. *Porites* (~ 10-cm diameter, n = 5 per treatment) and *Lithophyllum* (~ 8 cm in diameter, n = 4 per treatment) were chiselled off the back reef of the North Shore of Moorea²⁵. *Psammocora* and *Porites* were cleaned in the laboratory and the dead skeletons on which they were growing were sawed off, and the exposed skeletons were covered with epoxy-glue. Corals and *Lithophyllum* were glued to plastic bases with Z-spar epoxy to facilitate handling and labelling of the organisms. *Halimeda* were placed in black mesh cages positioned at the end of the flumes.

Experimental set-up

The experimental settings used in this experiment were the same as in a parallel experiment that tested the effects of four pCO₂ regimes on back reef communities assembled in 5 m flumes^{25,38}. Complete details of the experimental set-up are provided in Carpenter et al.²⁵.

Briefly, the organisms were maintained in four 5 x 0.3 x 0.3 m outdoor flumes²⁶ for 1 year. After an acclimation period of one week, seawater pCO₂ was

maintained at ambient value in one flume (360 μatm) and was ramped up to the other three levels (~ 360 , 560, 760, and 1060 μatm) over 24 hours. pCO_2 was maintained with a negative feedback pH controller that regulated the bubbling of pure CO_2 to maintain seawater pH at the target pH. pH varied daily by ~ 0.1 pH unit to simulate current levels of diurnal variability in the back reef of Moorea²⁵. This four pCO_2 level design was chosen to detect linear effects of pCO_2 on organisms³. Using this type of design can circumvent some of the issues around low replication of experimental tanks⁴⁰. Discrete measurements of pH_T and total alkalinity were made to check the carbonate chemistry in the flumes. pH_T was measured directly in the flume using a handheld pH meter that was mounted with a DG 115-SC electrode (Mettler Toledo, Columbus, OH, USA) calibrated with a TRIS buffer. Total alkalinity was measured using an open-cell titrator (T50, Mettler Toledo) and accuracy was checked using certified seawater.

Seawater was circulated in the flumes at 0.1 m s^{-1} to match the average yearly seawater velocity in the back reef of Moorea⁴¹. Freshly pumped sand filtered seawater (corresponding to a mesh size of 500 μm) was continuously delivered to the flumes at $\sim 5 \text{ L min}^{-1}$ to maintain constant A_T and dissolved oxygen levels within the flumes. The corals were not fed but small particles (i.e. $< 500 \mu\text{m}$) were not filtered and served as potential food source. Light levels were adjusted with shade cloths to match maximum intensities typically ranging between 1000 and 2000 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ in the back reef of Moorea. Because the flumes were exposed to natural sunlight, light varied strongly seasonally and daily. Temperature was controlled to approximate the mean monthly seawater temperature in the back reef of Moorea, which ranged from 29.3°C in April to 25.8°C in September³⁸.

Calcification

Calcification was investigated to determine its link with the calcifying fluid chemistry. Net calcification of the organisms was determined using the buoyant weight technique⁴². The difference in buoyant weight between the beginning and end of the one year-long incubation was converted to dry weight of aragonite for the corals and calcite for the *Lithophyllum* to calculate net calcification. Calcification rates were normalized to surface area (mg cm^{-2}) determined using the aluminium foil or the wax dipping method at the end of the incubation. Organisms that exhibited clear signs of damage (e.g. broken branches) that could have affected their weight

were excluded from the calcification analysis (4 *Psammocora*, 2 *Pocillopora*, and 1 *Acropora*).

