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 pCO_2 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 pCO_2 by determining their chemistry in the CF during a year-long experiment. We found no evidence of acclimatization to elevated pCO_2 across any of the tested taxa. The effects of increasing seawater pCO_2 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 organisms[1] as well as increased dissolution of existing calcium carbonate structures[2]. 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 precipitated[6]. Corals exert a strong control on their CF chemistry, with pH in the CF (pH_{cf}) maintained above seawater 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 available[14], 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 pH[7]. 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-specific[17]. 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 CF[11]. 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 employed[11].

Long-term OA experiments (several months to years) are rare[20], 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 CO_2 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 CO_2 seeps has been associated with their capacity to maintain elevated pH_{cf}[23]. However, it is difficult to draw conclusions from studies made at CO_2 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 flumes[25], 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 kotschyanum* and *Halimeda minima* (*Lithophyllum* and *Halimeda* thereafter). We assessed the calcification and CF responses of these organisms exposed to four different pCO_2 conditions over one year (2 months for *Halimeda*). We were able to maintain realistic flow conditions, natural irradiance, and stable pCO_2 by using outdoor flumes[26]. 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, pCO_2 treatments remained relatively stable. Overall, the average monthly mean pCO_2 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 ± 8 µmol quanta m^{-2} s^{-1}) to a maximum in November (478 ± 77 µmol quanta m^{-2} 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 ± 8.6 µmol kg^{-1} in the 561and 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). $Ca^{2+}_{cf}/Ca^{2+}_{SW}$ was only affected by the pCO_2 in *Pocillopora* (Fig. 4)(slope p-value = 0.009, Table S1) with maximum $Ca^{2+}_{cf}/Ca^{2+}_{SW}$ 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 µatm 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 pCO_2 treatment, particularly at the end of the incubation (Fig. S2). In contrast, DIC_{cf} (ANOVA, $p = 0.096$) and $Ca^{2+}_{cf} / Ca^{2+}_{SW}$ ($p = 0.107$) 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 $Ca^{2+}_{cf} / Ca^{2+}_{SW}$ 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 $Ca^{2+}_{cf}/Ca^{2+}_{SW}$ (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 pCO_2 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 pCO_2 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 pCO_2 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 pCO_2. 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 pCO_2, with *Porites* maintaining homeostatic conditions with changing pCO_2 and *Pocillopora*

compensating a decrease in pH_{cf} by increasing $[Ca^{2+}]_{cf.}$. It is these different mechanisms of maintaining favourable CF chemistry that enable constant calcification, observed here and in our previous work[10] (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 export[29] 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 months[10] 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 pH[14]. 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 experiment[19]. 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 Hawaii[17] and in some massive *Porites* sp. genotypes[32].

pH_{cf} homeostasis[24] 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 constant[18]. 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 $\Omega_{arag\ cf}$ with pCO_2. This result suggests that increasing $[Ca^{2+}]$ 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 $[Ca^{2+}]$ still need to be confirmed. However, previous evidence points toward an active transport of calcium by various intracellular pathways to the site of calcification[37].

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, Australia[11], and *Acropora youngei* and *Pocillopora damicornis* from Rottnest Island, Western Australia[30]. 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 studies[11], the presence (*Porites*) and absence (*Acropora*) of these seasonal trends in pH_{cf} and DIC_{cf} are related to species-specific effects, rather

than being artefacts arising from a lack of exposure to natural fluctuations of light and temperature that could ultimately alter interpretations of how seawater pH controls pH_{cf}. This is supported by the fact that decreases in pH_{cf} in response to seawater pH have also occurred in the presence of natural daily pH and oxygen variability in recent prior work[15].

In conclusion, our study demonstrates that despite the strong capacity of corals and coralline algae to modulate the chemistry at their site of calcification[10,15,17], the effects of ocean acidification were manifested after one year under realistic experimental conditions in three corals and in the algae. The magnitude of these effects is species-specific, and species' resistance to OA over one year can be linked to two main mechanisms studied here. Corals whose calcification was unaffected by OA either (1) maintained pH_{cf} homeostasis (*Porites*), or (2) increased Ca^{2+}_{cf} (*Pocillopora*) (Fig. 6). Increasing DIC_{cf} under OA is also a compensatory mechanism for decreasing pH_{cf}, but it was not sufficient here to provide resistance to OA. Our results thus suggest that these mechanisms are likely inherent characteristics, and that some corals and calcifying algae do not have the capacity to acclimate to OA, even over 1-year of experimental treatment. While acclimatization over one year did not occur in our study, further work assessing effects over multiple generations will be necessary to investigate the capacity to acclimate across generations. Finally, the results described here confirm that existing species-specific differences in sensitivities 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 kotschyanum* (*Lithophyllum*) and the green *Halimeda minima* (*Halimeda*). *Porites*, *Lithophyllum* and *Halimeda* were part of the back reef communities recreated in the flumes[25]. 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 Moorea[38]. 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 pCO_2 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 Moorea[25]. *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 pCO_2 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 flumes[26] for 1 year. After an acclimation period of one week, seawater pCO_2 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 Moorea[25]. This four pCO_2 level design was chosen to detect linear effects of pCO_2 on organisms[3]. Using this type of design can circumvent some of the issues around low replication of experimental tanks[40]. 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 Moorea[41]. Freshly pumped sand filtered seawater (corresponding to a mesh size of 500 µm) was continuously delivered to the flumes at ~ 5 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 µm) 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 µmol quanta m^{-2} 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 September[38].

Calcification

Calcification was investigated to determine its link with the calcifying fluid chemistry. Net calcification of the organisms was determined using the buoyant weight technique[42]. 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 $\delta^{11}B$ proxy method was used to estimate pH in the calcifying fluid of all taxa (pH_{cf})[7] and the $\delta^{11}B$ 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

494 the austral winter to early spring (July to October). The lowest Sr/Ca were recorded
495 for the period 0-4 and 4-8 months confirming that in these sections of the skeleton
496 corresponded to the months November to February and March to June, respectively,
497 when temperature where the highest in the flumes (see Table S1 in[38]). The Sr/Ca
498 proxy temperature reconstructions were not successful with *Acropora*, probably
499 because of its perforate skeleton.
500 The entire selected portions of the skeleton were crushed to powder in a
501 mortar and pestle. Therefore, all measurements represent an integration of the average
502 conditions in the calcifying fluid (over two months) when the selected portions of the
503 skeleton were formed. The measurements integrate the diel effects of light and pH
504 that varied similarly across treatments. All powders were processed subsequently
505 in the clean laboratory of the Advanced Geochemical Facility for Indian Ocean
506 Research (AGFIOR, University of Western Australia (UWA) for dissolution and
507 dilution to 10-ppm Ca solutions. Ten mg of each sample was placed in 6.25 % NaClO
508 for 15 mins, rinsed in Milli-Q water then dried for 24 h. Samples were then dissolved
509 in 0.51 N HNO_3, and the $\delta^{11}B$ was quantitatively separated on ion exchange columns.
510 $\delta^{11}B$ was measured on a multicollector inductively coupled plasma mass spectrometry
511 (NU II). Measurements of the international carbonate standard JCP-1 yielded a mean
512 value of 24.42 ± 0.05 ‰ (mean ± SE, n = 12), which is similar to the 24.33 ± 0.11 ‰
513 reported previously[44]. Calculations of pH_{cf} based on $\delta^{11}B$ were made in R using the
514 calculations of [45]:

515 $$pH_{cf} = pK_B - \log\left[\frac{(\delta^{11}B_{SW} - \delta^{11}B_{carb})}{\left(\alpha_{(B3-B4)}\delta^{11}B_{carb} - \delta^{11}B_{SW} + 1000\,(\alpha_{(B3-B4)} - 1)\right)}\right] \quad (1)$$

516 where pK_B is the dissociation constant dependent on temperature and salinity,
517 $\delta^{11}B_{sw} = 39.61$, and $\alpha_{B3\text{-}B4}$ is the boron isotopic fractionation factor for the pH
518 dependent equilibrium of the borate ($B(OH)_4^-$) relative to the boric acid ($B(OH)_3$)
519 species in the calcifying fluid, with a value of 1.0272[46]. Temperature and salinity
520 were representative of the average conditions in the flumes at which the selected
521 portions of the skeleton were grown.
522 B/Ca ratios, measured on the same material, and $\delta^{11}B$, were utilized to
523 determine [CO_3^{2-}] and [DIC] at the site of calcification $[DIC]_{cf}$ following[11] for corals.
524 B/Ca ratios were determined on the same aliquot of the solution used for pH_{cf}
525 estimates, and DIC_{cf} was calculated from estimates of carbonate ion concentrations
526 using the following equations described in McCulloch et al.[11]:

$$[CO_3^{2-}]_{cf} = K_D[B(OH)_4^-]_{cf}/\left(^{B}/_{Ca}\right)_{CaCO_3} \quad (2)$$

Where $K_D = K_{D.0}\exp(-k_{KD}[H^+]_T)$ with $K_{D,0} = 2.97 \pm 0.17\times10^{-3}$ (± 95% CI), $k_{K_D} = 0.0202 \pm 0.042$. The concentration of DIC_{cf} was then calculated from estimates of pH_{cf} and $[CO_3^{2-}]_{cf}$. 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 $[CO_3^{2-}]_{cf}$ have been made.

Raman spectroscopy

Confocal Raman spectroscopy was used to determine for the corals estimates of calcifying fluid aragonite saturation state $\Omega_{arag\ cf}$[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 $\Omega_{arag\ cf}$[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 lengths [47]. We used the abiogenic aragonite calibration equation of [47] to calculate $\Omega_{arag\ cf}$ 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 $\Omega_{arag\ cf}$ (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 $\Omega_{arag\ cf}$ of 12.3 (DeCarlo et al., 2017). The largest of these $\Omega_{arag\ cf}$ 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 $\Omega_{arag\ cf}$ 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.

$[Ca^{2+}]_{cf}$ determination

$[Ca^{2+}]_{cf}$ of corals was calculated as:

$$[Ca^{2+}]_{cf} = {\Omega_{Ar} * K_{sp}}/{[CO_3^{2-}]_{cf}} \quad (1)$$

where $[CO_3^{2-}]_{cf}$ and $\Omega_{arag\ cf}$ are derived from boron systematics and Raman spectroscopy, respectively[47]. $Ca_{cf}^{2+}/Ca_{sw}^{2+}$ ratios were calculated by normalizing to $[Ca^{2+}]_{sw}$, which was estimated from seawater salinity (average = 35.5). Estimates of $[Ca^{2+}]_{sw}$ were made based on the assumptions that metabolic activity (calcification) did not change the relationship seawater salinity $[Ca^{2+}]_{sw}$. 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 pCO_2 on the tested organisms, linear relationships between the dependant variables at the end of the experiment (calcification, pH_{cf}, DIC_{cf}, $\Omega_{arag\ cf}$, and $Ca^{2+}{}_{cf}$) and the explanatory variable (pCO_2) were investigated. To test for the effects of time on pH_{cf}, DIC_{cf}, $\Omega_{arag\ cf}$, and $Ca^{2+}{}_{cf}$ 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 pCO_2 on pH_{cf}, DIC_{cf}, $\Omega_{arag\ cf}$, and $Ca^{2+}{}_{cf}$ 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.

589 **References (Methods section)**
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Legends

Fig. 1. Effects of four pCO_2 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 kotschyanum* (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 $\delta^{11}B$ 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 kotschyanum* (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 ($p < 0.05$).