pH_{cf} and DIC_{cf}

Skeletons were stained by placing the organisms for 48 hours in seawater enriched with the fluorescent dye calcein at 50 mg L⁻¹ with a pH adjusted to ~8.1 by the addition of NaOH prior to the start of the experiment. The stain line was used to identify the part of the skeleton that grew during the experiment (Fig. S3). The $\delta^{11}\text{B}$ proxy method was used to estimate pH in the calcifying fluid of all taxa (pH_{cf})⁷ and the $\delta^{11}\text{B}$ and B/Ca method was used to estimate the dissolved inorganic carbon concentration in the calcifying fluid (DIC_{cf}) in the aragonitic taxa^{11,43}. Measurements of geochemistry were conducted on the dead skeleton after the experimental period. Skeleton was sampled from the tip of the branches or the top 1–2 mm of the skeleton for the 6 *Pocillopora* and *Psammocora* individuals, and 3 *Lithophyllum* per treatment. This portion of the skeleton was selected to sample material deposited during the last months of the experiment to detect any potential acclimatization to the experimental conditions. Because *Halimeda* degraded in the flumes, they were replaced several times during the experiment. The sampling for geochemistry was conducted on 3 *Halimeda* per treatment that were in the flumes for ~ 2 months. Only the top segments were used to ensure that they developed under the experimental conditions. Because the branches of *Acropora* extend linearly and quickly (i.e., 5-10 cm y⁻¹) it was possible to sample from four individuals per treatment skeleton parts that corresponded to different time points of the year of incubation (Fig. S3). The first section corresponded to the start of the experiment (i.e. the stain line). The rest of the 12 months linear extension was divided in four and was assumed to represent the growth after 0-3, 3-6, 6-9, and 9-12 months in the different treatments. Likewise, for three *Porites* individuals per treatment, four portions of the skeleton were sampled. One sample of the skeleton was taken below the stain line to represent the conditions prior to the experiment and the skeleton grown above the stain line was divided in three equal parts that were assumed to roughly represent the growth during the period 0–4, 4–8, and 8–12 months. Seasonal changes in temperature were confirmed using the inverse relationship between Sr/Ca and temperature for *Porites*. The sections prior to the start of the experiment and the one just below the tissue (section month 8-12) had the highest Sr/Ca corresponding to the coldest temperature recorded during

the austral winter to early spring (July to October). The lowest Sr/Ca were recorded for the period 0-4 and 4-8 months confirming that in these sections of the skeleton corresponded to the months November to February and March to June, respectively, when temperature where the highest in the flumes (see Table S1 in³⁸). The Sr/Ca proxy temperature reconstructions were not successful with *Acropora*, probably because of its perforate skeleton.

The entire selected portions of the skeleton were crushed to powder in a mortar and pestle. Therefore, all measurements represent an integration of the average conditions in the calcifying fluid (over two months) when the selected portions of the skeleton were formed. The measurements integrate the diel effects of light and pH that varied similarly across treatments. All powders were processed subsequently in the clean laboratory of the Advanced Geochemical Facility for Indian Ocean Research (AGFIOR, University of Western Australia (UWA) for dissolution and dilution to 10-ppm Ca solutions. Ten mg of each sample was placed in 6.25 % NaClO for 15 mins, rinsed in Milli-Q water then dried for 24 h. Samples were then dissolved in 0.51 N HNO₃, and the $\delta^{11}\text{B}$ was quantitatively separated on ion exchange columns. $\delta^{11}\text{B}$ was measured on a multicollector inductively coupled plasma mass spectrometry (NU II). Measurements of the international carbonate standard JCP-1 yielded a mean value of 24.42 ± 0.05 ‰ (mean $\pm$ SE, n = 12), which is similar to the 24.33 ± 0.11 ‰ reported previously⁴⁴. Calculations of pH_{cf} based on $\delta^{11}\text{B}$ were made in R using the calculations of⁴⁵:

$$\text{pH}_{\text{cf}} = \text{pK}_{\text{B}} - \log \left[\frac{(\delta^{11}\text{B}_{\text{SW}} - \delta^{11}\text{B}_{\text{carb}})}{(\alpha_{(\text{B3-B4})} \delta^{11}\text{B}_{\text{carb}} - \delta^{11}\text{B}_{\text{SW}} + 1000 (\alpha_{(\text{B3-B4})} - 1))} \right] \quad (1)$$

where pK_B is the dissociation constant dependent on temperature and salinity, $\delta^{11}\text{B}_{\text{sw}} = 39.61$, and $\alpha_{\text{B3-B4}}$ is the boron isotopic fractionation factor for the pH dependent equilibrium of the borate (B(OH)₄⁻) relative to the boric acid (B(OH)₃) species in the calcifying fluid, with a value of 1.0272⁴⁶. Temperature and salinity were representative of the average conditions in the flumes at which the selected portions of the skeleton were grown.

B/Ca ratios, measured on the same material, and $\delta^{11}\text{B}$, were utilized to determine [CO₃²⁻] and [DIC] at the site of calcification [DIC]_{cf} following¹¹ for corals. B/Ca ratios were determined on the same aliquot of the solution used for pH_{cf} estimates, and DIC_{cf} was calculated from estimates of carbonate ion concentrations using the following equations described in McCulloch et al.¹¹:

$$[\text{CO}_3^{2-}]_{\text{cf}} = K_D [\text{B}(\text{OH})_4^-]_{\text{cf}} / (\text{B}/\text{Ca})_{\text{CaCO}_3} \quad (2)$$

Where $K_D = K_{D,0} \exp(-k_{KD}[\text{H}^+]_T)$ with $K_{D,0} = 2.97 \pm 0.17 \times 10^{-3}$ ($\pm 95\%$ CI), $k_{KD} = 0.0202 \pm 0.042$. The concentration of DIC_{cf} was then calculated from estimates of pH_{cf} and $[\text{CO}_3^{2-}]_{\text{cf}}$. It was not possible to determine correct DIC_{cf} values for *Halimeda* likely because of dissolution and precipitation of calcium carbonate between the day and the night. DIC_{cf} was not determined on *Lithophyllum* because their skeleton is made of high Mg Calcite for which no inorganic precipitation experiment linking B/Ca and $[\text{CO}_3^{2-}]_{\text{cf}}$ have been made.

Raman spectroscopy

Confocal Raman spectroscopy was used to determine for the corals estimates of calcifying fluid aragonite saturation state $\Omega_{\text{arag cf}}^{47}$. Measurements were conducted on a WITec Alpha300RA+ using a 785 nm infrared laser, a 20x objective with 0.5 numerical aperture, and a 1200 mm^{-1} grating to achieve a spectral resolution of approximately 1.3 cm^{-1} . Skeleton powders were placed on glass slides and topography maps were made with the TrueSurface module. The automated stage followed the topography while conducting Raman measurements so that the optics were always in focus on the sample surfaces. 100 spectra were collected per sample in a 300 μm by 300 μm grid using 1 s integrations. Spectra with poor signal (< 50 intensity units) or contaminated by cosmic rays were excluded.

The widths of the ν_1 peaks were used as proxy measures of $\Omega_{\text{arag cf}}^{47}$. CaCO_3 minerals precipitating from more supersaturated solutions incorporate more impurities and are more disordered, which causes Raman peak broadening due to greater distributions of C-O bond lengths⁴⁷. We used the abiogenic aragonite calibration equation of⁴⁷ to calculate $\Omega_{\text{arag cf}}$ for the coral species from the ν_1 full width at half maximum intensity (FWHM). The mean and median of the standard errors for each individual $\Omega_{\text{arag cf}}$ ($n = 193$) were 0.19 and 0.16 units, respectively, where these uncertainties are based on the 100 replicate spectra collected for each individual. Repeated measurement of JCp-1 were used for instrument drift correction based on its $\Omega_{\text{arag cf}}$ of 12.3 (DeCarlo et al., 2017). The largest of these $\Omega_{\text{arag cf}}$ corrections, which were applied systematically to all samples measured in different analytical sessions, was 2 units. Analyses of *Porites*, *Psammocora*, and *Pocillopora* were all conducted within single analytical sessions, and therefore comparisons among treatments are not

sensitive to JCp-1 drift corrections. *Acropora* samples were analysed in two sessions, however, the $\Omega_{\text{arag cf}}$ means of the corrected *Acropora* data were indistinguishable between the two (10.65 ± 0.14 and 10.59 ± 0.08 , standard errors), making it unlikely that the JCp-1 corrections led to any artificial differences.

[Ca²⁺]_{cf} determination

[Ca²⁺]_{cf} of corals was calculated as:

$$[\text{Ca}^{2+}]_{\text{cf}} = \frac{\Omega_{\text{Ar}} * K_{\text{sp}}}{[\text{CO}_3^{2-}]_{\text{cf}}} \quad (1)$$

where $[\text{CO}_3^{2-}]_{\text{cf}}$ and $\Omega_{\text{arag cf}}$ are derived from boron systematics and Raman spectroscopy, respectively⁴⁷. $\text{Ca}_{\text{cf}}^{2+} / \text{Ca}_{\text{sw}}^{2+}$ ratios were calculated by normalizing to $[\text{Ca}^{2+}]_{\text{sw}}$, which was estimated from seawater salinity (average = 35.5). Estimates of $[\text{Ca}^{2+}]_{\text{sw}}$ were made based on the assumptions that metabolic activity (calcification) did not change the relationship seawater salinity $[\text{Ca}^{2+}]_{\text{sw}}$. This assumption was supported by the fact that seawater was pumped from Cook's Bay where there is no reef and only very few corals.