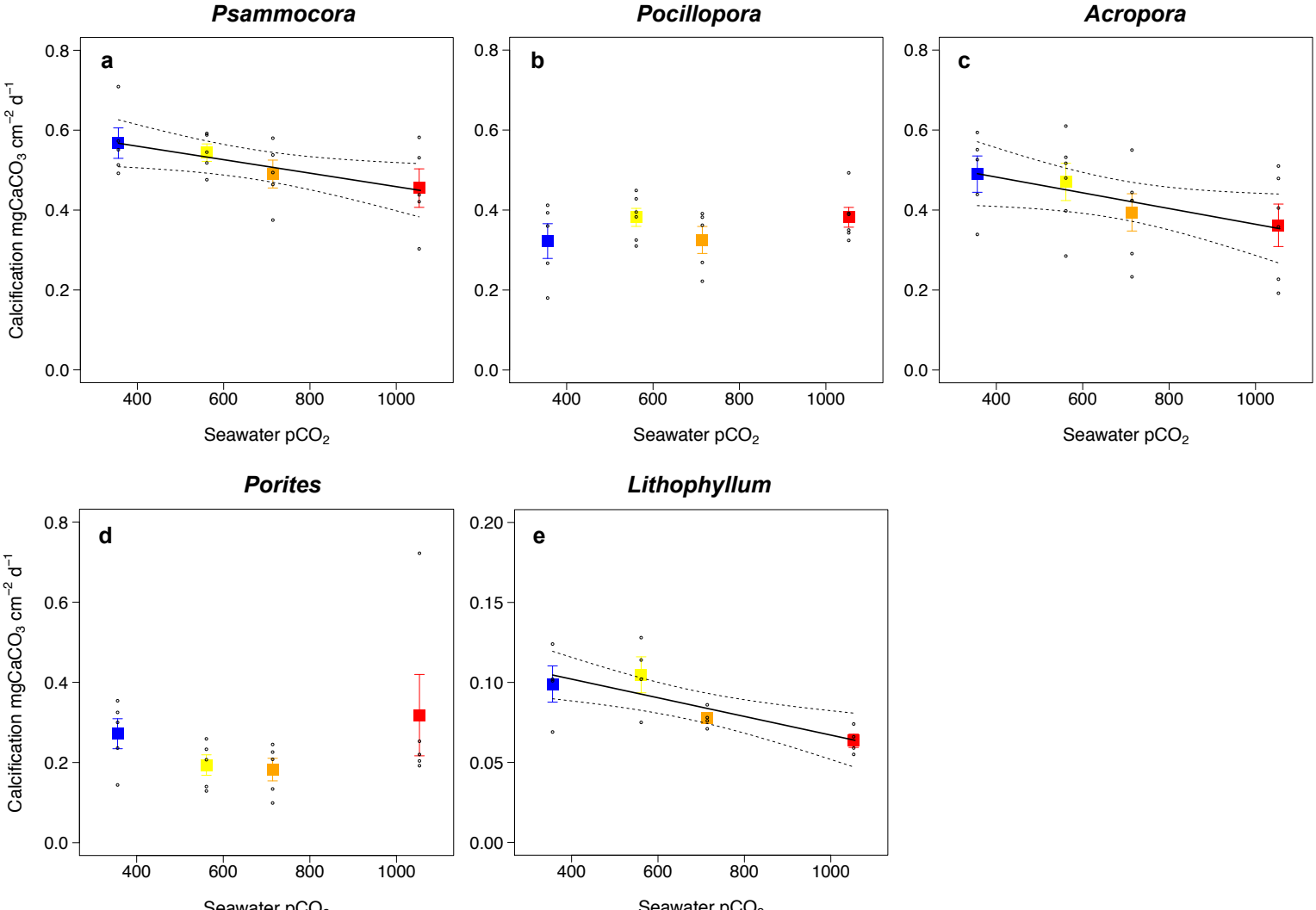
Fig. 3. Estimates of dissolved inorganic carbon in the calcifying fluid (DIC_{cf}) at the end of the 1-year incubation period. The geochemical proxy $\delta^{11}B$ and B/Ca were used to estimate DIC_{cf} of the corals *Psammocora profundacella* (A), *Pocillopora verrucosa* (B), *Acropora pulchra* (C), 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 ($p < 0.05$).

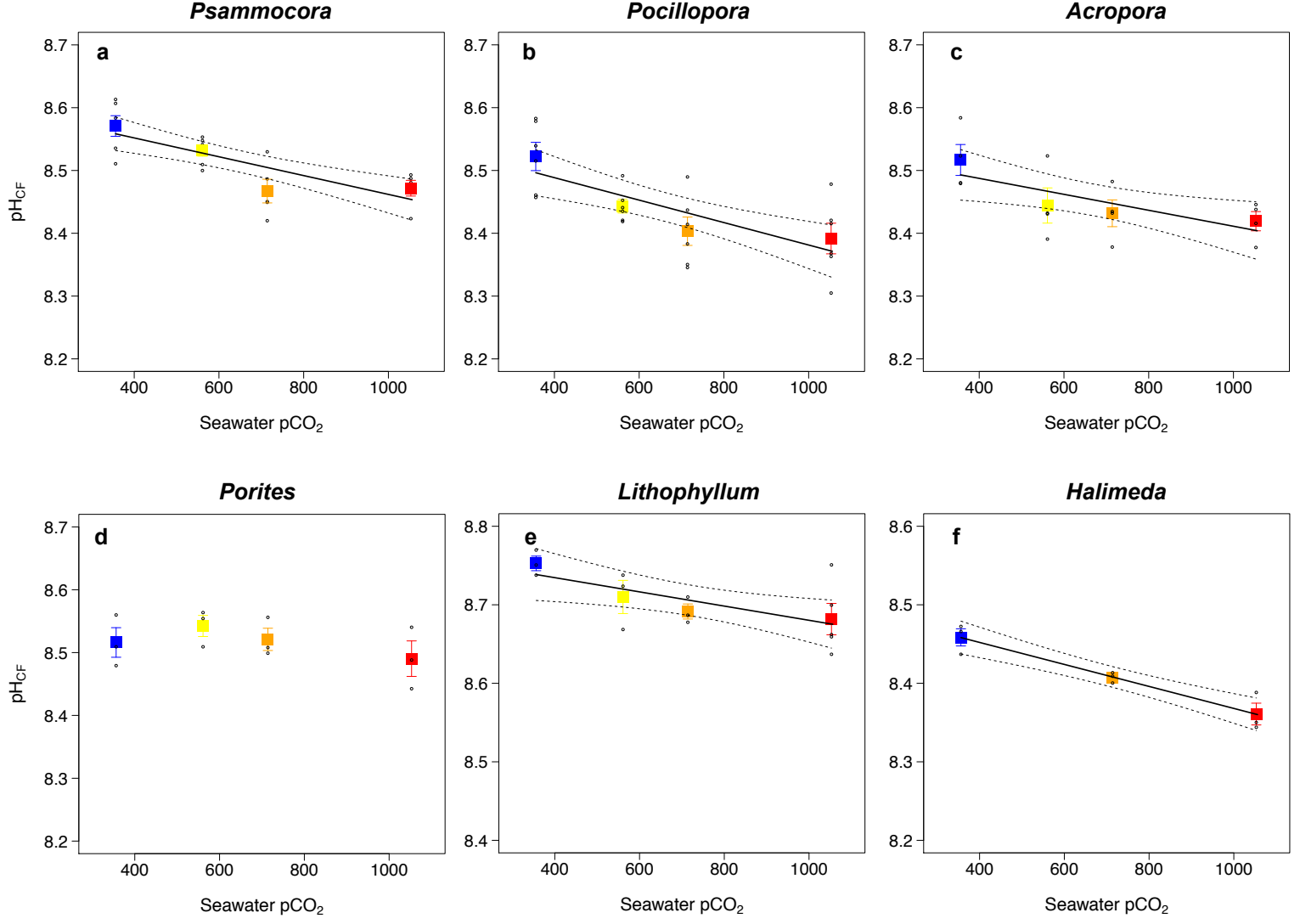
Fig 4. Ratio between the calcium in the calcifying fluid (Ca^{2+}_{cf}) and the calcium in seawater (Ca^{2+}_{SW}) in the four studied corals. Ca^{2+}_{cf} was calculated from estimates of aragonite saturation state and DIC_{cf}. Individual rates are shown as dots and the squares represent the mean ± 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 ($p < 0.05$).

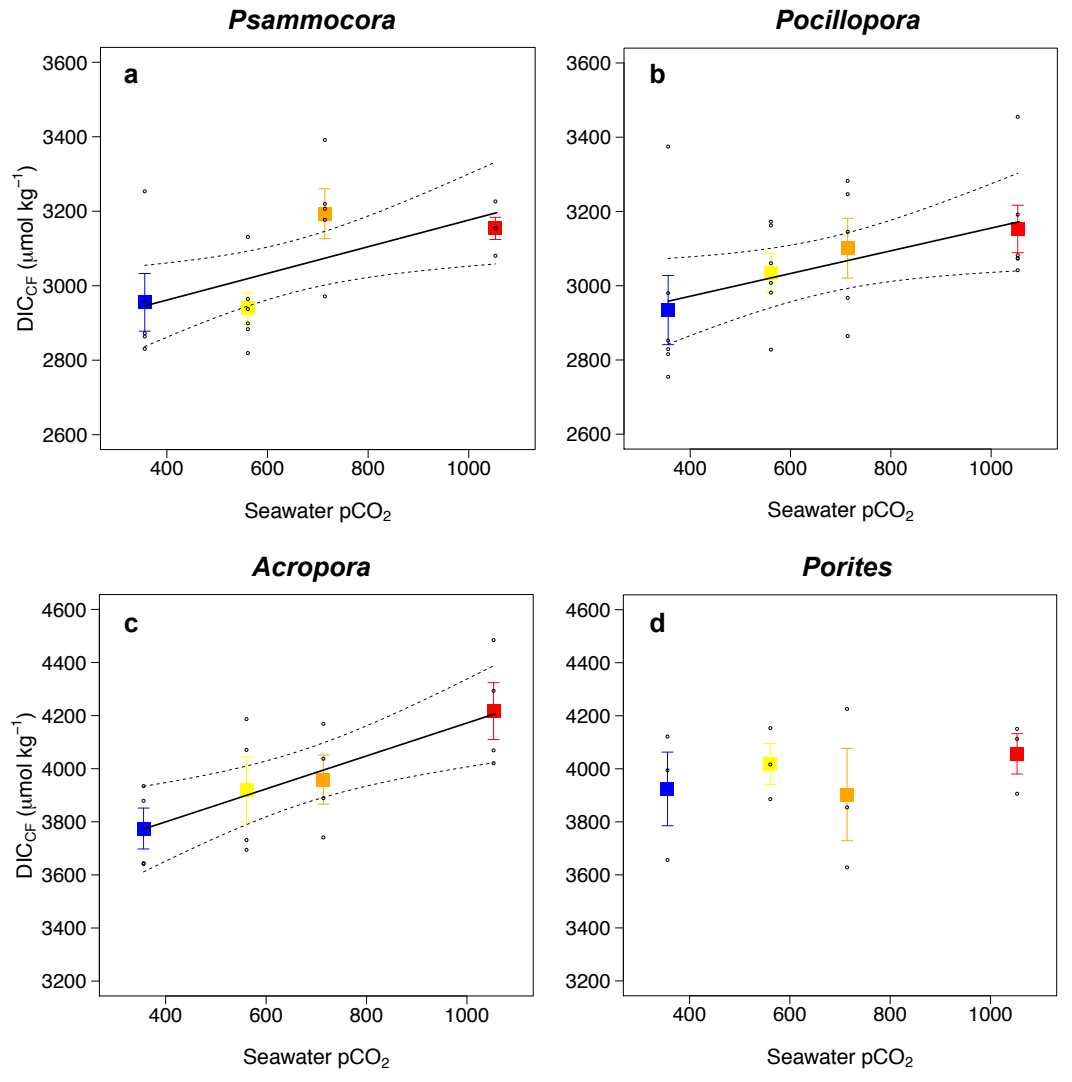
Fig. 5. Temporal variations of *A. pulchra* and massive *Porites* sp. calcifying fluid pH and DIC. The skeleton of *A. pulchra* was sampled to select sections grown at the start of the experiment, and after ~ 0-3, 3-6, 6-9 and 9-12 months of experiment (panels A and B), while the skeleton of massive *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.