Statistical analyses

The assumptions of normality and equality of variance were evaluated through graphical analyses of residuals using the R software. Because the experiment was designed to detect linear effects of pCO₂ on the tested organisms, linear relationships between the dependant variables at the end of the experiment (calcification, pH_{cf}, DIC_{cf}, $\Omega_{\text{arag cf}}$, and $\text{Ca}_{\text{cf}}^{2+}$) and the explanatory variable (pCO₂) were investigated. To test for the effects of time on pH_{cf}, DIC_{cf}, $\Omega_{\text{arag cf}}$, and $\text{Ca}_{\text{cf}}^{2+}$ of *Acropora* and *Porites*, repeated measured ANOVAs were used with individual coral as a random factor and the treatment and time as fixed effects. The effects of light, temperature and pCO₂ on pH_{cf}, DIC_{cf}, $\Omega_{\text{arag cf}}$, and $\text{Ca}_{\text{cf}}^{2+}$ of *Acropora* and *Porites* were tested using multiple linear regressions. All statistical analyses were done with R and the package nlme was used for the repeated measured ANOVAs.

References (Methods section)

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Legends

Fig. 1. Effects of four pCO₂ treatments (~360, 550, 700 and 1050 µatm) on the surface area-normalized net calcification rates. Calcification over the 1-year long experiment was measured on the corals *Psammocora profundacella* (A), *Pocillopora verrucosa* (B), *Acropora pulchra* (C), massive *Porites* sp. (D), and the coralline alga *Lithophyllum kotschyannum* (E). Individual rates are shown as dots and the squares represent the mean ± SE (n = 5 for *Psammocora*; n=6 or 5 for *Pocillopora* and *Acropora*; n = 5 for *Porites* and n = 4 for *Lithophyllum*). Linear regressions and the 95% confidence intervals are shown when the regressions had a statistically significant slope (p < 0.05).

Fig. 2. Estimates of pH in the calcifying fluid (pH_{cf}) at the end of the 1-year experiment. The geochemical proxy δ¹¹B was used to estimate pH_{cf} of the corals *Psammocora profundacella* (A), *Pocillopora verrucosa* (B), *Acropora pulchra* (C), and massive *Porites* sp. (D) and the algae *Lithophyllum kotschyannum* (E) and *Halimeda minima* (F). Individual rates are shown as dots and the squares represent the mean ± SE (n = 6 or 5 for *Psammocora* and *Pocillopora*; n = 4 for *Acropora*; n = 3 for *Porites* and *Halimeda*, and n = 4 for *Lithophyllum*). Linear regressions and the 95% confidence intervals are shown when the regressions had a statistically significant slope (p < 0.05).

Fig. 3. Estimates of dissolved inorganic carbon in the calcifying fluid (DIC_{cf}) at the end of the 1-year incubation period. The geochemical proxy δ¹¹B and B/Ca were used to estimate DIC_{cf} of the corals *Psammocora profundacella* (A), *Pocillopora verrucosa* (B), *Acropora pulchra* (C), and massive *Porites* sp.(D). Individual rates are shown as dots and the squares represent the mean ± SE (n = 6 or 5 for *Psammocora* and *Pocillopora*; n = 4 for *Acropora*; n = 3 for *Porites*). Linear regressions and the 95% confidence intervals are shown when the regressions had a statistically significant slope (p < 0.05).

Fig 4. Ratio between the calcium in the calcifying fluid (Ca²⁺_{cf}) and the calcium in seawater (Ca²⁺_{sw}) in the four studied corals. Ca²⁺_{cf} was calculated from estimates of aragonite saturation state and DIC_{cf}. Individual rates are shown as dots and the squares represent the mean ± SE (n = 6 or 5 for *Psammocora* and *Pocillopora*; n = 4 for *Acropora*; n = 3 for *Porites*). Linear regressions and the 95% confidence intervals are shown when the regressions had a statistically significant slope (p < 0.05).

Fig. 5. Temporal variations of *A. pulchra* and massive *Porites* sp. calcifying fluid pH and DIC. The skeleton of *A. pulchra* was sampled to select sections grown at the start of the experiment, and after ~ 0-3, 3-6, 6-9 and 9-12 months of experiment (panels A and B), while the skeleton of massive *Porites* sp. was sampled to select sections corresponding to the start of the experiment, and after ~ 0-4, 4-8, 8-12 months of experiment (panels C and D). Month 0 corresponds to November 2015. Error bars show SE.