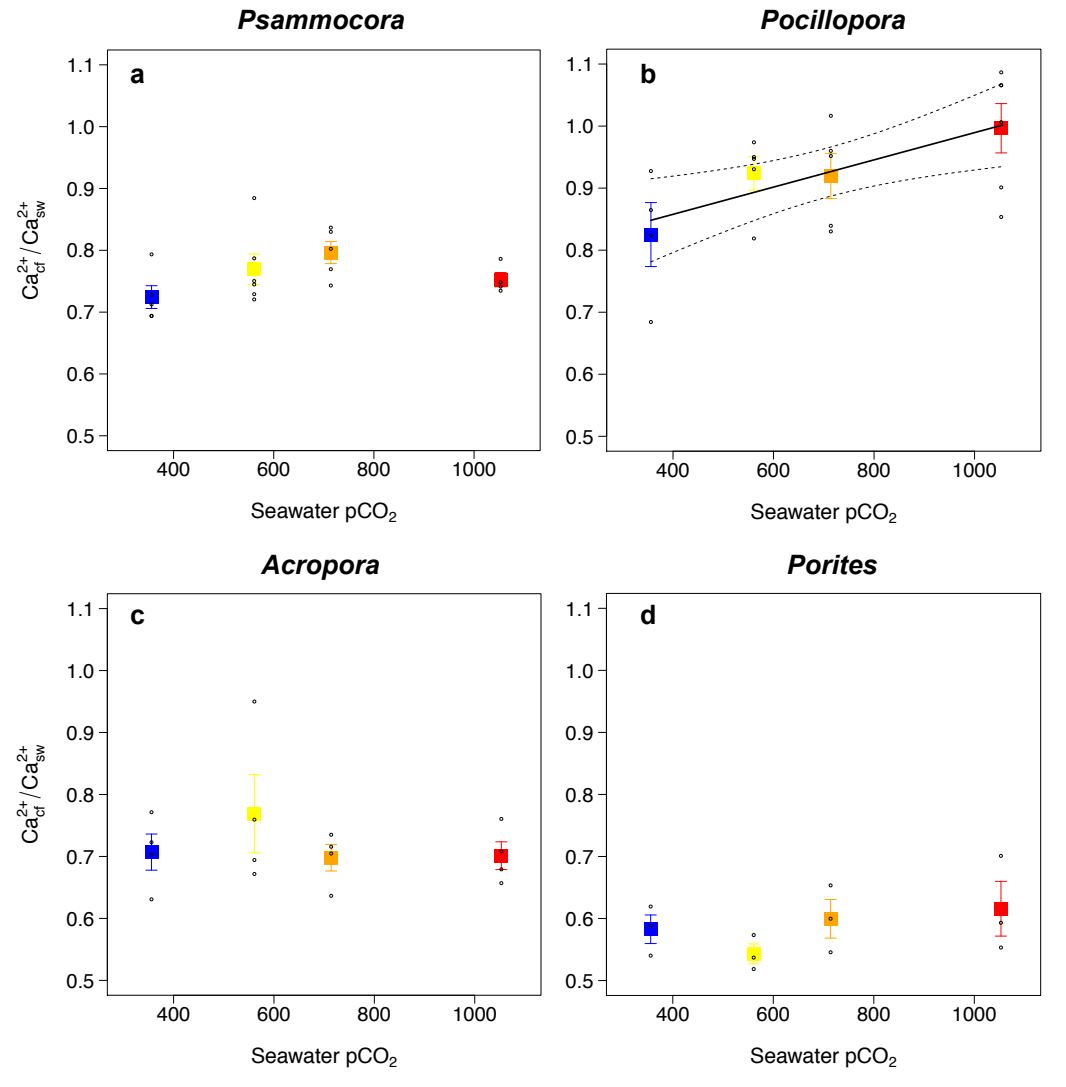
Fig. 6. Schematic summary of the responses to ocean acidification measured in the corals *Acropora pulchra*, *Psammocora profundacella*, massive *Porites* sp., and *Pocillopora verruca.* The calcifying fluid chemistry of the two corals whose calcification was affected by OA exhibited a decrease in pH_{cf}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

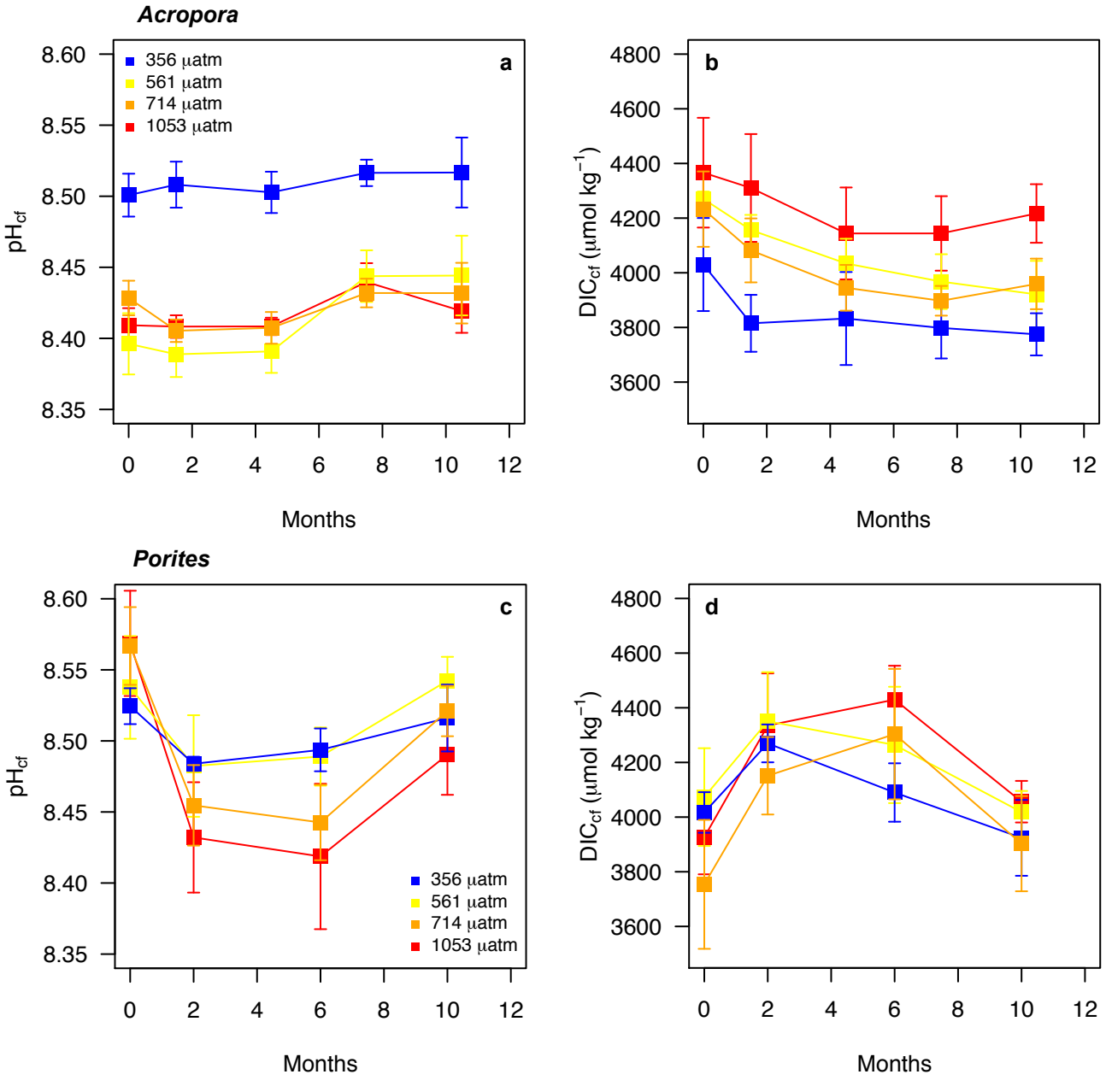
667 DIC_{cf}, while Ca^{2+}_{cf} was constant. In contrast, in the corals with calcification
668 unaffected by OA two different responses were found in *Porites* and *Pocillopora*.


Psammocora
Pocillopora
Acropora
Porites
Lithophyllum
a
b
c
d
e
Calcification mgCaCO3 cm−2 d−1
Seawater pCO2
0.0
0.2
0.4
0.6
0.8
0.00
0.05
0.10
0.15
0.20
400
600
800
1000


Psammocora
Pocillopora
Acropora
Porites
Lithophyllum
Halimeda
a
b
c
d
e
f
pH_CF
Seawater pCO_2
8.7
8.6
8.5
8.4
8.3
8.2
8.8
400
600
800
1000


Psammocora
a
Pocillopora
b
Acropora
c
Porites
d
DIC$_{CF}$ (µmol kg^{-1})
Seawater pCO$_2$
3600
3400
3200
3000
2800
2600
4600
4400
4200
4000
3800
400
600
800
1000


Psammocora
a
Pocillopora
b
Acropora
c
Porites
d
$Ca^{2+}_{cf} / Ca^{2+}_{sw}$
Seawater pCO_2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
400
600
800
1000


Acropora
a
356 µatm
561 µatm
714 µatm
1053 µatm
pHcf
8.60
8.55
8.50
8.45
8.40
8.35
0
2
4
6
8
10
12
Months
b
DICcf (µmol kg−1)
4800
4600
4400
4200
4000
3800
3600
Months
Porites
c
pHcf
356 µatm
561 µatm
714 µatm
1053 µatm
Months
d
DICcf (µmol kg−1)
Months

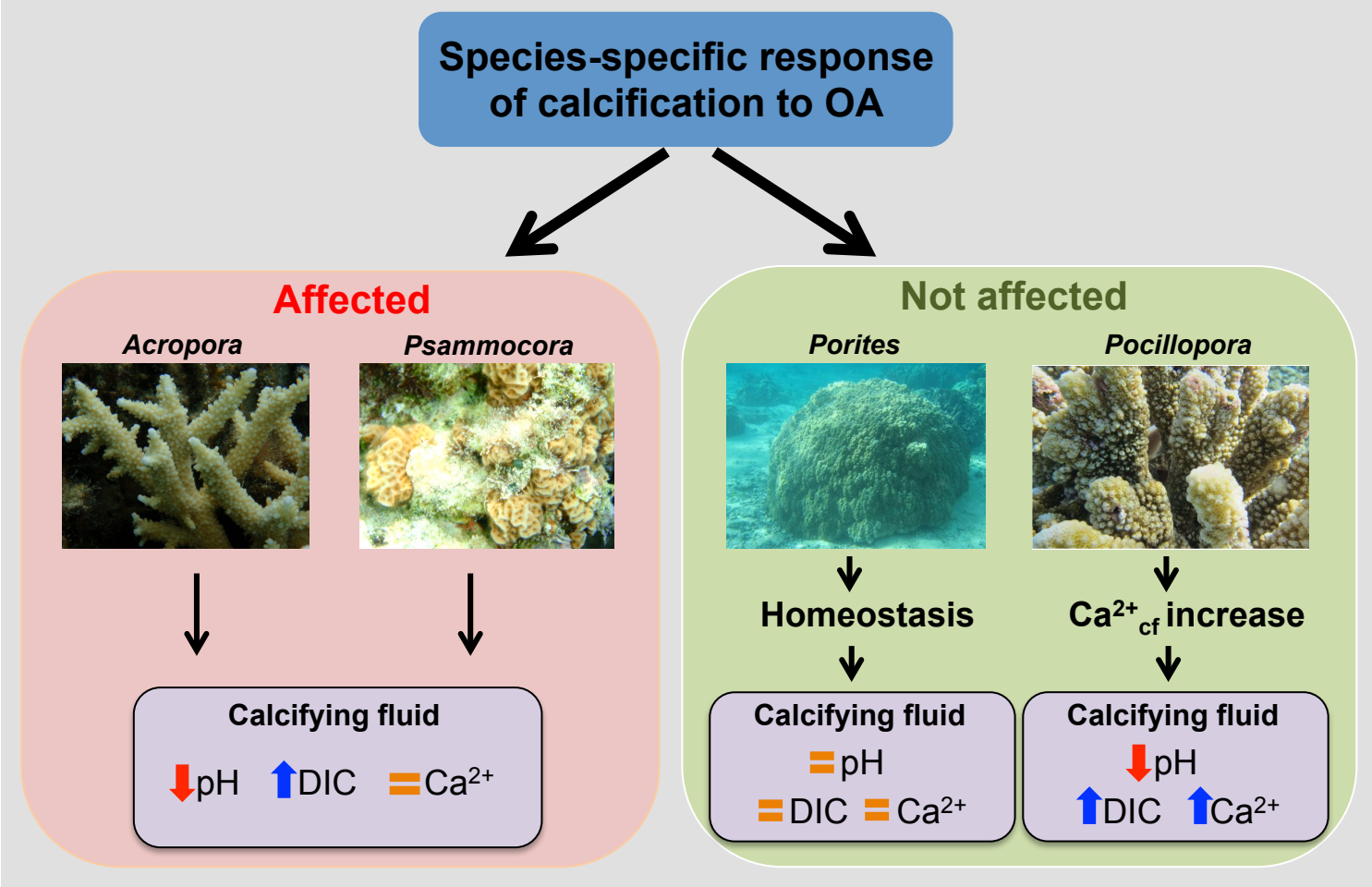

Species-specific response of calcification to OA
Affected
Acropora
Psammocora
Calcifying fluid
↓pH ↑DIC =Ca2+
Not affected
Porites
Pocillopora
Homeostasis
Ca2+cf increase
Calcifying fluid
=pH
=DIC =Ca2+
Calcifying fluid
↓pH
↑DIC ↑Ca2+

Fig. S1. Estimates of the aragonite saturation state in the calcifying fluid ($\Omega_{arag\ cf}$) of corals at the end of the 1-year incubation. Confocal Raman spectroscopy was used to estimate $\Omega_{arag\ cf}$ 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 ± 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 ($p < 0.05$).