Fig. 6. Schematic summary of the responses to ocean acidification measured in the corals *Acropora pulchra*, *Psammocora profundacella*, massive *Porites* sp., and *Pocillopora verruca*. The calcifying fluid chemistry of the two corals whose calcification was affected by OA exhibited a decrease in pH_{cf} and an increase in

667 DIC_{cf}, while Ca²⁺_{cf} was constant. In contrast, in the corals with calcification
668 unaffected by OA two different responses were found in *Porites* and *Pocillopora*.

Psammocora *Pocillopora* *Acropora* *Porites* *Lithophyllum*

Psammocora *Pocillopora* *Acropora* *Porites* *Lithophyllum* *Halimeda*

Psammocora *Pocillopora* *Acropora* *Porites*

Psammocora *Pocillopora* *Acropora* *Porites*

Acropora

Porites

Species-specific response of calcification to OA

Affected

Acropora

Psammocora

Calcifying fluid

pH

DIC

= Ca^{2+}

Not affected

Porites

Homeostasis

Calcifying fluid

= pH

= DIC

= Ca^{2+}

Pocillopora

$\text{Ca}^{2+}_{\text{cf}}$ increase

Calcifying fluid

pH

DIC

Ca^{2+}

Fig. S1. Estimates of the aragonite saturation state in the calcifying fluid ($\Omega_{\text{arag cf}}$) of corals at the end of the 1-year incubation. Confocal Raman spectroscopy was used to estimate $\Omega_{\text{arag cf}}$ of the corals *Psammocora profundacella* (A), *Pocillopora verrucosa* (B), *Acropora pulchra* (C), and massive *Porites* sp. (D). Individual rates are shown as dots and the squares represent the mean $\pm$ SE ($n = 6$ or 5 for *Psammocora* and *Pocillopora*; $n = 4$ for *Acropora*; $n = 3$ for *Porites*). Linear regressions and the 95% confidence intervals are shown when the regressions had a statistically significant slope ($p < 0.05$).

Fig S2. Temporal variations of *A. pulchra* and massive *Porites* sp. calcifying fluid Ω and $\text{Ca}^{2+}_{\text{cf}} / \text{Ca}^{2+}_{\text{sw}}$. The skeleton of *A. pulchra* was sampled to select sections grown at the start of the experiment, and after ~ 0 -3, 3-6, 6-9 and 9-12 months of experiment (panels A and B), while the skeleton of massive *Porites* sp. was sampled to select sections corresponding to the start of the experiment, and after ~ 0 -4, 4-8, 8-12 months of experiment (panels C and D). Month 0 corresponds to November 2015. Error bars show SE.

Fig. S3. Schematic showing the portions of the skeleton of *Acropora pulchra* that were selected to represent the conditions in the calcifying fluid at the start of the experiment (section 0), and after ~ 0-3 (section 1), 3-6 (section2), 6-9 (section 3), and 9-12 (section 4) months of experiment. The green lines represent the portions of the skeleton that were selected for each growth period.

Table S1: Parameters of the linear estimates of the relationship between calcification, pH_{cf}, DIC_{cf}, Ca²⁺_{cf}, and Ω_{cf}, and seawater pCO₂ (μatm).