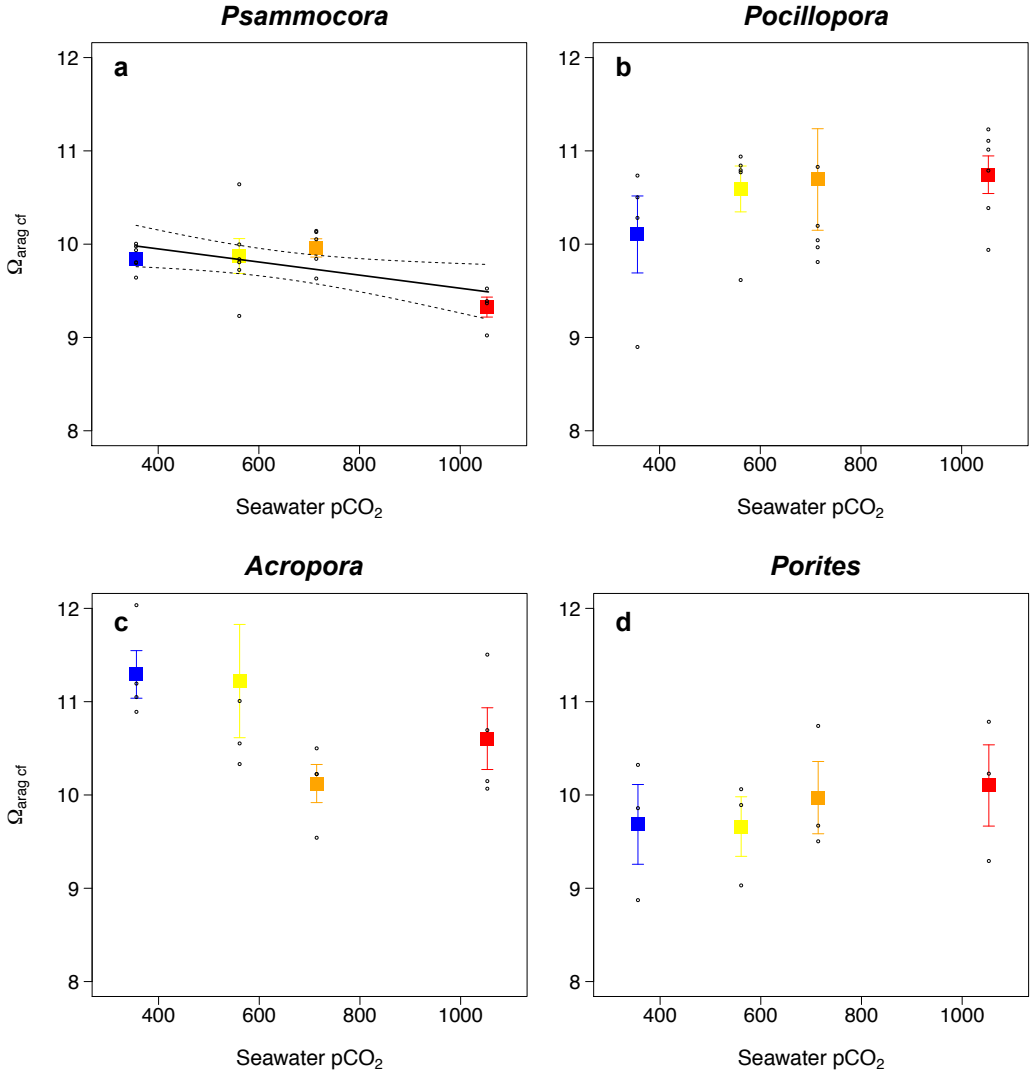


Fig S2. Temporal variations of *A. pulchra* and massive *Porites* sp. calcifying fluid Ω and Ca^{2+}_{cf}/ Ca^{2+}_{SW}. The skeleton of *A. pulchra* was sampled to select sections grown at the start of the experiment, and after ~ 0-3, 3-6, 6-9 and 9-12 months of experiment (panels A and B), while the 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.

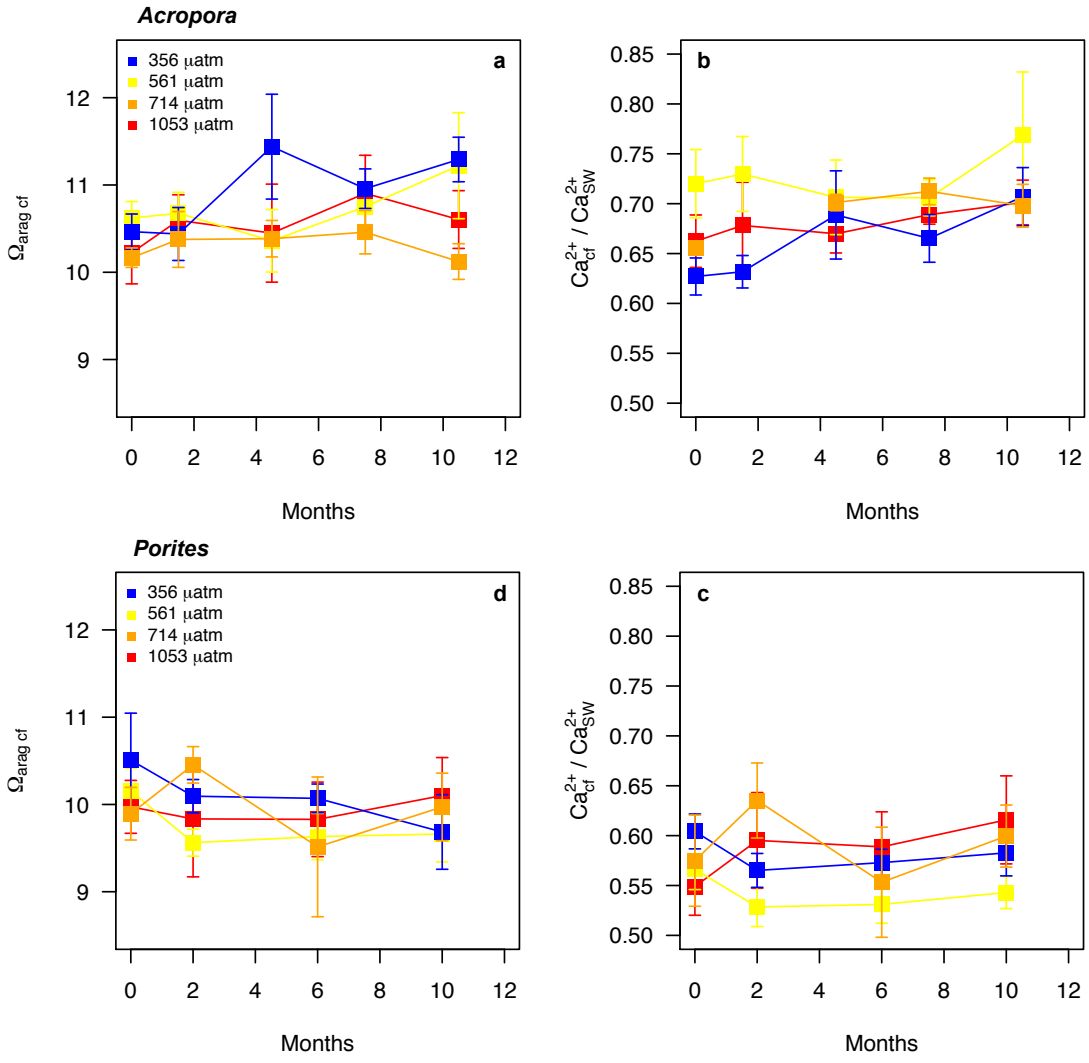


Fig. S3. Schematic showing the portions of the skeleton of *Acropora pulchra* that were selected to represent the conditions in the calcifying fluid at the start of the experiment (section 0), and after ~ 0-3 (section 1), 3-6 (section2), 6-9 (section 3), and 9-12 (section 4) months of experiment. The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

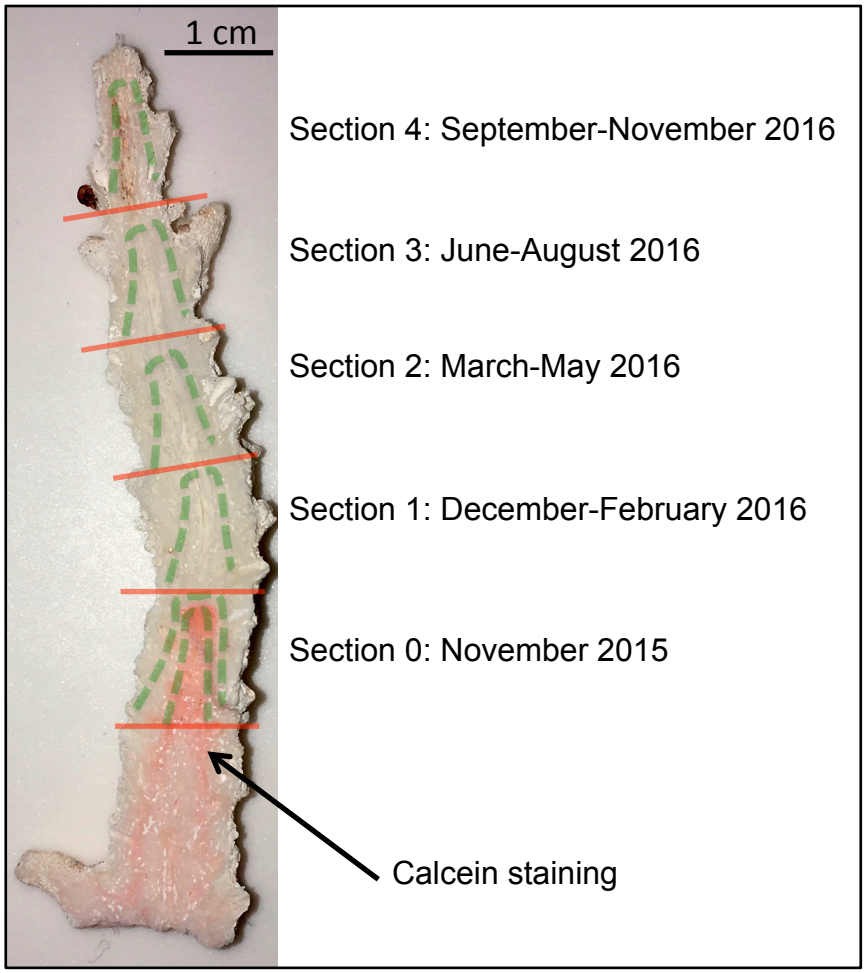


Table S1: Parameters of the linear estimates of the relationship between calcification, pH_{cf}, DICcf, Ca2+cf, and Ω_{cf}, and seawater pCO_2 (µatm).

Species	Parameter	Equation	Slope p-value	dF	F
Psammocora	Calcification	$0.63 - 1.68\ 10^{-4} * pCO_2$	**0.025**	18	5.958
	pH_{cf}	$8.61 - 1.49\ 10^{-4} * pCO_2$	**<0.001**	20	20.68
	DIC_{cf}	$2818 + 0.35 * pCO_2$	**0.0183**	18	6.738
	Ca^{2+}_{cf}	$0.73 + 1.10\ 10^{-5} * pCO_2$	0.39	18	0.775
	Ω_{cf}	$10.23 - 7.0\ 10^{-4} * pCO_2$	**0.025**	19	5.95
Pocillopora	Calcification	$0.31 + 6.12\ 10^{-5} * pCO_2$	0.326	20	1.015
	pH_{cf}	$8.56 - 1.79\ 10^{-4} * pCO_2$	**<0.001**	22	16.81
	DIC_{cf}	$2849 + 0.30 * pCO_2$	**0.0359**	21	5.026
	Ca^{2+}_{cf}	$0.77 + 2.19\ 10^{-4} * pCO_2$	**0.007**	19	8.934
	Ω_{cf}	$10.03 + 7.63\ 10^{-4} * pCO_2$	0.316	19	1.061
Acropora	Calcification	$0.56 - 1.97\ 10^{-4} * pCO_2$	**0.0465**	21	4.475
	pH_{cf}	$8.54 - 1.27\ 10^{-4} * pCO_2$	**0.016**	14	7.518
	DIC_{cf}	$3551 + 0.62 * pCO_2$	**0.005**	14	11.18
	Ca^{2+}_{cf}	$0.74 - 3.63\ 10^{-5} * pCO_2$	0.636	14	0.234
	Ω_{cf}	$11.6 - 1.2\ 10^{-3} * pCO_2$	0.162	14	2.18
Porites	Calcification	$0.18 + 8.68\ 10^{-5} * pCO_2$	0.466	18	0.5558
	pH_{cf}	$8.55 - 4.84\ 10^{-5} * pCO_2$	0.281	10	1.299
	DIC_{cf}	$3874 + 0.15 * pCO_2$	0.519	10	0.447
	Ca^{2+}_{cf}	$0.54 + 6.8\ 10^{-5} * pCO_2$	0.276	10	1.33
	Ω_{cf}	$9.40 + 6.7\ 10^{-4} * pCO_2$	0.36	10	0.921
Lithophyllum	Calcification	$0.12 - 5.83\ 10^{-5} * pCO_2$	**0.004**	14	11.64
	pH_{cf}	$8.77 - 9.05\ 10^{-5} * pCO_2$	**0.018**	12	7.489
Halimeda	pH_{cf}	$8.51 - 1.40\ 10^{-4} * pCO_2$	**<0.001**	7	51.12

Table S2: Parameters of the multiple linear estimates of the relationship between, pH_{cf}, DICcf, $Ca^{2+}{}_{cf}$, and Ω_{cf}, and seawater mean temperature, mean daily light, and pCO_2 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}, DICcf, $Ca^{2+}{}_{cf}$, and Ω_{cf}, and seawater mean temperature, mean daily light, and pCO_2 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