Species	Parameter	Equation	Slope p-value	dF	F
<i>Psammocora</i>	Calcification	$0.63 - 1.68 \cdot 10^{-4} \cdot \text{pCO}_2$	0.025	18	5.958
	pH _{cf}	$8.61 - 1.49 \cdot 10^{-4} \cdot \text{pCO}_2$	<0.001	20	20.68
	DIC _{cf}	$2818 + 0.35 \cdot \text{pCO}_2$	0.0183	18	6.738
	Ca ²⁺ _{cf}	$0.73 + 1.10 \cdot 10^{-5} \cdot \text{pCO}_2$	0.39	18	0.775
	Ω _{cf}	$10.23 - 7.0 \cdot 10^{-4} \cdot \text{pCO}_2$	0.025	19	5.95
<i>Pocillopora</i>	Calcification	$0.31 + 6.12 \cdot 10^{-5} \cdot \text{pCO}_2$	0.326	20	1.015
	pH _{cf}	$8.56 - 1.79 \cdot 10^{-4} \cdot \text{pCO}_2$	<0.001	22	16.81
	DIC _{cf}	$2849 + 0.30 \cdot \text{pCO}_2$	0.0359	21	5.026
	Ca ²⁺ _{cf}	$0.77 + 2.19 \cdot 10^{-4} \cdot \text{pCO}_2$	0.007	19	8.934
	Ω _{cf}	$10.03 + 7.63 \cdot 10^{-4} \cdot \text{pCO}_2$	0.316	19	1.061
<i>Acropora</i>	Calcification	$0.56 - 1.97 \cdot 10^{-4} \cdot \text{pCO}_2$	0.0465	21	4.475
	pH _{cf}	$8.54 - 1.27 \cdot 10^{-4} \cdot \text{pCO}_2$	0.016	14	7.518
	DIC _{cf}	$3551 + 0.62 \cdot \text{pCO}_2$	0.005	14	11.18
	Ca ²⁺ _{cf}	$0.74 - 3.63 \cdot 10^{-5} \cdot \text{pCO}_2$	0.636	14	0.234
	Ω _{cf}	$11.6 - 1.2 \cdot 10^{-3} \cdot \text{pCO}_2$	0.162	14	2.18
<i>Porites</i>	Calcification	$0.18 + 8.68 \cdot 10^{-5} \cdot \text{pCO}_2$	0.466	18	0.5558
	pH _{cf}	$8.55 - 4.84 \cdot 10^{-5} \cdot \text{pCO}_2$	0.281	10	1.299
	DIC _{cf}	$3874 + 0.15 \cdot \text{pCO}_2$	0.519	10	0.447
	Ca ²⁺ _{cf}	$0.54 + 6.8 \cdot 10^{-5} \cdot \text{pCO}_2$	0.276	10	1.33
	Ω _{cf}	$9.40 + 6.7 \cdot 10^{-4} \cdot \text{pCO}_2$	0.36	10	0.921
<i>Lithophyllum</i>	Calcification	$0.12 - 5.83 \cdot 10^{-5} \cdot \text{pCO}_2$	0.004	14	11.64
	pH _{cf}	$8.77 - 9.05 \cdot 10^{-5} \cdot \text{pCO}_2$	0.018	12	7.489
<i>Halimeda</i>	pH _{cf}	$8.51 - 1.40 \cdot 10^{-4} \cdot \text{pCO}_2$	<0.001	7	51.12

Table S2: Parameters of the multiple linear estimates of the relationship between, pH_{cf}, DIC_{cf}, Ca²⁺_{cf}, and Ω_{cf} , and seawater mean temperature, mean daily light, and pCO₂ treatment in *Acropora*.

Parameter	Explanatory variable	Estimate	Standard error	P-value
pH _{cf}	Temperature	-0.0109	0.0006	0.058
	Light	-0.0001	0.0001	0.151
	pCO ₂	0.0268	0.0004	<0.001
DIC _{cf}	Temperature	7.680	35.163	0.828
	Light	0.607	0.559	0.280
	pCO ₂	111.37	25.353	<0.001
Ω_{cf}	Temperature	-0.028	0.092	0.760
	Light	0.003	0.001	0.037
	pCO ₂	-0.143	0.065	0.032
Ca ²⁺ _{cf}	Temperature	-0.0073	0.0079	0.354
	Light	-0.0004	0.0001	0.002
	pCO ₂	0.0013	0.0056	0.817

Table S3: Parameters of the multiple linear estimates of the relationship between, pH_{cf}, DIC_{cf}, Ca²⁺_{cf}, and Ω_{cf}, and seawater mean temperature, mean daily light, and pCO₂ treatment in *Porites*.

Parameter	Explanatory variable	Estimate	Standard error	P-value
pH _{cf}	Temperature	-0.0278	0.0096	0.006
	Light	-0.0006	0.0002	0.004
	pCO ₂	-0.0103	0.0065	0.120
DIC _{cf}	Temperature	143.41	47.96	0.005
	Light	2.62	0.99	0.011
	pCO ₂	21.16	32.74	0.99
Ω _{cf}	Temperature	0.027	0.118	0.820
	Light	-0.002	0.002	0.390
	pCO ₂	-0.024	0.080	0.766
Ca ²⁺ _{cf}	Temperature	-0.0058	0.0102	0.575
	Light	0.00002	0.0002	0.942
	pCO ₂	0.0068	0.0069	0.335